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농학석사학위논문

Establishment of Analytical Method for Napropamide Residue in Korean Cabbage, Green Pepper, Apple, Mandarin, Potato and Soybean Using HPLC and LC-MS/MS

HPLC 와 LC-MS/MS 를 이용한 배추, 고추, 사과, 감귤, 감자, 대두에서의 Napropamide 의 잔류 분석법 확립

2014년 02월

서울대학교 대학원

농생명공학부 응용생명화학 전공

류 명 주

A THESIS FOR THE DEGREE OF MASTER OF SCIENCE

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A thesis submitted to the faculty of the Seoul National University Graduate School in partial fulfillment of the requirement for the degree of Master of Science in the School of Agricultural Biotechnology.

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위 원	노 희 명	(인)

ABSTRACT

Establishment of Analytical Method for Napropamide Residue in Korean Cabbage, Green Pepper, Apple, Mandarin, Potato and Soybean Using HPLC and LC-MS/MS

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The Graduate School

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This study was performed to develop a precise single residue analytical method of herbicide napropamide in representative crops for general residue analytical methods using HPLC and LC-MS/MS which could be applied to most of crops. Analytical steps of napropamide residue as follows : Korean cabbage, green pepper, apple, mandarin, potato and soybean were selected, macerated, extracted with acetone, concentrated and partitioned with *n*-hexane. Then it was concentrated and cleaned-up through Florisil column with ethyl acetate/*n*-hexane (20:80, v/v) before concentration and analysis with HPLC-UVD. LOQ (Limit of Quantification) of napropamide was 5 ng (S/N>10) and MLOQ (Method Limit of Quantitation) was 0.05 mg/kg. Recoveries were measured at three fortification

levels (MLOQ, 10MLOQ and 100MLOQ) on crop samples and ranged 85.2-105.4% (mean recoveries) and coefficients of variation were <10% regardless of sample type.

In order to development of analytical method using LC-MS/MS, the QuEChERS method was chosen for sample preparation. LOQ of napropamide was 0.05 ng and MLOQ was 0.01 mg/kg in QuEChERS-LC-MS/MS method. Recoveries were measure at two fortification levels (MLOQ and 10MLOQ) were reasonable (71.7-106.7%) and coefficients of variation were 1.4-11.9% at Korean cabbage, green pepper, apple, mandarin, potato and soybean.

Key words: Napropamide, HPLC, LC-MS/MS, LOQ, MLOQ, Recovery, QuEChERS

***Student number*: 2010-23448**

LIST OF FIGURES AND TABLES

1. INTRODUCTION

Pesticides are applied to fruits and vegetables at various stages of cultivation and during post-harvest storage. Definition of pesticides is any substance or mixture of substances intended for preventing, destroyling, repelling or migrating any pest. Pests are organisms that are competitive to mankind or his interests in some manner. The world's main source of food is plants. They are susceptible to 80,000 to 100,000 diseases caused by viruses, bacteria, mycoplasma like organisms, rickettsias, fungi, algae and parasitic higher plants and compete with many species of weeds (Ware 2004). Pesticide can be classified based on functional groups in their molecular structure (e.g. inorganic, organonitrogen, organohalogen or organosulfur compounds), or their specific biological activity on target species(e.g. insecticides, fungicides, herbicide, acaricide, etc.) (Ahmed 2001).

In the recent years, Many people have interests in food safety. Legislations were enacted in the USA, the EU, and other countries to regulate pesticides in food products (Ahmed 2001). Maximum residue limits (MRLs) in foodstuffs have been set by government agencies to guarantee consumer safety and regulate international trade.

Analysis of pesticide residues is extremely difficult because sample matrix is complex and pesticides consist of many types of compounds. And pesticide residues exist at ppm level or lower level than ppm. Therefore, analytical methodologies employed must be capable of residue measurement at very low

levels and must also provide unambiguous evidence to confirm both the identify and the magnitude of any residues detected (Taylor et al. 2002).

1. General methods for pesticide residue analysis

Pesticides residue methods may contain several discrete steps, as is true also for analytical methods for metal, drugs, and other agents of concern when present at relatively low levels. The steps are as follows.

1) Matrix modification : Various matrix pretreatment methods are employed for foods containing pesticides residues to ensure correct samples mass to take depending on the heterogeneity of matrix. Representative portions of the solid sample (e.g. whole fruits or vegetables) are weighed; chopped, homogenized in a mortar, blender or stirrer; or sonicator with a solvent (or a sorbent) to disintegrate the matrix (Ahmed 2001).

2) Extraction : To remove as much of the analyte from the matrix as practical, with a minimum extraction of extraneous materials that might interfere in the analysis (Fong et al. 1999). The necessity of using water-miscible solvents (acetone, methanol and acetonitrile) to extract pesticide residues for high moisture products has been established.

3) Liquid-liquid partitioning : To reduce the amount of polar impurities that partition into the organic phase. Therefore partitioning is used immiscible solvents

such as water and dichloromethane, *n*-hexane, ethyl acetate. Liquid-liquid partitioning can be improved by the addition of water-soluble salts such as sodium chloride. Adjust pH can increase efficiency of partitioning. If the analyte is acidic, adjustment to pH ~3 with acid will protonate the analyte and reduce its water solubility (Fong et al. 1999).

4) Solvent evaporation : Essentially residue analytical methods require removal of solvent at some point in order to increase the concentration of analytes in solution. Several different evaporation techniques are available, each with advantage and disadvantages. The best techniques in particular situation depend on the physical and chemical characteristics of the analyte and the solvent that must be evaporated (Kim 2008).

5) Clean-up : The analyte is concentrated and purified and the bulk of interfering coextractives are removed (Fong et al. 1999). Clean-up system can remove the coextractives which were not removed by liquid-liquid partitioning step, such as lipids and pigments

6) Derivatization : Conversion of the chemical of interest into a derivative, in order to enhance extractability, clean-up, or subsequent resolution and determination steps (Fong et al. 1999). This is an optional step, required for some chemicals and some methods, but not all.

7) Resolution : The analyte is resolved from remaining coextractives, so that it may be subsequently measured without significant interference. This is usually done by some form of refined chromatography (Kim 2008).

8) Detection : Obtaining a response (usually and electronic signal) that is proportional to the amount of analyte present. Selective detection infers that the analyte will produce a signal several times higher than those originating from the background (Kim 2008).

9) Determination : Calculating an amount of analyte present by reference to a standard, ether external or internal (Fong et al. 1999).

2. Method validation

The following parameters are extracted from the published papers the specify minimum analytical method validation requirements.

1) Accuracy : It is determined (average of a replicated set of trials) by use of certified reference materials, use of reference method of known uncertainty, or use of recovery from spiked sampled. Reference material and spiked samples should be carried through the entire procedure (from matrix modification to determination). The method of fortification of spiked samples should be described (Fong et al. 1999).

2) Recovery : It can be determined by the amount of recovered added analyte over an appropriate range of concentrations. The number of replicated samples per study varies (Fong et al. 1999).

$$\text{Percent of recovery} = \text{analyte recovered} / \text{analyte added} \times 100$$

3) Calibration curve and linearity : It defined as the responses of the method to a

number of concentrations, minimum of 5 not including zero, of the analyte standards. Responses at various concentrations in pure solvents and in matrix should be studied (Fong et al. 1999). Linearity is tested assessing signal responses of target analytes over a range of concentrations (Hernando et al. 2007). A minimum linear correlation is .99((주) 랩프런티어 2004).

4) Limit of detection (LOD) : There are several ways to define the LOD. Two examples are illustrated as follows a) the mean value of the matrix blank readings plus 3 standard deviations of the mean, expressed in analyte concentration. b) The amount, expressed in ppm or ppb, equivalent to 3 times the background signal contributed by the matrix blank (Fong et al. 1999, Miller 2005).

5) Limit of quantitation (LOQ) : There are several ways to define the LOQ. The values are established by repeated analysis of the appropriate samples, not by extrapolation. The examples are illustrated as follows a) The substrate blank plus 10 deviations b) The amount, expressed in ppm or ppb, equivalent to 10 times the background signal contributed by the matrix blank (Figure 1) (Fong et al. 1999, Miller 2005).

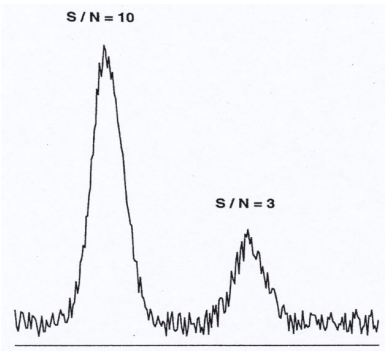


Figure 1. Illustration of two signal-to-noise(S/N) ratio, 10 and 3.(Miller 2005).

6) Precision : The precision of a method is assessed as the tightness of replicate fortifications measured by the relative standard deviation or coefficient of variation (CV). The precision of the method was evaluated by the determination of the intra- and inter-day variabilities. The precition calculated as RSC did not exceed the 15% for each concentration level tested (Hernando et al. 2007).

7) Sensitivity and MLOQ(Method Limit of Quantitation) : It is defined as the ability of the method to detect the analyte at the concentration of interest (Fong et al. 1999). To replace an existing method, the new method must be compared with the existing method.

8) Specificity : It is defined as the ability of the method to actually determine the analyte, not interfering with the compound. Chromatograms of reagent blanks and sample matrix blanks must be free of interfering peaks at the retention time(s) of interest (Fong et al. 1999).

10) Scope : Scope refers to the number of different sample matrix to which the method can be successfully applied. To extend the scope of the method, additional method validation work must be performed on the sample matrix of interest (Fong et al. 1999).

3. QuEChERS method

The QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method is an important sample preparation methodology for pesticide residue analysis that was developed in 2003 (Anastassiades et al. 2003). This methodology is based on the extraction of pesticides from the sample with acetonitrile. Removal of residual water and cleanup are performed simultaneously by using a rapid procedure, called dispersive solid-phase extraction, in which anhydrous magnesium sulfate ($MgSO_4$) and primary-secondary amine (PSA) sorbent are added before determination, reducing analysis cost, labour, waste, and glasswere and increasing sample throughput. This method, owing to many advantages over traditional techniques, has been introduced recently as an attractive alternative method for sample preparation (Wu et al. 2013).

In order to protect from a decomposition of pesticides during experimental processes because of pH of sample, many methods are studied. As most popular QuEChERS methods, CEN prEN15662 method (Anastassiades 2007) and AOAC 2007.01 method (Lehotay et al. 2007) were registered and introduced (Majors 2008, Lehotay et al. 2010, Lazartigues et al. 2011, Kwon et al. 2011).

However, this method requires high sensitivity instrument such as LC-MS/MS because of the use of small sample volume and many impurities compared to the conventional method (Lee et al. 2012).

4. Properties of napropamide

Napropamide [(R,S)-N,N-2-diethyl-2-(1-naphthyloxy)propionamide, Figure 2] is a selective systemic amide herbicide used to control a number of annual grasses and broad-leaved weeds. It inhibits root development and growth. Napropamide is applied to soils where vegetables, fruit trees and bushes, vines, strawberries, sunflowers, tobacco, olives, and mint or other crops are grown. It is available in emulsifiable concentrate, wettable powder, granules, and suspension concentrates.

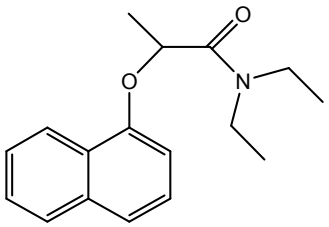


Figure 2. Structure of napropamide.

Napropamide, due to their physical and chemical properties(Table 1, (Tomlin 2006)), such as thermal instability and polarity, is difficult to determine using GC and GC/MS. Although amide group is exist in the structure, sensitivity was low because there is only one nitrogen. Napropamide is determined mainly by revered-phase HPLC with UV detection.

Table 1. Physico-chemical characteristic properties of napropamide

Common name	Napropamide (Herbicide)
Mode of action	Inhibition of cell division(inhibition of VLCFAs)
Physico-chemical Properties	Molecular weight : 271.4 Log P_{ow} : 3.3(25℃)
	Water solubility : 7.4 mg/L(25℃) Vapor pressure : 2.3×10^{-2} mPa(25℃) Stability : No decomposition occure over 16h at 100℃ Decomposed by sunlight; DT50 25.7 Stable to hydrolysis between pH4-10 at 40℃
Toxicology	LD_{50} for rats : >5000 mg/kg LC_{50} for rainbow trout : 9. 4 mg/L (96hr)
Residue	DT_{50} in aerobic lab. soil ≤ 230-670 days, in field ≤ 46-131 days MRLs : 0.1 mg/kg (Table 2)

The first order photodegradation half-life of napropamide in nine soils at 20℃ ranged from 72 to 150 days. Products were not identified. Photolysis in water was significantly faster than on soil. Photolysis in water at 25℃ and pH 7 using xenon arc irradiation gave three major photodegradation products in yield up to 20%, 27% and 9% (Chang et al., 1991). The photolysis half-life was 5.7 min and the rate

constant was 1.2×10^{-1} min^{-1}. Napropamide is stable to hydrolysis between pH 4 and 10 at 40℃(Roberts 1999).

Microbial degradation of napropamide in soil is slow. However, degradation rates may be enhanced in soils which have previously been treated with napropamide under conditions of normal agronomic use (Walker et al., 1993).

Table 2. Maximum Residue limits (MRLs) of napropamide in various crops

Crop	MRL (mg/kg)	Crop	MRL (mg/kg)
Mandarin	0.1	Korean cabbage	0.1
Potato	0.1	Cabbage	0.1
Green & red pepper	0.1	Brassica leafy vegetables	0.1
Bonnet bellflower	0.1	Sesame seed	0.1
Peanut	0.1	Chwinamul	0.1
Garlic	0.1	Tomato	0.1

While research about photodegradation and metabolism of napropamide have been studied in many field, only a few analytical studies for napropamide residue in crops were reported (Table 3).

Table 3. Analytical method of napropamide described in the literatures

Pesticide	Sample	Instrument	Reference
Napropamide	Corn and tamato	TLC	(Barrett et al. 1981)
Napropamide, bromacil	Soil	GLC	(Gerstl et al. 1983)
Napropamide	Rape, rape seed, rape straw	HPLC-UVD (265nm)	(Alawi 1984)
Napropamide	Cereals, maize, sugar beet, vegetable	GLC	(Rouchaud et al. 1991)
Napropamide	Herbicide enantiomer	HPLC-MS	(Muller et al. 1991)
Napropamide	Water	HPLC-UV-RAM (280nm)	(Chang et al. 1991)
Napropamide	Soil	HPLC-UVD (220nm) / GLC	(Walker et al. 1993)
Napropamide	Soil, water	HPLC-UVD (240nm)	(Donaldson et al. 1996)
Napropamide	Water	HPLC-UVD/ MS / NMR	(Aguer et al. 1998)

Napropamide, asulan, vamidothion, methomyl, benomyl, dimethoate, amitraz, thiophanate methyl, dichlorvos, propanil, cyanophos, fenobucarb, salithion, methidathion, pyridaphenthion, iprofenfos, isoprothiolane, malathion, fenitrothion, edifenfos, diazinon, pyrazolate, chlorpyrifos methyl, quintozene, isozathion, EPN	Serum	HPLC-DAD	(Mori et al. 1998)
Napropamide, propanil, fenobucarb, pyridaphenthion, isoprothiolane, malation, fenitrothion, edifenfos, diazinon, isoxathion	Serum, urine	HPLC-DAD	(Mori et al 1999)
Napropamide	Urine, feces, tissues	HPLC-UVD (280nm)	(Pahari et al. 2001)

Napropamide, carbendazim, diethofencarb, azoxystrobine, bupirimate	Strawberry	HPLC-DAD	(Falqui-Cao et al. 2001)
Napropamide, diphenamide, metolachlor	Tabacco leaves	HPLC-UVD (230nm)	(Liu et al. 2005)
Napropamide	Soil, made tea	HPLC-UVD (240nm)	(Biswas et al. 2007)
Napropamide	Cellulose, silica gel	HPLC-UVD (220nm, 300nm) / GC-MS	(Silva et al. 2008)
Napropamide	Rape seed	HPLC-UVD (230nm)	(Cui et al. 2010)
Napropamide	Soil, plant	HPLC-UVD (230nm)	(Zhang et al. 2010)
Napropamide, azoxystrobin, carbendazim, clomazone, diflufenican, dimethachlor, fluroxypyr, iprodion, isoproturon, mesosulfuron-methyl, metazachlor, quizalofop,	Fish muscle	LC-MS/MS	(Lazartigues et al. 2011)

thifensulfuron-methyl			
Napropamide, acibenzolar-S-methyl, metribuzin, propamocarb hydrochloride and thiamethoxam	Soil	LC-MS/MS	(Myresiotis et al. 2012)
Napropamide	Soil	HPLC-UVD (288nm)	(Sadegh-Zadeh et al. 2012

5. The purpose of the study

The purpose of the present study is to develop HPLC and LC-MS/MS method for determination of napropamide at concentration lower than maximum residue limits (MRLs). As crop samples for study, representative crops were selected among crop groups [fruits (apple and mandarin), vegetables (green pepper and Korean cabbage), beans and oil crops (soybean) and potatoes (potato)]. Extraction, partitioning, clean-up, and derivatization efficiency of napropamide in Korean cabbage, green pepper, apple, mandarin, potato and soybean were investigated by HPLC and LC-MS/MS.

2. MATERIALS AND METHODS

1. Materials

1.1 The subject pesticides

Napropamide (99.3%) was purchased from Dr. Ehrenstorfer GmbH.

1.2 Standard solutions

Each analytical standard was dissolved in acetonitrile to make concentrated stock solution at concentration of 1000 mg/L. The working solutions were prepared by appropriate dilutions of the stock solutions with acetonitrile.

1.3 Chemicals

Acetonitile and methanol were HPLC grade and purchased from Burdick and Jackson®. Acetone, dichloromethane, ethyl acetate and *n*-hexane were EP grade and purchased from Duksan reangent and chemical co., Ltd. Sodium sulfate (GR grade) and sodium chloride (GR grade) were from Junsei Chemical Co. Ltd. (Japan). Acetic acid (GR grade) was purchased from Sigma Aldrich co., Ltd. Florisil(60-100mesh) was purchased from Fluka™ and activated by drying at 130℃ over 5 hours. Filter papers (GF/A) were from Whatman International Ltd. (Maidstone, England). ULTRA QuECh™ Extraction Packet (EN, MM)(4 g MgSO4, 1 g NaCl, 1 g trisodium citrate dihydrate, 0.5 g disodium hydrogencitrate

sesquihydrate) and dSPE General (EN & MM – 1 mL Aliquot)(150 mg MgSO4, 25 mg PSA) were purchased from ULTRA Scientific (North Kingstown, RI, USA).

1.4 The subject crops

Korean cabbage, green pepper, apple, mandarin, potato and soybean of "residue-free (i.e. no pesticide residues are present above the detection limits of the multi-residue method)" grade were purchased from market. They were chopped, macerated and kept in a freezer at a temperature below -20℃ in polyethylene bags.

2. Method

2.1 Development of an improved analytical method for napropamide using HPLC-UVD

2.1.1 Establishment of sample preparation procedure of napropamide

2.1.1.1 Establishment of clean-up method with glass column chromatography

2.1.1.1.1 Preliminary experiment for clean-up solvent system

A glass column (35 × 1.5 i.d. cm) was filled with active Florisil (60-100 mesh, 10 g) and added anhydrous sodium sulfate (3 g). Then the column was conditioned with *n*-hexane (100 mL) before loading the napropamide standard solution (5 mL, 1 mg/L). The column was eluted with 50 mL of 5, 10, 15, 20, 25 %

acetone/*n*-hexane mixture in sequence. Ethyl acetate/*n*-hexane mixture was followed the same route. Each eluate was evaporated under 40℃ to dryness and the residue was dissolved with acetinitile (5 mL) and analyzed with HPLC.

2.1.1.1.2 Establishment of clean-up system

The Florisil column was conditioned with *n*-hexane (100 mL) (that is the Florisil column) before loading the napropamide standard solution (5 mL, 1 mg/kg). The column was eluted with washing solution and with elution solutions of different composition of acetone/*n*-hexane mixture in sequence (50 + 50 + 50 mL). And it was treated by same process with mixture of ethyl acetate/*n*-hexane (Table 4). Each eluate was evaporated under 40℃ to dryness and the residue was dissolved with acetonitrile (5 mL) and analyzed with HPLC.

Table 4. Condition of washing and elution solutions for column chromatography

Condition 1			Condition 2		
Acetone/*n*-Hexane		Volume(mL)	Ethyl acetate/*n*-Hexane		Volume(mL)
Washing	5:95	100mL	Washing	10:90	100mL
Elution	15:85	0-50mL	Elution	20:80	0-50mL
		50-100mL			50-100mL
		100-150mL			100-150mL

2.1.1.2 Selection of liquid-liquid partitioning system

An aliquot of napropamide solution (0.5 mL, 10 mg/L) was added to water (25 mL) and stood about 30 minutes. After that, water (50 mL) and saturated sodium chloride solution (50 mL) were added. The mixture were transferred in separatory funnel and extracted with each portion of three solvents (dichloromethane, *n*-hexane and ethyl acetate, 100 + 50 mL). Organic phases were dried with anhydrous sodium sulfate and evaporated under 40℃ to concentration. The residue was dissolved with acetonitrile (5 mL) and analyzed with HPLC.

2.1.2 Establishment of chromatographic condition

2.1.2.1 Selection of detection wavelength of HPLC

HPLC analysis was performed using an Agilent HPLC 1100 series system equipped with G1311A quaternary pump, G1322A degasser, G1313A autosampler, G1316A column oven, and G1315A DAD (diode-array detector). G1314A VWD (variable wavelength detector) was used and the detection wavelengths at 235 nm. The flow rate was 1 mL/min. The analytical column was an Agilent Eclipse XDB-C18 column (250 mm × 4.6 mm i.d., 5 μm particle) and column temperature was 35℃. The flow rate was 1 mL/min and injection volume was 20 μL.

Aliquot (20 μL) of napropamide standard solutions (1 mg/kg) was analyzed with HPLC-DAD (diode array detector, 190-400 nm) under isocratic elution for selection of detection wavelength.

2.1.2.2 Establishment of a HPLC condition for the separation of napropamide in Korean cabbage, green pepper, apple, mandarin, potato and soybean

For the separation of napropamide from interfere peak in sample matrices, crop samples were analyzed with two kind of mixture that acetonitrile-water [60:40 (v/v)] and methanol-water [75:25 (v/v)] as mobile phases.

2.1.2.3 Retention factor of napropamide of chromatogram

Retention factor(capacity factor, *k*) was calculated from equation using retention time (t_r) and adjusted retention time (t_r') (Equation 1).

Equation 1. $k = t_r' / t_m$

t_r = retention time (min)

t_m = retention time of a non-retained compound (min)

$t_r' = t_r - t_m$ = adjusted retention time (min)

2.1.2.4 Measurement of column efficiency

: Number of theoretical plate (*N*) and Height equivalent to a theoretical plate (*H*)

N was calculated using t_r and peak width (Rood 2007) (Equation 2). *N* and column length was used for calculation of *H* (Rood 2007) (Equation 3).

Equation 2. $N = 5.545(t_r / W_h)^2$

W_h = peak width at half height

Equation 3. H (mm) = column length (mm) / N

2.1.3 Method validation

2.1.3.1 Measurement of LOD and LOQ of napropamide for HPLC-UVD

LOD and LOQ were determined as the minimum concentration of analyte providing S/N ratio of 3 and 10, respectively. Napropamide standard solutions (0.01, and 0.05 mg/L) were analysis by HPLC-UVD. And the chromatograms were used to calculate the S/N ratio.

2.1.3.2 Assessment of reproducibility

Napropamide standard solution (0.25 mg/L) was analyzed by seven replicates. Variations of retention time (t_r), peak area and peak height were examined.

2.1.3.3 Calibration curve and linearity

The standard solution at concentration of 0.05, 0.1, 0.5, 1 and 5 mg/L were analyzed by HPLC and linearity was measured.

2.1.3.4 Calculation of MLOQ (Method Limit of Quantitation)

MLOQ is calculated by Equation 4 according to the sample amount, extraction procedure, rate of dilution and instrumental system.

Equation 4.

$$\text{MLOQ (mg/kg)} = \frac{\text{LOQ (ng)} \times \text{Final volume (mL)} \times \text{Dilution factor}}{\text{Injection volume (}\mu\text{L)} \times \text{Initial sample weight (g)}}$$

2.1.3.5 Recovery test of napropamide in crop samples

The macerated crop samples (25 g) of Korean cabbage, green pepper, apple, mandarin, potato and soybean were fortified with napropamide standard solution 0.05 (MLOQ), 0.5 (10 MLOQ) and 5 (100MLOQ) mg/kg levels and the samples were extracted with shaking at 200 rpm for 1 hour with acetone (100 mL). The mixture was filtered under reduced pressure through a Whatman GF/A filter paper and the filter cake was rinsed with acetone (30mL). The filtrates were combined and concentrated under vacuum at 40℃. The concentrate was dissolved in *n*-hexane (100 + 50 mL) and partitioned with water (50 mL) and saturated sodium chloride solution (50 mL). The upper layer was dried over anhydrous sodium sulfate and concentrated under vacuum at 40℃. The residue was dissolved in *n*-hexane (5 mL). The Florisil column was conditioned with *n*-hexane (100 mL).

The column was washed with 100 mL of ethyl acetate/*n*-hexane (10 : 90, v/v), after loading the extract and eluted with 100 mL of ethyl acetate/*n*-hexane (20 : 80, v/v). The eluate was concentrated with evaporator under 40℃ and dissolved with acetonitrile (5 mL) and analyzed with HPLC-UVD.

2.2 Development of an improved analytical method for napropamide using LC-MS/MS by QuEChERS

2.2.1 Optimization of ESI(+) (electrospray ionization, positive) and MS/MS condition

In order to optimize the best MS/MS condition, the capillary voltage, RF loading, CID excitation voltage, needle voltage were changed from 0 to 300 volts, from 0 to 300%, from 0 to 5.0 volts, from 0 to 5000 volts, respectively, while standard solution of napropamide (1 mg/L) was introduced into the system by direct flow injection mode.

2.2.2 LC-MS/MS analysis

The HPLC system was connected to ion trap mass spectrometer (Varian. 500-MS IT Mass Spectrometer) equipped with an electrospray ionization (ESI) source.

Napropamide was separated using HPLC (Agilent 1100 series, G1311A quaternary pump, G1322A degasser, G1313A autosampler, G1316A column oven,

USA) equipped with a C18 column (Phenomenex, 50 × 2.1 mm, 2.6 μm, USA). Column temperature was maintained 35℃. The HPLC mobile phase consisted of 0.1% formic acid (used as proton source) in acetonitrile and water at a flow rate of 0.2 mL/min and injection volume was 5 μL. The gradient elution of the mobile phase was performed to analyze napropamide (Table 5) with LC-MS/MS (Table 6). Total ion chromatogram (TIC) for napropamide under ESI(+) full scan mode was obtained by scanning from 100 to 300 *m/z*.

Table 5. HPLC condition of MS/MS for napropamide

Column	Phenomenex Kinetex C18 2.6μ (50 × 2.1 I.D. mm)	
Column temperature	35℃	
Mobile phase	A : 0.1% formic acid in water	
	B : 0.1% formic acid in acetonitrile	
	Time (min)	%B
	1.5	60
	3	90
	9	90
	10	60
	15	60
Injection volume	5 μL	
Flow rate	0.2 mL/min	

Table 6. Full scan mode condition for napropamide on LC-MS/MS

Capillary voltage	31.9 volts
RF Loading	82.9 %
Needle voltage	3500 volts
Nebulizer gas pressure	40 psi
Drying gas pressure	30 psi
Drying gas temperature	350℃

2.2.3 Method validation

2.2.3.1 Measurement of LOD and LOQ of napropamide for LC-MS/MS

Matrix matched standard solutions (0.003, 0.005 and 0.01 mg/L) of Korean cabbage, green pepper, apple, mandarin, potato and soybean were analyzed by LC-MS/MS. LOD and LOQ were determined as the minimum concentration of analyte providing S/N ratio of 3 and 10, respectively.

2.2.3.2 Calibration curve and linearity

Matrix matched standard solutions(0.003, 0.005, 0.01, 0.02, 0.1 and 0.2 mg/L) were prepared from serial diluted standards of napropamide with acetonitrile (0.015, 0.01, 0.05, 0.1, 0.5 and 1 mg/L) and then analyzed with LC-MS/MS. For making the matrix matched standard, 40 μL of each level of standards diluted with

acetonitrile were mixed with 160 µL of unfortified crop samples which were processed by QuEChERS method.

The linearity was examined by R^2 value.

2.2.3.3 Calculation of MLOQ (Method Limit of Quantitation)

MLOQ is calculated by Equation 4 according to the sample amount, extraction procedure, rate of dilution and instrumental system.

2.2.3.4 Recovery test of napropamide in crop samples by using QuEChERS method

The crop samples were fortified with napropamide standard solution to reach at 0.01 and 0.1 mg/L (MLOQ and 10MLOQ) level of concentration. The QuEChERS method was chosen as a sample preparation method for LC-MS/MS analysis (5 µL) with optimized MS/MS mode.

Homogenized crop samples (10g) were weighed into a 50 mL Teflon centrifuge tube and 10 mL of acetonitrile were added. The tubes were shacked for 10 min and then 4 g MgSO4, 1 g NaCl, 1 g trisodium citrate dihydrate, 0.5 g disodium hydrogencitrate sesquihydrate were added. The tubes were capped immediately and shacked for 10 min and centrifuged at 3000 rpm for 10 min. Then 1 mL of the upper layer (acetonitrile) was transferred into a 2.0 mL dispersive-SPE tubes containing 150 mg MgSO4 and 25 mg PSA for cleanup. Then the tubes were capped and vortexed for 2 min. The tubes were centrifuged for 5 min at 15000

rpm. 160μL of the supernatants were transferred into 400 μL insert tube in analytical vial and 40 μL of acetonitrle was added.

From the full scan spectra, the most abundant ion (base ion) was selected as precursor ion for MS/MS.

3. RESUTS AND DISCUSSION

1. Development of an improved analytical method for napropamide using HPLC-UVD

1.1 Establishment of sample prepare procedure of napropamide

1.1.1 Establishment of clean-up method with glass column chromatography of napropamide

As a first step for sample preparation procedure clean-up procedure was examined with glass column chromatography. Adsorption chromatography is generally used for clean-up method in pesticide residue analysis. It depends on the existence of weaker van der Waals forced and/or hydrogen bonding (Fong et al. 1999). The interfering coextractives (e.g. lipids and pigments) which were not removed by liquid-liquid partitioning step could be removed by column chromatography step.

Florisil, silica gel and alumina were used traditionally as column chromatography sorbents. In this study, Florisil, the most popular metarial for clean-up in pesticide analysis, was used for absorption column chromatography. In preliminary tests, 5, 10, 15, 20, 25% acetone (or ethyl acetate)/*n*-hexane solution were eluted in sequence after loading of napropamide. As a result, 15-20% ethyl acetate/*n*-hexane solution gave good recovery (105.3%) (Table 7).

Table 7. Recovery rate by sequential elution of acetone/*n*-hexane and ethyl acetate/*n*-hexane

Acetone/*n*-Hexane		Recovery (%)	Ethyl acetate/*n*-Hexane		Recovery (%)
5 : 95	50 mL	-	5 : 95	50 mL	-
10 : 90	50 mL	89.4	10 : 90	50 mL	-
15 : 85	50 mL	6.1	15 : 85	50 mL	12.2
20 : 80	50 mL	-	20 : 80	50 mL	93.1
25 : 75	50 mL	-	25 : 75	50 mL	-
Total		95.5	Total		105.3

For the selection of washing and elution solutions in actual procedure, the experiment was performed with two types of solvent conditions (Table 9).

Acetone/*n*-hexane mixture was too strong so the pesticide was eluted in washing step. On the other hand ethyl acetate/*n*-hexane elution solvent condition gave good recovery (Table 8). And washing solution with 10% ethyl acetate/*n*-hexane mixture removed impurities enough. Therefore, clean-up conditions by washing with 10% ethyl acetate/*n*-hexane and elution with 20% ethyl acetate/*n*-hexane was chosen for recovery test.

Table 8. Recovery rate of two condition of eluents

Acetone/*n*-Hexane		Recovery(%)	Ethyl acetate/*n*-Hexane		Recovery(%)
Washing	100mL	0.8	Washing	100mL	-
Elution	0-50mL	89.8	Elution	0-50mL	57.6
	50-100mL	-		50-100mL	37.7
	100-150mL	-		100-150mL	-
Total		90.6	Total		95.3

1.1.2 Liquid-liquid partitioning of napropamide

After establishment of clean-up procedure successfully, liquid-liquid partitioning system was examined. Liquid-liquid partitioning of sample extract between immiscible solvent, such as water versus dichloromethane, *n*-hexane and ethyl acetate removes the potentially interfering coextactives (e.g. carbohydrates) (Fong et al. 1999). Such partitioning can be improved by the addition of water-soluble salts such as sodium chloride. As more 'salt' dissolves in the aqueous phase, more of the pesticide is partitioned into the organic phase (Fong et al. 1999).

In this study, three organic solvents such as dichloromethane, ethyl acetate and *n*-hexane, were used with water. The three solvents were partitioned 100 mL first and than 50 mL additionally (Table 7). As a result, napropamide was well partitioned with *n*-hexane, enough to give recovery 98.0%. Therefore, *n*-hexane (100 + 50 mL) was selected as organic solvent for liquid-liquid partitioning

system.

Table 9. Efficiency of liquid-liquid partitioning with three different solvents

Solvents	Recovery (%)
n-Hexane	98.0
Ethyl acetate	94.4
Dichloromethane	94.1

1.2 Establishment of chromatographic condition

1.2.1 Establishment of a detection wavelength HPLC condition for the analysis of napropamide

Most analytical methodologies for residue analysis are based on the use of gas chromatography and liquid chromatography. However, GC determinations were proper due to poor resolution because of the chemical properties of napropamide.

Napropamide was analysed various wavelength in previous studies. To find out suitable detection wavelength, full UV spectrum of napropamide was recorded through DAD. In this study, considering UV cutoff of mobile phases 235 nm was selected for detection, even though λmax was 214 nm from DAD spectrum (Figure 3).

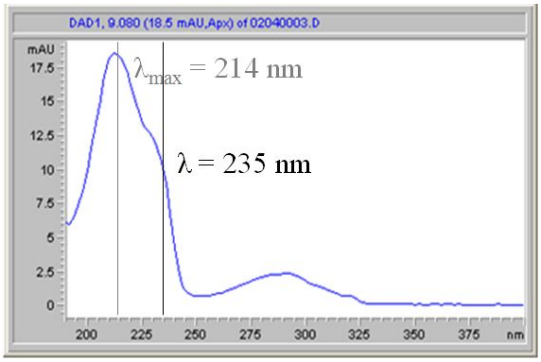


Figure 3. UV spectrum of napropamide.

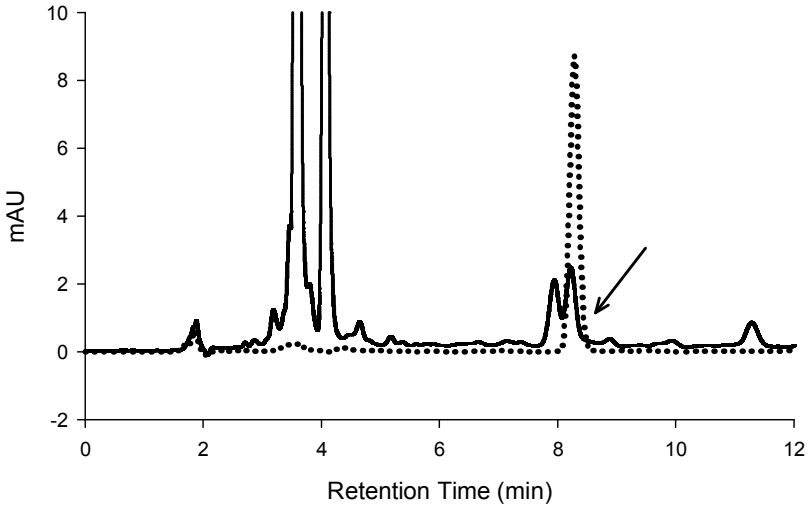
1.2.2 Establishment of a HPLC condition for the separation of napropamide in Korean cabbage, green pepper, apple, mandarin, potato and soybean

Selected mobile phase conditions were acetonitrile-water of 60:40 (v/v) was for Korean cabbage, apple, mandarin, potato and soybean samples, methanol-water of 75:25 (v/v) was for green pepper samples.

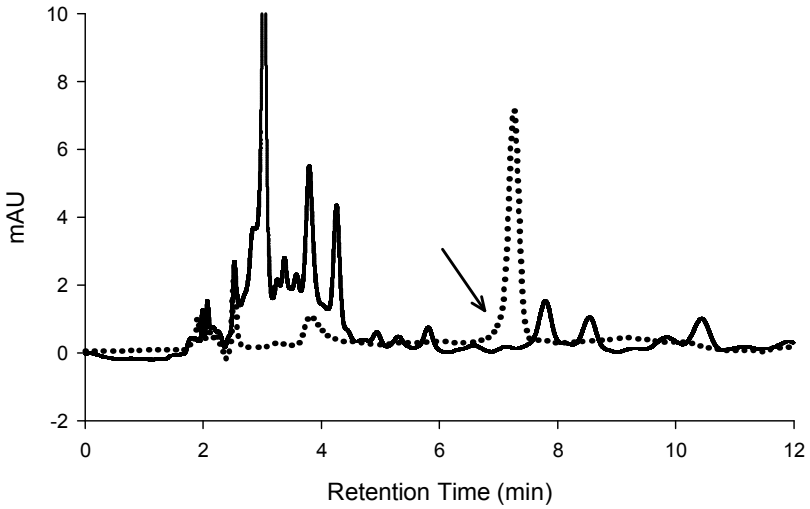
Because napropamide peak was overlapped with coextractives´ peak. For example, in the green pepper samples napropamide peak was overlapped with other peaks when eluted by acetonitrile-water 60:40 (v/v) (Figure 4).

1.2.3 Efficiency of napropamide peak in HPLC chromatogram

The retention times were 8.47 min in Korean cabbage, apple, mandarin, potato and soybean, 7.08 min in green pepper of napropamide. There were not shown interfered matrix peaks (Figure 11-14).



(A)



(B)

Figure 4. Chromatogram of napropamide (broken line) and green pepper control (straight line). (A) Acetonitrle-water 60:40, (B) methanol-water 75:25.

Retention factor (*k*) is commonly called the partition ratio or capacity factor, and is proportional to the time a compound spends in the stationary phase (t_r') relative to the time it spends in the mobile phase (t_m) (Rood 2007). There were 77.7 and 150.3 in green pepper and the others, respectively (Table 10).

1.2.4 Column efficiency for napropamide

: Number of theoretical plate (*N*) and height equivalent to a theoretical plate (*H*)

N and *H* were shown the efficiency (Rood 2007, McNair and Miller 1998). The shorter each theoretical plate, the greater the number that fits into a unit length of column, thus the greater the number of total theoretical plate per meter. High efficiency columns have small values of *H* (Rood 2007).

N were 27171 in Korean cabbage, apple, mandarin, potato and soybean, and 14181 in green pepper. Thus, *H* were 0.009 and 0.018 mm in each crop samples (Table 10).

Table 10. Retention times (t_r), retention factor (*k*), number of plates (*N*) and height of theoretical plate (*H*) of napropamide(each analytical condition)

Crops	t_r (min)	t_m (min)	t_r'	*k*	N	H (mm)
Korean cabbage, apple, mandarin, potato and soybean	8.47	0.056	8.414	150.3	27171	0.009
Green pepper	7.08	0.09	6.99	77.7	14181	0.018

1.3 Method validation

1.3.1 LOD (Limit of Detection) and LOQ (Limit of Quantitation) of napropamide

LOD and LOQ express the sensitivity of instruments (Fong et al. 1999, Miller 2005). From the results of analysis of several concentrations, 1ng was observed as practicable LOQ. However, in the light of many interfering substance from various crops, and further research, 1 ng was determined as LOD (Figure 5). LOQ could be calculated by multiply LOD by 5.

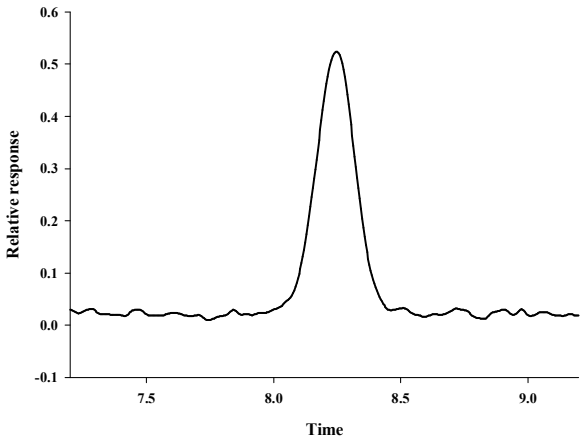


Figure 5. LOD of napropamide (0.05 mg/kg).

1.3.2 Reproducibility of napropamide

Amount of LOQ level (1 LOQ) of napropamide solution (5 ng) was analyzed 7 times for reproducibility study (Table 11). Good reproducibility was observed with small coefficient of variation (0.11-1.26%) for retention time (t_r), peak area and peak height, providing the stability and the reproducibility of instrument and analysis.

Table 11. LOQ and reproducibility of napropamide

LOQ	Reproducibility		
		Average	C.V (%)[a)]
5 ng	t_r (min)	8.24	0.11
	Area	5.54	1.71
	Height	5.07	1.26

[a)]C.V (Coefficient of variation, %) = Standard deviation / Average × 100

1.3.3 Linearity of calibration curve of napropamide

Good linearities were achieved between 0.05 and 5 mg/kg of napropamide standard solutions, with coefficients of determination 0.9999 (Figure 6-7). The regression equations were y = 103.3581x – 1.4408 for Korean cabbage, apple, mandarin, potato and soybean, y = 95.7015x – 2.3327 for green pepper.

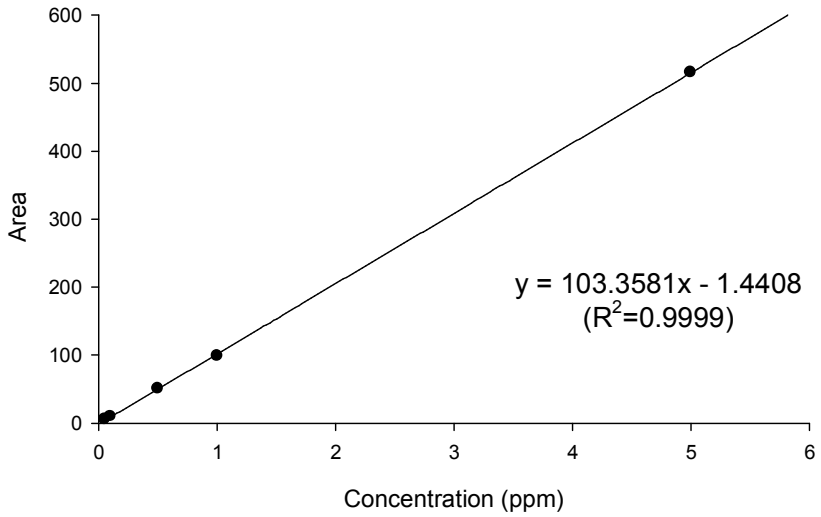


Figure 6. Calibration curve of napropamide for the analysis of Korean cabbage, apple, mandarin, potato and soybean samples.

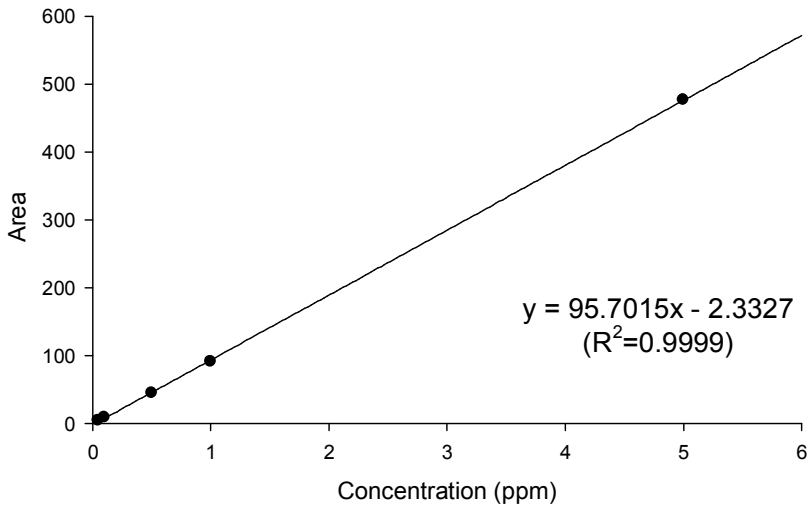


Figure 7. Calibration curve of napropamide for the analysis of green pepper samples

1.3.4 Calculation of MLOQ (Method Limit of Quantitation)

MLOQ (Method Limit of Quantitation) is calculated using LOQ, sample size and dilution factor of analytical method (Equation 4).

$$\text{MLOQ (mg/kg)} = \frac{5\ \text{ng} \times 5\ \text{mL}}{20\ \mu\text{L} \times 25\ \text{g}} = 0.05\ \text{mg/kg}$$

MLOQ value (0.05 mg/kg) satisfied criteria of KFDA(Korea Food and Drug Administration) which are below 0.05 mg/kg or half of MRL (이영득 2009).

1.3.5 Recoveries of napropamide from crop samples (accuracy and precision)

Recovery test can provide accuracy and precision of method validation by recovered rate(%) and C.V(coefficient of variation, %) (Fong et al. 1999).

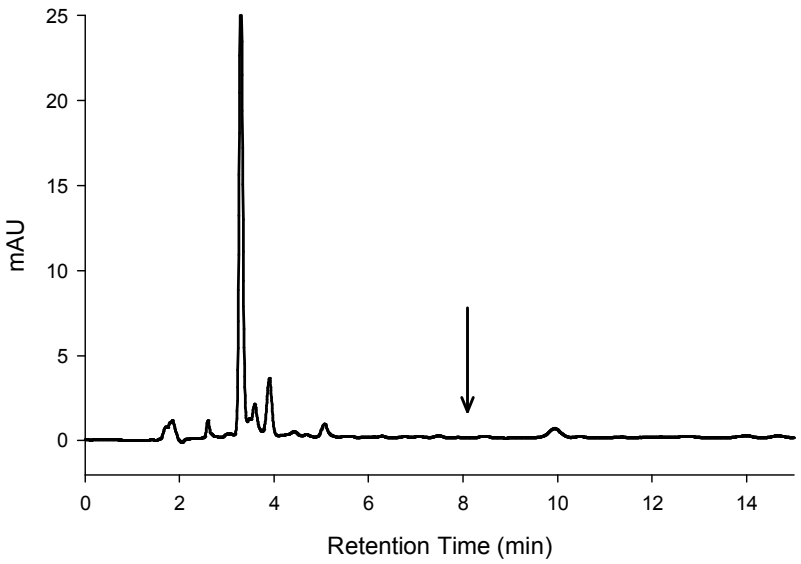
Untreated samples were spiked with MLOQ, 10MLOQ and 100MLOQ (0.05, 0.5 and 5 mg/kg) of napropamide standard solutions, and the analysis was performed using the established method of extraction, partitioning, and clean-up to give reasonable recoveries (85.2-105.4%) and C.V (0.3-4.2%) (Table 12, Figure 8-13).

Table 12. Recovery and MLOQ for napropamide in crops

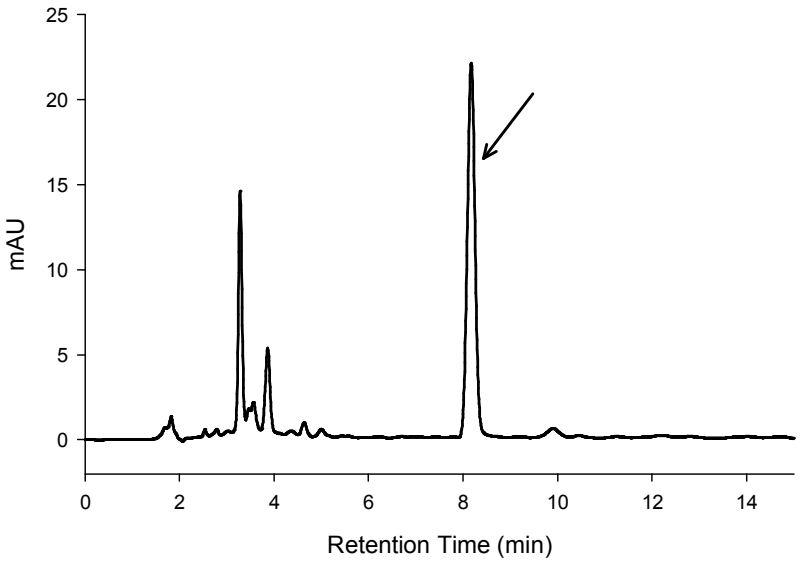
Fortified level (mg/kg)	Recovery (%)a) / CV (%)b)						MLOQ (mg/kg)
	Korean Cabbage	Green Pepper	Apple	Mandarin	Potato	Soybean	
0.05	94.9/1.9	87.0/1.8	95.1/2.8	95.3/1.3	105.4/1.7	95.7/1.2	0.05
0.5	96.0/1.1	91.1/1.0	96.6/3.1	90.4/4.2	96.2/0 6	88.9/1.6	
5	95.7/0.8	88.7/1.4	92.6/0.6	90.6/0.3	91.8/1.8	85.2/1.9	

a) Average of triplicate

b) Coefficient of variation, standard deviation / mean × 100

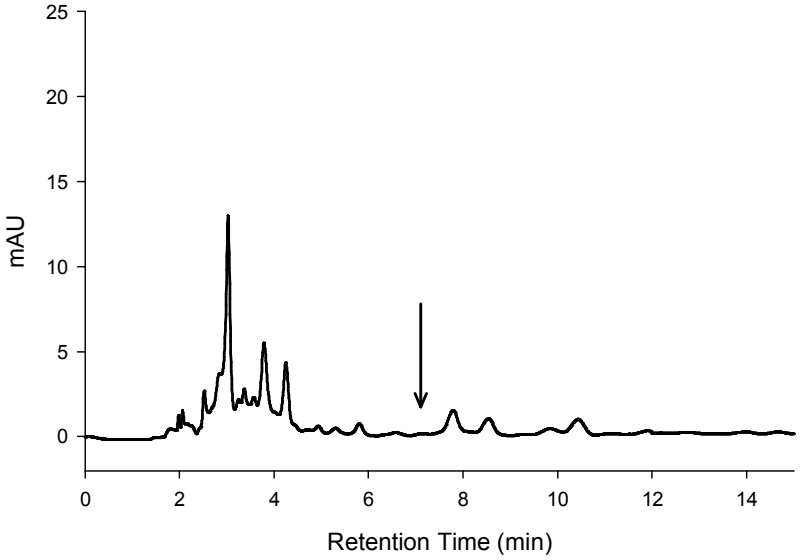


(A)

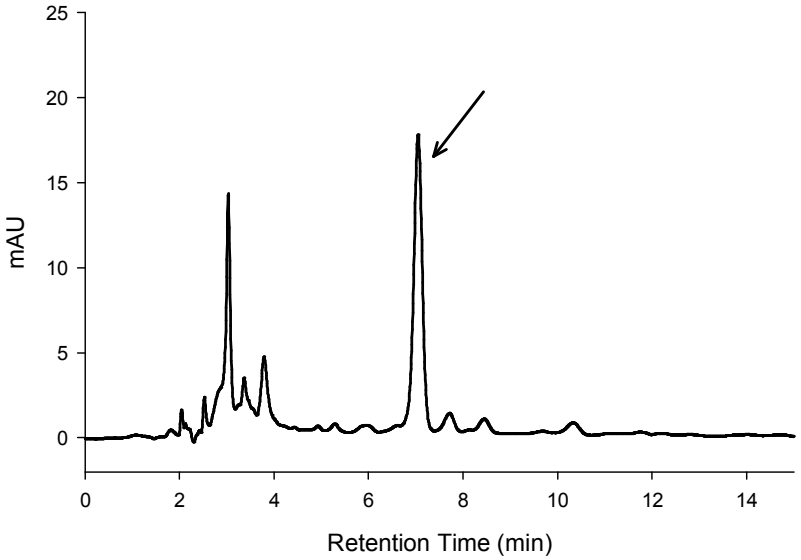


(B)

Figure 8. Chromatograms of control (A) and recovery (B) napropamide in Korean cabbage extracts (fortified at 0.5 mg/kg).

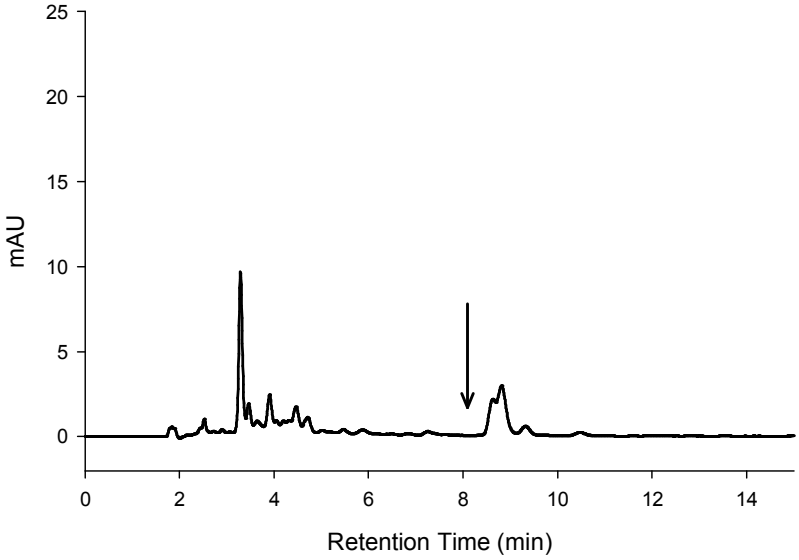


(A)

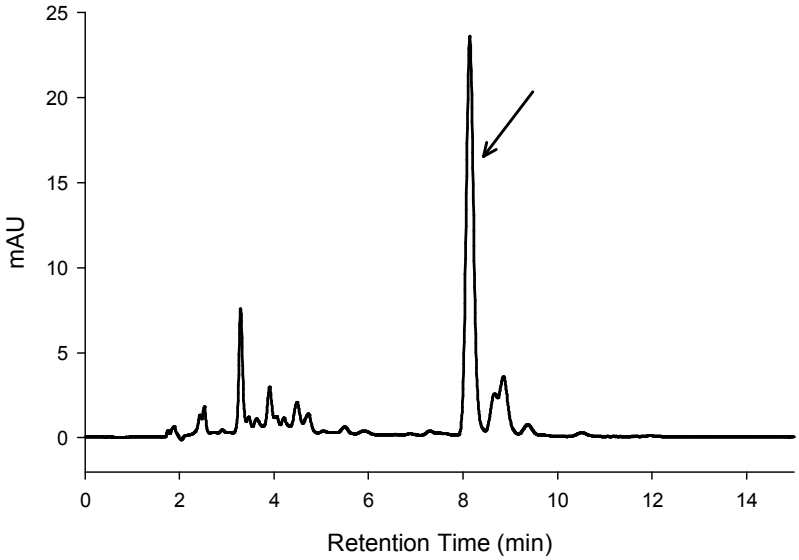


(B)

Figure 9. Chromatograms of control (A) and recovery (B) napropamide in green pepper extracts (fortified at 0.5 mg/kg).

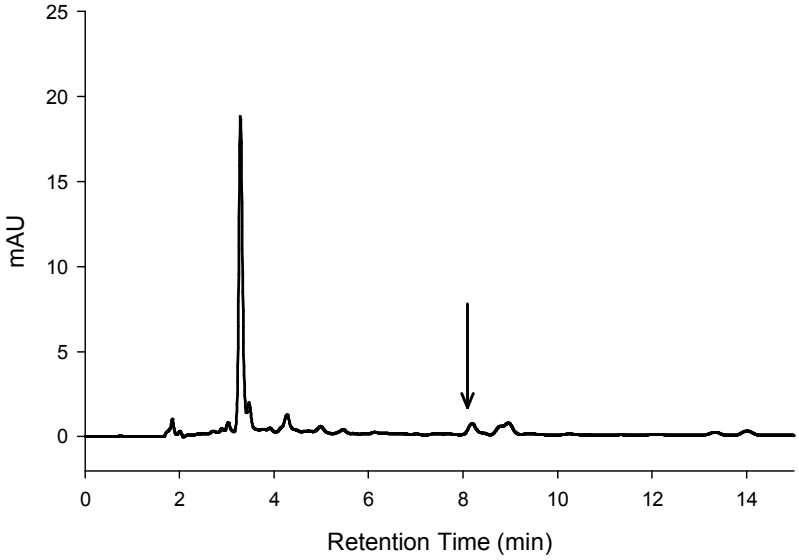


(A)

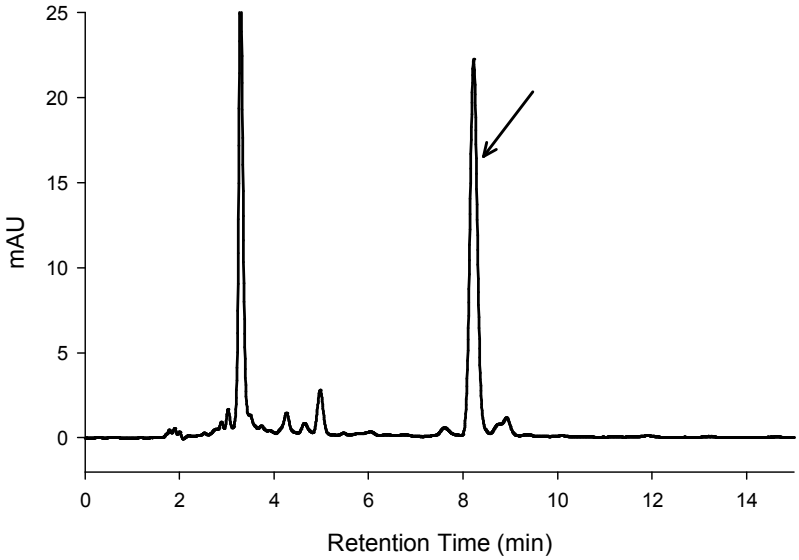


(B)

Figure 10. Chromatograms of control (A) and recovery (B) napropamide in apple extracts (fortified at 0.5 mg/kg).

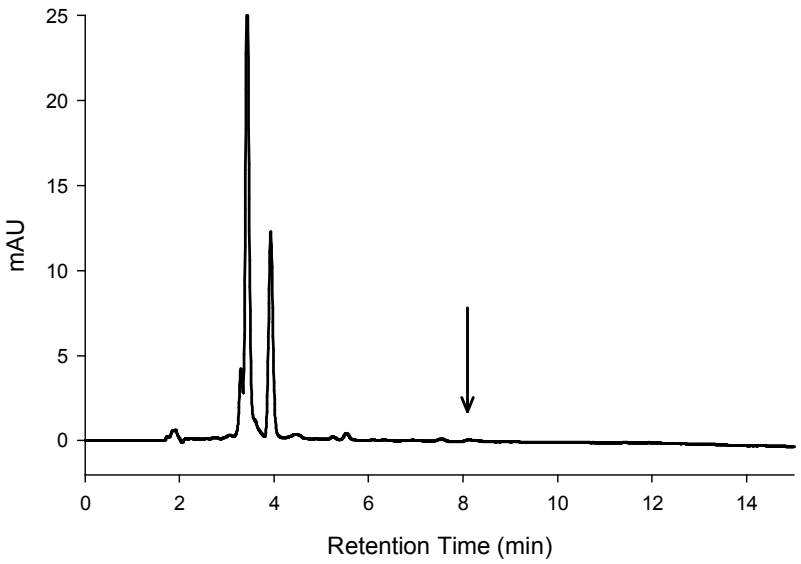


(A)

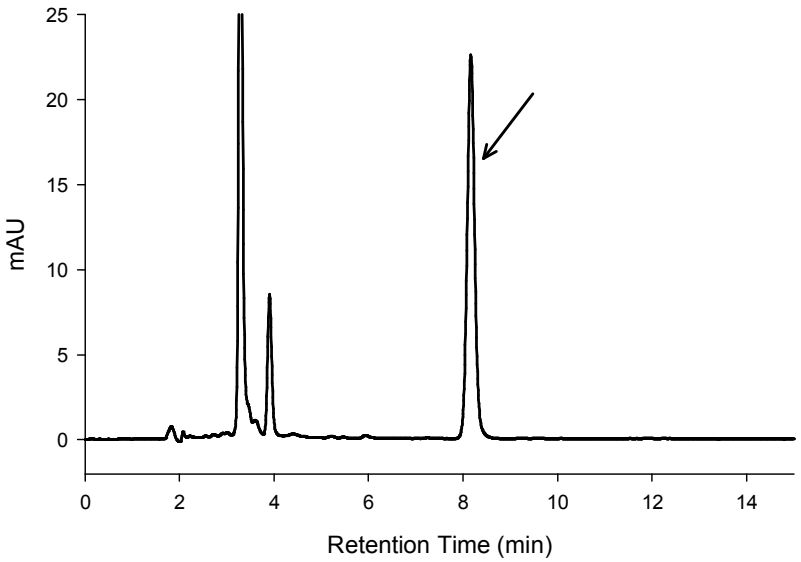


(B)

Figure 11. Chromatograms of control (A) and recovery (B) napropamide in mandarin extracts (fortified at 0.5 mg/kg).

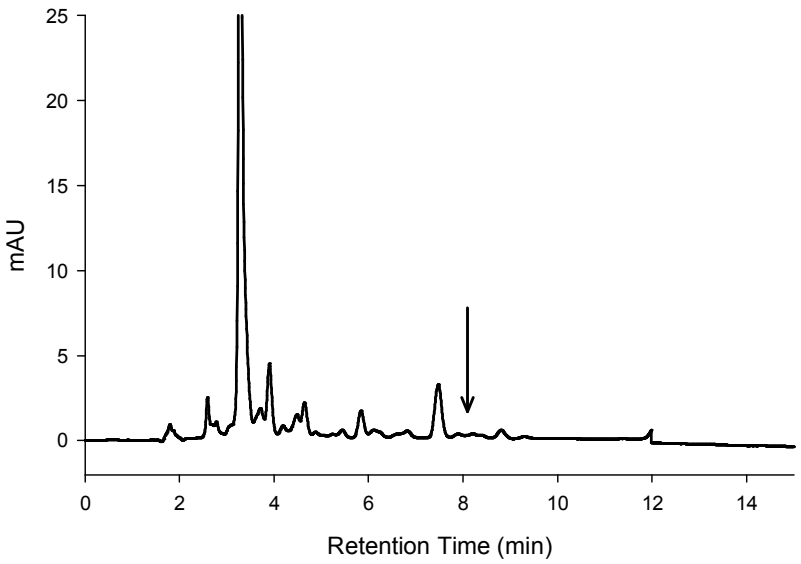


(A)

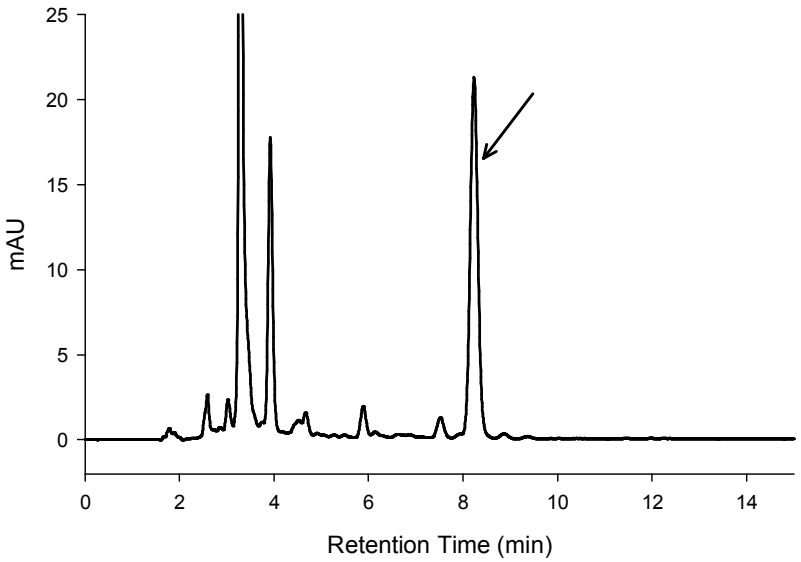


(B)

Figure 12. Chromatograms of control (A) and recovery (B) napropamide in potato extracts (fortified at 0.5 mg/kg).



(A)



(B)

Figure 13. Chromatograms of control (A) and recovery (B) napropamide in soybean extracts (fortified at 0.5 mg/kg).

2. Development of an improved analytical method for napropamide using LC-MS/MS by QuEChERS

2.1 Optimization of MS/MS condition for napropamide (VARIAN; LC-MS/MS III Operation Manual; MS Workstation version 6)

For optimum performance of MS/MS, four parameters (Capillary Voltage, RF Loading, Excitation Amplitude and Needle Voltage) need to be tuned.

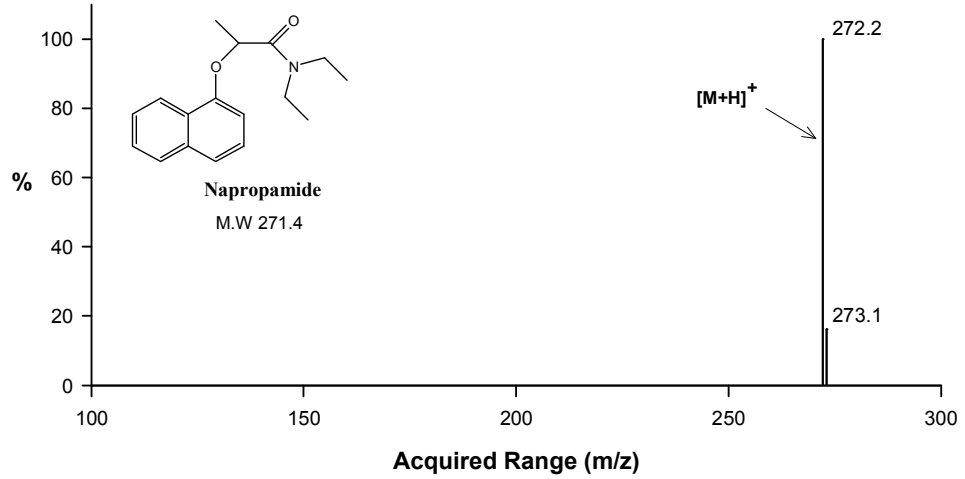
From full scan spectra, base ion of napropamide was selected as precursor ions (Figure 14; A). It was the protonated molecule ion of $[M+H]^+$ for napropamide (*m/z* 271).

In order to optimize the best MS/MS condition for napropamide, solution of napropamide was injected directly in the system and the products ion from MS/MS for napropamide from the precursor ion was identified (Figure 14; B). Optimized condition for capillary voltage, RF loading, excitation amplitude and needle voltage was established (Table 13).

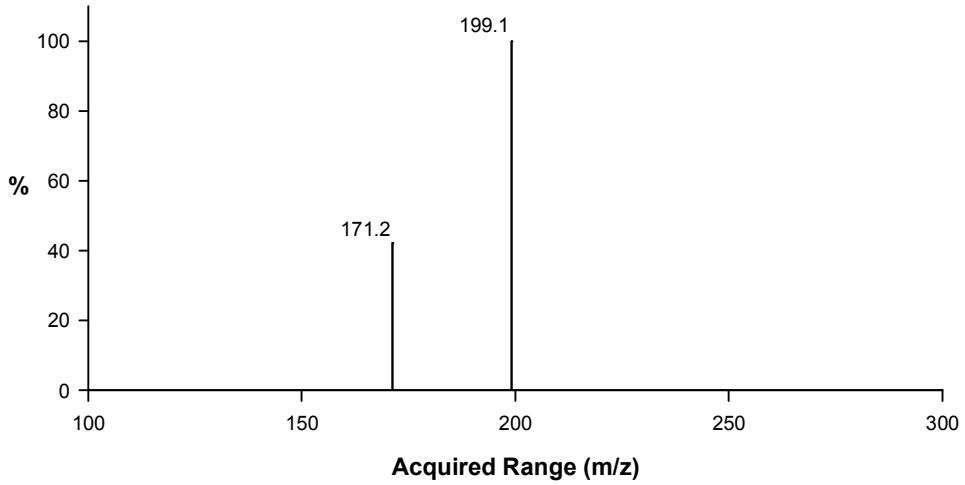
199.1 was chosen as quantification ion when analyzed samples, and 171.2 was as qualitative ion.

Table 13. Optimization condition of MS/MS for the analysis of napropamide

Capillary voltage (volts)	RF loading (%)	Excitation amplitude (volts)	Needle voltage (positive)	Precursor ions (*m/z*)	Product ion (*m/z*)
31.9	82.9	1.0	3500	272.2	199.1



(A)



(B)

Figure 14. Full scan spectrum (A) and MS/MS spectrum (B) of napropamide.

2.2 Establishment of a HPLC condition for LC-MS/MS

The separation of napropamide with crops was carried out with a solvent gradient consisting of 0.1% formic acid in acetonitrile and 0.1% formic acid in water on C18 column. Formic acid was used as a proton source. Detection was made by ESI(+) MS parameter to obtain total ion chromatogram (TIC) (Figure 15).

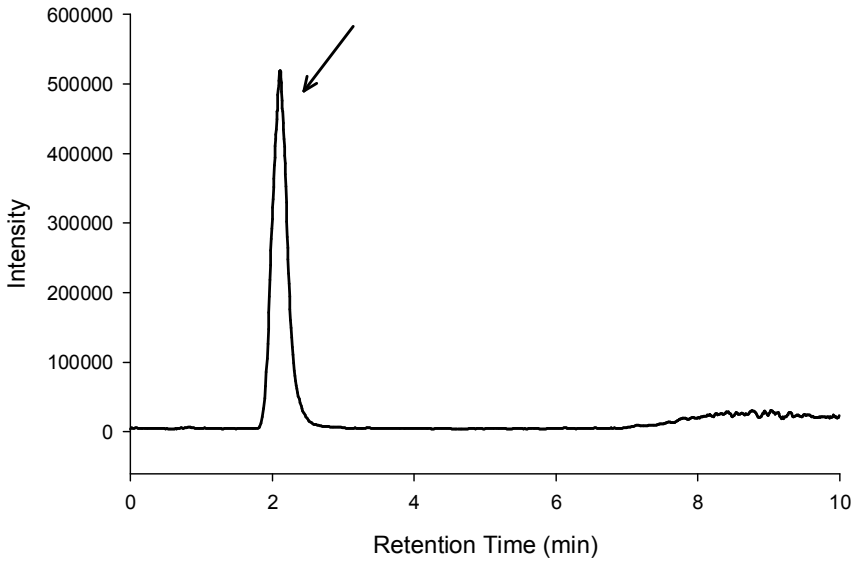


Figure 15. Total ion chromatogram (TIC) of napropamide. (5ng injection)

2.3 Matrix effect

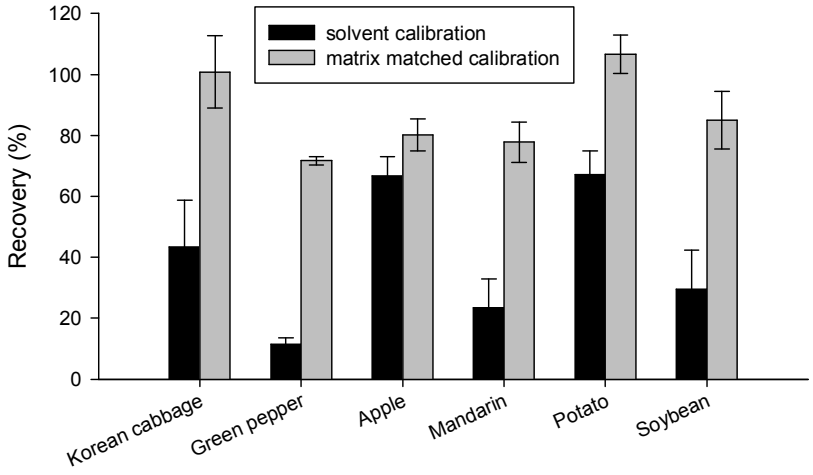
The matrix effects of the target analytes may result in positive or negative responses compared with those produced by solvent solutions and may greatly affect the method's accuracy. The occurrence of matrix-induced effects depends on whether or not the extracts contain compounds that will significantly influence the quantity of ionized analyte molecules of reaching the MS/MS path (Hajslova

et al. 2003, Wu et al. 2013).

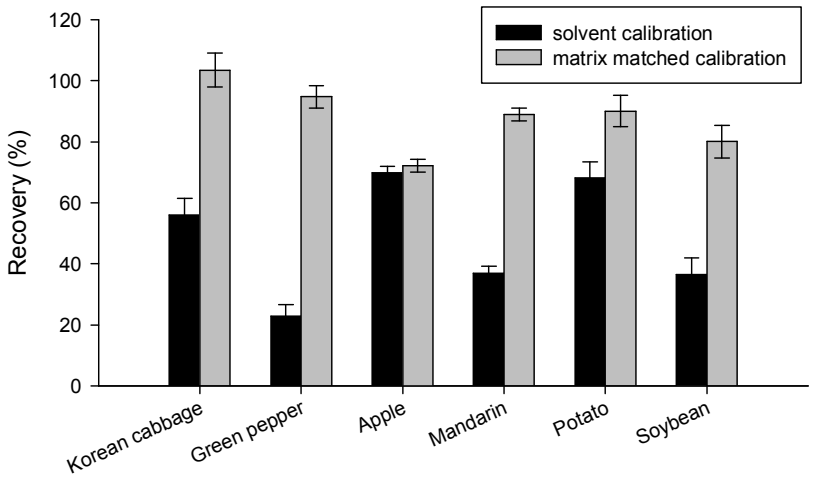
Six different matrixes at 0.01 and 0.1 mg/kg spiked levels was compared standards in solvent with matrix-matched standards (Figure 16).

When samples were processed in solvent calibration, the recoveries were 43.5-56.0%, 11.4-23.0%, 66.8-69.8%, 23.6-37.0%, 67.0-68.1% and 29.5-36.5% for Korean cabbage, green pepper, apple, mandarin, potato and soybean.

Therefore, calibration was performed by external matrix-matched standards to eliminate the matrix effect and to obtain a more realistic determination (Wu et al. 2013).



(A)



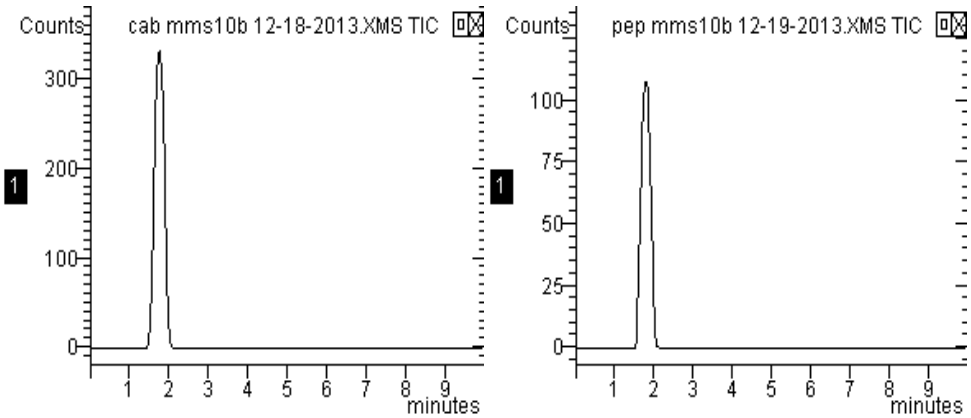
(B)

Figure 16. Accuracy of data obtained by LC-MS analysis of napropamide in six different matrixes extract; two alternative calibration techniques used; at (A)-0.01, and (B)-0.1 mg/kg.

2.4 Method validation

2.4.1 LOD (Limit of Detection) and LOQ (Limit of Quantitation) of napropamide for LC-MS/MS

LOQ were 0.05 ng (S/N>>10) respectively on each matrix matched standards (Figure 17-18). Because of matrix effect, intensity of concentrate level at LOQ were show distinction. LOD were calculated as 0.015 ng.



(A) (B)

Figure 17. LOQ of napropamide (0.05 ng) matrix matched standard for MS/MS analysis in Korean cabbage(A), green pepper(B).

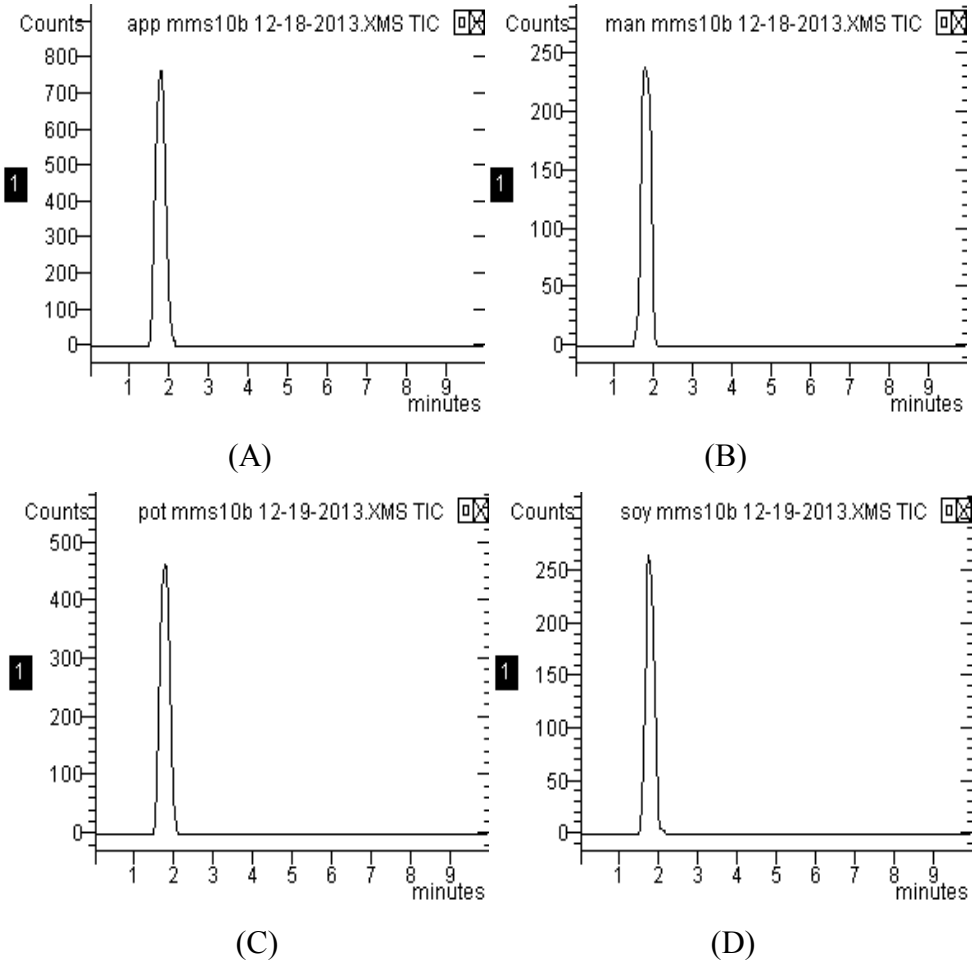


Figure 18. LOQ of napropamide (0.05 ng) matrix matched standard for MS/MS analysis in apple(A), mandarin(B), potato(C), soybean(D).

2.4.2 Linearity of calibration curve of napropamide

The linear regression equations obtained in range of 3 to 200 μg/L were y = 998.7619x – 2107.9863 for Korean cabbage, y = 450.7235x – 1053.6849 for green pepper, y = 1779.8738x – 2105.7285 for apple, y = 771.8916x – 1646.8806

for mandarin, y = 1397.9182x – 2696.0999 for potato, and y = 846.9555x – 1757.4191 for soybean (Figure 19-24). And coefficients of determination were within acceptable limits ($R^2 > 0.99$) ((주) 랩프런티어 2004).

Calibration data, LOD, and LOQ were arranged by table 14.

Table 14. Calibration data, LOD and LOQ for napropamide in different matrixes

Matrix	Calibration equation	Relative coefficient	LOD (ng/kg)	LOQ (ng/kg)
Korean cabbage	y = 998.7619x – 2107.9863	0.9999	3	10
Green pepper	y = 450.7235x – 1053.6849	0.9961		
Apple	y = 1779.8738x – 2105.7285	0.9991		
Mandarin	y = 771.8916x – 1646.8806	0.9996		
Potato	y = 1397.9182x – 2696.0999	0.9994		
Soybean	y = 846.9555x – 1757.4191	0.9989		

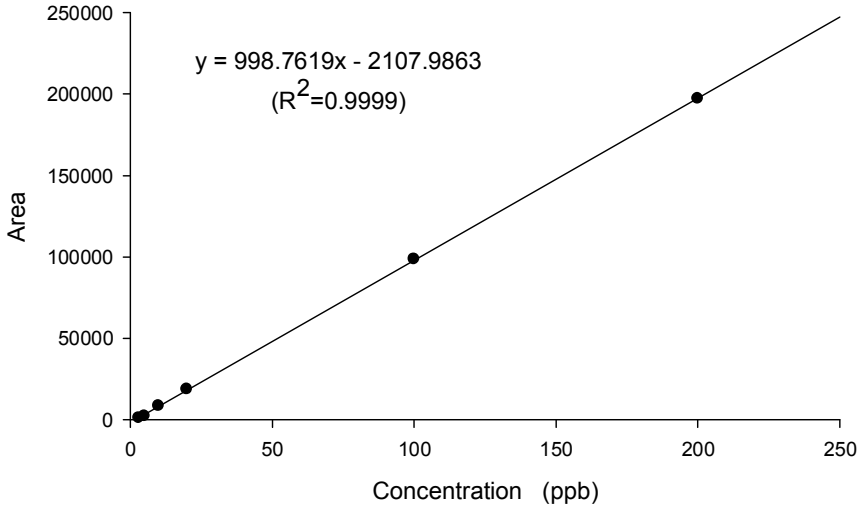


Figure 19. Calibration curve of napropamide for the MS/MS analysis of Korean cabbage sample.

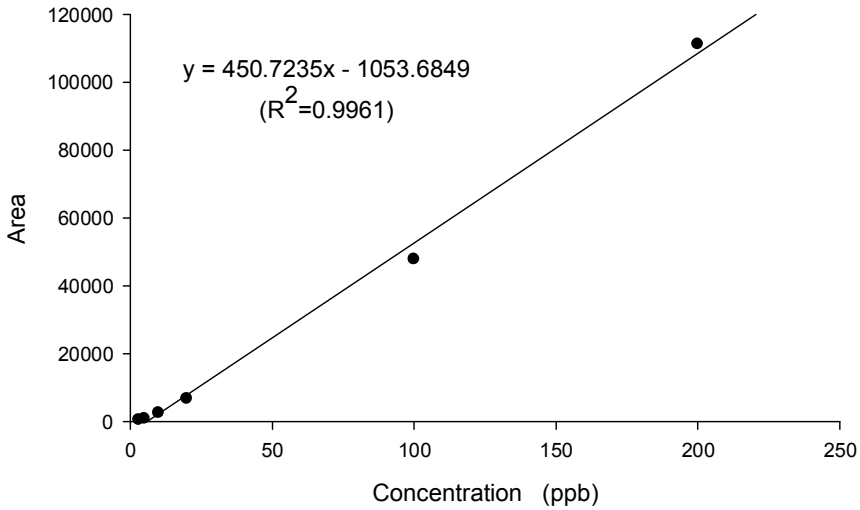


Figure 20. Calibration curve of napropamide for the MS/MS analysis of green pepper sample.

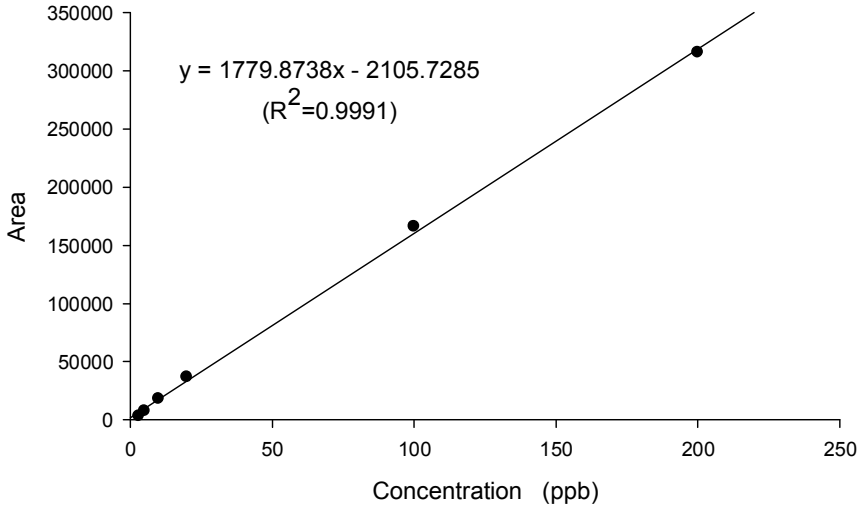


Figure 21. Calibration curve of napropamide for the MS/MS analysis of apple sample.

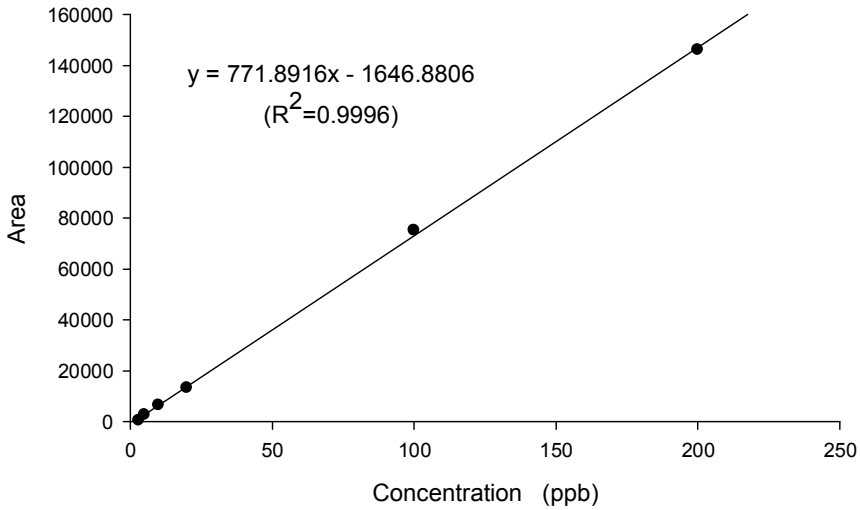


Figure 22. Calibration curve of napropamide for the MS/MS analysis of mandarin sample.

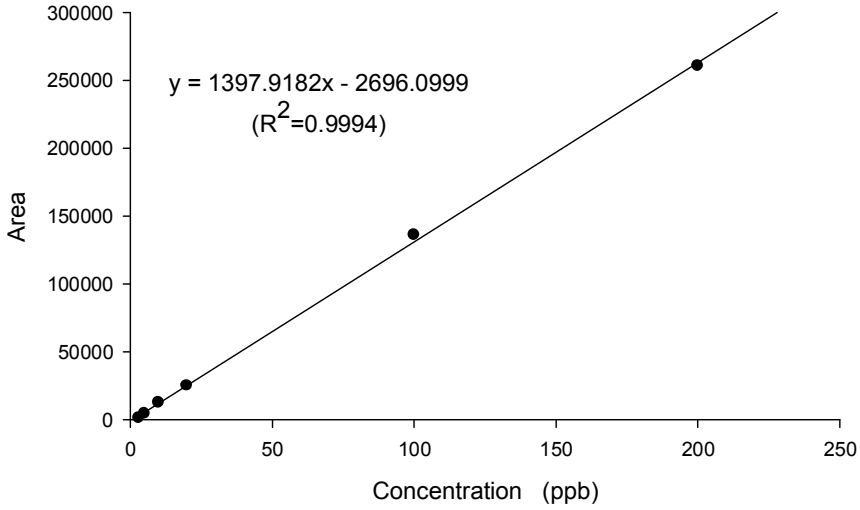


Figure 23. Calibration curve of napropamide for the MS/MS analysis of potato sample.

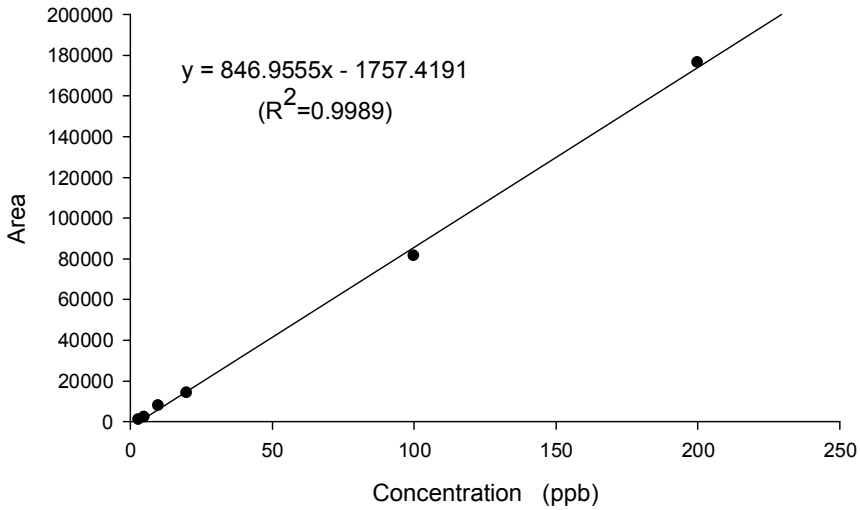


Figure 24. Calibration curve of napropamide for the MS/MS analysis of soybean sample.

2.4.3 Calculation of MLOQ (Method Limit of Quantitation)

MLOQ (Method Limit of Quantitation) is calculated using LOQ, sample size and dilution factor of analytical method (Equation 4).

$$\text{MLOQ (mg/kg)} = \frac{0.05\ \text{ng} \times 10\ \text{mL}}{5\ \mu\text{L} \times 10\ \text{g}} = 0.01\ \text{mg/kg}$$

MLOQ value (0.01 mg/kg) satisfied criteria of KFDA(Korea Food and Drug Administration) which are below 0.05 mg/kg or half of MRL (이영득 2009).

2.4.4 Recoveries of napropamide from crop samples by QuEChERS (accuracy and precision)

Recovery test can provide accuracy and precision of method validation by recovered rate(%) and C.V(coefficient of variation, %) (Fong et al. 1999).

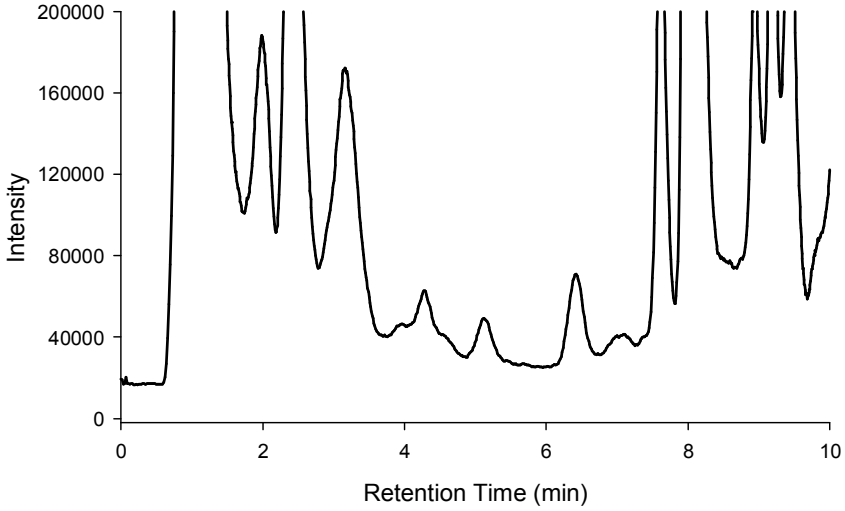
Untreated samples were spiked with MLOQ and 10MLOQ (0.01 and 0.1 mg/kg) of napropamide standard solutions, and the analysis was performed using the QuEChERS method to give reasonable recoveries (71.7-106.7%) and C.V (1.4-11.9%) at Korean cabbage, green pepper, apple, mandarin, potato and soybean(Table 15, Figure 25-30).

Table 15. Recovery and MLOQ for napropamide of QuEChERS-LC-MS/MS method in crops

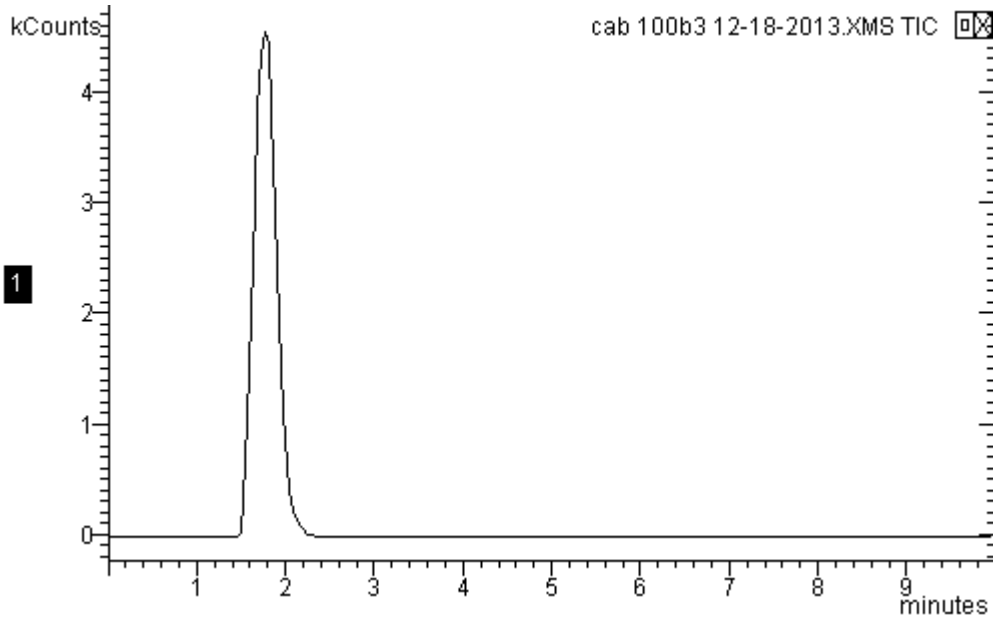
Fortified level (mg/kg)	Recovery (%)a) / CV (%)b)						MLOQ (mg/kg)
	Korean Cabbage	Green Pepper	Apple	Mandarin	Potato	Soybean	
0.01	100.8/11.9	71.7/1.4	80.2/5.3	77.8/6.6	106.7/6.3	84.9/9.4	0.01
0.1	103.5/5.5	94.7/3.7	72.1/2.1	88.9/2.1	90.1/5.2	80.1/5.4	

a) Average of triplicate

b) Coefficient of variation, standard deviation / mean × 100

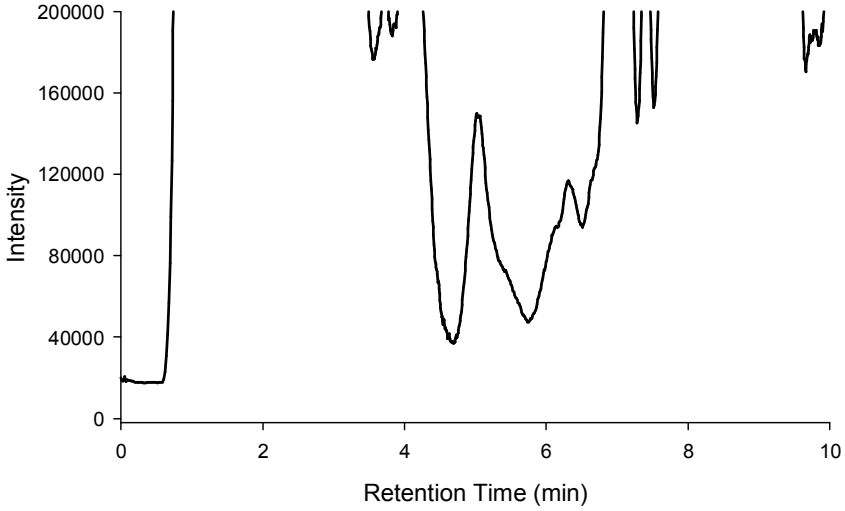


(A)

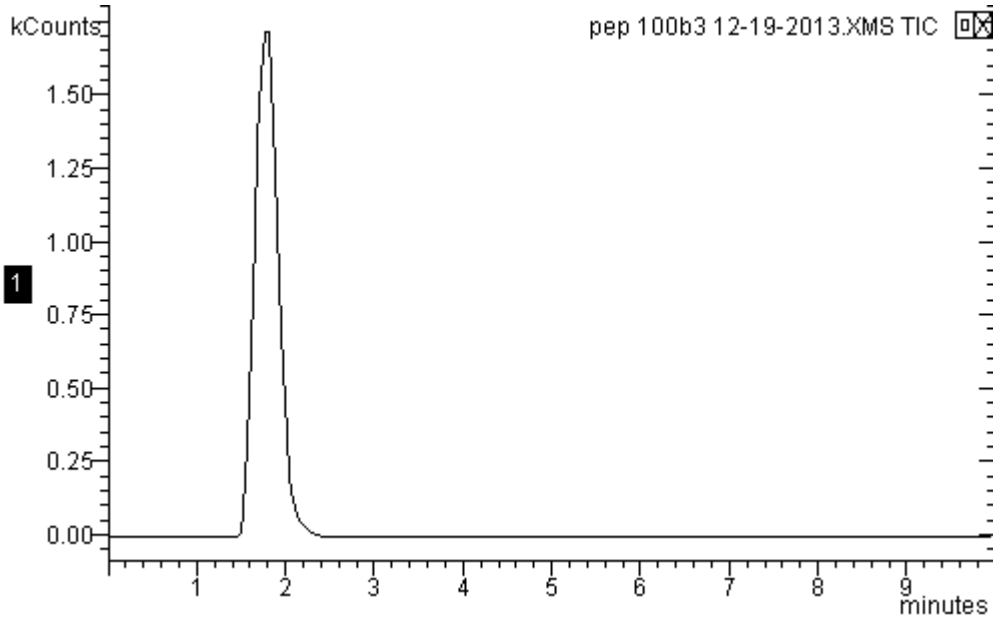


(B)

Figure 25. TIC (A), MS/MS (B) of Korean cabbage extracts (fortified at 0.1 mg/kg).

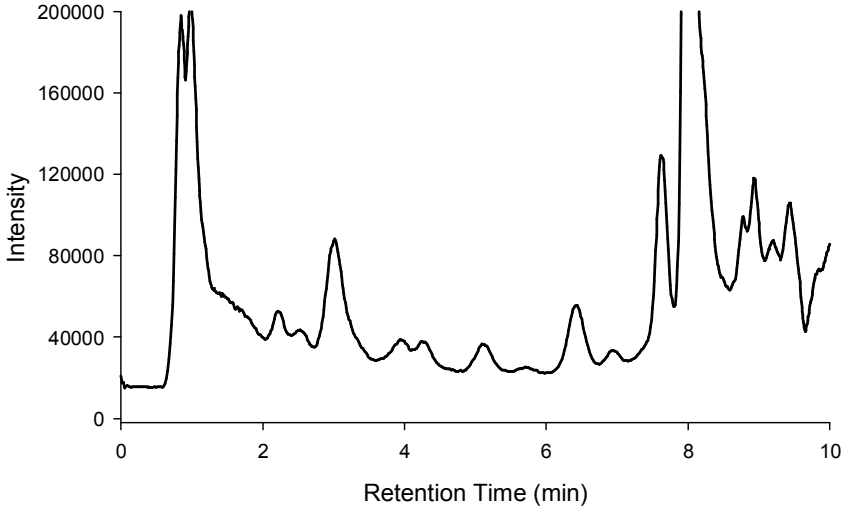


(A)

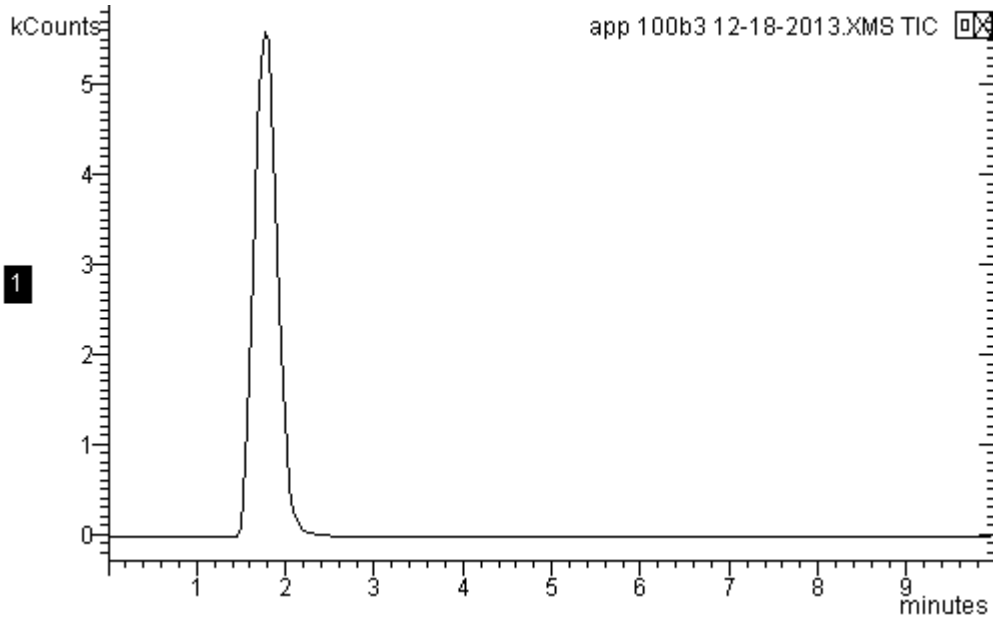


(B)

Figure 26. TIC (A), MS/MS (B) of green pepper extracts (fortified at 0.1 mg/kg).

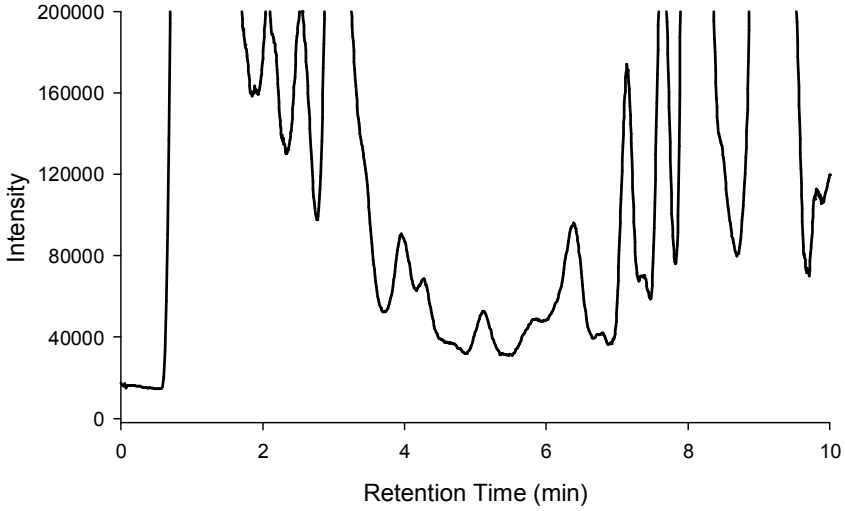


(A)

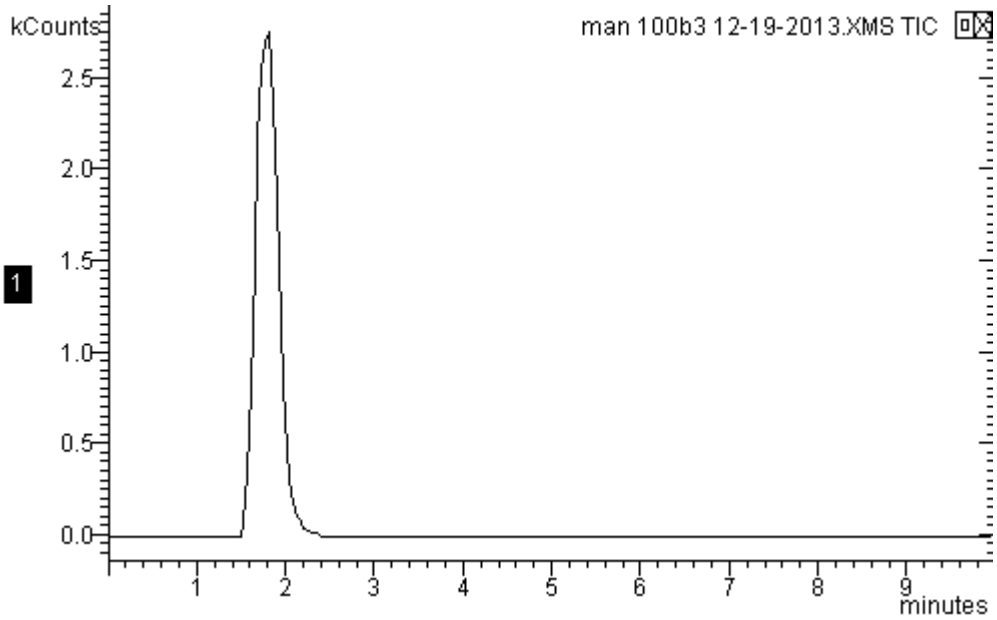


(B)

Figure 27. TIC (A), MS/MS (B) of apple extracts (fortified at 0.1 mg/kg).

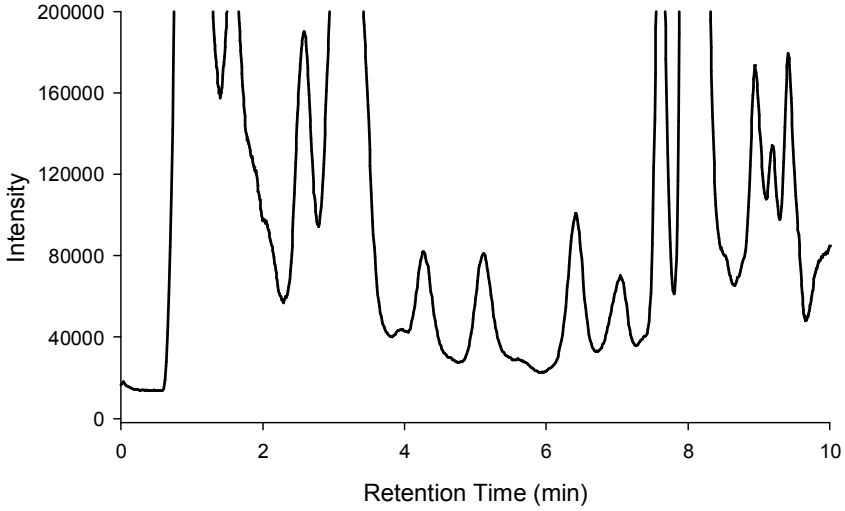


(A)

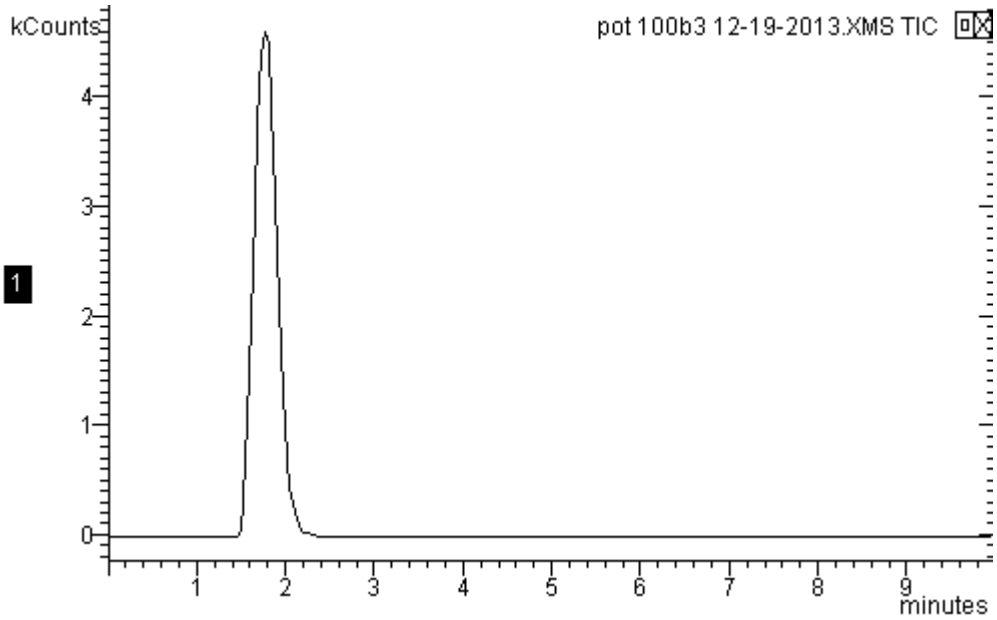


(B)

Figure 28. TIC (A), MS/MS (B) of mandarin extracts (fortified at 0.1 mg/kg).

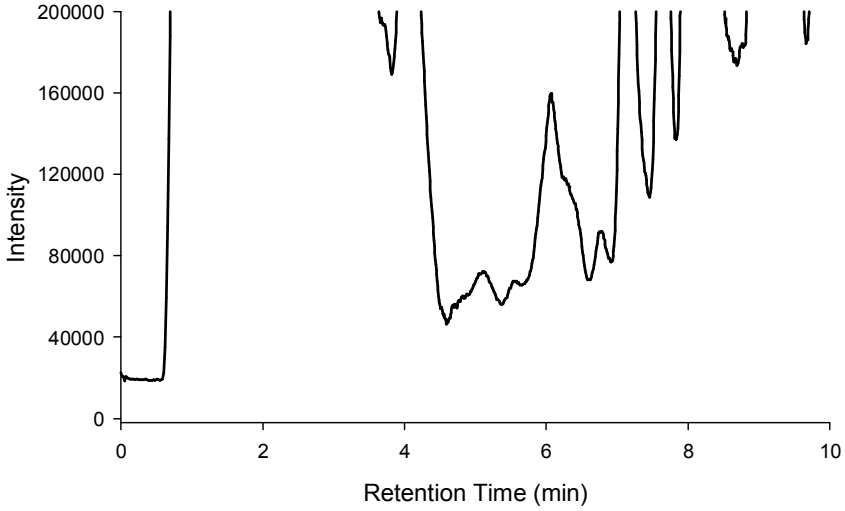


(A)

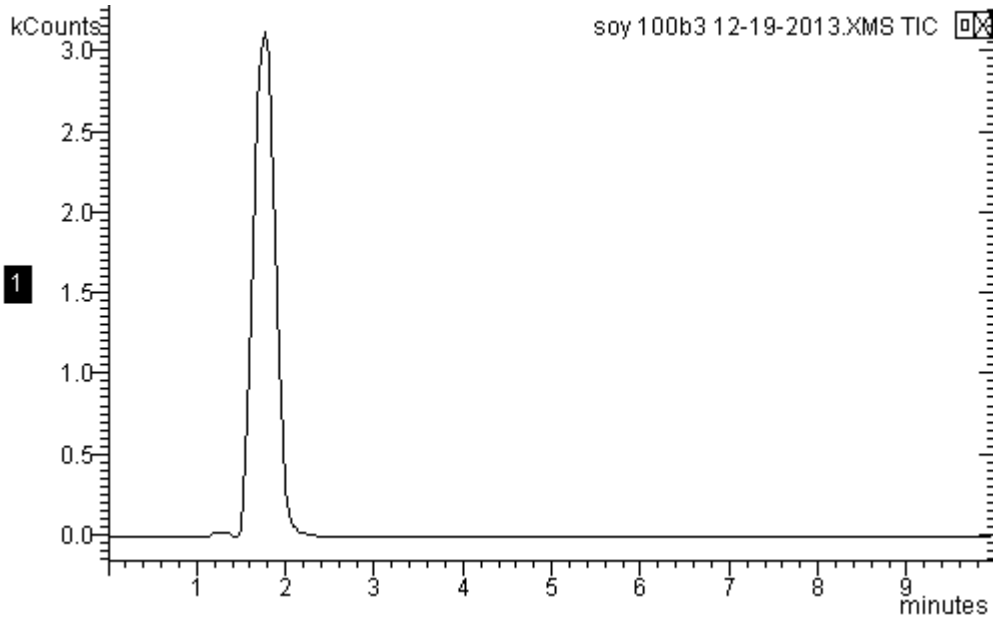


(B)

Figure 29. TIC (A), MS/MS (B) of potato extracts (fortified at 0.1 mg/kg).



(A)



(B)

Figure 30. TIC (A), MS/MS (B) of soybean extracts (fortified at 0.1 mg/kg).

4. CONCLUTION

To develop an improved analytical methods for napropamide residues in representative crops (Korean cabbage, green pepper, apple, mandarin, potato and soybean) were selected and the analytical methods were verified using HPLC and LC-MS/MS.

For analysis method using HPLC-UVD the extraction of napropamide with acetone, liquid-liquid partitioning with *n*-hexane, and clean-up with Florisil column chromatography procedures were established and applied to recovery test with crop samples. LOQ for napropamide was 5 ng and MLOQ was 0.05 mg/kg. Recoveries of napropamide at MLOQ, 10MLOQ and 100MLOQ were reasonable (85.2-105.4%).

In order to development of analysis method using LC-MS/MS, the QuEChERS method was chosen for sample preparation. As the result, LOQ of napropamide was 0.05 ng and MLOQ was 0.01 mg/kg in QuEChERS-LC-MS/MS method. Recoveries of napropamide at MLOQ, 10MLOQ were reasonable (71.7-106.7%) at Korean cabbage, green pepper, apple, mandarin, potato and soybean.

Therefore, the analytical methods established in this study can be employed as standard analytical method of napropamide in most of the fruits and vegetables.

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국문 요약

HPLC 와 LC-MS/MS 를 이용한 배추, 고추, 사과, 감귤, 감자, 대두에서의 Napropamide 의 잔류 분석법 확립

류 명 주

본 연구는 제초제 napropamide 의 잔류분석을 대부분의 농작물에 적용할 수 있도록 대표 작물을 선정하여 HPLC 및 LC-MS/MS 를 이용한 단성분 분석법을 개발하고자 하였다. 대표 작물은 배추, 고추, 사과, 감귤, 감자, 대두를 선정하였다. Napropamide 의 HPLC 잔류분석은 마쇄한 작물 시료에 acetone 으로 추출한 뒤, 농축하고 *n*-hexane 으로 분배하였다. 추출물을 Florisil 칼럼 크로마토그래피로 20% ethyl acetate 함유 *n*-hexane 으로 용리하는 방법으로 정제한 후 농축한 다음 HPLC-UVD 로 분석하는 방법을 확립하였다. Napropamid 의 정량한계(LOQ)는 5 ng 이었고, 분석정량한계(MLOQ)는 0.05 mg/kg 이었다. 무처리 시료에 napropamide 표준용액을 3 수준(MLOQ, 10MLOQ 와 100MLOQ) 3 반복으로 처리하여, 확립한

전체 분석과정을 거친 후, 회수율을 산출한 결과는 각각 85.2-105.4% 이었고, 농산물 시료에 관계없이 반복 간 분석오차는 10% 미만이었다.

LC-MS/MS 를 이용한 고감도 정밀분석법 확립을 위하여 전처리법으로는 최근 농약분석분야에서 널리 사용되고 있는 QuEChERS 법을 사용하였다. QuEChERS-LC-MS/MS 법에서 napropamide 의 정량한계는 0.05 ng 이었고, 분석정량한계(MLOQ)는 0.01 mg/kg 이었다. 대표작물로 선정한 배추, 고추, 사과, 감귤, 감자, 대두 대상으로 2 수준(MLOQ, 10MLOQ) 3 반복으로 회수율 시험을 한 결과, 모든 작물에서 71.7-106.7%의 회수율과 1.4-11.9%의 분석오차(C.V.)가 산출되었다.

주요어: 나프로파미드, HPLC, LC-MS/MS, 정량한계, 분석정량한계, 회수율, QuEChERS

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이 시간 동안 배운 것을 가슴에 지니고 살아가겠습니다.