이학석사 학위논문

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2018 년 2월

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Abstract

Sensitive determination of nonsteroidal anti-inflammatory drugs using 3-phase direct immersion single drop microextraction in-line coupled with capillary electrophoresis

Ji Eun Choi

Analytical Chemistry, School of Chemistry

The Graduate School

Seoul National University

A direct immersion (DI)-single drop microextraction (SDME) in the 3-phase mode was in-line coupled with capillary electrophoresis to improve the detection sensitivity for weakly acidic nonsteroidal anti-inflammatory drugs, such as ketoprofen, ibuprofen, and naproxen in solution. The acidic analytes in an acidified sample donor solution were extracted into a basic acceptor drop covered with a thin organic layer attached to the tip of a separation capillary. Due to the small dimensions of the acceptor and the organic phases, quite high enrichment factors of 1,600-2,600 were obtained from a 10-min

DI-SDME at 25°C with stirring. The limits of detections obtained by monitoring the absorbance at 214 nm were about 0.9-3 nM.

Keywords :

Single drop microextraction / Capillary electrophoresis /Direct immersion / Nonsteroidal anti-inflammatory drugs

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μM analytes enriched by 10-min DI-SDME with stirring. Others as in Fig. 1.

Figure 4. Effect of the extraction time. Donor phase: 1 μM analytes in HCl solution of pH 2. Extraction time: 5, 10, 15, and 30 min. Others as in Fig. 1.

Table 1 Analytical performance of DI-SDME-CE for NSAIDs

1 INTRODUCTION

Sample pretreatment methods that are often coupled with capillary electrophoresis (CE) for the cleanup and preconcentration of a sample before injection include solid-phase microextraction (SPME) [1-3] and liquid-phase microextraction (LPME) [4, 5]. SPME takes a short run time and consumes little amounts of sorbent and solvent but usually relies on commercial SPME fibers that may suffer from limited choices and cross-contamination with multiple uses [6]. LPME, on the contrary, utilizes various modes and extracting solvents, and easily avoids cross-contamination by using a fresh solvent each time [7, 8]. Widely used schemes of LPME include dispersive liquid-liquid microextraction (DLLME) [9, 10], hollow-fiber liquid phase microextraction (HF-LPME) [11-13], and single-drop microextraction (SDME) [14-16].

SDME-CE, where an acceptor drop is formed at the capillary inlet tip, is very convenient since the extraction and the sample injection are coupled in-line [17]. In the 3-phase mode of SDME, acidic analytes, for example, are enriched from an acidic aqueous donor phase to a basic aqueous acceptor drop through a thin organic layer covering the acceptor drop. Due to the small volume of the acceptor drop and a thin organic phase, high sample enrichment

factors (EFs) can be obtained in a short extraction time with minimal solvent consumption [18]. In this report, 3-phase SDME-CE has been applied to a direct immersion (DI) extraction of weakly acidic nonsteroidal anti-inflammatory drugs (NSAIDs), such as ketoprofen (KTP), ibuprofen (IBU), and naproxen (NAP) in solution. The EFs for the NSAIDs obtained by a 10-min DI-SDME were 1,600-2,600 and the limits of detections (LODs) were about 0.9-3 nM using a built-in UV detector of a commercial CE instrument.

2 MATERIALS AND METHODS

2.1 Reagents

KTP, IBU, NAP, sodium hydroxide, sodium tetraborate decahydrate, hydrochloric acid, ethanol, 1-octanol, and methanol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetic acid was from Merck (Darmstadt, Germany). Octadecyl trimethoxysilane (ODTS) was from Aldrich (Milwaukee, WI, USA). Deionized water was prepared with a Labtower EDI Water unit (Thermo Fisher Scientific, Langenselbold, Germany). Three 5-mM stock solutions of KTP, IBU, and NAP were prepared in methanol and stored in the dark at 4°C. Run

buffers were prepared by titrating sodium tetraborate solutions with 2 M NaOH. Standard samples for CE were prepared by diluting the corresponding stock solutions with a run buffer. Sample donor solutions were prepared by diluting the corresponding stock solutions with a HCl solution of pH 2. Every solution except for the donor phase was filtered through a 0.45-μm syringe filter (Whatman, Clifton, NY, USA) before use.

2.2 Capillary electrophoresis

CE was performed with a P/ACE MDQ CE system (Beckman, Fullerton, CA, USA) equipped with a UV detector monitoring at 214 nm. A 60-cm (50-cm to detector) bare fused silica capillary (Polymicro Technologies, Phoenix, AZ, USA) with a 50-μm inner diameter (ID) and 360-μm outer diameter was used. After comparing 240-400 mM sodium borate buffers of pH 9.2-10.5, a 320 mM sodium borate buffer of pH 9.8 was chosen as the run buffer for CE. The capillary was conditioned between runs by rinsing with a sequence of methanol, 0.1 M NaOH, water, and run buffer for 2, 3, 3, and 7 min, respectively, at 60 psi. The capillary temperature was set to 25°C.

2.3 3-Phase direct immersion single drop microextraction

Using a coating solution (5 vol% ODTS and 0.1 vol% acetic acid in ethanol) as described in our previous report [19], the inlet tip surface of the capillary was hydrophobically coated so that an acceptor drop covered with an organic phase could be stably attached during extraction. After filling the capillary with a run buffer by rinsing at 60 psi for 7 min, octanol was injected at 2 psi for a set time. Then, the capillary inlet was transferred to the sample solution and a backpressure of 0.7 psi for a set time was applied from the outlet to inlet to form a drop of an acceptor phase (run buffer) covered with a thin octanol layer hanging to the capillary inlet tip. To vary the octanol layer volume, the times for the octanol injection and drop formation were adjusted based on the Poiseuille equation [20]. To maintain the shape of the drop, a backpressure of 0.1 psi was applied for 0.43 min for every 1.56 min of extraction while monitoring with a video camera. A homemade microstirrer, as in our previous study [19], was used to agitate the donor phase. After extraction at 25°C, a portion of the enriched acceptor drop was injected hydrodynamically into the capillary at 0.5 psi for 3 s. Then, the capillary inlet was placed in a run buffer vial and electrophoresis was carried out.

3 RESULT AND DISCUSSION

3.1 Donor and acceptor phases

In a 3-phase liquid extraction for an ionizable analyte, the pH difference between the donor and acceptor phases is the driving force for the sample enrichment. Thus, for our weakly acidic analytes, KTP, IBU, and NAP, the sample donor solution was acidified to pH 2 using HCl while the basic run buffer of pH 9.8 was used as the acceptor phase. Although the EF could be increased with a higher pH acceptor, we chose to use the run buffer for convenience in operation.

For a three-phase extraction of an analyte from a donor phase (d) to an acceptor phase (a), through an organic phase (o), the EF at equilibrium is given by [21]:

$$\mathrm{EF}_{eq} = \frac{1}{(D_2/D_1)+(D_2 V_o/V_d)+(V_a/V_d)}, \qquad (1)$$

where V_d, V_o, and V_a are the volumes for the respective phases denoted by the subscripts, and $D_1 = C_o/C_d$ and $D_2 = C_o/C_a$ where C_d, C_o, and C_a are thee quilibrium analytical concentrations of the analyte. If the extraction time t is short, the EF can be approximated as [22]

$$\mathrm{EF}(t) \approx \frac{V_d}{V_a}\left\{1-\exp(-kt)\right\} \approx \frac{V_d}{V_a} kt, \qquad (2)$$

where the lag time was omitted for the thin (<100 *m*m) octanol layer in our DI-SDME and k is the first order rate constant given as

$$k \approx \frac{A_d A_a D_1 \bar{\beta}_d \bar{\beta}_a}{V_d (A_d D_2 \bar{\beta}_d + A_a \bar{\beta}_a)}, \qquad (3)$$

where A_d and A_a are the organic-donor and the organic-acceptor interfacial areas, respectively, and $\overline{\beta_d}$ and $\overline{\beta_a}$ are the overall mass-transfer coefficients through the organic-donor and the organic-acceptor interfaces, respectively. Then substituting Eq. (3) into Eq. (2),

$$\mathrm{EF}(t) \approx \frac{D_1 \bar{\beta}_d \bar{\beta}_a}{D_2 \bar{\beta}_d + (A_a / A_d) \bar{\beta}_a} \left(\frac{A_a}{V_a} \right) t. \qquad (4)$$

Therefore, higher EF values are expected for an acceptor phase with a smaller volume. To investigate the effect of the acceptor phase volume, the acceptor drop volume was varied from 5 to 12 nL while holding the octanol volume at 30 nL (Fig. 1). Considering the EFs and the stability of the drop, the acceptor drop volume of 8 nL was chosen as optimal.

3.2 Octanol layer

When an extraction is carried out from a donor phase to an acceptor phase through an organic phase for a finite time, higher EFs will be obtained by reducing the volume (or thickness) of the organic

phase. For a given volume of the acceptor phase (8 nL), the volume of the octanol layer was increased from 18 to 40 nL (the organic layer thickness estimated roughly from the 2-layer drop images varied from 35 to 85 μm). Fig. 2 shows the increase in EFs as the octanol layer volume was decreased. The drop became unstable at an octanol volume smaller than 20 nL with an increase in the extraction time and agitation. Hence the 20-nL octanol was deemed optimal.

3.3 Agitation of the sample

Agitation will increase the mass transfer coefficients by decreasing the Nernst diffusion film thickness ($\overline{\beta} = \kappa/\delta$, where κ is the diffusion coefficient and δ is the film thickness) and thus higher EFs will be obtained for a given extraction time. To investigate the effect of agitation, the EFs from 10-min DI-SDMEs at 25°C with and without stirring were compared, as shown in Fig. 3. The agitation of the sample increased the EFs by 9-12 times: from 270 to 2,500 for KTP, from 130 to 1,600 for IBU, and from 300 to 2,600 for NAP.

3.4 Extraction time

Under the optimal conditions chosen so far, the extraction times for DI-SDME were increased up to 30 min. As shown in Fig.

4, the EFs from DI-SDME with stirring at 25°C increased almost linearly with time up to 10 min and increased less significantly afterwards. Meanwhile, the relative standard deviations (RSDs) of EFs gradually increased with time. Considering the EFs and RSDs, the extraction time of 10 min was chosen as optimal.

3.5 Analytical performance

Table 1 summarizes the analytical performance of our DI-SDME-CE in the analysis of the three NSAIDs. When the extraction was performed for 10 min at 25°C with stirring, the EFs for KTP, IBU, and NAP were 2500, 1600, and 2600, respectively. The LODs for KTP, IBU, and NAP were 1, 3, and 0.9 nM, respectively.

Recently, Garcia-Vazquez *et al.* reported 3-phase DI-SDME-CE of NSAIDs [23]. After an off-line extraction of the analytes from a 400-μL sample of pH 2 into a 300-μL organic phase (ethyl acetate), a 10-min backextraction into a 510-nL acceptor drop of 0.001 M NaOH yielded EFs of 27 for KTP, 12 for IBU, and 44 for NAP. The main difference between DI-SDMEs of Ref. [23] and ours are the dimensions of the extraction system. Assuming the partition coefficients of the analytes between water and octanol [24] are the same as those between water and ethyl acetate, the equilibrium EFs from Eq. (1) are 500-600 for Ref [23] and 50,000-150,000 for our work. 60-130 times higher EFs obtained from our 10-min DI-SDME

were also due to the small acceptor drop volume (see Eq. 4) and the thin organic phase (see section 3.2).

4 Concluding remarks

3-Phase DI-SDME and the subsequent CE analysis were in-line coupled in an automatic manner without modifying the existing CE instrument. Taking advantage of the extremely small acceptor drop covered with a thin organic layer, very high EFs of few thousands were obtained for NSAIDs in a short extraction time of 10 min. Thus 3-phase DI-SDME is a promising technique for determining of NSAIDs and similar compounds in water and biological samples.

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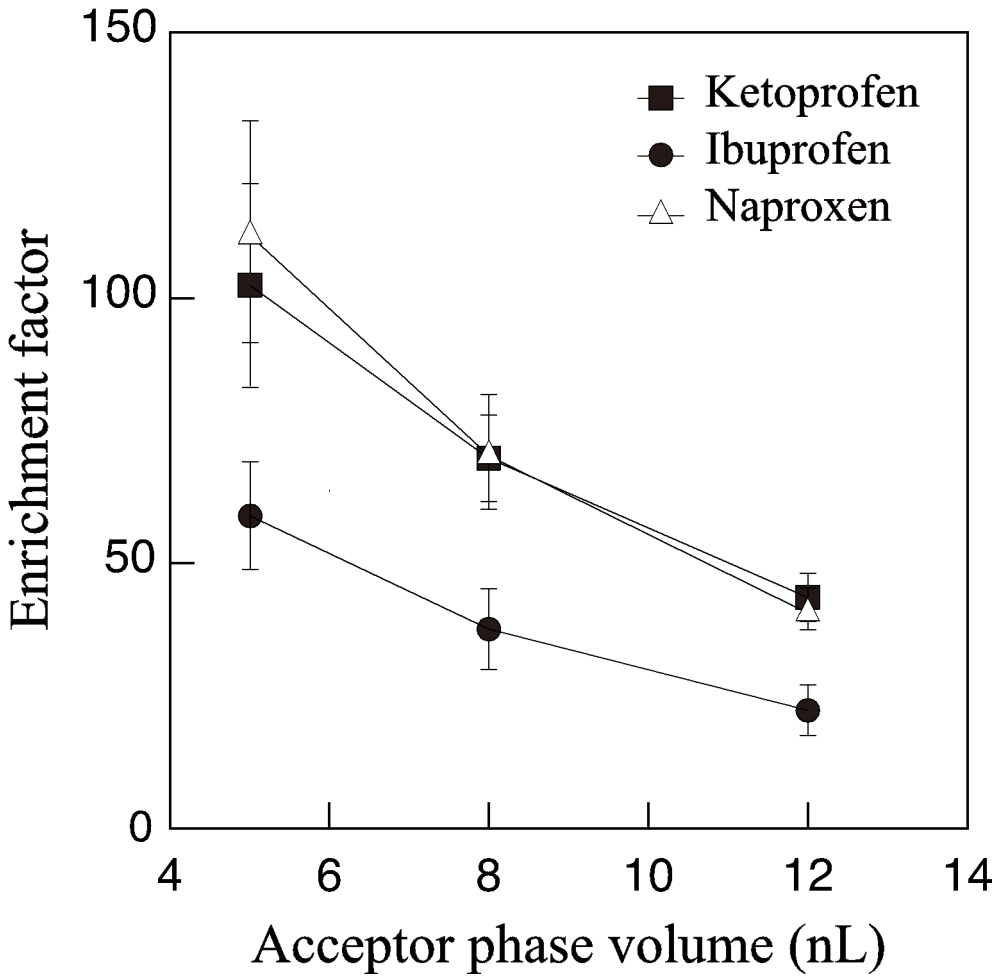
150
100
50
0
Enrichment factor
Ketoprofen
Ibuprofen
Naproxen
4
6
8
10
12
14
Acceptor phase volume (nL)

Figure 1

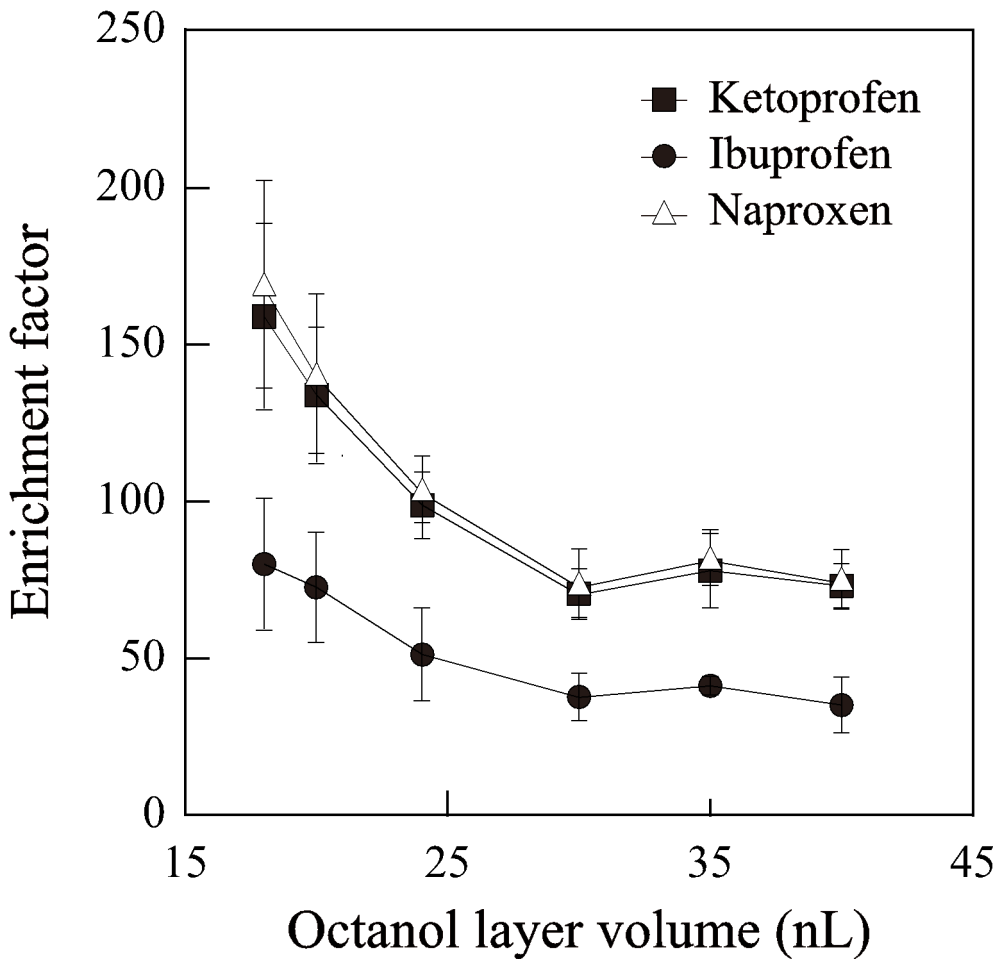
250
200
150
100
50
0
Enrichment factor
Ketoprofen
Ibuprofen
Naproxen
15
25
35
45
Octanol layer volume (nL)

Figure 2

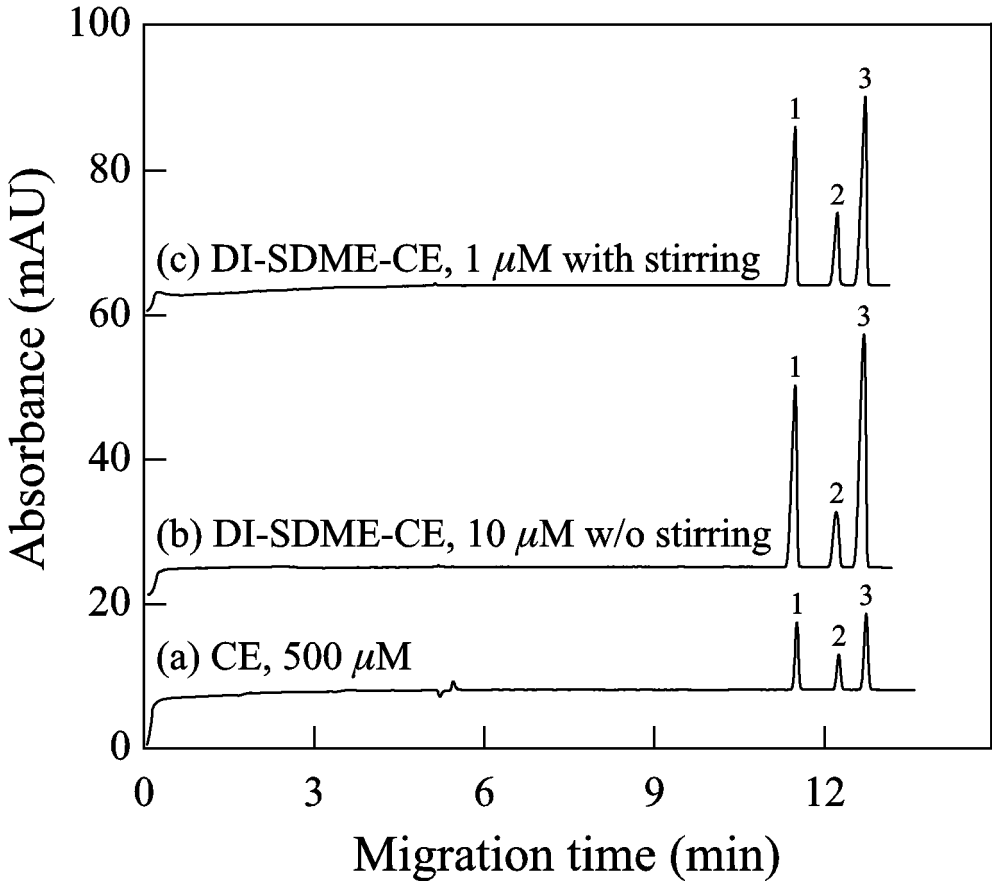
100
80
60
40
20
0
Absorbance (mAU)
(c) DI-SDME-CE, 1 μM with stirring
(b) DI-SDME-CE, 10 μM w/o stirring
(a) CE, 500 μM
1
2
3
0
3
6
9
12
Migration time (min)

Figure 3

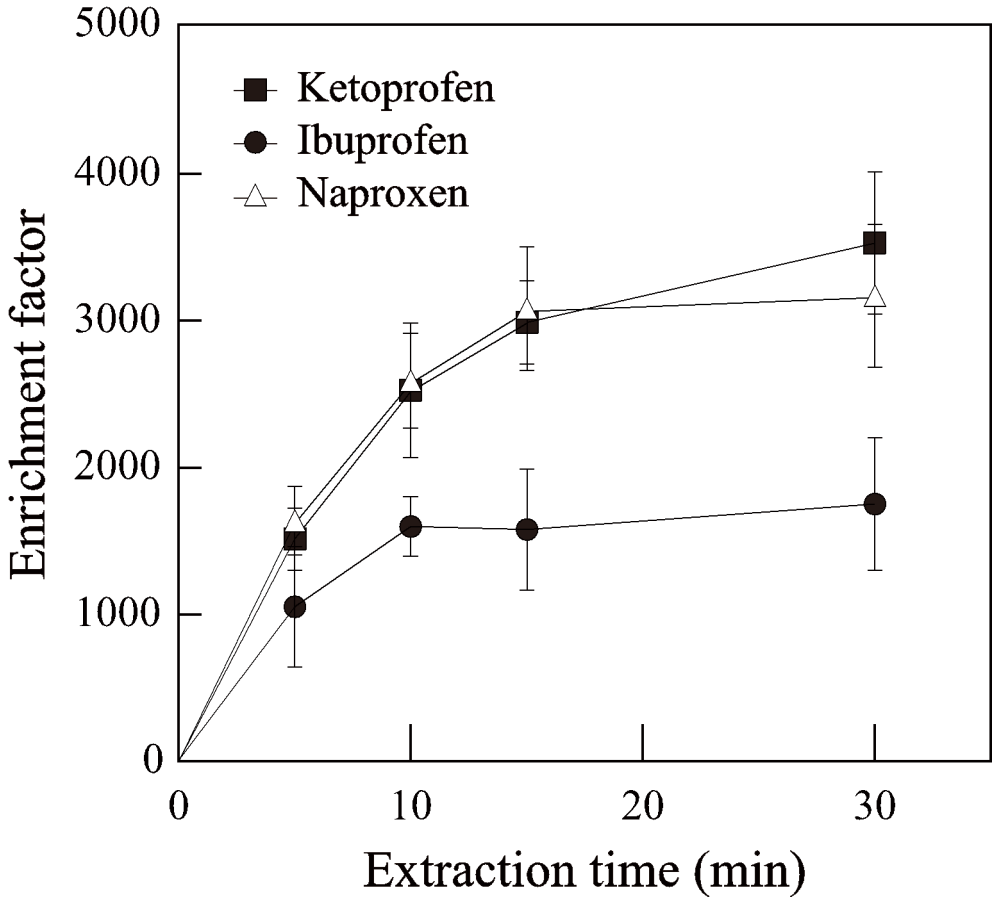
5000
4000
3000
2000
1000
0
Enrichment factor
Ketoprofen
Ibuprofen
Naproxen
0
10
20
30
Extraction time (min)

Figure 4

Table 1

Analyte	RSD (n = 4)		EF	LOD (S/N = 3)	Linear range	Linearity (r^2)	Calibration curve[b]
	MT[a]	CPA[a]					
KTP	2.8%	18%	2,500	1 nM	10-3000 nM	0.9972	y = 9.7787x – 216.25
IBU	2.6%	13%	1,600	3 nM	10-3000 nM	0.9817	y = 3.5583x + 466.47
NAP	2.8%	12%	2,600	0.9 nM	10-3000 nM	0.9976	y = 11.445x + 405.55

Donor phase: 1 μM analytes in HCl solution of pH 2. Extraction for 10 min at 25°C with stirring. Others as in Fig. 1.

a) MT; migration time, CPA; corrected peak area

b) y; corrected peak area (μAU s), x; concentration (nM)

국문초록

상용화된 모세관 전기영동 기기를 이용하여 ketoprofen, ibuprofen, 그리고 naproxen과 같은 약산인 비스테로이드성 소염진통제를 분석하는데 있어서 검출 감도를 향상시키기 위하여 3-상 액체상 미세방울 추출법을 수행하였다. 산성 수용성 주개층에서 산성 분석물질이 모세관 입구에서 얇은 유기층으로 둘러싸인 염기성 받개층 방울로 추출된다. 적은 양의 받개층과 유기층을 사용하기 때문에 25°C에서 교반을 하면서 10분 추출했을 때 1,600-2,600의 높은 농축지수를 얻을 수 있었고 214 nm에서 0.9-3 nM의 검출한계를 얻을 수 있었다.

주요어 : 미세방울 추출법, 모세관 전기영동, 비스테로이드성 소염진통제
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