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On-line sample extraction and enrichment of non-steroidal anti-inflammatory drugs by pre-column in capillary liquid chromatography mass spectrometry

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Abstract

A rapid and sensitive analytical method has been developed for the simultaneous determination of 16 non-steroidal anti-inflammatory drugs (NSAIDs) in human plasma by capillary liquid chromatography (LC) and quadrupole mass spectrometry with electrospray ionization operated in the negative ion mode. The sample clean-up and enrichment on a pre-column were accomplished on-line to improve the sensitivity. This method greatly reduced sample preparation time and sample volume compared with off-line sample extraction methods and conventional LC methods, respectively. The recoveries of NSAIDs from human plasma were 56.7–96.9%. The total analytical time for a single analytical run was approximately 15 min. The detection limits of NSAIDs were 0.001–0.075 μg ml^{-1} using a selected ion monitoring mode.

Keywords: Non-steroidal anti-inflammatory drugs; Capillary column; Liquid chromatography-mass spectrometry; On-line enrichment

1. Introduction

Capillary liquid chromatography (LC) was developed more than 30 years ago. Capillary LC methods and related techniques have been increasingly developed and are becoming more user-friendly because injection valves with low dead volume, pumping systems working at low flow rates, high-resolution columns and novel detection systems have been developed by manufacturers and research institutes, and capillary LC systems and their accessories are commercially available from a number of manufacturers. Compared to conventional LC columns, the use of capillary LC columns has some advantages. The lower flow rate and smaller column size save solvents, reagents, and column packing materials. This situation is environmentally friendly. Improved mass sensitivity can be expected owing to small-volume detection in capillary LC. This is especially of benefit when the sample amounts available are restricted as in the case of biological samples. In forensic toxicology, sample is limited and sample volume is often very small. When LC is combined with an electrospray ionization-mass spectrometer, it is desirable to have narrow bore columns, since this implies reduced flow rates [1]. The efficiency of ionization improves as the flow rate is lowered because less volume of mobile phase passes through the electrospray emitter, and thus producing smaller charged droplets.

However, a general problem in microcolumn LC techniques is the loss of detection sensitivity due to the small injection volumes. The optimum injection volume is reduced proportionally to the square of the column diameter. For the LC determination of analytes in sample matrix, tedious and time-consuming pre-treatment procedures such as the removal of matrix by liquid–liquid extraction or solid phase extraction were required. These problems can be overcome by the use of on-line capillary pre-column enrichment system. In this system, the sample clean-up and enrichment are accomplished on-line, improving sensitivity. We had developed an on-line capillary pre-column enrichment system for aromatic hydrocarbons in water [2,3], bromate in drinking water [4], and ethanol in breath [5].

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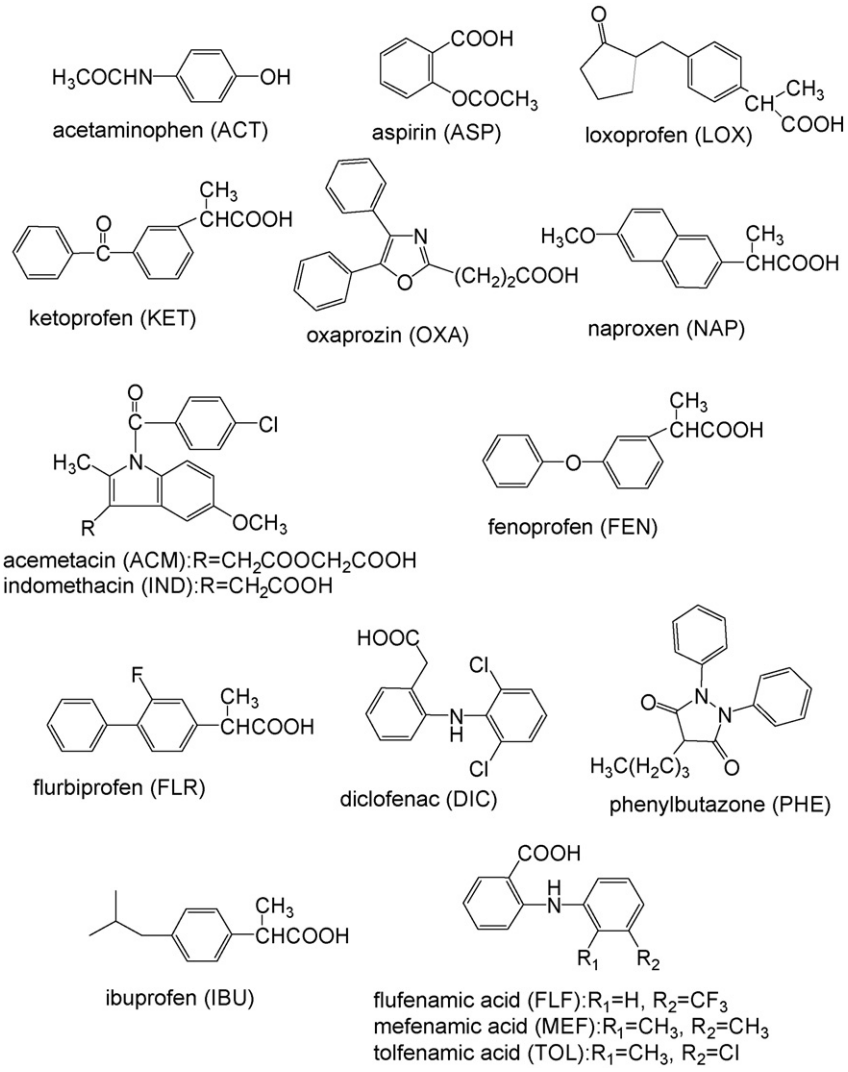

Fig. 1. Structures and abbreviations of NSAIDs used in this study.

Various methods reported analysis of non-steroidal anti-inflammatory drugs (NSAIDs) determined either single or a few compounds [6–21]. In forensic toxicology, it is important to identify the causative agent of poisoning rapidly. As many kinds of NSAIDs are commercially available and two or more kinds of NSAIDs might be used at one time in a suicidal attempt, rapid and simultaneous methods are therefore required for a screening test. The methods of simultaneous determination of NSAIDs form of unchanged drug without detecting its metabolites have been reported, including LC with UV detector [22–27] and gas chromatography-mass spectrometry [28,29]. UV detection cannot provide information about the molecular masses and structures of analytes. And due to their polar nature, it is difficult to separate most underivatized NSAIDs by gas chromatography. The determination of 4 NSAIDs by LC–mass spectrometry (MS) with atmospheric pressure chemical ionization (APCI) was also reported [30]. But this LC–MS with APCI method was applied to only dosage form. Therefore, we previously reported simultaneous determination of 16 NSAIDs in human plasma by LC–MS with SPE [31]. We chose LC–MS because it is more widespread, simpler to optimize and more affordable than LC–tandem mass spectrometry.

Since acetaminophen is hydrophilic compound, it is retained poorly by ODS column. In order to retain all analytes strongly on a pre-column, we employed Oasis HLB as the pre-column stationary phase. Oasis HLB is a macroporous copolymer made from a balance ratio of two monomers, the lipophilic divinylbenzene and the hydrophilic *N*-vinylpyrrolidone. Due to the fact that this macroporous copolymer exhibits both lipophilic and hydrophilic retention characteristics, it retains wide spectrum of both polar and non polar compounds.

Aspirin is rapidly metabolized to salicylic acid. However, it has been reported that aspirin could be determined in human plasma after oral administration [32]. In forensic toxicology, to identify the causative agent of poisoning it is important to detect form of unchanged drug. Therefore, we chose aspirin as the analyte in this method.

This study aimed to develop a rapid, selective and sensitive method for the simultaneous determination of 16 NSAIDs (Fig. 1) using a pre-column in capillary LC–MS. In this method,

the sample clean-up is accomplished on-line. Furthermore, it required a smaller sample volume compared with conventional LC method; the sensitivity of the system was nevertheless improved due to the reduced column size and pre-column enrichment. Spiked samples were analyzed to confirm that this method could be applied to NSAIDs in complex matrix such as biological fluid. We chose plasma as the sample matrix because plasma is one of complex matrices.

2. Experimental

2.1. Materials and reagents

Acetaminophen (ACT), aspirin (ASP), loxoprofen (LOX), ketoprofen (KET), acemetacin (ACM), oxaprozin (OXA), fenoprofen (FEN), flurbiprofen (FLR), indomethacin (IND), diclofenac (DIC), ibuprofen (IBU), phenylbutazone (PHE), flufenamic acid (FLF), mefenamic acid (MEF) and tolfenamic acid (TOL) were purchased from Sigma (St. Louis, MO, USA). Naproxen (NAP) was purchased from Aldrich (Milwaukee, WI, USA). Acetonitrile used in the study was of HPLC grade, and other common chemicals used were of analytical grade. Water was purified using the Milli-Q-System from Millipore Corp (Bedford, MA, USA).

2.2. Solutions

Stock solution was prepared by dissolving appropriate amount of each NSAID in acetonitrile to yield concentrations of 100 μg ml^{-1} and stored at 4 °C in a refrigerator. Standard solutions were prepared by diluting the stock solution with water. Plasma samples were prepared by spiking the standard solutions into drug-free human plasma and vortex mixed. Quality control samples were prepared separately in the same manner as plasma samples. The concentration of quality control samples was adjusted to 0.05, 0.1, 0.25, 0.5 or 1 μg ml^{-1}, and three different concentrations were selected for each compound considering its sensitivity.

2.3. Sample preparation

Phosphoric acid (30 μl) was added to the plasma sample (0.5 ml) and then diluted with water (0.5 ml) and vortex-mixed. And 10 μl aliquot was injected into the pre-column LC–MS system.

Since most of NSAIDs also bind to proteins in plasma, phosphoric acid was used to disrupt the drug-protein binding.

2.4. Sample extraction and enrichment procedure

An in-house assembled pre-column LC–MS system was used (Fig. 2). In valve loading position (Fig. 2A), sample was injected onto the pre-column by using a micro syringe and subsequently 60 μl of Milli-Q water was injected as washing solvent. The analytes were extracted and enriched on the pre-column and sample matrix compounds had been fully washed out of the pre-column. The valve was then switched to the position as in

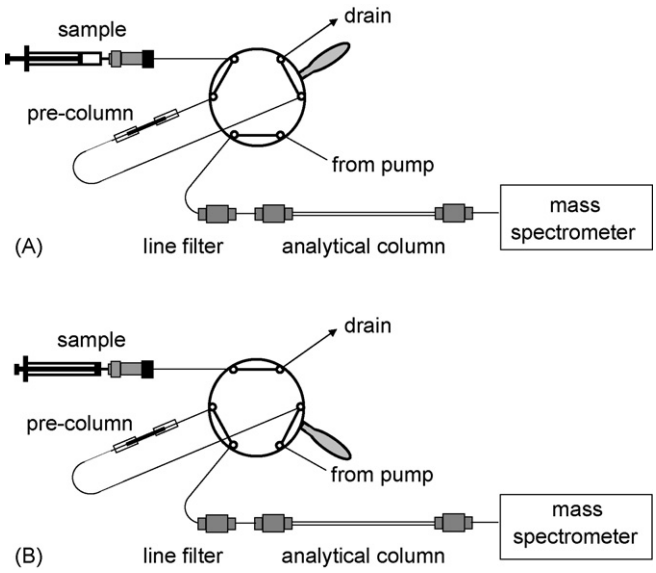

Fig. 2. A schematic diagram of the pre-column LC–MS system. (A) Sample loading position. The analytes were extracted and enriched on a pre-column. (B) The analytes were back flushed to the analytical column from pre-column.

Fig. 2B, and the analytes were back flushed to the analytical column. The valve was returned to loading position (Fig. 2A) after analysis. Acetonitrile (100 μl) and Milli-Q water (100 μl) were successively injected in order to rinse and equilibrate the pre-column.

2.5. Instrumentation

The pre-column LC–MS system was performed using a Waters Alliance 2690 HPLC pump, an ACURATE IC-100VAR splitter (LC Packings, Amsterdam, The Netherlands), an M-435 micro injection valve (Upchurch Scientific, Oak Harbor, WA, USA), laboratory-made analytical column and pre-column, and a Micromass ZMD quadrupole mass spectrometer. The ACURATE IC-100-VAR splitter with 0.3 mm i.d. column calibrator was placed between the pump and micro injection valve. The flow rate of mobile phase, which was pumped at 12.5 μl min^{-1}, went to the micro-injection valve after passing the splitter. The mobile phase was a mixture of 10 mM ammonium formate aqueous solution (adjusted to pH 3.25 with formic acid) and acetonitrile (40:60, v/v). The analytical column was prepared by using fused silica capillary (150 mm × 0.53 mm i.d.; GL Sciences) as reported previously [33]. The stationary phase of analytical column employed was L-column ODS (5 μm particle size; Chemicals Evaluation and Research Institute, Tokyo, Japan). And laboratory-made line filter (stainless steel frit, 2 μm) was used to protect the analytical column. The pre-column was prepared by using fused silica capillary (12 mm × 0.32 mm i.d.; GL Sciences), and the stationary phase employed was Oasis HLB (30 μm particle size; Waters). The connection capillary tubes attached to the M-435 micro injection valve were prepared from fused silica tubing with 50 or 75 μm i.d. (GL Sciences).

The mass spectrometry conditions for electrospray ionization operated in the negative ion mode were as follows: the cone and

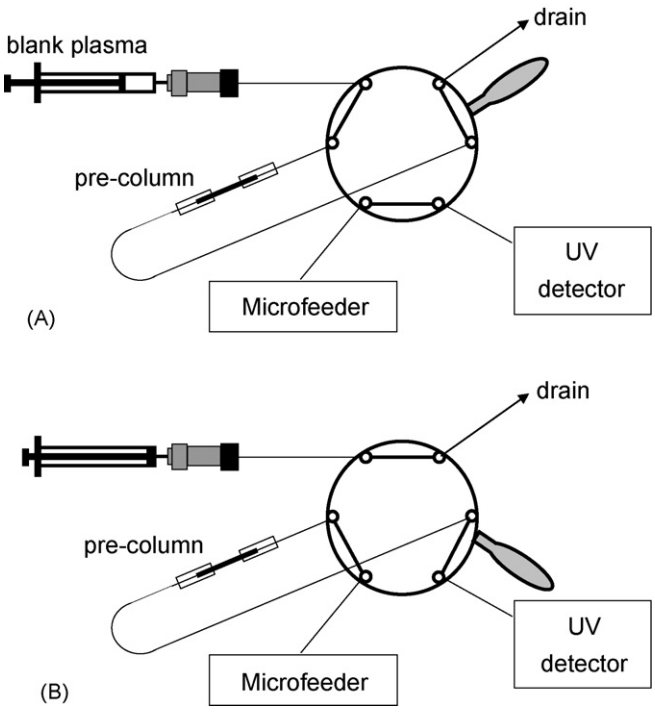

Fig. 3. A schematic diagram of measurement the elution profile of the sample matrix. (A) Blank plasma (10 μl) was injected into pre-column. (B) Valve was switched and matrix compounds from plasma were eluted out with Milli-Q water and detected UV detector.

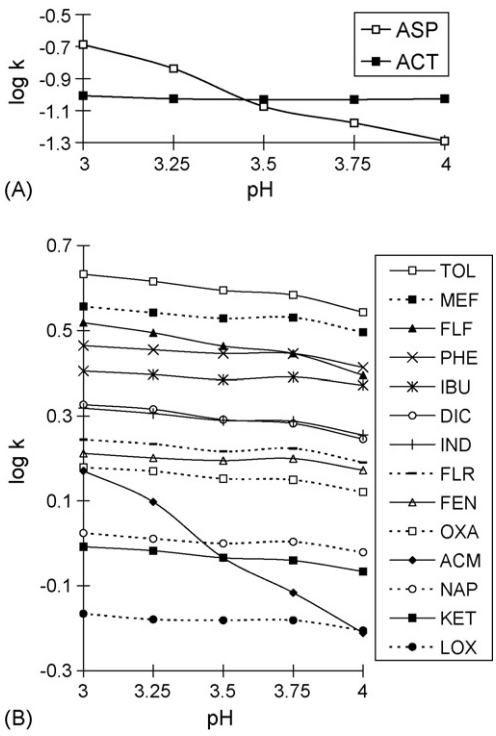

Fig. 4. (A, B) Relationship between the logarithms of retention factors (k) and the pH of buffer in the mobile phase. Column: L-column ODS (150 mm × 0.53 mm i.d.). Mobile phase: acetonitrile/10 mM ammonium formate aqueous solution (60:40), 12.5 μl min^{-1}. Detection: ESI negative ion mode (cone voltage 30 V, capillary voltage 3.0 kV).

probe capillary voltages were 30 V and 3.0 kV, respectively; the source block and desolvation temperatures were 80 and 150 °C, respectively. The cone and probe capillary voltages were set based on our previous report [31].

In order to measure the elution profile of the sample matrix (human plasma), pre-column was connected via the M-435 micro injection valve to the UV-2070 UV detector (Jasco, Tokyo, Japan) and MF-2 Microfeeder syringe pump (Azumadenki Kogyo, Tokyo, Japan) (Fig. 3). The UV detector was set at 220 nm. The Microfeeder was equipped with an MS-GAN 050 gas-tight syringe (0.5 ml; Ito, Fuji, Japan) and operated at flow rate of 11.1 μl min^{-1}.

3. Results and discussion

3.1. Optimization of chromatographic conditions

The chromatographic conditions were optimized with respect to the pH of the mobile phase and amount of organic modifier in the mobile phase. In the present study, the mobile phase was a mixture of an organic modifier and a buffer. Acetonitrile was used as the organic modifier, while ammonium formate aqueous solution was used as the buffer in which its pH was adjusted by addition of formic acid. In order to investigate the effect of pH, it was varied over the range of 3.0–4.0, while keeping the concentration of acetonitrile at 60%. Fig. 4A and B show relationships between the logarithms of retention factors (k) and the pH of buffer. Hold-up time (t_0) was determined with uracil as a non-retained marker compound. As shown in Fig. 4A and

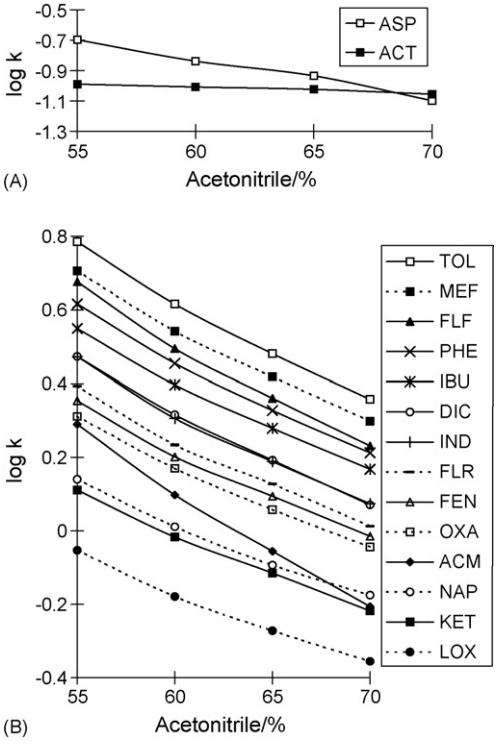

Fig. 5. (A, B) Relationship between the logarithms of retention factors (k) and the concentration of acetonitrile in the mobile phase. Column: L-column ODS (150 mm × 0.53 mm i.d.). Mobile phase: acetonitrile/10 mM ammonium formate aqueous solution (pH 3.25), 12.5 μl min^{-1}. Detection: ESI negative ion mode (cone voltage 30 V, capillary voltage 3.0 kV).

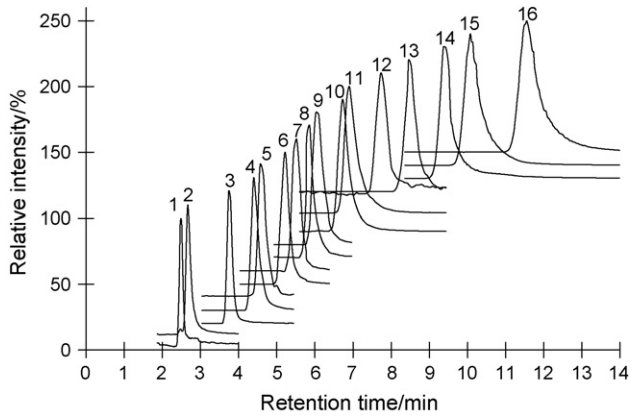

Fig. 6. Mass fragmentograms of 16 NSAIDs by using the pre-column in capillary LC–MS (overlay step: 10%). Mass fragmentograms were acquired in the selected ion monitoring mode at the *m/z* value of the base ions. The analytical conditions are given in Section 2. Compounds are: (1) = ACT, (2) = ASP, (3) = LOX, (4) = KET, (5) = NAP, (6) = ACM, (7) = OXA, (8) = FEN, (9) = FLR, (10) = DIC, (11) = IND, (12) = IBU, (13) = PHE, (14) = FLF, (15) = MEF, (16) = TOL.

B, the logarithms of *k* values in ASP, ACM and FLF decreased significantly with increasing pH compared to the others because of their pK_a values (ASP: 3.5, ACM: 2.9, FLF: 3.9). When the pH was 3.25, all of the analytes were separated.

In order to investigate the effect of the organic modifier, the concentration of acetonitrile was varied over the range of 55–70%, while keeping the pH of buffer at 3.25. Fig. 5A and B show relationships between the logarithms of *k* and the concentration of acetonitrile in the mobile phase. The logarithms of *k* values in all analytes were decreased with increasing concentration of acetonitrile in the mobile phase. All analytes were best separated when the concentration of acetonitrile was 60%.

Therefore, we finally chose the concentration of acetonitrile at 60% and the pH of buffer at 3.25.

Fig. 6 shows mass fragmentograms which were acquired in the selected ion monitoring (SIM) mode at the *m/z* value of the base ions (Table 1). Under these conditions, all analytes were separated by using the pre-column in capillary LC–MS. The total analytical time for a single analytical run was approximately 15 min.

3.2. Pre-column

Pre-columns were made using the same lot of packing materials. There was no significant difference in the recoveries between the pre-columns when standard solutions were injected into each pre-column ($n = 3$). Therefore, the differences in pre-column to pre-column reproducibility were almost negligible.

Pre-column showed no decrease in efficiency and analytical column showed no increase in back pressure and no deterioration of column performance after more than 50 injections of plasma samples.

3.3. Washing solvent

In order to wash the pre-column after sample injection, washing solvent was injected. Milli-Q water with no organic modifier

Table 1
The major ions observed in the spectrum at cone voltage of 30 V

Compound	MW	Base peak (*m/z*)	Other ion peak (*m/z*)
ACT	151	150	
ASP	180	137	
LOX	246	245	201
KET	254	209	253
NAP	230	185	170, 229
ACM	415	414	
OXA	293	292	
FEN	241	197	
FLR	244	199	
IND	357	312	356, 358
DIC	296	250	294
IBU	206	205	161
PHE	308	307	
FLF	281	280	
MEF	241	240	
TOL	261	260	

Operating conditions in this table—inlet system: direct (by syringe pump, 10 μl min^{-1}). Sample: 1 μg ml^{-1} NSAID dissolved in mobile phase. Electrospray ionization, negative ion mode. Capillary voltage: 3 kV. Cone voltage: 30 V. Source block temperature: 80 °C. Desolvation temperature: 150 °C.

was used as washing solvent. Milli-Q water with organic solvent (methanol or acetonitrile) as washing solvent did not result in better recoveries because of breakthrough. Therefore, we chose Milli-Q water with no organic modifier as washing solvent. To determine the adequate volume of washing solvent, blank plasma sample was injected onto the pre-column (Fig. 3A), and valve was switched and the elution profile of the sample matrix was measured by UV detector (Fig. 3B). UV detector was set to 220 nm to detect not only plasma proteins but also any other matrix compound. Fig. 7 shows an elution profile of blank plasma. As shown in Fig. 7, sample matrix compounds from plasma were removed from the pre-column within 5 min. Due to the pore diameter (about 8 nm) of the Oasis HLB, most protein interferences were extruded from the pore and not retained on the pre-column and directly flushed into the waste. Since the flow rate was 11.1 μl min^{-1} and it took ca. 5 min to remove almost all matrix compounds from the pre-column, a 55.5 μl of

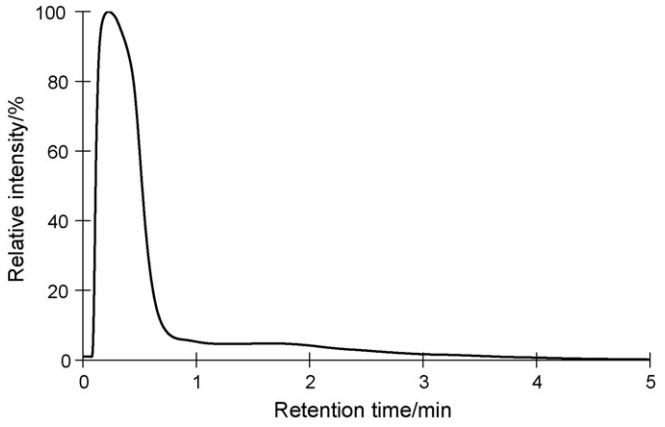

Fig. 7. Elution profile of plasma on the pre-column. Eluent: Milli-Q water, 11.1 μl min^{-1}. Detection: UV detector, 220 nm.

washing solvent was needed and sufficient for the removal of matrix compounds.

The volume of washing solvent was then examined up to 60 μl and breakthrough of analytes was not observed. Therefore, we determined 60 μl of Milli-Q water as the washing solvent.

3.4. Carryover

In order to reduce carryover, rinsing solvent was injected to pre-column between the analyses. The rinsing solvent was acetonitrile and Milli-Q water. Acetonitrile was used as the rinsing solvent because it has higher elution power. After rinsing with acetonitrile, Milli-Q water was injected to equilibrate the pre-column. No carryover from previous injections was observed when standard solutions and plasma samples were injected.

3.5. Method optimization

Quantitative measurements of the 16 analytes were carried out by LC–MS in the SIM mode. No significant interference peaks were observed from plasma taken from three different humans.

3.5.1. Stability of stock solution

The stability of stock solution was tested by comparing its response with that of freshly prepared solution after dilution with water. No significant difference (maximum 6.3% loss) in the instrument response was observed. The stock solution was stable for at least 2 months with storage at 4 °C.

Table 2
Linear range

Compound	Calibration range (μg ml^{-1})	r^2
ACT	0.25–5	0.9994
ASP	0.025–0.25	0.9991
LOX	0.05–1	0.9990
KET	0.025–1	0.9990
NAP	0.1–2.5	0.9994
ACM	0.01–1	0.9990
OXA	0.1–5	0.9994
FEN	0.01–1	0.9999
FLR	0.01–1	0.9994
IND	0.01–2.5	0.9991
DIC	0.1–2.5	0.9997
IBU	0.25–2.5	0.9990
PHE	0.025–0.75	0.9990
FLF	0.01–0.75	0.9990
MEF	0.01–0.25	0.9992
TOL	0.01–0.75	0.9990

Operating conditions in this table—column: L-column ODS (150 mm × 0.53 mm i.d., 5 μm particle size). Mobile phase: 10 mM ammonium formate aqueous solution (pH 3.25) and acetonitrile (40:60, v/v) flow rate: 12.5 μl min^{-1}. Pre-column: Oasis HLB (12 mm × 0.32 mm i.d., 30 μm particle size). Sample: plasma samples. Injection volume: 10 μl. Electrospray ionization, negative ion mode. Capillary voltage: 3 kV. Cone voltage: 30 V. Source block temperature: 80 °C. Desolvation temperature: 150 °C.

Table 3
Intra-day and inter-day precision

Compound	Concentration (μg ml^{-1})	R.S.D. (%, $n = 6$)	
		Intra-day	Inter-day
ACT	0.25	11.5	13.0
	0.5	8.9	10.0
	1	7.6	9.5
ASP	0.05	7.1	7.3
	0.5	5.9	7.1
	1	6.9	6.8
LOX	0.1	8.3	9.1
	0.5	6.5	9.0
	1	5.9	7.6
KET	0.05	5.7	7.4
	0.5	4.6	6.7
	1	5.8	6.9
NAP	0.25	8.0	7.9
	0.5	6.1	7.3
	1	6.9	7.2
ACM	0.05	6.0	6.2
	0.5	5.8	6.0
	1	6.1	7.8
OXA	0.25	6.7	7.2
	0.5	5.8	6.2
	1	5.4	7.4
FEN	0.05	6.1	6.9
	0.5	5.5	6.7
	1	5.2	6.0
FLR	0.05	5.8	6.4
	0.5	5.1	6.9
	1	6.0	6.2
IND	0.05	6.0	6.3
	0.5	6.2	8.1
	1	4.3	6.1
DIC	0.25	5.0	6.8
	0.5	3.8	6.0
	1	3.1	5.2
IBU	0.25	9.1	11.3
	0.5	6.5	8.1
	1	7.1	7.3
PHE	0.05	6.5	7.7
	0.25	3.6	4.6
	0.5	3.9	5.0
FLF	0.05	6.4	9.1
	0.25	5.3	6.0
	0.5	4.8	6.3
MEF	0.05	7.2	7.5
	0.1	5.6	7.3
	0.25	5.2	6.9
TOL	0.05	4.1	5.8
	0.25	3.3	5.1
	0.5	4.0	5.5

Operating conditions in this table—column: L-column ODS (150 mm × 0.53 mm i.d., 5 μm particle size). Mobile phase: 10 mM ammonium formate (pH 3.25) and acetonitrile (40:60, v/v). flow rate: 12.5 μl min^{-1}. Pre-column: Oasis HLB (12 mm × 0.32 mm i.d., 30 μm particle size). Sample: quality control samples. Injection volume: 10 μl. Electrospray ionization, negative ion mode. Capillary voltage: 3 kV. Cone voltage: 30 V. Source block temperature: 80 °C. Desolvation temperature: 150 °C.

Table 4
Detection and quantitation limits

Compound	Detection limit (μg ml^{-1})	Quantitation limit (μg ml^{-1})
ACT	0.075	0.25
ASP	0.001	0.005
LOX	0.01	0.05
KET	0.005	0.025
NAP	0.025	0.1
ACM	0.0025	0.01
OXA	0.025	0.1
FEN	0.0025	0.01
FLR	0.0025	0.01
IND	0.0025	0.01
DIC	0.025	0.1
IBU	0.05	0.25
PHE	0.0075	0.025
FLF	0.001	0.005
MEF	0.001	0.005
TOL	0.001	0.005

Operating conditions in this table—column: L-column ODS (150 mm × 0.53 mm i.d., 5 μm particle size). Mobile phase: 10 mM ammonium formate aqueous solution (pH 3.25) and acetonitrile (40:60, v/v). flow rate: 12.5 μl min^{-1}. Pre-column: Oasis HLB (12 mm × 0.32 mm i.d., 30 μm particle size). Sample: plasma samples. Injection volume: 10 μl. Electrospray ionization, negative ion mode. Capillary voltage: 3 kV. Cone voltage: 30 V. Source block temperature: 80 °C. Desolvation temperature: 150 °C.

Table 5
Recoveries of NSAIDs from plasma sample and the R.S.D.

Compound	Recovery (%)	R.S.D. (%, $n = 6$)
ACT	56.7	11.5
ASP	73.0	6.5
LOX	91.6	6.0
KET	83.8	5.2
NAP	80.1	8.0
ACM	88.8	6.0
OXA	96.9	6.7
FEN	73.2	5.5
FLR	86.7	6.0
IND	88.2	3.9
DIC	79.9	5.0
IBU	84.2	9.1
PHE	66.7	3.6
FLF	96.4	5.3
MEF	77.1	5.2
TOL	75.4	3.3

Operating conditions in this table—column: L-column ODS (150 mm × 0.53 mm i.d., 5 μm particle size). Mobile phase: 10 mM ammonium formate aqueous solution (pH 3.25) and acetonitrile (40:60, v/v) flow rate: 12.5 μl min^{-1}. Pre-column: Oasis HLB (12 mm × 0.32 mm i.d., 30 μm particle size). Sample: plasma samples (0.25 μg ml^{-1}). Injection volume: 10 μl. Electrospray ionization, negative ion mode. Capillary voltage: 3 kV. Cone voltage: 30 V. Source block temperature: 80 °C. Desolvation temperature: 150 °C.

3.5.2. Linearity

The linearity of the calibration lines was estimated by correlating the peak area in the mass fragmentograms of the base ion (Table 1) to sample concentration. The calibration curves for the NSAIDs were obtained by plotting the concentrations versus the peak areas. The linearity was investigated using plasma samples. Correlation coefficients (r^2) from 0.9990 to 0.9999 were found for all analytes showing a good linearity (Table 2).

3.5.3. Precision

Intra-day ($n = 6$) and inter-day ($n = 6$) precision of this method was determined by analyzing quality control samples with three different concentrations in linear range for each compound. The inter-day precision was assessed for consecutive days. The intra-day and inter-day precision results are shown in Table 3. The intra-day and inter-day precision (R.S.D.) was less than 11.5 and 13.0%, respectively.

3.5.4. Detection limit and quantitation limit

The detection limit and quantitation limit were calculated for plasma sample based on the signal-to-noise ratio equals to 3 and 10, respectively, and are summarized in Table 4. The detection limits were 0.001–0.075 μg ml^{-1}. From the results above, this method appears to be sufficiently sensitive to detect the NSAIDs at therapeutic level.

3.5.5. Recovery

Recoveries of 16 NSAIDs from plasma samples (0.25 μg ml^{-1}) were calculated by comparing the peak areas of each analyte to the standard solution, which injected directly into the analytical column. Table 5 shows the recoveries of 16 NSAIDs from human plasma. The recoveries of NSAIDs from human plasma were between 56.7 and 96.9%.

4. Conclusion

A rapid and sensitive analytical method was developed for the simultaneous determination of 16 NSAIDs using a pre-column in capillary LC–MS. In this system, the sample clean-up and enrichment were accomplished on-line, and thus resulted in improved sensitivity. Consequently, this method reduced sample preparation time and sample volume compared with off-line sample extraction methods and conventional LC methods, respectively. In addition, the usage of a capillary column saved solvent, reagent and column packing materials. Since this method could be applied to spiked samples, it may be applicable to NSAIDs in biological fluid. Our capillary LC–MS method appears to be sufficiently sensitive for detecting the NSAIDs at their therapeutic levels.

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