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Tandem Mass Spectrometry를 활용한 혈청, 소변, 양봉 시료 및 대표작물 중 다종농약다성분 동시분석

Simultaneous Analysis of Pesticide Multiresidues in Human Serum, Urine, Apiculture Samples, and Representative Crops Using Tandem Mass Spectrometry

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서울대학교 대학원
농생명공학부 응용생명화학전공
신 용 호

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Simultaneous Analysis of Pesticide Multiresidues in Human Serum, Urine, Apiculture Samples, and Representative Crops Using Tandem Mass Spectrometry

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Yongho Shin
Applied Life Chemistry Major
Department of Agricultural Biotechnology
Seoul National University

Abstract

Pesticides are used for the effective control of pests, microorganisms, and weeds from crops and have contributed to food security. It is necessary to determine as many pesticides as possible in human, the environment, and agricultural products due to the intrinsic toxicity and ecotoxicity of pesticides. In this study, a tandem mass spectrometry coupled to a gas chromatography (GC-MS/MS) or liquid chromatography (LC-MS/MS) was utilized to determine approximately four hundreds of pesticides in biological samples (serum and urine), apiculture samples (bee, pollen, and honey), and representative crops (pepper, orange, brown rice, and soybean). The scheduled multiple reaction monitoring (MRM) of the tandem mass spectrometer was employed in all methodologies to achieve rapid and simultaneous analysis and to obtain optimal sensitivity and selectivity of target analytes. The preparation methods for serum and urine were selected by comparing with the three versions of “Quick, Easy, Cheap, Effective, Rugged, and Safe” (QuEChERS) procedures. The optimized method was validated for 379 (serum) and 380 (urine) pesticides using LC-MS/MS. As a result, 94.5% (serum) and 95.8% (urine) of the total pesticides satisfied a limit of quantitation of 10 ng/mL. The established analytical method was applied to GC-MS/MS amenable pesticides (54 for serum and 55 for urine) and 53 analytes showed a limit of quantitation of 10 ng/mL. It was enough low sensitivity to determine pesticides in biological samples for forensic, clinical, and occupational exposure application. Apiculture samples, that is, bee (dead, healthy imago, and larva), pollen, and

honey were treated by optimized QuEChERS methods. Among the pesticide multiresidues, three neonicotinoids (clothianidin, imidacloprid, and thiamethoxam), which are expected to be totally banned for outdoor use in the European Union (EU) by the end of 2018, were subjected to method validation. The limit of quantitation of each analyte was 1 ng/g and it was sufficiently low to determine pesticide residues below the levels of acute oral toxicity (LD_{50}) of the bee. The field monitoring was conducted in two area near the apple orchard and pepper field in 2014. The analysis of the neonicotinoids and 391 multiresidue pesticides in apiculture samples were carried out. Based on residue levels, comprehensive honey bee exposure near farmland was able to be understood. Four representative crops were treated using miniaturized Multiclass Pesticide Multiresidue Method (No. 2) of the Korea Food Code, and the analytical method was evaluated for 384 pesticides using GC-MS/MS. As a result, 95.1-99.5% of the total pesticides satisfied the method limit of quantitation <10 ng/g in the crops, therefore the analytical method obtained the sufficient detection ability required by positive list system.

Key words: bee product, crop, GC-MS/MS, honey bee, LC-MS/MS, multiresidue, pesticide, serum, urine

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Table of Contents

Abstract.....i

Table of Contents.....iii

List of Tables.....x

List of Figures.....xiv

List of Supplementary Information.....xx

Preface.....1

Chapter I. Development and Validation of Pesticide Multiresidue Analysis in Human Serum and Urine Using LC-MS/MS and GC-MS/MS.....3

Introduction.....4

Pesticide intoxication.....4

Pesticide analysis in biological samples.....5

Advantage of the tandem mass spectrometry.....10

Preparation methodology for biological sample.....10

Purpose of the present study.....12

Part 1. Development and Validation of Pesticide Multiresidue Analysis in Human Serum and Urine Using LC-MS/MS.....13

Materials and Methods.....14

Chemicals and reagents.....14

Preparation of standard solutions.....14

LC-MS/MS parameters.....15

Comparison of three versions of QuEChERS.....16

Final established sample preparation.....17

Validation of analytical methods.....17

Safety information.....19

Results and Discussion.....20

Optimization of multiple reaction monitoring (MRM) in LC-MS/MS.....20

Relationship between partition-coefficient and retention time.....21

Optimization of sample extraction step.....26

Method validation.....38

Limit of quantitation (LOQ).....38

Linearity of calibration.....44

Accuracy and precision.....50

Recovery.....64

Matrix effect.....79

Conclusions.....90

Part 2. Development and Validation of Pesticide Multiresidue Analysis in Human Serum and Urine Using GC-MS/MS.....91

Materials and Methods.....92

Chemicals and reagents.....92

GC-MS/MS instrumental conditions.....92

Establishment of scheduled MRM.....93

Sample preparation using modified QuEChERS.....94

Validation of methodology.....94

Safety information.....95

Results and Discussion.....96

Characteristics of pesticide to be studied.....96

Optimization of MRM.....99

Determination of final selected pesticides to be validated.....102

Validation of analytical method.....103

Limit of quantitation (LOQ) and linearity of calibration.....103

Accuracy and precision.....110

Recovery.....111

Matrix effect.....114

Conclusions.....115

Chapter II. Analysis of Neonicotinoids (Clothianidin, Imidacloprid, and Thiamethoxam) and Pesticide Multiresidues in Honey Bee, Pollen, and Honey Using LC-MS/MS and GC-MS/MS.....119

Introduction.....120

Benefits from honey bee.....120

Honey bee Colony Collapse Disorder (CCD) 120

Neonicotinoid, a suspicious chemical leading to CCD.....121

Analysis of pesticide residues in apiculture samples.....122

Purpose of the present study.....123

Materials and Methods.....126

Chemicals and reagents.....126

Preparation of matrix-matched standards.....127

Sample collection.....127

Instrumental conditions of LC-MS/MS and GC-MS/MS.....133

LC-MS/MS.....133

GC-MS/MS.....134

MRM optimization in LC-MS/MS and GC-MS/MS.....135

Sample preparation.....136

Method validation for clothianidin, imidacloprid, and thiamethoxam.....137

Pesticide multiresidue screening in bee, pollen, and honey.....137

Statistical analysis.....138

Safety information.....138

Results and Discussion.....139

Body weights of honey bees.....139

MRM optimization.....141

Method validation for neonicotinoids.....143

Analysis of neonicotinoids (clothianidin, imidaclprid, and thiamethoxam) in bee, pollen, and honey.....145

Bee.....145

Pollen.....154

Honey.....162

Analysis of pesticide multiresidues in bee, pollen, and honey.....164

Conclusions.....180

Chapter III. Multiresidue Analysis for 384 Pesticides in Pepper, Orange, Brown Rice, and Soybean Using Florisil Solid-phase Extraction and GC-MS/MS.....183

Introduction.....184

Introduction of positive list system.....184

Tandem mass spectrometry for pesticide multiresidue analysis.....186

Solid-phase extraction for pesticide purification.....192

Multiclass Pesticide Multiresidue Method (No. 2) 193

Purpose of the present study.....195

Materials and Methods.....196

Chemicals and reagents.....196

Preparation of matrix-matched standard.....196

Instrumental conditions of GC-MS/MS.....197

Multiple reaction monitoring (MRM) profile optimization.....198

Sample preparation of pepper, orange, brown rice, and soybean.....198

Defatting procedure in soybean using *n*-hexane/acetonitrile partitioning...199

Method validation.....199

Results and Discussion.....201

MRM optimization and selection of pesticides to be validated.....201

Characteristics of 384 pesticides.....202

Comparison of the preparation procedures with/without *n*-hexane/acetonitrile partitioning.....203

Method limit of quantitation (MLOQ).....206

Instrumental repeatability.....209

Linearity of calibration.....210

Recovery.....215

Matrix effect.....224

Conclusions.....225

Supplementary Information.....228

References.....255

초록.....273

List of Tables

Table 1. **Representative pesticide analytical methods in biological samples.....7**

Table 2. **List of the 379 pesticides classified by chemical groups for the optimized analytical method in serum.....28**

Table 3. **List of representative chemical groups and 380 pesticides selected for the final method validation in urine.....30**

Table 4. **The number of pesticides with recoveries between 70-120% with RSDs below 20% in the recovery test from different extraction methods for 379 Pesticides in 100 μL of human serum (fortification Level at 250 ng/mL, n = 3).....36**

Table 5. **The number of pesticides with recoveries between 70-120% with RSDs below 20% in the recovery test from different extraction methods for 379 Pesticides in 100 μL of human urine (fortification Level at 250 ng/mL, n = 3).....37**

Table 6. **Distribution of linear ranges for 379 pesticides in serum for the final established analytical method.....46**

Table 7. **Distribution of correlation coefficients (r^2) for 379 pesticides in serum for the final established analytical method.....47**

Table 8. **Distribution of linear ranges for 380 pesticides in urine for the final established analytical method.....48**

Table 9. Distribution of correlation coefficients (r^2) for 380 pesticides in urine for the final established analytical method.....49

Table 10. Distribution of recovery and RSD range for 379 pesticides at fortification levels of 10, 50, and 250 ng/mL in serum for the final established analytical method.....68

Table 11. Pesticides for which recovery test results were not within 70-120% (RSD ≤20%) at all treated levels (10, 50, and 250 ng/mL), and intra-day accuracy results with RSD (serum).....70

Table 12. Distribution of recovery and RSD range for 380 pesticides at fortification levels of 10, 50, and 250 ng/mL in urine for the final established analytical method.....78

Table 13. List of pesticides to be studied and their chemical groups.....97

Table 14. The optimized GC-MS/MS parameters including retention times (t_R), MRM transitions for each pesticide.....100

Table 15. Representative pesticide multiresidue analytical method in apiculture samples.....124

Table 16. Sampling results in Giran during investigation period on April 24 to June 6, 2014.....131

Table 17. Sampling results in Yeongyang during investigation period on July 6 to August 7, 2014.....132

Table 18. The numbers of dead and healthy imago collected in the two areas and their total and average body weights.....140

Table 19. The established retention times (t_R), monoisotopic masses, quasi-molecular ion types, and MRM transitions of LC-MS/MS for the neonicotinoid pesticides.....142

Table 20. The limit of quantitation (LOQ), correlation coefficients (r^2), recovery results for neonicotinoid pesticides in bee, pollen, and honey samples.....144

Table 21. Distribution of neonicotinoid residues in dead imago at three sites in Giran.....147

Table 22. Distribution of neonicotinoid residues in dead imago at two sites in Yeongyang.....148

Table 23. Distribution of neonicotinoid residues in pollen at two sites in Giran.....155

Table 24. Distribution of neonicotinoid residues in pollen at two sites in Yeongyang.....156

Table 25. Distribution of neonicotinoid residues in honey in Giran and Yeongyang.....163

Table 26. Positive detection frequency for bee, pollen, and honey samples in Giran.....166

Table 27. Distribution of median values and residue ranges for pesticide multiresidues in Giran.....170

Table 28. Positive detection frequency for bee, pollen, and honey samples in Yeongyang.....174

Table 29. Distribution of median values and residue ranges for pesticide multiresidues in Yeongyang.....178

Table 30. The current pesticide regulation in crops and PLS to be introduced in the Republic of Korea (Ministry of Food and Drug Safety).....185

Table 31. Review of tandem mass spectrometry for pesticide multiresidues in agricultural products during three-year publication (2016-2018).....188

Table 32. Representative analytical methods for pesticide multiresidues including solid-phase extraction (SPE) cleanup procedure.....194

Table 33. Distribution of MLOQs for 384 pesticides in pepper, orange, brown rice, and soybean.....208

Table 34. Summary of instrumental repeatability to show distribution of RSD of area for 384 pesticides in pepper, orange, brown rice, and soybean (*n* = 7).....211

Table 35. Distribution of correlation coefficients (r^2) for 384 pesticides in pepper, orange, brown rice, and soybean.....214

Table 36. Distribution of recoveries for 384 pesticides in pepper, orange, brown rice, and soybean.....216

List of Figures

Fig. 1. **Scatter plot to show retention time (t_R) and partition-coefficient (log *P*) for 359 among 383 pesticides.....22**

Fig. 2. **Comparison of log *P* values calculated using the 1st order regression model and values calculated in other works.....24**

Fig. 3. **TIC obtained by LC-MS/MS analysis of (a) matrix-matched standard in human serum with 379 pesticides at 100 ng/mL (4 μL injection) and (b) TIC of control (non-fortified) serum sample.....32**

Fig. 4. **TIC obtained by LC-MS/MS analysis of (a) matrix-matched standard in human urine with 380 pesticides at 100 ng/mL (4 μL injection) and (b) TIC of control (non-fortified) urine sample.....34**

Fig. 5. **Pie chart showing distribution of LOQs (ng/mL) for 379 pesticides in serum for the final optimized analytical method. Light gray bar, 10 ng/mL; gray bar, 25 ng/mL; dark gray bar, 50 ng/mL; black bar, 100 ng/mL.....40**

Fig. 6. **Pie chart showing distribution of LOQs (ng/mL) for 380 pesticides in urine for the final optimized analytical method. Light gray bar, 10 ng/mL; gray bar, 25 ng/mL; dark gray bar, 50 ng/mL; black bar, 100 ng/mL.....42**

Fig. 7. Scatter plots for 379 pesticides in serum to show accuracies and precisions (RSD) in (a) intra-day and (b) inter-day tests (at 150 ng/mL of QC level).....52

Fig. 8. Percentage of 379 pesticides satisfying the accuracy values within 80-120% (RSD ≤20%) at 10 ng/mL and within 85-115% (RSD ≤15%) at 50, 150, and 250 ng/mL in the intra-day (grey bars) and inter-day (dark grey bars) tests using the final established method in serum sample.....56

Fig. 9. Scatter plots for 380 pesticides in urine to show accuracies and precisions (RSD) in (a) intra-day and (b) inter-day tests (at 150 ng/mL of QC level).....60

Fig. 10. The number of pesticides satisfying the accuracy range of 80-120% with RSD ≤20% at a QC level of 10 ng/mL and the accuracy range of 85-115 with RSD ≤ 15% at 50, 150, and 250 ng/mL levels under intra-day (grey bars) and inter-day (dark grey bars) conditions in urine sample.....62

Fig. 11. Distribution to show recovery values for 379 pesticides classified into the representative chemical groups (treated at 50 ng/mL in serum).....66

Fig. 12. Recovery results (treated at 250 ng/mL in serum) of three different QuEChERS extraction methods for pH-dependent pesticides that showed lower recovery rate in the validation test.....72

Fig. 13. Distribution of recovery rates for 380 pesticides by representative chemical groups at fortification levels of 50 ng/mL in urine.....76

Fig. 14. Scatter plot to show t_R and matrix effect of 379 pesticides in serum.....80

Fig. 15. Distribution of matrix effects (%) for 379 pesticides classified into soft effect (light grey bars, -20% to 0% and 0% to 20%), middle effect (grey bars, -50% to -20% and 20% to 50%), and strong effect (dark grey bars, <-50% and >50%) in human serum samples.....84

Fig. 16. Scatter plot between retention time (t_R) and matrix effect for 380 target pesticides in urine.....86

Fig. 17. Summary of matrix effects for 380 pesticides classified into soft effect (light grey bars, -20% to 0% and 0% to 20%), middle effect (grey bars, -50% to -20% and 20% to 50%), and strong effect (dark grey bars, <-50% and >50%) in human urine samples.....88

Fig. 18. Structures for phthalimide organochlorines, (a) captafol, (b) captan, and (c) folpet. MRM chromatograms for matrix-matched standards of (d) captafol, (e) captan, (f) folpet, and (g)-(i) these recovery samples in serum, and MRM chromatograms for matrix-matched standards of (j) captafol, (k) captan, (l) folpet, and (m)-(o) these recovery samples in urine.....104

Fig. 19. MRM chromatograms of (a) solvent-only standard, (b) matrix-matched standard in serum, and (c) matrix-matched standard in urine for binapacryl.....106

Fig. 20. Individual LOQs and correlation coefficients (r^2) of 55 pesticides for the final established analytical method in serum and urine.....108

Fig. 21. The number of pesticides satisfying the accuracy range of 80-120% with RSD ≤20% at a QC level of 10 ng/mL and the accuracy range of 85-115 with RSD ≤ 15% at QC levels of 50, 150, and 250 ng/mL in (a) serum and (b) urine under intra-day (grey bars) and inter-day (dark grey bars) conditions.....112

Fig. 22. Distribution of matrix effects for 380 pesticides in (a) serum and (b) urine. The matrix effect was classified into soft effect (light grey bars, -20% to 0% and 0% to 20%), middle effect (grey bars, -50% to -20% and 20% to 50%), and strong effect (dark grey bars, <-50% and >50%).....116

Fig. 23. Distribution of monitoring sites in the Republic of Korea.....128

Fig. 24. Distribution of residues for (a) clothianidin, (b) imidacloprid, and (c) thiamethoxam in dead imago samples at three sites in Giran.....150

Fig. 25. Distribution of residues for (a) clothianidin, (b) imidacloprid, and (c) thiamethoxam in dead imago samples at two sites in Yeongyang.....152

Fig. 26. Distribution of residues for (a) clothianidin, (b) imidacloprid, and (c) thiamethoxam in pollen samples at three sites in Giran.....158

Fig. 27. Distribution of residues for (a) clothianidin, (b) imidacloprid, and (c) thiamethoxam in pollen samples at two sites in Yeongyang.....160

Fig. 28. Distribution of the numbers of detection frequencies for fluvalinate, etofenprox, carbaryl, acetamiprid, and spiromesifen, which ranked first to fifth among the pesticide multiresidues by the detection frequency.....168

Fig. 29. Distribution of the numbers of detection frequencies for fluvalinate, etofenprox, acephate, etofenprox, flubendiamide, and flonicamid, which ranked first to fifth among the pesticide multiresidues by the detection frequency.....176

Fig. 30. Scan chromatograms (m/z 50-500) for control soybean samples of (a) partitioned and (b) non-partitioned procedures.....204

Fig. 31. Relative peak area (100 at 1st injection) of DDT-p,p', fenfuram, folpet, methoxychlor (pepper), and chlorothalonil (orange) at 50 ng/mL to show peak decreases as the number of injections increases.....212

Fig. 32. Percentages of pesticides satisfying recovery 70-120% (RSD ≤20%) classified by activity groups.....218

Fig. 33. Percentages of pesticides satisfying recovery 70-120% (RSD ≤20%) classified by chemical groups at (a) 0.01 mg/kg and (b) 0.05 mg/kg.....222

Fig. 34. Distribution of matrix effects for 384 pesticides in pepper, orange, brown rice, and soybean. Group 3 and 4 are included in soft matrix effect, Group 2 and 5 in medium effect, and Group 1 and 6 in strong effect.....226

List of Supplementary Information

Table S1. **The retention times (t_R), monoisotopic masses, quasi-molecular ion types, and MRM transitions of LC-MS/MS for the multiresidual pesticides.....228**

Table S2. **The optimized GC-MS/MS parameters including retention times (t_R), MRM transitions for each pesticide.....233**

Table S3. **List of general pesticide information for 384 pesticides.....239**

Preface

Pesticides are used worldwide for the control of insects, microorganisms, fungi, and other harmful pests in order to protect agricultural products. According to a U.S. Environmental Protection Agency (EPA) report, world pesticide expenditure at the producer level was $55,921 million in 2012 (Atwood and Paisley-Jones, 2017). In the United States, $8,866 million was reported on the same basis, corresponding to 16% of the world pesticide market (Atwood and Paisley-Jones, 2017). In the Republic of Korea, the Korea Crop Protection Association's agrochemical book reported that the amount of pesticide shipment was 19,798 tons in 2016 (Korea Crop Protection Association, 2017).

Use of pesticide has contributed to improving productivity, protection of crop losses/yield reduction, and food quality (Aktar et al., 2009). Cooper and Dobson (2007) reported that the use of pesticides has contributed to the improvement of crop/livestock yields and quality, increased shelf life of produce, and prevention of harmful organisms from interfering in human activities and structures, from which secondary benefits such as national agricultural economic development, reduced maintenance costs, or quality of life improvement have followed (Cooper and Dobson, 2007).

Although pesticide has been a great influence on food security over the decades, its toxicological/ecotoxicological effects on human and the ecosystem also cannot be ignored. It is important to maintain pesticide residues below sustainable levels in crops and the environment and to monitor in human, environmental indicators, and food for safety management.

The purpose of the study is the analysis of pesticide multiresidues in biological, apiculture samples, and representative crops. For the effective and high-throughput multiresidue analysis, the scheduled multiple reaction monitoring (MRM) mode of gas or liquid chromatography-tandem mass spectrometry (GC-MS/MS or LC-MS/MS) was employed in every methodology.

The study comprises three chapters. In **Chapter I**, novel bioanalytical methods for multiresidual pesticides in serum and urine were developed using LC-MS/MS (**Part 1**) after the comparison of three scaled-down QuEChERS methods and validated with various parameters. These methodologies were applied for GC-MS/MS amenable pesticides and the validation results in serum and urine were discussed in **Part 2**. In **Chapter II**, modified QuEChERS methods for neonicotinoids (clothianidin, imidacloprid, and thiamethoxam) were validated in honey bee, pollen, and honey. With this analytical method and multiresidue screening method, pesticide residues in apiculture samples were determined and risk assessment was attempted for some pesticides in an aspect of ecotoxicology. In the last chapter, Multiclass Pesticide Multiresidue Method (No. 2) of the Korea Food Code was modified by scaling-down, and approximately two hundreds of pesticides were newly verified with an original GC-MS/MS list (about 200 pesticides) in the method (**Chapter III**). The reinforced analytical method was validated and evaluated in four representative crops (pepper, orange, brown rice, and soybean).

This pesticide multiresidue research provides a comprehensive methodology for the residue determination in three major fields such as forensic/clinical sciences, ecotoxicology, and agricultural/food chemistry.

Chapter I

Development and Validation of Pesticide Multiresidue Analysis in Human Serum and Urine Using LC-MS/MS and GC-MS/MS

Introduction

Pesticide intoxication

One of the major disadvantages of pesticides affected by human is that these chemicals cause acute poisoning problems. Acute intoxication symptoms caused by pesticides range from mild symptoms such as nausea, headache, and paresthesia to fatalities (Thundiyil et al., 2008). Pesticide intoxication resulting from intentional intake or misuse is a major social issue. Gunnell et al. (2007) investigated the global distribution of suicide by pesticide and estimated that there are 258,234 (plausible range from 233,997 to 325,907) suicides from pesticide poisoning each year, representing 30% (27% to 37%) of all suicides worldwide (Gunnell et al., 2007). In the United States, 234 deaths by pesticide poisoning were identified over a 10 year span (1999 to 2008) according to the Centers for Disease Control and Prevention's Wide-ranging Online Data for Epidemiologic Research (CDC WONDER) report, and an average of 20,116 people were exposed to pesticides annually, accounting for 17.8% of treatment in healthcare facilities from 2006 to 2010 (Langley and Mort, 2012). In the Republic of Korea, 16,161 reports of mortality and 45,291 reports of inpatient and outpatient treatment related to pesticide intoxication were reported during 5 years (2006 to 2010) (Cha et al., 2014).

Various occupational researches have revealed that a large number of farmers have experienced pesticide intoxication. Calvert et al. (2008) investigated 3,271 cases of acute pesticide poisoning in the United States from 1998 to 2005 and reported that 2,334 (71%) were employed as farmworkers (Calvert et al., 2008). It was reported that up to 25 million cases of pesticide

intoxication may be experienced by agricultural workers in the Asian developing country (Jeyaratnam, 1990). In the Republic of Korea, it was reported that 22.9% of 1,958 male farmers had experienced acute intoxication symptoms within 48 h after using pesticides in 2010 (Kim et al., 2013). More recently, Lee and coworkers in 2015 have surveyed 663 farmers in Gyeong-gi province, South Korea, and 44 (6.63%) of them responded that they had experienced acute poisoning within 24 h of spraying pesticide directly or indirectly during 2013-2014 (Lee et al., 2015).

Pesticide analysis in biological samples

Biological monitoring of pesticide poisoning is useful for identifying evidence of health problems in the environment/ecotoxicology, in the agricultural and forensic fields, or for detoxification in a medical institution. Human biological samples such as blood, urine, hair, and saliva have been primary sources for determination of pesticides (**Table 1**).

Among the biological samples, blood is a regulated fluid, which means that its volume does not vary substantially with water intake or other factors (Barr et al., 2002). Therefore, blood is available without further dilutions for determination of the internal concentration of pesticides. It is also advantageous that pesticides are present in blood as parent compounds instead of their metabolites as usually found in urine (Wessels et al., 2003), and blood has less risk of exogenous or endogenous contamination compared to hair (Altshul et al., 2004). Because only a few milliliters of blood from adults or less in the case of children can be obtained, analytical methods for a few tens or several hundreds of microliters of blood samples have been developed and validated to

overcome the sample volume problem (Mostafa et al., 2011; Saito et al., 2013; Wittsiepe et al., 2014). Serum analysis is usually preferred over whole blood analysis, because serum has a minor matrix complexity, and is a more homogenous material (Gill et al., 1996; Hernández et al., 2002). Therefore, one or more cleanup steps can be reduced with serum samples compared to whole blood (Lacassie et al., 2001a; Hernández et al., 2002).

Urine also has several advantages over other samples. Urine is easier to obtain than invasive samples such as blood, and larger amounts of urine are available compared with blood, hair, and saliva. Because urine is a homogeneous biological fluid composed of 95% water (Cortéjade et al., 2016), complex preparation steps for purification of target pesticides are not needed. Although most pesticides are metabolized rapidly in the body and excreted in urine as free metabolites, mercapturate detoxification products, and/or glucuronide or sulfate-bound compounds within 48 h (Hernández et al., 2005), various chemical groups of pesticides still remain intact and present in urine (Montesano et al., 2007; Usui et al., 2012; Quansah et al., 2016). It is easier and less costly to obtain analytical standards of pesticides rather than those of metabolites.

Screening of as many parent compounds as possible is also needed in many applications because there have been deaths resulting from various chemical groups of pesticides (Lee et al., 2010), some of them (e.g., benzoximate and etofenprox) showing very low acute toxicity (LD_{50} >10,000 mg/kg; oral acute for rat) (Turner, 2015).

Table 1. Representative pesticide analytical methods in biological samples

No.	Matrix	Instrument	Sample preparation	Number of analytes	Reference
1	Blood	GC-MS	LLE[1)]	11	(Papoutsis et al., 2012)
2	Saliva	TD-ESI[2)]/MS	LLE	5	(Lee et al., 2016)
3	Serum	GC-MS/MS	SPE[3)]	20	(Chang et al., 2016)
4	Blood	LC-MS/MS, LC-(IT[4)])MS/Orbitrap, and GC-MS	QuEChERS[5)]	64	(Plassmann et al., 2015)
5	Serum and urine	LC-MS/MS	SPE	3	(Watanabe et al., 2014)
6	Urine	LC-MS/MS	SPE	6	(Ueyama et al., 2014)
7	Blood	LC-MS/MS and LC-MS/TOF[6)]	QuEChERS	215	(Kim et al., 2014)
8	Serum and urine	LC-(ICP[7)])MS	Dilute-and-shoot	4	(Kazui et al., 2014)
9	Urine	LC-MS/MS	LLE	1	(Garner and Jones, 2014)
10	Serum	GC-MS/TOF	SPE	50	(Fan et al., 2014)
11	Serum	LC-MS/MS	PP[8)]	29	(Dong et al., 2014)
12	Serum	GC-MS	CC[9)]	4	(Azandjeme et al., 2014)

Table 1. *(Continued)*

No.	Matrix	Instrument	Sample preparation	Number of analytes	Reference
13	Serum	LC-MS/MS	Monolithic spin column	16	(Saito et al., 2013)
14	Hair and Urine	LC-MS	SLE[10)] and LLE	2	(Kavvalakis et al., 2013)
15	Blood and urine	LC-MS/MS	QuEChERS	6	(Usui et al., 2012)
16	Blood and urine	GC-MS	SPE	1	(Takayasu et al., 2012)
17	Hair	GC-MS/MS	SPME[11)]	50	(Schummer et al., 2012)
18	Serum and urine	LC-FLD	Dilute-and-shoot	2	(Esteve-Romero et al., 2012)
19	Serum and urine	GC-MS	Monolithic spin column	3	(Saito et al., 2011)
20	Plasma	LC-MS/MS	PP	3	(Mostafa et al., 2011)
21	Serum	GC-MS	SPME	2	(Kasiotis et al., 2011)
22	Urine	GC-(IT)MS/MS and LC-MS/MS	SPE	>200	(Cazorla-Reyes et al., 2011)
23	Urine	LC-MS/MS	LLE	6	(Montesano et al., 2007)
24	Blood	GC-(IT)MS/MS	SPME	11	(Hernández et al., 2002)

Table 1. *(Continued)*

No.	Matrix	Instrument	Sample preparation	Number of analytes	Reference
25	Serum and plasma	GC-HR[12)]MS	SPE	29	(Barr et al., 2002)
26	Blood, serum	GC-MS	SPE	29	(Lacassie et al., 2001a)
27	Serum	LC-MS and GC-MS	SPE	61	(Lacassie et al., 2001b)
28	Serum and urine	LC-MS/MS	PP and Direct injection	2	(Sancho et al., 2000)

[1)]Liquid-liquid extraction

[2)]Thermal desorption electrospray

[3)]Solid-phase extraction

[4)]Ion-trap

[5)]Quick, Easy, Cheap, Effective, Rugged, and Safe

[6)]Time-of-flight

[7)]Inductively coupled plasma

[8)]Protein precipitation

[9)]Column chromatography

[10)]Solid-liquid extraction

[11)]Solid-phase microextraction

[12)]High resolution

Advantage of the tandem mass spectrometry

In the case of pesticide intoxication, analysis of multiresidue pesticides with high reliability and speed is important in order to identify unknown compounds for further medical treatment and forensic investigation. Traditional liquid chromatography (LC) and gas chromatography (GC) have many limitations in specificity, sensitivity, and speed for multiresidue analysis (Aysal et al., 2007; Moliner-Martínez et al., 2011). Furthermore, both conventional instruments require partitioning or cleanup procedures to remove interference, which takes a long time, uses high volumes of solvents, and may remove target compounds during extensive cleanup steps. A single quadrupole (SQ) mass filter overcomes some problems by performing selected ion monitoring (SIM) but still can fail to distinguish a target pesticide from other pesticides or interferences with a similar retention time (t_R) and m/z. Tandem mass spectrometry coupled with liquid chromatography (LC) or gas chromatography (GC) has been widely utilized. Among the tandem mass spectrometry, triple quadrupole (TQ) analyzers is a powerful analytical technique for quantitative detection of a broad range of pesticides in short time with simultaneous manner by operating in multiple reaction monitoring (MRM) mode in biological monitoring.

Preparation methodology for biological sample

In various alternative cleanup procedures for serum and urine sample, column chromatography (CC), solid-phase extraction (SPE), liquid-liquid extraction (LLE) have been reported as representative preparation methods (**Table 1**). Protein precipitation (PP) with acetonitrile solvent is specific for blood (serum) sample due to its protein molecule (Sancho et al., 2000; Dong et al., 2014). CC

and SPE are advantageous for a few specific target compounds, but take much time and effort to conduct and have some difficulty finding optimum washing/elution conditions covering various chemical properties. LLE and PP are more convenient than CC or SPE, but have similar drawbacks to CC or SPE and interferences of serum or urine may remain in the extract to cause a serious matrix effect or lead to low extraction efficiency.

The aqueous characteristic of urine and advanced separation techniques such as LC- or GC-MS/MS make urine preparation relatively convenient and easy. Direct injection or dilute-and-shoot procedures are the simplest ways to identify pesticides in urine (Esteve-Romero et al., 2012; Kazui et al., 2014; Cortéjade et al., 2016). Nevertheless, these processes have major problems in that urinary salts or macromolecules may decrease the sensitivity of an instrument or cause severe clogging on the injection syringe or ESI probe.

The QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method is a preparation method with strong extraction efficiency and convenience in multiresidue analysis. Since the first unbuffered QuEChERS analytical method for crops was developed in 2003, a number of improved QuEChERS methods have been validated and applied (Anastassiades et al., 2003). Among these methods, AOAC 2007.01 and EN 15662 methods, in which buffer reagents are contained for adjustment of sample pH, have been used widely with advantages in extraction rate (recovery) of pH-dependent pesticides (Lehotay, 2007; EN 15662, 2008). Recently, QuEChERS methods have been used for biological samples in clinical and forensic toxicology (Usui et al., 2012; Kim et al., 2014; Plassmann et al., 2015).

Purpose of the present study

In this study, a simultaneous multiresidue screening analytical method in human serum and urine was developed and validated using LC- and GC-MS/MS. The scheduled MRMs and retention times (t_R) for each analyte were optimized for qualification and quantitation within 15 minutes (LC-MS/MS) and 30 minutes (GC-MS/MS) per sample. This chapter of the study is comprised of two part. In **Part 1**, 379 pesticides in serum and 380 in urine were investigated using LC-MS/MS. Three different versions of QuEChERS extraction methods were compared and modified to use a very small sample volume (100 μL) without using dispersive SPE (dSPE) in the cleanup procedure. Using the established method in **Part 1**, 54 pesticide in serum and 55 in urine were evaluated using GC-MS/MS in **Part 2**. It was found that acceptable validation data (limit of quantitation (LOQ), linearity of calibration, accuracy and precision, recovery, and matrix effect) for most pesticides were obtained. This fast and convenient analytical method is applicable for biomonitoring of pesticide multiresidues in serum and urine samples from food toxicology, agricultural operator exposure, clinical and forensic studies and investigation.

Part 1

Development and Validation of Pesticide Multiresidue Analysis in Human Serum and Urine Using LC-MS/MS

Materials and Methods

Chemicals and reagents

Individual pesticide standards (purity >98%) or stock solutions (1,000 mg/L) for quality control (QC) were obtained from ChemService (West Chester, PA), Dr. Ehrenstorfer (Augsburg, Germany), Sigma-Aldrich (St, Louis, MO), Wako Pure Chemical Industries (Osaka, Japan), and ULTRA Scientific (North Kingstown, RI). Ammonium formate (≥99.0%), formic acid (LC-MS grade), acetic acid (HOAc, ≥99.7%), magnesium sulfate anhydrous ($MgSO_4$, ≥99.5%), sodium acetate anhydrous (NaOAc, ≥99.0%), sodium citrate dibasic sesquihydrate ($Na_2HCitr{\cdot}1.5H_2O$, ≥99.0%), and sodium citrate tribasic dihydrate ($Na_3Citrate{\cdot}2H_2O$, ≥99.0%) were purchased from Sigma-Aldrich. Sodium chloride (NaCl, 99.0%) was obtained from Samchun (Gyeonggi-do, South Korea). Methanol and acetonitrile (HPLC grade) were purchased from Fisher Scientific (Seoul, South Korea). Ceramic homogenizers (2 mm) were purchased from Ultra Scientific. Deionized water was prepared in house using LaboStar TWF UV 7 (Siemens, MA). Serum from human male was obtained from Sigma-Aldrich. Human urine was collected from healthy volunteers with the permission of the Institutional Review Board (IRB) at Seoul National University, Seoul, the Republic of Korea. Samples were stored at -70 °C until preparation and analysis.

Preparation of standard solutions

Individual pesticide stock solutions (1,000 mg/L) were prepared in acetonitrile. For pesticides that were difficult to dissolve at this concentration level (e.g.,

carbendazim), acetone, methanol, or water were used instead of acetonitrile or lower concentrations of stock solutions were prepared so that these components could be sufficiently dissolved. To prepare four groups of intermediate mixed stock solutions at 10 mg/L, a portion of each stock solution was brought up with acetonitrile in a 25-mL volumetric flask. The aliquots of intermediates were again mixed to make a final mixed standard solution at 2.5 mg/L. This was diluted with acetonitrile to make the mixed working standard solutions of lower concentrations for preparing calibration curves and using in several validation procedures.

LC-MS/MS parameters

LC-MS/MS analysis was carried out on a Shimadzu Nexera X2 UHPLC system coupled to a Shimadzu LCMS-8050 triple quadrupole mass spectrometer (Kyoto, Japan). The UHPLC system comprised a solvent delivery module (LC-30AD), column oven (CTO-20A), autosampler (SIL-30AC), and degassing unit (DGU-20A5R). A Kinetex C18 column (100 × 2.1 mm, 2.6 µm, Phenomenex, Torrance, CA) was used for analyte separation, and a SecurityGuard Ultra guard column (Phenomenex) was connected to the column to prevent contamination. The oven temperature was maintained at 40 °C. The total flow rate of the mobile phase was 0.2 mL/min. For the mobile phases, solvent A was 5 mM ammonium formate and 0.1% formic acid in water and B was 5 mM ammonium formate and 0.1% formic acid in methanol. For the gradient program, mobile phase B was initialized at 5%, and after 0.5 minutes, B was raised to 55% for 0.5 min, ramped to 95% for 7 min, held for 3 min, raised to 100% for 1 min, then dropped sharply to 5% for 0.1 min, and held for

2.9 min. The total analytical time was 15.0 min, and the injection volume was 4 μL. LabSolutions software (version 5.72) was used for multiresidue MRM data processing.

In the mass spectrometer system, ionization of target analytes was performed by a heated electrospray ionization (ESI) with positive/negative switching mode. The interface, desolvation line (DL), and heat block temperature were 300, 250, and 400 °C, respectively. The heating gas (air), nebulizing (nitrogen), and drying gas (nitrogen) flow were 10, 3, and 15 L/min, respectively. The collision-induced dissociation (CID) gas was argon. For MS/MS analysis, each standard solution (0.1-1 mg/L) was injected without the column to obtain a full scan spectrum (m/z 50-500 or m/z 100-1,000). A precursor ion (e.g., $[M+H]^+$) was selected from the spectrum data and subjected to collision with several collision energy (CE) voltages to find the two product ions that showed the highest and the second highest detection intensity. The former ion transition was used as a quantifier for quantitation of the target compound, and the latter was used as a qualifier for its reference. This MRM was scheduled by the retention time of each compound, and the MRM detection window was ±0.5 min. Finally, dwell times (≥ 2.0 ms) were adjusted automatically based upon loop time (0.12 s) for the maximized data acquisition. LabSolutions (version 5.72) as LCMS software was utilized for data processing.

Comparison of three versions of QuEChERS

Human serum and urine (0.1 mL) were extracted with three different QuEChERS extraction reagents scaled-down as follows: (A) original QuEChERS (Anastassiades et al., 2003) procedure (0.4 mL of acetonitrile, 40

mg of $MgSO_4$, and 10 mg of NaCl); (B) QuEChERS of AOAC 2007.01 (Lehotay, 2007) procedure (1% HOAc in acetonitrile (0.4 mL), 40 mg of $MgSO_4$, and 10 mg of NaOAc); and (C) QuEChERS of EN 15662 (EN 15662, 2008) procedure (0.4 mL of acetonitrile, 40 mg of $MgSO_4$, 10 mg of NaCl, 10 mg of $Na_3Citrate \cdot 2H_2O$, and 5 mg of $Na_2HCitr \cdot 1.5H_2O$). The extract from each method was centrifuged and 0.2 mL of supernatants were mixed with 0.05 mL of acetonitrile for matrix-matching. Each of serum and urine sample was equivalent to 0.2 mL per mL of final extract. Finally, 4 μL of the sample was analyzed by LC-MS/MS.

Final established sample preparation

Each serum and urine (0.1 mL) sample in a 2-mL microcentrifuge tube was extracted respectively, with 0.4 mL of acetonitrile by shaking for 1 min at 1,200 rpm using a Geno Grinder (1600 MiniG SPEX Sample Prep, Metuchen, NJ). Forty milligrams of $MgSO_4$ and 10 mg of NaCl were added under ice bath conditions to prevent heat caused by $MgSO_4$. The tube was centrifuged for 5 min at 13,000 rpm using microcentrifuge (17TR, Hanil Science, Seoul, the Republic of Korea). The supernatant (0.2 mL) was transferred into a 2-mL amber glass vial and mixed with 0.05 mL of acetonitrile for matrix-matching. Without further cleanup steps, 4 μL of the final extraction sample was taken into LC-MS/MS for analysis of target analytes.

Validation of analytical methods

For determination of the LOQ and linearity of calibration, matrix-matched procedure standard solutions at 10, 20, 50, 100, 150, and 250 ng/mL were

analyzed. The minimum concentration satisfying a signal to noise ratio (S/N) greater than 10 on the chromatogram was selected as the LOQ.

The linearity of calibration was evaluated (n = 5) by the correlation coefficient (r^2) of the calibration curve from 10 to 250 ng/mL. The r was calculated using the following equation (Almeida et al., 2002):

$$r = \frac{\sum w_i \cdot \sum w_i x_i y_i - \sum w_i x_i \cdot \sum w_i y_i}{\sqrt{\sum w_i \cdot \sum w_i x_i^2 - (\sum w_i x_i)^2} \cdot \sqrt{\sum w_i \cdot \sum w_i y_i^2 - (\sum w_i y_i)^2}}$$

where: w_i = a weighting regression factor

x_i and y_i = ith data pair of n total data pairs

A weighting regression factor of *1/x* ($w_i = 1/x_i$) was adopted to minimize calculation error at low concentrations. By using the weighting method, a 1st order linear regression model ($y = a + bx$) from the least squares approximation was converted adding weighting factor w_i (Almeida et al., 2002).

$$b = \frac{\sum w_i \cdot \sum w_i x_i y_i - \sum w_i x_i \cdot \sum w_i y_i}{\sum w_i \cdot \sum w_i x_i^2 - (\sum w_i x_i)^2}$$

$$a = \frac{\sum w_i x_i^2 \cdot \sum w_i y_i - \sum w_i x_i \cdot \sum w_i x_i y_i}{\sum w_i \cdot \sum w_i x_i^2 - (\sum w_i x_i)^2}$$

where: b = slope of the regression equation

$a = y$ intercept of the regression equation

Accuracy and precision tests were performed using a QC sample (a sample with a known quantity of analyte (US FDA, 2013)) at 10, 50, 150, and 250 ng/mL levels. The intra-day tests were conducted by analyzing five replicates of each

treated level in a single day. The inter-day tests were carried out by analyzing one QC sample of each treated level per day for five separate days.

To verify the extraction efficiency of the preparation process, recovery tests at fortification levels of 10, 50, and 250 ng/mL were conducted. Five μL of mixed working standard solutions (200, 1,000, and 5,000 ng/mL) in acetonitrile were fortified in 0.1 mL of each blank serum or urine, respectively and the treated samples were prepared as the final established preparation procedures (n = 3). Recovery of target compounds was determined using calibration curves of matrix-matched standards to compensate for matrix effects in LC-MS/MS analysis.

The matrix effect was also calculated by comparing the slope of the calibration curve of the matrix-matched standards with that of the calibration curve of the solvent-based standards using the following equation:

$$\text{Matrix effect}, \% = \left(\frac{\text{Slope of matrix-matched standard calibration}}{\text{Slope of solvent-based standard calibration}} - 1\right) \times 100$$

Safety information

All pesticide standards and reagents used in this study were handled according to the Material Safety Data Sheet (MSDS)'s safety instructions. For all instrumentation, the manufacturer's safety information was followed and implemented.

Results and Discussion

Optimization of multiple reaction monitoring (MRM) in LC-MS/MS

For the determination of MRM transition profiles, a full scan analysis was first performed with 400 pesticides. In this step, 17 compounds (binapacryl, bromophos-methyl, chlorpropham, cyanophos, cyfluthrin, dichlofluanid, dicofol, disulfoton, endosulfan-sulfate, ethalfluralin, isofenphos, isofenphos-methyl, nitrothal-isopropyl, oxyfluorfen, parathion-methyl, silafluofen, and spiromesifen) did not give a suitable quasi-molecular ion (precursor ion) and were excluded. These compounds were analyzed using GC-MS/MS in **Part 2**. The remaining 383 components successfully produced precursor ions. Among them, 326 target compounds were $[M+H]^+$ quasi-molecular ion form, 24 compounds were $[M+NH_4]^+$ form, seven compounds (abamectin B1a, alanycarb, aldicarb, butocarboxim, lepimectin A3, lepimectin A4, and pyribenzoxim) were $[M+Na]^+$ form, and two compounds (milbemectin A3 and milbemectin A4) were $[M+H-H_2O]^+$ form in the ESI positive mode. Twenty three pesticides were $[M-H]^-$ form and dithianon showed an ion form $[M\cdot]^-$ in the ESI negative mode. After CID step, quantifier and qualifier ions were selected depending on intensity. After MRM optimization steps, retention time and sensitivity of target compounds were verified using both the solvent-based standards (acetonitrile) and matrix-matched standard of serum and urine. However, folpet was rejected after this step due to its poor response in all standard types (further discussed in **Part 2**).

Relationship between partition-coefficient and retention time

The partition-coefficient, abbreviated *P*, is the ratio of the concentration of a compound in liquid A and B when the two-layer solution is equilibrium at a constant pH and temperature. Usually, un-ionized water and octanol are used as liquid A and B. In this case, *P* value is a parameter of hydrophobicity. The *P* value is expressed as the logarithm and calculated using the following equation:

$$\log P = \log(\frac{[solute]_{octanol}}{[solute]_{water}})$$

There have been attempts to measure log *P* from the retention times (t_R) of various compounds using HPLC (Valko et al., 2001). In this study, log *P* and retention time (t_R) established from MRM profiles were investigated and the relationship between the two parameters was verified. Among the 383 pesticides, log *P* values for 359 compounds were found (MacBean, 2012; Turner, 2015). The correlation coefficient (*r*, unweighted) value between t_R and log *P* was +0.8737 (**Fig. 1**). The results showed that t_R and log *P* have strong positive correlations. Using the 1st order regression model (y = 0.8701x - 2.3548), log *P* values for the remaining 24 pesticides that have no log *P* data were predicted (**Fig. 2**). The results were compared to the calculated log *P* data from other works (IUPAC; Chemicalize.org, 2017). The results showed that the log *P* values of most pesticides from the two data sources were similar. Unlike these compounds, cartap exhibited a significant difference (>3) between the two data. In order to establish a more elaborate model to explain well between the two variables, more information on pesticides such as pKa is needed in addition to log *P*.

Fig. 1. Scatter plot to show retention time (t_R) and partition-coefficient (log *P*) for 359 among 383 pesticides

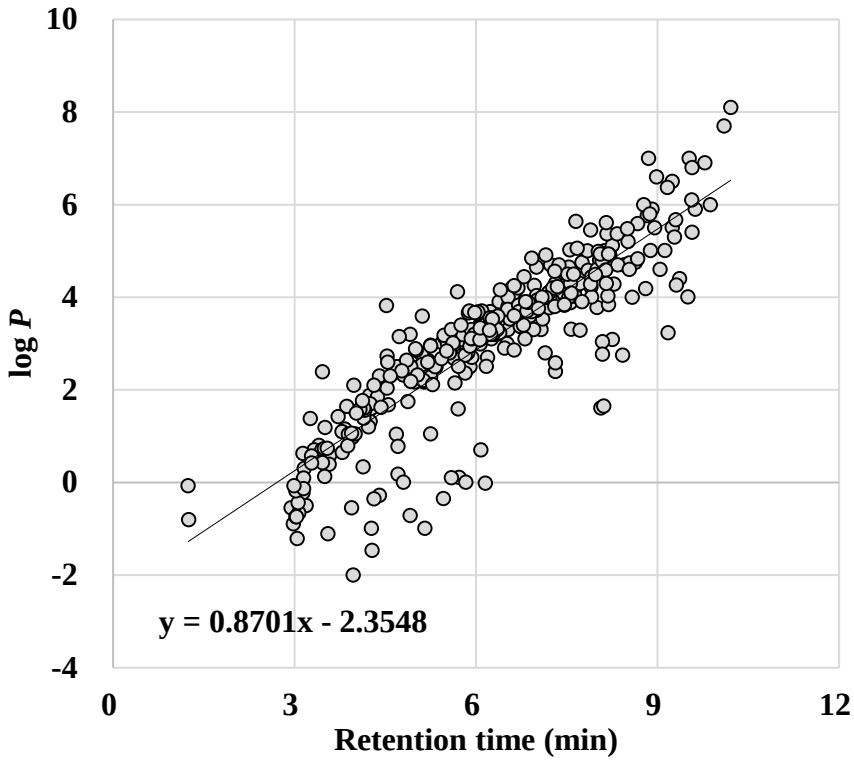
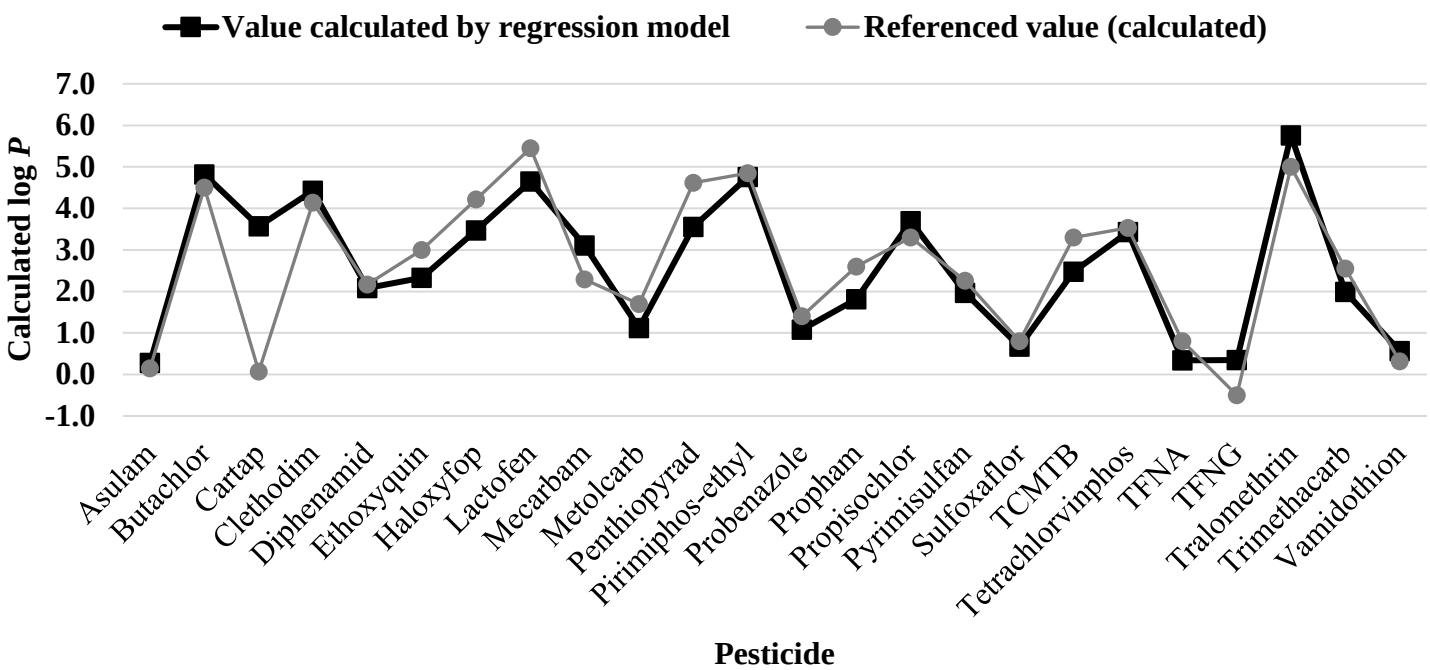

10
8
6
4
2
0
-2
-4
log P
y = 0.8701x - 2.3548
0
3
6
9
12
Retention time (min)

Fig. 2. Comparison of log *P* values calculated using the 1st order regression model and values calculated in other works


Value calculated by regression model
Referenced value (calculated)
Calculated log P
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
-1.0
Asulam
Butachlor
Cartap
Clethodim
Diphenamid
Ethoxyquin
Haloxyfop
Lactofen
Mecarbam
Metolcarb
Penthiopyrad
Pirimiphos-ethyl
Probenazole
Propham
Propisochlor
Pyrimisulfan
Sulfoxaflor
TCMTB
Tetrachlorvinphos
TFNA
TFNG
Tralomethrin
Trimethacarb
Vamidothion
Pesticide

Optimization of sample extraction step

Serum and urine are liquid-based samples, so it is appropriate to prepare the sample using QuEChERS methods for high extraction efficiency (recovery) of multiresidual pesticides. Since the first QuEChERS method for crops was developed using GC-MS in 2003 (Anastassiades et al., 2003), there have been preparation procedure improvements for LC amenable pesticides or lower recovery rate compounds such as pH-dependent pesticides (Koesukwiwat et al., 2008; Ribeiro Begnini Konatu et al., 2017). The official procedures such as the AOAC 2007.01 method containing acetate buffers and the EN 15662 method containing citrate buffers have been developed to improve recovery efficiency for pH-dependent pesticides (Lehotay, 2007; EN 15662, 2008). The entire or a portion of these types of QuEChERS methods have been utilized or modified depending on the characteristics of the pesticides and sample matrices in many analytical studies (Rejczak and Tuzimski, 2015).

In this study, the sample size was reduced to 0.1 mL, and optimization of the final preparation step was established by comparing the scaled-down methods from three different QuEChERS procedures (Anastassiades et al., 2003; Lehotay, 2007; EN 15662, 2008).

For the serum sample, bensultap, dithianon, and the acidic flonicamid metabolite TFNG [N-(4-trifluoromethylnicotinoyl)glycine] were again rejected because they could not be recovered. Except for the rejected three analytes (bensultap, dithianon, and TFNG), the remaining 379 pesticides were selected as the final research analytes in serum (**Table 2**). For the urine sample, aldicarb and bensultap were not recovered at all in any of the three preparation methods.

Therefore, these two compounds were excluded, and the remaining 380 pesticides were selected for final validation in urine (**Table 3**).

The total ion chromatogram (TIC) for the 379 target analytes in serum and the 380 analytes urine sample is shown in **Fig. 3 and 4**. There were no false positives in non-fortified serum samples, and no overlaps were observed between pesticides in fortified samples.

The number of pesticides that satisfied the recovery range from 70 to 120% with relative standard deviation (RSD) below 20% based on the criteria of SANTE/11813/2017 (European Commission, 2017) and their percentage ratio for each extraction method in serum and urine are shown in **Table 4 and 5**. There was no significant difference in the number of analytes satisfying the recovery criteria between the three methods in both matrices. For the serum sample, 344 (90.8%), 341 (90.0%), and 341 (90.0%) of the total 379 pesticides satisfied the recovery criteria for methods (A), (B), and (C), respectively. Method (A), the unbuffered condition, showed slightly higher number of pesticides than the others. For the urine sample, 360 (94.7%), 359 (94.5%), and 357 (93.9%) of total pesticides for methods (A), (B), and (C) fell within the criteria, respectively. From the optimization experiment results, the final preparation method using method (A) (downsized original QuEChERS) was established in both matrices. In addition, further cleanup steps, such as dSPE, were discarded in this treatment method to prevent the loss of labile target analytes and minimize the analysis time.

Table 2. List of the 379 pesticides classified by chemical groups for the optimized analytical method in serum

Chemical group (No. of compounds)	**Compound name**
Aryloxyalkanoic/ Aryloxyphenoxypropionic acid (12)	2,4-D, Clomeprop, Cyhalofop-butyl, Diclofop-methyl, Fenoxaprop-p-ethyl, Haloxyfop, Haloxyfop-R-Methyl, MCPA, Mecoprop-P, Metamifop, Propaquizafop, Quizalofop-ethyl
Avermectin/ Spinosyn (11)	Abamectin B1a, Emamectin B1a, Emamectin B1b, Lepimectin A3, Lepimectin A4, Milbemectin A3, Milbemectin A4, Spinetoram (XDE-175-J), Spinetoram (XDE-175-L), Spinosyn A, Spinosyn D
Carbamate (42)	Alanycarb, Aldicarb, Asulam, Bendiocarb, Benfuracarb, Benthiavalicarb-isopropyl, Butocarboxim, Carbaryl, Carbofuran, Carbosulfan, Cycloate, Dazomet, Di-allate, Diethofencarb, Dimepiperate, Esprocarb, Ethiofencarb, Fenobucarb (BPMC), Fenothiocarb, Fenoxycarb, Furathiocarb, Iprovalicarb, Isoprocarb, Methiocarb, Methomyl, Metolcarb, Molinate, Oxamyl, Pebulate, Phenmedipham, Pirimicarb, Promecarb, Propamocarb, Propham, Propoxur, Pyributicarb, Thiobencarb, Thiodicarb, Tri-allate, Trimethacarb, Vernolate, XMC
Imidazolinone (5)	Fenamidone, Imazamox, Imazapic, Imazaquin, Imazethapyr
Neonicotinoid (7)	Acetamiprid, Clothianidin, Dinotefuran, Imidacloprid, Nitenpyram, Thiacloprid, Thiamethoxam
Organophosphate (64)	Acephate, Anilofos, Azamethiphos, Azinphos-ethyl, Azinphos-methyl, Bensulide, Cadusafos, Carbophenothion, Chlorfenvinphos, Chlorpyrifos, Chlorpyrifos-methyl, Demeton-S-methyl, Diazinon, Dichlorvos, Dicrotophos, Dimethoate, Dimethylvinphos, Edifenphos, EPN, Ethion, Ethoprophos, Etrimfos, Fenamiphos, Fenthion, Fonofos, Fosthiazate, Imicyafos, Iprobenfos, Isazofos, Isoxathion, Malathion, Mecarbam, Methamidophos, Methidathion, Mevinphos, Monocrotophos, Omethoate, Oxydemeton-methyl, Parathion, Phenthoate, Phorate, Phosalone, Phosmet, Phosphamidon, Phoxim, Piperophos, Pirimiphos-ethyl, Pirimiph;os-methyl, Profenofos, Prothiofos, Pyraclofos, Pyrazophos, Pyridaphenthion, Quinalphos, Sulprofos, Tebupirimfos, Terbufos, Tetrachlorvinphos, Thiometon, Tolclofos-methyl, Triazophos, Tribufos, Trichlorfon, Vamidothion
Pyrethroid (14)	Bifenthrin, Cycloprothrin, Cyhalothrin-lambda, Cypermethrin, Deltamethrin, Etofenprox, Fenpropathrin, Fenvalerate, Flucythrinate, Fluvalinate, Halfenprox, Permethrin, Phenothrin, Tralomethrin
Strobilurin (8)	Azoxystrobin, Fluacrypyrim, Kresoxim-methyl, Metominostrobin, Orysastrobin, Picoxystrobin, Pyraclostrobin, Trifloxystrobin
Triazine (12)	Ametryn, Atrazine, Cyanazine, Dimethametryn, Hexazinone, Metribuzin, Prometryn, Propazine, Simazine, Simetryn, Terbuthylazine, Terbutryn
Triazole (26)	Amisulbrom, Azaconazole, Bitertanol, Cafenstrole, Carfentrazone-ethyl, Cyproconazole, Difenoconazole, Diniconazole, Epoxiconazole, Fenbuconazole, Fluquinconazole, Flusilazole, Hexaconazole, Imibenconazole, Metconazole, Myclobutanil, Paclobutrazol, Penconazole, Propiconazole, Simeconazole, Tebuconazole, Tetraconazole, Triadimefon, Triadimenol, Triticonazole, Uniconazole

Table 2. *(Continued)*

Chemical group (No. of compounds)	**Compound Name**
Urea (34)	Azimsulfuron, Bensulfuron-methyl, Chlorfluazuron, Chlorimuron-ethyl, Chlorotoluron, Chlorsulfuron, Cyclosulfamuron, Daimuron, Diafenthiuron, Diflubenzuron, Diuron, Ethametsulfuron-methyl, Ethoxysulfuron, Flucetosulfuron, Flufenoxuron, Forchlorfenuron, Halosulfuron-methyl, Hexaflumuron, Imazosulfuron, Isoproturon, Linuron, Lufenuron, Metazosulfuron, Methabenzthiazuron, Metobromuron, Nicosulfuron, Novaluron, Pencycuron, Rimsulfuron, Teflubenzuron, Thidiazuron, Thifensulfuron-methyl, Tribenuron-methyl, Triflumuron
Others/ Unclassified (144)	Acibenzolar-S-methyl, Alachlor, Allidochlor, Ametoctradin, Amitraz, Benfuresate, Bentazone, Benzobicyclon, Benzoximate, Bifenazate, Bifenox, Boscalid, Bromacil, Bromobutide, Bromoxynil, Bupirimate, Buprofezin, Butachlor, Butafenacil, Carbendazim, Carboxin, Carpropamid, Cartap, Chinomethionat, Chlorantraniliprole, Chloridazon, Chromafenozide, Cinmethylin, Clethodim, Clofentezine, Clomazone, Cyazofamid, Cyflufenamid, Cymoxanil, Cyprodinil, Cyromazine, Diflufenican, Dimethachlor, Dimethenamid, Dimethomorph, Diphenamid, Diphenylamine, Dithiopyr, Ethaboxam (EBX), Ethoxyquin, Etoxazole, Famoxadone, Fenarimol, Fenazaquin, Fenhexamid, Fenoxanil, Fenpyroximate, Fentrazamide, Ferimzone, Fipronil, Flonicamid, Fluazinam, Flubendiamide, Fludioxonil, Flufenacet, Flumiclorac-pentyl, Flumioxazin, Fluopicolide, Fluopyram, Flusulfamide, Flutolanil, Fluxapyroxad, Hexythiazox, Imazalil, Inabenfide, Indanofan, Indoxacarb, Iprodione, Isoprothiolane, Isopyrazam, Lactofen, Mandipropamid, Mefenacet, Mefenpyr-diethyl, Mepanipyrim, Mepronil, Metalaxyl, Methoxyfenozide, Metolachlor, Metrafenone, Napropamide, Nitrapyrin, Nuarimol, Ofurace, Oxadiazon, Oxadixyl, Oxaziclomefone, Pendimethalin, Penoxsulam, Penthiopyrad, Picolinafen, Pretilachlor, Probenazole, Prochloraz, Propachlor, Propanil, Propisochlor, Propyzamide, Pymetrozine, Pyrazolynate, Pyrazoxyfen, Pyribenzoxim, Pyridaben, Pyridalyl, Pyridate, Pyrifenox, Pyrifluquinazon, Pyrimethanil, Pyrimidifen, Pyriminobac-methyl E, Pyriminobac-methyl Z, Pyrimisulfan, Pyriproxyfen, Pyroquilon, Quinmerac, Quinoclamine, Saflufenacil, Sethoxydim, Spirodiclofen, Spirotetramat, Sulfoxaflor, TCMTB, Tebufenozide, Tebufenpyrad, TFNA [4-trifluoromethyl nicotinic acid], Thenylchlor, Thiabendazole, Thiazopyr, Thifluzamide, Thiocyclam, Thiophanate-methyl, Tiadinil, Tolfenpyrad, Tolylfluanid, Triclopyr, Tricyclazole, Triflumizole, Trifluralin, Zoxamide

Table 3. List of representative chemical groups and 380 pesticides selected for the final method validation in urine

Chemical group (No. of compounds)	Compound name
Aryloxyalkanoic/ Aryloxyphenoxy-propionic acid (12)	2,4-D, Clomeprop, Cyhalofop-butyl, Diclofop-methyl, Fenoxaprop-p-ethyl, Haloxyfop, Haloxyfop-R-Methyl, MCPA, Mecoprop-P, Metamifop, Propaquizafop, Quizalofop-ethyl
Avermectin/ Spinosyn (11)	Abamectin B1a, Emamectin B1a, Emamectin B1b, Lepimectin A3, Lepimectin A4, Milbemectin A3, Milbemectin A4, Spinetoram (XDE-175-J), Spinetoram (XDE-175-L), Spinosyn A, Spinosyn D
Carbamate (41)	Alanycarb, Asulam, Bendiocarb, Benfuracarb, Benthiavalicarb-isopropyl, Butocarboxim, Carbaryl, Carbofuran, Carbosulfan, Cycloate, Dazomet, Di-allate, Diethofencarb, Dimepiperate, Esprocarb, Ethiofencarb, Fenobucarb (BPMC), Fenothiocarb, Fenoxycarb, Furathiocarb, Iprovalicarb, Isoprocarb, Methiocarb, Methomyl, Metolcarb, Molinate, Oxamyl, Pebulate, Phenmedipham, Pirimicarb, Promecarb, Propamocarb, Propham, Propoxur, Pyributicarb, Thiobencarb, Thiodicarb, Tri-allate, Trimethacarb, Vernolate, XMC
Imidazolinone (5)	Fenamidone, Imazamox, Imazapic, Imazaquin, Imazethapyr
Neonicotinoid (7)	Acetamiprid, Clothianidin, Dinotefuran, Imidacloprid, Nitenpyram, Thiacloprid, Thiamethoxam
Organophosphate (64)	Acephate, Anilofos, Azamethiphos, Azinphos-ethyl, Azinphos-methyl, Bensulide, Cadusafos, Carbophenothion, Chlorfenvinphos, Chlorpyrifos, Chlorpyrifos-methyl, Demeton-S-methyl, Diazinon, Dichlorvos, Dicrotophos, Dimethoate, Dimethylvinphos, Edifenphos, EPN, Ethion, Ethoprophos, Etrimfos, Fenamiphos, Fenthion, Fonofos, Fosthiazate, Imicyafos, Iprobenfos, Isazofos, Isoxathion, Malathion, Mecarbam, Methamidophos, Methidathion, Mevinphos, Monocrotophos, Omethoate, Oxydemeton-methyl, Parathion, Phenthoate, Phorate, Phosalone, Phosmet, Phosphamidon, Phoxim, Piperophos, Pirimiphos-ethyl, Pirimiphos-methyl, Profenofos, Prothiofos, Pyraclofos, Pyrazophos, Pyridaphenthion, Quinalphos, Sulprofos, Tebupirimfos, Terbufos, Tetrachlorvinphos, Thiometon, Tolclofos-methyl, Triazophos, Tribufos, Trichlorfon, Vamidothion
Pyrethroid (14)	Bifenthrin, Cycloprothrin, Cyhalothrin-lambda, Cypermethrin, Deltamethrin, Etofenprox, Fenpropathrin, Fenvalerate, Flucythrinate, Fluvalinate, Halfenprox, Permethrin, Phenothrin, Tralomethrin
Strobilurin (8)	Azoxystrobin, Fluacrypyrim, Kresoxim-methyl, Metominostrobin, Orysastrobin, Picoxystrobin, Pyraclostrobin, Trifloxystrobin
Triazine (12)	Ametryn, Atrazine, Cyanazine, Dimethametryn, Hexazinone, Metribuzin, Prometryn, Propazine, Simazine, Simetryn, Terbuthylazine, Terbutryn
Triazole (26)	Amisulbrom, Azaconazole, Bitertanol, Cafenstrole, Carfentrazone-ethyl, Cyproconazole, Difenoconazole, Diniconazole, Epoxiconazole, Fenbuconazole, Fluquinconazole, Flusilazole, Hexaconazole, Imibenconazole, Metconazole, Myclobutanil, Paclobutrazol, Penconazole, Propiconazole, Simeconazole, Tebuconazole, Tetraconazole, Triadimefon, Triadimenol, Triticonazole, Uniconazole

Table 3. *(Continued)*

Chemical group (No. of compounds)	Compound name
Urea (34)	Azimsulfuron, Bensulfuron-methyl, Chlorfluazuron, Chlorimuron-ethyl, Chlorotoluron, Chlorsulfuron, Cyclosulfamuron, Daimuron, Diafenthiuron, Diflubenzuron, Diuron, Ethametsulfuron-methyl, Ethoxysulfuron, Flucetosulfuron, Flufenoxuron, Forchlorfenuron, Halosulfuron-methyl, Hexaflumuron, Imazosulfuron, Isoproturon, Linuron, Lufenuron, Metazosulfuron, Methabenzthiazuron, Metobromuron, Nicosulfuron, Novaluron, Pencycuron, Rimsulfuron, Teflubenzuron, Thidiazuron, Thifensulfuron-methyl, Tribenuron-methyl, Triflumuron
Others/ Unclassified (146)	Acibenzolar-S-methyl, Alachlor, Allidochlor, Ametoctradin, Amitraz, Benfuresate, Bentazone, Benzobicyclon, Benzoximate, Bifenazate, Bifenox, Boscalid, Bromacil, Bromobutide, Bromoxynil, Bupirimate, Buprofezin, Butachlor, Butafenacil, Carbendazim, Carboxin, Carpropamid, Cartap, Chinomethionat, Chlorantraniliprole, Chloridazon, Chromafenozide, Cinmethylin, Clethodim, Clofentezine, Clomazone, Cyazofamid, Cyflufenamid, Cymoxanil, Cyprodinil, Cyromazine, Diflufenican, Dimethachlor, Dimethenamid, Dimethomorph, Diphenamid, Diphenylamine, Dithianon, Dithiopyr, Ethaboxam (EBX), Ethoxyquin, Etoxazole, Famoxadone, Fenarimol, Fenazaquin, Fenhexamid, Fenoxanil, Fenpyroximate, Fentrazamide, Ferimzone, Fipronil, Flonicamid, Fluazinam, Flubendiamide, Fludioxonil, Flufenacet, Flumiclorac-pentyl, Flumioxazin, Fluopicolide, Fluopyram, Flusulfamide, Flutolanil, Fluxapyroxad, Hexythiazox, Imazalil, Inabenfide, Indanofan, Indoxacarb, Iprodione, Isoprothiolane, Isopyrazam, Lactofen, Mandipropamid, Mefenacet, Mefenpyr-diethyl, Mepanipyrim, Mepronil, Metalaxyl, Methoxyfenozide, Metolachlor, Metrafenone, Napropamide, Nitrapyrin, Nuarimol, Ofurace, Oxadiazon, Oxadixyl, Oxaziclomefone, Pendimethalin, Penoxsulam, Penthiopyrad, Picolinafen, Pretilachlor, Probenazole, Prochloraz, Propachlor, Propanil, Propisochlor, Propyzamide, Pymetrozine, Pyrazolynate, Pyrazoxyfen, Pyribenzoxim, Pyridaben, Pyridalyl, Pyridate, Pyrifenox, Pyrifluquinazon, Pyrimethanil, Pyrimidifen, Pyriminobac-methyl E, Pyriminobac-methyl Z, Pyrimisulfan, Pyriproxyfen, Pyroquilon, Quinmerac, Quinoclamine, Saflufenacil, Sethoxydim, Spirodiclofen, Spirotetramat, Sulfoxaflor, TCMTB, Tebufenozide, Tebufenpyrad, TFNA [4-trifluoromethyl nicotinic acid], TFNG [N-(4-trifluoromethylnicotinoyl)glycine], Thenylchlor, Thiabendazole, Thiazopyr, Thifluzamide, Thiocyclam, Thiophanate-methyl, Tiadinil, Tolfenpyrad, Tolylfluanid, Triclopyr, Tricyclazole, Triflumizole, Trifluralin, Zoxamide

Fig. 3. TIC obtained by LC-MS/MS analysis of (a) matrix-matched standard in human serum with 379 pesticides at 100 ng/mL (4 μL injection) and (b) TIC of control (non-fortified) serum sample

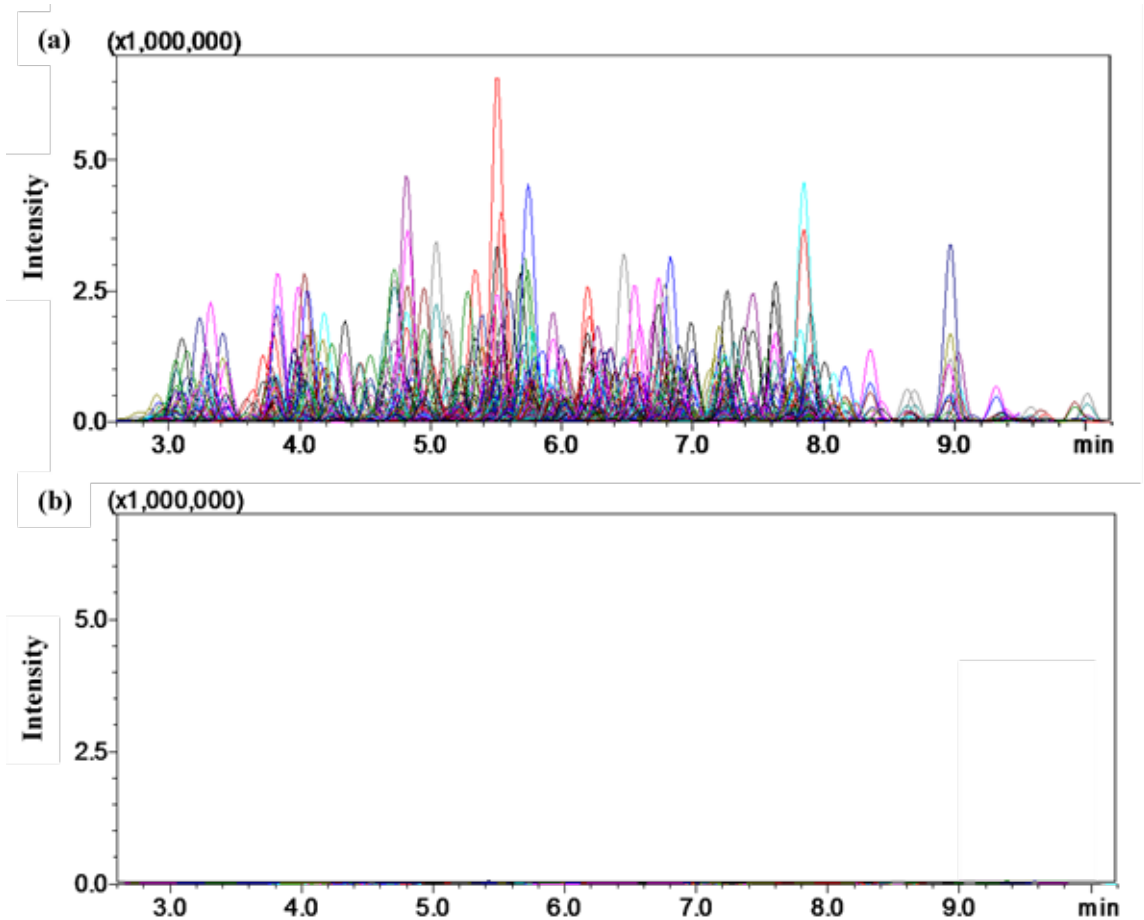

(a)
(x1,000,000)
Intensity
5.0
2.5
0.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
min
(b)
(x1,000,000)
Intensity
5.0
2.5
0.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
min

Fig. 4. TIC obtained by LC-MS/MS analysis of (a) matrix-matched standard in human urine with 380 pesticides at 100 ng/mL (4 μL injection) and (b) TIC of control (non-fortified) urine sample

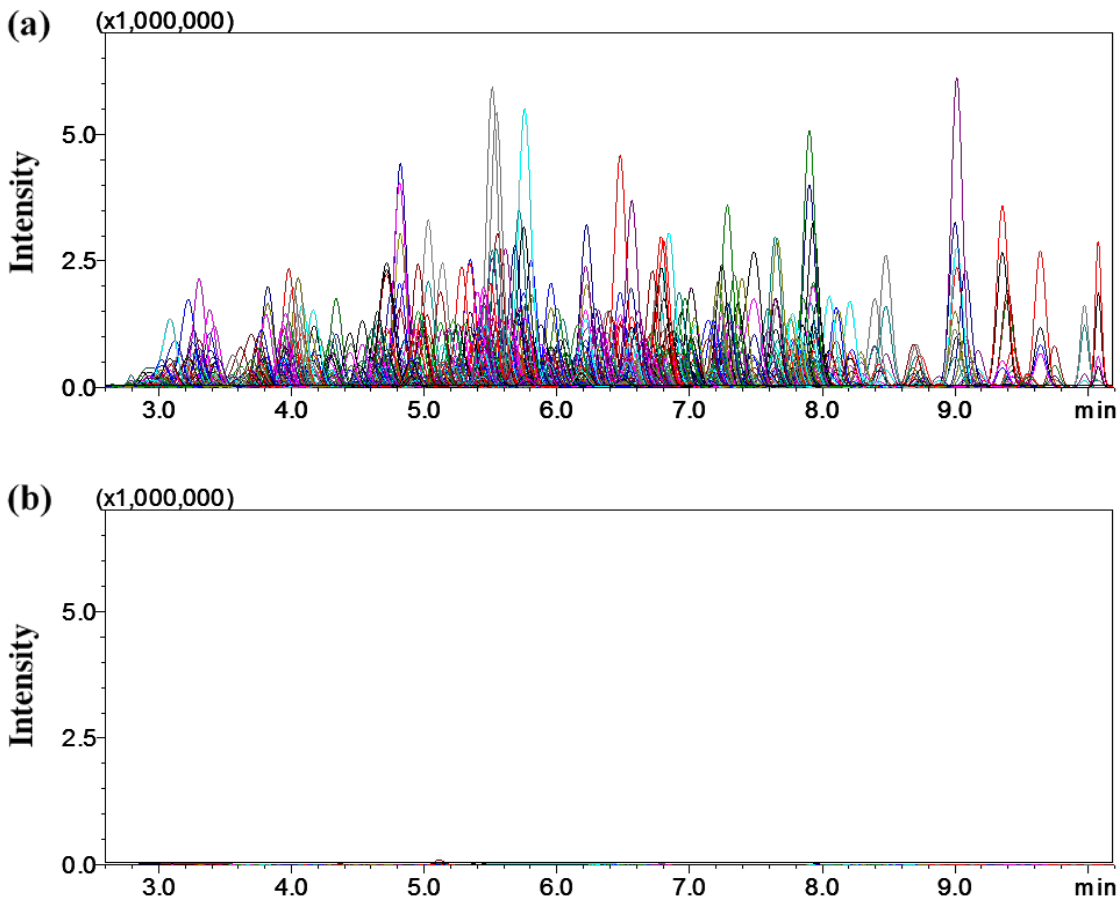

(a)
(x1,000,000)
Intensity
5.0
2.5
0.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
min
(b)
(x1,000,000)
Intensity
5.0
2.5
0.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
min

Table 4. The number of pesticides with recoveries between 70-120% with RSDs below 20% in the recovery test from different extraction methods for 379 Pesticides in 100 μL of human serum (fortification Level at 250 ng/mL, n = 3)

Type of method	Preparation method scaled-down from	Extraction solvent	Extraction reagent	No. of analytes	% of analytes
A	Original method	Acetonitrile (400 μL)	$MgSO_4$ (40 mg) NaCl (10 mg)	344	90.8
B	AOAC 2007.01	1% HOAc in acetonitrile (400 μL)	$MgSO_4$ (40 mg) NaOAc (10 mg)	341	90.0
C	EN 15662	Acetonitrile (400 μL)	$MgSO_4$ (40 mg) NaCl (10 mg) $Na_3Citrate{\cdot}2H_2O$ (10 mg) $Na_2HCitr{\cdot}1.5H_2O$ (5mg)	341	90.0

Table 5. The number of pesticides with recoveries between 70-120% with RSDs below 20% in the recovery test from different extraction methods for 379 Pesticides in 100 μL of human urine (fortification Level at 250 ng/mL, n = 3)

Type of method	Preparation method scaled-down from	Extraction solvent	Extraction reagent	No. of analytes	% of analytes
A	Original method	Acetonitrile (400 μL)	$MgSO_4$ (40 mg) NaCl (10 mg)	360	94.7
B	AOAC 2007.01	1% HOAc in acetonitrile (400 μL)	$MgSO_4$ (40 mg) NaOAc (10 mg)	359	94.5
C	EN 15662	Acetonitrile (400 μL)	$MgSO_4$ (40 mg) NaCl (10 mg) $Na_3Citrate{\cdot}2H_2O$ (10 mg) $Na_2HCitr{\cdot}1.5H_2O$ (5mg)	357	93.9

Method validation

With the final established analytical method, several validation tests were conducted, and the result of each parameter was verified with adequate criteria. The five validation parameters to be determined were limit of quantitation (LOQ), linearity of calibration, accuracy and precision, and recovery and matrix effect.

Limit of quantitation (LOQ). The LOQ was defined as the lowest concentration or mass of the analyte that was validated with acceptable accuracy (European Commission, 2017). One way to determine the LOQ is to verify that the S/N on the chromatogram is greater than 10 (De Bièvre et al., 2005). In this study, the LOQs ranged from 10 to 250 ng/mL for the pesticide multiresidues in serum and urine were verified.

In the serum sample, 358 compounds of the total 379 pesticides (94.5% of the total) had LOQs of 10 ng/mL (**Fig. 5**). It is remarkable that a sufficiently low LOQ level was obtained with reliable selectivity while analyzing 379 pesticides simultaneously. Most of the pesticides were detectable at very low concentration, even though almost 400 pesticides were analyzed simultaneously. The ratio of pesticides satisfying LOQ 10 ng/mL was higher than Dulaurent et al. (2010)'s screening analysis of more than 300 pesticides in blood using MS^2 and MS^3 mode of LC-IT-MS (Dulaurent et al., 2010). Among the remaining 21 (5.5%) components with LOQs higher than 10 ng/mL, the LOQ levels of 11 (2.9%), 7 (1.8%), and 3 (0.8%) analytes were determined at 25, 50, and 100 ng/mL, respectively. These LOQs are sufficiently low enough to detect cases of acute pesticide poisoning because pesticide concentrations in

blood have been reported to be from a few tens of ng/mL to several tens of μg/mL in most acute poisoning cases (Miyazaki et al., 1989; Lee et al., 1999; Lacassie et al., 2001b; Hikiji et al., 2013). These results demonstrate that this analytical method can sufficiently determine pesticides from an unknown sample without further concentration of the sample.

In the urine sample, the large majority of pesticides (364 compounds, 95.8% of the total 380 pesticides) were found to have an LOQ at 10 ng/mL, the minimum level in the analytical methods (**Fig. 6**). Among the remaining 16 (4.3%) pesticides, nine (benfuresate, bifenox, fenvalerate, inabenfide, milbemectin A3, parathion, terbufos, trifluralin, and a flonicamid metabolite TFNG [N-(4-trifluoromethylnicotinoyl)glycine]) showed LOQ at 25 ng/mL. Six pesticides (diphenylamine, dithianon, flonicamid, nitrapyrin, thiocyclam, and a flonicamid metabolite TFNA [4-trifluoromethyl nicotinic acid]) showed LOQ at 50 ng/mL, and only butocarboxim had an LOQ at 100 ng/mL. No pesticide showed a higher (150 and 250 ng/mL) LOQ. Those compounds with LOQ > 10 ng/mL also had sufficiently low detectability for forensic applications because urinary concentrations of parent compounds have been reported to range from sub to hundreds of ng/mL in cases of acute pesticide intoxication or in some biomonitoring investigations (Hattori et al., 1982; Montesano et al., 2007; Cazorla-Reyes et al., 2011; Usui et al., 2012; Quansah et al., 2016). Therefore, with the established analytical method, 380 pesticides can be determined in a urinary sample without further concentration of the sample extract.

Fig. 5. Pie chart showing distribution of LOQs (ng/mL) for 379 pesticides in serum for the final optimized analytical method. Light gray bar, 10 ng/mL; gray bar, 25 ng/mL; dark gray bar, 50 ng/mL; black bar, 100 ng/mL

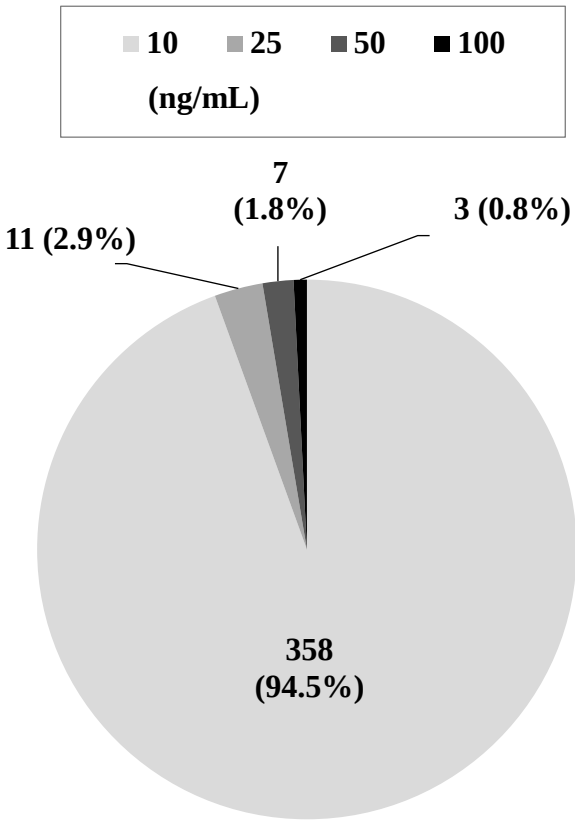
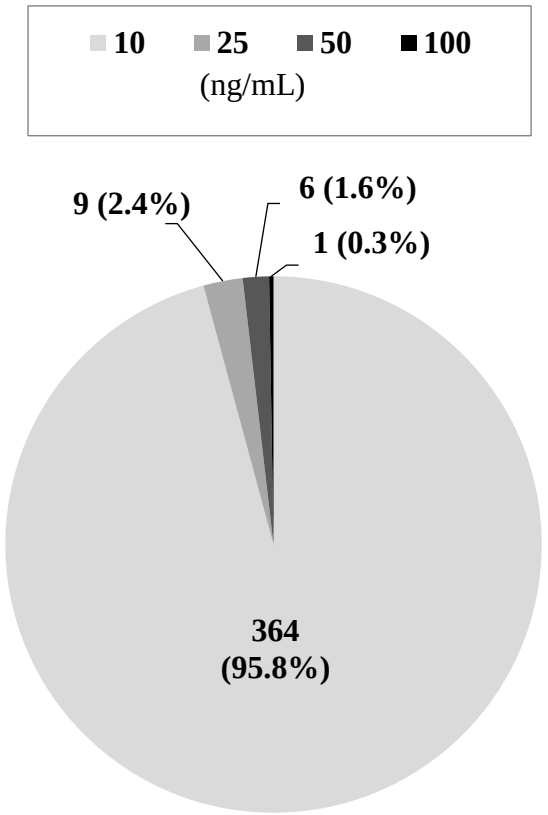

10
25
50
100
(ng/mL)
7
(1.8%)
3 (0.8%)
11 (2.9%)
358
(94.5%)

Fig. 6. Pie chart showing distribution of LOQs (ng/mL) for 380 pesticides in urine for the final optimized analytical method. Light gray bar, 10 ng/mL; gray bar, 25 ng/mL; dark gray bar, 50 ng/mL; black bar, 100 ng/mL


10
25
50
100
(ng/mL)
9 (2.4%)
6 (1.6%)
1 (0.3%)
364
(95.8%)

Linearity of calibration. Calibration was defined as the determination of the relationship between the observed signal from the target analyte in the sample extract and known quantities of the analyte prepared as standard solutions (European Commission, 2017). The degree of dependence established between the two variables can be expressed by the correlation coefficient (r^2). The closer the r^2 value is to 1, the better is the fit between signals and their concentrations (quantitative information).

Before the correlation coefficients of target analytes were determined, linear ranges were investigated. For the serum sample, there were various linear ranges (**Table 6**) because each analyte had a different LOQ and upper limit of quantitation (concentration of the highest calibration standard). Most of the compounds (92.4%) had a linear range from 10 to 250 ng/mL, which was the largest range in the analytical method. However, 19 compounds (5.0%) showed linear ranges from their LOQ to 250 ng/mL (9 for 25-250 ng/mL, 7 for 50-250 ng/mL, and 3 for 100-250 ng/mL). For the remaining 10 (2.6%) components, a linear range could not be drawn to 250 ng/mL due to the saturation effect of the signal at higher concentrations, resulting in linear ranges from their LOQ to 150 ng/mL for 8 analytes (6 for 10-150 ng/mL and 2 for 25-150 ng/mL) and 2 for 10-100 ng/mL.

For the correlation coefficient (r^2) of the 379 target compounds in serum (**Table 7**), 356 pesticides (93.9%) had r^2 greater than 0.990, indicating that most of the pesticides had a quantitative property with good linearity within these linear ranges. Correlation coefficients for 17 compounds (4.5%) were within 0.980-0.990, and four pesticides were within 0.900-0.980. It was expected that these ranges of correlation coefficients were also acceptable for the

multiresidue screening method. Diafenthiuron and tolyfluanid had somewhat poor correlation coefficients (0.835 and 0.879, respectively).

For the urine sample, Most of the pesticides (95.3%) had a linear range over 10-250 ng/mL concentrations, the largest linear range in this analytical method (**Table 8**). Linear ranges of 16 compounds (4.3%) were from LOQ to 250 ng/mL (nine for 25-250 ng/mL, six for 50-250 ng/mL, and one for 100-250 ng/mL). Dazomet and carbosulfan had linear ranges of 10-150 ng/mL and 10-100 ng/mL, respectively, due to signal saturation at higher concentration. The linear ranges for those two compounds were reduced by excluding higher concentrations to maintain quantitative properties at lower level concentrations.

Of the 380 target pesticides in urine, correlation coefficients of 366 (96.3%) were $r^2 \geq 0.990$ (**Table 9**), meaning that most of the compounds had excellent quantitative properties with good linearity within their linear ranges. Correlation coefficients of 10 (2.6%) compounds were within 0.980-0.990, adequate to maintain quantitative properties for screening purposes. The remaining 4 (1.1%) compounds (benfuracarb, diphenylamine, flumioxazin, and trifluralin) showed somewhat poor linearities (r^2 within 0.9771-0.9790).

Table 6. Distribution of linear ranges for 379 pesticides in serum for the final established analytical method

Linear range (ng/mL)	No. of analytes	% of analytes	Remarks
10-250	350	92.4	-
25-250	9	2.4	Abamectin B1a, Aldicarb, Butocarboxim, Imazamox, Iprodione, MCPA, Milbemectin A3, Propham, Terbufos
50-250	7	1.8	Diphenylamine, Fenvalerate, TFNA, Thiometon, Tolylfluanid, Tralomethrin, Trifluralin
100-250	3	0.8	Inabenfide, Nitrapyrin, Thiocyclam
10-150	6	1.6	Asulam, Benfuracarb, Bentazone, Cafenstrole, Fluxapyroxad, Mecoprop-P
25-150	2	0.5	Bifenox, Diafenthiuron
10-100	2	0.5	Carbosulfan, Dazomet
Sum	379	100	-

Table 7. Distribution of correlation coefficients (r^2) for 379 pesticides in serum for the final established analytical method

r^2	No. of analytes	% of analytes	Remarks
≥0.990	356	93.9	-
0.980-0.990	17	4.5	Abamectin B1a, Aldicarb, Benfuracarb, Bentazone, Bromacil, Cyazofamid, Cypermethrin, Demeton-S-methyl, Flufenacet, Fluopicolide, Iprodione, Methomyl, Metribuzin, Pyrimisulfan, Sulfoxaflor, Thiamethoxam, Thiocyclam
0.900-0.980	4	1.1	Cyhalofop-butyl, Cyhalothrin-lambda, Diphenylamine, Parathion
<0.900	2	0.5	Diafenthiuron, Tolylfluanid
Sum	379	100	-

Table 8. Distribution of linear ranges for 380 pesticides in urine for the final established analytical method

Linear range (ng/mL)	No. of pesticides (%)	% of analytes	Remarks
10-250	362	95.3	-
25-250	9	2.4	Benfuresate, Bifenox, Fenvalerate, Inabenfide, Milbemectin A3, Parathion, Terbufos, TFNG, Trifluralin
50-250	6	1.6	Diphenylaimine, Dithianon, Flonicamid, Nitrapyrin, TFNA, Thiocyclam
100-250	1	0.3	Butocarboxim
10-150	1	0.3	Dazomet
10-100	1	0.3	Carbosulfan
Sum	380	100	-

Table 9. Distribution of correlation coefficients (r^2) for 380 pesticides in urine for the final established analytical method

r^2	No. of analytes	% of analytes	Remarks
≥0.990	366	96.3	-
0.980-0.990	10	2.6	Abamectin B1a, Benfuresate, Bifenox, Butocarboxim, Cafenstrole, Cyromazine, Dazomet, Fluxapyroxad, Nitrapyrin, Thiometon
<0.980	4	1.1	Benfuracarb, Diphenylamine, Flumioxazin, Trifluralin
Sum	380	100	-

Accuracy and precision. Accuracy was defined as the degree of closeness of the determined value to the nominal or known true value, and precision as the closeness of agreement among a series of measurements obtained from multiple sampling of the same homogenous sample (US FDA, 2013).The accuracy value was calculated as the average of the measured values (%) for the replicates (*n* = 5) at each level, and its precision was expressed as RSD (%):

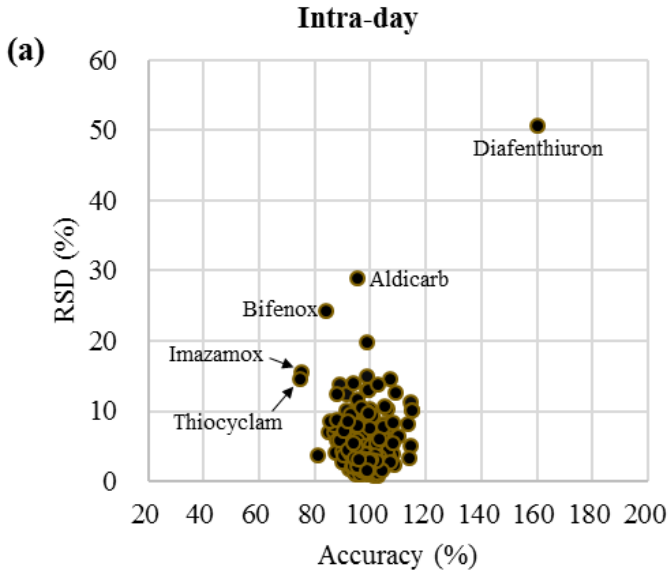
$$\text{RSD}, \% = \frac{\text{Standard deviation}}{\text{Average}} \times 100$$

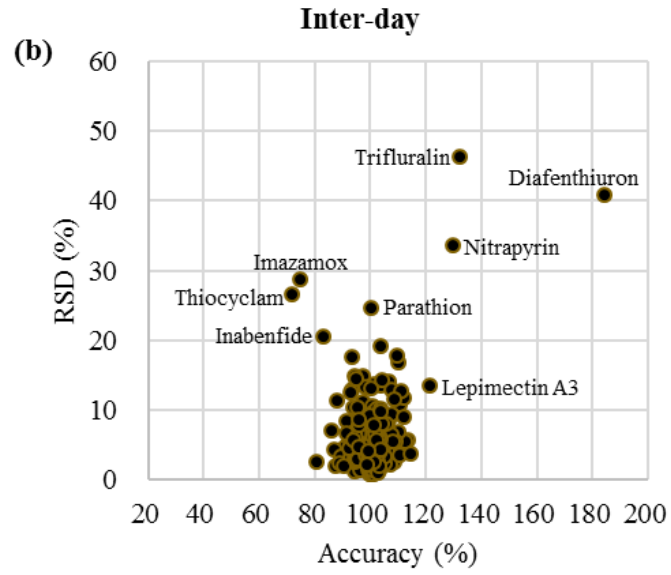
For the serum sample, the representative results at a QC level of 150 ng/mL were given using scatter plots of intra- and inter-day tests to verify accuracy and precision values of 379 pesticides at a glance (**Fig. 7**). In both of intra- and inter-day, most of the pesticides were located in a square zone of accuracy; 80-120% and RSD; 0-20% showing excellent accuracies and precisions. Only a few pesticide such as aldicarb, bifenox, diafenthiuron, imazamox, thiocyclam in intra-day and diafenthiuron, imazamox, inabenfide, lepimectin A3, nitrapyrin, parathion, thiocyclam, trifluralin in inter-day were out of the zone. There was no relationship between there compounds and those of chemical group or t_R.

To summarize and evaluate accuracy and precision results including all QC levels, data were statistically processed based on reasonable criteria. According to the criteria of US FDA (2013), accuracy values are 80-120% (RSD $\leq$20%) at the LOQ level and 85-115% (RSD $\leq$15%) at higher levels (US FDA, 2013). In this study, there were four LOQs (10, 25, 50, and 100 ng/mL) for each analyte depending on their sensitivity and the LOQ for most of the

pesticides (94.5%) was 10 ng/mL. Therefore, the former criterion of accuracy and precision was applied for the 10 ng/mL QC level, and the latter was applied for the other (50, 150, and 250 ng/mL) QC levels to reduce the complexity of reorganizing data.

Fig. 7. Scatter plots for 379 pesticides in serum to show accuracies and precisions (RSD) in (a) intra-day and (b) inter-day tests (at 150 ng/mL of QC level)

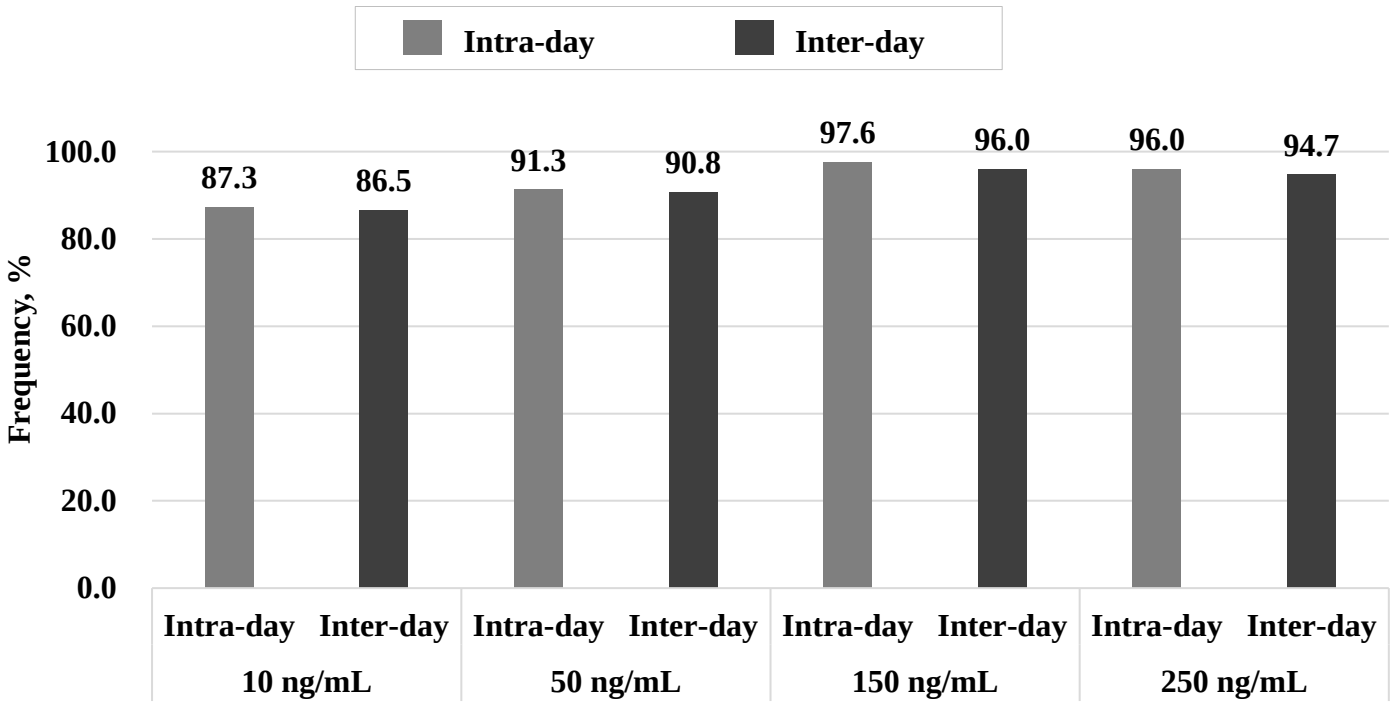

(a)
Intra-day
RSD (%)
Accuracy (%)
Diafenthiuron
Aldicarb
Bifenox
Imazamox
Thiocyclam
0
10
20
30
40
50
60
20
40
60
80
100
120
140
160
180
200


(b)
Inter-day
RSD (%)
Accuracy (%)
Trifluralin
Diafenthiuron
Nitrapyrin
Imazamox
Thiocyclam
Parathion
Inabenfide
Lepimectin A3
0
10
20
30
40
50
60
20
40
60
80
100
120
140
160
180
200

As shown in **Fig. 8**, the percentages of pesticides satisfying the criteria at 10 ng/mL were 87.3 and 86.5% in the intra- and inter-day tests, respectively. At the 50, 150, and 250 ng/mL levels, the percentages meeting the criteria were 91.3, 97.6, and 96.0% in the intra-day test and 90.8, 96.0, and 94.7% in the inter-day test, respectively. Most of the pesticides satisfied the accuracy and precision (RSD) criteria, and the proportions of pesticides meeting the criteria in intra-day were slightly higher than those of inter-day. In the case of tolyfluanid, its poor linearity did not affect its quantitation property, showing an excellent accuracy (85.1-106.0%) with an acceptable precision (6.0-14.7%) in intra- and inter-day tests. Four pesticides (bifenox, diafenthiuron, imazamox, and nitrapyrin) did not satisfy the criteria at all QC levels in the intra- and inter-day tests. In the case of bifenox, nitrapyrin, and imazamox, the accuracy values were 68.8-129.8% (RSD; 10.1-33.6%) within those of linear ranges. This indicated that those pesticides had acceptable accuracy and precision ranges in the screening analysis. Diafenthiuron had poor accuracy (116.5-184.1%) and precision results (RSD; 40.8-62.3%) in both tests, due to the poor linearity of the calibration (r^2 = 0.835) and instrumental reproducibility. Therefore, diafenthiuron should be determined by qualitative confirmation rather than quantitative confirmation when analyzing an unknown serum sample. The accuracy and precision of diafenthiuron has been reported as excellent with buffered QuEChERS approaches in tomatoes, evaluated with recovery test, by using LC-MS/MS45. Because there is no literature on the analysis of diafenthiuron in serum or blood to our knowledge, further research to improve accuracy and precision of diafenthiuron in serum is needed.

The results confirmed that the true concentration value for most pesticides in serum can be determined excellently and reliably, and is also valid over several days. Therefore, using the established preparation method and instrumental condition, biomonitoring for pesticide multiresidues can be conducted simultaneously in agricultural or other cases of pesticide intoxication.

Fig. 8. Percentage of 379 pesticides satisfying the accuracy values within 80-120% (RSD ≤20%) at 10 ng/mL and within 85-115% (RSD ≤15%) at 50, 150, and 250 ng/mL in the intra-day (grey bars) and inter-day (dark grey bars) tests using the final established method in serum sample


Intra-day
Inter-day
Frequency, %
100.0
80.0
60.0
40.0
20.0
0.0
87.3
86.5
91.3
90.8
97.6
96.0
96.0
94.7
Intra-day
Inter-day
Intra-day
Inter-day
Intra-day
Inter-day
Intra-day
Inter-day
10 ng/mL
50 ng/mL
150 ng/mL
250 ng/mL

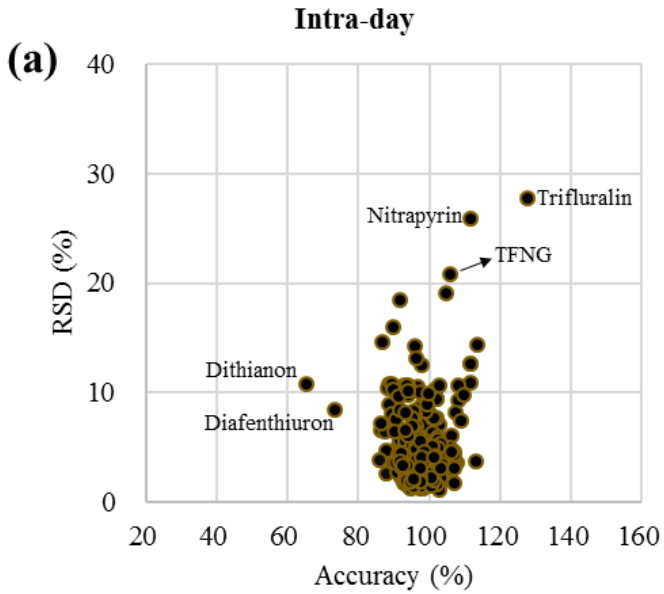
For the urine sample, the representative results at a QC level of 150 ng/mL were given using scatter plots of intra- and inter-day tests to verify accuracy and precision values of 380 pesticides at a glance, (**Fig. 9**). In both plots, most of the pesticides were within the square zone of accuracy; 80-120% and RSD; 0-20%, showing the excellent accuracy and precision values at a QC level of 150 ng/mL. Only a few compounds in the intra-day (diafenthiuron, dithianon, nitrapyrin, TFNG, and trifluralin) and inter-day (dithianon, TFNG, and trifluralin) were out of the zone, still within the square zone of accuracy; 60-140% and RSD; 0-30%. Individual accuracy results of inter-day were closer to 100% than those of intra-day.

The accuracy and precision data were statistically processed to summarize and evaluate the results including all QC levels based on reasonable reference criteria. For the QC level of 10 ng/mL, 89.7% and 87.1% of 380 analytes satisfied the accuracy criteria in the intra- and inter-day measurements, respectively (**Fig. 10**). For QC levels of 50, 150, and 250 ng/mL, 92.1-97.6% of analytes in intra-day and 90.8-97.4% in inter-day fell within the acceptable criteria. The ratio satisfying the accuracy criteria was highest at 150 ng/mL in both intra- and inter-day testing, and the number of pesticides meeting the criteria under the intra-day condition was slightly higher than that under inter-day conditions at all QC levels. Although nearly 400 pesticides were extracted together from a QC sample and analyzed simultaneously in only 15 minutes, most of the compounds did not lose their chemical properties or react with each other, and LC-MS/MS showed excellent throughput abilities to select, detect, and quantify hundreds of pesticides with high reliability. Furthermore, this bioanalytical method was verified as valid by inter-day results.

Only a few compounds showed poor accuracy and precision at all QC levels in urine. One compound (TFNG) in the intra-day testing and two compounds (benfuresate and nitrapyrin) in the inter-day testing did not satisfy acceptable accuracy or precision criteria at all QC levels. Four compounds (butocarboxim, dithianon, parathion, and trifluralin) did not satisfy the same criteria in both the intra- and inter-day test. Those seven compounds were not detectable at 10 ng/mL, the lowest concentration level in this analytical method, and so had somewhat poor sensitivity compared with other pesticides. Accuracy ranges for these compounds at all QC levels were within 62.1-145.9%, sufficiently valid to identify and quantify for rapid screening purposes.

From these results, biomonitoring for multiresidual pesticides using this analytical method can be performed with high reliability in forensic and clinical applications.

Fig. 9. Scatter plots for 380 pesticides in urine to show accuracies and precisions (RSD) in (a) intra-day and (b) inter-day tests (at 150 ng/mL of QC level)


(a)
Intra-day
RSD (%)
Accuracy (%)
Trifluralin
Nitrapyrin
TFNG
Dithianon
Diafenthiuron
0
10
20
30
40
20
40
60
80
100
120
140
160

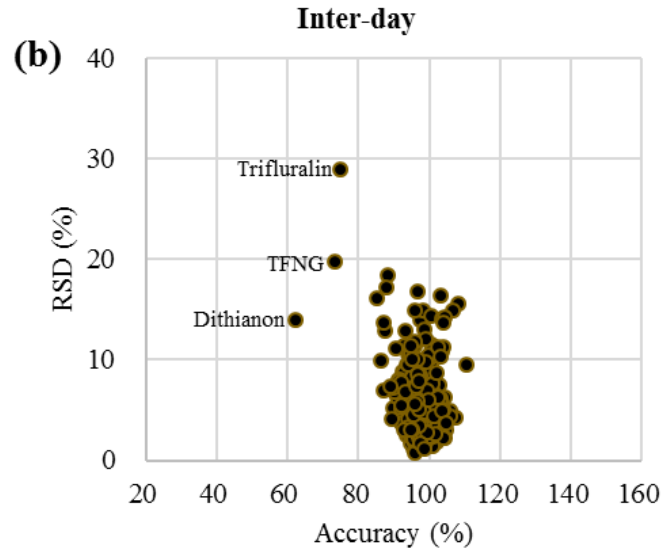
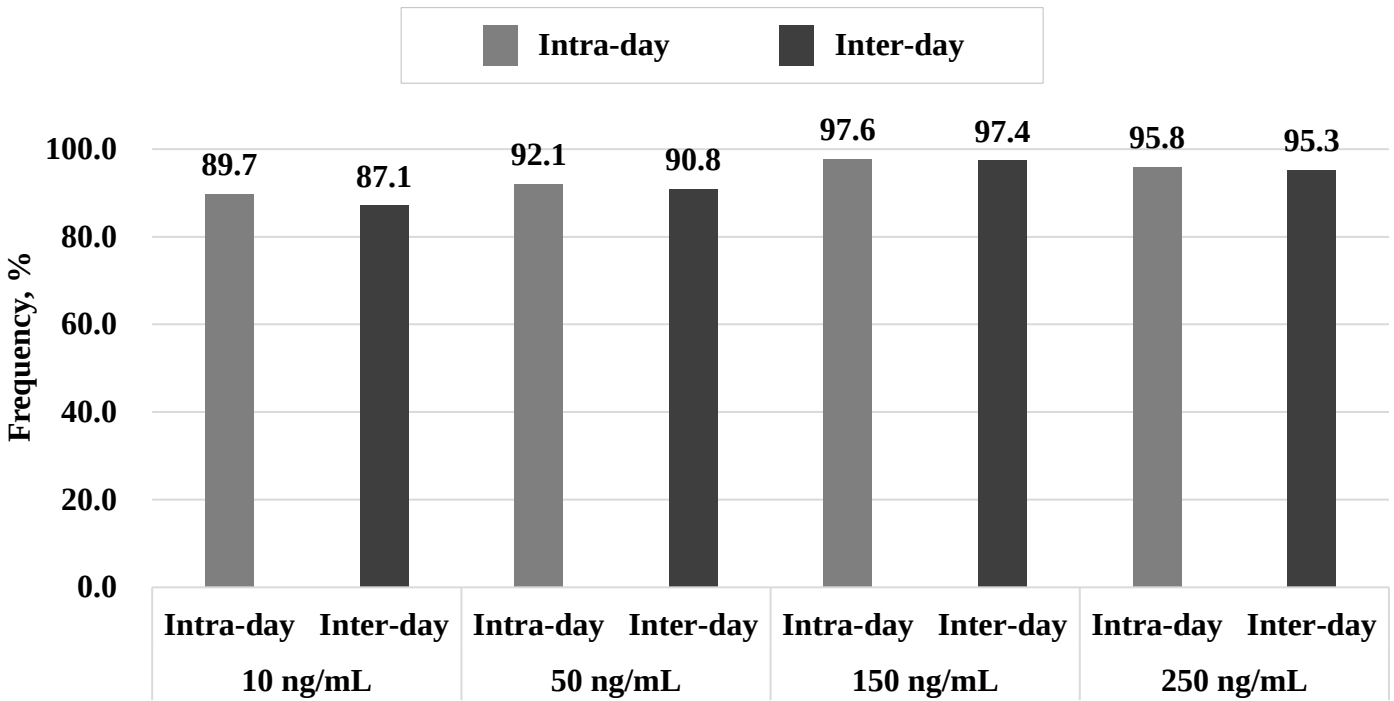

(b)
Inter-day
RSD (%)
Accuracy (%)
Trifluralin
TFNG
Dithianon
0
10
20
30
40
20
40
60
80
100
120
140
160

Fig. 10. The number of pesticides satisfying the accuracy range of 80-120% with RSD ≤20% at a QC level of 10 ng/mL and the accuracy range of 85-115 with RSD ≤ 15% at 50, 150, and 250 ng/mL levels under intra-day (grey bars) and inter-day (dark grey bars) conditions in urine sample


Intra-day
Inter-day
Frequency, %
100.0
80.0
60.0
40.0
20.0
0.0
89.7
87.1
92.1
90.8
97.6
97.4
95.8
95.3
Intra-day
Inter-day
10 ng/mL
50 ng/mL
150 ng/mL
250 ng/mL

Recovery. Recovery was defined as the proportion of the analyte remaining at the point of final determination, following its addition immediately prior to extraction (European Commission, 2017). The extraction efficiency of the preparation step is excellent when the recovery rate of a compound is close to 100%. A greater recovery rate could increase the sensitivity of target analytes. Recovery can also be a parameter of trueness (accuracy) for the analytical method. Recovery and its variation (RSD) have been regarded as accuracy and precision parameters in many bioanalytical methods (Cazorla-Reyes et al., 2011; Kim et al., 2014). Generally, a recovery rate of 70-120% (RSD $\leq$20%) is an acceptable trueness range (European Commission, 2017). These criteria have already been utilized for the verification of extraction efficiency for multiresidual pesticides in which the original QuEChERS extraction solvent (acetonitrile) and unbuffered salts ($MgSO_4$ and NaCl) were superior to other buffered reagents, as described in the above section.

For the serum sample, the recovery data at a fortification level of 50 ng/mL were presented according to representative chemical groups (**Fig. 11**), for the verification of recovery rates of 379 pesticides at a glance. All of the compounds belonging to the neonicotinoid, strobilurin, triazine, triazole groups and most of the pesticides classified as the avermectin/spinsyn, carbamate, organophosphate, pyrethroid, urea, and others/unclassified groups showed excellent recovery range (70-120%). However, half of the pesticides belonging to the aryloxyalkanoic/aryloxyphenoxypropionic acid and most of the imidazolinone pesticides showed lower recovery ranges (<70%). Most of the pesticides in the two groups are acidic compounds or zwitterions, so the extraction efficiencies for these polar compounds were decreased.

To summarize and evaluate recovery data at all treated levels, the results were classified in accordance with Mol et al. (2008) and Jia et al. (2014) by five different recovery ranges (<30%, 30-50%, 50-70%, 70-120%, and >120%) for multiresidue pesticides (Mol et al., 2008; Jia et al., 2014). RSD results were also classified with two groups (0-20% and >20%). As shown in **Table 10**, 85.8, 90.2, and 91.8% of target analytes satisfied the recovery range of 70-120% with RSD ≤20% at fortification levels of 10, 50, and 250 ng/mL, respectively. The percentages of pesticides with a recovery rate of less than 70% were 3.4-6.6% at all fortification levels. Only 1.8% and 1.4% of pesticides had a recovery rate greater than 120% at 10 and 250 ng/mL of the treated level and no pesticide included at 50 ng/mL. The overall recovery results were similar with those in Kim et al. (2014) using mini-QuEChERS (AOAC 2007.1 buffer salts) for whole blood analysis, in which approximately 83% and 11% of 215 pesticides had a recovery range of 80-100% and 100-150% respectively (Kim et al., 2014). However, among the pesticides with a recovery rate under 60%, acephate, aldicarb, dimepiperate, diphenylamine, EPN, fluazinam, methamidophos, omethoate, pyridalyl, teflubenzuron, and triclopyr showed a better recovery rate (72.3-114.2%) in our study, most likely because cleanup was omitted after extraction in the sample preparation procedure.

Fig. 11. Distribution to show recovery values for 379 pesticides classified into the representative chemical groups (treated at 50 ng/mL in serum)

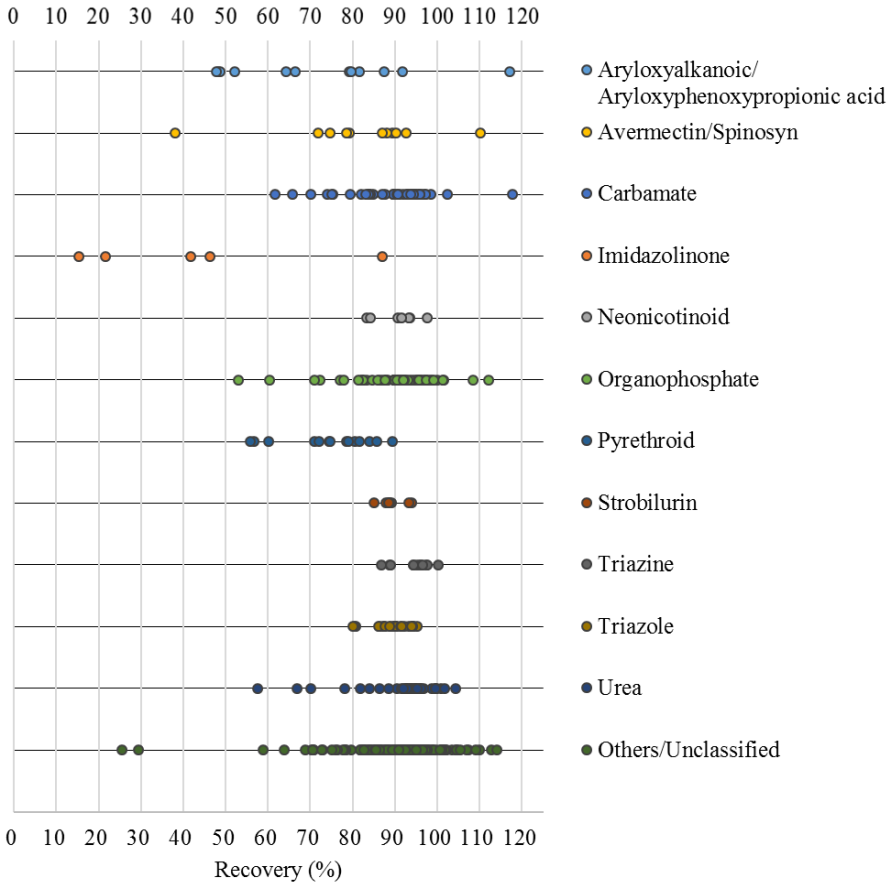

0 10 20 30 40 50 60 70 80 90 100 110 120
Aryloxyalkanoic/ Aryloxyphenoxypropionic acid
Avermectin/Spinosyn
Carbamate
Imidazolinone
Neonicotinoid
Organophosphate
Pyrethroid
Strobilurin
Triazine
Triazole
Urea
Others/Unclassified
0 10 20 30 40 50 60 70 80 90 100 110 120
Recovery (%)

Table 10. Distribution of recovery and RSD range for 379 pesticides at fortification levels of 10, 50, and 250 ng/mL in serum for the final established analytical method

Recovery Range	**RSD**	**Treated Level** No. of analytes (%)		
		10 ng/mL	**50 ng/mL**	**250 ng/mL**
<30%	0-20%	1 (0.3)	3 (0.8)	0 (0.0)
	>20%	0 (0.0)	1 (0.3)	0 (0.0)
30-50%	0-20%	1 (0.3)	3 (0.8)	6 (1.6)
	>20%	1 (0.3)	3 (0.8)	0 (0.0)
50-70%	0-20%	7 (1.8)	13 (3.4)	10 (2.6)
	>20%	3 (0.8)	2 (0.5)	0 (0.0)
70-120%	0-20%	325 (85.8)	342 (90.2)	348 (91.8)
	>20%	13 (3.4)	8 (2.1)	0 (0.0)
>120%	0-20%	5 (1.3)	0 (0.0)	4 (1.1)
	>20%	2 (0.5)	0 (0.0)	1 (0.3)
N.D.[1)]		21 (5.5)	4 (1.1)	10 (2.6)
Sum		379 (100)	379 (100)	379 (100)

[1)]Not determined due to out of linear range.

From the results, 18 compounds were verified as out of the recovery range of 70-120% with RSD ≤20% at all fortification levels (**Table 11**). Two compounds (inabenfide and nitrapyrin) had high recovery rates (123.4 and 173.9%, respectively). The reason for the recovery rates exceeding 120% despite the matrix-matching was that low sensitivity of inabenfide and nitrapyrin (LOQ; 100 ng/mL) and insufficient calibration points caused quantitation error. In contrast, 16 compounds showed a low recovery range of 15.4-66.2% or poor recovery RSD (20.5-52.7%). Among the pesticides with low recovery rates, nine compounds (2,4-D, imazamox, imazapic, imazaquin, imazethapyr, MCPA, mecoprop-P, quinmerac, and the flonicamid metabolite TFNA [4-trifluoromethyl nicotinic acid]) were acidic compounds or zwitterion (Turner, 2015; Chemicalize.org, 2017). Therefore, it is thought that those analytes were present as ionic forms in the serum at almost neutral pH, and some of them remained in the water layer at the partitioning step and were not recovered. It has been reported that the recovery rate for some of these pH-dependent compounds increased by extracting with an organic acid or an acidic buffer in many matrices including blood (Pareja et al., 2011; Carneiro et al., 2013; Kim et al., 2014). Our study verified that these pesticides obtained a greater recovery rate when using method (B) (AOAC 2007.1) or (C) (EN 15662) in the optimizing sample preparation step (**Fig. 12**). Although the pesticides listed in **Table 11** were out of the recovery criteria range, the accuracy and precision were excellent except for a few analytes, such that screening of the pesticides is not a problem.

Table 11. Pesticides for which recovery test results were not within 70-120% (RSD ≤20%) at all treated levels (10, 50, and 250 ng/mL), and intra-day accuracy results with RSD (serum)

		Treated level	10 ng/mL		50 ng/mL		250 ng/mL		
No.	Compound name	Chemical group	Recovery, % (RSD, %)	Accuracy, % (RSD, %)	Recovery, % (RSD, %)	Accuracy, % (RSD, %)	Recovery, % (RSD, %)	Accuracy, % (RSD, %)	Remarks
1	2,4-D	Aryloxy-alkanoic acid	58.7 (10.0)	107.0 (12.7)	52.2 (14.8)	93.9 (12.6)	60.7 (8.9)	104.1 (12.5)	pKa 2.73 (20-25 ℃) (Turner, 2015)
2	Abamectin B1a	Avermectin	N.D.[1)]	N.D.[1)]	38.2 (58.8)	92.2 (35.6)	60.0 (11.8)	98.1 (10.7)	-
3	Bifenox	Nitrophenyl Ether	N.D.[1)]	N.D.[1)]	58.9 (22.7)	83.8 (20.1)	N.D.[1)]	N.D.[1)]	-
4	Diafenthiuron	Urea	N.D.[1)]	N.D.[1)]	57.5 (26.0)	116.5 (62.3)	N.D.[1)]	N.D.[1)]	-
5	Etofenprox	Pyrethroid	52.3 (5.9)	96.8 (2.7)	55.8 (3.6)	88.2 (6.3)	59.5 (7.2)	85.2 (13.3)	-
6	Imazamox	Imidazolinone	N.D.[1)]	N.D.[1)]	15.4 (47.8)	68.8 (32.7)	34.9 (9.5)	72.6 (19.9)	Zwitterion (Turner, 2015)
7	Imazapic	Imidazolinone	31.4 (11.4)	105.0 (13.2)	21.6 (7.1)	85.3 (9.7)	31.8 (9.4)	80.9 (10.5)	Zwitterion (Turner, 2015)
8	Imazaquin	Imidazolinone	52.8 (27.0)	93.9 (14.0)	46.3 (6.3)	92.1 (7.9)	52.6 (0.7)	89.3 (6.0)	pKa 3.8 (20-25 ℃) (Turner, 2015)
9	Imazethapyr	Imidazolinone	56.6 (6.1)	97.0 (13.9)	41.7 (4.2)	92.0 (4.5)	54.1 (3.0)	88.1 (12.0)	Zwitterion (Turner, 2015)
10	Inabenfide	Unclassified	N.D.[1)]	N.D.[1)]	N.D.[1)]	N.D.[1)]	123.4 (16.5)	102.8 (8.0)	-
11	MCPA	Aryloxy-alkanoic acid	N.D.[1)]	N.D.[1)]	48.3 (20.5)	99.4 (9.8)	66.2 (10.9)	90.1 (4.4)	pKa 3.73 (20-25 ℃) (Turner, 2015)
12	Mecoprop-P	Aryloxy-alkanoic acid	60.0 (7.0)	97.0 (11.3)	48.7 (30.0)	74.8 (16.4)	N.D.[1)]	N.D.[1)]	pKa 3.78 (20-25 ℃) (Turner, 2015)
13	Nitrapyrin	Unclassified	N.D.[1)]	N.D.[1)]	N.D.[1)]	N.D.[1)]	173.9 (44.2)	110.3 (14.7)	-

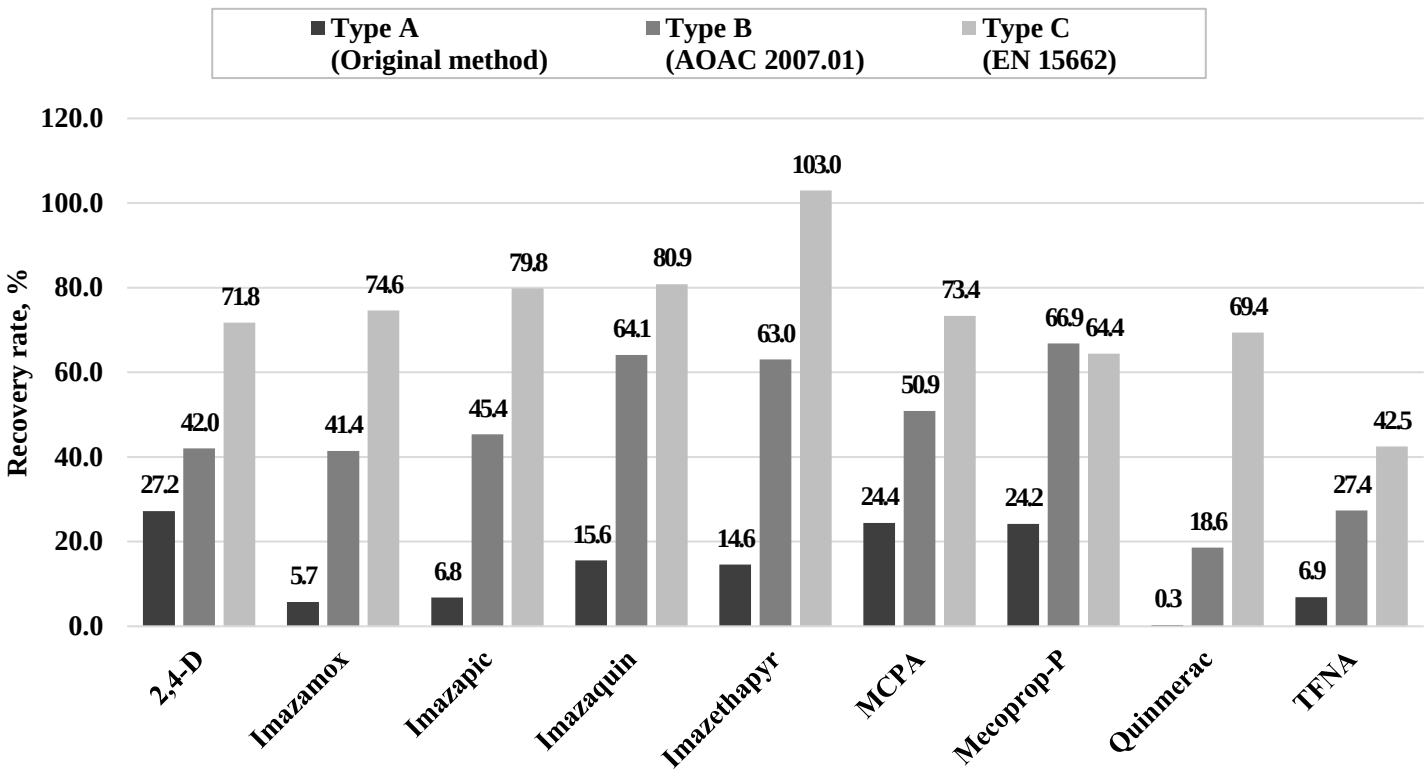
Table 11. *(Continued)*

No.	Compound name	Treated level / Chemical group	10 ng/mL Recovery, % (RSD, %)	10 ng/mL Accuracy, % (RSD, %)	50 ng/mL Recovery, % (RSD, %)	50 ng/mL Accuracy, % (RSD, %)	250 ng/mL Recovery, % (RSD, %)	250 ng/mL Accuracy, % (RSD, %)	Remarks
14	Quinmerac	Quinoline-carboxylic acid	25.1 (6.6)	88.9 (6.7)	29.3 (6.3)	98.8 (10.2)	35.3 (3.2)	85.1 (8.0)	pKa 4.32 (20-25 ℃) (Turner, 2015)
15	TFNA	Nicotinic acid	N.D.[1)]	N.D.[1)]	25.5 (15.9)	100.0 (14.5)	32.6 (2.3)	87.6 (14.3)	pKa 2.62[2)], 3.99[2)] (calculated) (Chemicalize.org, 2017)
16	Thiocyclam	Nereistoxin analogue	N.D.[a]	N.D.[a]	N.D.[a]	N.D.[a]	49.0 (13.0)	85.8 (12.1)	-
17	Tolylfluanid	Phenyl-sulfamide	N.D.[a]	N.D.[a]	N.D.[a]	104.6 (14.7)	31.0 (15.4)	85.3 (8.8)	-
18	Tralomethrin	Pyrethroid	N.D.[a]	N.D.[a]	72.0 (52.7)	131.2 (12.4)	63.2 (10.7)	99.6 (12.9)	-

[1)]Not determined due to out of linear range.

[2)]Calculated values using Chemicalize.org by ChemAxon.

Fig. 12. Recovery results (treated at 250 ng/mL in serum) of three different QuEChERS extraction methods for pH-dependent pesticides that showed lower recovery rate in the validation test


Type A
(Original method)
Type B
(AOAC 2007.01)
Type C
(EN 15662)
Recovery rate, %
120.0
100.0
80.0
60.0
40.0
20.0
0.0
27.2
42.0
71.8
5.7
41.4
74.6
6.8
45.4
79.8
15.6
64.1
80.9
14.6
63.0
103.0
24.4
50.9
73.4
24.2
66.9
64.4
0.3
18.6
69.4
6.9
27.4
42.5
2,4-D
Imazamox
Imazapic
Imazaquin
Imazethapyr
MCPA
Mecoprop-P
Quinmerac
TFNA

In conclusion, most of the pesticides were well recovered at all treated levels in this study. The recovery rate of pH-dependent compounds could be increased by adjusting the sample pH.

For the urine sample, a distribution chart of recovery was introduced in accordance with representative chemical groups (**Fig. 13**). All the pesticides belonging to carbamate, imidazolinone, neonicotinoid, strobilurin, triazine, and triazole groups were within the recovery range of 70-120% at a treated level of 50 ng/mL, showing excellent recovery. It was remarkable that, although most imidazolinone and aryloxyalkanoic/aryloxyphenoxypropionic acid compounds are acidic or zwitterions, their extraction efficiencies were not reduced under unbuffered conditions. The pyrethroid group showed the lowest percent recovery (71.4%) of the 70-120% recovery group.

The distribution chart (**Table 12**) to summarize recovery results showed that 328 (86.3%), 335 (88.2%), and 338 (88.9%) of the pesticides were within the recovery range of 70-120% (RSD $\leq$20%) at 10, 50, and 250 ng/mL, respectively. One to 21 (0.3 to 5.5%) pesticides were included in the same range with RSD >20%. Some pesticides (3.3-10.4%) belonged to the lower recovery rate group (30-70%), but no pesticide showed recovery rates less than 30%. Only 2.1% and 0.3% of pesticides showed a recovery rate greater than 120% at the 10 and 50 ng/mL levels, respectively, and no pesticide was included in this range at 250 ng/mL.

The recovery results were compared to the report of Cazorla-Reyes et al. (2011) in which 204 pesticides in urine samples were extracted and purified at once by SPE (C18 cartridge) and then analyzed using GC-IT-MS/MS (117 pesticides) and LC-MS/MS (87 pesticides) (Cazorla-Reyes et al., 2011). The

number of pesticides satisfying the recovery rate of 70-120% with RSD ≤20% in the Cazorla-Reyes et al. report is similar to our results (Cazorla-Reyes et al., 2011). However, some compounds (e.g., acephate, bendiocarb, flufenoxuron, and simazine) that fell outside of the criterion of 50 ng/mL in their report showed excellent recovery rates (86.9-98.6% with RSD 5.9-13.1%) at the same treated level in our study, and, vice versa, a few pesticides (e.g., bifenthrin, hexythiazox, lufenuron, parathion, permethrin, and tebufenpyrad) showed better recovery range (71-111% with RSD 2-8%) in the report (Cazorla-Reyes et al., 2011).

From the recovery data, most of the pesticides showed high extraction efficiency by this bioanalytical method. In spite of the diverse chemical properties of the different pesticides, strong extraction/partitioning reagents used in the preparation step maintained overall excellent recovery rates. Additionally, further cleanup steps were excluded to prevent the loss of target analytes.

Fig. 13. Distribution of recovery rates for 380 pesticides by representative chemical groups at fortification levels of 50 ng/mL in urine

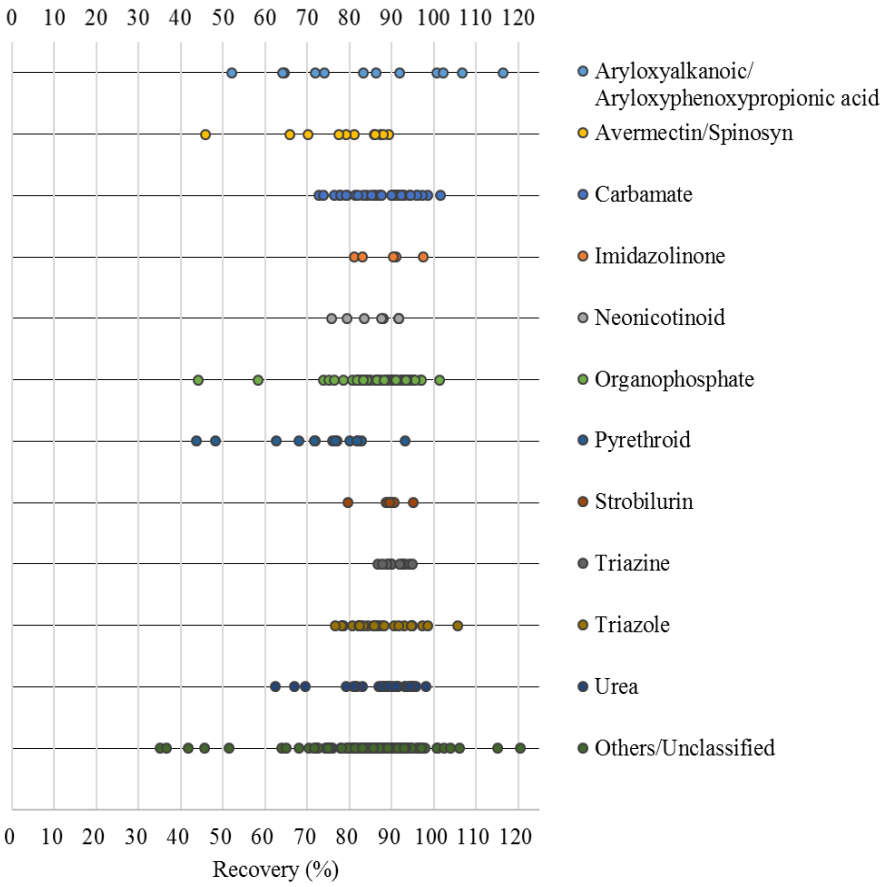

0 10 20 30 40 50 60 70 80 90 100 110 120
Aryloxyalkanoic/ Aryloxyphenoxypropionic acid
Avermectin/Spinosyn
Carbamate
Imidazolinone
Neonicotinoid
Organophosphate
Pyrethroid
Strobilurin
Triazine
Triazole
Urea
Others/Unclassified
0 10 20 30 40 50 60 70 80 90 100 110 120
Recovery (%)

Table 12. Distribution of recovery and RSD range for 380 pesticides at fortification levels of 10, 50, and 250 ng/mL in urine for the final established analytical method

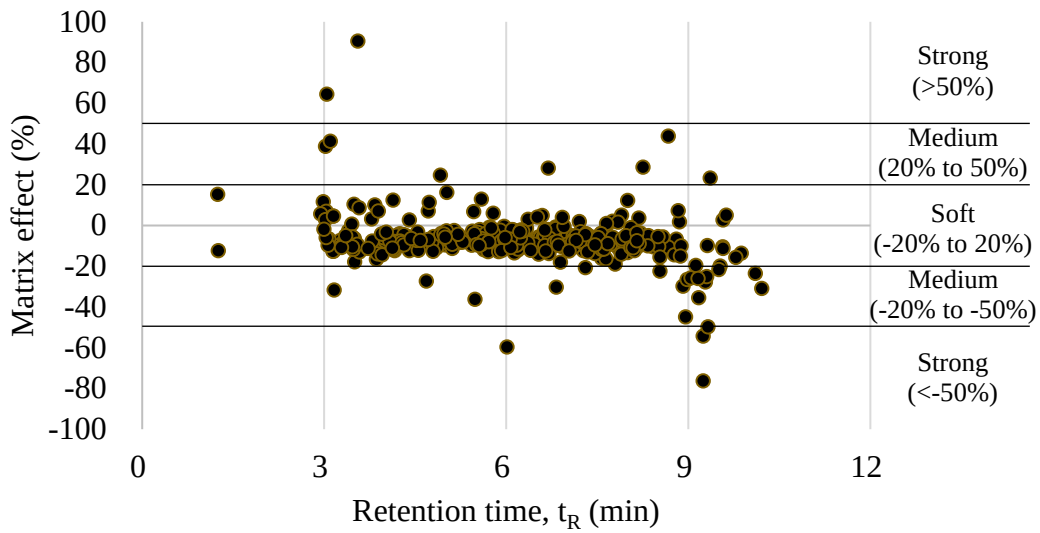
Recovery range	RSD	Treated level No. of pesticides (%)		
		10 ng/mL	50 ng/mL	250 ng/mL
<30%	0-20%	0 (0.0)	0 (0.0)	0 (0.0)
	>20%	0 (0.0)	0 (0.0)	0 (0.0)
30-50%	0-20%	3 (0.8)	2 (0.5)	6 (1.6)
	>20%	1 (0.3)	6 (1.6)	1 (0.3)
50-70%	0-20%	4 (1.1)	13 (3.4)	31 (8.2)
	>20%	4 (1.1)	1 (0.3)	1 (0.3)
70-120%	0-20%	328 (86.3)	335 (88.2)	338 (88.9)
	>20%	16 (4.2)	21 (5.5)	1 (0.3)
>120%	0-20%	6 (1.6)	0 (0.0)	0 (0.0)
	>20%	2 (0.5)	1 (0.3)	0 (0.0)
N.D.[1)]		16 (4.2)	1 (0.3)	2 (0.5)
Sum		380 (100)	380 (100)	380 (100)

[1)]Not determined due to out of linear range.

Matrix effect. The matrix effect was defined as the influence of one or more co-extracted compounds from the sample on the measurement of the analyte's concentration or mass (European Commission, 2017). The matrix effect when analyzing pesticides using LC with mass spectrometer is a common phenomenon (Hajšlová and Zrostlíková, 2003). Kebarle and Tang (1993) first reported the mechanism of matrix effect in ESI mode (Kebarle and Tang, 1993). One technique for minimizing the matrix effect is sample dilution (Hernández et al., 2005; Ferrer et al., 2011a; Panuwet et al., 2016). In this study, therefore, 0.1 mL of the serum and urine sample was extracted with four times larger extraction solvent volume (0.4 mL of acetonitrile). The extract solution was also partitioned into an organic solvent layer and water layer using $MgSO_4$ and NaCl to remove polar compounds from the organic solvent layer that may affect the matrix effect. According to equation described above, the matrix effect of each compound can be expressed as percentage enhancement (> 0%) or suppression (< 0%). The farther away the percentage is from zero (0%), the larger is the matrix effect.

For the serum sample, the average matrix effect for the 379 pesticides was -6.8%, which means that the response on LC-MS/MS for most compounds was somewhat suppressed by the matrix. The matrix effect data and t_R of each pesticide were plotted on a scatter plot (**Fig. 14**) for the verification of a relationship between the two variables. The scatter graph showed that matrix effects of most pesticides were within the soft effect zone during the time of first pesticide elution to 9 minutes. From 9 minutes to the time of the last pesticide elution, however, more than 50% of the compounds were out of soft effect zone, showing large instrumental signal suppression.

Fig. 14. Scatter plot to show t_R and matrix effect of 379 pesticides in serum


100
80
60
40
20
0
-20
-40
-60
-80
-100
Matrix effect (%)
0
3
6
9
12
Retention time, t_R (min)
Strong
(>50%)
Medium
(20% to 50%)
Soft
(-20% to 20%)
Medium
(-20% to -50%)
Strong
(<-50%)

According to Kmellár et al. (2008) and Ferrer et al. (2011), the results were divided into six ranges and three groups (**Fig. 15**), corresponding to a soft effect when the value was within -20% to 0% or 0% to 20%, a medium effect within -50% to -20% or 20% to 50%, and a strong effect below -50% or above 50% (Kmellár et al., 2008; Ferrer et al., 2011a; Ferrer et al., 2011b). The number of pesticides with a soft effect (between -20% and 20%) was 349 (92.1%), which was considered as no matrix effect (Ferrer et al., 2011a). Therefore, those compounds in this group were not prone to be affected by serum, indicating that solvent-based calibration could be possible for quantitation. The compounds within the medium and strong effect groups were 25 (6.6%) and 5 (1.3%), respectively. For those analytes susceptible to influences of the serum matrix, it is necessary to make quantitation data using matrix-matched calibration to avoid enhancement or suppression of responses.

For the urine sample, the average matrix effect of the 380 target pesticides in urine was -4.1%. This negative percentage indicates that the urine matrix tended to slightly suppress the signal intensity of target compounds in LC-MS/MS. For verification of matrix effect for each pesticide and correlation with retention time, the scatter plot of matrix effect and t_R were shown as **Fig. 16**. From the initiation time of pesticide elution to around 5 minutes, a large number of pesticides were located in below -20% of matrix effect. The matrix effects were weakened after approximately 5 minutes to end of the elution time. This results indicated that most of the polar urinary matrices were co-eluted with target pesticides in the early stages of analytical time (~5 min), so affected considerable signal suppression of target compounds. \

The summary of matrix effects for the 380 pesticides classified into three groups and 6 ranges (**Fig. 17**) showed that most of the pesticides (74.2%) were included in the soft effect group, in which 179 (47.1%) compounds fell between -20% and 0%, and 103 (27.1%) pesticides fell between 0% and 20%. Within the soft group, matrix effects are considered negligible on LC-MS/MS (Ferrer et al., 2011b). Therefore, it is possible to determine the concentration of real urine samples using solvent-only (matrix-free) standard solution rather than matrix-matched solution. The numbers of compounds in the medium and strong groups were 66 (17.3%) and 32 (8.4%), respectively. These groups were susceptible to interfering influences in urine, thus requiring matrix-matched calibration for correct quantitation.

In conclusion, using sample dilution in the preparation step, most of the pesticides showed a very small matrix effect, regarded as no effect by the human serum and urine matrix during quantitation. Only a few compounds with a large matrix effect need alternative approaches such as matrix-matching or standard addition method in the quantitative process in the biological samples.

Fig. 15. Distribution of matrix effects (%) for 379 pesticides classified into soft effect (light grey bars, -20% to 0% and 0% to 20%), middle effect (grey bars, -50% to -20% and 20% to 50%), and strong effect (dark grey bars, <-50% and >50%) in human serum samples

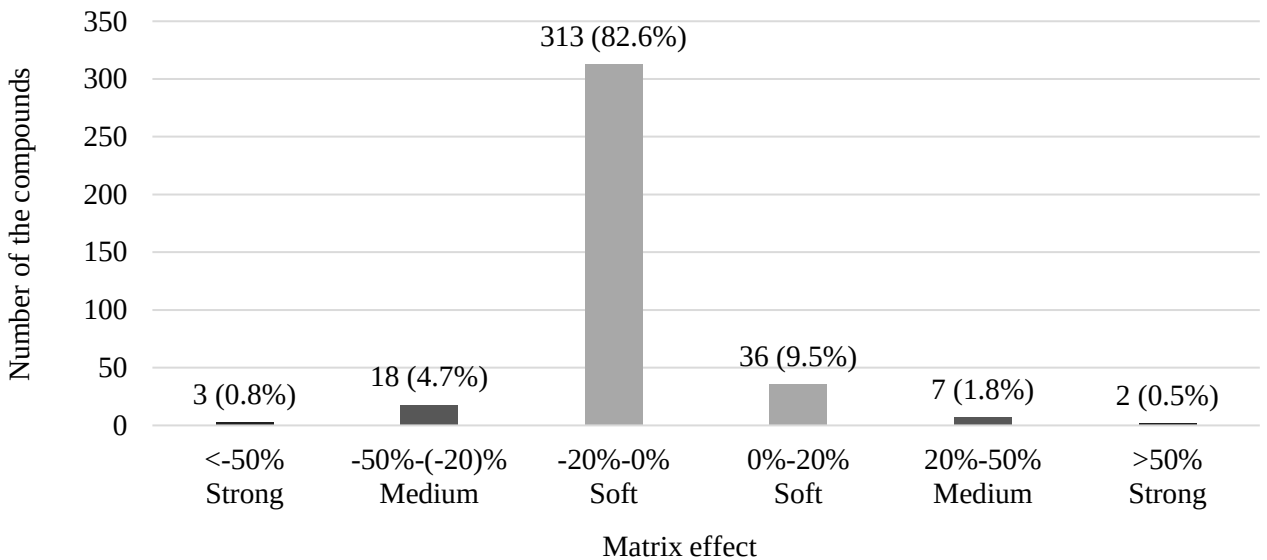

350
300
250
200
150
100
50
0
Number of the compounds
313 (82.6%)
36 (9.5%)
18 (4.7%)
7 (1.8%)
3 (0.8%)
2 (0.5%)
<-50%
Strong
-50%-(-20)%
Medium
-20%-0%
Soft
0%-20%
Soft
20%-50%
Medium
>50%
Strong
Matrix effect

Fig. 16. Scatter plot between retention time (t_R) and matrix effect for 380 target pesticides in urine

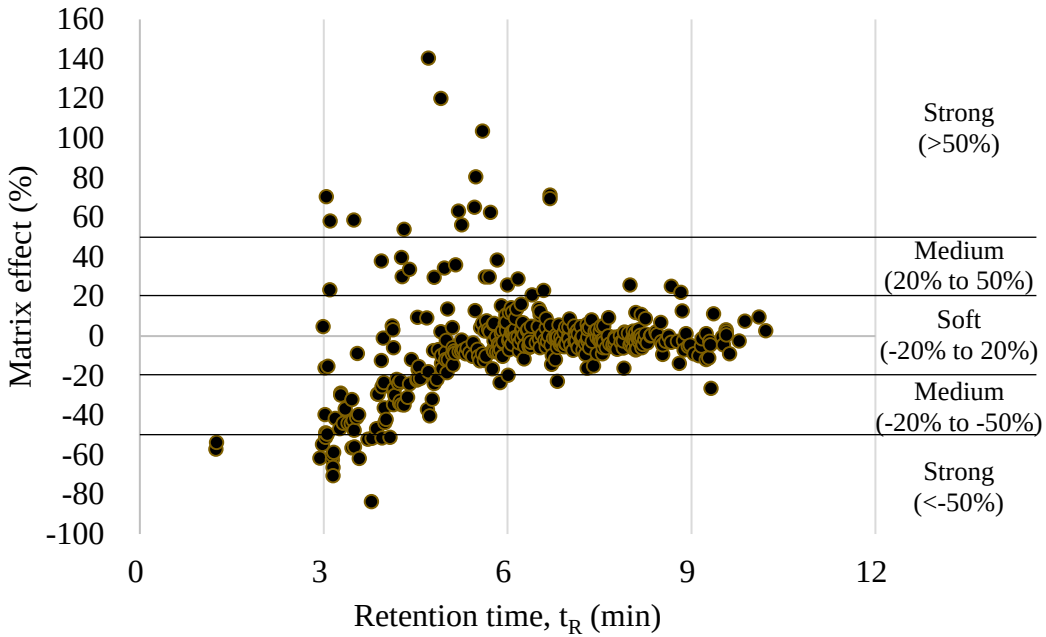
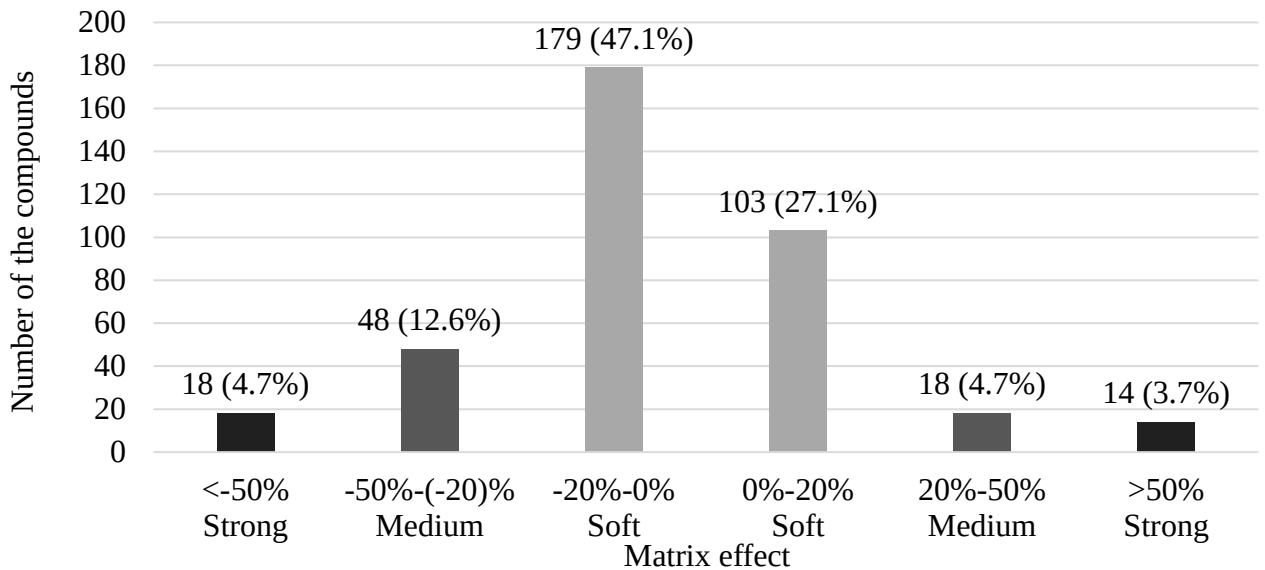

160
140
120
100
80
60
40
20
0
-20
-40
-60
-80
-100
Matrix effect (%)
0
3
6
9
12
Retention time, t_R (min)
Strong
(>50%)
Medium
(20% to 50%)
Soft
(-20% to 20%)
Medium
(-20% to -50%)
Strong
(<-50%)

Fig. 17. Summary of matrix effects for 380 pesticides classified into soft effect (light grey bars, -20% to 0% and 0% to 20%), middle effect (grey bars, -50% to -20% and 20% to 50%), and strong effect (dark grey bars, <-50% and >50%) in human urine samples


Number of the compounds
200
180
160
140
120
100
80
60
40
20
0
18 (4.7%)
48 (12.6%)
179 (47.1%)
103 (27.1%)
18 (4.7%)
14 (3.7%)
<-50%
Strong
-50%-(-20)%
Medium
-20%-0%
Soft
0%-20%
Soft
20%-50%
Medium
>50%
Strong
Matrix effect

Conclusions

A quantitative screening method for rapid and simultaneous analysis of 379 pesticides in serum and 380 pesticides in urine was developed using LC-MS/MS. High speed positive/negative switching electrospray ionization (ESI) mode was utilized, and scheduled multiple reaction monitoring (MRM) was employed. The limit of quantitation was 10 ng/mL for more than 94% of target compounds in both matrices, showing sufficiently low to detect multiresidues at trace levels. The scaled-down QuEChERS procedure was optimized and used for sample preparation after three versions of QuEChERS were compared for recovery. The established method was fully validated for important analytical parameters such as linearity of calibration, accuracy and precision, recovery, and matrix effect. The correlation coefficients (r^2) of calibration were ≥0.990 for 93.9% (serum) and 96.3% (urine) of target compounds. In the accuracy and precision tests, most of the pesticides showed excellent results in intra- and inter-day conditions. In the recovery tests at 10, 50, and 250 ng/mL, 85.8-91.8% of all target compounds in serum and 86.3-88.9% in urine satisfied the recovery range of 70-120% (RSD ≤20%). The average matrix effect for all target compounds in serum and urine were -6.8% and -4.1%, respectively. The established analytical methods in this study can be applied to the identification of pesticide intoxication cases and biomonitoring in total diet study, food toxicology, agricultural, forensic and clinical sciences.

Part 2

Development and Validation of Pesticide Multiresidue Analysis in Human Serum and Urine Using GC-MS/MS

Materials and Methods

Chemicals and reagents

Reference standards (analytical grade) or stock solutions (1,000 mg/L) of each pesticide were purchased from Sigma-Aldrich (St. Louis, MO, USA), Dr. Ehrenstorfer (Augsburg, Germany), and Ultra Scientific (North Kingstown, RI, USA). Acetonitrile and acetone (HPLC grade) were obtained from Fisher Scientific (Seoul, South Korea). Magnesium sulfate anhydrous ($MgSO_4$, purity ≥99.5%) were purchased from Sigma-Aldrich. Sodium chloride (NaCl, 99.0%) was obtained from Samchun (Gyeonggi-do, South Korea). Ceramic homogenizers (2 mm) were provided from Ultra Scientific.

Individual reference standards were subjected to dissolving with a solvent such as acetonitrile or acetone to give each stock solution of 1,000 mg/L. These solutions and commercial stock solutions were mixed so that the concentration of the mixed standard solution was 10 mg/L. This solution was further diluted to make the working solutions using in MS/MS profiling, preparing calibration curves, and validation studies. All the prepared solutions were stored at -20 ℃ until the study was conducted.

GC-MS/MS instrumental conditions

GC-MS/MS analysis was carried out on a Shimadzu GCMS-TQ8040 triple quadrupole mass spectrometer coupled to a GC-2010 plus equipped with an AOC-20i autosampler (Kyoto, Japan). For the mass spectrometer, electron energy of the EI was 70 eV and temperature values of ion source and interface were 230 and 250 ℃, respectively. Detector voltage was maintained at 1.4 kV

during the entire instrumental performance. Argon (≥99.999%) was used as collision inductive dissociation (CID) gas. For the gas chromatograph, a 3.5-mm Topas GC glass liner with wool (Restek, Bellefonte, PA, USA) was inserted in the inlet. The inlet temperature was 280 °C and the pulsed injection at a pressure of 250 kPa was used. Injection mode was splitless and the injection volume was 2 μL. A capillary column was Rxi-5Sil MS (30 m × 0.25 mm i.d., 0.25 μm d_f, Restek, Bellefonte, PA, USA). The oven temperature program (30 min in total) was initialized at 90 °C (held for 3 min), ramped to 120 °C at 20 °C/min, and then to 300 °C at 8 °C/min (held for 3 min). Helium (≥99.999%) was used as carrier gas and total column flow was 1.50 mL/min (constant). For the multiresidue MRM data processing, GCMS solution version 4.30 was utilized.

Establishment of scheduled MRM

Each standard solution of 1 to 10 mg/L was injected (2 μL) to obtain a full scan spectrum in the range of mass to charge ratio (m/z) 50-500. One or two of precursor ion(s) were selected in the spectrum and then a product scan with various collision energies (CE; 3-42 V) was conducted. Among the product ions fragmented, two of them with optimum CE were finally selected as a quantifier and a qualifier ion based on those of selectivity from other compounds, signal intensity, and peak shape on the chromatogram. The loop time of MRM mode was 0.30 sec and the minimum MRM window was set to ±0.30 min from retention time (t_R) so that the dwell time was at least 15 ms.

Sample preparation using modified QuEChERS

Pesticides in human serum and urine sample were extracted by the previously established procedures in **Part 1**. In brief, 100 μL of an aliquot was extracted with 400 μL of acetonitrile in 2-mL of microcentrifuge tube (Eppendorf, Hamburg, Germany). The extract was shaken with two ceramic homogenizer beads for 1 min at 1,200 rpm using a Geno Grinder (1600 MiniG SPEX Sample Prep, Metuchen, NJ, USA). $MgSO_4$ (40 mg) and NaCl (10 mg) were added to the tube for solvent-water layer partitioning. This step was exothermic by $MgSO_4$, so the extract was subjected to cooling on an ice bath. The tube was centrifuged for 5 min at 13,000 rpm (16,800 g) using a microcentrifuge (17TR, Hanil Science, Seoul, Korea), and then without dSPE cleanup, 200 μL of organic supernatant was matrix-matched with 50 μL acetonitrile. The aliquot was equivalent to 0.2 mL per one mL of the final extract. Two μL of the final extract was injected into GC-MS/MS for the target compound analysis.

Validation of methodology

For the determination of LOQ, different concentrations at 10, 25, 50, 100, 150, and 250 ng/mL of matrix-matched procedure standards of serum and urine were injected into GC-MS/MS, respectively. The result of each MRM chromatogram was investigated whether satisfying the signal to noise ratio (S/N) greater than 10 at the LOQ level. If a compound did not meet the S/N criterion, higher concentration satisfying S/N criteria was selected as LOQ. The linearity of calibration was determined (n = 5) by the correlation coefficient (r^2) of the calibration curve within a range from 10 to 250 ng/mL. To correct quantitation properties at low concentrations, a weighting factor of *1/x* was adopted.

Accuracy and precision tests were performed using four different levels of serum or urine quality control (QC) samples (10, 50, 150, and 250 ng/mL). The tests were evaluated under intra-day and inter-day conditions. The intra-day condition was that 5 replicates of each QC level were subjected to analysis in a day. The inter-day condition was that one QC sample of each level was analyzed per day during five successive days. The recovery test was conducted at treated levels of 10, 50, and 250 ng/mL (n = 3). Blank samples of serum or urine was fortified with pesticides, respectively, and treated as the same preparation procedure as described above. In the GC-MS/MS analysis, some target pesticides were affected by the matrix effect severely, so the recovery rate for each pesticide was corrected by using matrix-matched standard calibration. The matrix effect of each target analyte was also calculated by comparing a slope of the calibration curve of the matrix-matched standards and that of the calibration curve of the solvent-only standards using the following equation:

$$\text{Matrix effect}, \% = \left(\frac{\text{Slope of matrix-matched standard calibration}}{\text{Slope of solvent-based standard calibration}} - 1\right) \times 100$$

Safety information

All pesticide standards and reagents used in this study were handled according to the Material Safety Data Sheet (MSDS)'s safety instructions. For all instrumentation, the manufacturer's safety information was followed and implemented.

Results and Discussion

Characteristics of pesticide to be studied

A total of 58 pesticides was selected as research compounds at first (**Table 13**). Among the pesticides, 41 compounds are generally undetectable or have very low sensitivity on LC/MS system. The other 17 pesticides (binapacryl, bromophos, chlorpropham, cyanophos, cyfluthrin, dichlofluanid, dicofol, disulfoton, endosulfan-sulfate, ethalfluralin, isofenphos, isofenphos-methyl, nitrothal-isopropyl, oxyfluorfen, parathion-methyl, silafluofen, and spiromesifen) are known to be amenable on LC/MS (EU Reference Laboratories for Residues of Pesticides), however, could not ionized on LC-MS/MS in **Part 1**. For the chemical group of pesticides, Most of the pesticides were organochlorine (40 compounds). Major pesticide groups such as organophosphate (7), pyrethroid (3), carbamate (1) were also included, and the remaining of 7 pesticides was included in minor groups or unclassified. Among the 56 pesticides, 8 compounds were metabolites of organochlorines such as DDT (o,p'-DDD, p,p'-DDD, o,p'-DDE, and p,p'-DDE), endosulfan (endosulfan-sulfate), heptachlor (heptachlor-epoxide), quintozene (pentachloroaniline and pentachlorothioanisole). These metabolites were included as target analytes because they have already been detected in human biological samples (Zhou et al., 2011; Genuis et al., 2016) or have been identified as major metabolites in experiments with apes (Müller et al., 1978).

Table 13. List of pesticides to be studied and their chemical groups

No.	Compound name	Chemical group	Remarks
1	Aldrin	Organochlorine	-
2	BHC-alpha	Organochlorine	-
3	BHC-beta	Organochlorine	-
4	BHC-delta	Organochlorine	-
5	BHC-gamma	Organochlorine	-
6	Binapacryl[1)]	Others/Unclassified	-
7	Bromophos	Organophosphate	-
8	Bromopropylate	Organochlorine	-
9	Chlordane-cis	Organochlorine	-
10	Chlordane-trans	Organochlorine	-
11	Chlorfenapyr	Organochlorine	-
12	Chlorobenzilate	Organochlorine	-
13	Chlorothalonil	Organochlorine	-
14	Chlorpropham	Carbamate	-
15	Chlorthal-dimethyl	Others/Unclassified	-
16	Cyanophos	Organophosphate	-
17	Cyfluthrin	Pyrethroid	Isomer mixture (4 peaks)
18	DDD-o,p'	Organochlorine	DDT metabolite
19	DDD-p,p'	Organochlorine	DDT metabolite
20	DDE-o,p'	Organochlorine	DDT metabolite
21	DDE-p,p'	Organochlorine	DDT metabolite
22	DDT-o,p'	Organochlorine	-
23	DDT-p,p'	Organochlorine	-
24	Dichlobenil	Organochlorine	-
25	Dichlofluanid	Organochlorine	-
26	Dicloran	Organochlorine	-
27	Dicofol	Organochlorine	-
28	Dieldrin	Organochlorine	-
29	Disulfoton	Organophosphate	-
30	Endosulfan-alpha	Organochlorine	-
31	Endosulfan-beta	Organochlorine	-

Table 13. *(Continued)*

No.	Compound name	Chemical group	Remarks
32	Endosulfan-sulfate	Organochlorine	Endosulfan metabolite
33	Endrin	Organochlorine	-
34	Ethalfluralin	Others/Unclassified	-
35	Etridiazole	Organochlorine	-
36	Fenclorim	Others/Unclassified	-
37	Fenitrothion	Organophosphate	-
38	Fthalide	Organochlorine	-
39	Heptachlor	Organochlorine	-
40	Heptachlor-epoxide	Organochlorine	Heptachlor metabolite
41	Isofenphos	Organophosphate	-
42	Isofenphos-methyl	Organophosphate	-
43	Methoxychlor	Organochlorine	-
44	Nitrothal-isopropyl	Others/Unclassified	-
45	Oxyfluorfen	Others/Unclassified	-
46	Parathion-methyl	Organophosphate	-
47	Pentachloroaniline	Organochlorine	Quintozene metabolite
48	Pentachlorothioanisole	Organochlorine	Quintozene metabolite
49	Procymidone	Organochlorine	-
50	Quintozene	Organochlorine	-
51	Silafluofen	Pyrethroid	-
52	Spiromesifen	Others/Unclassified	-
53	Tefluthrin	Pyrethroid	-
54	Tetradifon	Organochlorine	-
55	Vinclozolin	Organochlorine	-
-	Captafol[2)]	Organochlorine	-
-	Captan[2)]	Organochlorine	-
-	Folpet[2)]	Organochlorine	-

[1)]Compound excluded from the final analytical method validation study in serum.

[2)]These compounds were excluded from the list of the final validation in serum and urine

Optimization of MRM

MRM optimization on GC-MS/MS was conducted in the order of 1) mass full scan with m/z 50-500, 2) precursor ion selection, 3) product ion scan under CID, and 4) determination of product ion. At first, all of the pesticides to be studied were subjected to full scan analysis and successfully obtained their specific spectrum patterns. According to each pesticide spectrum data, most of the target analytes were fragmented in the EI source and existed abundantly as various fragment ions in mass analyzers. Therefore, the precursor ions were selected from one or two of the fragment ions as well as the molecular ion. Each selected precursor ion was subjected to further fragmentation with various CE voltages, and then its product ions were determined depending on intensity or selectivity. Because the resolution of the triple quadrupole mass spectrometer was unit mass, two transition profiles of each pesticide were finally established according to the guideline of SANTE/11813/2017 (European Commission, 2017). Each MRM transition was selected respectively as a quantifier ion for quantitation processing and a qualifier ion for verification of the false positive (**Table 14**).

Table 14. The optimized GC-MS/MS parameters including retention times (t_R), MRM transitions for each pesticide

No.	Pesticide Name	t_R (min)	Transition	
			Precursor ion > Product ion (CE, V)	
			Qualifier	Qualifier
1	Aldrin	16.35	263 > 193 (30)	263 > 191 (30)
2	BHC-alpha	12.78	181 > 145 (15)	219 > 183 (9)
3	BHC-beta	13.40	181 > 145 (18)	219 > 183 (9)
4	BHC-delta	14.32	181 > 145 (15)	219 > 183 (9)
5	BHC-gamma	13.64	181 > 145 (15)	219 > 183 (12)
6	Binapacryl	19.08	83 > 55 (9)	83 > 53 (15)
7	Bromophos	16.81	331 > 93 (30)	329 > 93 (27)
8	Bromopropylate	21.71	341 > 183 (21)	183 > 76 (27)
9	Chlordane-cis	18.17	377 > 268 (27)	377 > 266 (24)
10	Chlordane-trans	17.86	377 > 268 (27)	377 > 266 (24)
11	Chlorfenapyr	19.08	247 > 227 (15)	247 > 200 (27)
12	Chlorobenzilate	19.47	251 > 111 (27)	139 > 75 (27)
13	Chlorothalonil	14.04	264 > 168 (27)	266 > 168 (24)
14	Chlorpropham	12.20	127 > 65 (21)	213 > 171 (9)
15	Chlorthal-dimethyl	16.40	301 > 223 (24)	332 > 301 (18)
16	Cyanophos	13.75	243 > 109 (15)	125 > 79 (9)
17-1	Cyfluthrin_1	24.53	163 > 127 (6)	226 > 206 (15)
17-2	Cyfluthrin_2	24.65	163 > 127 (6)	226 > 206 (15)
17-3	Cyfluthrin_3	24.72	163 > 127 (6)	226 > 206 (15)
17-4	Cyfluthrin_4	24.78	163 > 127 (6)	226 > 206 (15)
18	DDD-o,p'	18.85	235 > 165 (24)	165 > 163 (30)
19	DDD-p,p'	19.74	235 > 165 (24)	235 > 199 (18)
20	DDE-o,p'	17.93	246 > 176 (30)	318 > 246 (27)
21	DDE-p,p'	18.70	246 > 176 (27)	318 > 246 (21)
22	DDT-o,p'	19.67	235 > 165 (24)	235 > 199 (18)
23	DDT-p,p'	20.55	235 > 165 (24)	235 > 199 (18)
24	Dichlobenil	7.73	171 > 100 (27)	136 > 100 (9)
25	Dichlofluanid	16.05	224 > 123 (15)	224 > 77 (30)
26	Dicloran	13.10	206 > 176 (12)	206 > 124 (27)
27	Dicofol	16.63	139 > 111 (15)	250 > 139 (15)
28	Dieldrin	18.81	279 > 207 (27)	263 > 193 (30)

Table 14. *(Continued)*

No.	Pesticide Name	t_R (min)	Transition	
			Precursor ion > Product ion (CE, V)	
			Qualifier	Qualifier
29	Disulfoton	14.20	142 > 109 (6)	186 > 153 (6)
30	Endosulfan-alpha	18.17	241 > 206 (15)	339 > 160 (21)
31	Endosulfan-beta	19.54	339 > 160 (18)	339 > 267 (9)
32	Endosulfan-sulfate	20.43	272 > 237 (18)	272 > 235 (15)
33	Endrin	19.30	263 >191 (30)	263 >193 (28)
34	Ethalfluralin	12.10	276 > 202 (15)	316 > 276 (12)
35	Etridiazole	9.29	211 > 183 (12)	183 > 140 (15)
36	Fenclorim	12.81	189 > 104 (15)	224 > 189 (15)
37	Fenitrothion	15.88	277 > 260 (6)	260 > 125 (15)
38	Fthalide	16.67	243 > 215 (18)	272 > 243 (18)
39	Heptachlor	15.47	272 > 237 (18)	272 > 235 (18)
40	Heptachlor-epoxide	17.29	353 > 263 (18)	353 > 282 (15)
41	Isofenphos	17.30	213 > 121 (15)	185 > 121 (15)
42	Isofenphos-methyl	16.95	199 > 121 (12)	199 > 93 (27)
43	Methoxychlor	21.82	227 > 169 (27)	227 > 212 (18)
44	Nitrothal-isopropyl	16.68	236 > 194 (12)	194 > 148 (12)
45	Oxyfluorfen	18.84	252 > 146 (30)	252 > 170 (30)
46	Parathion-methyl	15.25	263 > 109 (15)	263 > 246 (6)
47	Pentachloroaniline	14.75	263 > 192 (21)	265 > 194 (21)
48	Pentachlorothioanisole	15.99	296 > 263 (18)	263 > 193 (30)
49	Procymidone	17.55	283 > 96 (12)	285 > 96 (12)
50	Quintozene	13.52	295 > 237 (18)	237 > 143 (24)
51	Silafluofen	25.50	286 > 258 (12)	286 > 207 (15)
52	Spiromesifen	21.28	272 > 254 (9)	272 > 185 (24)
53	Tefluthrin	14.35	177 > 127 (18)	197 > 141 (15)
54	Tetradifon	22.29	356 > 159 (15)	356 > 229 (9)
55	Vinclozolin	15.22	212 > 172 (15)	285 > 212 (15)
-	Captafol[1)]	21.03	183>79 (15)	313>79 (21)
-	Captan[1)]	17.63	149 > 79 (18)	149 > 105 (6)
-	Folpet[1)]	17.77	260 > 130 (15)	260 >232 (9)

[1)]These compounds were excluded from the list of the final validation in serum and urine.

Determination of final selected pesticides to be validated

After MRM optimization and determination of t_R, recovery samples and matrix-matched standard solutions of serum and urine at 250 ng/mL were injected respectively on GC-MS/MS to select final pesticides for validation tests. As a result, 54 of the total 58 pesticides were found to be free of serious degradation or false positives. Captafol, captan, and folpet which are phthalimide (PI) organochlorines, however, were not recovered at all in both of recovery samples (**Fig. 18**). Because these PI organochlorines are very unstable, so hydrolyzed in aqueous conditions or in broad ranges of pH (Turner, 2015). Furthermore, it has been reported that captan and folpet were rapidly degraded into tetrahydrophthalimide (THPI) and PI, respectively, in the blood (half-lives; 0.97 s for captan and 4.9 s for folpet) (Gordon et al., 2001). Therefore, captan, captafol, and folpet were excluded from the list of pesticide to be developed. Instead of determination of captan, captafol, and folpet, THPI and PI could be biomarkers of these pesticides in serum and urine (Berthet et al., 2011).

In another case, binapacryl, a dinitrophenol pesticide, was found to be interfered severely by serum matrix (**Fig. 19**). The MRM transition of this compound was comprised of very small m/z (quantifier; 83 > 55 and qualifier; 83 > 53, see **Table 14**), so it could be easily overlaid by small molecule or fragment with similar t_R. On the other hand, there was no interference or overlay for binapacryl in urine. Therefore, binapacryl was excluded from the final list of the pesticide only in serum.

In summary, among the 58 pesticides, 54 analytes in serum and 55 analytes in urine were selected as final validation compounds.

Validation of analytical method

Limit of quantitation (LOQ) and linearity of calibration. There are various criteria for the determination of LOQ (Kruve et al., 2015). Among them, S/N approach has been widely used for development of bioanalytical methodologies (Pauwels et al., 1999; Pozzebon et al., 2003). LOQs were determined for the minimum concentration of S/N ≥10 in serum or urine sample. For the 55 target pesticides, 53 satisfied LOQ criterion at a concentration of 10 ng/mL in both samples (**Fig. 20**). Endosulfan-beta did not satisfy this criterion in both samples, thus higher concentration (25 ng/mL, S/N ≥10) was selected as LOQ. The LOQ of binapacryl was also determined at 25 ng/mL in urine sample, but could not be determined in serum sample due to overlaps with interferences (see **Fig. 19**). In many application reports including acute and intoxication, local monitoring, and exposure from agricultural field, some pesticides and their metabolites studied in this study have been detected higher than 10 ng/mL in blood or urine (Beltran et al., 2001; Columé et al., 2001; Lacassie et al., 2001b; López et al., 2001; Sharma et al., 2015). Therefore, this bioanalytical method had sufficient sensitivity for the determination of pesticides in agricultural and forensic fields.

The linearity of calibration for each pesticide was verified by preparing a calibration curve ranging from LOQ to 250 ng/mL. The correlation coefficient (r^2) of calibration tells how strong a relationship between the two variables (concentration and signal) is. The closer the r^2 value is to 1, the stronger the positive relationship. The r^2 of target compounds were greater than 0.9935 in serum and 0.9925 in urine (**Fig. 20**). It indicates that relationship between concentration and signal of all target pesticides was highly strong, thus ensuring quantitative properties.

Fig. 18. Structures for phthalimide organochlorines, (a) captafol, (b) captan, and (c) folpet. MRM chromatograms for matrix-matched standards of (d) captafol, (e) captan, (f) folpet, and (g)-(i) these recovery samples in serum, and MRM chromatograms for matrix-matched standards of (j) captafol, (k) captan, (l) folpet, and (m)-(o) these recovery samples in urine

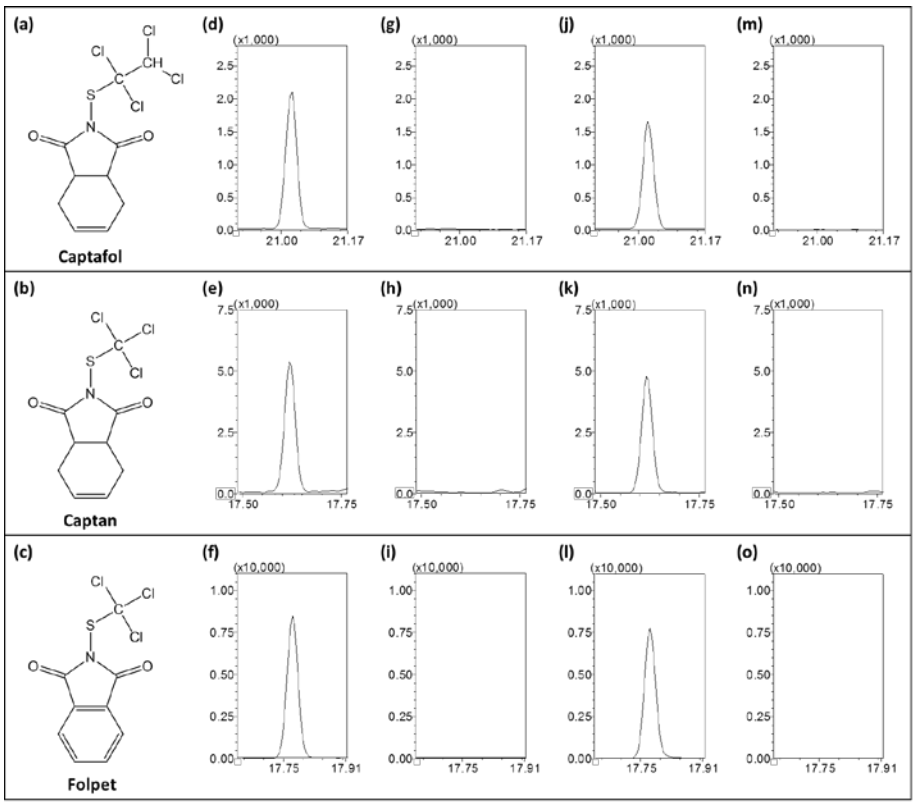

(a)
Captafol
(b)
Captan
(c)
Folpet
(d)
(e)
(f)
(g)
(h)
(i)
(j)
(k)
(l)
(m)
(n)
(o)
(x1,000)
(x10,000)
21.00
21.17
17.50
17.75
17.75
17.91

Fig. 19. MRM chromatograms of (a) solvent-only standard, (b) matrix-matched standard in serum, and (c) matrix-matched standard in urine for binapacryl.

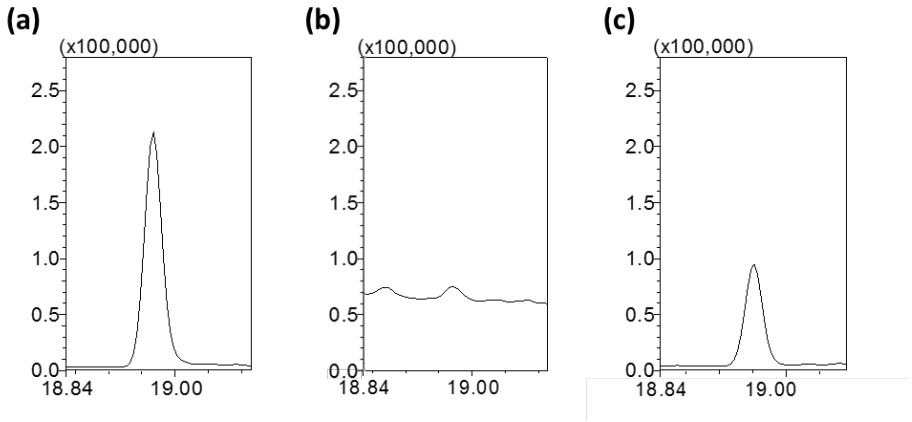
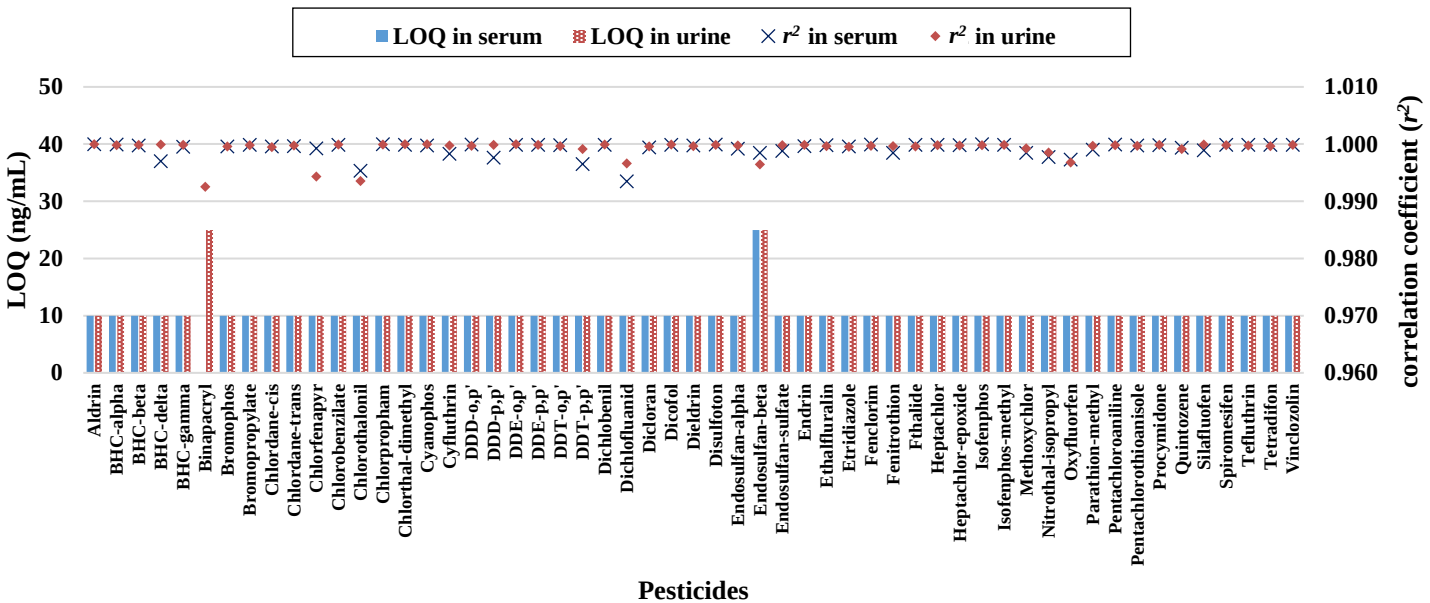

(a)
(x100,000)
2.5
2.0
1.5
1.0
0.5
0.0
18.84
19.00
(b)
(x100,000)
2.5
2.0
1.5
1.0
0.5
0.0
18.84
19.00
(c)
(x100,000)
2.5
2.0
1.5
1.0
0.5
0.0
18.84
19.00

Fig. 20. Individual LOQs and correlation coefficients (r^2) of 55 pesticides for the final established analytical method in serum and urine

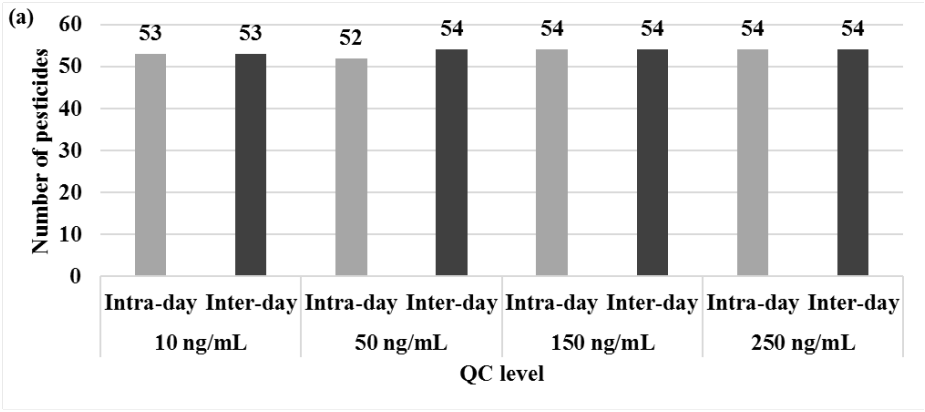

LOQ in serum
LOQ in urine
r² in serum
r² in urine
LOQ (ng/mL)
correlation coefficient (r²)
50
40
30
20
10
0
1.010
1.000
0.990
0.980
0.970
0.960
Aldrin
BHC-alpha
BHC-beta
BHC-delta
BHC-gamma
Binapacryl
Bromophos
Bromopropylate
Chlordane-cis
Chlordane-trans
Chlorfenapyr
Chlorobenzilate
Chlorothalonil
Chlorpropham
Chlorthal-dimethyl
Cyanophos
Cyfluthrin
DDD-o,p'
DDD-p,p'
DDE-o,p'
DDE-p,p'
DDT-o,p'
DDT-p,p'
Dichlobenil
Dichlofluanid
Dicloran
Dicofol
Dieldrin
Disulfoton
Endosulfan-alpha
Endosulfan-beta
Endosulfan-sulfate
Endrin
Ethalfluralin
Etridiazole
Fenclorim
Fenitrothion
Fthalide
Heptachlor
Heptachlor-epoxide
Isofenphos
Isofenphos-methyl
Methoxychlor
Nitrothal-isopropyl
Oxyfluorfen
Parathion-methyl
Pentachloroaniline
Pentachlorothioanisole
Procymidone
Quintozene
Silafluofen
Spiromesifen
Tefluthrin
Tetradifon
Vinclozolin
Pesticides

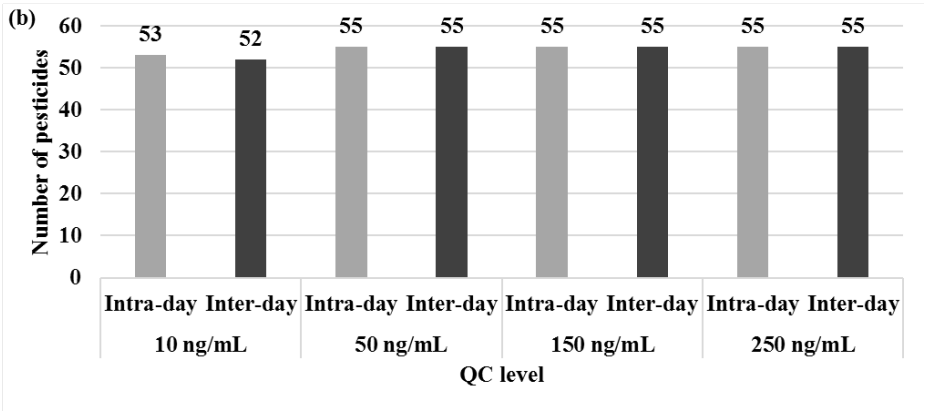
Accuracy and precision. The accuracy and precision tests at 10, 50, 150, and 250 ng/mL were conducted under intra-day and inter-day conditions through the QC sample (a sample with a known quantity of analyte (US FDA, 2013)) analysis. According to US FDA, the acceptable criteria of accuracy ranges with precision ranges (expressed as RSD) were 80-120% with RSD ≤20% at an LOQ and 85-115% with RSD ≤15% at higher concentration (US FDA, 2013). In this study, the LOQ criteria were applied at the QC level of 10 ng/mL and the other criteria at 50, 150, and 250 ng/mL. The accuracy ranges of the target pesticides in serum were 83.5-119.3% with RSD 1.1-19.8% in the intra-day condition and 81.4-118.4% with RSD 0.6-14.4% in the inter-day condition. The accuracy ranges in urine were 91.5-114.2% in the intra-day with RSD 0.5-19.1% in the intra-day and 91.8%-120.4% with RSD 0.9-16.8%. For the serum QC samples, the numbers of 54 target pesticides satisfying the criteria were 53 (98.1% of the total) at the QC level of 10 ng/mL, and 52 to 54 (96.3% to 100%) at 50, 150, and 250 ng/mL, respectively (**Fig. 21, a**). Except for LOQ issues, p,p'-DDT and methoxychlor (accuracy 108.7% and 114.8%, respectively) at 50 ng/mL were slightly out of the RSD criteria (19.8% and 19.1%, respectively) in intra-day. For the urine QC samples, the numbers of 55 target pesticides satisfying the criteria were 52 to 53 (94.5% to 96.4% of the total) at the QC level of 10 ng/mL, and 55 (100%) at 50, 150, and 250 ng/mL, respectively (**Fig. 21, b**). Except for LOQ issues, chlorothalonil at 10 ng/mL were slightly out of the accuracy criteria (120.4% with RSD 14.7%) in the inter-day. From the results, the most of the pesticides obtained excellent and robust bioanalytical methods in serum and urine using GC-MS/MS. A few pesticides slightly out of the criteria are also available for screening purpose. Therefore, this analytical methods can be

performed with high reliability in forensic investigation, clinical biomonitoring, or for occupational/non-occupational exposure.

Recovery. The recovery not only indicates the extraction efficiency of a pesticide in the sample treatment but also it can be another accuracy parameter. The European Commission recommended an acceptable recovery range from 70 to 120% with RSD ≤20% (European Commission, 2017). The recovery tests were performed at fortification levels of 10, 50, and 250 ng/mL in serum and urine sample. As a result, recovery ranges were 70.4-118.2% (RSD; 0.3-14.8%) in serum and 70.5-119.2% (RSD; 0.2-17.5%) in urine at all treated levels. These results indicate that all target pesticides fell the acceptable recovery criteria within their detection ranges. The scaled-down QuEChERS procedures established for LC amenable pesticides also exhibited strong and rugged extraction efficiencies for relatively non-polar GC amenable pesticides.

Fig. 21. The number of pesticides satisfying the accuracy range of 80-120% with RSD ≤20% at a QC level of 10 ng/mL and the accuracy range of 85-115 with RSD ≤ 15% at QC levels of 50, 150, and 250 ng/mL in (a) serum and (b) urine under intra-day (grey bars) and inter-day (dark grey bars) conditions


(a)
Number of pesticides
60
50
40
30
20
10
0
53
53
52
54
54
54
54
54
Intra-day
Inter-day
10 ng/mL
50 ng/mL
150 ng/mL
250 ng/mL
QC level


(b)
Number of pesticides
60
50
40
30
20
10
0
53
52
55
55
55
55
55
55
Intra-day
Inter-day
10 ng/mL
50 ng/mL
150 ng/mL
250 ng/mL
QC level

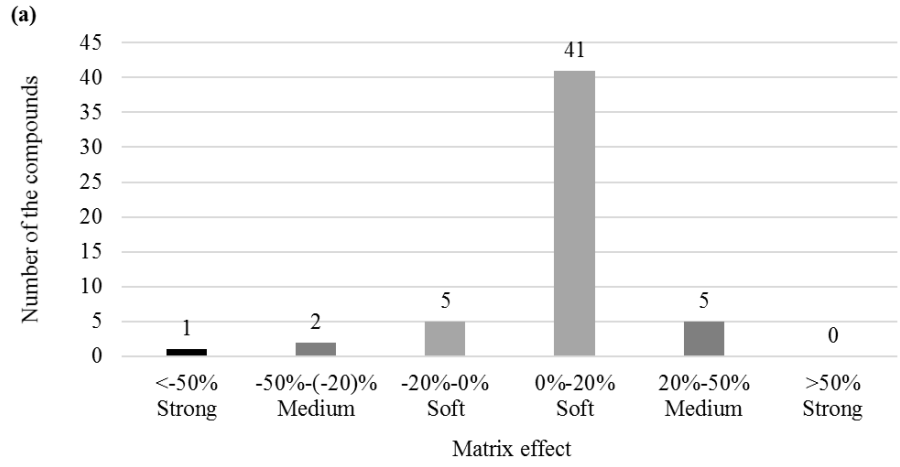
Matrix effect. The matrix effect is a common effect on GC and mass spectrometer system. This phenomenon in biological samples can be reduced by sample dilution (López et al., 2001) and be corrected by internal standard or preparing calibration curve with matrix-matched standards (Saito et al., 2012; Luzardo et al., 2015). In this study, 100 μL of serum or urine samples were extracted with four times larger volumes of acetonitrile (400 μL) and matrix-matched standards were used to overcome matrix effect.

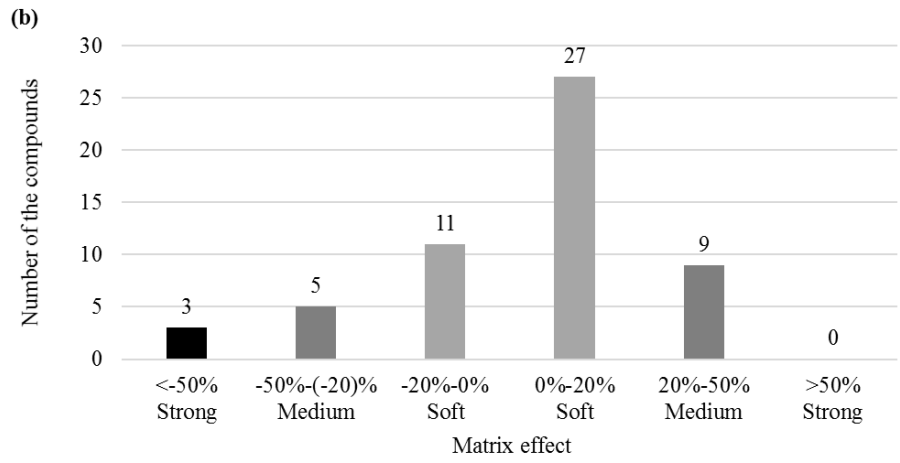
The matrix effect was expressed as percentage by the equation as described above. If a percentage is 0%, not matrix effect is observed. A value farther from 0% tells that matrices contribute to more enhancement or suppression of the detector response. The average matrix effects for target pesticides in serum and urine were 8.8% and 1.7%, respectively. Serum matrices had a greater effect than urine matrices. Matrix effect values were divided into 6 ranges and three groups (soft, medium, and strong matrix effect, see **Fig 22**). Most of the matrix effects for the target pesticides (46 compounds for serum and 38 for urine) were included in soft effect range (between -20 and 20% of the percentages). Within the range, matrices are considered to not affect detector responses of target analytes, thus negligible (He et al., 2015). The other groups of pesticides (8 for serum and 17 for urine) including medium (-50% to -20% or 20% to 50%) and strong (<-50% or >50%) showed larger matrix effect. These pesticides within the ranges need matrix-matched standard calibration to correct the quantitation.

Conclusions

A rapid and simultaneous bioanalytical method for 54 pesticides in human serum and for 55 pesticides in human urine was established and validated using GC-MS/MS. A pulsed injection at a high pressure (250 kPa) on the GC injector and an EI mode on the ion source of MS/MS were used and a scheduled MRM of each target compound were established for an effective and high-throughput analysis. For the sample preparation, a modified QuEChERS procedure without dSPE cleanup was adopted for application in small volumes (100 μL) of aliquot. Except for binapacryl in serum, the false positive was not found on the MRM window of each pesticide in both of matrix. The target LOQ of the established methodology was at 10 ng/mL and 53 of all the pesticides met the criteria in both of serum and urine, showing sufficiently low to detect multiresidues at trace levels in both of serum and urine samples. The correlation coefficients (r^2) were ≥0.990 for all target analytes within the linear range from LOQ to 250 ng/mL. For ruggedness of the method, the accuracy and precision were conducted under the intra- and inter-day conditions and most of the compounds showed excellent validation results. The recovery rates were 70.4-119.2% with the RSD of 0.2-17.5% at fortification levels of LOQ, 50, and 250 ng/mL, showing a high extraction efficiency in this preparation procedure. The averages of the matrix effect (%) in serum and urine samples were 8.8% and 1.7%, respectively. This determination method of screening multiresidual pesticides can be useful in forensic or medical case of acute pesticide intoxication where highly fast monitoring is essential.

Fig. 22. Distribution of matrix effects for 380 pesticides in (a) serum and (b) urine. The matrix effect was classified into soft effect (light grey bars, -20% to 0% and 0% to 20%), middle effect (grey bars, -50% to -20% and 20% to 50%), and strong effect (dark grey bars, <-50% and >50%)


(a)
Number of the compounds
45
40
35
30
25
20
15
10
5
0
1
2
5
41
5
0
<-50% Strong
-50%-(-20)% Medium
-20%-0% Soft
0%-20% Soft
20%-50% Medium
>50% Strong
Matrix effect


(b)
Number of the compounds
30
25
20
15
10
5
0
3
5
11
27
9
0
<-50% Strong
-50%-(-20)% Medium
-20%-0% Soft
0%-20% Soft
20%-50% Medium
>50% Strong
Matrix effect

Chapter II

Analysis of Neonicotinoids (Clothianidin, Imidacloprid, and Thiamethoxam) and Pesticide Multiresidues in Honey Bee, Pollen, and Honey Using LC-MS/MS and GC-MS/MS

Introduction

Benefits from honey bee

Honey bee is an important pollinator and considerably contribute to the ecosystem and agriculture in the earth. Pollination is essential to reproductive system of wild flowers, and bees mediate pollination by their foraging behavior (Corbet et al., 1991). It was reported that almost all of pollination (90-100%) for many agricultural crops such as apple, almond, onion, and carrot is carried out by honey bee (Johnson, 2010). The value of pollination by insect is estimated at more than $200 billion, accounting for 9.5% of the total value of global agricultural production (vanEngelsdorp and Meixner, 2010). Fruit productivity by honey bee pollination is superior to that by artificial pollination. In the Republic of Korea, the rate of apple fruit set was 40.9% for honey bee (*Apis mellifera*) whereas 26.7% for artificial (hand) method (Lee et al., 2008). Honey bee also provide various apicultural products such as honey, pollen, and wax.

Honey bee Colony Collapse Disorder (CCD)

Recently, inexplicable and massive honey bee disappearances were observed. This phenomenon began to be a serious issue from fall 2006, as the beekeeping industry in the USA experienced catastrophic losses (Johnson et al., 2009). A similar disaster was also reported in Europe (Benjamin et al., 2012). This syndrome was named Colony Collapse Disorder (CCD) and its definition is that (1) the apparent rapid loss of adult bee workers resulting in weak or dead colonies with excess brood populations compared to adult bee populations; (2)

the noticeable lack of dead bee workers both within and surrounding the hive; and (3) the delayed invasion of hive pests (e.g., small hive beetles and wax moths) and kleptoparasitism from neighboring honey bee colonies (Cox-Foster et al., 2007; vanEngelsdorp et al., 2009). CCD caused a 50-90% loss of the beekeeping colonies in the United States (Cox-Foster et al., 2007).

There are various possible causes such as larger animal predatory damage, mite (*Acarapis woodi and Varroa destructor*), microorganism, virus, global warming, urbanization, abuse of pesticide, genetically modified (GM) crop, and electromagnetic radiation (Stankus, 2008; Sainudeen Sahib, 2011).

Neonicotinoid, a suspicious chemical leading to CCD

There are some opinions that pesticide poisoning, especially caused by neonicotinoids is related to CCD. Neonicotinoid is a relatively modern pesticide introduced in the early 1990s. Its safety than other pesticides made it popular and neonicotinoid became one of the most commonly used insecticide globally. In 2008, the agrochemical market share of neonicotinoids was 24% (€1.5 billion) of total volume (€6.3 billion) and neonicotinoid had gained an 80% (€0.77 billion) share of a total insecticidal seed treatment market (€0.96 million) (Jeschke et al., 2011). Recently, however, various honey bee malfunctions caused by neonicotinoids have been reported, such as impaired winterization, decreased immunity, promotion of a viral pathogen replication which may lead to CCD (Di Prisco et al., 2013; Lu et al., 2014).

Among the neonicotinoids, clothianidin, imidacloprid, and thiamethoxam are highly neurotoxic to bees (European Commission, 2005; European Commission, 2006; European Food Safety Authority, 2008). The

European Union (EU) restricted the temporary use of these controversial neonicotinoids in crops attractive to pollinators since 2013 (European Commission, 2013). After the prohibition, the European Commission again asked the European Food Safety Authority (EFSA) for an updated risk assessment of the neonicotinoids, and the EFSA confirmed the risks of these three pesticides to honey bees as well as wild bees on February, 2018 (European Food Safety Authority, 2018a; European Food Safety Authority, 2018b; European Food Safety Authority, 2018c; European Food Safety Authority, 2018d). The EU approved the ban on the neonicotinoids on April, 2018 and clothianidin, imidacloprid, and thiamethoxam are expected to be totally banned for all outdoor uses since the end of 2018 (Carrington, 2018).

Analysis of pesticide residues in apiculture samples

As numerous beekeepers and related industry have suffered from a decline in honey bee population, researches have tried to find more accurate and significant relationship between bee death incidents (including CCD) and neonicotinoids as well as multiresidues by developing analytical method and monitoring residue levels in various apiculture samples. Honey bee is one of the complex matrix consisting of protein and fat. Pollen, which is one of the bee products has high protein and sugar (Komosinska-Vassev et al., 2015). The major composition of honey is sugar. Therefore, the challenge is to determine pesticides without overlaps between target analytes and matrices from protein, fat, and sugar. Introduction of mass spectrometry coupled to a liquid chromatography or gas chromatography makes it possible to analyze many pesticides without matrix interferences (**Table 15**). In particular, tandem mass

spectrometry allows high selective sensitive and analysis. Jovanov et al. (2013) reported a trace level of detection (limit of detection; 0.5-1.0 ng/g) for seven neonicotinoids in honey using LC-MS/MS (Jovanov et al., 2013). In addition, tandem mass spectrometry enables a simultaneous analysis of hundreds of target analytes. Many recent literature have developed multiresidue methodology for more than two hundred pesticides in apiculture sample such as honeybee and pollen (Vázquez et al., 2015; Kiljanek et al., 2016). With the development of analytical technique, simple and convenient sample treatment procedure such as the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) is applicable in beekeeping samples (Wiest et al., 2011; Kasiotis et al., 2014).

Purpose of the present study

In this study, three neonicotinoids (clothianidin, imidacloprid, and thiamethoxam) and 391 pesticides in honey bee (dead imago, healthy imago, and larva), pollen, and honey were analyzed using LC-MS/MS or GC-MS/MS. The modified QuEChERS method was used for treatments of bee, honey, and pollen samples and the methodologies of the three neonicotinoids was fully validated with parameters of limit of quantitation (LOQ), linearity of calibration, and recovery. The residue levels of neonicotinoids and pesticide multiresidues were determined in the samples collected near two areas of an apple orchard and a pepper field to improve knowledge of honey bee exposure and to carry out risk assessment for some suspicious pesticides using acute oral LD_{50} of bee.

Table 15. Representative pesticide multiresidue analytical method in apiculture samples

No.	Matrix	Instrument	Sample preparation	Number of analytes	Reference
1	Honey bee	LC-MS/MS	QuEChERS[1)]	200	(Kiljanek et al., 2016)
2	Pollen	LC-MS/MS and GC-MS/MS	QuEChERS	253	(Vázquez et al., 2015)
3	Honey bee, pollen, and wax	GC-MS	dSPE[2)] (Z-Sep[3)])	11	(Li et al., 2015)
4	Honey bee, pollen, and honey	LC-MS/MS	QuEChERS	115	(Kasiotis et al., 2014)
5	Honey	LC-MS/MS	DLLME[4)]	7	(Jovanov et al., 2013)
6	Pollen and nectar	LC-MS/MS	SPE[5)]	12	(Dively and Kamel, 2012)
7	Honey bee, pollen, bee bread, nectar, and honey	LC-MS/MS	QuEChERS	5	(Pohorecka et al., 2012)
8	Honey bee	LC-MS/MS	SPE	5	(Martel and Lair, 2011)
9	Honey bee, pollen, and honey	LC-MS/MS and GC-TOF[6)]	QuEChERS	80	(Wiest et al., 2011)
10	Honey bee, pollen, and honey	LC-MS/MS	QuEChERS +SPE	12	(Kamel, 2010)

Table 15. *(Continued)*

No.	Matrix	Instrument	Sample preparation	Number of analytes	Reference
11	Honey	GC-MS	SPE	48	(Rissato et al., 2007)
12	Honey	LC-MS/MS	OCLLE[7)]	17	(Pirard et al., 2007)
13	Pollen	LC-MS/MS	SPE and LLE[8)]	41	(Chauzat et al., 2006)

[1)]Quick, Easy, Cheap, Effective, Rugged, and Safe

[2)]Dispersive solid-phase extraction

[3)]Zirconium based sorbent

[4)]Dispersive liquid-liquid microextraction

[5)]Solid-phase extraction

[6)]Time-of-flight

[7)]On-column liquid-liquid extraction

[8)]Liquid-liquid extraction

Materials and Methods

Chemicals and reagents

Reference standards of clothianidin (purity; 99.6%), imidacloprid (99.5%), and thiamethoxam (99.0%) were obtained from Wako Pure Chemical Industries (Osaka, Japan), ChemService (West Chester, PA), and Dr. Ehrenstorfer (Augsburg, Germany), respectively. Pesticide standards (>98%) and stock solutions (1,000 mg/L) for multiresidue analysis were purchased from Wako Pure Chemical Industries, ChemService, Dr. Ehrenstorfer, Sigma-Aldrich (St. Louis, MO), Tokyo Chemical Industry (Tokyo, Japan), AccuStandard (New Haven, CT), and ULTRA Scientific (North Kingstown, RI, USA). Formic acid (LC-MS grade) and ammonium formate (≥99.0%) magnesium sulfate anhydrous ($MgSO_4$, ≥99.5%), sodium acetate anhydrous (NaOAc, ≥99.0%), sodium citrate dibasic sesquihydrate ($Na_2HCitr{\cdot}1.5H_2O$, ≥99.0%), and sodium citrate tribasic dihydrate ($Na_3Citrate{\cdot}2H_2O$, ≥99.0%) was sourced from Sigma-Aldrich. Solvents (acetonitrile, acetone, and methanol) for HPLC grade was bought from Fisher Scientific (Seoul, Republic of Korea). Sodium chloride (NaCl, 99.0%) was purchased from Samchun (Gyeonggi-do, South Korea). QuEChERS extraction packet (4 g $MgSO_4$, 1 g NaCl, 1 g $Na_3Citrate{\cdot}2H_2O$, 0.5 g $Na_2HCitr{\cdot}1.5H_2O$) was purchased from ULTRA Scientific. Dispersive SPE in a 2-mL microcentrifuge tube (150 mg $MgSO_4$ and 25 mg PSA) was obtained from Agilent Technologies (Santa Clara, CA). Ceramic homogenizers for 15-mL tube were purchased from Agilent Technologies.

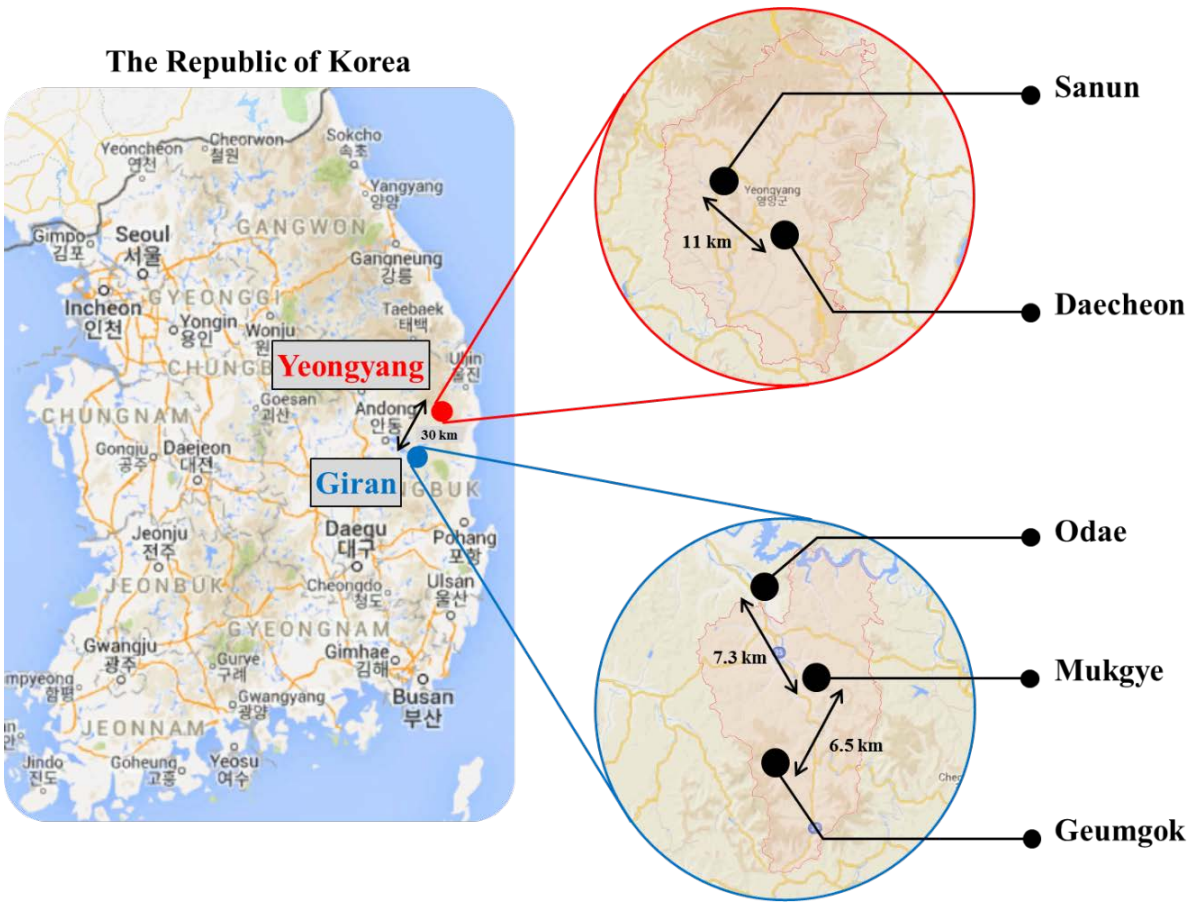
Preparation of matrix-matched standards

Stock solutions (≤1,000 mg/L) was prepared from reference standards using acetonitrile, acetone, and methanol. Aliquots of solutions for clothianidin, imidacloprid, and thiamethoxam were mixed to give a concentration of 10 mg/L. A portion of each multiresidual pesticide were also mixed to make a concentration of 2.5 mg/L. Each standard mixture was further diluted with acetonitrile, respectively. Standard solutions were stored at -20°C before use. Matrix-matched standards were prepared by mixing 0.1 mL standard solutions and 0.4 mL of the blank matrix solutions of bee, pollen, and honey.

Sample collection

Blank bee samples without pesticides were thankfully obtained from beekeepers of Seoul National University (the Republic of Korea) and blank pollen and honey were purchased from a commercial market. Bee (*Apis mellifera L.*) colonies for the field monitoring were obtained from the beekeepers near monitoring areas. The colonies (n = 5) were placed in Giran (three monitoring sites; Geumgok, Mukgye, and Odae, the areas of apple orchards) and Yeongyang (two sites; Sanun and Daecheon, the areas of pepper fields), the Republic of Korea, respectively (**Fig. 23**). Before the investigation, all colonies were treated with fluvalinate to control mites and additional pesticide treatments around colonies were not conducted during the investigation.

Fig. 23. Distribution of monitoring sites in the Republic of Korea


The Republic of Korea
Yeoncheon
Cheorwon
Sokcho
Yangyang
GANGWON
Gimpo
Seoul
Gangneung
GYEONGGI
Incheon
Yongin
Wonju
Taebaek
Yeongyang
CHUNGB
CHUNGNAM
Goesan
Andong
30 km
Gongju
Daejeon
Giran
Daegu
Pohang
Jeonju
JEONBUK
Cheongdo
Ulsan
GYEONGNAM
Gwangju
Gurye
Gimhae
Busan
Gwangyang
JEONNAM
Jindo
Goheung
Yeosu
Yeongyang
11 km
Sanun
Daecheon
7.3 km
6.5 km
Odae
Mukgye
Geumgok

The monitoring periods for dead imago and pollen were at approximately 7 days intervals between before and after full bloom seasons of apple (from April 24 to June 6, 2014 in Giran) and pepper (from July 6 to August 6, 2014 in yeongyang), respectively (**Table 16 and 17**). In each area, baskets and pollen traps were installed in front of the five colony entries to collect dead imagos and pollen, respectively. Healthy imagos, larvae (including the pupal stages), and honey were collected near or in colonies on June 10 in Giran and August 7 in Yeongyang, respectively. All samples were stored at -20 °C until sample treatment and analysis. The dead and pollen samples from 5 different colonies in each day investigation were combined together, resulting in one dead imago and pollen sample per apiary, respectively. The healthy, larva, and honey samples collected from each colony were collected and analyzed respectively.

Table 16. Sampling results in Giran during investigation period on April 24 to June 6, 2014

Sample	Site (Giran)	Monitoring data, 2014												
		21-Apr	25-Apr	3-May	10-May	17-May	24-May	30-May	6-Jun	10-Jun, colony				
										No. 1	No. 2	No. 3	No. 4	No. 5
Dead imago	**Geumgok**	O	O	O	O	O	O	O	O					
	Mukgye	O	O	O	O	O	O	O	O					
	Odae	O	O	O	O	O	O	O	O					
Healthy imago	**Geumgok**									O	O	O	O	O
	Mukgye									O	O	O	O	O
	Odae									O	O	O	O	O
Larva	**Geumgok**									O		O	O	O
	Mukgye									O		O	O	
	Odae										O	O		
Pollen	**Geumgok**	O	O	O	O	O	O	O	O					
	Mukgye	O	O	O	O	O	O	O	O					
	Odae	O	O	O	O	O	O	O	O					
Honey	**Geumgok**									O	O	O	O	O
	Mukgye									O	O	O	O	O
	Odae									O	O	O	O	O

Table 17. Sampling results in Yeongyang during investigation period on July 6 to August 7, 2014

Sample	Site (Yeongyang)	Monitoring data, 2014										
		6-Jul	14-Jul	20-Jul	27-Jul	1-Aug	6-Aug	7-Aug, colony				
								No. 1	No. 2	No. 3	No. 4	No. 5
Dead imago	Sanun	O	O	O	O	O	O					
	Daecheon	O	O	O	O	O	O					
Healthy imago	Sanun							O	O	O	O	O
	Daecheon							O	O	O	O	O
Larva	Sanun							O	O	O	O	O
	Daecheon							O	O	O	O	O
Pollen	Sanun	O		O	O	O	O					
	Daecheon	O		O	O	O	O					
Honey	Sanun							O	O	O	O	O
	Daecheon							O	O	O	O	O

Instrumental conditions of LC-MS/MS and GC-MS/MS

LC-MS/MS. For the determination of three neonicotinoids (clothianidin, imidacloprid, and thiamethoxam) and multiresidual pesticides selected to an LC analysis, a Shimadzu LCMS-8040 triple quadrupole mass spectrometer coupled to a Shimadzu Nexera UHPLC (Kyoto, Japan) was utilized. The UHPLC system was comprised of a degasser (DGU-20A5), two of solvent delivery module (LC-30AD), an autosampler (SIL-30AC), and a column oven (CTO-20A). These instruments were connected by communications bus module (CBM-20A).

For the MS/MS conditions, nebulizing gas and drying gas flow rates were 3 L/min and 15 L/min, respectively. Desolvation line (DL) and heat block temperature values were 250 °C and 400 °C, respectively. A polarity switching electrospray ionization (ESI) mode was employed for an ionization of target analytes. Argon gas (≥99.999%) was used in collision-induced dissociation (CID) during a product scan or multiple reaction monitoring (MRM). Auto dwell time allocation was adopted and detection window for each pesticide was ±1.0 min. LabSolution version 5.60 was utilized as a LCMS software during data processing.

For the UHPLC conditions during analysis of three neonicotinoids in bee, and pollen, the separation was performed on a Luna C18 column (100 × 2.0 mm, 3 μm, Phenomenex, Torrance, CA) coupled with SecurityGuard Ultra guard column (Phenomenex) at 40 °C oven temperature. Mobile phases were 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B), and the total flow rate was 0.2 mL/min. The gradient program for mobile phases B was initialized at 5% for 0.5 min, ramped to 95% for 2 min, held at 95% for 1.5 min,

and then raised to 100% for 0.5 min. After the elution, the percentage of B was sharply reduced to 5% for 0.5 min and held at 5% for 2 min for initialization of the mobile phases. Total of the runtime was 7 min and the injection volume was 5 μL.

For the UHPLC conditions during analysis of three neonicotinoids in honey and pesticide multiresidues in bee, pollen, and honey, the separation was conducted on a Kinetex C18 column (100 × 2.1 mm, 2.6 μm, Phenomenex, Torrance, CA) coupled with SecurityGuard Ultra guard column at 40 °C oven temperature. Mobile phases were 5 mM ammonium formate and 0.1% formic acid in water (A) and 5 mM ammonium formate and 0.1% formic acid in acetonitrile (B), and the total flow rate was 0.2 mL/min. The gradient program for mobile phases B was initialized at 5% for 0.5 min, ramped to 95% for 6.5 min, held at 95% for 3 min, and then raised to 100% for 0.5 min. After the elution, the percentage of B was sharply reduced to 5% for 1 min and held at 5% for 3 min for initialization of the mobile phases. Total of the runtime was 15 min and the injection volume was 5 μL. Deionized water was prepared in house using LaboStar TWF UV 7 (Siemens, MA).

GC-MS/MS. For the determination multiresidual pesticides selected to a GC analysis, a Bruker SCION TQ triple quadrupole mass spectrometer coupled to a Bruker SCION 451 GC gas chromatograph (Billerica, MA) was utilized. The GC was furnished with an autosampler (CP-8400, Bruker). In the GC, a Zebron ZB-SemiVolatiles (30 m × 0.25 mm i.d., 0.25 μm d_f, Phenomenex) capillary column was installed. Helium (≥99.999%) was used as a carrier gas and total constant flow rate was 1.0 mL/min. The injection mode was splitless with a

pulsed pressure at 40 psi, and inlet temperature was 260 °C. The oven temperature was initialized at 90 °C for 3 min, ramped to 150 °C (20 °C/min), raised 300 °C (5 °C/min), and then held at 300 °C for 4 min. The total run time was 40 min and the injection volume was 2 μL. For the MS/MS conditions, transfer line, manifold, and ion source temperature values were 280, 40, and 230 °C, respectively. The electron ionization (EI) mode at 70 eV was employed for an ionization of target analytes. Argon gas (≥99.999%) was used in collision-induced dissociation (CID) and the collision pressure was 1.50 mTorr. The Dynamic mode (EDR) was used for the detector signal gain. MS Workstation (version 8.2) was utilized as a software during data processing.

MRM optimization in LC-MS/MS and GC-MS/MS

Each standard solution at 1-10 mg/L was injected into an instrument to obtain a full scan spectrum (m/z 50-1,000 for LC-MS/MS and m/z 50-500 for GC-MS/MS). For LC-MS/MS, a quasi-molecular ion (e.g., $[M+H]^+$, $[M-H]^-$) was selected as a precursor ion. The precursor ion was subjected to a product scan under CID with various collision energies (CE). From a product scan spectrum, two product ions were chosen in a consideration of selectivity and sensitivity. For GC-MS/MS, one molecular ion, or fragment ions were selected as a precursor ion(s) and the selection of product ion followed the procedures described above. Among the two MRM transitions, one was appointed as a quantifier and the other as a qualifier. Using the established MRM conditions, retention time (t_R) for each pesticide was verified.

Sample preparation

Bee (dead imago, healthy imago, and larva) was homogenized with dry ice using a mini blender. The QuEChERS EN 15662 (EN 15662, 2008) procedure was modified as the sample amounts. For bee and pollen, 2 g of aliquots in a 15-mL centrifuge tube was treated with 2 mL (bee) or 5 mL (pollen) of water, respectively. The sample was left for approximately 15 min to let the sample absorb the water entirely. A ceramic homogenizer and acetonitrile (2 mL) were added to the sample and the tube was shaken for 10 min at 300 rpm. The extract was treated with $MgSO_4$ (0.8 g) NaCl (0.2 g), $Na_3Citrate{\cdot}2H_2O$ (0.2 g), and $Na_2HCitr{\cdot}1.5H_2O$ (0.1 g), and then shaken vigorously for 1 min. During the entire partitioning procedures, the sample was cooled down with an ice bath. The tube was centrifuged for 10 min at 3,000 rpm, and 1 mL of the upper layer was added into a 2-mL microcentrifuge tube containing 150 mg $MgSO_4$ and 25 mg PSA (dSPE) under an ice bath. After the sample was mixed for 1 min using a vortex mixer, and then centrifuged for 5 min at 13,000 rpm. The upper layer (0.4 mL) was matrix-matched with 0.1 mL acetonitrile.

For a honey sample, 5 g of aliquots in a 50-mL centrifuge tube was treated with 10 mL water, extracted with 5 mL of acetonitrile, and then partitioned with a QuEChERS extraction packet (4 g $MgSO_4$, 1 g NaCl, 1 g $Na_3Citrate{\cdot}2H_2O$, and 0.5 g $Na_2HCitr{\cdot}1.5H_2O$). The organic layer (1 mL) was treated with dSPE and centrifuged, and then the upper layer (0.4 mL) was matrix-matched with 0.1 mL acetonitrile.

Each sample of bee, pollen, and honey was equivalent to 0.8 g per 1 mL of the final extract. The final extract was divided into four 2-mL amber vials

(two for neonicotinoid and two for pesticide multiresidue analysis) and injected into the LC-MS/MS (5 μL) or GC-MS/MS (2 μL), respectively.

Method validation for clothianidin, imidacloprid, and thiamethoxam

Analytical methods for neonicotinoids in bee, pollen, and honey were subjected to validation with parameters of the limit of quantitation (LOQ), linearity of calibration, and recovery in bee, pollen, and honey samples. The LOQ was determined by selecting the lowest level of the concentrations from matrix-matched standards satisfying signal to noise ratio (S/N) $\geq$10 (De Bièvre et al., 2005). The linearity of calibration was investigated using matrix-matched standards with linear ranges of 1-50 ng/g. A weighting regression factor (*1/x*) was employed to correct quantitation near lower concentrations of the calibration curve. The linearity of each calibration curve was expressed as the correlation coefficient (r^2). The recovery test was conducted at treated levels of 1, 5, and 10 ng/g. Each sample was spiked with neonicotinoids and prepared as the procedures described above (n = 3). The LC-MS/MS responses from recovery samples were compared with those of matrix-matched standard to calculate recovery rates of neonicotinoids.

Pesticide multiresidue screening in bee, pollen, and honey

Pesticide screening and quantitation for 391 pesticides (205 for LC-MS/MS and 186 for GC-MS/MS) were conducted. The matrix-matched standard calibration was employed to correct a matrix effect (a phenomenon in which a signal intensity is enhanced or suppressed by matrices on the LC- and GC-MS/MS). Linear range was 1-200 ng/g for bee and pollen, and 1-500 ng/g for honey and

the weighting regression factor (*1/x*) for each pesticide calibration was applied during residue determination.

Statistical analysis

The percentile method was utilized to summarize residue dataset (Kiljanek et al., 2016). For a few suspicious residue values (e.g., exceeding LD_{50}), outlier test was conducted with the Dixon's Q test to determine whether the values are statistically acceptable. The values <LOQ were assigned to 1/2 LOQ of each pesticide (Jaraczewska et al., 2006; Mercadante et al., 2013). The difference was considered to be significant if p value was less than 0.05 ($p < 0.05$).

Safety information

All pesticide standards and reagents used in this study were handled according to the Material Safety Data Sheet (MSDS)'s safety instructions. For all instrumentation, the manufacturer's safety information was followed and implemented.

Results and Discussion

Body weights of honey bees

During the monitoring period, a total of 8,325 honey bee imagos (6,615 for the dead and 1,710 for the healthy) was collected and weighted (**Table 18**). The total average of body weights in Giran was higher than that in Yeongyang in both statuses. The total averages of bee body weights were 0.06 g/bee for the dead and 0.11 g/bee for the healthy. Zoltowska et al. (2011) reported the body weights of newly emerged bee workers, average 0.1 g, similar to our results (Zoltowska et al., 2011). The average body weight of dead bees was 55% of that of healthy imagos due to dehydration. Therefore, each measured value from dead or healthy imago sample was corrected by the average body weight (bw) of the healthy imago (0.11 g) to reduce an over- or underestimation, as following equation:

$$\text{Residue level } (ng \cdot g_{bw}^{-1}) = \frac{\text{Measured value in a sample } (ng \cdot g^{-1}) \times A}{\text{Total average of healthy bee bw } (g \cdot bee_{bw})}$$

where: $A =$ average of dead bee bw in corresponding date $(g \cdot bee_{bw})$

Table 18. The numbers of dead and healthy imago collected in the two areas and their total and average body weights

Status[1)]		Dead imago			Healthy imago		
Area/site		No. of Bees (n)	Total body weight (g)	Average body weight (g/bee)	No. of Bees (n)	Total Body weight (g)	Average body weight (g/bee)
Giran	Geumgok	1,069	94.86	0.09	384	47.04	0.12
	Mukgye	1,480	68.36	0.05	315	38.88	0.12
	Odae	2,053	147.96	0.07	318	37.56	0.12
	Total	**4,602**	**311.18**	**0.07**	**1,017**	**123.48**	**0.12**
Yeongyang	Sanun	1,448	73.44	0.05	381	37.88	0.10
	Daewon	565	37.07	0.07	312	33.50	0.11
	Total	**2,013**	**110.51**	**0.05**	**693**	**71.38**	**0.10**
Total		**6,615**	**421.69**	**0.06**	**1,710**	**194.86**	**0.11**

[1)]The weights of the larvae were not measured.

MRM optimization

The MRM profiles of the three neonicotinoids (clothianidin, imidacloprid, and thiamethoxam) were optimized on LC-MS/MS. The precursor ions for the compounds were ionized positively by adducting proton ($[M+H]^+$). With these precursor ions, product ions under optimum CE were selected. Because of different LC conditions, retention times (t_R) in honey samples were slightly faster than those in bee and pollen samples. The detailed MRM transitions and t_R were in **Table 19**. The MRM profiles of 391 pesticides were also successfully established using LC-MS/MS (205 compounds) or GC-MS/MS (186 compounds). The detailed MRM transitions and retention times on LC- and GC-MS/MS were in **Table S1 and S2**.

Table 19. The established retention times (t_R), monoisotopic masses, quasi-molecular ion types, and MRM transitions of LC-MS/MS for the neonicotinoid pesticides

Matrix	Neonicotinoid	t_R (min)	Mono Isotopic mass	Quasi-molecular ion	Precursor ion > Product ion (CE, eV)	
					Quantification	Identification
Bee and pollen	Clothianidin	3.36	249	$[M+H]^+$	250 > 169 (-12)	250 > 132 (-16)
	Imidacloprid	3.41	255	$[M+H]^+$	256 > 209 (-14)	256 > 175 (-19)
	Thiamethoxam	3.23	291	$[M+H]^+$	292 > 211 (-12)	292 > 181 (-22)
Honey	Clothianidin	3.19	249	$[M+H]^+$	250 > 169 (-12)	250 > 132 (-16)
	Imidacloprid	3.23	255	$[M+H]^+$	256 > 209 (-14)	256 > 175 (-19)
	Thiamethoxam	3.09	291	$[M+H]^+$	292 > 211 (-12)	292 > 181 (-22)

Method validation for neonicotinoids

For the three neonicotinoids (clothianidin, imidacloprid, and thiamethoxam), analytical methods in bee, pollen, and honey were validated. The validation parameters were the LOQ, linearity of calibration, and recovery. The LOQ of clothianidin, imidacloprid, and thiamethoxam was 1 ng/g, respectively, in all matrices (**Table 20**). Because the average body weight of healthy imagos was 0.11 g (see **Table 18**), the minimum of 0.11 ng per a bee can be detectable for neonicotinoids. The acute oral (LD_{50}) toxicities for clothianidin, imidacloprid, and thiamethoxam are 3.79, 3.7, and 5 ng/bee (acute contact LD_{50}; 44.3, 81, and 24 ng/bee), respectively (European Commission, 2005; European Commission, 2006; European Food Safety Authority, 2008). These values were 33.6-45.5 times (acute contact LD_{50}; 306-413 times) higher than LOQ (0.11 ng $_{bw}$ /bee), thus the sensitivity in the methodology is sufficiently low for the ecotoxicological risk assessment. The correlation coefficients (r^2) for neonicotinoids were greater than 0.990 in all matrices, showing excellent linearity of calibration.

The recovery ranges for the neonicotinoids were 74.4-98.2% (RSD 0.9-17.0%) in bee, 79.9-102.5% in pollen (RSD 0.9-14.4%) and 78.0-116.2% (RSD 0.4-17.1%) in honey at treated levels of 1, 5, and 10 ng/g, respectively. According to the guidance of SANTE/11813/2017, the acceptable criteria for recovery is 70-120% with RSD $\leq$20% (European Commission, 2017). All the recovery results fell within the criteria, thus these analytical methods for neonicotinoids had the reliable trueness and precision including the LOQ level in bee, pollen, and honey.

Table 20. The limit of quantitation (LOQ), correlation coefficients (r^2), recovery results for neonicotinoid pesticides in bee, pollen, and honey samples

Matrix	Neonicotinoid	LOQ ng/g	r^2	Recovery, % (RSD, %)		
				1 ng/g	5 ng/g	10 ng/g
Bee	Clothianidin	1	0.9992	74.4 (3.5)	97.1 (4.4)	92.7 (1.3)
	Imidacloprid	1	0.9963	94.9 (17.0)	91.0 (5.6)	94.8 (8.1)
	Thiamethoxam	1	0.9985	92.9 (5.9)	98.2 (6.3)	94.2 (0.9)
Pollen	Clothianidin	1	0.9981	94.0 (14.4)	79.9 (3.9)	81.1 (6.2)
	Imidacloprid	1	0.9962	102.5 (11.3)	95.4 (9.0)	90.9 (5.4)
	Thiamethoxam	1	0.9983	90.5 (7.4)	95.0 (3.9)	94.8 (0.9)
Honey	Clothianidin	1	0.9962	78.0 (17.1)	100.4 (6.2)	105.3 (6.8)
	Imidacloprid	1	0.9964	111.4 (4.8)	106.2 (8.0)	90.9 (2.9)
	Thiamethoxam	1	0.9920	85.1 (14.5)	116.2 (0.4)	114.2 (4.5)

Analysis of neonicotinoids (clothianidin, imidaclprid, and thiamethoxam) in bee, pollen, and honey

Five field sites were investigated (three in Giran and two in Yeongyang) on April 24 to June 10, 2014 (Giran), and on July 6 to August 7, 2014 (Yeongyang). The average foraging distance of honey bees from a bee colony is about 2 km (Visscher and Seeley, 1982). Therefore, the distances between the areas were at least 5 km not to overlap the spheres of activities of honey bee workers from different areas (see **Fig. 23**). The monitoring periods were between before and after full bloom seasons of apple in Giran or pepper in Yeongyang so that bee workers could sufficiently pollinate apple or pepper and do foraging activities. There has been a guidance for applications of clothianidin, imidacloprid, and thiamethoxam in apple and pepper (Korea Crop Protection Association, 2012; Korea Crop Protection Association, 2015). It is expected that these neonicotinoid ingredients were conventionally sprayed on apple orchards in Giran and on pepper fields in Yeongyang. The total numbers of available samples collected during the monitoring periods were 36 (dead imago), 25 (healthy imago), 19 (larva), 34 (pollen), and 25 (honey), respectively, and these samples were analyzed and residues of neonicotinoids were determined.

Bee. The residue concentrations from measured values for bee imago samples were corrected by the average body weight (bw) of the healthy imago (0.11 g) to reduce an over- or underestimation, as the equation described above.

In Giran, the area of apple orchards, at least one of the three neonicotinoids were detected positively in 15 (62.5%) of the 24 dead imago samples (**Table 21**). Among the three sites (Geumgok, Mukgye, and Odae) in

Giran, the smallest detection frequency was observed in Odae (3 samples, 37.5% of the total). The narrowest determination range within 75th to 95th percentile was observed in Geumgok (0.8-4.9 ng/g $_{bw}$) and the highest residue was found in Odae (15.3 ng/g $_{bw}$, clothianidin). When the results are sorted by ingredients, clothianidin showed the largest detection frequency (11 samples, 45.8% of the total) and the highest residue range (2.9 to 13.5 ng/g $_{bw}$) within 75th and 95th at the three sites. On the other hand, thiamethoxam showed the lowest residues in the dead among the three neonicotinoids. No neonicotinoid was detected in healthy imago and larva samples.

In Yeongyang, the area of pepper fields, at least one of the three neonicotinoids were detected in 10 (83.3%) of 12 dead imago samples (**Table 22**). The two sites (Sanun and Daecheon) in Yeongyang exhibited the same detection frequencies (5 samples, 83.3% of the total, respectively). Determination ranges within 50th to 95th percentile were lower in Sanun (0.8-6.1 ng/g $_{bw}$) than in Daecheon (1.5-22.4 ng/g $_{bw}$) and the highest residue was found in Daecheon (56.9 ng/g $_{bw}$, clothianidin). When the results are sorted by ingredients, clothianidin showed the largest detection frequency (9 samples, 75.0% of the total) and the highest residue range (3.0 to 34.6 ng/g $_{bw}$) within 50th and 95th at the two sites. No neonicotinoid was detected in healthy imago and larva samples.

Table 21. Distribution of neonicotinoid residues in dead imago at three sites in Giran

Dead imagos in Giran (No. of the total samples)		Frequency of positive detection (%)	Min ng/g bw	Percentile, ng/g bw				Max ng/g bw
				50th	75th	90th	95th	
Total (24)		15 (62.5%)	<LOQ	<LOQ	0.9	3.5	10.4	15.3
Sorted by area	**Geumgok (8)**	6 (75.0%)	<LOQ	<LOQ	0.8	3.1	4.9	9.1
	Mukgye (8)	6 (75.0%)	<LOQ	<LOQ	0.9	1.4	10.9	13.7
	Odae (8)	3 (37.5%)	<LOQ	<LOQ	0.7	4.6	11.0	15.3
Sorted by ingredient	**Clothianidin (24)**	11 (45.8%)	<LOQ	<LOQ	2.9	11.5	13.5	15.3
	Imidacloprid (24)	6 (25.0%)	<LOQ	<LOQ	0.6	1.6	3.4	11.9
	Thiamethoxam (24)	3 (12.5%)	<LOQ	<LOQ	<LOQ	0.6	1.2	1.6

Table 22. Distribution of neonicotinoid residues in dead imago at two sites in Yeongyang

Dead imagos in Yeongyang (No. of the total samples)		Frequency of positive detection (%)	Min ng/g $_{bw}$	Percentile, ng/g $_{bw}$				Max ng/g $_{bw}$
				50th	75th	90th	95th	
	Total (12)	10 (83.3%)	<LOQ	1.0	5.1	7.9	15.0	56.9
Sorted by area	Sanun (6)	5 (83.3%)	<LOQ	0.8	2.7	5.7	6.1	6.9
	Daecheon (6)	5 (83.3%)	<LOQ	1.5	6.4	15.0	22.4	56.9
Sorted by ingredient	Clothianidin (12)	9 (75.0%)	<LOQ	3.0	5.5	15.4	34.6	56.9
	Imidacloprid (12)	6 (50.0%)	<LOQ	<LOQ	3.1	8.6	11.4	14.5
	Thiamethoxam (12)	7 (58.3%)	<LOQ	0.8	3.5	5.6	5.7	5.9

The positive detection ratio per a sample in Yeongyang was 1.3 times higher than that in Giran. In accordance with the statistics in **Table 21 and 22**, clothianidin showed higher residues in all percentile parameters and maximum values than thiamethoxam and imidacloprid. Thiamethoxam is easily converted into clothianidin in insects and plants metabolism (Nauen et al., 2003), thus it is possible that thiamethoxam was biotransformed rapidly into clothianidin by bee, apple, pepper, or other biotas.

To evaluate ecotoxicology, neonicotinoid residues were compared to bee acute oral LD_{50}. Because the average bodyweight of healthy imago was 0.11 g (see **Table 18**), Therefore, LD_{50}s of 3.79, 3.7, and 5 ng/bee for clothianidin, thiamethoxam, and imidacloprid (European Commission, 2005; European Commission, 2006; European Food Safety Authority, 2008) correspond with 34.5, 33.6, and 45.5 ng/g $_{bw}$, respectively.

In Giran, residues of three neonicotinoids in the dead imago samples were under LD_{50} values (**Fig. 24**) during the investigation period. The Maximum residues of neonicotinoids were 44.3% (clotianidin), 35.4% (imidacloprid), and 3.5% (thiamethoxam) of LD_{50}, respectively. In Yeongyang, residues of neonicotinoids in the dead imago samples were under LD_{50} values except for clothianidin (**Fig. 25**). The Maximum residues of neonicotinoids were 164.9% (clotianidin), 43.2% (imidacloprid), and 13.0% (thiamethoxam) of LD_{50}, respectively. The only one sample collected on June 20 showed exceeding LD_{50} of clothianidin 56.9 ng/g $_{bw}$. For this value, the Dixon's Q test was conducted and it was significant outlier ($p < 0.05$). Therefore, the effects of neonicotinoid residues in bee were not lethal, considering individual toxicity only.

Fig. 24. Distribution of residues for (a) clothianidin, (b) imidacloprid, and (c) thiamethoxam in dead imago samples at three sites in Giran

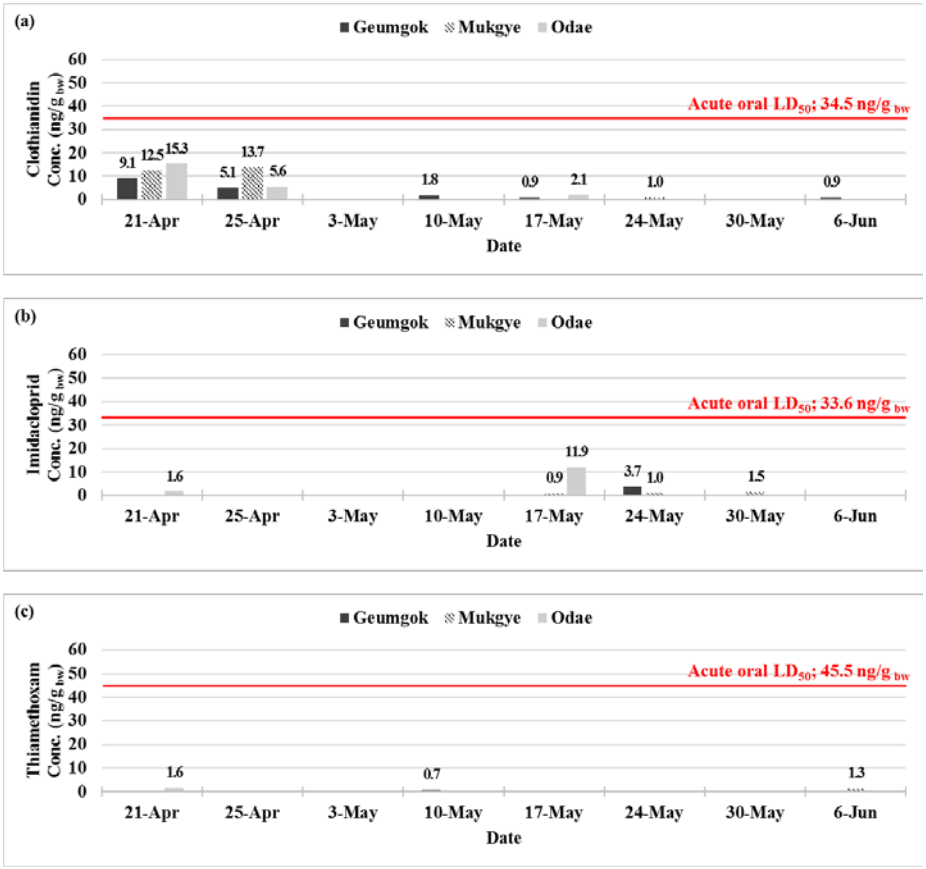
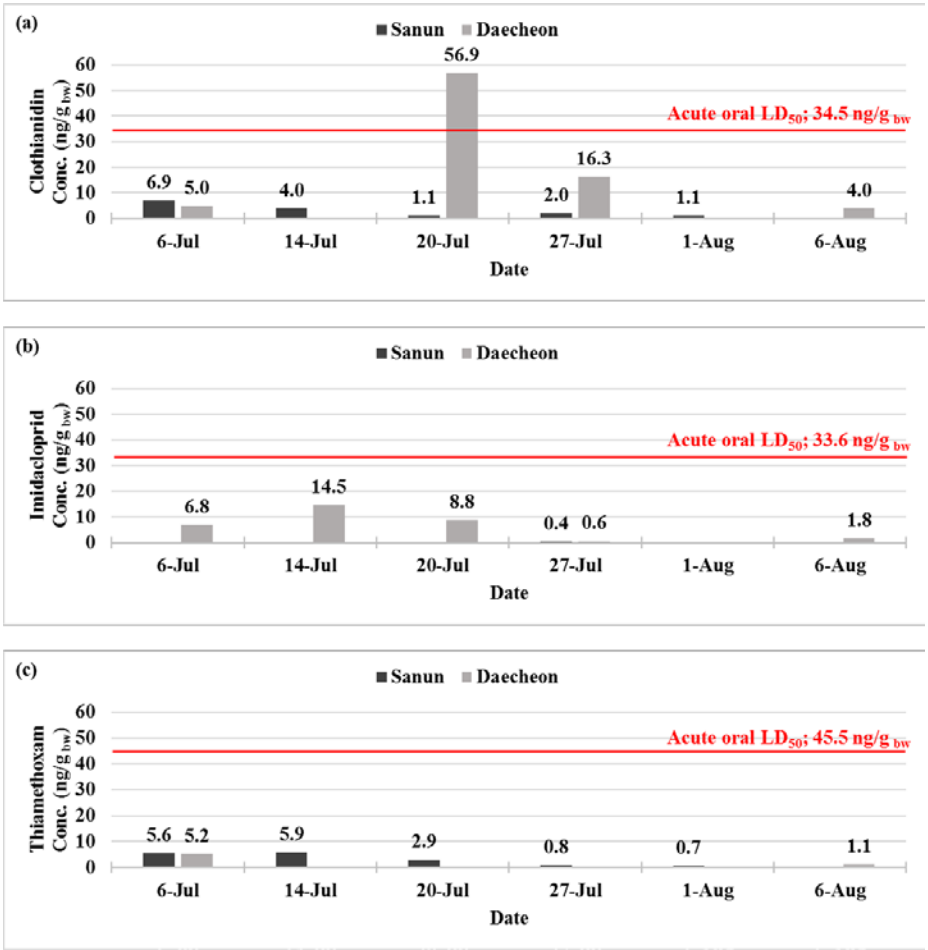

(a)
Geumgok
Mukgye
Odae
Clothianidin Conc. (ng/g bw)
60
50
40
30
20
10
0
Acute oral LD50; 34.5 ng/g bw
9.1
12.5
15.3
5.1
13.7
5.6
1.8
0.9
2.1
1.0
0.9
21-Apr
25-Apr
3-May
10-May
17-May
24-May
30-May
6-Jun
Date
(b)
Geumgok
Mukgye
Odae
Imidacloprid Conc. (ng/g bw)
60
50
40
30
20
10
0
Acute oral LD50; 33.6 ng/g bw
1.6
0.9
11.9
3.7
1.0
1.5
21-Apr
25-Apr
3-May
10-May
17-May
24-May
30-May
6-Jun
Date
(c)
Geumgok
Mukgye
Odae
Thiamethoxam Conc. (ng/g bw)
60
50
40
30
20
10
0
Acute oral LD50; 45.5 ng/g bw
1.6
0.7
1.3
21-Apr
25-Apr
3-May
10-May
17-May
24-May
30-May
6-Jun
Date

Fig. 25. Distribution of residues for (a) clothianidin, (b) imidacloprid, and (c) thiamethoxam in dead imago samples at two sites in Yeongyang


(a)
Sanun
Daecheon
Clothianidin Conc. (ng/g bw)
60
50
40
30
20
10
0
Acute oral LD50; 34.5 ng/g bw
6.9
5.0
4.0
56.9
1.1
2.0
16.3
1.1
4.0
6-Jul
14-Jul
20-Jul
27-Jul
1-Aug
6-Aug
Date
(b)
Sanun
Daecheon
Imidacloprid Conc. (ng/g bw)
60
50
40
30
20
10
0
Acute oral LD50; 33.6 ng/g bw
6.8
14.5
8.8
0.4
0.6
1.8
6-Jul
14-Jul
20-Jul
27-Jul
1-Aug
6-Aug
Date
(c)
Sanun
Daecheon
Thiamethoxam Conc. (ng/g bw)
60
50
40
30
20
10
0
Acute oral LD50; 45.5 ng/g bw
5.6
5.2
5.9
2.9
0.8
0.7
1.1
6-Jul
14-Jul
20-Jul
27-Jul
1-Aug
6-Aug
Date

Pollen. In Giran, at least one of the three neonicotinoids were detected positively in 14 (58.3%) of the 24 pollen samples (**Table 23**). Among the three sites (Geumgok, Mukgye, and Odae) in Giran, the smallest detection frequency was observed in Geumgok (3 samples, 37.5% of the total), whereas the highest residue level was found at this site (17.0 ng/g, thiamethoxam). The lowest residue range within 75th to 95th percentile was observed in Odae (<LOQ to 3.1 ng/g). When the results are sorted by ingredients, imidacloprid showed the largest detection frequency (8 samples, 33.3% of the total). Thiamethoxam showed the lowest residue range within 75th to 95th percentile (<LOQ to 0.9 ng/g), whereas indicated the highest residue level (17.0 ng/g) among the neonicotinoids.

In Yeongyang, samples collected on July 14 in both of Sanun and Daecheon were not able to be measured due to insufficient sample amounts. At least one of the three neonicotinoids were detected positively in 5 (62.5%) of the 8 pollen samples (**Table 24**). The percentile values were similar in Sanun and Daecheon. When the results are sorted by ingredients, imidacloprid showed the largest detection frequency (4 samples, 50.0% of the total), the highest percentile values (1.4-4.3 ng/g within 50th to 95th percentile), and the highest maximum residue (4.5 ng/g) among the neonicotinoids. In contrast, clothianidin exhibited the smallest detection frequency (1 sample, 12.5% of the total), the lowest percentile ranges (<LOQ to 0.9 ng/g within 50th to 95th percentile), and the lowest maximum residue (1.1 ng/g) among the neonicotinoids.

Table 23. Distribution of neonicotinoid residues in pollen at two sites in Giran

Pollen in Giran (No. of the total samples)		Frequency of positive detection (%)	Min ng/g	Percentile, ng/g				Max ng/g
				50th	75th	90th	95th	
Total (24)		14 (58.3%)	<LOQ	<LOQ	<LOQ	2.3	6.3	17.0
Sorted by area	**Geumgok (8)**	3 (37.5%)	<LOQ	<LOQ	<LOQ	3.8	15.0	17.0
	Mukgye (8)	6 (75.0%)	<LOQ	<LOQ	1.0	1.8	4.8	7.6
	Odae (8)	5 (62.5%)	<LOQ	<LOQ	<LOQ	2.3	3.1	8.2
Sorted by ingredient	**Clothianidin (24)**	7 (29.2%)	<LOQ	<LOQ	1.2	4.5	5.3	8.2
	Imidacloprid (24)	8 (33.3%)	<LOQ	<LOQ	1.2	2.1	6.8	16.8
	Thiamethoxam (24)	2 (8.3%)	<LOQ	<LOQ	<LOQ	<LOQ	0.9	17.0

Table 24. Distribution of neonicotinoid residues in pollen at two sites in Yeongyang

Pollen in Yeongyang (No. of the total samples)		Frequency of positive detection (%)	Min ng/g	Percentile, ng/g				Max ng/g
				50th	75th	90th	95th	
Total (8)		5 (62.5%)	<LOQ	<LOQ	1.2	2.4	3.6	4.5
Sorted by area	**Sanun (4)**	2 (50.0%)	<LOQ	<LOQ	0.8	2.4	3.4	4.5
	Daecheon (4)	3 (75.0%)	<LOQ	<LOQ	1.3	2.2	3.0	3.8
Sorted by ingridient	**Clothianidin (8)**	1 (12.5%)	<LOQ	<LOQ	<LOQ	0.7	0.9	1.1
	Imidacloprid (8)	4 (50.0%)	<LOQ	1.4	2.8	4.0	4.3	4.5
	Thiamethoxam (8)	2 (25.0%)	<LOQ	<LOQ	0.8	1.6	1.6	1.7

The positive detection ratio per a sample was similar in both of Giran and Yeongyang. In accordance with the statistics in **Table 23 and 24**, imidacloprid showed higher residues in all percentile parameters and maximum values than clothianidin and thiamethoxam. To evaluate ecotoxicology of bee, neonicotinoid residues in pollen were compared to bee acute oral LD_{50} values. In Giran, residue levels of three neonicotinoids were under LD_{50}s (**Fig. 26**). The Maximum residues of neonicotinoids were 23.8% (clotianidin), 50.0% (imidacloprid), and 37.4% (thiamethoxam) of LD_{50} values, respectively. In Yeongyang, residue concentrations of neonicotinoids were also under LD_{50} (**Fig. 27**). The Maximum residues of neonicotinoids were 3.2% (clotianidin), 13.4% (imidacloprid), and 3.7% (thiamethoxam) of LD_{50} values, respectively. Therefore, the effects of neonicotinoid residues in pollen were not lethal to bees, considering individual toxicity only.

Fig. 26. Distribution of residues for (a) clothianidin, (b) imidacloprid, and (c) thiamethoxam in pollen samples at three sites in Giran

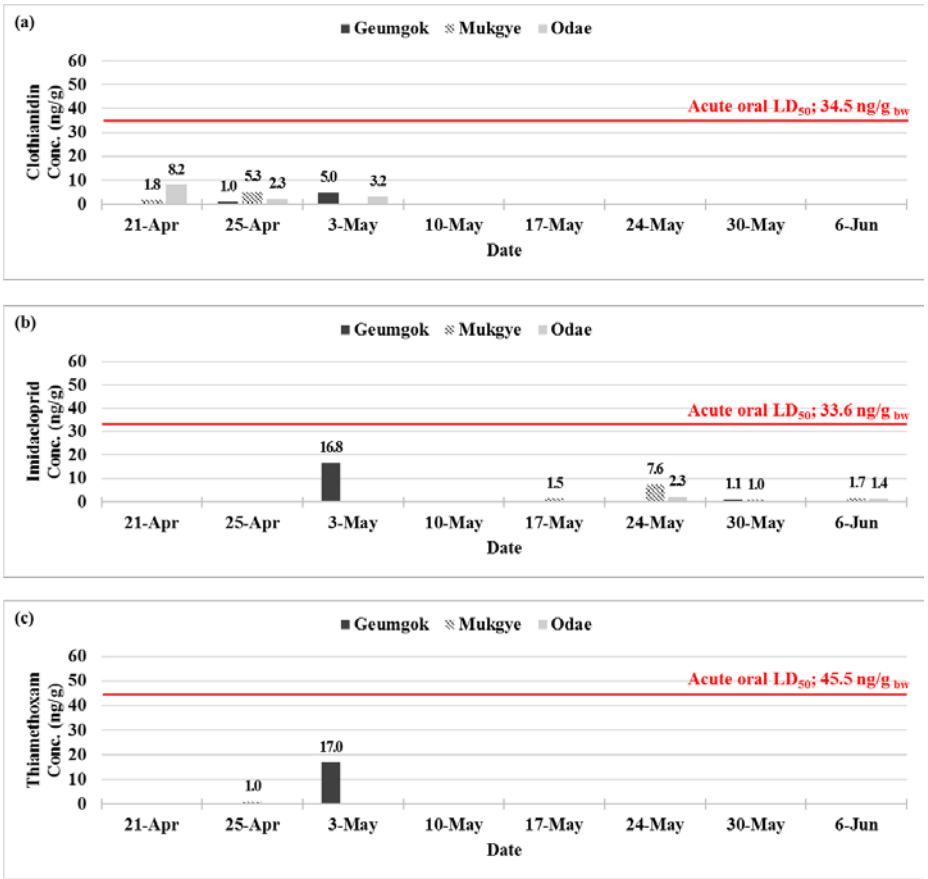
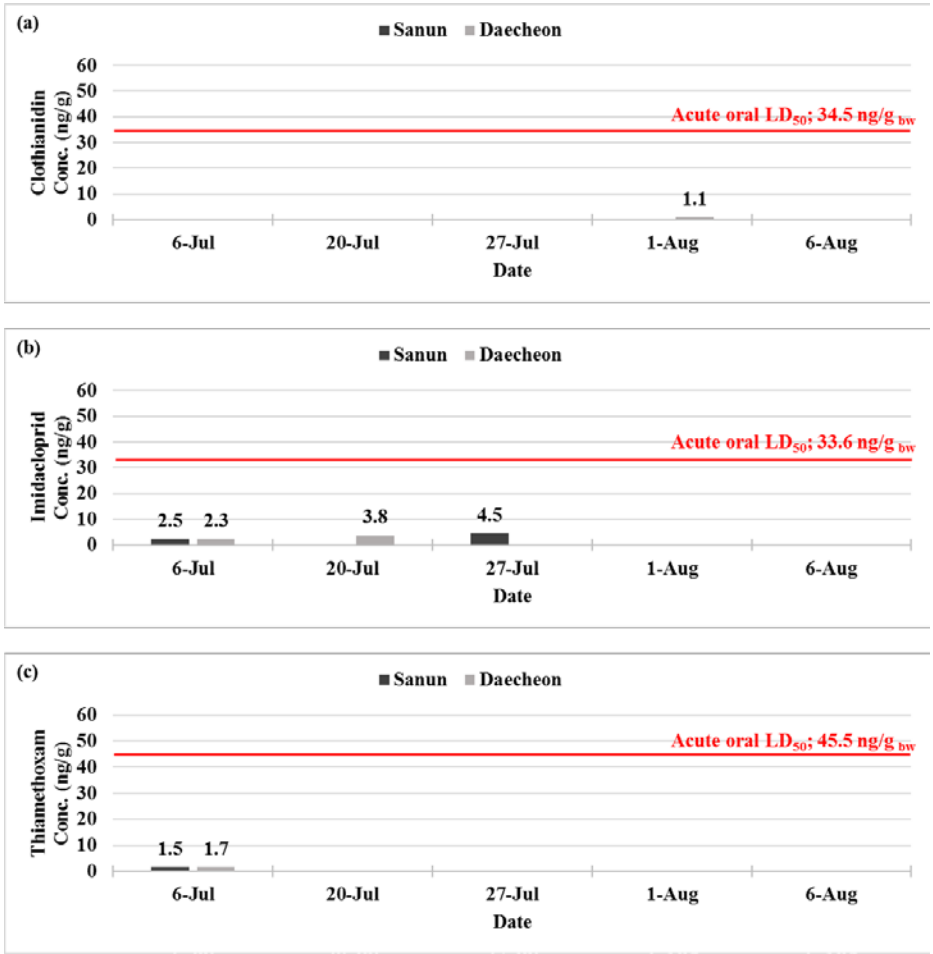

(a)
Geumgok
Mukgye
Odae
Clothianidin Conc. (ng/g)
60
50
40
30
20
10
0
Acute oral LD50; 34.5 ng/g bw
1.8
8.2
1.0
5.3
2.3
5.0
3.2
21-Apr
25-Apr
3-May
10-May
17-May
24-May
30-May
6-Jun
Date
(b)
Geumgok
Mukgye
Odae
Imidacloprid Conc. (ng/g)
60
50
40
30
20
10
0
Acute oral LD50; 33.6 ng/g bw
16.8
1.5
7.6
2.3
1.1
1.0
1.7
1.4
21-Apr
25-Apr
3-May
10-May
17-May
24-May
30-May
6-Jun
Date
(c)
Geumgok
Mukgye
Odae
Thiamethoxam Conc. (ng/g)
60
50
40
30
20
10
0
Acute oral LD50; 45.5 ng/g bw
1.0
17.0
21-Apr
25-Apr
3-May
10-May
17-May
24-May
30-May
6-Jun
Date

Fig. 27. Distribution of residues for (a) clothianidin, (b) imidacloprid, and (c) thiamethoxam in pollen samples at two sites in Yeongyang


(a)
Sanun
Daecheon
Clothianidin Conc. (ng/g)
60
50
40
30
20
10
0
Acute oral LD50; 34.5 ng/g bw
1.1
6-Jul
20-Jul
27-Jul
1-Aug
6-Aug
Date
(b)
Sanun
Daecheon
Imidacloprid Conc. (ng/g)
Acute oral LD50; 33.6 ng/g bw
2.5
2.3
3.8
4.5
6-Jul
20-Jul
27-Jul
1-Aug
6-Aug
Date
(c)
Sanun
Daecheon
Thiamethoxam Conc. (ng/g)
Acute oral LD50; 45.5 ng/g bw
1.5
1.7
6-Jul
20-Jul
27-Jul
1-Aug
6-Aug
Date

Honey. In Giran, residue ranges of clothianidin and imidacloprid were <LOQ-1.4 ng/g and <LOQ-1.3 ng/g, respectively, and thiamethoxam was <LOQ (**Table 25**). In Yeongyang, residue ranges of imidacloprid and thiamethoxam were <LOQ-2.6 ng/g and <LOQ-1.9 ng/g, respectively, and clothianidin was <LOQ. These residue levels were ≤7.7% of the three neonicotinoid LD_{50}s. Therefore, the effects of neonicotinoid residues in honey were not lethal to bees, considering individual toxicity only. The residue ranges were also lower than the MRLs of three neonicotinoids (0.05 mg/kg, respectively), accounting for ≤5.2% of the MRLs. This indicate that honey produced in these area is sufficiently safe in aspect of human health and available for a food stuff.

Table 25. Distribution of neonicotinoid residues in honey in Giran and Yeongyang

Giran	Date	10-Jun, Colony (ng/g)				
	Neonicotinoid	No.1	No.2	No.3	No.4	No.5
Geumgok	Clothianidin	<LOQ	1.4	<LOQ	1.3	<LOQ
	Imidacloprid	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	Thiamethoxam	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Mukgye	Clothianidin	<LOQ	<LOQ	1.4	<LOQ	<LOQ
	Imidacloprid	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	Thiamethoxam	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Odae	Clothianidin	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	Imidacloprid	1.1	<LOQ	<LOQ	1.3	<LOQ
	Thiamethoxam	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Yeongyang	**Date**	**7-Aug, Colony (ng/g)**				
	Neonicotinoid	**No.1**	**No.2**	**No.3**	**No.4**	**No.5**
Sanun	Clothianidin	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	Imidacloprid	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	Thiamethoxam	<LOQ	<LOQ	1.9	1.3	1.1
Daecheon	Clothianidin	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	Imidacloprid	1.9	<LOQ	<LOQ	1.1	2.6
	Thiamethoxam	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

Analysis of pesticide multiresidues in bee, pollen, and honey

To evaluate variable ecotoxicological effects as well as neonicotinoids, 391 pesticides in bee, pollen, and honey samples were screened using MRM mode of LC- and GC-MS/MS. The samples to be measured were the same as the samples for the neonicotinoids analysis. Among the target pesticides, 52 analytes were positively detected in at least one of bee (dead imago, healthy imago, and larva), pollen, and honey samples. Fluvalinate, its numbers of detection frequencies are the largest among the pesticides in both of Giran (49 of the 87 samples) and Yeongyang (30 of the 52 samples), is an acaricide that was treated in bee colonies before the investigation to control mites. Diphenylamine is a post-harvest deterioration inhibitor for apple or a naturally producted compound in some crops (Drzyzga, 2003). Atrazine, ethiofencarb were not registered to application in apple and pepper as well as in any crops neither, and the others were banned or suspended from sales recently or seemed to come during treatment of other crops (Korea Crop Protection Association, 2013).

In Giran, the area of apple orchard, 46 pesticides were determined above LOQ and 38 (82.6%) of them have been acceptable to treat on apple orchard in the Republic of Korea (**Table 26**) (Korea Crop Protection Association, 2012; Korea Crop Protection Association, 2015). Because these pesticides also have been registered in various crops, some of the pesticides might come from other crop residues. Fluvalinate (pyrethroid), etofenprox (pyrethroid), carbaryl (carbamate), acetamiprid (neonicotinoid), and spiromesifen (tetronic acid) ranked first to fifth among the pesticides in detection frequencies in this area (**Fig. 28**). Etofenprox and spiromesifen were not determined in honey samples

and acetamiprid in honey samples showed larger detection ratio than the others. Acetamiprid and etofenprox exhibited lower toxicity (acute oral LD_{50} 14,500 and 270 ng/bee, corresponding with 132,000 and 2,500 ng/g $_{bw}$ in this study) than neonicotinoids (thiamethoxam; 5 ng/bee), but highly toxic to bee (World Health Organization; Tomlin, 2009). The residue levels for these pesticides are lower than acute oral LD_{50}s in all samples (**Table 27**). Fluvalinate and spiromesifen are not hazardous to bee (acute oral LD_{50} 163,000 ng/bee; 1,480,000 ng/g $_{bw}$ and 790,000 ng/bee; 7,180,000 ng/g $_{bw}$, respectively), their maximum residue levels were negligible in aspect of ecotoxicology (Tomlin, 2009). For carbaryl (acute oral LD_{50} 230 ng/bee; 2,100 ng/g $_{bw}$), only one pollen sample collected on May 3 in Odae slightly exceeded LD_{50} value (2,114 ng/g $_{bw}$, 100.7% of LD_{50}) (Food and Agriculture Organization). For this value, the Dixon's Q test was conducted and it was significant outlier ($p < 0.05$). Chlorantraniliprole, difenoconazole, diflubenzuron, fluazinam, indoxacarb, terflubenzuron, and thiophanate-methyl showed higher maximum concentrations (1,300-7,048 ng/g) in pollen. Except for indoxacarb, these pesticides are non-toxic to bee (acute oral LD_{50} >100,000 ng/bee; >909,000 ng/g $_{bw}$) (Tomlin, 2009). Indoxacarb is highly toxic (acute oral LD_{50} 260 ng/bee; 2,400 ng/g $_{bw}$), and the maximum residue in pollen was 2.5 times higher than LD_{50} (Tomlin, 2009). Residue ranges in honey samples were not hazardous to bee, whereas fluvalinate in one honey sample was exhibited a higher residue level (966.7 ng/g) than its MRL (50 ng/g) (European Commission, 2018). These values exceeding LD_{50} of indoxacarb or MRL of fluvalinate are turned out to be significant outliers ($p < 0.05$).

Table 26. Positive detection frequency for bee, pollen, and honey samples in Giran

No.	Compound name	Total (%) *n* = 87	Bee[1)]				Pollen				Honey				Registered for apple
			Total (%) *n* = 48	Geum-gok	Muk-gye	Odae	Total (%) *n* = 24	Geum-gok	Muk-gye	Odae	Total (%) *n* = 15	Geum-gok	Muk-gye	Odae	
1	Abamectin B1a	2 (2.3)	-	-	-	-	2 (8.3)	1	1	-	-	-	-	-	yes
2	Acetamiprid	35 (40.2)	2 (4.2)	-	2	-	19 (79.2)	7	8	4	14 (93.3)	4	5	5	yes
3	Acrinathrin	3 (3.4)	-	-	-	-	3 (12.5)	2	1	-	-	-	-	-	yes
4	Atrazine	6 (6.9)	-	-	-	-	6 (25.0)	2	2	2	-	-	-	-	no[2)]
5	Bifenazate	17 (19.5)	8 (16.7)	1	4	3	9 (37.5)	2	2	5	-	-	-	-	yes
6	Carbaryl	37 (42.5)	14 (29.2)	4	6	4	22 (91.7)	6	8	8	1 (6.7)	-	-	1	yes
7	Carbendazim	2 (2.3)	-	-	-	-	-	-	-	-	2 (13.3)	-	2	-	yes
8	Carbofuran	8 (9.2)	2 (4.2)	1	-	1	6 (25.0)	1	4	1	-	-	-	-	no
9	Chlorantraniliprole	26 (29.9)	10 (20.8)	4	4	2	16 (66.7)	5	6	5	-	-	-	-	yes
10	Chlorpyrifos	20 (23.0)	13 (27.1)	1	5	7	7 (29.2)	2	2	3	-	-	-	-	yes
11	Cyhalothrin-lambda	8 (9.2)	3 (6.3)	-	2	1	5 (20.8)	3	2	-	-	-	-	-	yes
12	Cyprodinil	17 (19.5)	14 (29.2)	5	4	5	3 (12.5)	2	1	-	-	-	-	-	yes
13	Deltamethrin	1 (1.1)	-	-	-	-	1 (4.2)	-	-	1	-	-	-	-	yes
14	Difenoconazole	11 (12.6)	9 (18.8)	5	2	2	2 (8.3)	1	-	1	-	-	-	-	yes
15	Diflubenzuron	30 (34.5)	22 (45.8)	7	6	9	8 (33.3)	4	2	2	-	-	-	-	yes
16	Diphenylamine	12 (13.8)	4 (8.3)	1	2	1	8 (33.3)	2	4	2	-	-	-	-	no[3)]
17	Emamectin B1a	5 (5.7)	-	-	-	-	5 (20.8)	1	3	1	-	-	-	-	yes
18	Emamectin B1b	2 (2.3)	-	-	-	-	2 (8.3)	1	1	-	-	-	-	-	yes
19	Ethiofencarb	1 (1.1)	-	-	-	-	1 (4.2)	-	1	-	-	-	-	-	no[2)]
20	Etofenprox	38 (43.7)	24 (50.0)	7	9	8	14 (58.3)	4	6	4	-	-	-	-	yes
21	Fenazaquin	1 (1.1)	1 (2.1)	1	-	-	-	-	-	-	-	-	-	-	yes
22	Fenvalerate	1 (1.1)	1 (2.1)	1	-	-	-	-	-	-	-	-	-	-	yes
23	Flonicamid	11 (12.6)	-	-	-	-	7 (29.2)	3	2	2	4 (26.7)	3	-	1	yes
24	Fluacrypyrim	1 (1.1)	-	-	-	-	1 (4.2)	-	1	-	-	-	-	-	no[4)]
25	Fluazinam	14 (16.1)	5 (10.4)	3	2	-	9 (37.5)	3	2	4	-	-	-	-	yes
26	Flubendiamide	27 (31.0)	10 (20.8)	4	3	3	17 (70.8)	5	7	5	-	-	-	-	yes
27	Flufenoxuron	11 (12.6)	2 (4.2)	-	2	-	9 (37.5)	1	4	4	-	-	-	-	yes
28	Fluquinconazole	17 (19.5)	9 (18.8)	3	3	3	8 (33.3)	3	2	3	-	-	-	-	yes

Table 26. *(Continued)*

No.	Compound name	Total (%) *n* = 87	Bee[1)]				Pollen				Honey				Registered for apple
			Total (%) *n* = 48	Geum-gok	Muk-gye	Odae	Total (%) *n* = 24	Geum-gok	Muk-gye	Odae	Total (%) *n* = 15	Geum-gok	Muk-gye	Odae	
29	Fluvalinate	49 (56.3)	37 (77.1)	14	8	15	8 (33.3)	3	4	1	4 (26.7)	-	2	2	no[5)]
30	Hexythiazox	11 (12.6)	-	-	-	-	11 (45.8)	3	4	4	-	-	-	-	yes
31	Indoxacarb	6 (6.9)	3 (6.3)	3	-	-	3 (12.5)	2	1	-	-	-	-	-	yes
32	Kresoxim-methyl	12 (13.8)	1 (2.1)	-	-	1	11 (45.8)	4	4	3	-	-	-	-	yes
33	Mepronil	1 (1.1)	-	-	-	-	1 (4.2)	-	1	-	-	-	-	-	no
34	Methomyl	23 (26.4)	7 (14.6)	1	5	1	16 (66.7)	5	5	6	-	-	-	-	no[4)]
35	Methoxyfenozide	21 (24.1)	4 (8.3)	1	2	1	17 (70.8)	5	7	5	-	-	-	-	yes
36	Novaluron	30 (34.5)	24 (50.0)	5	8	11	6 (25.0)	1	2	3	-	-	-	-	yes
37	Picoxystrobin	17 (19.5)	9 (18.8)	-	7	2	8 (33.3)	2	3	3	-	-	-	-	yes
38	Pyraclostrobin	5 (5.7)	-	-	-	-	5 (20.8)	2	1	2	-	-	-	-	yes
39	Pyrimethanil	5 (5.7)	-	-	-	-	5 (20.8)	3	-	2	-	-	-	-	yes
40	Spirodiclofen	4 (4.6)	-	-	-	-	4 (16.7)	-	1	3	-	-	-	-	yes
41	Spiromesifen	35 (40.2)	20 (41.7)	7	7	6	15 (62.5)	5	6	4	-	-	-	-	yes
42	Sulfoxaflor	7 (8.0)	2 (4.2)	2	-	-	4 (16.7)	-	3	1	1 (6.7)	-	1	-	yes
43	Teflubenzuron	12 (13.8)	4 (8.3)	4	-	-	8 (33.3)	4	3	1	-	-	-	-	yes
44	Thiodicarb	13 (14.9)	4 (8.3)	-	4	-	9 (37.5)	2	3	4	-	-	-	-	yes
45	Thiophanate-methyl	30 (34.5)	10 (20.8)	1	6	3	20 (83.3)	6	8	6	-	-	-	-	yes
46	Trifloxystrobin	5 (5.7)	-	-	-	-	5 (20.8)	2	3	-	-	-	-	-	yes

[1)]Dead and healthy imago, and larva.

[2)]Not included in Pesticide Registration Status of the Republic of Korea.

[3)]Post-harvest deterioration inhibitor for apple or naturally producted compound in some crops.

[4)]Banned or suspended from sales in 2011 (methomyl) and in 2013 (fluacrypyrim).

[5)]Pre-treated in colony before monitoring to coltrol mites.

Fig. 28. Distribution of the numbers of detection frequencies for fluvalinate, etofenprox, carbaryl, acetamiprid, and spiromesifen, which ranked first to fifth among the pesticide multiresidues by the detection frequency

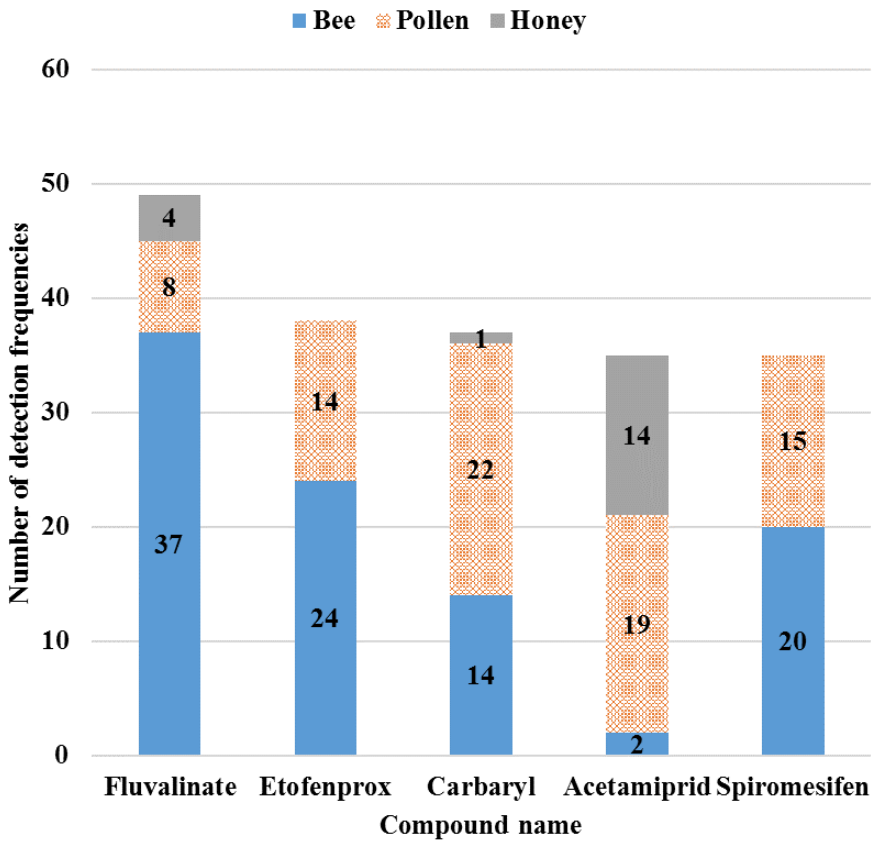

Bee
Pollen
Honey
60
50
40
30
20
10
0
Number of detection frequencies
4
8
37
14
24
1
22
14
14
19
2
15
20
Fluvalinate
Etofenprox
Carbaryl
Acetamiprid
Spiromesifen
Compound name

Table 27. Distribution of median values and residue ranges for pesticide multiresidues in Giran

No.	Pesticides	Honey bee						Pollen ng/g		Honey ng/g	
		Dead imago ng/g $_{bw}$		Healty imago ng/g $_{bw}$		Larva ng/g					
		Median	Residue range	Median	Residue range	Median	Residue range	Median	Residue range	Median	Residue range
1	Abamectin B1a	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ-86.8	<LOQ	<LOQ
2	Acetamiprid	<LOQ	<LOQ-42.7	<LOQ	<LOQ	<LOQ	<LOQ	5.2	<LOQ-103.6	5.5	<LOQ-21.8
3	Acrinathrin	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ-22.5	<LOQ	<LOQ
4	Atrazine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ-5.7	<LOQ	<LOQ
5	Bifenazate	<LOQ	<LOQ-184.9	<LOQ	<LOQ-33.7	<LOQ	<LOQ	<LOQ	<LOQ-493.1	<LOQ	<LOQ
6	Carbaryl	8.3	<LOQ-1960.9	<LOQ	<LOQ	<LOQ	<LOQ	7.2	<LOQ-2114	<LOQ	<LOQ-1.2
7	Carbendazim	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ-5.7
8	Carbofuran	<LOQ	<LOQ-11.7	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ-26.8	<LOQ	<LOQ
9	Chlorantraniliprole	<LOQ	<LOQ-42.9	<LOQ	<LOQ	<LOQ	<LOQ-11.2	6.7	<LOQ-2414	<LOQ	<LOQ
10	Chlorpyrifos	<LOQ	<LOQ-91.3	<LOQ	<LOQ-40.0	<LOQ	<LOQ	<LOQ	<LOQ-748.4	<LOQ	<LOQ
11	Cyhalothrin-lambda	<LOQ	<LOQ-112.6	<LOQ	<LOQ-48.3	<LOQ	<LOQ	<LOQ	<LOQ-202.8	<LOQ	<LOQ
12	Cyprodinil	11.7	<LOQ-166.4	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ-729.4	<LOQ	<LOQ
13	Deltamethrin	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ-25.1	<LOQ	<LOQ
14	Difenoconazole	<LOQ	<LOQ-81.3	<LOQ	<LOQ-39.6	<LOQ	<LOQ	<LOQ	<LOQ-1821	<LOQ	<LOQ
15	Diflubenzuron	<LOQ	<LOQ-554.2	25.6	10.4-68.6	<LOQ	<LOQ-30.8	<LOQ	<LOQ-1335	<LOQ	<LOQ
16	Diphenylamine	<LOQ	<LOQ-27.8	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ-130.9	<LOQ	<LOQ
17	Emamectin B1a	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ-30.6	<LOQ	<LOQ
18	Emamectin B1b	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ-33.3	<LOQ	<LOQ
19	Ethiofencarb	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ-5.0	<LOQ	<LOQ
20	Etofenprox	<LOQ	<LOQ-234.6	6.4	<LOQ-15.2	<LOQ	<LOQ-2.5	5.7	<LOQ-133.6	<LOQ	<LOQ
21	Fenazaquin	<LOQ	<LOQ-11.6	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
22	Fenvalerate	<LOQ	<LOQ-11.0	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
23	Flonicamid	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ-68.7	<LOQ	<LOQ-12.8

Table 27. *(Continued)*

No.	Pesticides	Honey bee						Pollen ng/g		Honey ng/g	
		Dead imago ng/g $_{bw}$		Healty imago ng/g $_{bw}$		Larva ng/g $_{bw}$					
		Median	Residue range	Median	Residue range	Median	Residue range	Median	Residue range	Median	Residue range
24	Fluacrypyrim	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ-1.6	<LOQ	<LOQ
25	Fluazinam	<LOQ	<LOQ	<LOQ	<LOQ-102.9	<LOQ	<LOQ	<LOQ	<LOQ-7048	<LOQ	<LOQ
26	Flubendiamide	<LOQ	<LOQ-75.4	<LOQ	<LOQ	<LOQ	<LOQ	2.9	<LOQ-126.8	<LOQ	<LOQ
27	Flufenoxuron	<LOQ	<LOQ-19.2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ-199.8	<LOQ	<LOQ
28	Fluquinconazole	<LOQ	<LOQ-130.2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ-326.6	<LOQ	<LOQ
29	Fluvalinate	42.4	<LOQ-302.0	26.0	<LOQ-103.3	47.2	<LOQ-179.5	<LOQ	<LOQ-874.2	<LOQ	<LOQ-966.7
30	Hexythiazox	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ-43.0	<LOQ	<LOQ
31	Indoxacarb	<LOQ	<LOQ	<LOQ	<LOQ-177.3	<LOQ	<LOQ	<LOQ	<LOQ-5987	<LOQ	<LOQ
32	Kresoxim-methyl	<LOQ	<LOQ-574.9	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ-355.3	<LOQ	<LOQ
33	Mepronil	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ-30.1	<LOQ	<LOQ
34	Methomyl	<LOQ	<LOQ-42.1	<LOQ	<LOQ	<LOQ	<LOQ	23.9	<LOQ-725.3	<LOQ	<LOQ
35	Methoxyfenozide	<LOQ	<LOQ-36.7	<LOQ	<LOQ	<LOQ	<LOQ	5.5	<LOQ-58.5	<LOQ	<LOQ
36	Novaluron	<LOQ	<LOQ-63.0	32.1	<LOQ-62.4	<LOQ	<LOQ-10.3	<LOQ	<LOQ-712.6	<LOQ	<LOQ
37	Picoxystrobin	<LOQ	<LOQ-67.1	<LOQ	<LOQ-162.2	<LOQ	<LOQ	<LOQ	<LOQ-110.1	<LOQ	<LOQ
38	Pyraclostrobin	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ-371.2	<LOQ	<LOQ
39	Pyrimethanil	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ-480.6	<LOQ	<LOQ
40	Spirodiclofen	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ-31.4	<LOQ	<LOQ
41	Spiromesifen	<LOQ	<LOQ-52.4	65.0	<LOQ-261.8	<LOQ	<LOQ-38.4	14.3	<LOQ-2544	<LOQ	<LOQ
42	Sulfoxaflor	<LOQ	<LOQ-19.1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ-14.3	<LOQ	<LOQ-1.9
43	Teflubenzuron	<LOQ	<LOQ	<LOQ	<LOQ-289.4	<LOQ	<LOQ-65.6	<LOQ	<LOQ-1300	<LOQ	<LOQ
44	Thiodicarb	<LOQ	<LOQ-11.4	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ-5.4	<LOQ	<LOQ
45	Thiophanate-methyl	<LOQ	<LOQ-342.9	<LOQ	<LOQ-112.6	<LOQ	<LOQ	25.7	<LOQ-1886	<LOQ	<LOQ
46	Trifloxystrobin	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ-73.2	<LOQ	<LOQ

In Yeongyang, the area of pepper field, 36 pesticides were detected and 30 (83.3%) of them have been acceptable to treat on pepper field (**Table 28**) (Korea Crop Protection Association, 2012; Korea Crop Protection Association, 2015). Because these pesticides also have been registered in other crops, some of the pesticides might come from other sources. Fluvalinate (pyrethroid), acephate (organophosphate), etofenprox (pyrethroid), flubendiamide (diamide), and flonicamid (selective feeding blocker) ranked first to fifth among the pesticides in detection frequencies in this area (**Fig. 29**). Etofenprox and flubendiamide were not determined in honey samples and residue of Flonicamid was below LOQ in bee (imago and larva) samples. The residue ranges of these five pesticides investigated were lower than acute oral LD_{50}s (≥270 ng/bee; ≥2,500 ng/g $_{bw}$) in all samples (**Table 29**) (Marletto et al., 2003; Tomlin, 2009). The residue levels of the pesticides in the dead and healthy imago samples were lower than 100 ng/g $_{bw}$. In the larva samples, only fluvalinate was determined and its maximum concentration (565.8 ng/g) was higher than that in the imago samples. However, fluvalinate is not hazardous to bee (acute oral LD_{50} 163,000 ng/bee; 1,480,000 ng/g $_{bw}$), and its maximum level was only 0.04% of LD_{50} (Tomlin, 2009). In contrast to Giran, there was no target analyte in pollen sample above 500 ng/g. Spirodiclofen (acute oral LD_{50} >196,000 ng/bee; >1,780,000 ng/g $_{bw}$) and thiophanate-methyl (>100,000 ng/bee; >909,000 ng/g $_{bw}$) showed the highest residue levels in pollen (365.1 and 320.1 ng/g, respectively) but negligible in aspect of ecotoxicology (Tomlin, 2009). Residue ranges in honey samples were not hazardous to bee and lower than those of MRLs (20-1,000 ng/g) (European Commission, 2018).

In conclusion, residue levels of pesticide multiresidues which were detected at higher concentrations in honey bee, pollen, and honey samples were not lethal to honey bee. Although there were a few cases exceeding acute oral LD_{50} of pesticides, these concentrations turned out to be significant outliers ($p < 0.05$). The other pesticides which were positively detected at minor levels but not evaluated in this study may possess lethal toxicity. There are no ecotoxicity data available for these pesticides to our knowledge. Therefore, further systemic investigation and research are required to carry out comprehensive risk assessment to honey bee.

Table 28. Positive detection frequency for bee, pollen, and honey samples in Yeongyang

No.	Compound name	Total (%) *n* = 52	Bee[1)]			Pollen			Honey			Registered for pepper
			Total (%) *n* = 32	Sanun	Dae-cheon	Total (%) *n* = 10	Sanun	Dae-cheon	Total (%) *n* = 10	Sanun	Dae-cheon	
1	Abamectin B1a	1 (1.9)	-	-	-	1 (10.0)	1	-	-	-	-	yes
2	Acephate	17 (32.7)	3 (9.4)	-	3	7 (70.0)	4	3	7 (70.0)	4	3	yes
3	Acetamiprid	13 (25.0)	-	-	-	8 (80.0)	3	5	5 (50.0)	5	-	yes
4	Boscalid	1 (1.9)	-	-	-	1 (10.0)	-	1	-	-	-	yes
5	Carbendazim	7 (13.5)	-	-	-	-	-	-	7 (70.0)	4	3	yes
6	Carbofuran	2 (3.8)	-	-	-	2 (20.0)	1	1	-	-	-	no
7	Chlorantraniliprole	7 (13.5)	1 (3.1)	-	1	6 (60.0)	2	4	-	-	-	yes
8	Chlorpyrifos	1 (1.9)	-	-	-	1 (10.0)	1	-	-	-	-	yes
9	Cyhalothrin-lambda	3 (5.8)	3 (9.4)	1	2	-	-	-	-	-	-	yes
10	Difenoconazole	1 (1.9)	1 (3.1)	-	1	-	-	-	-	-	-	yes
11	Diflubenzuron	2 (3.8)	2 (6.3)	1	1	-	-	-	-	-	-	yes
12	Dimethomorph	5 (9.6)	1 (3.1)	-	1	4 (40.0)	2	2	-	-	-	yes
13	Diphenylamine	8 (15.4)	8 (25.0)	4	4	-	-	-	-	-	-	no[2)]
14	Emamectin B1a	1 (1.9)	-	-	-	1 (10.0)	1	-	-	-	-	yes
15	Etofenprox	16 (30.8)	9 (28.1)	6	3	7 (70.0)	4	3	-	-	-	yes
16	Ferimzone	1 (1.9)	-	-	-	1 (10.0)	-	1	-	-	-	no
17	Flonicamid	14 (26.9)	-	-	-	6 (60.0)	5	1	8 (80.0)	5	3	yes
18	Fluacrypyrim	1 (1.9)	-	-	-	1 (10.0)	1	-	-	-	-	no
19	Fluazinam	6 (11.5)	1 (3.1)	-	1	5 (50.0)	1	4	-	-	-	yes
20	Flubendiamide	15 (28.8)	7 (21.9)	5	2	8 (80.0)	4	4	-	-	-	yes
21	Flufenoxuron	5 (9.6)	2 (6.3)	-	2	3 (30.0)	2	1	-	-	-	yes
22	Fluvalinate	30 (57.7)	26 (81.3)	11	15	3 (30.0)	1	2	1 (10.0)	1	-	no[3)]

Table 28. *(Continued)*

No.	Compound name	Total (%) *n* = 52	Bee[1)]			Pollen			Honey			Registered for pepper
			Total (%) *n* = 32	Sanun	Dae-cheon	Total (%) *n* = 10	Sanun	Dae-cheon	Total (%) *n* = 10	Sanun	Dae-cheon	
23	Metalaxyl	6 (11.5)	3 (9.4)	2	1	1 (10.0)	1	-	2 (20.0)	2	-	yes
24	Methomyl	5 (9.6)	1 (3.1)	1	-	4 (40.0)	2	2	-	-	-	no[4)]
25	Methoxyfenozide	2 (3.8)	1 (3.1)	-	1	1 (10.0)	-	1	-	-	-	yes
26	Metrafenone	2 (3.8)	-	-	-	2 (20.0)	-	2	-	-	-	yes
27	Novaluron	11 (21.2)	10 (31.3)	5	5	1 (10.0)	-	1	-	-	-	yes
28	Picoxystrobin	1 (1.9)	-	-	-	1 (10.0)	-	1	-	-	-	yes
29	Pyraclostrobin	8 (15.4)	1 (3.1)	-	1	7 (70.0)	2	5	-	-	-	yes
30	Spirodiclofen	2 (3.8)	1 (3.1)	-	1	1 (10.0)	1	-	-	-	-	yes
31	Spiromesifen	1 (1.9)	1 (3.1)	-	1	-	-	-	-	-	-	yes
32	Sulfoxaflor	12 (23.1)	1 (3.1)	1	-	4 (40.0)	3	1	7 (70.0)	5	2	yes
33	Teflubenzuron	2 (3.8)	-	-	-	2 (20.0)	-	2	-	-	-	yes
34	Thiodicarb	1 (1.9)	-	-	-	1 (10.0)	1	-	-	-	-	yes
35	Thiophanate-methyl	8 (15.4)	2 (6.3)	-	2	6 (60.0)	3	3	-	-	-	yes
36	Trifloxystrobin	6 (11.5)	-	-	-	6 (60.0)	2	4	-	-	-	yes

[1)]Dead and healthy imago, and larva.

[2)]Post-harvest deterioration inhibitor for apple or naturally producted compound in some crops.

[3)]Pre-treated in colony before monitoring to coltrol mites.

[4)]Banned in 2011.

Fig. 29. Distribution of the numbers of detection frequencies for fluvalinate, etofenprox, acephate, etofenprox, flubendiamide, and flonicamid, which ranked first to fifth among the pesticide multiresidues by the detection frequency

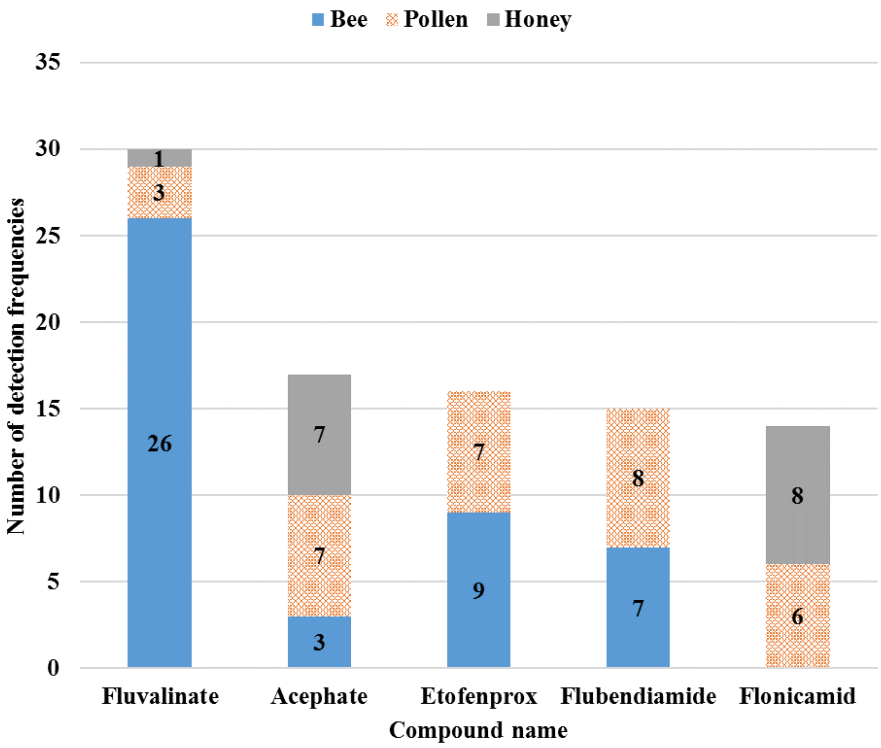

Bee
Pollen
Honey
35
30
25
20
15
10
5
0
Number of detection frequencies
1
3
26
7
7
3
7
9
8
7
8
6
Fluvalinate
Acephate
Etofenprox
Flubendiamide
Flonicamid
Compound name

Table 29. Distribution of median values and residue ranges for pesticide multiresidues in Yeongyang

No.	Pesticides	Honey bee						Pollen ng/g		Honey ng/g	
		Dead imago ng/g $_{bw}$		Healty imago ng/g $_{bw}$		Larva ng/g					
		Median	Residue range	Median	Residue range	Median	Residue range	Median	Residue range	Median	Residue range
1	Abamectin B1a	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ-8.5	<LOQ	<LOQ
2	Acephate	<LOQ	<LOQ-55.9	<LOQ	<LOQ	<LOQ	<LOQ	3.7	<LOQ-16.1	4.4	<LOQ-14.0
3	Acetamiprid	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	6.8	<LOQ-78.6	2.0	3.4-12.7
4	Boscalid	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ-13.0	<LOQ	<LOQ
5	Carbendazim	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.3	<LOQ-4.8
6	Carbofuran	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ-1.4	<LOQ	<LOQ
7	Chlorantraniliprole	<LOQ	<LOQ-14.9	<LOQ	<LOQ	<LOQ	<LOQ	2.0	<LOQ-11.5	<LOQ	<LOQ
8	Chlorpyrifos	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ-10.7	<LOQ	<LOQ
9	Cyhalothrin-lambda	<LOQ	<LOQ-19.9	<LOQ	<LOQ-25.6	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
10	Difenoconazole	<LOQ	<LOQ-20.9	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
11	Diflubenzuron	<LOQ	<LOQ-37.9	<LOQ	<LOQ-9.2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
12	Dimethomorph	<LOQ	<LOQ-14.4	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ-62.7	<LOQ	<LOQ
13	Diphenylamine	34.0	<LOQ-96.7	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
14	Emamectin B1a	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ-1.1	<LOQ	<LOQ
15	Etofenprox	2.3	<LOQ-12.3	<LOQ	<LOQ-9.5	<LOQ	<LOQ	2.5	<LOQ-29.7	<LOQ	<LOQ
16	Ferimzone	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ-5.9	<LOQ	<LOQ
17	Flonicamid	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	2.3	<LOQ-57.0	8.6	<LOQ-39.3
18	Fluacrypyrim	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ-67.5	<LOQ	<LOQ

Table 29. *(Continued)*

No.	Pesticides	Honey bee						Pollen ng/g		Honey ng/g	
		Dead imago ng/g $_{bw}$		Healty imago ng/g $_{bw}$		Larva ng/g					
		Median	Residue range	Median	Residue range	Median	Residue range	Median	Residue range	Median	Residue range
19	Fluazinam	<LOQ	<LOQ-19.7	<LOQ	<LOQ	<LOQ	<LOQ	1.3	<LOQ-152.8	<LOQ	<LOQ
20	Flubendiamide	<LOQ	<LOQ-20.6	<LOQ	<LOQ	<LOQ	<LOQ	2.7	<LOQ-71.7	<LOQ	<LOQ
21	Flufenoxuron	<LOQ	<LOQ-54.1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ-6.9	<LOQ	<LOQ
22	Fluvalinate	12.1	6.2-70.4	17.5	<LOQ-43.4	39.4	<LOQ-565.8	<LOQ	<LOQ-139.4	<LOQ	<LOQ-4.9
23	Metalaxyl	<LOQ	<LOQ-4.1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ-189.9	<LOQ	<LOQ-1.3
24	Methomyl	<LOQ	<LOQ-9.9	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ-12.2	<LOQ	<LOQ
25	Methoxyfenozide	<LOQ	<LOQ-15.9	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ-2.3	<LOQ	<LOQ
26	Metrafenone	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ-8.2	<LOQ	<LOQ
27	Novaluron	10.8	<LOQ-55.3	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ-58.2	<LOQ	<LOQ
28	Picoxystrobin	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ-5.6	<LOQ	<LOQ
29	Pyraclostrobin	<LOQ	<LOQ-15.3	<LOQ	<LOQ	<LOQ	<LOQ	2.8	<LOQ-58.4	<LOQ	<LOQ
30	Spirodiclofen	<LOQ	<LOQ	<LOQ	<LOQ-24.4	<LOQ	<LOQ	<LOQ	<LOQ-365.1	<LOQ	<LOQ
31	Spiromesifen	<LOQ	<LOQ-52.1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
32	Sulfoxaflor	<LOQ	<LOQ-4.8	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ-123.5	5.0	<LOQ-14.3
33	Teflubenzuron	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ-112.9	<LOQ	<LOQ
34	Thiodicarb	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ-1.5	<LOQ	<LOQ
35	Thiophanate-methyl	<LOQ	<LOQ-72.5	<LOQ	<LOQ	<LOQ	<LOQ	5.1	<LOQ-320.1	<LOQ	<LOQ
36	Trifloxystrobin	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	4.6	<LOQ-22.1	<LOQ	<LOQ

Conclusions

Three neonicotinoids (clothianidin, imdacloprid, and thiamethoxam) and 391 pesticide multiresidues in honey bee (dead imago, healthy imago, and larva), pollen, and honey were analyzed using the LC-MS/MS or GC-MS/MS. The scheduled MRM modes of LC- and GC-MS/MS were employed for high-throughput analysis and QuEChERS with a citrate buffer were used to sample treatments. For the three neonicotinoids, their LOQs in LC-MS/MS were 1 ng/g, respectively, thus the analytical method sufficiently determined pesticide residues below acute oral LD_{50}. The recovery range of the neonicotinoids was 74.4-116.2% (RSD 0.4-17.1%) at 1, 5, and 10 ng/g in bee, pollen, and honey samples, which indicate that the established method exhibited excellent accuracy and precision.

In the field monitoring near the area of apple orchard and pepper field, three neonicotinids and 52 of 391 pesticides were determined in a total of 139 apiculture samples. The residue levels of the neonicotinoids and mainly detected multiresidues were not lethal to honey bee except for a few compounds. The compounds exceeding acute oral LD_{50} were turned to be outliers in aspect of statistics. To explain these outliers, an exposure model should be established based on vast regional statistics of multiresidues. Some minor pesticides cannot evaluated in an aspect of ecotoxicology due to no ecotoxicity data available to our knowledge. Synergistic toxicity between pesticides is also an important factor to be considered, but data related are also insufficient. Therefore, further systemic investigation and research including are should be conducted to conclude comprehensive risk assessment to honey bee. Nevertheless, this study

is valuable itself as the first attempt to determine controversial neonicotinoids as well as pesticide multiresidues in honey bees and their products in the Republic of Korea. This field monitoring result can be an important information to improve knowledge of honey bee exposure and on how pesticides move from agricultural fields to the environment.

Chapter III

Multiresidue Analysis for 384 Pesticides in Pepper, Orange, Brown Rice, and Soybean Using Florisil Solid-phase Extraction and GC-MS/MS

Introduction

Introduction of positive list system

Pesticide residues in agricultural products are regulated by governmental authorities to secure the health of populations. The Republic of Korea is one of the countries with lower self-sufficiency rate of food, thus a more endeavor is required to investigate various kinds of imported agricultural products. The current food safety system for pesticide residues in the Republic of Korea is based on the Negative List System (NLS), in which the pesticide residue in Maximum Residue Levels (MRLs) list is regulated (Ministry of Food and Drug Safety); however, the Positive List System (PLS) will be enforced from 2019 onward instead of the NLS.

The PLS is one of the food safety systems in which strict safety management of food from all pesticides with/without MRL is performed (**Table 30**). In the PLS, the levels of pesticide residue should be below 0.01 mg/kg when an MRL has not been established in corresponding crops and food (Iwasaki et al., 2007). The PLS has been successfully implemented for many years in various countries including the United States, Australia, Canada, Hong Kong, Japan, Taiwan, and the European Union (EU). In Korea, the PLS has been implemented for tropical and subtropical fruits, nuts, and seeds since December 31, 2016, and will be applied to all agricultural products starting on January 1, 2019 (Ministry of Food and Drug Safety).

Table 30. The current pesticide regulation in crops and PLS to be introduced in the Republic of Korea (Ministry of Food and Drug Safety)

MRL established for the pesticide	Before introduction for the PLS	After introduction of the PLS
Established	Apply the MRL that is set	Apply the MRL that is set (Same as before enforcement of the PLS)
Not established	1. Apply the CODEX standards for the particular agricultural product (excluding crop groupings). 2. Apply the lowest of the standards set for similar agricultural products. 3. Apply the lowest limit set for the pesticide concerned.	Apply the uniform level of 0.01 mg/kg

Tandem mass spectrometry for pesticide multiresidue analysis

Introduction of the PLS requires high-level analytical techniques to determine a trace concentration of hundreds of pesticides in various food matrices. Tandem mass spectrometry has been widely used as an analytical tool to simultaneously detect and quantity pesticide multiresidues in various matrix origins (Soler and Picó, 2007; LeDoux, 2011). In general, most of tandem mass spectrometers utilized in quantitative analysis of multiresidue is a triple quadrupole mass spectrometer (*QqQ*), which consist of two quadrupole analyzers (*Q*) to purify molecular mass ion by m/z and one collision quadrupole (*q*) aligned between the quadrupole analyzers. Tandem mass spectrometry also can be applicable to a combination of quadrupole (*Q*) and high-resolution mass (HRMS) analyzer such as time-of-flight (TOF) or orbitrap (Cheng et al., 2017; Goon et al., 2018).

The multiple reaction monitoring (MRM) of *QqQ* is one of the recent mass spectrometric techniques that is an improved concept of a selected ion monitoring (SIM) in single mass spectrometry. The two analyzers (*Q*) are conducted as SIM, whereas the second analyzer (*Q3*) select one of the fragments (product ions) of a precursor ion that is purified from the first analyzer (*Q1*). Precursor ion is fragmented by collision induced dissociation (CID) during passing through the *q* (*Q2*). The MRM is superior to SIM in an aspect of sensitivity and selectivity. Wong et al. established multiresidue analysis for 168 pesticides in dried ginseng powders using GC-MS and GC-MS/MS and outstanding specificity and sensitivity of target analytes were observed in tandem mass spectrometry than in single mass spectrometry (Wong et al., 2010).

Many works of literatures have reported the *QqQ* coupled with liquid chromatography (LC) or gas chromatography (GC) (Vázquez et al., 2016; Han et al., 2017; Wu, 2017). The LC and GC served as an compound purification and separation technique to prevent contamination of mass spectrometer by sample matrices and to distribute massive spectrometric data by retention time. Supercritical fluid chromatography (SFC) and ion chromatography (IC) are also available with mass spectrometry for specific types of pesticides (Adams et al., 2017; Cutillas et al., 2018).

Table 31 showed recently established analytical methods for pesticide multiresidues by the tandem mass spectrometry in various type of agricultural product matrices during three-year period (2016-2018).

Table 31. Review of the tandem mass spectrometry for pesticide multiresidues in agricultural products during three-year publication (2016-2018)

No.	Matrix	Instrument	Number of analytes	Reference
1	Tomato, orange, and leek	SFC[1)]-MS/MS	164	(Cutillas et al., 2018)
2	Sweet pepper	LC-MS/MS	21	(da Costa Morais et al., 2018)
3	Spices	LC-MS/Orbitrap	199	(Goon et al., 2018)
4	Kiwifruit	LC-MS/MS	49	(Kim et al., 2018)
5	Brown rice, orange, and spinach	LC-MS/MS	310	(Lee et al., 2018b)
6	Teas	GC-MS/MS	128	(Li et al., 2018a)
7	Mango	GC-MS/MS and LC-MS/MS	113	(Li et al., 2018b)
8	Lettuce	LC-MS/MS	16	(Ribeiro Begnini Konatu and Sales Fontes Jardim, 2018)
9	Cardamom	GC-MS/MS	243	(Shabeer et al., 2018)
10	Flour and grape	IC[2)]-MS/MS	12	(Adams et al., 2017)

Table 31. *(Continued)*

No.	Matrix	Instrument	Number of analytes	Reference
11	Apple, pear, tomato, cucumber, and cabbage	GC-MS/TOF[3)]	15	(Cheng et al., 2017)
12	Rice, wheat, and corn	GC-MS/MS	124	(Han et al., 2017)
13	Vegetable oils	GC-MS/MS	255	(He et al., 2017)
14	Cardamom	LC-MS/MS	154	(Jadhav et al., 2017)
15	Wheat, rye, oat	LC-MS/MS	23	(Kaczyński and Łozowicka, 2017)
16	Pear	LC-MS/MS	170	(Kemmerich et al., 2018)
17	Brown rice, spinach, orange, and potato	GC-MS/MS	360	(Lee et al., 2017)
18	Tomato, sweet pepper	LC-MS/MS	21	(Martins et al., 2017)
19	Rice	GC-MS/MS	31	(Mondal et al., 2017)
20	Apple, citrus fruit, peanut, wheat, tea, and spinach	LC-MS/MS	23	(Qin et al., 2017)
21	Lettuce	LC-MS/MS	16	(Ribeiro Begnini Konatu et al., 2017)
22	Tomato, leek	LC-MS/MS	41	(Robles-Molina et al., 2017)

Table 31. *(Continued)*

No.	Matrix	Instrument	Number of analytes	Reference
23	Currants, raspberries, cherries, strawberries, blackberries, cauliflowers, and broccoli	LC-MS/MS	60	(Stachniuk et al., 2017)
24	Crop plants	LC-MS/MS	72	(Viera et al., 2017)
25	Oolong tea	GC-MS/MS	89	(Wu, 2017)
26	Rice	LC-MS/MS	20	(Cabrera et al., 2016)
27	Olive oil	LC-MS/MS	165	(Dias et al., 2016)
28	Wheat	LC-MS/MS	42	(Friedrich et al., 2016)
29	Rice and wheat flour	GC-MS/MS	100	(Grande-Martínez et al., 2016)
30	Cowpea	GC-MS/MS	171	(Han et al., 2016)
31	Green tea	GC-MS/MS	101	(Hou et al., 2016)
32	Olive oil, olives, and avocado	LC-MS/MS	67	(López-Blanco et al., 2016)
33	Lettuce and orange	GC-MS/MS and LC-MS/MS	175	(Lozano et al., 2016)

Table 31. *(Continued)*

No.	Matrix	Instrument	Number of analytes	Reference
34	Sugar beet and beet molasses	GC-MS/MS and LC-MS/MS	>400	(Lozowicka et al., 2016)
35	Spinach	LC-MS/MS	44	(Qin et al., 2016a)
36	Apple, citrus fruit, peanut, wheat, tea, and spinach	LC-MS/MS	25	(Qin et al., 2016b)
37	Vegetable oils	GC-MS/MS	213	(Vázquez et al., 2016)
38	Tomato	GC-MS/MS	>140	(Walorczyk et al., 2016)

[1)]Supercritical fluid chromatography

[2)]Ion chromatography

[3)]Time-of-flight

Solid-phase extraction for pesticide purification

With the tandem mass spectrometry, sample treatment is an important factor to conduct pesticide analysis. Well-established sample preparation exert the best extraction efficiency with rugged results, thus trace levels of detection limit of target analytes can be obtained in the same instrument condition. The ideal methodology to preserve the integrity of the target pesticide is sample extraction without purification step. When using this treatment, however, it is difficult to distinguish between unremoved matrices and multi-analytes no matter how a mass spectrometer is superior. The interferences also cause a severe matrix effects, so that target analytes may not be detected at all. The cleaning and maintanance cycle of mass spectrometric parts such as an ion source also become shorter due to numerous matrices. A proper purification procedure removing matrices from pesticides is, therefore, required.

Solid-phase extraction (SPE) is one of the cleanup techniques first introduced since the mid-1970s (Sabik et al., 2000). General SPE is performed by passing sample extract or liquid sample itself through a solid sorbent in a glass or polypropylene cartridge. It is advantageous in that it can be purified strongly with a small amount of solvent, thus, alternative to liquid-liquid extraction or column chromatography. Since a SPE cartridge was commercially available in 1978, numerous types of sorbent in difference cartridge sizes allow analysts to have a chance to analyze pesticides with various chemical properties (Picó et al., 2007).

The SPE is also applicable in pesticide multiresidue analysis. It is a challenge to establish optimum washing/elution conditions for multi-analyte characteristics. Many of literatures overcome this issue in various ways (**Table**

32). Yang et al. (2011) tried dual SPE with two different types of SPE and obtained excellent recoveries for 88 pesticides in raspberries, strawberries, blueberries, and grapes (Yang et al., 2011). SPE can be combined with the "Quick, Easy, Cheap, Effective, Rugged, and Safe" (QuEChERS) procedure that is a strong extraction and partitioning methodology (Anastassiades et al., 2003). Chen et al. (2011) and Hou et al. (2016) established analytical methods using QuEChERS for the sample extraction and partitioning and then SPE for purification (Chen et al., 2011; Hou et al., 2016).

Multiclass Pesticide Multiresidue Method (No. 2)

The Multiclass Pesticide Multiresidue Method (No. 2) of the Korea Food Code is an official analytical method for analysis of pesticide multiresidues in crops (Ministry of Food and Drug Safety). The method has powerful sample treatment procedures using an amino-propyl (NH_2) for LC-amenable pesticides and a forisil for GC-amenable pesticides. Among the sorbents, florisil consists of a magnesium silicate with a high polarity. Florisil has strong purification characteristics for pesticides from fatty samples due to its ability to preferentially retain some lipids (Żwir-Ferenc and Biziuk, 2006). Polar matrices such as chlorophylls triglycerides and phytosterols, common substances of fruits and vegetables are also easily removed from the sample extract by associating with the surface of florisil (Torres et al., 1996). Thus, the Multiclass Pesticide Multiresidue Method (No. 2) is a golden standard to determine pesticides in various kinds of agricultural products and food.

Table 32. Representative analytical methods for pesticide multiresidues including solid-phase extraction (SPE) cleanup procedures

No.	Matrix	SPE type	Instrument	Number of analytes	Reference
1	Green tea	GCB/PSA	GC-MS/MS	101	(Hou et al., 2016)
2	Tea	Sep-Pak Carbon NH_2	LC-MS/MS	65	(Chen et al., 2011)
3	Raspberries, strawberries, blueberries, and grapes	Envi-Carb & NH_2-LC coupled	GC-MS	88	(Yang et al., 2011)
4	Dried ginseng powders	C_8 and GCB/PSA	GC-MS and GC-MS/MS	168	(Wong et al., 2010)
5	Honey, fruit juice, and wine	Envi-Carb & Sep-Pak NH_2 coupled	GC-MS and LC-MS/MS	450	(Pang et al., 2006)
6	Juice	Silica Bondesil-C_{18}	GC-MS	50	(Albero et al., 2005)
7	White grapes	Sep-Pak Silica	LC-DAD	14	(Rial Otero et al., 2003)
8	Egg	GCB NH_2 Florisil	GC-ECD and FPD	36	(Schenck and Donoghue, 2000)

Purpose of the present study

In this study, a simultaneous multiresidue analytical method was developed using GC-MS/MS. A scheduled MRM mode of GC-MS/MS was employed for an effective throughput of target pesticides. Four representative crops popular in Korea were selected as matrices; pepper (high pigment and chlorophyll), orange (high acidic compounds), brown rice (high starch), and soybean (high protein and fat). The official Multiclass Pesticide Multiresidue Method (No. 2) of the Korea Food Code was scaled down and validated with 384 pesticides. For removing fat in soybean sample, liquid-liquid partitioning method using *n*-hexane/acetonitrile was also investigated for comparing to non-partitioning method. The evaluated analytical method is applicable for the PLS as well as the rapid and sensitive monitoring of pesticide multiresidues in pepper, orange, brown rice, and soybean and their related agriculture products.

Materials and Methods

Chemicals and reagents

Each pesticide standard (analytical grade) or stock solution (10, 100, 500 or 1,000 mg/L) was purchased from ChemService (West Chester, PA), Wako Pure Chemical Industries (Osaka, Japan), Dr. Ehrenstorfer (Augsburg, Germany), Sigma-Aldrich (St. Louis, MO), Tokyo Chemical Industry (Tokyo, Japan), AccuStandard (New Haven, CT) and ULTRA Scientific (North Kingstown, RI), or thankfully obtained from Laboratory of Environmental Chemistry of Kyungpook National University (the Republic of Korea), and Ministry of Food and Drug Safety (Republic of Korea). HPLC grades of acetonitrile, acetone, methanol were sourced from Fisher Scientific (Seoul, Republic of Korea). Sodium chloride (NaCl, 99.0%) was obtained from Samchun (Gyeonggi-do, the Republic of Korea). Diethylene glycol (≥99.0%) was purchased from Sigma-Aldrich. Strata-FL-PR florisil cartridge (500 mg/6 mL) was obtained from Phenomenex (Torrance, CA).

Preparation of matrix-matched standard

Each analytical standard was dissolved in acetonitrile, acetone, or methanol in accordance with their solubility to make 1,000 mg/L of stock solutions. A portion of each stock solution from standards or commercial products was then mixed and diluted with acetonitrile so that the concentration of four groups of intermediate mixed stock solutions became 10 mg/L in each mixed standard solution. The aliquots of intermediates were again mixed to make a final mixed standard solution at 2.5 mg/L. This solution was subjected to further serial

dilution using acetonitrile to prepare working solutions at 1, 0.5, 0.25, 0.1, 0.05, 0.025, and 0.01 mg/L. These solutions were finally mixed with matrix solutions from blank samples at a ratio of 1:4 (v/v) to prepare matrix-matched standards at 0.2, 0.1, 0.05, 0.02, 0.01, 0.005, and 0.002 mg/L.

Instrumental conditions of GC-MS/MS

Pesticide multiresidues were separated by a Shimadzu GC-2010 plus furnished with an AOC-20i autosampler (Kyoto, Japan) and analyzed by a Shimadzu GCMS-TQ8040 triple quadrupole mass spectrometer (Kyoto, Japan). The GC conditions followed our previous work (Lee et al., 2017). Briefly, the inlet temperature was 280 °C and the injection volume was 2 μL. A Topaz glass liner (3.5-mm) with wool (Restek, Bellefonte, PA) was installed within the inlet and splitless mode was used during sample injection. The capillary column was Rxi-5Sil MS (30 m × 0.25 mm i.d., 0.25 μm d_f, Restek, Bellefonte, PA). The oven temperature program was initialized with 70 °C (held for 2 min), ramped to 160 °C at 15 °C/min, then increased to 260 °C at 5 °C/min, and finally ramped to 300 °C at 15 °C/min (held for 8 min). The total program time was 38.7 min. Helium (≥99.999%) was used as carrier gas and flow rate (constant) was 1.0 mL/min.

For the MS/MS conditions, the electron ionization (EI) mode was selected for ionization and electron voltage was 70 eV. The ion source and transfer line temperature were 230 and 280 °C, respectively. The collision inductive dissociation (CID) was assisted with argon (≥99.999%) gas. The detector voltage was 1.4 kV (constant). The data processing was conducted using LabSolutions (GCMS solution, version 4.30).

Multiple reaction monitoring (MRM) profile optimization

Each pesticide standard solution was injected into GC-MS/MS, respectively, and full scan spectrum of each target was obtained (m/z range; 50-500). From the spectrum, one or two of a fragment(s) or molecular ion were selected as a precursor ion(s) considering intensity and selectivity. Each precursor ion was subjected to product scan using CID with various collision energy (CE), and two optimum product ions were selected as a quantifier ion and a qualifier ion, respectively.

Sample preparation of pepper, orange, brown rice, and soybean

Before sample preparation, pepper, orange, brown rice, and soybean were homogenized respectively with dry ice using a blender. Preparation of each crop was conducted using modified Multiclass Pesticide Multiresidue Method (No. 2) from the Korea Food Code (Ministry of Food and Drug Safety). Ten gram of an aliquot was transferred into a 50-mL centrifuge tube. For brown rice and soybean, 6 mL water was added to the tube to let the entire sample soak the water sufficiently. The aliquot was extracted with 20 mL of acetonitrile. The tube was shaken for 2 min at 1,200 rpm using Geno Grinder (1600 MiniG SPEX Sample Prep, Metuchen, NJ), and then the sample was subjected to suction filtration under vacuum. The extract was treated with 3 g of NaCl and then centrifuged for 5 min at 3,500 rpm using Combi 408 (Hanil Science Industrial Co., Ltd., Korea). The supernatant (8 mL) was treated with 0.2 mL of 2% diethylene glycol in acetone and evaporated with a rotary evaporator (40 °C) and reconstructed in 4 mL of 20% acetone in *n*-hexane (v/v).

For SPE cleanup step, a florisil cartridge (500 mg) was conditioned with 5 mL *n*-hexane and 5 mL of 20% acetone in *n*-hexane. Reconstructed extract (4 mL) was loaded and the loaded cartridge was eluted without washing step. The cartridge was eluted again with 5 mL of 20% acetone in *n*-hexane. The eluted extracts were combined and then evaporated to dryness under a stream of nitrogen gas. The sample was reconstructed in 2 mL acetonitrile, and 0.8 mL of the extract was matrix-matched with 0.2 mL of acetonitrile. Finally, the aliquot (2 μL) was injected into GC-MS/MS for target analytes separation and analysis. The sample was equivalent to 1.6 g per 1 mL in the final extract.

Defatting procedure in soybean using *n*-hexane/acetonitrile partitioning

Partitioning step with *n*-hexane and acetonitrile was conducted before the SPE cleanup, and its extraction and cleanup efficiency were compared to the method without the partitioning procedure. Briefly, 10 g of soybean was subjected to extraction, filtration, NaCl partitioning, and evaporation, as described above. The extract was then dissolved in 30 mL of *n*-hexane saturated with acetonitrile and partitioned with 30 mL of acetonitrile saturated with *n*-hexane (twice). The lower layers from each partition were collected, and treated with 0.2 mL of 2% diethylene glycol in acetone, and then evaporated with rotary evaporator (40 °C) for the next cleanup step, as described above.

Method validation

The method limit of quantitation (MLOQ) was calculated from the instrumental limit of quantitation (ILOQ), injection volume, and sample equivalent in the final extract as following equation:

$$\text{MLOQ}\ \ (mg \cdot kg^{-1}) = \frac{\text{ILOQ}\ \ (ng)}{\text{injection volume}\ \ (\mu L)} \times \frac{1}{\text{sample equivalent}\ \ (g \cdot mL^{-1})}$$

ILOQ was determined by the signal to noise ratio (S/N) method (De Bièvre et al., 2005). Matrix-matched standards in pepper, orange, brown rice, and soybean samples were injected into GC-MS/MS, respectively, and the lowest amount of chromatogram which satisfied the S/N value ≥10 for each pesticide was selected as ILOQ. Instrumental repeatability for each pesticide was verified with RSD of peak area by injecting and analyzing matrix-matched standard seven times. The linearity of calibration was determined using matrix-matched standards in each crop sample. Instrumental linear range was investigated from 0.002 mg/L to 0.2 mg/L (equivalent to 0.00125-0.125 mg/kg of method linear range). A weighting regression factor (*1/x*) was employed to minimize calculation errors at low concentrations. The correlation coefficient (r^2) of calibration was calculated for each target analyte. Recovery of each pesticide was determined at 0.01 and 0.05 mg/kg. To conduct the recovery tests, 10 g of each blank sample was treated with mixed standard solutions, respectively, and prepared as described above (n = 3). The recovery samples were compared with matrix-matched standards to verify extraction efficiencies. The matrix effect was verified to compare a slope of calibration form a matrix-matched standard and from a solvent-based standard. Matrix effect values (%) for each pesticide were calculated as following equation:

$$\text{Matrix effect}, \% = \left(\frac{\text{Slope of matrix-matched standard calibration}}{\text{Slope of solvent-based standard calibration}} - 1\right) \times 100$$

Results and Discussion

MRM optimization and selection of pesticides to be validated

A total of 397 pesticides was selected to be studied. Using standard solutions of each pesticide, MRM profiles were successfully established. Retention times were also verified under GC conditions. It is noticeable that deltamethrin and tralomethrin had the same MRM conditions (precursor ion > product ion; 253 > 172 for quantifier and 253 > 174 for qualifier) with the same retention times (32.01 and 32.27 min), thus they could not be distinguished. It is possible that tralomethrin was debrominated at the inlet of GC and converted to deltamethrin (Woudneh and Oros, 2006). According to Pesticide MRLs in Food published in the Republic of Korea , MRLs of tralomethrin follow that of deltamethrin (Ministry of Food and Drug Safety, 2017). Therefore, deltamethrin and tralomethrin shared the same validation results.

Using the matrix-matched standards of pepper, orange, brown rice, and soybean at 0.2 mg/L MRM chromatograms for 397 pesticides were verified. Anilazine, daimuron, and pyrazosulfuron-ethyl were not detected at all and captafol and captan had a poor sensitivity in all crop samples. Therefore, these five pesticides were discarded from the final method validation. Recovery samples were also investigated and three compounds (naled, oxydemeton-methyl, and schradan) were not recovered in all samples. Benzyladenine, nicotine, tecloftalam, triflusulfuron-methyl, and trinexapac-ethyl showed the low recovery values below 30% in all crops. Thus, these eight pesticides were also excluded from the list of target analytes according to the guidance of

SANTE/11813/2017 (European Commission, 2017). Finally, the remaining of 384 analytes from 397 pesticides was investigated in further validation steps.

Characteristics of 384 pesticides

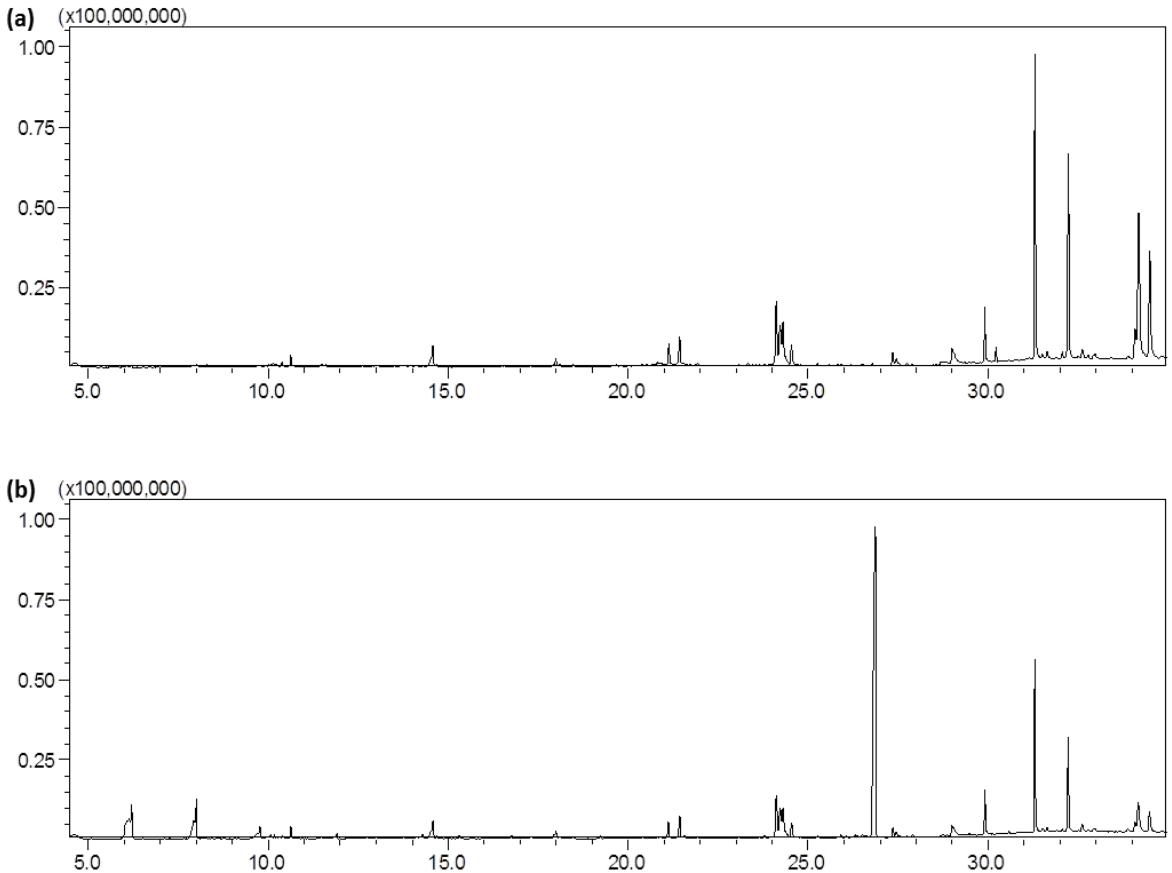
Among the pesticides to be established, 57.6% was listed in Pesticide MRLs in Food, accounting for 45.9% of 466 MRL entries in 2017 (Ministry of Food and Drug Safety, 2017). For pesticide activities, 163 (42.4%) of 384 pesticides was insecticide (covering acaricide and nematicide), 86 (22.4%) was fungicide (covering bactericide), 108 (28.1%) was herbicide, and 6 (1.6%) was plant growth regulator. Inter-activity pesticides that exhibit activities in two or more different groups were accounted for 13 (3.4%) of the total. In addition, the pesticide chemical groups were divided into eight, which were carbamate (25 compounds), organochlorine (66), organophosphate (87), pyrethroid (19), triazine (16), triazole (22), urea (7), and others/unclassified (142). Among the 384 pesticides, 13 compounds were major metabolites of alachlor (alachlor-2-hydroxy, [2',6'-diethyl-N-2-hydroxy(methoxymethyl)acetanilide]), amitraz (BTS 27919 [2,4-dimethylformanilide]), chlordane (oxychlordane), chlorothalonil (pentachlorobenzonitrile), DDT (o,p'-DDD, p,p'-DDD, o,p'-DDE, and p,p'-DDE), endosulfan (endosulfan-sulfate), heptachlor (heptachlor-epoxide), and quintozene (pentachloroaniline, pentachlorobenzene, and pentachlorothioanisole). These metabolites have been found in various environmental matrices or crop residues (Dejonckheere et al., 1976; el-Nabarawy and Carey, 1988; Kross et al., 1992; Eitzer et al., 2001; Golfinopoulos et al., 2003; Gong et al., 2004; Ziaei and Amini, 2013).

Individual characteristics of information of the 384 pesticides is given in **Table S3**.

Comparison of the preparation procedures with/without *n*-hexane/ acetonitrile partitioning

The optimum soybean preparation method was selected between the procedures with/without *n*-hexane/acetonitrile partitioning. To verify extraction efficiency, the recovery test at 0.05 mg/kg was conducted in both procedures. The percentages of 203 pesticides satisfying the criteria of recovery 70-120% (RSD ≤20%) were 74.5% for partitioning and 84.4% for non-partitioning (European Commission, 2017). Some of the pesticides with higher log *P* were remained in the hexane layer during partitioning. To verify the cleanup efficiency, scan chromatograms of control soybean samples were compared (**Fig. 30**). At t_R values of 5.0-10.0 min and 26.5-27.0 min, more impurities were detected when the non-partitioning procedure was applied than when the partitioned procedure was applied, whereas larger peaks were observed when the partitioning procedure was applied at a t_R value of 31.0 min or greater. There were no significant differences in the cleanup between the procedures. From the results, the preparation procedures without n-hexane/acetonitrile partitioning (non-partitioning) was considered as the most appropriate soybean treatment method for GC–MS/MS analysis.

Fig. 30. Scan chromatograms (m/z 50-500) for control soybean samples of (a) partitioned and (b) non-partitioned procedures


(a)
(x100,000,000)
1.00
0.75
0.50
0.25
5.0
10.0
15.0
20.0
25.0
30.0
(b)
(x100,000,000)
1.00
0.75
0.50
0.25
5.0
10.0
15.0
20.0
25.0
30.0

Method limit of quantitation (MLOQ)

Matrix-matched standard mixtures at 0.004, 0.01, 0.02, 0.04, 0.1, 0.2, and 0.4 ng (equivalent to 0.002, 0.005, 0.01, 0.02, 0.05, 0.1, and 0.2 mg/L matrix-matched standard solutions) in pepper, orange, brown rice, and soybean were injected into the GC-MS/MS and the lowest amount satisfying S/N ≥10 on the chromatogram for each pesticide was selected as ILOQ. The MLOQ was derived from the ILOQ, injection volume (2 μL), and sample equivalent (1.6 g/mL) in the final extract. If ILOQ is 0.004 ng, MLOQ can be calculated using equation as described above.

$$\text{MLOQ}\ \ (mg \cdot kg^{-1}) = \frac{\text{ILOQ}\ \ (ng)}{\text{injection volume}\ \ (\mu L)} \times \frac{1}{\text{sample equivalent}\ \ (g \cdot mL^{-1})}$$

$$\text{MLOQ}\ \ (mg \cdot kg^{-1}) = 0.004\ \ (ng)/2\ \ (\mu L) \times 1/1.6\ \ (g \cdot mL^{-1})$$

$$= 0.0013\ \ (mg \cdot kg^{-1})$$

Among the 384 pesticides, 380 (99.0%), 381 (99.2%), 365 (95.1%), and 382 (99.5%) were MLOQ below 0.01 mg/kg in pepper, orange, brown rice, and soybean, respectively, showing excellent sensitivity in the analytical method (**Table 33**). Only 2 (0.5%) to 3 (0.8%) compounds showed MLOQ >0.01 mg/kg in some crops. Carbosulfan and propargite were not detectable in pepper. The percentage was slightly lower in brown rice than other crops because 16 compounds were not determined at all investigation ranges. Among them, eight compounds were azines (ametryn, bupirimate, cyanazine, cyprazine, dimethametryn, prometryn, simetryn, and terbutryn) and four were azoles (azaconazole, cyproconazole, flusilazole, myclobutanil). They exhibited severely broaden peaks only in brown rice samples. Nevertheless, the overall

percentages satisfying MLOQ below 0.01 mg/kg were similar or greater than those in our previous study using QuEChERS method with GC-MS/MS (Lee et al., 2017). Thus, this analytical method can determine pesticide multiresidues in pepper, orange, brown rice, soybean, and related crop with sufficient sensitivity.

Table 33. Distribution of MLOQs for 384 pesticides in pepper, orange, brown rice, and soybean

MLOQ	**Crop** No. of analytes			
	Pepper	Orange	Brown rice	Soybean
≤ 0.01	380 (99.0%)	381 (99.2%)	365 (95.1%)	382 (99.5%)
> 0.01	2 (0.5%)	3 (0.8%)	3 (0.8%)	2 (0.5%)
N.D.[1)]	2 (0.5%)	0 (0.0%)	16 (4.2%)	0 (0.0%)
Sum	384 (100%)	384 (100%)	384 (100%)	384 (100%)

[1)]Not determined.

Instrumental repeatability

The instrumental repeatability is a parameter to ensure the integrity of instrumental performance for target compounds (Zhao and Lee, 2001; Lee et al., 2018a). Repeatability test was conducted by consecutively injecting matrix-matched standard solutions at 0.01 and 0.05 mg/L (n = 7) and verified RSD of area for each pesticide. Average RSDs of the target pesticides were 2.3-4.3% at 0.01 mg/L and 1.3-2.6% at 0.05 mg/L, showing better repeatability at higher concentration (0.05 mg/L) in all crops. For the 384 pesticides in pepper, orange, brown rice, and soybean samples, RSDs for 372 (96.9%), 377 (98.2%), 362 (94.3%), and 378 (98.4%) at 0.01 mg/L and 375 (97.7%), 383 (99.7%), 364 (94.8%), and 384 (100.0%) at 0.05 mg/L were below than 10% (**Table 34**), respectively. This indicates that GC-MS/MS exerted excellent performance for the most pesticides during sample injection and analysis. RSDs for 3 (0.8%) to 5 (1.3%) at 0.01 mg/L and 0 to 4 (1.0%) at 0.05 mg/L of total pesticides fell within 10 to 20% in all crops. These analytes still showed acceptable repeatability considering that RSD criterion for recovery is ≤20% according to the SANTE guidance document (European Commission, 2017).

Fenfuram, folpet, methoxychlor, and p,p'-DDT in pepper, and chlorothalonil in orange obtained RSDs more than 20% at 0.01 or 0.05 mg/L. These compounds tended to decrease in area as the number of injections increased (**Fig. 31**), thus require auxiliary approaches such as internal standard calibration (Nguyen et al., 2008) to correct quantitation. Except for the compounds, target pesticides in all crops presented the excellent instrumental repeatability on established GC-MS/MS conditions.

Linearity of calibration

The linearity of matrix-matched calibration was determined with the linear range from LOQ to 0.2 mg/L. Among the 384 target analytes, 377 (98.2%), 375 (97.7%), 364 (94.8%), and 379 (98.7%) showed correlation coefficient (r^2) ≥0.990 in pepper, orange, brown rice, soybean, respectively (**Table 35**). Only r^2 of 2 (0.5%) to 3 (0.8%) pesticides were within 0.980-0.990 and r^2 of 2 (0.5%) to 6 (1.6%) were <0.980. Therefore, most of the pesticides obtained precise quantitative analysis properties.

Table 34. Summary of instrumental repeatability to show distribution of RSD of area for 384 pesticides in pepper, orange, brown rice, and soybean (n = 7)

Crop	**Pepper** No. of analytes		**Orange** No. of analytes		**Brown rice** No. of analytes		**Soybean** No. of analytes	
RSD (area)	**0.01 mg/L**	**0.05 mg/L**	**0.01 mg/L**	**0.05 mg/L**	**0.01 mg/L**	**0.05 mg/L**	**0.01 mg/L**	**0.05 mg/L**
≤10%	372 (96.9%)	375 (97.7%)	377 (98.2%)	383 (99.7%)	362 (94.3%)	364 (94.8%)	378 (98.4%)	384 (100.0%)
10-20%	5 (1.3%)	4 (1.0%)	3 (0.8%)	0 (0.0%)	4 (1.0%)	4 (1.0%)	4 (1.0%)	0 (0.0%)
>20%	2 (0.5%)	3 (0.8%)	0 (0.0%)	1 (0.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
N.D.	5 (1.3%)	2 (0.5%)	4 (1.0%)	0 (0.0%)	18 (4.7%)	16 (4.2%)	2 (0.5%)	0 (0.0%)
Sum	384 (100%)	384 (100%)	384 (100%)	384 (100%)	384 (100%)	384 (100%)	384 (100%)	384 (100%)

N.D.; Not determined.

Fig. 31. Relative peak area (100 at 1st injection) of DDT-p,p', fenfuram, folpet, methoxychlor (pepper), and chlorothalonil (orange) at 50 ng/mL to show peak decreases as the number of injections increases

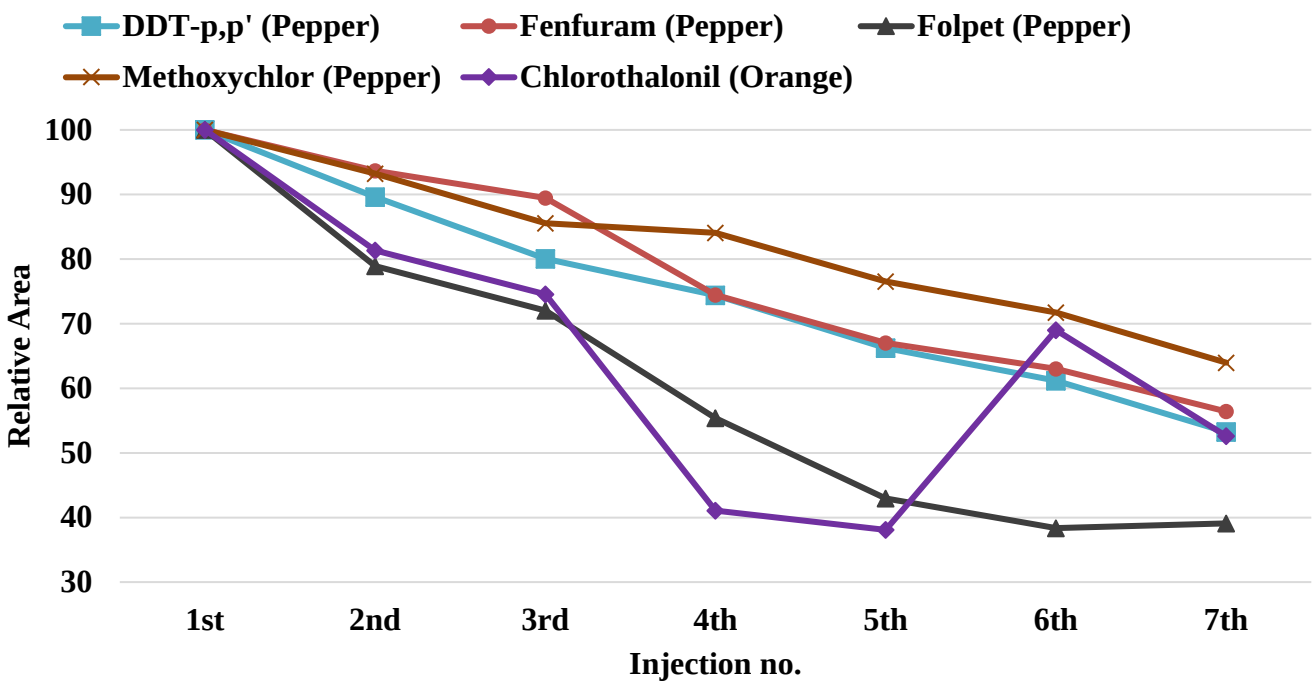

DDT-p,p' (Pepper)
Fenfuram (Pepper)
Folpet (Pepper)
Methoxychlor (Pepper)
Chlorothalonil (Orange)
Relative Area
100
90
80
70
60
50
40
30
1st
2nd
3rd
4th
5th
6th
7th
Injection no.

Table 35. Distribution of correlation coefficients (r^2) for 384 pesticides in pepper, orange, brown rice, and soybean

r^2	**Crop** No. of pesticides			
	Pepper	**Orange**	**Brown rice**	**Soybean**
≥0.990	377 (98.2%)	375 (97.7%)	364 (94.8%)	379 (98.7%)
0.980-0.990	3 (0.8%)	3 (0.8%)	2 (0.5%)	3 (0.8%)
<0.980	2 (0.5%)	6 (1.6%)	2 (0.5%)	2 (0.5%)
N.D.	2 (0.5%)	0 (0.0%)	16 (4.2%)	0 (0.0%)
Sum	384 (0.0%)	384 (0.0%)	384 (0.0%)	384 (0.0%)

N.D.; Not determined.

Recovery

For the efficient sample treatment, sample and extraction solvent of Multiclass Pesticide Multiresidue Method (No. 2) of the Korea Food Code (Ministry of Food and Drug Safety) were reduced from 50 to 10 g and 100 to 20 mL acetonitrile. During the evaporation at 40 °C, diethylene glycol in acetone, a keeper solution (Gunther et al., 1962), was added in each sample to prevent some volatile pesticides from evaporation with the solvent. To maintain the optimal and rugged performance of the florisil SPE, the sorbent was prevented from drying out during the conditioning, sample-loading, and elution steps, as Mutavdžić et al. (2006)'s instruction (Mutavdžić et al., 2006).

The recovery test was conducted at 0.01 and 0.05 mg/kg. As a result, 323 (84.1%) to 354 (92.2%) pesticides at 0.01 mg/kg and 324 (84.4%) to 354 (92.2%) at 0.05 mg/kg satisfied the acceptable recovery criteria of 70-120% with RSD ≤20% (**Table 36**) in pepper, orange, brown rice, and soybean (European Commission, 2017). The number of pesticides satisfying the criteria at 0.05 mg/kg was slightly higher than that at 0.01 mg/kg in orange, brown rice, and soybean. The recovery of pepper indicated the same percentage (92.2%) at both treated levels and showed the largest number of pesticides among the crops. These compounds can be detectable with highly reliable trueness and precision properties.

Table 36. Distribution of recoveries for 384 pesticides in pepper, orange, brown rice, and soybean

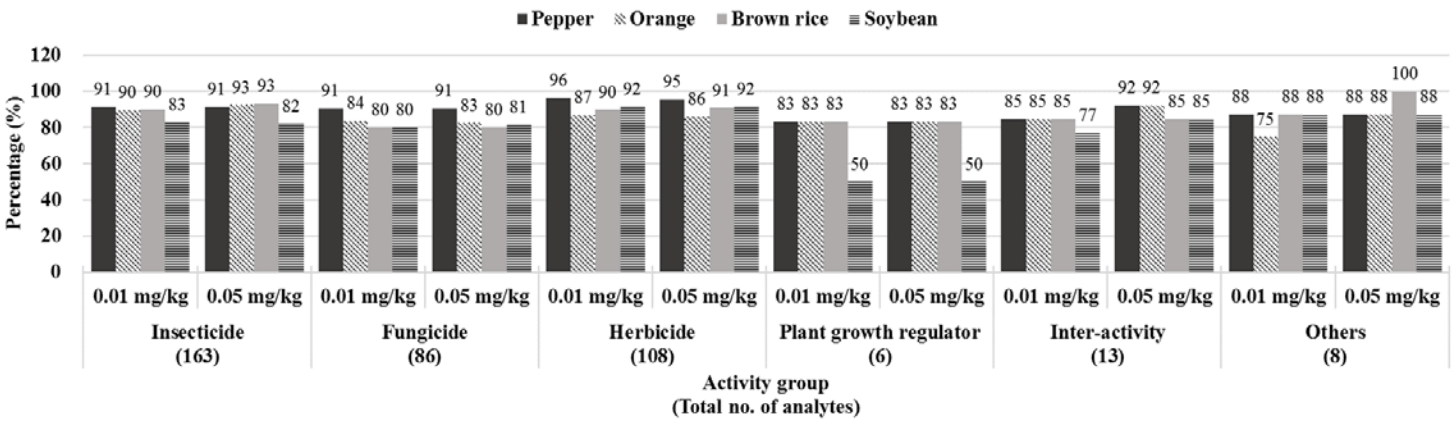
Recovery range %	RSD %	Pepper No. of analytes (%)		Orange No. of analytes (%)		Brown rice No. of analytes (%)		Soybean No. of analytes (%)	
		0.01 mg/kg	**0.05 mg/kg**	**0.01 mg/kg**	**0.05 mg/kg**	**0.01 mg/kg**	**0.05 mg/kg**	**0.01 mg/kg**	**0.05 mg/kg**
<30	**≤0**	2 (0.5)	2 (0.5)	6 (1.6)	8 (2.1)	2 (0.5)	4 (1.0)	3 (0.8)	5 (1.3)
30-70	**≤20**	23 (6.0)	26 (6.8)	34 (8.9)	35 (9.1)	24 (6.3)	20 (5.2)	51 (13.3)	54 (14.1)
	>20	0 (0.0)	0 (0.0)	3 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)	3 (0.8)	1 (0.3)
70-120	**≤20**	354 (92.2)	354 (92.2)	334 (87.0)	339 (88.3)	336 (87.5)	343 (89.3)	323 (84.1)	324 (84.4)
	>20	1 (0.3)	0 (0.0)	2 (0.5)	0 (0.0)	1 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)
120-140	**≤20**	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	>20	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
>140	**≤0**	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)	0 (0.0)	0 (0.0)
N.D.		4 (1.0)	2 (0.5)	5 (1.3)	1 (0.3)	21 (5.5)	16 (4.2)	4 (1.0)	0 (0.0)
Sum		384 (100)	384 (100)	384 (100)	384 (100)	384 (100)	384 (100)	384 (100)	384 (100)

N.D.; Not determined.

The percentage of pesticides out of the criteria (recovery 30-70% or 120-140%) but still within RSD ≤20% were 5.2 to 14.1% in all crops. The recoveries of these compounds are consistent (European Commission, 2017), so still acceptable for a screening purpose. The remaining of pesticides (0.5-2.1%; recovery <30% or 140%, or RSD >20%) requires alternative ways to correct recoveries such as employment of internal standard calibration (Nguyen et al., 2008) or isotope dilution (Bravo et al., 2002; Focant et al., 2004).

For more detailed information, recovery results were divided by pesticide activities. **Fig. 32** showed the percentages of pesticides which fell recovery 70-120% (RSD ≤20%) by six groups of activities in pepper, orange, brown rice, and soybean. As a result, 75-100% of target pesticides satisfied the criteria in insecticide, fungicide, herbicide, inter-activity, and others. Plant growth regulator also showed the excellent percentages (83%) in pepper, orange, and brown rice, whereas half of the pesticides were not included in the criteria in soybean. These plant growth regulators (2-(1-naphthyl)acetamide, 2,6-diisopropylnaphthalene, and ethychlozate) can be acceptable for screening purpose due to the constant recovery results (RSD ≤5.0%).

Fig. 32. Percentages of pesticides satisfying recovery 70-120% (RSD ≤20%) classified by activity groups

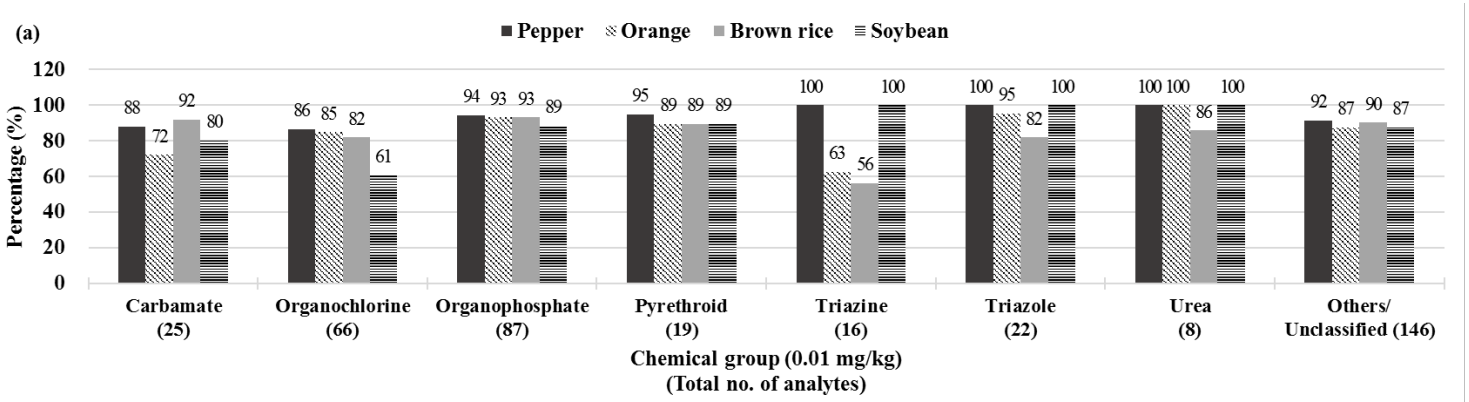

Pepper
Orange
Brown rice
Soybean
Percentage (%)
120
100
80
60
40
20
0
91 90 90 83
91 93 93 82
91 84 80 80
91 83 80 81
96 87 90 92
95 86 91 92
83 83 83 50
83 83 83 50
85 85 85 77
92 92 85 85
88 75 88 88
88 88 100 88
0.01 mg/kg
0.05 mg/kg
Insecticide (163)
Fungicide (86)
Herbicide (108)
Plant growth regulator (6)
Inter-activity (13)
Others (8)
Activity group
(Total no. of analytes)

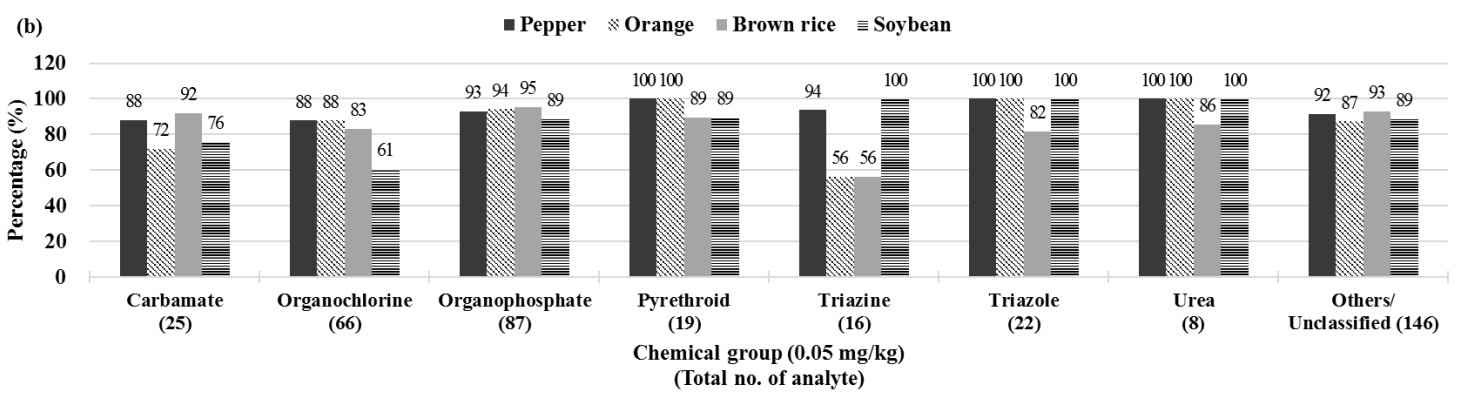
When the recovery results were classified as chemical group covering the recovery 70-120% (RSD ≤20%), six groups (carbamate, organophosphate, pyrethroid, triazole, urea, and others/unclassified) showed relatively excellent percentage ranges from 72% to 100% at treated levels of 0.01 and 0.05 mg/kg. (**Fig. 33**) Organochlorine pesticides also indicated high percentages from target pesticides in pepper, orange, and brown rice (82-88%), but showed the low percentage (61%) in soybean at both treated level. The organochlorines out of the recovery criteria only in soybean was non-polar pesticides such as aldrin, DDE (both of o,p'- and p,p'-), heptachlor, cis-nonachlor, pentachloroaniline, pentachlorothioanisole, quintozene, and tecnazene. The issues were not caused by cleanup steps but by extraction steps. The fat content of soybean is 20% (20 g/100 g total weight) of the total (USDA). It is possible that some of the non-polar pesticides were strongly adsorbed on by non-polar fat, thus there pesticides were not extracted sufficiently with acetonitrile. This problem can be solved by separating the pesticides from the fat by freezing. In the established method using freezing extraction and florisil dispersive-SPE (dSPE), 95 pesticides covering the problematic pesticides in our study obtained acceptable recoveries in soybean oil (Nguyen et al., 2010). The percentages of triazine pesticides were accounted for 56-63% in orange and brown rice. Because some of triazines in brown rice were rejected to be determined by severe peak broadening, thus this contributed to the low percentages. Further investigation is needed for problematic triazines in orange because there was no problem with validation parameters such as LOQ, linearity of calibration, and repeatability. These pesticides showed excellent recoveries with the same crop in Lee et al.

(2017)’s report using acidified extraction solvent (0.1% formic acid in acetonitrile) and PSA dSPE (Lee et al., 2017).

In conclusion, although the sample amount and extraction solvent volumes were reduced from the original method, this analytical method is still valid for determining most of the pesticide in representative crops and applicable for 93.8-99.0% of the total pesticides for screening purpose. For a few pesticides with recovery problems in some crops, alternative methods will be useful.

Fig. 33. Percentages of pesticides satisfying recovery 70-120% (RSD ≤20%) classified by chemical groups at (a) 0.01 mg/kg and (b) 0.05 mg/kg


(a)
Pepper
Orange
Brown rice
Soybean
Percentage (%)
120
100
80
60
40
20
0
88 72 92 80
86 85 82 61
94 93 93 89
95 89 89 89
100 63 56 100
100 95 82 100
100 100 86 100
92 87 90 87
Carbamate (25)
Organochlorine (66)
Organophosphate (87)
Pyrethroid (19)
Triazine (16)
Triazole (22)
Urea (8)
Others/ Unclassified (146)
Chemical group (0.01 mg/kg)
(Total no. of analytes)


(b)
Pepper
Orange
Brown rice
Soybean
Percentage (%)
120
100
80
60
40
20
0
88 72 92 76
88 88 83 61
93 94 95 89
100 100 89 89
94 56 56 100
100 100 82 100
100 100 86 100
92 87 93 89
Carbamate (25)
Organochlorine (66)
Organophosphate (87)
Pyrethroid (19)
Triazine (16)
Triazole (22)
Urea (8)
Others/ Unclassified (146)
Chemical group (0.05 mg/kg)
(Total no. of analyte)

Matrix effect

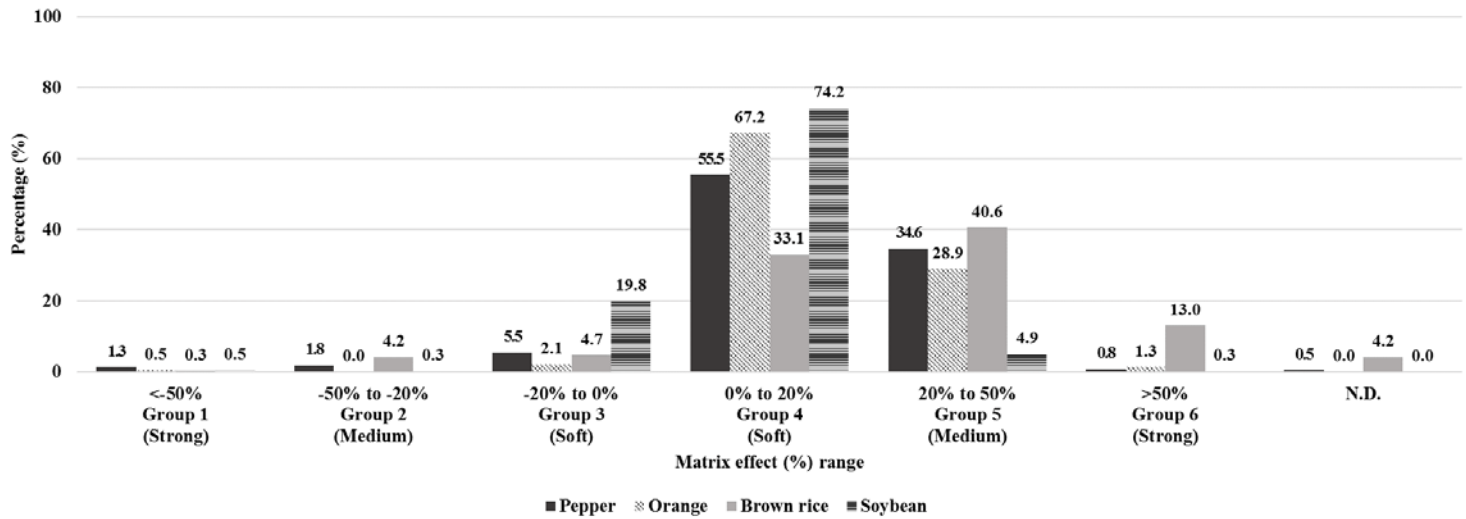
The matrix effect when analyzing pesticides using GC is a common phenomenon (Hajšlová and Zrostlíková, 2003). Erney et al. (1993) did first discuss matrix-induced signal enhancement of organophosphate pesticides on GC (Erney et al., 1993). One of the causes inducing matrix effects in GC is a masking effect in which matrices and target analytes competitively interact with active sites of a liner during injection (Hajšlová and Zrostlíková, 2003). Many works of literature have reported the signal enhancement by crop matrices on GC-MS or GC-MS/MS (Schenck and Lehotay, 2000; Lehotay et al., 2010; He et al., 2015; Lee et al., 2017).

The averages of matrix effects for target pesticides were 14.4%, 17.0%, 25.2%, and 5.9% in pepper, orange, brown rice, and soybean, respectively. Signals of most of the pesticides were enhanced by matrices in all crops. Brown rice and orange matrices gave the greatest and second greatest influence between the crops, respectively, but showed lower matrix effects than those in other multiresidue works (He et al., 2015; Lee et al., 2017). Matrix effects distribution classified as six group ranges (**Fig. 34**) indicated that most of the pesticides were included in Group 4 (0% to 20% of matrix effect range) or Group 5 (20% to 50%), exhibiting soft to medium enhancement effects. Pesticides in the soft group (61.0%, 69.3%, 37.8%, and 94.0% of the total in each four crop) are not required to matrix-matching because matrix effect is negligible (Rajski et al., 2013; He et al., 2015). The medium (Group 2 and 4) and strong (Group 1 and 6) group should employ matrix-matching to correct quantitation on GC-MS/MS.

Conclusions

A simultaneous multiresidue method of 384 pesticides using solid-phase extraction (SPE) was validated in pepper, orange, brown rice, and soybean by gas chromatography-tandem mass spectrometry (GC-MS/MS). The MRM on GC-MS/MS was optimized with electron ionization (EI) mode. Among the total pesticides, 95.1-99.5% satisfied the MLOQ below than 0.01 mg/kg in all crops. This indicates that most of the pesticides are applicable in PLS in which a residue level should be under 0.01 mg/kg for the pesticide without MRL lists of each crop. The numbers of pesticides satisfying recovery range of 70-120% with relative standard deviation (RSD) ≤20% were 84.1-92.2% at 0.01 mg/kg and 84.4-92.2% at 0.05 mg/kg showing excellent trueness and precision in the analytical method. Furthermore, 93.8-99.0% of 384 pesticides (recovery 30-140% and RSD ≤20%) was applicable for multiresidue screening purpose. The average matrix effect values (%) for four crops were 5.9% to 25.2%, indicating that crop matrices caused a signal enhancement on GC-MS/MS. This established methods can be sufficiently applied for the rapid and sensitive monitoring of pesticide multiresidues in pepper, orange, brown rice, soybean, and their related crops.

Fig. 34. Distribution of matrix effects for 384 pesticides in pepper, orange, brown rice, and soybean. Group 3 and 4 are included in soft matrix effect, Group 2 and 5 in medium effect, and Group 1 and 6 in strong effect


100
80
60
40
20
0
Percentage (%)
1.3
0.5
0.3
0.5
1.8
0.0
4.2
0.3
5.5
2.1
4.7
19.8
55.5
67.2
33.1
74.2
34.6
28.9
40.6
4.9
0.8
1.3
13.0
0.3
0.5
0.0
4.2
0.0
<-50%
Group 1
(Strong)
-50% to -20%
Group 2
(Medium)
-20% to 0%
Group 3
(Soft)
0% to 20%
Group 4
(Soft)
20% to 50%
Group 5
(Medium)
>50%
Group 6
(Strong)
N.D.
Matrix effect (%) range
Pepper
Orange
Brown rice
Soybean

Supplementary Information

Table S1. The retention times (t_R), monoisotopic masses, quasi-molecular ion types, and MRM transitions of LC-MS/MS for the multiresidual pesticides

No.	Compound name	t_R (min)	Mono Isotopic mass	Quasi-molecular ion	Precursor ion > Product ion (CE, eV)	
					Quantification	Identification
1	2,4-D	4.56	220	$[M-H]^-$	219 > 161 (12)	219 > 125 (27)
2	Abamectin B1a	9.39	872	$[M+NH_4]^+$	890 > 305 (-28)	890 > 567 (-15)
3	Acephate	2.82	183	$[M+H]^+$	184 > 143 (-9)	184 > 49 (-21)
4	Acetamiprid	3.27	222	$[M+H]^+$	223 > 126 (-19)	223 > 56 (-16)
5	Acibenzolar-S-methyl	5.58	210	$[M+H]^+$	211 > 135 (-31)	211 > 91 (-20)
6	Aldicarb	3.70	190	$[M+NH_4]^+$	208 > 116 (-7)	208 > 89 (-16)
7	Allidochlor	3.82	173	$[M+H]^+$	174 > 98 (-13)	174 > 41 (-22)
8	Ametoctradin	7.37	275	$[M+H]^+$	276 > 176 (-37)	276 > 149 (-38)
9	Ametryn	5.05	227	$[M+H]^+$	228 > 186 (-15)	228 > 68 (-40)
10	Amisulbrom	7.67	465	$[M+H]^+$	466 > 227 (-22)	466 > 226 (-14)
11	Amitraz	8.91	293	$[M+H]^+$	294 > 163 (-16)	294 > 122 (-31)
12	Asulam	2.91	230	$[M+H]^+$	231 > 156 (-10)	231 > 92 (-23)
13	Atrazine	4.77	215	$[M+H]^+$	216 > 174 (-16)	216 > 68 (-35)
14	Azaconazole	4.83	299	$[M+H]^+$	300 > 159 (-29)	300 > 231 (-17)
15	Azamethiphos	3.87	324	$[M+H]^+$	325 > 182 (-18)	325 > 112 (-40)
16	Azimsulfuron	4.66	424	$[M+H]^+$	425 > 182 (-19)	425 > 139 (-39)
17	Azinphos-ethyl	6.12	345	$[M+H]^+$	346 > 132 (-15)	346 > 77 (-37)
18	Bendiocarb	4.02	223	$[M+H]^+$	224 > 167 (-9)	224 > 109 (-18)
19	Benfuracarb	7.83	410	$[M+H]^+$	411 > 195 (-25)	411 > 190 (-12)
20	Bensulfuron-methyl	5.00	410	$[M+H]^+$	411 > 149 (-20)	411 > 182 (-21)
21	Bensulide	6.48	397	$[M+H]^+$	398 > 158 (-24)	398 > 314 (-11)
22	Bentazone	3.81	240	$[M-H]^-$	239 > 132 (25)	239 > 133 (24)
23	Benthiavalicarb-isopropyl	5.76	381	$[M+H]^+$	382 > 180 (-29)	382 > 116 (-22)
24	Benzobicyclon	5.63	446	$[M+H]^+$	447 > 257 (-25)	447 > 229 (-38)
25	Benzoximate	7.21	363	$[M+H]^+$	364 > 199 (-12)	364 > 105 (-23)
26	Bifenazate	5.91	300	$[M+H]^+$	301 > 198 (-10)	301 > 170 (-20)
27	Boscalid	5.47	342	$[M+H]^+$	343 > 139 (-19)	343 > 112 (-40)
28	Bromacil	4.10	260	$[M+H]^+$	261 > 205 (-14)	261 > 188 (-28)
29	Bromoxynil	4.52	275	$[M-H]^-$	274 > 79 (27)	274 > 167 (32)
30	Bupirimate	6.23	316	$[M+H]^+$	317 > 166 (-26)	317 > 108 (-27)
31	Butafenacil	6.00	474	$[M+NH_4]^+$	492 > 331 (-22)	492 > 180 (-45)
32	Butocarboxim	3.64	190	$[M+NH_4]^+$	208 > 75 (-8)	208 > 47 (-30)
33	Cafenstrole	5.81	350	$[M+H]^+$	351 > 100 (-11)	351 > 72 (-29)
34	Carbaryl	4.22	201	$[M+H]^+$	202 > 145 (-10)	202 > 127 (-27)
35	Carbendazim	3.14	191	$[M+H]^+$	192 > 160 (-17)	192 > 132 (-29)

No.	Compound name	t_R (min)	Mono Isotopic mass	Quasi-molecular ion	Precursor ion > Product ion (CE, eV)	
					Quantification	Identification
36	Carbofuran	4.05	221	$[M+H]^+$	222 > 165 (-13)	222 > 123 (-22)
37	Carboxin	4.25	235	$[M+H]^+$	236 > 142 (-16)	236 > 87 (-25)
38	Carfentrazone-ethyl	6.63	411	$[M+NH_4]^+$	429 > 412 (-12)	429 > 346 (-23)
39	Chlorantraniliprole	4.95	481	$[M+H]^+$	482 > 283 (-15)	482 > 450 (-17)
40	Chlorfenvinphos	6.89	358	$[M+H]^+$	359 > 155 (-13)	359 > 99 (-28)
41	Chloridazon	3.37	221	$[M+H]^+$	222 > 77 (-36)	222 > 104 (-23)
42	Chlorimuron-ethyl	5.58	414	$[M+H]^+$	415 > 186 (-19)	415 > 185 (-24)
43	Chlorotoluron	4.54	212	$[M+H]^+$	213 > 72 (-23)	213 > 46 (-15)
44	Chromafenozide	6.05	394	$[M+H]^+$	395 > 175 (-16)	395 > 339 (-8)
45	Cinmethylin	8.11	274	$[M+H]^+$	275 > 105 (-21)	275 > 153 (-7)
46	Clofentezine	7.22	302	$[M+H]^+$	303 > 138 (-14)	303 > 102 (-35)
47	Clomeprop	7.94	323	$[M+H]^+$	324 > 120 (-21)	324 > 203 (-16)
48	Cyanazine	3.84	240	$[M+H]^+$	241 > 214 (-17)	241 > 104 (-30)
49	Cyazofamid	6.20	324	$[M+H]^+$	325 > 108 (-14)	325 > 261 (-10)
50	Cycloate	7.43	215	$[M+H]^+$	216 > 54 (-38)	216 > 133 (-18)
51	Cycloprothrin	8.80	481	$[M+NH_4]^+$	499 > 257 (-15)	499 > 181 (-36)
52	Cyclosulfamuron	5.95	421	$[M+H]^+$	422 > 261 (-17)	422 > 217 (-27)
53	Cymoxanil	3.46	198	$[M+H]^+$	199 > 128 (-7)	199 > 111 (-17)
54	Cyromazine	2.71	166	$[M+H]^+$	167 > 85 (-20)	167 > 60 (-21)
55	Deltamethrin	9.05	505	$[M+NH_4]^+$	523 > 506 (-12)	523 > 281 (-18)
56	Demeton-S-Methyl	4.11	230	$[M+H]^+$	231 > 89 (-8)	231 > 61 (-31)
57	Diafenthiuron	8.83	384	$[M+H]^+$	385 > 329 (-20)	385 > 278 (-34)
58	Dicrotophos	3.09	237	$[M+H]^+$	238 > 112 (-12)	238 > 193 (-9)
59	Diethofencarb	5.29	267	$[M+H]^+$	268 > 226 (-9)	268 > 124 (-32)
60	Diflubenzuron	6.35	310	$[M+H]^+$	311 > 158 (-14)	311 > 141 (-30)
61	Diflufenican	7.55	394	$[M+H]^+$	395 > 266 (-25)	395 > 246 (-35)
62	Dimethachlor	4.98	255	$[M+H]^+$	256 > 223 (-14)	256 > 148 (-27)
63	Dimethoate	3.33	229	$[M+H]^+$	230 > 199 (-9)	230 > 125 (-20)
64	Dimethomorph	5.61	387	$[M+H]^+$	388 > 301 (-21)	388 > 165 (-32)
65	Dimethylvinphos	5.87	332	$[M+H]^+$	333 > 127 (-13)	333 > 207 (-19)
66	Diuron	4.85	232	$[M+H]^+$	233 > 72 (-21)	233 > 160 (-25)
67	Emamectin B1a	7.99	886	$[M+H]^+$	886 > 158 (-42)	886 > 82 (-55)
68	Emamectin B1b	7.70	872	$[M+H]^+$	872 > 158 (-36)	872 > 82 (-55)
69	Ethaboxam	4.39	320	$[M+H]^+$	321 > 183 (-23)	321 > 200 (-26)
70	Ethametsulfuron-methyl	4.27	410	$[M+H]^+$	411 > 196 (-19)	411 > 168 (-31)
71	Ethiofencarb	4.39	225	$[M+H]^+$	226 > 107 (-14)	226 > 77 (-43)
72	Ethoxyquin	5.20	217	$[M+H]^+$	218 > 148 (-22)	218 > 174 (-29)
73	Ethoxysulfuron	5.69	398	$[M+H]^+$	399 > 261 (-16)	399 > 218 (-25)
74	Etofenprox	9.83	376	$[M+NH_4]^+$	394 > 177 (-14)	394 > 359 (-12)
75	Famoxadone	6.89	374	$[M+NH_4]^+$	392 > 331 (-11)	392 > 238 (-18)
76	Fenhexamid	6.02	301	$[M+H]^+$	302 > 97 (-25)	302 > 55 (-40)
77	Fenobucarb	5.26	207	$[M+H]^+$	208 > 95 (-14)	208 > 152 (-8)
78	Fenoxaprop-P-ethyl	7.75	361	$[M+H]^+$	362 > 288 (-18)	362 > 121 (-28)
79	Fenoxycarb	6.47	301	$[M+H]^+$	302 > 88 (-21)	302 > 116 (-12)

No.	Compound name	t_R (min)	Mono Isotopic mass	Quasi-molecular ion	Precursor ion > Product ion (CE, eV)	
					Quantification	Identification
80	Fenpyroximate	8.85	421	$[M+H]^+$	422 > 366 (-17)	422 > 138 (-32)
81	Fentrazamide	6.77	349	$[M+H]^+$	350 > 197 (-8)	350 > 83 (-22)
82	Ferimzone	5.24	254	$[M+H]^+$	255 > 91 (-33)	255 > 132 (-19)
83	Flonicamid	3.05	229	$[M-H]^-$	228 > 81 (10)	228 > 146 (20)
84	Fluacrypyrim	7.43	426	$[M+H]^+$	427 > 205 (-11)	427 > 145 (-25)
85	Fluazinam	8.14	464	$[M-H]^-$	463 > 416 (19)	463 > 398 (17)
86	Flubendiamide	6.58	682	$[M+H]^+$	683 > 408 (-9)	683 > 273 (-32)
87	Flufenacet	6.15	363	$[M+H]^+$	364 > 151 (-21)	364 > 194 (-12)
88	Flufenoxuron	8.57	488	$[M+H]^+$	489 > 158 (-19)	489 > 141 (-45)
89	Flumiclorac-pentyl	7.95	423	$[M+NH_4]^+$	441 > 308 (-23)	441 > 354 (-15)
90	Flumioxazin	4.96	354	$[M+H]^+$	355 > 327 (-14)	355 > 299 (-27)
91	Fluopicolide	5.70	382	$[M+H]^+$	383 > 173 (-22)	383 > 145 (-50)
92	Fluopyram	5.99	396	$[M+H]^+$	397 > 173 (-28)	397 > 208 (-22)
93	Fluquinconazole	5.96	375	$[M+H]^+$	376 > 349 (-19)	376 > 307 (-26)
94	Flusulfamide	7.04	414	$[M-H]^-$	413 > 171 (38)	413 > 349 (23)
95	Fluvalinate	9.36	502	$[M+H]^+$	503 > 181 (-29)	503 > 208 (-13)
96	Fonofos	6.88	246	$[M+H]^+$	247 > 109 (-20)	247 > 137 (-11)
97	Forchlorfenuron	4.75	247	$[M+H]^+$	248 > 129 (-15)	248 > 93 (-33)
98	Furathiocarb	7.93	382	$[M+H]^+$	383 > 194 (-16)	383 > 251 (-16)
99	Halosulfuron-methyl	6.01	434	$[M+H]^+$	435 > 182 (-20)	435 > 139 (-45)
100	Haloxyfop	6.55	361	$[M+H]^+$	362 > 316 (-17)	362 > 91 (-32)
101	Hexaflumuron	7.56	460	$[M+H]^+$	461 > 158 (-16)	461 > 141 (-41)
102	Hexazinone	4.08	252	$[M+H]^+$	253 > 170 (-15)	253 > 71 (-35)
103	Hexythiazox	8.38	352	$[M+H]^+$	353 > 228 (-16)	353 > 168 (-24)
104	Imazamox	3.37	305	$[M+H]^+$	306 > 261 (-22)	306 > 193 (-27)
105	Imazapic	3.44	275	$[M+H]^+$	276 > 231 (-21)	276 > 163 (-27)
106	Imazaquin	3.99	311	$[M+H]^+$	312 > 267 (-21)	312 > 199 (-29)
107	Imazethapyr	3.77	289	$[M+H]^+$	290 > 245 (-21)	290 > 177 (-28)
108	Imazosulfuron	5.53	412	$[M+H]^+$	413 > 156 (-21)	413 > 153 (-14)
109	Imibenconazole	8.07	410	$[M+H]^+$	411 > 125 (-29)	411 > 171 (-20)
110	Imicyafos	3.72	304	$[M+H]^+$	305 > 201 (-21)	305 > 235 (-17)
111	Iprovalicarb	6.06	320	$[M+H]^+$	321 > 119 (-15)	321 > 203 (-9)
112	Isofenphos-methyl	6.67	331	$[M+H]^+$	332 > 230 (-15)	332 > 121 (-33)
113	Isoprocarb	4.64	193	$[M+H]^+$	194 > 95 (-15)	194 > 137 (-9)
114	Isoproturon	4.76	206	$[M+H]^+$	207 > 72 (-16)	207 > 46 (-16)
115	Isopyrazam	7.36	359	$[M+H]^+$	360 > 244 (-24)	360 > 340 (-15)
116	Isoxathion	7.12	313	$[M+H]^+$	314 > 105 (-17)	314 > 286 (-9)
117	Lactofen	8.00	461	$[M+NH_4]^+$	479 > 344 (-15)	479 > 223 (-35)
118	Lepimectin A3	9.27	705	$[M+H]^+$	706 > 153 (-17)	706 > 688 (-11)
119	Lepimectin A4	9.58	719	$[M+H]^+$	720 > 167 (-17)	720 > 702 (-11)
120	Linuron	5.37	248	$[M+H]^+$	249 > 182 (-14)	249 > 160 (-19)
121	Mandipropamid	5.51	411	$[M+H]^+$	412 > 328 (-15)	412 > 125 (-33)
122	Mefenpyr-diethyl	7.00	372	$[M+H]^+$	373 > 327 (-13)	373 > 160 (-33)
123	Mepanipyrim	6.12	223	$[M+H]^+$	224 > 77 (-41)	224 > 106 (-26)

No.	Compound name	t_R (min)	Mono Isotopic mass	Quasi-molecular ion	Precursor ion > Product ion (CE, eV)	
					Quantification	Identification
124	Metalaxyl	4.72	279	$[M+H]^+$	280 > 220 (-14)	280 > 192 (-19)
125	Metamifop	7.81	440	$[M+H]^+$	441 > 288 (-19)	441 > 123 (-29)
126	Metazosulfuron	5.32	475	$[M+H]^+$	476 > 182 (-21)	476 > 156 (-21)
127	Methiocarb	5.43	225	$[M+H]^+$	226 > 169 (-10)	226 > 121 (-19)
128	Methomyl	3.04	162	$[M+H]^+$	163 > 88 (-9)	163 > 106 (-10)
129	Methoxyfenozide	5.75	368	$[M+H]^+$	369 > 149 (-19)	369 > 313 (-8)
130	Metolachlor	6.33	283	$[M+H]^+$	284 > 252 (-15)	284 > 176 (-27)
131	Metolcarb	3.86	165	$[M+H]^+$	166 > 109 (-11)	166 > 94 (-32)
132	Metrafenone	7.20	408	$[M+H]^+$	409 > 209 (-15)	409 > 227 (-19)
133	Milbemectin A3	9.29	528	$[M+H-H_2O]^+$	511 > 95 (-32)	511 > 113 (-19)
134	Nicosulfuron	3.84	410	$[M+H]^+$	411 > 182 (-21)	411 > 213 (-18)
135	Novaluron	7.66	492	$[M+H]^+$	493 > 158 (-20)	493 > 141 (-41)
136	Omethoate	2.88	213	$[M+H]^+$	214 > 183 (-11)	214 > 125 (-21)
137	Oxamyl	2.95	219	$[M+NH_4]^+$	237 > 72 (-15)	237 > 90 (-8)
138	Oxaziclomefone	7.84	375	$[M+H]^+$	376 > 190 (-16)	376 > 161 (-29)
139	Pebulate	7.34	203	$[M+H]^+$	204 > 57 (-17)	204 > 128 (-10)
140	Pencycuron	7.26	328	$[M+H]^+$	329 > 125 (-28)	329 > 218 (-15)
141	Penoxsulam	4.18	483	$[M+H]^+$	484 > 195 (-31)	484 > 194 (-40)
142	Pentoxazone	7.85	353	$[M+NH_4]^+$	371 > 286 (-17)	371 > 354 (-9)
143	Phosmet	5.10	317	$[M+H]^+$	318 > 160 (-14)	318 > 77 (-53)
144	Phoxim	7.10	298	$[M+H]^+$	299 > 77 (-29)	299 > 129 (-10)
145	Picolinafen	8.10	376	$[M+H]^+$	377 > 238 (-26)	377 > 359 (-20)
146	Picoxystrobin	6.51	367	$[M+H]^+$	368 > 145 (-21)	368 > 205 (-10)
147	Pirimicarb	4.10	238	$[M+H]^+$	239 > 72 (-30)	239 > 182 (-13)
148	Promecarb	5.62	207	$[M+H]^+$	208 > 109 (-15)	208 > 151 (-9)
149	Propachlor	4.76	211	$[M+H]^+$	212 > 170 (-15)	212 > 94 (-27)
150	Propamocarb	2.89	188	$[M+H]^+$	189 > 102 (-20)	189 > 74 (-26)
151	Propaquizafop	8.00	443	$[M+H]^+$	444 > 100 (-20)	444 > 56 (-25)
152	Propazine	5.45	229	$[M+H]^+$	230 > 146 (-22)	230 > 188 (-16)
153	Propham	4.64	179	$[M+H]^+$	180 > 138 (-9)	180 > 120 (-14)
154	Propisochlor	6.82	283	$[M+H]^+$	284 > 224 (-10)	284 > 148 (-20)
155	Propoxur	4.02	209	$[M+H]^+$	210 > 111 (-13)	210 > 168 (-7)
156	Propyzamide	5.77	255	$[M+H]^+$	256 > 190 (-14)	256 > 173 (-21)
157	Pymetrozine	2.86	217	$[M+H]^+$	218 > 105 (-20)	218 > 78 (-43)
158	Pyraclostrobin	7.03	387	$[M+H]^+$	388 > 194 (-12)	388 > 163 (-25)
159	Pyrazolynate	7.20	438	$[M+H]^+$	439 > 91 (-35)	439 > 173 (-19)
160	Pyribenzoxim	8.04	609	$[M+H]^+$	610 > 413 (-12)	610 > 180 (-21)
161	Pyributicarb	8.29	330	$[M+H]^+$	331 > 181 (-16)	331 > 108 (-29)
162	Pyridate	9.52	378	$[M+H]^+$	379 > 207 (-18)	379 > 351 (-11)
163	Pyrifenox	6.07	294	$[M+H]^+$	295 > 93 (-22)	295 > 67 (-54)
164	Pyrimethanil	5.33	199	$[M+H]^+$	200 > 107 (-24)	200 > 82 (-26)
165	Pyrimisulfan	4.83	419	$[M+H]^+$	420 > 370 (-20)	420 > 255 (-28)
166	Pyriproxyfen	8.27	321	$[M+H]^+$	322 > 96 (-14)	322 > 78 (-51)
167	Pyroquilon	3.97	173	$[M+H]^+$	174 > 132 (-22)	174 > 117 (-33)

No.	Compound name	t_R (min)	Mono Isotopic mass	Quasi-molecular ion	Precursor ion > Product ion (CE, eV)	
					Quantification	Identification
168	Quinalphos	6.66	298	$[M+H]^+$	299 > 97 (-31)	399 > 163 (-23)
169	Quinmerac	3.42	221	$[M+H]^+$	222 > 204 (-14)	222 > 141 (-33)
170	Quinoclamine	3.94	207	$[M+H]^+$	208 > 105 (-25)	208 > 77 (-39)
171	Quizalofop-ethyl	7.80	372	$[M+H]^+$	373 > 299 (-18)	373 > 91 (-31)
172	Rimsulfuron	4.15	431	$[M+H]^+$	432 > 182 (-20)	432 > 325 (-15)
173	Saflufenacil	5.06	500	$[M+H]^+$	501 > 198 (-44)	501 > 349 (-26)
174	Sethoxydim	8.04	327	$[M+H]^+$	328 > 178 (-19)	328 > 282 (-12)
175	Simazine	4.16	201	$[M+H]^+$	202 > 132 (-19)	202 > 124 (-18)
176	Spinosyn A	6.89	731	$[M+H]^+$	732 > 142 (-32)	732 > 98 (-55)
177	Spinosyn D	7.32	745	$[M+H]^+$	746 > 142 (-32)	746 > 98 (-55)
178	Spirodiclofen	8.84	410	$[M+H]^+$	411 > 71 (-21)	411 > 313 (-12)
179	Spiromesifen	8.57	370	$[M+H]^+$	371 > 273 (-11)	371 > 255 (-23)
180	Sulfoxaflor	3.35	277	$[M+H]^+$	278 > 174 (-9)	278 > 154 (-27)
181	Sulprofos	8.46	322	$[M+H]^+$	323 > 219 (-16)	323 > 247 (-12)
182	TCMTB	5.37	238	$[M+H]^+$	239 > 180 (-12)	239 > 136 (27)
183	Tebufenozide	6.50	352	$[M+H]^+$	353 > 133 (-19)	353 > 297 (-9)
184	Teflubenzuron	8.09	380	$[M-H]^-$	379 > 339 (11)	379 > 359 (7)
185	Tetrachlorvinphos	6.51	366	$[M+H]^+$	367 > 127 (-15)	367 > 206 (-37)
186	Thenylchlor	6.13	323	$[M+H]^+$	324 > 127 (-14)	324 > 53 (-54)
187	Thiabendazole	3.32	201	$[M+H]^+$	202 > 175 (-23)	202 > 131 (-33)
188	Thiacloprid	3.41	252	$[M+H]^+$	253 > 126 (-20)	253 > 99 (-43)
189	Thidiazuron	3.99	220	$[M+H]^+$	221 > 102 (-15)	221 > 30 (-30)
190	Thifensulfuron-methyl	3.81	387	$[M+H]^+$	388 > 167 (-16)	388 > 205 (-26)
191	Thiodicarb	4.29	354	$[M+H]^+$	355 > 88 (-16)	355 > 108 (-16)
192	Thiometon	4.57	246	$[M+H]^+$	247 > 89 (-10)	247 > 61 (-31)
193	Thiophanate-methyl	3.90	342	$[M+H]^+$	343 > 151 (-20)	343 > 311 (-11)
194	Tiadinil	5.87	269	$[M+H]^+$	270 > 101 (-20)	270 > 103 (-20)
195	Tolfenpyrad	8.08	383	$[M+H]^+$	384 > 197 (-26)	384 > 154 (-43)
196	Tribenuron-methyl	4.56	395	$[M+H]^+$	396 > 155 (-14)	396 > 181 (-20)
197	Tribufos	9.17	314	$[M+H]^+$	315 > 57 (-25)	315 > 169 (-16)
198	Tricyclazole	3.58	189	$[M+H]^+$	190 > 163 (-20)	190 > 136 (-26)
199	Trifloxystrobin	7.52	408	$[M+H]^+$	409 > 186 (-19)	409 > 145 (-43)
200	Trimethacarb	4.84	193	$[M+H]^+$	194 > 137 (-11)	194 > 122 (-25)
201	Triticonazole	6.07	317	$[M+H]^+$	318 > 70 (-17)	318 > 125 (-32)
202	Uniconazole	5.95	291	$[M+H]^+$	292 > 70 (-24)	292 > 125 (-30)
203	Vamidothion	3.24	287	$[M+H]^+$	288 > 146 (-14)	288 > 58 (-40)
204	Vernolate	7.33	203	$[M+H]^+$	204 > 128 (-10)	204 > 43 (-19)
205	XMC	4.43	179	$[M+H]^+$	180 > 123 (-10)	180 > 108 (-19)

Table S2. The optimized GC-MS/MS parameters including retention times (t_R) and MRM transitions for each pesticide

No.	Name	t_R (min)	Precursor ion > Product ion (CE, eV)	
			Quantification	Identification
1-1	Acrinathrin_1	28.03	181 > 152 (-30)	208 > 181 (-15)
1-2	Acrinathrin_2	28.38	181 > 152 (-30)	208 > 181 (-15)
2	Alachlor	16.57	188 > 160 (-10)	160 > 130 (-25)
3	Aldrin	18.02	263 > 193 (-30)	293 > 220 (-30)
4	Anilofos	26.62	226 > 157 (-15)	184 > 157 (-10)
5	Azinphos-methyl	28.47	132 > 77 (-15)	160 > 77 (-15)
6	Azoxystrobin	34.41	344 > 329 (-15)	388 > 300 (-15)
7	BHC-alpha	12.81	181 > 145 (-15)	219 > 183 (-10)
8	BHC-beta	13.54	181 > 145 (-15)	219 > 183 (-15)
9	BHC-delta	14.77	181 > 145 (-15)	219 > 183 (-10)
10	BHC-gamma	13.83	181 > 145 (-15)	219 > 183 (-10)
11	Bifenox	26.68	341 > 310 (-10)	343 > 312 (-10)
12	Bifenthrin	26.11	181 > 165 (-20)	181 > 166 (-15)
13	Bromobutide	16.30	119 > 91 (-10)	232 > 176 (-10)
14	Bromophos	18.68	331 > 316 (-15)	331 > 286 (-25)
15	Bromopropylate	26.13	183 > 155 (-15)	341 > 185 (-20)
16	Buprofezin	21.75	105 > 104 (-10)	105 > 77 (-15)
17	Butachlor	20.52	160 > 130 (-25)	176 > 147 (-15)
18	Cadusafos	13.08	159 > 97 (-20)	158 > 97 (-15)
19	Captan	19.64	79 > 77 (-10)	151 > 79 (-10)
20	Carbophenothion	23.90	199 > 143 (-10)	342 > 157 (-5)
21	Chinomethionat	20.25	234 > 206 (-10)	206 > 148 (-20)
22	Chlordane-cis	20.21	373 > 266 (-20)	375 > 266 (-20)
23	Chlordane-trans	20.66	373 > 266 (-20)	375 > 266 (-20)
24	Chlorfenapyr	22.10	59 > 31 (-5)	59 > 29 (-10)
25	Chlorfluazuron	20.83	321 > 304 (-30)	323 > 306 (-20)
26	Chlorobenzilate	22.66	251 > 139 (-15)	139 > 111 (-15)
27	Chlorothalonil	15.45	264 > 168 (-30)	266 > 231 (-20)
28	Chlorpropham	12.67	213 > 127 (-10)	127 > 65 (-20)
29	Chlorpyrifos	17.93	314 > 258 (-10)	316 > 260 (-10)
30	Chlorpyrifos-methyl	16.31	286 > 93 (-20)	288 > 93 (-25)
31	Chlorthal-dimethyl	18.10	301 > 223 (-20)	332 > 301 (-20)

No.	Name	t_R (min)	Precursor ion > Product ion (CE, eV)	
			Quantification	Identification
32	Clomazone	14.22	125 > 289 (-15)	204 > 107 (-20)
33	Cyanophos	14.07	243 > 109 (-10)	109 > 79 (-10)
34	Cyflufenamid	22.12	91 > 65 (-15)	412 > 295 (-10)
35-1	Cyfluthrin_1	30.02	163 > 127 (-10)	163 > 91 (-20)
35-2	Cyfluthrin_2	30.14	163 > 127 (-10)	163 > 91 (-20)
35-3	Cyfluthrin_3	30.21	163 > 127 (-10)	163 > 91 (-20)
35-4	Cyfluthrin_4	30.27	163 > 127 (-10)	163 > 91 (-20)
36	Cyhalofop-butyl	27.72	357 > 256 (-10)	229 > 109 (-20)
37	Cyhalothrin-lambda	28.03	181 > 152 (-25)	197 > 141 (-15)
38-1	Cypermethrin_1	30.41	163 > 127 (-5)	181 > 152 (-20)
38-2	Cypermethrin_2	30.52	163 > 127 (-5)	181 > 152 (-20)
38-3	Cypermethrin_3	30.58	163 > 127 (-5)	181 > 152 (-20)
38-4	Cypermethrin_4	30.64	163 > 127 (-5)	181 > 152 (-20)
39	Cyproconazole	22.20	222 > 125 (-20)	139 > 111 (-20)
40	Cyprodinil	19.13	225 > 224 (-10)	224 > 208 (-20)
41	Daimuron	5.51	146 > 77 (-15)	146 > 105 (-10)
42	DDD-o,p'	21.71	235 > 165 (-20)	237 > 165 (-20)
43	DDD-p,p'	23.04	235 > 165 (-20)	237 > 165 (-20)
44	DDE-o,p'	20.32	246 > 176 (-35)	318 > 248 (-15)
45	DDE-p,p'	21.48	246 > 176 (-35)	318 > 248 (-15)
46	DDT-o,p'	22.72	235 > 165 (-20)	237 > 165 (-20)
47	DDT-p,p'	23.99	235 > 165 (-20)	237 > 165 (-20)
48	Di-allate	13.21	234 > 150 (-20)	234 > 192 (-15)
49	Diazinon	14.28	199 > 93 (-15)	179 > 122 (-25)
50	Dichlofluanid	17.60	224 > 123 (-10)	167 > 124 (-15)
51	Dichlorvos	7.59	109 > 79 (-5)	185 > 127 (-30)
52	Diclofop-methyl	24.89	340 > 253 (-15)	253 > 162 (-10)
53	Dicloran	13.79	206 > 176 (-10)	176 > 148 (-15)
54	Dicofol	18.46	139 > 111 (-15)	139 > 75 (-30)
55	Dieldrin	21.61	263 > 193 (-25)	279 > 207 (-25)
56-1	Difenoconazole_1	32.04	323 > 265 (-20)	323 > 202 (-25)
56-2	Difenoconazole_2	32.13	323 > 265 (-20)	323 > 202 (-25)
57	Dimepiperate	19.76	119 > 91 (-15)	145 > 112 (-10)
58	Dimethametryn	19.32	212 > 122 (-15)	212 > 94 (-25)
59	Dimethenamid	14.24	230 > 154 (-15)	154 > 111 (-15)
60	Diniconazole	22.82	268 > 232 (-15)	270 > 232 (-15)

No.	Name	t_R (min)	Precursor ion > Product ion (CE, eV)	
			Quantification	Identification
61	Diphenamid	18.69	167 > 165 (-25)	239 > 167 (-10)
62	Diphenylamine	12.24	168 > 167 (-20)	169 > 168 (-20)
63	Disulfoton	15.09	88 > 60 (-10)	186 > 97 (-20)
64	Dithiopyr	17.01	354 > 286 (-15)	306 > 286 (-10)
65	Edifenphos	23.96	173 > 109 (-10)	310 > 173 (-15)
66	Endosulfan-alpha	20.66	241 > 206 (-20)	195 > 125 (-25)
67	Endosulfan-beta	22.73	241 > 206 (-15)	195 > 159 (-10)
68	Endosulfan-sulfate	24.09	272 > 237 (-15)	237 > 143 (-30)
69	Endrin	21.98	263 > 193 (-30)	281 > 245 (-10)
70	EPN	26.05	169 > 141 (-10)	157 > 110 (-15)
71	Esprocarb	17.65	222 > 91 (-15)	162 > 91 (-15)
72	Ethalfluralin	12.49	316 > 276 (-5)	333 > 276 (-15)
73	Ethion	22.96	231 > 129 (-20)	231 > 175 (-15)
74	Ethoprophos	11.63	158 > 97 (-20)	200 > 158 (-5)
75	Etoxazole	26.39	300 > 270 (-20)	359 > 187 (-25)
76	Etridiazole	9.85	211 > 183 (-10)	183 > 140 (-15)
77	Etrimfos	15.29	292 > 153 (-20)	277 > 125 (-15)
78	Fenamidone	26.45	268 > 180 (-25)	238 > 103 (-20)
79	Fenamiphos	20.91	303 > 195 (-10)	288 > 260 (-5)
80	Fenarimol	26.44	139 > 111 (-15)	251 > 139 (-15)
81	Fenazaquin	26.72	145 > 117 (-15)	160 > 145 (-10)
82	Fenbuconazole	29.95	129 > 102 (-15)	198 > 129 (-10)
83	Fenitrothion	17.37	277 > 260 (-10)	260 > 125 (-15)
84	Fenothiocarb	20.54	160 > 72 (-15)	160 > 106 (-10)
85	Fenpropathrin	26.44	181 > 152 (-25)	181 > 127 (-30)
86	Fenthion	18.06	278 > 109 (-15)	169 > 121 (-15)
87-1	Fenvalerate_1	31.45	167 > 125 (-10)	225 > 119 (-20)
87-2	Fenvalerate_2	31.72	167 > 125 (-10)	225 > 119 (-20)
88	Fipronil	19.34	367 > 213 (-25)	369 > 215 (-30)
89-1	Flucythrinate_1	30.59	199 > 157 (-10)	199 > 107 (-20)
89-2	Flucythrinate_2	30.82	199 > 157 (-10)	199 > 107 (-20)
90	Fludioxonil	21.22	248 > 127 (-25)	182 > 127 (-15)
91	Flutolanil	21.08	173 > 145 (-15)	281 > 173 (-15)
92	Folpet	19.89	260 > 130 (-15)	262 > 130 (-20)
93-1	Fosthiazate_1	18.69	195 > 103 (-10)	195 > 139 (-5)
93-2	Fosthiazate_2	18.78	195 > 103 (-10)	195 > 139 (-5)

No.	Name	t_R (min)	Precursor ion > Product ion (CE, eV)	
			Quantification	Identification
94	Fthalide	18.52	243 > 215 (-20)	241 > 213 (-20)
95	Halfenprox	30.47	265 > 237 (-10)	265 > 117 (-10)
96	Heptachlor	16.80	272 > 237 (-15)	272 > 235 (-15)
97	Heptachlor-epoxide	19.37	353 > 263 (-15)	355 > 265 (-25)
98	Hexaconazole	21.12	214 > 172 (-20)	216 > 161 (-20)
99	Indanofan	26.54	139 > 111 (-20)	310 > 139 (-20)
100	Indoxacarb	33.46	218 > 203 (-10)	264 > 176 (-15)
101	Iprobenfos	15.58	204 > 91 (-10)	246 > 91 (-15)
102	Iprodione	25.38	314 > 245 (-15)	316 > 247 (-15)
103	Isazofos	15.13	161 > 119 (-25)	257 > 162 (-10)
104	Isofenphos	19.39	213 > 121 (-15)	255 > 121 (-25)
105	Isoprothiolane	21.21	231 > 189 (-10)	290 > 118 (-15)
106	Kresoxim-methyl	21.81	131 > 89 (-30)	206 > 131 (-10)
107	Lufenuron	9.39	353 > 203 (-15)	203 > 111 (-20)
108	Malathion	17.68	173 > 99 (-15)	158 > 125 (-10)
109	Mecarbam	19.53	131 > 74 (-20)	329 > 159 (-10)
110	Mefenacet	27.69	192 > 136 (-15)	192 > 109 (-25)
111	Mepronil	23.50	119 > 91 (-10)	269 > 119 (-20)
112	Metconazole	26.70	125 > 89 (-20)	125 > 99 (-15)
113	Methabenzthiazuron	12.18	164 > 136 (-15)	136 > 109 (-25)
114	Methidathion	20.12	145 > 85 (-5)	145 > 58 (-10)
115	Methoxychlor	26.32	227 > 169 (-25)	228 > 227 (-20)
116	Metobromuron	15.22	197 > 90 (-25)	199 > 171 (-15)
117	Metribuzin	15.84	198 > 82 (-25)	199 > 184 (-15)
118	Mevinphos	9.47	192 > 127 (-10)	127 > 95 (-15)
119	Molinate	11.05	126 > 55 (-10)	187 > 126 (-10)
120	Myclobutanil	21.63	179 > 125 (-15)	150 > 123 (-15)
121	Napropamide	20.95	128 > 72 (-10)	271 > 128 (-10)
122	Nitrapyrin	9.82	194 > 133 (-25)	194 > 158 (-20)
123	Nitrothal-isopropyl	18.51	236 > 194 (-15)	194 > 148 (-15)
124	Nuarimol	24.71	235 > 139 (-15)	314 > 139 (-15)
125	Ofurace	23.57	232 > 158 (-20)	281 > 158 (-5)
126	Oxadiazon	21.53	258 > 175 (-10)	258 > 112 (-25)
127	Oxadixyl	22.88	163 > 132 (-15)	163 > 117 (-25)
128	Oxyfluorfen	21.75	252 > 146 (-30)	361 > 300 (-15)
129	Paclobutrazol	20.43	236 > 125 (-10)	236 > 167 (-10)

No.	Name	t_R (min)	Precursor ion > Product ion (CE, eV)	
			Quantification	Identification
130	Parathion	18.18	291 > 109 (-15)	261 > 109 (-10)
131	Parathion-methyl	16.51	263 > 109 (-15)	263 > 246 (-5)
132	Penconazole	19.29	248 > 157 (-25)	159 > 123 (-20)
133	Pendimethalin	19.09	252 > 162 (-15)	281 > 252 (-5)
134	Pentachloroaniline	15.88	263 > 193 (-25)	265 > 193 (-25)
135	Pentachlorothioanisole	17.54	296 > 263 (-10)	296 > 281 (-15)
136	Penthiopyrad	22.88	302 > 177 (-5)	177 > 101 (-20)
137-1	Permethrin_1	29.28	183 > 153 (-15)	183 > 168 (-15)
137-2	Permethrin_2	29.47	183 > 153 (-15)	183 > 168 (-15)
138	Phenthoate	19.61	274 > 125 (-20)	246 > 121 (-10)
139	Phorate	13.22	231 > 175 (-10)	260 > 75 (-10)
140	Phosalone	27.24	182 > 111 (-20)	367 > 182 (-10)
141-1	Phosphamidon_1	14.75	127 > 109 (-15)	264 > 127 (-20)
141-2	Phosphamidon_2	16.01	127 > 109 (-15)	264 > 127 (-20)
142	Piperophos	26.13	320 > 122 (-15)	140 > 98 (-10)
143	Pirimiphos-ethyl	18.70	318 > 166 (-15)	333 > 180 (-15)
144	Pirimiphos-methyl	17.30	290 > 125 (-20)	305 > 180 (-10)
145-1	Probenazole_1	14.35	159 > 130 (-15)	130 > 103 (-20)
145-2	Probenazole_2	16.57	159 > 130 (-15)	130 > 103 (-20)
146	Prochloraz	29.54	180 > 138 (-15)	180 > 69 (-15)
147	Procymidone	19.79	283 > 96 (-10)	285 > 96 (-15)
148	Profenofos	21.31	339 > 269 (-15)	337 > 267 (-15)
149	Prometryn	16.93	241 > 184 (-10)	226 > 184 (-10)
150	Propanil	16.23	161 > 99 (-25)	163 > 101 (-20)
151-1	Propiconazole_1	24.05	173 > 145 (-20)	259 > 173 (-10)
151-2	Propiconazole_2	24.27	173 > 145 (-20)	259 > 173 (-10)
152	Prothiofos	21.17	309 > 239 (-15)	267 > 239 (-10)
153	Pyraclofos	28.76	194 > 138 (-20)	360 > 194 (-15)
154	Pyrazophos	28.31	221 > 193 (-10)	232 > 204 (-10)
155	Pyridaben	29.48	147 > 117 (-20)	147 > 132 (-15)
156	Pyridalyl	30.82	204 > 148 (-20)	164 > 146 (-15)
157	Pyridaphenthion	25.70	340 > 199 (-15)	199 > 77 (-20)
158	Pyrimidifen	31.24	184 > 169 (-20)	161 > 135 (-15)
159	Pyriminobac-methyl E	23.93	302 > 256 (-20)	302 > 230 (-20)
160	Pyriminobac-methyl Z	22.64	302 > 256 (-20)	256 > 188 (-20)
161	Quintozene	14.29	295 > 237 (-15)	237 > 143 (-25)

No.	Name	t_R (min)	Precursor ion > Product ion (CE, eV)	
			Quantification	Identification
162	Silafluofen	30.93	179 > 151 (-10)	179 > 169 (-10)
163	Simeconazole	16.55	211 > 121 (-15)	278 > 135 (-15)
164	Simetryn	16.69	213 > 170 (-10)	170 > 155 (-10)
165	Tebuconazole	24.79	250 > 125 (-25)	125 > 99 (-15)
166	Tebufenpyrad	26.63	333 > 171 (-25)	318 > 131 (-15)
167	Tebupirimfos	15.52	234 > 126 (-15)	276 > 234 (-10)
168	Tefluthrin	15.22	177 > 127 (-15)	197 > 141 (-10)
169	Terbufos	14.54	231 > 175 (-10)	288 > 231 (-5)
170	Terbuthylazine	14.24	214 > 104 (-20)	229 > 173 (-10)
171	Terbutryn	17.36	241 > 185 (-5)	226 > 96 (-25)
172	Tetraconazole	18.32	336 > 204 (-30)	338 > 206 (-30)
173	Tetradifon	27.03	159 > 131 (-10)	356 > 229 (-10)
174	Thiazopyr	17.76	327 > 277 (-25)	396 > 327 (-20)
175	Thifluzamide	21.61	194 > 166 (-10)	166 > 125 (-20)
176	Thiobencarb	17.96	100 > 72 (-5)	257 > 100 (-10)
177	Tolclofos-methyl	16.55	265 > 250 (-15)	250 > 220 (-15)
178	Tolylfluanid	19.39	238 > 137 (-15)	137 > 91 (-20)
179	Triadimefon	17.96	208 > 181 (-10)	181 > 99 (-20)
180	Triadimenol	19.80	168 > 70 (-10)	128 > 65 (-20)
181	Triazophos	23.50	161 > 134 (-10)	257 > 162 (-15)
182	Triflumizole	19.89	278 > 73 (-10)	287 > 218 (-15)
183	Triflumuron	8.32	139 > 111 (-15)	155 > 139 (-10)
184	Trifluralin	12.73	306 > 264 (-10)	264 > 160 (-20)
185	Vinclozolin	16.45	285 > 212 (-15)	287 > 214 (-10)
186-1	Zoxamide_1	25.05	187 > 159 (-15)	189 > 161 (-15)
186-2	Zoxamide_2	25.36	187 > 159 (-15)	189 > 161 (-15)

Table S3. List of general pesticide information for 384 pesticides

No.	Compound name	Activity group	Chemical group	Remarks
1	2-(1-naphthyl)acetamide	Plant growth regulator	Others/Unclassified	
2	2,6-Diisopropylnaphthalene	Plant growth regulator	Others/Unclassified	
3	2-phenylphenol	Fungicide	Others/Unclassified	Korea MRLs in food (2017)
4	Acetochlor	Herbicide	Others/Unclassified	Korea MRLs in food (2017)
5	Acibenzolar-S-methyl	Others	Others/Unclassified	Korea MRLs in food (2017)
6	Acrinathrin	Insecticide	Pyrethroid	Korea MRLs in food (2017)
7	Alachlor	Herbicide	Others/Unclassified	Korea MRLs in food (2017)
8	Alachlor-2-hydroxy	Herbicide	Others/Unclassified	Alachlor metabolite
9	Aldrin	Insecticide	Organochlorine	Korea MRLs in food (2017)
10	Allethrin	Insecticide	Pyrethroid	
11	Allidochlor	Herbicide	Others/Unclassified	
12	Ametryn	Herbicide	Triazine	
13	Anilofos	Herbicide	Organophosphate	Korea MRLs in food (2017)
14	Aramite	Insecticide	Others/Unclassified	
15	Aspon	Insecticide	Organophosphate	
16	Atrazine	Herbicide	Triazine	
17	Azaconazole	Fungicide	Triazole	
18	Azinphos-ethyl	Insecticide	Organophosphate	
19	Azinphos-methyl	Insecticide	Organophosphate	Korea MRLs in food (2017)
20	Benalaxyl	Fungicide	Others/Unclassified	Korea MRLs in food (2017)
21	Benfluralin	Herbicide	Others/Unclassified	
22	Benodanil	Fungicide	Others/Unclassified	
23	Benoxacor	Others	Others/Unclassified	

No.	Compound name	Activity group	Chemical group	Remarks
24	Benzoylprop-ethyl	Herbicide	Others/Unclassified	
25	BHC-alpha	Insecticide	Organochlorine	Korea MRLs in food (2017)
26	BHC-beta	Insecticide	Organochlorine	Korea MRLs in food (2017)
27	BHC-delta	Insecticide	Organochlorine	Korea MRLs in food (2017)
28	BHC-gamma	Insecticide	Organochlorine	Korea MRLs in food (2017)
29	Bifenox	Herbicide	Others/Unclassified	Korea MRLs in food (2017)
30	Bifenthrin	Insecticide	Pyrethroid	Korea MRLs in food (2017)
31	Binapacryl	Inter-activity	Others/Unclassified	
32	Biphenyl	Fungicide	Others/Unclassified	
33	Bromacil	Herbicide	Others/Unclassified	Korea MRLs in food (2017)
34	Bromobutide	Herbicide	Others/Unclassified	Korea MRLs in food (2017)
35	Bromophos	Insecticide	Organophosphate	
36	Bromophos-ethyl	Insecticide	Organophosphate	
37	Bromopropylate	Insecticide	Organochlorine	Korea MRLs in food (2017)
38	BTS 27919	Insecticide	Others/Unclassified	Amitraz metabolite
39	Bupirimate	Fungicide	Others/Unclassified	
40	Buprofezin	Insecticide	Others/Unclassified	Korea MRLs in food (2017)
41	Butachlor	Herbicide	Others/Unclassified	Korea MRLs in food (2017)
42	Butafenacil	Herbicide	Others/Unclassified	
43	Butralin	Inter-activity	Others/Unclassified	
44	Butylate	Herbicide	Carbamate	
45	Cadusafos	Insecticide	Organophosphate	Korea MRLs in food (2017)
46	Carbophenothion	Insecticide	Organophosphate	Korea MRLs in food (2017)
47	Carbosulfan	Insecticide	Carbamate	Korea MRLs in food (2017)
48	Chinomethionat	Inter-activity	Others/Unclassified	Korea MRLs in food (2017)

No.	Compound name	Activity group	Chemical group	Remarks
49	Chlorbenside	Insecticide	Organochlorine	
50	Chlorbufam	Herbicide	Carbamate	
51	Chlordane-cis	Insecticide	Organochlorine	Korea MRLs in food (2017)
52	Chlordane-trans	Insecticide	Organochlorine	Korea MRLs in food (2017)
53	Chlordimeform	Insecticide	Others/Unclassified	
54	Chlorethoxyfos	Insecticide	Organophosphate	
55	Chlorfenapyr	Insecticide	Organochlorine	Korea MRLs in food (2017)
56	Chlorfenson	Insecticide	Organochlorine	
57	Chlorfluazuron	Insecticide	Urea	Korea MRLs in food (2017)
58	Chlorflurenol-methyl	Inter-activity	Others/Unclassified	
59	Chloridazon	Herbicide	Organochlorine	
60	Chlormephos	Insecticide	Organophosphate	
61	Chlornitrofen	Herbicide	Organochlorine	
62	Chlorobenzilate	Insecticide	Organochlorine	Korea MRLs in food (2017)
63	Chloroneb	Fungicide	Organochlorine	
64	Chloropropylate	Insecticide	Organochlorine	
65	Chlorothalonil	Fungicide	Organochlorine	Korea MRLs in food (2017)
66	Chloroxuron	Herbicide	Urea	
67	Chlorpropham	Inter-activity	Carbamate	Korea MRLs in food (2017)
68	Chlorpyrifos	Insecticide	Organophosphate	Korea MRLs in food (2017)
69	Chlorpyrifos-methyl	Insecticide	Organophosphate	Korea MRLs in food (2017)
70	Chlorthal-dimethyl	Herbicide	Organochlorine	
71	Chlorthion	Insecticide	Organophosphate	
72	Chlorthiophos	Insecticide	Organophosphate	
73	Chlozolinate	Fungicide	Organochlorine	

No.	Compound name	Activity group	Chemical group	Remarks
74	Cinidon-ethyl	Herbicide	Others/Unclassified	
75	Cinmethylin	Herbicide	Others/Unclassified	
76	Clomazone	Herbicide	Others/Unclassified	Korea MRLs in food (2017)
77	Clomeprop	Herbicide	Others/Unclassified	
78	Coumaphos	Insecticide	Organophosphate	
79	Crotoxyphos	Insecticide	Organophosphate	
80	Crufomate	Insecticide	Organophosphate	
81	Cyanazine	Herbicide	Triazine	
82	Cyanofenphos	Insecticide	Organophosphate	
83	Cyanophos	Insecticide	Organophosphate	
84	Cycloate	Herbicide	Carbamate	
85	Cycloxydim	Herbicide	Others/Unclassified	
86	Cyenopyrafen	Insecticide	Others/Unclassified	Korea MRLs in food (2017)
87	Cyflufenamid	Fungicide	Others/Unclassified	Korea MRLs in food (2017)
88	Cyflumetofen	Insecticide	Others/Unclassified	Korea MRLs in food (2017)
89	Cyfluthrin	Insecticide	Pyrethroid	Korea MRLs in food (2017)
90	Cyhalofop-butyl	Herbicide	Others/Unclassified	Korea MRLs in food (2017)
91	Cyhalothrin-lambda	Insecticide	Pyrethroid	Korea MRLs in food (2017)
92	Cypermethrin	Insecticide	Pyrethroid	Korea MRLs in food (2017)
93	Cyprazine	Herbicide	Triazine	
94	Cyproconazole	Fungicide	Triazole	Korea MRLs in food (2017)
95	Cyprodinil	Fungicide	Others/Unclassified	Korea MRLs in food (2017)
96	DDD-o,p'	Insecticide	Organochlorine	DDT metabolite
97	DDD-p,p'	Insecticide	Organochlorine	DDT metabolite
98	DDE-o,p'	Insecticide	Organochlorine	DDT metabolite

No.	Compound name	Activity group	Chemical group	Remarks
99	DDE-p,p'	Insecticide	Organochlorine	DDT metabolite
100	DDT-o,p'	Insecticide	Organochlorine	Korea MRLs in food (2017)
101	DDT-p,p'	Insecticide	Organochlorine	Korea MRLs in food (2017)
102	Deltamethrin	Insecticide	Pyrethroid	Korea MRLs in food (2017)
103	Demeton-O	Insecticide	Organophosphate	
104	Demeton-S	Insecticide	Organophosphate	
105	Demeton-S-methyl-sulfone	Insecticide	Organophosphate	
106	Desmedipham	Herbicide	Carbamate	
107	Desmetryn	Herbicide	Triazine	
108	Dialifor	Insecticide	Organophosphate	
109	Di-allate	Herbicide	Carbamate	
110	Diazinon	Insecticide	Organophosphate	Korea MRLs in food (2017)
111	Dichlobenil	Herbicide	Organochlorine	Korea MRLs in food (2017)
112	Dichlofenthion	Insecticide	Organophosphate	
113	Dichlofluanid	Fungicide	Organochlorine	Korea MRLs in food (2017)
114	Dichlormid	Others	Others/Unclassified	
115	Dichlorvos	Insecticide	Organophosphate	Korea MRLs in food (2017)
116	Diclobutrazol	Fungicide	Triazole	
117	Diclofop-methyl	Herbicide	Organochlorine	Korea MRLs in food (2017)
118	Dicloran	Fungicide	Organochlorine	Korea MRLs in food (2017)
119	Dicofol	Insecticide	Organochlorine	Korea MRLs in food (2017)
120	Dieldrin	Insecticide	Organochlorine	Korea MRLs in food (2017)
121	Diethatyl-ethyl	Herbicide	Others/Unclassified	
122	Diethofencarb	Fungicide	Carbamate	Korea MRLs in food (2017)
123	Difenoconazole	Fungicide	Triazole	Korea MRLs in food (2017)

No.	Compound name	Activity group	Chemical group	Remarks
124	Diflufenican	Herbicide	Others/Unclassified	
125	Dimepiperate	Herbicide	Carbamate	Korea MRLs in food (2017)
126	Dimethachlor	Herbicide	Others/Unclassified	
127	Dimethametryn	Herbicide	Triazine	Korea MRLs in food (2017)
128	Dimethenamid	Herbicide	Others/Unclassified	Korea MRLs in food (2017)
129	Dimethoate	Insecticide	Organophosphate	Korea MRLs in food (2017)
130	Dimethylvinphos	Insecticide	Organophosphate	Korea MRLs in food (2017)
131	Diniconazole	Fungicide	Triazole	Korea MRLs in food (2017)
132	Dinitramine	Herbicide	Others/Unclassified	
133	Dinobuton	Inter-activity	Others/Unclassified	
134	Dioxabenzofos	Insecticide	Organophosphate	
135	Dioxacarb	Insecticide	Carbamate	
136	Dioxathion	Insecticide	Organophosphate	
137	Diphenamid	Herbicide	Others/Unclassified	Korea MRLs in food (2017)
138	Diphenylamine	Fungicide	Others/Unclassified	Korea MRLs in food (2017)
139	Disulfoton	Insecticide	Organophosphate	Korea MRLs in food (2017)
140	Dithiopyr	Herbicide	Others/Unclassified	Korea MRLs in food (2017)
141	Edifenphos	Fungicide	Organophosphate	Korea MRLs in food (2017)
142	Endosulfan-alpha	Insecticide	Organochlorine	Korea MRLs in food (2017)
143	Endosulfan-beta	Insecticide	Organochlorine	Korea MRLs in food (2017)
144	Endosulfan-sulfate	Insecticide	Organochlorine	Endosulfan metabolite
145	Endrin	Insecticide	Organochlorine	Korea MRLs in food (2017)
146	EPN	Insecticide	Organophosphate	Korea MRLs in food (2017)
147	Epoxiconazole	Fungicide	Triazole	Korea MRLs in food (2017)
148	EPTC	Herbicide	Carbamate	

No.	Compound name	Activity group	Chemical group	Remarks
149	Esprocarb	Herbicide	Carbamate	Korea MRLs in food (2017)
150	Etaconazole	Fungicide	Triazole	
151	Ethalfluralin	Herbicide	Others/Unclassified	Korea MRLs in food (2017)
152	Ethion	Insecticide	Organophosphate	Korea MRLs in food (2017)
153	Ethofumesate	Herbicide	Others/Unclassified	
154	Ethoprophos	Insecticide	Organophosphate	Korea MRLs in food (2017)
155	Ethychlozate	Plant growth regulator	Others/Unclassified	Korea MRLs in food (2017)
156	Etofenprox	Insecticide	Pyrethroid	Korea MRLs in food (2017)
157	Etoxazole	Insecticide	Others/Unclassified	Korea MRLs in food (2017)
158	Etridiazole	Fungicide	Organochlorine	Korea MRLs in food (2017)
159	Etrimfos	Insecticide	Organophosphate	Korea MRLs in food (2017)
160	Famphur	Insecticide	Organophosphate	
161	Fenamidone	Fungicide	Others/Unclassified	Korea MRLs in food (2017)
162	Fenamiphos	Insecticide	Organophosphate	Korea MRLs in food (2017)
163	Fenarimol	Fungicide	Organochlorine	Korea MRLs in food (2017)
164	Fenazaquin	Insecticide	Others/Unclassified	Korea MRLs in food (2017)
165	Fenbuconazole	Fungicide	Triazole	Korea MRLs in food (2017)
166	Fenchlorphos	Insecticide	Organophosphate	
167	Fenfuram	Fungicide	Others/Unclassified	
168	Fenitrothion	Insecticide	Organophosphate	Korea MRLs in food (2017)
169	Fenobucarb	Insecticide	Carbamate	Korea MRLs in food (2017)
170	Fenothiocarb	Insecticide	Carbamate	Korea MRLs in food (2017)
171	Fenoxanil	Fungicide	Others/Unclassified	Korea MRLs in food (2017)
172	Fenoxycarb	Insecticide	Carbamate	Korea MRLs in food (2017)
173	Fenpropathrin	Insecticide	Pyrethroid	Korea MRLs in food (2017)

No.	Compound name	Activity group	Chemical group	Remarks
174	Fenpropidin	Fungicide	Others/Unclassified	
175	Fenpropimorph	Fungicide	Others/Unclassified	
176	Fenpyrazamine	Fungicide	Others/Unclassified	Korea MRLs in food (2017)
177	Fenson	Insecticide	Organochlorine	
178	Fensulfothion	Insecticide	Organophosphate	Korea MRLs in food (2017)
179	Fenthion	Insecticide	Organophosphate	Korea MRLs in food (2017)
180	Fenvalerate	Insecticide	Pyrethroid	Korea MRLs in food (2017)
181	Fipronil	Insecticide	Others/Unclassified	Korea MRLs in food (2017)
182	Flamprop-isopropyl	Herbicide	Others/Unclassified	
183	Flamprop-methyl	Herbicide	Others/Unclassified	
184	Flonicamid	Insecticide	Others/Unclassified	Korea MRLs in food (2017)
185	Fluazifop-butyl	Herbicide	Others/Unclassified	Korea MRLs in food (2017)
186	Fluchloralin	Herbicide	Others/Unclassified	
187	Flucythrinate	Insecticide	Pyrethroid	Korea MRLs in food (2017)
188	Fludioxonil	Fungicide	Others/Unclassified	Korea MRLs in food (2017)
189	Flufenpyr-ethyl	Herbicide	Others/Unclassified	
190	Flumetralin	Plant growth regulator	Others/Unclassified	
191	Flumiclorac-pentyl	Herbicide	Others/Unclassified	
192	Flumioxazin	Herbicide	Others/Unclassified	Korea MRLs in food (2017)
193	Fluopyram	Fungicide	Others/Unclassified	Korea MRLs in food (2017)
194	Fluorodifen	Herbicide	Others/Unclassified	
195	Flupyradifurone	Insecticide	Others/Unclassified	Korea MRLs in food (2017)
196	Flurochloridone	Herbicide	Others/Unclassified	
197	Flurtamone	Herbicide	Others/Unclassified	
198	Flusilazole	Fungicide	Triazole	Korea MRLs in food (2017)

No.	Compound name	Activity group	Chemical group	Remarks
199	Fluthiacet-methyl	Herbicide	Others/Unclassified	
200	Flutianil	Fungicide	Others/Unclassified	Korea MRLs in food (2017)
201	Flutolanil	Fungicide	Others/Unclassified	Korea MRLs in food (2017)
202	Flutriafol	Fungicide	Triazole	
203	Fluvalinate	Insecticide	Pyrethroid	Korea MRLs in food (2017)
204	Folpet	Fungicide	Organochlorine	Korea MRLs in food (2017)
205	Fonofos	Insecticide	Organophosphate	
206	Formothion	Insecticide	Organophosphate	Korea MRLs in food (2017)
207	Fosthiazate	Insecticide	Organophosphate	Korea MRLs in food (2017)
208	Fthalide	Fungicide	Organochlorine	Korea MRLs in food (2017)
209	Furathiocarb	Insecticide	Carbamate	Korea MRLs in food (2017)
210	Furilazole	Others	Others/Unclassified	
211	Halfenprox	Insecticide	Pyrethroid	Korea MRLs in food (2017)
212	Heptachlor	Insecticide	Organochlorine	Korea MRLs in food (2017)
213	Heptachlor-epoxide	Insecticide	Organochlorine	Heptachlor metabolite
214	Heptenophos	Insecticide	Organophosphate	
215	Hexachlorobenzene	Fungicide	Organochlorine	
216	Hexaconazole	Fungicide	Triazole	Korea MRLs in food (2017)
217	Imazalil	Fungicide	Organochlorine	Korea MRLs in food (2017)
218	Imazamethabenz-methyl	Herbicide	Others/Unclassified	
219	Indanofan	Herbicide	Others/Unclassified	Korea MRLs in food (2017)
220	Indoxacarb	Insecticide	Others/Unclassified	Korea MRLs in food (2017)
221	Iprobenfos	Fungicide	Organophosphate	Korea MRLs in food (2017)
222	Iprodione	Inter-activity	Organochlorine	Korea MRLs in food (2017)
223	Iprovalicarb	Fungicide	Carbamate	Korea MRLs in food (2017)

No.	Compound name	Activity group	Chemical group	Remarks
224	Isazofos	Insecticide	Organophosphate	Korea MRLs in food (2017)
225	Isofenphos	Insecticide	Organophosphate	Korea MRLs in food (2017)
226	Isofenphos-methyl	Insecticide	Organophosphate	
227	Isopropalin	Herbicide	Others/Unclassified	
228	Isoprothiolane	Inter-activity	Others/Unclassified	Korea MRLs in food (2017)
229	Isotianil	Inter-activity	Others/Unclassified	Korea MRLs in food (2017)
230	Isoxadifen-ethyl	Others	Others/Unclassified	
231	Isoxathion	Insecticide	Organophosphate	
232	Kresoxim-methyl	Fungicide	Others/Unclassified	Korea MRLs in food (2017)
233	Lactofen	Herbicide	Others/Unclassified	
234	Leptophos	Insecticide	Organophosphate	
235	Malathion	Insecticide	Organophosphate	Korea MRLs in food (2017)
236	Mecarbam	Insecticide	Organophosphate	Korea MRLs in food (2017)
237	Mefenacet	Herbicide	Others/Unclassified	Korea MRLs in food (2017)
238	Mefenpyr-diethyl	Others	Others/Unclassified	
239	Mephosfolan	Insecticide	Organophosphate	
240	Mepronil	Fungicide	Others/Unclassified	Korea MRLs in food (2017)
241	Metazachlor	Herbicide	Organochlorine	
242	Metconazole	Fungicide	Triazole	Korea MRLs in food (2017)
243	Methidathion	Insecticide	Organophosphate	Korea MRLs in food (2017)
244	Methoprotryne	Herbicide	Triazine	
245	Methoxychlor	Insecticide	Organochlorine	Korea MRLs in food (2017)
246	Methyl 3,5-dichlorobenzoate	Inter-activity	Others/Unclassified	
247	Methyl trithion	Insecticide	Organophosphate	
248	Metolachlor	Herbicide	Others/Unclassified	Korea MRLs in food (2017)

No.	Compound name	Activity group	Chemical group	Remarks
249	Metoxuron	Herbicide	Urea	
250	Metrafenone	Fungicide	Others/Unclassified	Korea MRLs in food (2017)
251	Metribuzin	Herbicide	Triazine	Korea MRLs in food (2017)
252	Mevinphos	Insecticide	Organophosphate	Korea MRLs in food (2017)
253	MGK-264	Others	Others/Unclassified	
254	Mirex	Insecticide	Organochlorine	
255	Molinate	Herbicide	Carbamate	Korea MRLs in food (2017)
256	Monolinuron	Herbicide	Urea	
257	Myclobutanil	Fungicide	Triazole	Korea MRLs in food (2017)
258	Napropamide	Herbicide	Others/Unclassified	Korea MRLs in food (2017)
259	Neburon	Herbicide	Urea	
260	N-Ethyl-p-toluene sulfonamide	Herbicide	Others/Unclassified	
261	Nitralin	Herbicide	Others/Unclassified	
262	Nitrapyrin	Fungicide	Organochlorine	Korea MRLs in food (2017)
263	Nitrothal-isopropyl	Fungicide	Others/Unclassified	
264	Nonachlor-cis	Insecticide	Organochlorine	
265	Nonachlor-trans	Insecticide	Organochlorine	
266	Norflurazon	Herbicide	Others/Unclassified	Korea MRLs in food (2017)
267	Noruron	Herbicide	Urea	
268	Nuarimol	Fungicide	Organochlorine	Korea MRLs in food (2017)
269	Octhilinone	Fungicide	Others/Unclassified	
270	Ofurace	Fungicide	Others/Unclassified	Korea MRLs in food (2017)
271	Oryzalin	Herbicide	Others/Unclassified	Korea MRLs in food (2017)
272	Oxadiazon	Herbicide	Others/Unclassified	Korea MRLs in food (2017)
273	Oxadixyl	Fungicide	Others/Unclassified	Korea MRLs in food (2017)

No.	Compound name	Activity group	Chemical group	Remarks
274	Oxycarboxin	Fungicide	Others/Unclassified	
275	Oxychlordane	Insecticide	Organochlorine	Chlordane metabolite
276	Oxyfluorfen	Herbicide	Others/Unclassified	Korea MRLs in food (2017)
277	Paclobutrazol	Plant growth regulator	Triazole	Korea MRLs in food (2017)
278	Parathion	Insecticide	Organophosphate	Korea MRLs in food (2017)
279	Parathion-methyl	Insecticide	Organophosphate	Korea MRLs in food (2017)
280	Pebulate	Herbicide	Carbamate	
281	Penconazole	Fungicide	Triazole	Korea MRLs in food (2017)
282	Pendimethalin	Herbicide	Others/Unclassified	Korea MRLs in food (2017)
283	Penflufen	Fungicide	Others/Unclassified	Korea MRLs in food (2017)
284	Pentachloroaniline	Fungicide	Organochlorine	Quintozene metabolite
285	Pentachlorobenzene	Fungicide	Organochlorine	Quintozene metabolite
286	Pentachlorobenzonitrile	Fungicide	Organochlorine	Chlorothalonil metabolite
287	Pentachlorothioanisole	Fungicide	Organochlorine	Quintozene metabolite
288	Penthiopyrad	Fungicide	Others/Unclassified	Korea MRLs in food (2017)
289	Pentoxazone	Herbicide	Others/Unclassified	Korea MRLs in food (2017)
290	Permethrin	Insecticide	Pyrethroid	Korea MRLs in food (2017)
291	Perthane	Insecticide	Organochlorine	
292	Phenothrin	Insecticide	Pyrethroid	Korea MRLs in food (2017)
293	Phenthoate	Insecticide	Organophosphate	Korea MRLs in food (2017)
294	Phorate	Insecticide	Organophosphate	Korea MRLs in food (2017)
295	Phosalone	Insecticide	Organophosphate	Korea MRLs in food (2017)
296	Phosfolan	Insecticide	Organophosphate	
297	Phosmet	Insecticide	Organophosphate	Korea MRLs in food (2017)
298	Phosphamidon	Insecticide	Organophosphate	Korea MRLs in food (2017)

No.	Compound name	Activity group	Chemical group	Remarks
299	Picolinafen	Herbicide	Others/Unclassified	
300	Picoxystrobin	Fungicide	Others/Unclassified	Korea MRLs in food (2017)
301	Piperophos	Herbicide	Organophosphate	Korea MRLs in food (2017)
302	Pirimicarb	Insecticide	Carbamate	Korea MRLs in food (2017)
303	Pirimiphos-ethyl	Insecticide	Organophosphate	Korea MRLs in food (2017)
304	Pirimiphos-methyl	Insecticide	Organophosphate	Korea MRLs in food (2017)
305	Pretilachlor	Herbicide	Others/Unclassified	Korea MRLs in food (2017)
306	Probenazole	Others	Others/Unclassified	Korea MRLs in food (2017)
307	Prochloraz	Fungicide	Organochlorine	Korea MRLs in food (2017)
308	Procymidone	Fungicide	Organochlorine	Korea MRLs in food (2017)
309	Profenofos	Insecticide	Organophosphate	Korea MRLs in food (2017)
310	Profluralin	Herbicide	Others/Unclassified	
311	Prometon	Herbicide	Triazine	
312	Prometryn	Herbicide	Triazine	Korea MRLs in food (2017)
313	Propachlor	Herbicide	Others/Unclassified	
314	Propanil	Herbicide	Others/Unclassified	Korea MRLs in food (2017)
315	Propargite	Insecticide	Others/Unclassified	Korea MRLs in food (2017)
316	Propazine	Herbicide	Triazine	
317	Propetamphos	Insecticide	Organophosphate	
318	Propham	Inter-activity	Carbamate	
319	Propiconazole	Fungicide	Triazole	Korea MRLs in food (2017)
320	Propisochlor	Herbicide	Others/Unclassified	Korea MRLs in food (2017)
321	Propyzamide	Herbicide	Others/Unclassified	
322	Proquinazid	Fungicide	Others/Unclassified	
323	Prosulfocarb	Herbicide	Carbamate	

No.	Compound name	Activity group	Chemical group	Remarks
324	Prothiofos	Insecticide	Organophosphate	Korea MRLs in food (2017)
325	Pyracarbolid	Fungicide	Others/Unclassified	
326	Pyraclofos	Insecticide	Organophosphate	Korea MRLs in food (2017)
327	Pyrazophos	Fungicide	Organophosphate	Korea MRLs in food (2017)
328	Pyridaben	Insecticide	Others/Unclassified	Korea MRLs in food (2017)
329	Pyridalyl	Insecticide	Others/Unclassified	Korea MRLs in food (2017)
330	Pyridaphenthion	Insecticide	Organophosphate	Korea MRLs in food (2017)
331	Pyrifenox	Fungicide	Others/Unclassified	
332	Pyrifluquinazon	Insecticide	Others/Unclassified	Korea MRLs in food (2017)
333	Pyrimidifen	Insecticide	Others/Unclassified	Korea MRLs in food (2017)
334	Pyriminobac-methyl E	Herbicide	Others/Unclassified	Korea MRLs in food (2017)
335	Pyriminobac-methyl Z	Herbicide	Others/Unclassified	Korea MRLs in food (2017)
336	Quinalphos	Insecticide	Organophosphate	Korea MRLs in food (2017)
337	Quinoxyfen	Fungicide	Others/Unclassified	
338	Quintozene	Fungicide	Organochlorine	Korea MRLs in food (2017)
339	Secbumeton	Herbicide	Triazine	
340	Silafluofen	Insecticide	Pyrethroid	Korea MRLs in food (2017)
341	Simeconazole	Fungicide	Triazole	Korea MRLs in food (2017)
342	Simetryn	Herbicide	Triazine	Korea MRLs in food (2017)
343	Spiroxamine	Fungicide	Others/Unclassified	Korea MRLs in food (2017)
344	Sulfallate	Herbicide	Carbamate	
345	Sulfotep	Insecticide	Organophosphate	
346	Sulprofos	Insecticide	Organophosphate	
347	TCMTB	Fungicide	Others/Unclassified	
348	Tebuconazole	Fungicide	Triazole	Korea MRLs in food (2017)

No.	Compound name	Activity group	Chemical group	Remarks
349	Tebufenpyrad	Insecticide	Others/Unclassified	Korea MRLs in food (2017)
350	Tebupirimfos	Insecticide	Organophosphate	Korea MRLs in food (2017)
351	Tecnazene	Inter-activity	Organochlorine	Korea MRLs in food (2017)
352	Tefluthrin	Insecticide	Pyrethroid	Korea MRLs in food (2017)
353	Terbacil	Herbicide	Others/Unclassified	
354	Terbufos	Insecticide	Organophosphate	Korea MRLs in food (2017)
355	Terbumeton	Herbicide	Triazine	
356	Terbuthylazine	Herbicide	Triazine	Korea MRLs in food (2017)
357	Terbutryn	Herbicide	Triazine	Korea MRLs in food (2017)
358	Tetrachlorvinphos	Insecticide	Organophosphate	
359	Tetraconazole	Fungicide	Triazole	Korea MRLs in food (2017)
360	Tetradifon	Insecticide	Organochlorine	Korea MRLs in food (2017)
361	Tetramethrin	Insecticide	Pyrethroid	
362	Tetrasul	Insecticide	Organochlorine	
363	Thiazopyr	Herbicide	Others/Unclassified	Korea MRLs in food (2017)
364	Thifluzamide	Fungicide	Others/Unclassified	Korea MRLs in food (2017)
365	Thiometon	Insecticide	Organophosphate	Korea MRLs in food (2017)
366	Thionazin	Insecticide	Organophosphate	
367	Tolclofos-methyl	Fungicide	Organophosphate	Korea MRLs in food (2017)
368	Tolfenpyrad	Insecticide	Others/Unclassified	Korea MRLs in food (2017)
369	Tolylfluanid	Fungicide	Organochlorine	Korea MRLs in food (2017)
370	Tralomethrin	Insecticide	Pyrethroid	Korea MRLs in food (2017)
371	Triadimefon	Fungicide	Triazole	Korea MRLs in food (2017)
372	Triadimenol	Fungicide	Triazole	Korea MRLs in food (2017)
373	Tri-allate	Herbicide	Carbamate	Korea MRLs in food (2017)

No.	Compound name	Activity group	Chemical group	Remarks
374	Triazophos	Insecticide	Organophosphate	Korea MRLs in food (2017)
375	Tribufos	Inter-activity	Organophosphate	
376	Trichloronat	Insecticide	Organophosphate	
377	Tridiphane	Herbicide	Others/Unclassified	
378	Triflumizole	Fungicide	Others/Unclassified	Korea MRLs in food (2017)
379	Triflumuron	Insecticide	Urea	Korea MRLs in food (2017)
380	Trifluralin	Herbicide	Others/Unclassified	Korea MRLs in food (2017)
381	Uniconazole	Plant growth regulator	Triazole	
382	Vernolate	Herbicide	Carbamate	
383	Vinclozolin	Fungicide	Organochlorine	Korea MRLs in food (2017)
384	Zoxamide	Fungicide	Others/Unclassified	Korea MRLs in food (2017)

References

Adams S, Guest J, Dickinson M, Fussell RJ, Beck J, Schoutsen F (2017) Development and Validation of Ion Chromatography–Tandem Mass Spectrometry-Based Method for the Multiresidue Determination of Polar Ionic Pesticides in Food. *J Agric Food Chem.* 65:7294-7304.

Aktar MW, Sengupta D, Chowdhury A (2009) Impact of pesticides use in agriculture: Their benefits and hazards. *Interdiscip Toxicol.* 2:1-12.

Albero B, Sánchez-Brunete C, Tadeo JL (2005) Multiresidue determination of pesticides in juice by solid-phase extraction and gas chromatography–mass spectrometry. *Talanta.* 66:917-924.

Almeida AM, Castel-Branco MM, Falcão AC (2002) Linear regression for calibration lines revisited: weighting schemes for bioanalytical methods. *J Chromatogr B.* 774:215-222.

Altshul L, Covaci A, Hauser R (2004) The relationship between levels of PCBs and pesticides in human hair and blood: Preliminary results. *Environ Health Perspect.* 112:1193-1199.

Anastassiades M, Lehotay SJ, Štajnbaher D, Schenck FJ (2003) Fast and easy multiresidue method employing acetonitrile extraction/partitioning and “dispersive solid-phase extraction” for the determination of pesticide residues in produce. *J AOAC Int.* 86:412-431.

Atwood D, Paisley-Jones C. Pesticides Industry Sales and Usage: 2008-2012 Market Estimates. Washington, D.C.: United States Environmental Protection Agency (U.S. EPA)2017.

Aysal P, Ambrus Á, Lehotay SJ, Cannavan A (2007) Validation of an efficient method for the determination of pesticide residues in fruits and vegetables using ethyl acetate for extraction. *J Environ Sci Health Part B.* 42:481-490.

Azandjeme CS, Delisle H, Fayomi B, Ayotte P, Djrolo F, Houinato D et al. (2014) High serum organochlorine pesticide concentrations in diabetics of a cotton producing area of the Benin Republic (West Africa). *Environ Int.* 69:1-8.

Barr DB, Barr JR, Maggio VL, Whitehead RD, Sadowski MA, Whyatt RM et al. (2002) A multi-analyte method for the quantification of contemporary pesticides in human serum and plasma using high-resolution mass spectrometry. *J Chromatogr B: Anal Technol Biomed Life Sci.* 778:99-111.

Beltran J, Pitarch E, Egea S, López FJ, Hernández F (2001) Gas chromatographic determination of selected pesticides in human serum by head-space solid-phase microextraction. *Chromatographia.* 54:757-763.

Benjamin D, Dennis v, Peter N (2012) Colony collapse disorder in Europe. *Environ Microbiol Rep.* 4:123-125.

Berthet A, Bouchard M, Schüpfer P, Vernez D, Danuser B, Huynh CK (2011) Liquid

chromatography–tandem mass spectrometry (LC/APCI-MS/MS) methods for the quantification of captan and folpet phthalimide metabolites in human plasma and urine. *Anal Bioanal Chem.* 399:2243-2255.

Bravo R, Driskell WJ, Whitehead JRD, Needham LL, Barr DB (2002) Quantitation of dialkyl phosphate metabolites of organophosphate pesticides in human urine using GC-MS-MS with isotopic internal standards. *J Anal Toxicol.* 26:245-252.

Cabrera LdC, Caldas SS, Prestes OD, Primel EG, Zanella R (2016) Evaluation of alternative sorbents for dispersive solid-phase extraction clean-up in the QuEChERS method for the determination of pesticide residues in rice by liquid chromatography with tandem mass spectrometry. *J Sep Sci.* 39:1945-1954.

Calvert GM, Karnik J, Mehler L, Beckman J, Morrissey B, Sievert J et al. (2008) Acute pesticide poisoning among agricultural workers in the United States, 1998–2005. *Am J Ind Med.* 51:883-898.

Carneiro RP, Oliveira FAS, Madureira FD, Silva G, de Souza WR, Lopes RP (2013) Development and method validation for determination of 128 pesticides in bananas by modified QuEChERS and UHPLC–MS/MS analysis. *Food Control.* 33:413-423.

Carrington D (2018) EU agrees total ban on bee-harming pesticides. The Guardian. https://www.theguardian.com/environment/2018/apr/27/eu-agrees-total-ban-on-bee-harming-pesticides. Accessed June 9.

Cazorla-Reyes R, Fernández-Moreno JL, Romero-González R, Frenich AG, Vidal JLM (2011) Single solid phase extraction method for the simultaneous analysis of polar and non-polar pesticides in urine samples by gas chromatography and ultra high pressure liquid chromatography coupled to tandem mass spectrometry. *Talanta.* 85:183-196.

Cha ES, Khang Y-H, Lee WJ (2014) Mortality from and incidence of pesticide poisoning in South Korea: Findings from national death and health utilization data between 2006 and 2010. *PLoS One.* 9:e95299.

Chang C, Luo J, Chen M, Wu K, Dong T, He X et al. (2016) Determination of twenty organophosphorus pesticides in blood serum by gas chromatography-tandem mass spectrometry. *Anal Methods.* 8:4487-4496.

Chauzat M-P, Faucon J-P, Martel A-C, Lachaize J, Cougoule N, Aubert M (2006) A Survey of Pesticide Residues in Pollen Loads Collected by Honey Bees in France. *J Econ Entomol.* 99:253-262.

Chemicalize.org (2017). https://chemicalize.com. Accessed May, 16.

Chen G, Cao P, Liu R (2011) A multi-residue method for fast determination of pesticides in tea by ultra performance liquid chromatography–electrospray tandem mass spectrometry combined with modified QuEChERS sample preparation procedure. *Food Chem.* 125:1406-1411.

Cheng Z, Dong F, Xu J, Liu X, Wu X, Chen Z et al. (2017) Simultaneous determination of organophosphorus pesticides in fruits and vegetables using atmospheric pressure gas chromatography quadrupole-time-of-flight mass spectrometry. *Food Chem.* 231:365-373.

Columé A, Cárdenas S, Gallego M, Valcárcel M (2001) A solid phase extraction method for the screening and determination of pyrethroid metabolites and organochlorine pesticides in human urine. *Rapid Commun Mass Spectrom.* 15:2007-2013.

Cooper J, Dobson H (2007) The benefits of pesticides to mankind and the environment. *Crop Protection.* 26:1337-1348.

Corbet SA, Williams IH, Osborne JL (1991) Bees and the Pollination of Crops and Wild Flowers in the European Community. *Bee World.* 72:47-59.

Cortéjade A, Kiss A, Cren C, Vulliet E, Buleté A (2016) Development of an analytical method for the targeted screening and multi-residue quantification of environmental contaminants in urine by liquid chromatography coupled to high resolution mass spectrometry for evaluation of human exposures. *Talanta*. 146:694-706.

Cox-Foster DL, Conlan S, Holmes EC, Palacios G, Evans JD, Moran NA et al. (2007) A Metagenomic Survey of Microbes in Honey Bee Colony Collapse Disorder. *Science.* 318:283-287.

Cutillas V, Galera MM, Rajski Ł, Fernández-Alba AR (2018) Evaluation of supercritical fluid chromatography coupled to tandem mass spectrometry for pesticide residues in food. *J Chromatogr A.* 1545:67-74.

da Costa Morais EH, Collins CH, Jardim ICSF (2018) Pesticide determination in sweet peppers using QuEChERS and LC–MS/MS. *Food Chem.* 249:77-83.

De Bièvre P, Günzler H, Eds. (2005) Validation in Chemical Measurement. Springer, New York

Dejonckheere W, Steurbaut W, Kips RH (1976) Residues of quintozene, its contaminants and metabolites in soil, lettuce, and witloof-chicory, Belgium--1969-74. *Pestic Monit J.* 10:68-73.

Di Prisco G, Cavaliere V, Annoscia D, Varricchio P, Caprio E, Nazzi F et al. (2013) Neonicotinoid clothianidin adversely affects insect immunity and promotes replication of a viral pathogen in honey bees. *Proc Natl Acad Sci.* 110:18466-18471.

Dias JV, Cutillas V, Lozano A, Pizzutti IR, Fernández-Alba AR (2016) Determination of pesticides in edible oils by liquid chromatography-tandem mass spectrometry employing new generation materials for dispersive solid phase extraction clean-up. *J Chromatogr A.* 1462:8-18.

Dively GP, Kamel A (2012) Insecticide Residues in Pollen and Nectar of a Cucurbit Crop and Their Potential Exposure to Pollinators. *J Agric Food Chem.* 60:4449-4456.

Dong X, Shi Z, Liang S, Sun H (2014) Rapid simultaneous determination of herbicides in human serum by UPLC-ESI-MS. *Anal Methods.* 6:6939-6947.

Drzyzga O (2003) Diphenylamine and derivatives in the environment: a review. *Chemosphere.* 53:809-818.

Dulaurent S, Moesch C, Marquet P, Gaulier J-M, Lachâtre G (2010) Screening of

pesticides in blood with liquid chromatography–linear ion trap mass spectrometry. *Anal Bioanal Chem.* 396:2235-2249.

Eitzer BD, Mattina MI, Iannucci-Berger W (2001) Compositional and chiral profiles of weathered chlordane residues in soil. *Environ Toxicol Chem.* 20:2198-2204.

el-Nabarawy IM, Carey WF (1988) Improved method for determination of chlorothalonil and related residues in cranberries. *J Assoc Off Anal Chem.* 71:358-360.

EN 15662 (2008) Foods of plant origin-determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE-QuEChERS-method. *EN 15662*.EN 15662:2008.

Erney DR, Gillespie AM, Gilvydis DM, Poole CF (1993) Explanation of the matrix-induced chromatographic response enhancement of organophosphorus pesticides during open tubular column gas chromatography with splitless or hot on-column injection and flame photometric detection. *J Chromatogr A.* 638:57-63.

Esteve-Romero J, Marco-Peiro S, Rambla-Alegre M, Durgbanshi A, Bose D, Mourya SK (2012) A micellar liquid chromatographic method for the determination of carbaryl and 1-naphthol in biological samples. *J Liq Chromatogr Relat Technol.* 35:355-361.

EU Reference Laboratories for Residues of Pesticides EURL Data Pool. http://www.eurl-pesticides-datapool.eu/. Accessed May 31.

European Commission (2005) SANCO/10533/05 - final; January 2005. http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=activesubstance.ViewReview&id=368. Accessed May 20.

European Commission (2006) SANCO/10390/2002 - rev. final; July 2006. http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=activesubstance.ViewReview&id=399. Accessed May 20.

European Commission (2013) Commission implementing regulation (EU) No 485/2013. *Official Journal of the European Union.*

European Commission (2017) Guidance document on analytical quality control and validation procedures for pesticide residues and analysis in food and feed (SANTE/11813/2017). https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_mrl_guidelines_wrkdoc_2017-11813.pdf. Accessed May 16.

European Commission (2018) EU Pesticides database (files updated on 09/03/2018). http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=download.MRL. Accessed June 1.

European Food Safety Authority (2008) Conclusion regarding the peer review of the pesticide risk assessment of the active substance imidacloprid. *EFSA J.* 6:1-120.

European Food Safety Authority (2018a) Evaluation of the data on clothianidin,

imidacloprid and thiamethoxam for the updated risk assessment to bees for seed treatments and granules in the EU. *EFSA.*EN-1378.

European Food Safety Authority (2018b) Peer review of the pesticide risk assessment for bees for the active substance clothianidin considering the uses as seed treatments and granules. *EFSA J.* 16:5177.

European Food Safety Authority (2018c) Peer review of the pesticide risk assessment for bees for the active substance imidacloprid considering the uses as seed treatments and granules. *EFSA J.* 16:5178.

European Food Safety Authority (2018d) Peer review of the pesticide risk assessment for bees for the active substance thiamethoxam considering the uses as seed treatments and granules. *EFSA J.* 16:5179.

Fan R, Zhang F, Wang H, Zhang L, Zhang J, Zhang Y et al. (2014) Reliable screening of pesticide residues in maternal and umbilical cord sera by gas chromatography-quadrupole time of flight mass spectrometry. *Sci China: Chem.* 57:669-677.

Ferrer C, Lozano A, Agüera A, Girón AJ, Fernández-Alba AR (2011a) Overcoming matrix effects using the dilution approach in multiresidue methods for fruits and vegetables. *J Chromatogr A.* 1218:7634-7639.

Ferrer C, Martínez-Bueno MJ, Lozano A, Fernández-Alba AR (2011b) Pesticide residue analysis of fruit juices by LC–MS/MS direct injection. One year pilot survey. *Talanta*. 83:1552-1561.

Focant J-F, Sjödin A, Turner WE, Patterson DG (2004) Measurement of selected polybrominated diphenyl ethers, polybrominated and polychlorinated biphenyls, and organochlorine pesticides in human serum and milk using comprehensive two-dimensional gas chromatography isotope dilution time-of-flight mass spectrometry. *Anal Chem.* 76:6313-6320.

Food and Agriculture Organization FAO specifications and evaluations for agricultural pesticides: Carbaryl. http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/Specs/Carbaryl07.pdf. Accessed May 26.

Friedrich MT, Martins ML, Prestes OD, Zanella R (2016) Use of Factorial Design in the Development of Multiresidue Method for Determination of Pesticide Residues in Wheat by Liquid Chromatography-Tandem Mass Spectrometry. *Food Anal Method.* 9:2541-2551.

Garner F, Jones K (2014) Biological monitoring for exposure to methamidophos: A human oral dosing study. *Toxicol Lett.* 231:277-281.

Genuis SJ, Lane K, Birkholz D (2016) Human elimination of organochlorine pesticides: Blood, urine, and sweat study. *Biomed Res Int.* 2016:1624643.

Gill US, Schwartz HM, Wheatley B (1996) Development of a method for the analysis of PCB congeners and organochlorine pesticides in blood/serum. *Chemosphere*. 32:1055-1061.

Golfinopoulos SK, Nikolaou AD, Kostopoulou MN, Xilourgidis NK, Vagi MC, Lekkas

DT (2003) Organochlorine pesticides in the surface waters of Northern Greece. *Chemosphere.* 50:507-516.

Gong ZM, Tao S, Xu FL, Dawson R, Liu WX, Cui YH et al. (2004) Level and distribution of DDT in surface soils from Tianjin, China. *Chemosphere.* 54:1247-1253.

Goon A, Khan Z, Oulkar D, Shinde R, Gaikwad S, Banerjee K (2018) A simultaneous screening and quantitative method for the multiresidue analysis of pesticides in spices using ultra-high performance liquid chromatography-high resolution (Orbitrap) mass spectrometry. *J Chromatogr A.* 1532:105-111.

Gordon EB, Ehrlich T, Mobley S, Williams M (2001) Measurement of the reaction between the fungicides captan or folpet and blood thiols. *Toxicol Methods.* 11:209-223.

Grande-Martínez Á, Arrebola-Liébanas FJ, Martínez-Vidal JL, Hernández-Torres ME, Garrido-Frenich A (2016) Optimization and validation of a multiresidue pesticide method in rice and wheat flour by modified QuEChERS and GC–MS/MS. *Food Anal Method.* 9:548-563.

Gunnell D, Eddleston M, Phillips MR, Konradsen F (2007) The global distribution of fatal pesticide self-poisoning: Systematic review. *BMC Public Health.* 7:357.

Gunther FA, Blinn RC, Carman GE (1962) Insecticide residues - residue of sevin on and in lemons and oranges. *J Agric Food Chem.* 10:222-223.

Hajšlová J, Zrostlíková J (2003) Matrix effects in (ultra)trace analysis of pesticide residues in food and biotic matrices. *J Chromatogr A.* 1000:181-197.

Han Y, Song L, Zou N, Chen R, Qin Y, Pan C (2016) Multi-residue determination of 171 pesticides in cowpea using modified QuEChERS method with multi-walled carbon nanotubes as reversed-dispersive solid-phase extraction materials. *J Chromatogr B.* 1031:99-108.

Han Y, Song L, Zou N, Qin Y, Li X, Pan C (2017) Rapid multiplug filtration cleanup method for the determination of 124 pesticide residues in rice, wheat, and corn. *J Sep Sci.* 40:878-884.

Hattori H, Suzuki O, Yasuoka T, Asano M, Katsumata Y (1982) Identification and quantitation of disulfoton in urine and blood of a cadaver by gas chromatography/mass spectrometry. *Jpn J Legal Med.* 36:411-413.

He Z, Wang L, Peng Y, Luo M, Wang W, Liu X (2015) Multiresidue analysis of over 200 pesticides in cereals using a QuEChERS and gas chromatography–tandem mass spectrometry-based method. *Food Chem.* 169:372-380.

He Z, Wang Y, Wang L, Peng Y, Wang W, Liu X (2017) Determination of 255 pesticides in edible vegetable oils using QuEChERS method and gas chromatography tandem mass spectrometry. *Anal Bioanal Chem.* 409:1017-1030.

Hernández F, Pitarch E, Beltran J, López FJ (2002) Headspace solid-phase microextraction in combination with gas chromatography and tandem mass spectrometry for the determination of organochlorine and organophosphorus pesticides in whole human blood. *J Chromatogr B: Anal Technol Biomed Life*

Sci. 769:65-77.

Hernández F, Sancho JV, Pozo OJ (2005) Critical review of the application of liquid chromatography/mass spectrometry to the determination of pesticide residues in biological samples. *Anal Bioanal Chem.* 382:934-946.

Hikiji W, Yamaguchi K, Saka K, Hayashida M, Ohno Y, Fukunaga T (2013) Acute fatal poisoning with Tolfenpyrad. *J Forensic Leg Med.* 20:962-964.

Hou X, Lei S, Guo L, Qiu S (2016) Optimization of a multi-residue method for 101 pesticides in green tea leaves using gas chromatography tandem mass spectrometry. *Rev Bras Farmacogn.* 26:401-407.

IUPAC Pesticide Properties Database. https://sitem.herts.ac.uk/aeru/iupac/. Accessed July, 19.

Iwasaki M, Sato I, Jin Y, Saito N, Tsuda S (2007) Problems of positive list system revealed by survey of pesticide residue in food. *J Toxicol Sci.* 32:179-184.

Jadhav M, Thekkumpurath AS, Nakade M, Gadgil M, Oulkar D, Arimboor R et al. (2017) Multiresidue Method for Targeted Screening of Pesticide Residues in Spice Cardamom (Elettaria cardamomum) by Liquid Chromatography with Tandem Mass Spectrometry. *J AOAC Int.* 100:603-609.

Jaraczewska K, Lulek J, Covaci A, Voorspoels S, Kaluba-Skotarczak A, Drews K et al. (2006) Distribution of polychlorinated biphenyls, organochlorine pesticides and polybrominated diphenyl ethers in human umbilical cord serum, maternal serum and milk from Wielkopolska region, Poland. *Sci Total Environ.* 372:20-31.

Jeschke P, Nauen R, Schindler M, Elbert A (2011) Overview of the Status and Global Strategy for Neonicotinoids. *J Agric Food Chem.* 59:2897-2908.

Jeyaratnam J (1990) Acute pesticide poisoning: a major global health problem. *World Health Stat Q.* 43:139-144.

Jia W, Chu X, Ling Y, Huang J, Chang J (2014) High-throughput screening of pesticide and veterinary drug residues in baby food by liquid chromatography coupled to quadrupole Orbitrap mass spectrometry. *J Chromatogr A.* 1347:122-128.

Johnson R (2010) Honey Bee Colony Collapse Disorder. Congressional Research Service (7-5700, RL33938). Accessed June 12.

Johnson RM, Evans JD, Robinson GE, Berenbaum MR (2009) Changes in transcript abundance relating to colony collapse disorder in honey bees (Apis mellifera). *Proc Natl Acad Sci.* 106:14790-14795.

Jovanov P, Guzsvány V, Franko M, Lazić S, Sakač M, Šarić B et al. (2013) Multi-residue method for determination of selected neonicotinoid insecticides in honey using optimized dispersive liquid–liquid microextraction combined with liquid chromatography-tandem mass spectrometry. *Talanta.* 111:125-133.

Kaczyński P, Łozowicka B (2017) One-Step QuEChERS-Based Approach to Extraction and Cleanup in Multiresidue Analysis of Sulfonylurea Herbicides in Cereals by Liquid Chromatography–Tandem Mass Spectrometry. *Food Anal Method.* 10:147-160.

Kamel A (2010) Refined Methodology for the Determination of Neonicotinoid Pesticides and Their Metabolites in Honey Bees and Bee Products by Liquid Chromatography−Tandem Mass Spectrometry (LC-MS/MS). *J Agric Food Chem.* 58:5926-5931.

Kasiotis KM, Souki H, Carageorgiou H, Machera K (2011) Determination of dimethoate and omethoate in human serum samples. Risk assessment for the operator. *Int J Environ Anal Chem.* 91:876-883.

Kasiotis KM, Anagnostopoulos C, Anastasiadou P, Machera K (2014) Pesticide residues in honeybees, honey and bee pollen by LC–MS/MS screening: Reported death incidents in honeybees. *Sci Total Environ.* 485-486:633-642.

Kavvalakis MP, Tzatzarakis MN, Theodoropoulou EP, Barbounis EG, Tsakalof AK, Tsatsakis AM (2013) Development and application of LC–APCI–MS method for biomonitoring of animal and human exposure to imidacloprid. *Chemosphere.* 93:2612-2620.

Kazui Y, Seto Y, Inoue H (2014) Phosphorus-specific determination of glyphosate, glufosinate, and their hydrolysis products in biological samples by liquid chromatography–inductively coupled plasma–mass spectrometry. *Forensic Toxicol.* 32:317-322.

Kebarle P, Tang L (1993) From ions in solution to ions in the gas phase - the mechanism of electrospray mass spectrometry. *Anal Chem.* 65:972A-986A.

Kemmerich M, Bernardi G, Prestes OD, Adaime MB, Zanella R (2018) Comprehensive Method Validation for the Determination of 170 Pesticide Residues in Pear Employing Modified QuEChERS Without Clean-Up and Ultra-High Performance Liquid Chromatography Coupled to Tandem Mass Spectrometry. *Food Anal Method.* 11:556-577.

Kiljanek T, Niewiadowska A, Semeniuk S, Gaweł M, Borzęcka M, Posyniak A (2016) Multi-residue method for the determination of pesticides and pesticide metabolites in honeybees by liquid and gas chromatography coupled with tandem mass spectrometry—Honeybee poisoning incidents. *J Chromatogr A.* 1435:100-114.

Kim H-s, Kim J, Suh JH, Han SB (2014) General unknown screening for pesticides in whole blood and Korean gastric contents by liquid chromatography–tandem mass spectrometry. *Arch Pharm Res.* 37:1317-1324.

Kim J-H, Kim J, Cha ES, Ko Y, Kim DH, Lee WJ (2013) Work-related risk factors by severity for acute pesticide poisoning among male farmers in South Korea. *Int J Environ Res Public Health.* 10:1100-1112.

Kim Y-A, Abd El-Aty AM, Rahman MM, Jeong JH, Shin H-C, Wang J et al. (2018) Method development, matrix effect, and risk assessment of 49 multiclass pesticides in kiwifruit using liquid chromatography coupled to tandem mass spectrometry. *J Chromatogr B.* 1076:130-138.

Kmellár B, Fodor P, Pareja L, Ferrer C, Martínez-Uroz MA, Valverde A et al. (2008) Validation and uncertainty study of a comprehensive list of 160 pesticide residues in multi-class vegetables by liquid chromatography–tandem mass

spectrometry. *J Chromatogr A.* 1215:37-50.

Koesukwiwat U, Sanguankaew K, Leepipatpiboon N (2008) Rapid determination of phenoxy acid residues in rice by modified QuEChERS extraction and liquid chromatography–tandem mass spectrometry. *Anal Chim Acta.* 626:10-20.

Komosinska-Vassev K, Olczyk P, Ka, #x17a, mierczak J, Mencner L et al. (2015) Bee Pollen: Chemical Composition and Therapeutic Application. *Evid-based Complement Altern Med.* 2015:6.

Korea Crop Protection Association (2012) Guigance for Pesticide Application *[in Korean]*. KCPA, Seoul, the Republic of Korea

Korea Crop Protection Association (2013) 2013 Pesticide Handbook. KCPA, Seoul, the Republic of Korea

Korea Crop Protection Association (2015) Guigance for Pesticide Application *[in Korean]*. KCPA, Seoul, the Republic of Korea

Korea Crop Protection Association (2017) Agrochemical Year Book *[in Korean]*. KCPA, Seoul, the Republic of Korea

Kross BC, Selim MI, Hallberg GR, Bruner DR, Cherryholmes K (1992) Pesticide contamination of private well water, a growing rural health concern. *Environ Int.* 18:231-241.

Kruve A, Rebane R, Kipper K, Oldekop M-L, Evard H, Herodes K et al. (2015) Tutorial review on validation of liquid chromatography–mass spectrometry methods: Part I. *Anal Chim Acta.* 870:29-44.

Lacassie E, Dreyfuss MF, Gaulier JM, Marquet P, Daguet JL, Lachâtre G (2001a) Multiresidue determination method for organophosphorus pesticides in serum and whole blood by gas chromatography–mass-selective detection. *J Chromatogr B: Biomed Sci Appl.* 759:109-116.

Lacassie E, Marquet P, Gaulier J-M, Dreyfuss M-F, Lachâtre G (2001b) Sensitive and specific multiresidue methods for the determination of pesticides of various classes in clinical and forensic toxicology. *Forensic Sci Int.* 121:116-125.

Langley RL, Mort SA (2012) Human exposures to pesticides in the United States. *J Agromedicine.* 17:300-315.

LeDoux M (2011) Analytical methods applied to the determination of pesticide residues in foods of animal origin. A review of the past two decades. *J Chromatogr A.* 1218:1021-1036.

Lee C-W, Su H, Chen P-Y, Lin S-J, Shiea J, Shin S-J et al. (2016) Rapid identification of pesticides in human oral fluid for emergency management by thermal desorption electrospray ionization/mass spectrometry. *J Mass Spectrom.* 51:97-104.

Lee HS, Lee JH, Roh SY, Kim HG, Lee KJ, Nam-gung SJ et al. (2015) Symptom prevalence and work-related risk factors of acute pesticide poisoning among Korean farmers in Gyeong-gi province. *J Agric Med Community Health.* 40:228-239.

Lee J, Kim L, Shin Y, Lee J, Lee J, Kim E et al. (2017) Rapid and simultaneous analysis of 360 pesticides in brown rice, spinach, orange, and potato using microbore GC-MS/MS. *J Agric Food Chem.* 65:3387-3395.

Lee J, Kim E, Shin Y, Lee J, Lee J, Maasfeld W et al. (2018a) Validation protocol for whole-body dosimetry in an agricultural exposure study. *Appl Biol Chem.* 61:125-130.

Lee J, Shin Y, Lee J, Lee J, Kim BJ, Kim J-H (2018b) Simultaneous analysis of 310 pesticide multiresidues using UHPLC-MS/MS in brown rice, orange, and spinach. *Chemosphere.* 207:519-526.

Lee S-B, Seo D-K, Choi K-H, Lee S-W, Yoon H-J, Park H-C et al. (2008) The visited insects on apple flowers, and the characteristics on pollinating activity of pollinators released for pollination of apple orchards. *J Apic.* 23:275-282.

Lee S-K, Ameno K, In S-W, Yang W-K, Koo K-S, Yoo Y-C et al. (1999) Acute fatal poisoning cases due to furathiocarb ingestion. *Forensic Sci Int.* 101:65-70.

Lee SK, Rhee JS, Jung JM, Lee HS (2010) Pesticide poisoning deaths detected at the National Forensic Service Headquarters in Seoul of Korea: A five-year survey (2005-2009). *Environ Health Toxicol.* 25:263-271.

Lehotay SJ (2007) Determination of pesticide residues in foods by acetonitrile extraction and partitioning with magnesium sulfate: Collaborative study. *J AOAC Int.* 90:485-520.

Lehotay SJ, Son KA, Kwon H, Koesukwiwat U, Fu W, Mastovska K et al. (2010) Comparison of QuEChERS sample preparation methods for the analysis of pesticide residues in fruits and vegetables. *J Chromatogr A.* 1217:2548-2560.

Li J, Zhang Z, Sun M, Zhang B, Fan C (2018a) Use of a Headspace Solid-Phase Microextraction-Based Methodology Followed by Gas Chromatography–Tandem Mass Spectrometry for Pesticide Multiresidue Determination in Teas. *Chromatographia.* 81:809-821.

Li P, Duan Y, Ge H, Zhang Y, Wu X (2018b) Multiresidue Analysis of 113 Pesticides in Different Maturity Levels of Mangoes Using an Optimized QuEChERS Method with GC-MS/MS and UHPLC-MS/MS. *Food Anal Method.*

Li Y, Kelley RA, Anderson TD, Lydy MJ (2015) Development and comparison of two multi-residue methods for the analysis of select pesticides in honey bees, pollen, and wax by gas chromatography–quadrupole mass spectrometry. *Talanta.* 140:81-87.

López-Blanco R, Nortes-Méndez R, Robles-Molina J, Moreno-González D, Gilbert-López B, García-Reyes JF et al. (2016) Evaluation of different cleanup sorbents for multiresidue pesticide analysis in fatty vegetable matrices by liquid chromatography tandem mass spectrometry. *J Chromatogr A.* 1456:89-104.

López FJ, Pitarch E, Egea S, Beltran J, Hernández F (2001) Gas chromatographic determination of organochlorine and organophosphorus pesticides in human fluids using solid phase microextraction. *Anal Chim Acta.* 433:217-226.

Lozano A, Kiedrowska B, Scholten J, de Kroon M, de Kok A, Fernández-Alba AR

(2016) Miniaturisation and optimisation of the Dutch mini-Luke extraction method for implementation in the routine multi-residue analysis of pesticides in fruits and vegetables. *Food Chem.* 192:668-681.

Lozowicka B, Ilyasova G, Kaczynski P, Jankowska M, Rutkowska E, Hrynko I et al. (2016) Multi-residue methods for the determination of over four hundred pesticides in solid and liquid high sucrose content matrices by tandem mass spectrometry coupled with gas and liquid chromatograph. *Talanta.* 151:51-61.

Lu C, Warchol KM, Callahan RA (2014) Sub-lethal exposure to neonicotinoids impaired honey bees winterization before proceeding to colony collapse disorder. *Bull Insectology.* 67:125-130.

Luzardo OP, Almeida-González M, Ruiz-Suárez N, Zumbado M, Henríquez-Hernández LA, Meilán MJ et al. (2015) Validated analytical methodology for the simultaneous determination of a wide range of pesticides in human blood using GC–MS/MS and LC–ESI/MS/MS and its application in two poisoning cases. *Sci Justice.* 55:307-315.

MacBean C (2012) The Pesticide Manual: A World Compendium (Supplementary Entries - Extended) (16th ed). British Crop Production Council, Alton, Hampshire, England

Marletto F, Patetta A, Manino A (2003) Laboratory assessment of pesticide toxicity to bumblebees. *Bull Insectology.* 56:155-158.

Martel A-C, Lair C (2011) Validation of a highly sensitive method for the determination of neonicotinoid insecticides residues in honeybees by liquid chromatography with electrospray tandem mass spectrometry. *Int J Environ Anal Chem.* 91:978-988.

Martins ML, Kemmerich M, Prestes OD, Maldaner L, Jardim ICSF, Zanella R (2017) Evaluation of an alternative fluorinated sorbent for dispersive solid-phase extraction clean-up of the quick, easy, cheap, effective, rugged, and safe method for pesticide residues analysis. *J Chromatogr A.* 1514:36-43.

Mercadante R, Polledri E, Bertazzi PA, Fustinoni S (2013) Biomonitoring short- and long-term exposure to the herbicide terbuthylazine in agriculture workers and in the general population using urine and hair specimens. *Environ Int.* 60:42-47.

Ministry of Food and Drug Safety Positive List System for pesticide residues in food. http://www.mfds.go.kr/. Accessed June 1.

Ministry of Food and Drug Safety Korea Food Code *[in Korean]*. http://www.foodsafetykorea.go.kr. Accessed May 16.

Ministry of Food and Drug Safety (2017) Pesticide MRLs in Food [*in Korean*]. http://www.mfds.go.kr/. Accessed May 16.

Miyazaki T, Yashiki M, Kojima T, Chikasue F, Ochiai A, Hidani Y (1989) Fatal and non-fatal methomyl intoxication in an attempted double suicide. *Forensic Sci Int.* 42:263-270.

Mol HGJ, Plaza-Bolaños P, Zomer P, de Rijk TC, Stolker AAM, Mulder PPJ (2008) Toward a generic extraction method for simultaneous determination of

pesticides, mycotoxins, plant toxins, and veterinary drugs in feed and food matrixes. *Anal Chem.* 80:9450-9459.

Moliner-Martínez Y, Molins-Legua C, Verdú-Andrés J, Herráez-Hernández R, Campíns-Falcó P (2011) Advantages of monolithic over particulate columns for multiresidue analysis of organic pollutants by in-tube solid-phase microextraction coupled to capillary liquid chromatography. *J Chromatogr A.* 1218:6256-6262.

Mondal R, Kole RK, Bhttacharyya A (2017) Validation of Multiresidue Method for Analysis of 31 Pesticides in Rice Using Gas Chromatography-Tandem Mass Spectrometry. *J AOAC Int.* 100:1094-1101.

Montesano MA, Olsson AO, Kuklenyik P, Needham LL, Bradman A, Barr DB (2007) Method for determination of acephate, methamidophos, omethoate, dimethoate, ethylenethiourea and propylenethiourea in human urine using high-performance liquid chromatography-atmospheric pressure chemical ionization tandem mass spectrometry. *J Exposure Sci Environ Epidemiol.* 17:321-330.

Mostafa A, Medley G, Roberts DM, Mohamed MS, Elshanawani AA, Roberts MS et al. (2011) Simultaneous quantification of carbamate insecticides in human plasma by liquid chromatography/tandem mass spectrometry. *J Chromatogr B: Anal Technol Biomed Life Sci.* 879:2234-2238.

Müller WF, Scheunert I, Rozman K, Kögel W, Freitag D, Richter E et al. (1978) Comparative metabolism of hexachlorobenzene and pentachloronitrobenzene in plants, rats, and rhesus monkeys. *Ecotoxicol Environ Saf.* 2:437-445.

Mutavdžić D, Babić S, Ašperger D, Horvat AJM, Kaštelan-Macan M (2006) Comparison of different solid-phase extraction materials for sample preparation in the analysis of veterinary drugs in water samples. *JPC-J Planar Chromat-Mod TCL.* 19:454-462.

Nauen R, Ebbinghaus-Kintscher U, Salgado VL, Kaussmann M (2003) Thiamethoxam is a neonicotinoid precursor converted to clothianidin in insects and plants. *Pestic Biochem Physiol.* 76:55-69.

Nguyen TD, Yu JE, Lee DM, Lee G-H (2008) A multiresidue method for the determination of 107 pesticides in cabbage and radish using QuEChERS sample preparation method and gas chromatography mass spectrometry. *Food Chem.* 110:207-213.

Nguyen TD, Lee MH, Lee GH (2010) Rapid determination of 95 pesticides in soybean oil using liquid–liquid extraction followed by centrifugation, freezing and dispersive solid phase extraction as cleanup steps and gas chromatography with mass spectrometric detection. *Microchem J.* 95:113-119.

Pang GF, Fan CL, Liu YM, Cao YZ, Zhang JJ, Fu BL et al. (2006) Multi-residue method for the determination of 450 pesticide residues in honey, fruit juice and wine by double-cartridge solid-phase extraction/gas chromatography-mass spectrometry and liquid chromatography-tandem mass spectrometry. *Food Addit Contam.* 23:777-810.

Panuwet P, Hunter RE, D'Souza PE, Chen X, Radford SA, Cohen JR et al. (2016)

Biological matrix effects in quantitative tandem mass spectrometry-based analytical methods: Advancing biomonitoring. *Crit Rev Anal Chem.* 46:93-105.

Papoutsis I, Mendonis M, Nikolaou P, Athanaselis S, Pistos C, Maravelias C et al. (2012) Development and validation of a simple GC–MS method for the simultaneous determination of 11 anticholinesterase pesticides in blood—clinical and forensic toxicology applications. *J Forensic Sci.* 57:806-812.

Pareja L, Cesio V, Heinzen H, Fernández-Alba AR (2011) Evaluation of various QuEChERS based methods for the analysis of herbicides and other commonly used pesticides in polished rice by LC–MS/MS. *Talanta.* 83:1613-1622.

Pauwels A, Wells DA, Covaci A, Schepens PJC (1999) Improved sample preparation method for selected persistent organochlorine pollutants in human serum using solid-phase disk extraction with gas chromatographic analysis. *J Chromatogr B: Biomed Sci Appl.* 723:117-125.

Picó Y, Fernández M, Ruiz MJ, Font G (2007) Current trends in solid-phase-based extraction techniques for the determination of pesticides in food and environment. *J Biochem Biophys Methods.* 70:117-131.

Pirard C, Widart J, Nguyen BK, Deleuze C, Heudt L, Haubruge E et al. (2007) Development and validation of a multi-residue method for pesticide determination in honey using on-column liquid–liquid extraction and liquid chromatography–tandem mass spectrometry. *J Chromatogr A.* 1152:116-123.

Plassmann MM, Schmidt M, Brack W, Krauss M (2015) Detecting a wide range of environmental contaminants in human blood samples—combining QuEChERS with LC-MS and GC-MS methods. *Anal Bioanal Chem.* 407:7047-7054.

Pohorecka K, Skubida P, Miszczak A, Semkiw P, Sikorski P, Zagibajło K et al. (2012) Residues of Neonicotinoid Insecticides in Bee Collected Plant Materials from Oilseed Rape Crops and their Effect on Bee Colonies. 56:115.

Pozzebon JM, Queiroz SCN, Melo LFC, A. Kapor M, Jardim ICSF (2003) Application of new high-performance liquid chromatography and solid-phase extraction materials to the analysis of pesticides in human urine. *J Chromatogr A.* 987:381-387.

Qin Y, Huang B, Zhang J, Han Y, Li Y, Zou N et al. (2016a) Analytical method for 44 pesticide residues in spinach using multi-plug-filtration cleanup based on multiwalled carbon nanotubes with liquid chromatography and tandem mass spectrometry detection. *J Sep Sci.* 39:1757-1765.

Qin Y, Zhang J, Zhang Y, Li F, Han Y, Zou N et al. (2016b) Automated multi-plug filtration cleanup for liquid chromatographic–tandem mass spectrometric pesticide multi-residue analysis in representative crop commodities. *J Chromatogr A.* 1462:19-26.

Qin Y, Zhang J, Li Y, Wang Q, Wu Y, Xu L et al. (2017) Automated multi-filtration cleanup with nitrogen-enriched activated carbon material as pesticide multi-residue analysis method in representative crop commodities. *J Chromatogr A.* 1515:62-68.

Quansah R, Bend JR, Abdul-Rahaman A, Armah FA, Luginaah I, Essumang DK et al.

(2016) Associations between pesticide use and respiratory symptoms: A cross-sectional study in Southern Ghana. *Environ Res.* 150:245-254.

Rajski Ł, Lozano A, Uclés A, Ferrer C, Fernández-Alba AR (2013) Determination of pesticide residues in high oil vegetal commodities by using various multi-residue methods and clean-ups followed by liquid chromatography tandem mass spectrometry. *J Chromatogr A.* 1304:109-120.

Rejczak T, Tuzimski T (2015) A review of recent developments and trends in the QuEChERS sample preparation approach. *Open Chem.* 13:980-1010.

Rial Otero R, Cancho Grande B, Simal Gándara J (2003) Multiresidue method for fourteen fungicides in white grapes by liquid–liquid and solid-phase extraction followed by liquid chromatography–diode array detection. *J Chromatogr A.* 992:121-131.

Ribeiro Begnini Konatu F, Breitkreitz MC, Sales Fontes Jardim IC (2017) Revisiting quick, easy, cheap, effective, rugged, and safe parameters for sample preparation in pesticide residue analysis of lettuce by liquid chromatography–tandem mass spectrometry. *J Chromatogr A.* 1482:11-22.

Ribeiro Begnini Konatu F, Sales Fontes Jardim IC (2018) Development and validation of an analytical method for multiresidue determination of pesticides in lettuce using QuEChERS–UHPLC–MS/MS. *J Sep Sci.* 41:1726-1733.

Rissato SR, Galhiane MS, de Almeida MV, Gerenutti M, Apon BM (2007) Multiresidue determination of pesticides in honey samples by gas chromatography–mass spectrometry and application in environmental contamination. *Food Chem.* 101:1719-1726.

Robles-Molina J, Gilbert-López B, García-Reyes JF, Molina-Díaz A (2017) Simultaneous liquid chromatography/mass spectrometry determination of both polar and "multiresidue" pesticides in food using parallel hydrophilic interaction/reversed-phase liquid chromatography and a hybrid sample preparation approach. *J Chromatogr A.* 1517:108-116.

Sabik H, Jeannot R, Rondeau B (2000) Multiresidue methods using solid-phase extraction techniques for monitoring priority pesticides, including triazines and degradation products, in ground and surface waters. *J Chromatogr A.* 885:217-236.

Sainudeen Sahib S (2011) Impact of mobile phones on the density of honey bees. *Mun Ent Zool.* 6:396-399.

Saito T, Fukushima T, Yui Y, Miyazaki S, Nakamoto A, Namera A et al. (2011) Monolithic spin column extraction and GC-MS for the simultaneous assay of diquat, paraquat, and fenitrothion in human serum and urine. *Anal Bioanal Chem.* 400:25-31.

Saito T, Miura N, Namera A, Oikawa H, Miyazaki S, Nakamoto A et al. (2012) Mixed-mode C–C18 monolithic spin-column extraction and GC–MS for simultaneous assay of organophosphorus compounds, glyphosate, and glufosinate in human serum and urine. *Forensic Toxicol.* 30:1-10.

Saito T, Miura N, Namera A, Miyazaki S, Ohta S, Oikawa H et al. (2013) Rapid

determination of polar and non-polar pesticides in human serum, using mixed-mode C-C18 monolithic spin column extraction and LC–MS/MS. *Chromatographia.* 76:781-789.

Sancho JV, Pozo OJ, Hernández F (2000) Direct determination of chlorpyrifos and its main metabolite 3,5,6-trichloro-2-pyridinol in human serum and urine by coupled-column liquid chromatography/electrospray-tandem mass spectrometry. *Rapid Commun Mass Spectrom.* 14:1485-1490.

Schenck FJ, Donoghue DJ (2000) Determination of Organochlorine and Organophosphorus Pesticide Residues in Eggs Using a Solid Phase Extraction Cleanup. *J Agric Food Chem.* 48:6412-6415.

Schenck FJ, Lehotay SJ (2000) Does further clean-up reduce the matrix enhancement effect in gas chromatographic analysis of pesticide residues in food? *J Chromatogr A.* 868:51-61.

Schummer C, Salquèbre G, Briand O, Millet M, Appenzeller BMR (2012) Determination of farm workers' exposure to pesticides by hair analysis. *Toxicol Lett.* 210:203-210.

Shabeer TPA, Girame R, Utture S, Oulkar D, Banerjee K, Ajay D et al. (2018) Optimization of multi-residue method for targeted screening and quantitation of 243 pesticide residues in cardamom (*Elettaria cardamomum*) by gas chromatography tandem mass spectrometry (GC-MS/MS) analysis. *Chemosphere.* 193:447-453.

Sharma A, Gill JPS, Bedi JS (2015) Monitoring of pesticide residues in human blood from Punjab, India. *Bull Environ Contam Toxicol.* 94:640-646.

Soler C, Picó Y (2007) Recent trends in liquid chromatography-tandem mass spectrometry to determine pesticides and their metabolites in food. *TrAC Trends Anal Chem.* 26:103-115.

Stachniuk A, Szmagara A, Czeczko R, Fornal E (2017) LC-MS/MS determination of pesticide residues in fruits and vegetables. *J Environ Sci Health Part B.* 52:446-457.

Stankus T (2008) A Review and Bibliography of the Literature of Honey Bee Colony Collapse Disorder: A Poorly Understood Epidemic that Clearly Threatens the Successful Pollination of Billions of Dollars of Crops in America. *J Agric Food Inf.* 9:115-143.

Takayasu T, Ishida Y, Nosaka M, Kawaguchi M, Kuninaka Y, Kimura A et al. (2012) High concentration of methidathion detected in a fatal case of organophosphate-poisoning. *Leg Med.* 14:263-266.

Thundiyil JG, Stober J, Besbelli N, Pronczuk J (2008) Acute pesticide poisoning: A proposed classification tool. *Bull W H O.* 86:205-209.

Tomlin CDS (2009) The Pesticide Manual: A World Compendium Alton, Hampshire, England. British Crop Production Council

Torres CM, Picó Y, Mañes J (1996) Determination of pesticide residues in fruit and vegetables. *J Chromatogr A.* 754:301-331.

Turner JA (2015) The Pesticide Manual: A World Compendium (17th ed). British Crop Production Council, Alton, Hampshire, England

Ueyama J, Nomura H, Kondo T, Saito I, Ito Y, Osaka A et al. (2014) Biological monitoring method for urinary neonicotinoid insecticides using LC-MS/MS and its application to Japanese adults. *J Occup Health.* 56:461-468.

US FDA (2013) Guidance for Industry: Bioanalytical Method Validation. https://www.fda.gov/downloads/drugs/guidances/ucm368107.pdf. Accessed May 16.

USDA USDA Food Composition Databases. https://ndb.nal.usda.gov/ndb/search/list. Accessed May 15.

Usui K, Hayashizaki Y, Minagawa T, Hashiyada M, Nakano A, Funayama M (2012) Rapid determination of disulfoton and its oxidative metabolites in human whole blood and urine using QuEChERS extraction and liquid chromatography–tandem mass spectrometry. *Leg Med.* 14:309-316.

Valko K, Du C, Bevan C, Reynolds DP, Abraham MH (2001) Rapid Method for the Estimation of Octanol / Water Partition Coefficient (Log Poct) from Gradient RP-HPLC Retention and a Hydrogen Bond Acidity Term (Sigma alpha2H). *Curr Med Chem.* 8:1137-1146.

vanEngelsdorp D, Evans JD, Saegerman C, Mullin C, Haubruge E, Nguyen BK et al. (2009) Colony Collapse Disorder: A Descriptive Study. *PLoS One.* 4:e6481.

vanEngelsdorp D, Meixner MD (2010) A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them. *J Invertebr Pathol.* 103:S80-S95.

Vázquez PP, Lozano A, Uclés S, Ramos MMG, Fernández-Alba AR (2015) A sensitive and efficient method for routine pesticide multiresidue analysis in bee pollen samples using gas and liquid chromatography coupled to tandem mass spectrometry. *J Chromatogr A.* 1426:161-173.

Vázquez PP, Hakme E, Uclés S, Cutillas V, Galera MM, Mughari AR et al. (2016) Large multiresidue analysis of pesticides in edible vegetable oils by using efficient solid-phase extraction sorbents based on quick, easy, cheap, effective, rugged and safe methodology followed by gas chromatography–tandem mass spectrometry. *J Chromatogr A.* 1463:20-31.

Viera MS, Rizzetti TM, de Souza MP, Martins ML, Prestes OD, Adaime MB et al. (2017) Multiresidue determination of pesticides in crop plants by the quick, easy, cheap, effective, rugged, and safe method and ultra-high-performance liquid chromatography tandem mass spectrometry using a calibration based on a single level standard addition in the sample. *J Chromatogr A.* 1526:119-127.

Visscher PK, Seeley TD (1982) Foraging strategy of honeybee colonies in a temperate deciduous forest. *Ecology.* 63:1790-1801.

Walorczyk S, Kopeć I, Szpyrka E (2016) Pesticide Residue Determination by Gas Chromatography-Tandem Mass Spectrometry as Applied to Food Safety Assessment on the Example of Some Fruiting Vegetables. *Food Anal Method.* 9:1155-1172.

Watanabe D, Ohta H, Yamamuro T (2014) Solid-phase extraction of phosphorous-containing amino acid herbicides from biological specimens with a zirconia-coated silica cartridge. *J Chromatogr B: Anal Technol Biomed Life Sci.* 969:69-76.

Wessels D, Barr DB, Mendola P (2003) Use of biomarkers to indicate exposure of children to organophosphate pesticides: Implications for a longitudinal study of children's environmental health. *Environ Health Perspect.* 111:1939-1946.

Wiest L, Buleté A, Giroud B, Fratta C, Amic S, Lambert O et al. (2011) Multi-residue analysis of 80 environmental contaminants in honeys, honeybees and pollens by one extraction procedure followed by liquid and gas chromatography coupled with mass spectrometric detection. *J Chromatogr A.* 1218:5743-5756.

Wittsiepe J, Nestola M, Kohne M, Zinn P, Wilhelm M (2014) Determination of polychlorinated biphenyls and organochlorine pesticides in small volumes of human blood by high-throughput on-line SPE-LVI-GC-HRMS. *J Chromatogr B: Anal Technol Biomed Life Sci.* 945:217-224.

Wong JW, Zhang K, Tech K, Hayward DG, Krynitsky AJ, Cassias I et al. (2010) Multiresidue Pesticide Analysis of Ginseng Powders Using Acetonitrile- or Acetone-Based Extraction, Solid-Phase Extraction Cleanup, and Gas Chromatography−Mass Spectrometry/Selective Ion Monitoring (GC-MS/SIM) or −Tandem Mass Spectrometry (GC-MS/MS). *J Agric Food Chem.* 58:5884-5896.

World Health Organization WHO specifications and evaluations for public health pesticides: Etofenprox. http://www.who.int/whopes/quality/en/Etofenprox_eval_WHO_july_2007.pdf. Accessed May 26.

Woudneh MB, Oros DR (2006) Pyrethroids, pyrethrins, and piperonyl butoxide in sediments by high-resolution gas chromatography/high-resolution mass spectrometry. *J Chromatogr A.* 1135:71-77.

Wu C-C (2017) Multiresidue method for the determination of pesticides in Oolong tea using QuEChERS by gas chromatography-triple quadrupole tandem mass spectrometry. *Food Chem.* 229:580-587.

Yang X, Zhang H, Liu Y, Wang J, Zhang YC, Dong AJ et al. (2011) Multiresidue method for determination of 88 pesticides in berry fruits using solid-phase extraction and gas chromatography–mass spectrometry: Determination of 88 pesticides in berries using SPE and GC–MS. *Food Chem.* 127:855-865.

Zhao L, Lee HK (2001) Application of static liquid-phase microextraction to the analysis of organochlorine pesticides in water. *J Chromatogr A.* 919:381-388.

Zhou P, Wu Y, Yin S, Li J, Zhao Y, Zhang L et al. (2011) National survey of the levels of persistent organochlorine pesticides in the breast milk of mothers in China. *Environ Pollut.* 159:524-531.

Ziaei MM, Amini M (2013) Risk assessment process of amitraz on environment and human health. *WebmedCentral TOXICOLOGY.* 4:WMC004121.

Zoltowska K, Frączek R, Lipiński Z (2011) Hydrolases of developing worker brood

and newly emerged worker of Apis mellifera carnica. *J Apic Sci.* 55:27-36.

Żwir-Ferenc A, Biziuk M (2006) Solid phase extraction technique – trends, opportunities and applications. *Pol J Environ Stud.* 15:677-690.

초 록

서울대학교 대학원
농생명공학부 응용생명화학전공
신용호

농약은 지난 수십 년 동안 작물 중 해충, 선충, 미생물 및 잡초 등의 방제에 활용되었으며 인류의 식량 안보에 지대한 공헌을 하였다. 그러나 농약은 본질적으로 인체독성 및 생태독성을 가지고 있다. 따라서 다종(多種)·다수(多數)의 농약에 대해 인체 내, 환경 및 농산물 중 농약 잔류 수준을 지속적으로 모니터링하여 그 양상을 신속하고 정확하게 파악할 필요가 있다. 본 연구에서는 기체 및 액체크로마토그래피-탠덤질량분석법을 활용하여 생체 시료(혈청 및 소변), 양봉 시료(꿀벌, 화분 및 꿀) 및 대표작물(고추, 오렌지, 현미 및 대두) 중 약 사백여 개의 다종농약다성분을 동시분석하였다. 개별 농약성분에 대한 최적의 감도 및 선택성을 확보하고자 탠덤질량분석기의 multiple reaction monitoring (MRM) 방법을 활용하였다. 생체 시료인 혈청 및 소변에 대한 전처리는 세 가지 형태의 “Quick, Easy, Cheap, Effective, Rugged, and Safe” (QuEChERS)법을 시료량 및 용매량을 축소하여 비교하였다. 이중 최적의 전처리법을 선택한 후, LC-MS/MS를 활용하여 혈청 중 379성분 및 소변 중 380성분에 대한 분석법을 검증하였으며, 그 결과 전체 농약 성분 중 94.5% (혈청) 및 95.8% (소변)가 정량한계 10 ng/mL를 만족하였다. 또한 확립된 전처리법을 GC-MS/MS 대상 농약 성분(혈청; 54개, 소변; 55개)에 적용하여 이중 각각 53성분에 대해 10 ng/mL를 만족하여, 농약 중독 또는 농작업자 농약 살포시 생체시료를 통한 원인 규명 및 체내 노출량을 파악할 수 있는 충분한 감도를 확보하였다. 양봉시료인 꿀벌(사충, 생충 및 유충), 화분 및 꿀은 QuEChERS법을 최적화하여 전처리하였다.

유럽연합(EU)에서 2018년 말부터 옥외 사용이 전면 금지될 것으로 예상되는 네오니코티노이드(neonicotinoid) 계열 농약 클로티아니딘(clothianidin), 이미다클로프리드(imidacloprid) 및 티아메톡삼(thiamethoxam)에 대해 분석법을 검증하였으며, 3종 시료에서 모두 정량한계 1 ng/g을 만족하였다. 이는 벌의 급성경구독성 (LD_{50})보다 충분히 낮은 수준으로 농약에 의한 생태독성을 충분히 모니터링할 수 있었다. 2014년 두 지역(사과과수원 및 고추밭 일대)에서 모니터링을 수행하여 수집한 양봉 시료에 대해 네오니코티노이드 분석 및 다종농약 391성분 스크리닝 분석을 실시하였다. 분석 결과를 바탕으로 꿀벌이 잔류 농약에 노출되는 양상을 확인하고 검출된 농약 중 일부 성분에 대해 위해성 평가(risk assessment)를 실시하였다. 대표작물 4종은 식품공전 다종농약다성분 분석법-제2법의 시료량 및 용매량을 축소하여 전처리하였으며, GC-MS/MS를 사용하여 농약 384성분에 대해 분석법을 평가하였다. 그 결과 전체 농약 성분 중 95.1-99.5%가 분석법상 정량한계(method limit of quantitation) 10 ng/g 이하를 만족하여 농약 허용물질목록 관리제도(positive list system)가 요구하는 잔류농약 분석 감도(≤10 ng/g)를 확보하였다.

주요어 : 기체크로마토그래피-탠덤질량분석, 꿀, 꿀벌, 농산물, 농약, 다종농약다성분, 소변, 액체크로마토그래피-탠덤질량분석, 화분, 혈청

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