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Rapid screening for acidic non-steroidal anti-inflammatory drugs in urine by gas chromatography–mass spectrometry in the selected-ion monitoring mode

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Abstract

A rapid screening procedure is described for the simultaneous determination of various acidic non-steroidal anti-inflammatory drugs (NSAIDs) at sub-nanogram levels. The procedure involves solid-phase extraction (SPE) of NSAIDs using Chromosorb P as the adsorbent in partition mode, with subsequent single-step conversion to *tert.*-butyldimethylsilyl (TBDMS) derivatives, followed by direct analysis by gas chromatography–mass spectrometry (GC–MS). The characteristic $[M-57]^+$ high-mass ions constituting the base peaks in the electron-impact mass spectra of most TBDMS derivatives permitted sensitive detection of NSAIDs by GC–MS in selected-ion monitoring (SIM) mode, even in the presence of higher levels of coextracted urinary organic acids. The detection limit for SIM of each drug was in the range 0.03–0.9 pg. When applied to urine samples (250 μl) spiked with NSAIDs, the present GC–SIM–MS method allowed simultaneous screening for various NSAIDs with good overall precision and accuracy in the range of 10–40 ng.

Keywords: Non-steroidal anti-inflammatory drugs

1. Introduction

The carboxylated acidic non-steroidal anti-inflammatory drugs (NSAIDs) constitute the principal class of agents for controlling pain and inflammation of rheumatic diseases. A combination of these drugs is also used for analgesic abuse in horses and other animals [1]. Simultaneous detection and identification of these various NSAIDs is important for clinical and toxicological screening, as well as for controlling illegal use in animals [1–9].

In the literature, the application of high-resolution capillary gas chromatography (GC) to NSAID profiling analysis has been extensively investigated [1,3,7–10]. We have previously reported a simple profiling method which can simultaneously screen for various acidic NSAIDs [8,10]. The major advantage of our method is the use of solid-phase extraction (SPE) in partition mode using hydrophilic Chromosorb P as the adsorbent to recover NSAIDs from small amounts of biological samples. The recovered NSAIDs are then directly converted to their corresponding stable *tert.*-butyldimethylsilyl (TBDMS) derivatives in a single step for dual-capillary column GC analyses. Our dual-capillary

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column GC system provides accurate measurements of retention index (I) and area ratio (AR) values on dual columns of different polarity, thus enabling positive peak identification through computer library matching based on the two I sets and AR comparison [11].

A major problem with simultaneous screening for various acidic NSAIDs present at sub-nanogram levels in biological samples, especially urine specimens, is the coextraction of multiple endogenous organic acids at much higher concentrations which interfere with the resolution of individual NSAIDs upon GC analyses [10]. Therefore, a very sensitive method which can selectively monitor NSAIDs is required, such as gas chromatography–mass spectrometry (GC–MS) in selected-ion monitoring (SIM) mode.

GC–SIM–MS methods have been applied to trimethylsilylated [1] and methylated [9] NSAIDs, but not to the TBDMS derivatives to date. However, it is well known that TBDMS derivatives possess superior SIM properties, because of the characteristic $[M-57]^+$ high mass ions that constitute base peaks in their electron-impact ionization (EI) mass spectra [8,12,13].

In continuation of NSAID screening research [8,10], the present study was undertaken to investigate the optimal conditions of GC–SIM–MS analyses. The optimal SIM conditions were applied to the accurate quantitative screening of urine samples for trace NSAIDs that were either spiked or ingested.

2. Experimental

2.1. Materials

Standards of twenty NSAIDs obtained from Sigma (St. Louis, MO, USA) and various pharmaceutical companies were as follows: ibuprofen, alclofenac, fenoprofen, flufenamic acid, naproxen, niflumic acid, pirprofen, ketoprofen, mefenamic acid, fenclofenac, tolfenamic acid, diclofenac, suprofen, tolmetin, zomepirac, indoprofen, fentiazac, indomethacin, flubiprofen and lonazolac. Silylating reagent, N-methyl-N-(*tert.*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) was purchased from Pierce (Rockford, IL, USA), and triethylamine (TEA) from Aldrich (Milwaukee, WI, USA). All other chemicals were of analytical grade and used as received. Chromosorb P (acid-washed, 80–100 mesh) was purchased from Supelco (Bellefonte, PA, USA). A glass tube (5 mm I.D.) packed with Chromosorb P (400 mg) was washed successively with methanol, acetone, dichloromethane and diethyl ether, and activated under vacuum (150°C, 3 h) prior to being used as a SPE tube.

2.2. NSAID solutions

NSAID stock solution containing eighteen NSAIDs in their free acid form was prepared at a concentration of 0.1 μg/μl in methanol. The stock solution was used to prepare working solutions of varying concentrations (0.5 to 20 ng/μl) in methanol. Flubiprofen and lonazolac, used as the internal standards (I.S.), were dissolved in methanol at a concentration of 5 ng/μl. Flubiprofen was used as the I.S. for drugs eluted prior to lonazolac, and lonazolac was used as the I.S. for drugs eluted after lonazolac.

2.3. tert.-Butyldimethylsilylation

Prior to GC–MS analyses, NSAIDs were converted to their corresponding TBDMS derivatives as follows: NSAID solutions were evaporated to dryness under a gentle stream of nitrogen at 50°C; to the residues 5 μl of TEA and 5 μl of dodecane were added, and the mixtures were then silylated with 10 μl of MTBSTFA at 60°C for 1 h. The reaction mixture was directly examined by GC–MS. The standard samples containing twenty NSAIDs at 1 ng each were prepared for determining detection limits. The standard samples for testing linearity of GC–SIM–MS responses were prepared with five NSAID standard solutions containing 10, 20, 100, 200 and 400 ng of each NSAID and 100 ng of the I.S.

2.4. Sample preparation

All the biological samples used for this study were first morning urine samples collected in the early

morning and stored frozen before use: Nine healthy male subjects were divided to three groups, and urine samples were collected on the first day following the oral administration of ibuprofen (1200 mg), mefenamic acid (1500 mg) and naproxen (500 mg) to each group. Also, urine samples were collected on the first, second and third days following the oral administration of naproxen (250 mg) to a single subject.

Aliquots of 250 μl of blank urine without spiking or after spiking with increasing amounts of NSAIDS (20, 100 and 200 ng each), and urine containing unknown amounts of administered NSAIDs, were mixed with 100 ng of I.S. so that the concentration of I.S. was 5 ng/μl in the final TBDMS reaction mixture. Each solution was made alkaline (pH 13) with 5 *M* sodium hydroxide, followed by washing with diethyl ether (2×1 ml). The aqueous layer was acidified (pH<1) with concentrated sulphuric acid, and saturated with sodium chloride, and then subjected to SPE in partition mode as described previously [10]. Briefly, the mixture was loaded onto an activated Chromosorb P column, followed by elution with diethyl ether (1 ml) and dichloromethane (1 ml) in sequence, using a solid-phase extractor (Supelco). The combined eluates were then evaporated and derivatized as described above. Samples for intra-day and inter-day assay tests were prepared by derivatizing blank urine extracts after spiking with increasing amounts of NSAIDs (10, 20, 80, 160 and 240 ng each) and fixed amount (100 ng) of I.S.. All samples were individually prepared in triplicate. They were analyzed in a day for intra-day assays, and on every other day for inter-day assays.

2.5. Gas chromatography–mass spectrometry

A Hewlett-Packard HP 5890A series II gas chromatograph, interfaced to an HP 5970B MSD (70 eV, EI mode) which was on-line to an HP 59940A MS ChemStation system, was used with a Ultra-2 (25 m×0.20 mm I.D., 0.11 μm d_f) cross-linked methyl silicone capillary column (Hewlett-Packard, Avondale, PA, USA). The column head pressure of helium as the carrier gas was set to 82.7 kPa. Aliquots of 0.5 μl of samples were introduced in split injection mode (5:1) at the injector temperature of 270°C, and the oven temperature was initially 190°C (held for 0.5 min), programmed to 230°C at a rate of 4°C/min then to 290°C at a rate of 30°C/min; the run time was 20 min. The interface and ion source temperatures were 300 and ~200°C, respectively.

2.6. Data acquisition

In scanning mode, mass range was 60 to 500 u at a rate of 1.29 scan/s, and the voltage of electron multiplier (EM) detector was 1400 V. In SIM mode, a base peak ion for each drug was selected and the start time for ion monitoring was programmed from 3.0 to 17.0 min to set up ten groups of ions to be monitored as listed in Table 1. A dwell time of 80 ms and the EM voltage of 2000 V were chosen for each ion monitored. The effect of dwell time such as 30, 50, 80, 100, 150 and 200 ms on the ion abundances were investigated.

2.7. Calculation

The detection limit for each NSAID was calculated based on the weight giving a signal three times the peak-to-peak noise of the background signal. Least-squares regression analysis was performed on the measured peak-area ratios against increasing weight ratios of NSAIDs to I.S. in order to test linearity of SIM responses and to plot calibration curves for the quantitative measurements of NSAIDs. Recoveries of NSAIDs added at three different levels to urine samples were calculated using I.S. method in order to test overall efficiency of the combined SPE, TBDMS derivatization and SIM measurement.

3. Results and discussion

3.1. Optimal GC–SIM–MS conditions

In a previous report [8], we verified that the TBDMS derivatives of twenty-six acidic NSAIDs possess superior GC and MS properties. In most of

Table 1
GC–SIM–MS data of NSAID standards as their TBDMS derivatives

No.	NSAID	Retention time (min)	Start time (min)	$[M-57]^+$ ion selected (m/z)	Detection limit (pg)
1	Ibuprofen	4.330	3.0	263.15	0.3
2	Alclofenac	6.614		283.05	0.9
3	Fenoprofen	8.568	7.5	299.15	0.3
4	Flufenamic acid	8.579		338.15	0.4
5	Flubiprofen	8.722		301.10	0.1
6	Naproxen	9.870	9.0	287.15	0.2
7	Niflumic acid	9.958		339.05	0.2
8	Pirprofen	10.533	10.3	308.15	0.6
9	Ketoprofen	11.665	11.5	311.15	0.3
10	Mefenamic acid	11.671		298.15	0.2
11	Fenclofenac	11.988		353.05	0.1
12	Tolfenamic acid	12.330	12.2	318.05	0.1
13	Diclofenac	12.538		352.05	0.2
14	Suprofen	12.729		317.05	0.2
15	Tolmetin	13.059	12.9	314.15	0.04
16	Zomepirac	13.544		348.15	0.9
17	Lonazolac	15.986	15.0	369.10	0.03
18	Indoprofen	16.609	16.3	338.25	0.2
19	Fentiazac	16.737		386.15	0.2
20	Indomethacin	17.630	17.0	370.25[a]	0.1

Analyzed on a Ultra-2 capillary column (25 m×0.20 mm I.D., 0.11 μm d_f), temperature programmed from 190°C (2 min) to 230°C at 4°C/min, then to 290°C at 30°C/min, in SIM mode at dwell time of 80 ms and EM voltage of 2000 V.
[a] Corresponds to $[M-101]^+$.

their EI mass spectra, $[M-57]^+$ high-mass ions generated by preferential cleavage of the labile *tert.*-butyl function from molecular ions constituted base peaks with a few exceptions.

Thus, for profiling analysis of NSAID mixtures in SIM acquisition mode, $[M-57]^+$ ions were selected as the single ion to be monitored for all NSAIDs except for indomethacin, where its second most abundant $[M-101]^+$ ion at m/z 370 was chosen rather than the third most abundant $[M-57]^+$ ion (Table 1). Under the present GC–MS condition, the retention time of each drug was very precise with reproducibility of 0.20% or better. Therefore, the start time of SIM activation was programmed from 3.0 to 17.0 min to set up ten groups of ions to be monitored (Table 1). Among the dwell times tested, 80 ms was found to yield the highest ion abundance for most of the drugs. With a single ion selected for SIM of each drug, the detection limits were in the range of 0.03–0.9 pg, proving the excellent sensitivity of our GC–SIM–MS conditions.

The typical total ion chromatogram of a standard mixture containing 200 ng each of twenty NSAIDs (Fig. 1A) shows that most drugs were resolved within 20 min with good sensitivity. Incomplete resolution between fenoprofen (peak 3) and flufenamic acid (peak 4), between naproxen (peak 6) and niflumic acid (peak 7), and between ketoprofen (peak 9) and mefenamic acid (peak 10) were observed. However, their peaks were clearly resolved in the individual ion chromatograms (Fig. 1B–G), because of the distinctive differences between the masses of their selected-ions. When the linearity and precision of the SIM response to each ion selected were examined, linear responses for the range 10–400 ng in 20 μl of final reaction mixtures were obtained with correlation coefficients varying from 0.992–0.999 (Table 2).

3.2. Urine matrix effects

Previously, we reported that determination of trace NSAIDs in urine was difficult due to the presence of

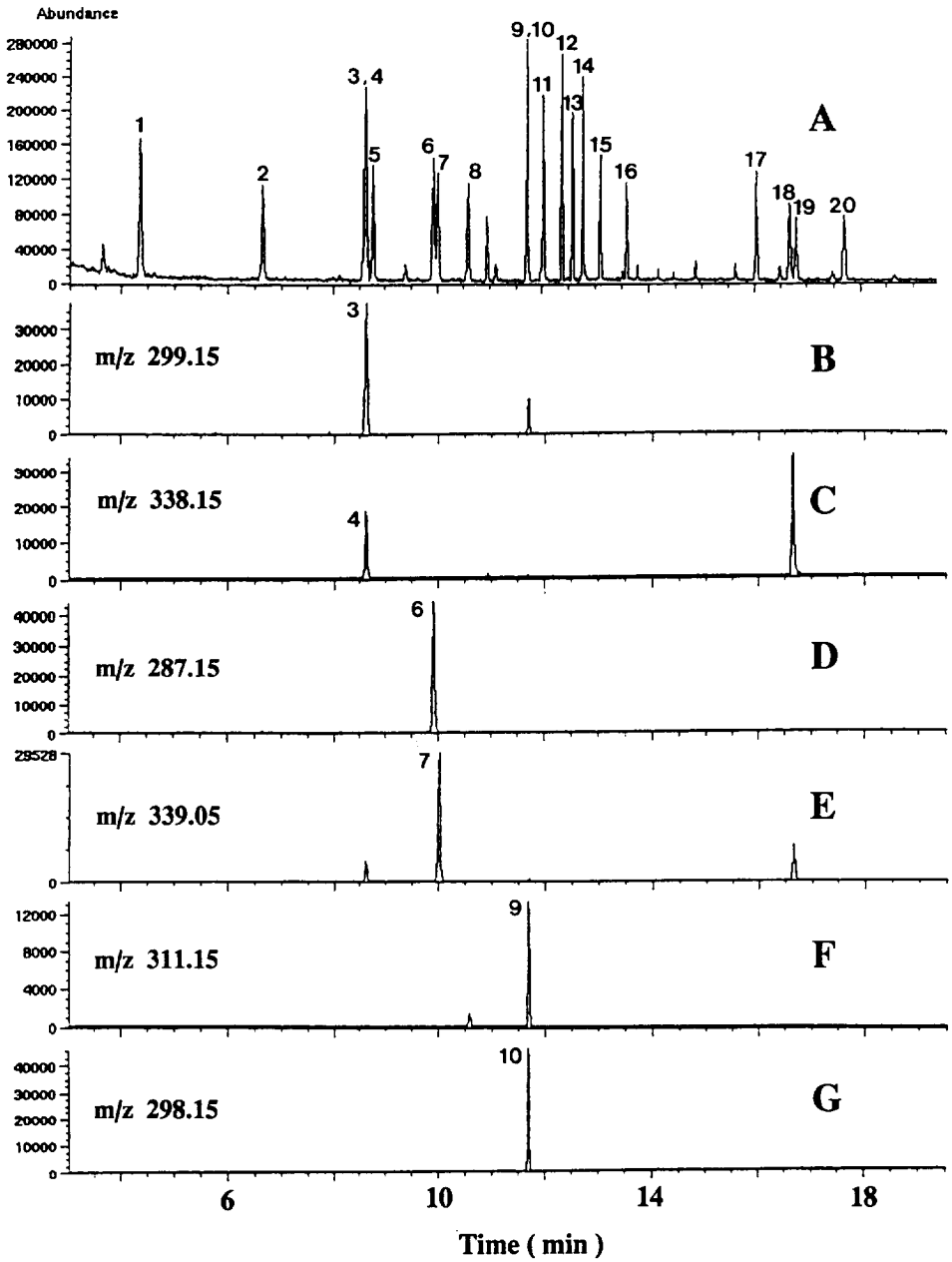

Fig. 1. Total ion chromatogram (A) of a standard mixture containing 200 ng each of 20 NSAIDs and their selected-ion chromatograms (B–G). Peak numbers correspond to those in Table 1. GC–SIM–MS conditions are given in Section 2.5 and Section 2.6.

interfering biogenic organic acids at much higher concentrations [10]. Therefore, urine matrix effects on SIM detection were examined with urine extracts prepared from blank urine using our SPE method in partition mode [10]. When the inner diameter of SPE tube was reduced to 5 mm, urinary organic acids

Table 2
Linearity of GC–SIM–MS responses to NSAID standards as their TBDMS derivatives

NSAID	Regression line[a]		Correlation coefficient (r)
	m	b	
Ibuprofen	1.130	0.0022	0.997
Alclofenac	0.364	−0.0112	0.990
Fenoprofen	1.010	−0.0121	0.998
Flufenamic acid	0.365	0.0230	0.990
Naproxen	0.889	−0.0159	0.997
Niflumic acid	0.374	0.0149	0.997
Pirprofen	0.275	0.0033	0.997
Ketoprofen	0.613	−0.0175	0.993
Mefenamic acid	0.644	0.1350	0.996
Fenclofenac	0.471	0.0484	0.992
Tolfenamic acid	0.364	0.0035	0.995
Diclofenac	0.221	−0.0070	0.994
Suprofen	0.545	0.0217	0.995
Tolmetin	0.151	0.0039	0.997
Zomepirac	0.093	0.0041	0.996
Indoprofen	0.251	0.0571	0.997
Fentiazac	0.131	0.0222	0.998
Indomethacin	0.096	0.0121	0.996

Standards containing 10, 20, 100, 200 and 400 ng of each NSAID at 100 ng of I.S. were analyzed on a Ultra-2 capillary column (25 m×0.20 mm I.D., 0.11 μm d_f), temperature programmed from 190°C (2 min) to 230°C at 4°C/min, then to 290°C at 30°C/min, in SIM mode at dwell time of 80 ms and EM voltage of 2000 V.
[a] m=slope=relative weight response=mean area ratio of NSAID×weight of I.S./weight of NSAID; b=y-intercept.

were recovered more efficiently within a shorter time (20 min), and required smaller amounts of urine (250 μl), Chromosorb P (400 mg) and eluting solvent (2 ml), for the quantitative analyses compared to the previous procedure [10]. The typical total ion chromatogram of blank urine extracts obtained in scanning mode shows a good urinary organic acid profile (Fig. 2A). When the same extract was analyzed in SIM mode, only a few organic acids such as 4-hydroxy-3-methoxyphenylacetic acid (peak a), *p*-hydroxyphenylacetic acid (peak b), *m*-hydroxyphenylacetic acid (peak c), *o*-hydroxyhippuric acid (peak d) and docosahexanoic acid (peak e) were detected (Fig. 2B). The total ion chromatogram in SIM mode of blank urine extract spiked with 240 ng each of NSAIDs demonstrates that each drug was well resolved from these interfering organic acids (Fig. 2C). Complete resolutions with no interferences are more clearly depicted in the individual ion chromatograms (Fig. 3).

When intra-day (Table 3) and inter-day (Table 4) assay tests were performed, linear relationships were observed between the area ratios and amount ratios of NSAIDs. As the levels decreased, the precision was generally lowered. However, the overall reproducibility appears to be satisfactory for the quantification of trace NSAIDs in unknown urine samples.

When the whole procedure of SPE and TBDMS derivatization with subsequent GC–SIM–MS analysis was applied to urine samples spiked with NSAIDs at three different levels, efficient and selective detection of each drug was possible (Table 5). The recoveries ranged from 74.6–114.9% with precision within 11.3% except for tolmetine (21.2%). The extraction efficiencies decreased with a decrease in the amount of the drugs added, indicating the occurrence of endogenous interference at low drug concentrations. The overall efficiency of the three combined steps for the simultaneous screening of NSAIDs appear to be sufficient for their quantitative measurements in unknown urine samples.

3.3. Screening of urine for NSAIDs

The present method was applied to first morning urine samples collected from healthy subjects following the oral administration of ibuprofen (1200 mg), mefenamic acid (1500 mg) or naproxen (500 or 250 mg). A series of selected-ion chromatograms of naproxen (Fig. 4) demonstrate the usefulness of our method in rapid screening for naproxen in urine samples collected on the first (Fig. 4A), second (Fig. 4B), and third (Fig. 4C) days after oral administration. We observed that the peak height of naproxen decreased with time, while the unknown peak adjacent to naproxen relatively increased. Each NSAID excreted in 250-μl aliquots of urine from ten subjects was quantitatively measured (Table 6). Large variations in the excreted amounts of each drug from subject to subject were observed. For the naproxen administered to the single subject, the amount of naproxen in the second day urine sample decreased rapidly relative to the first day sample. However, on

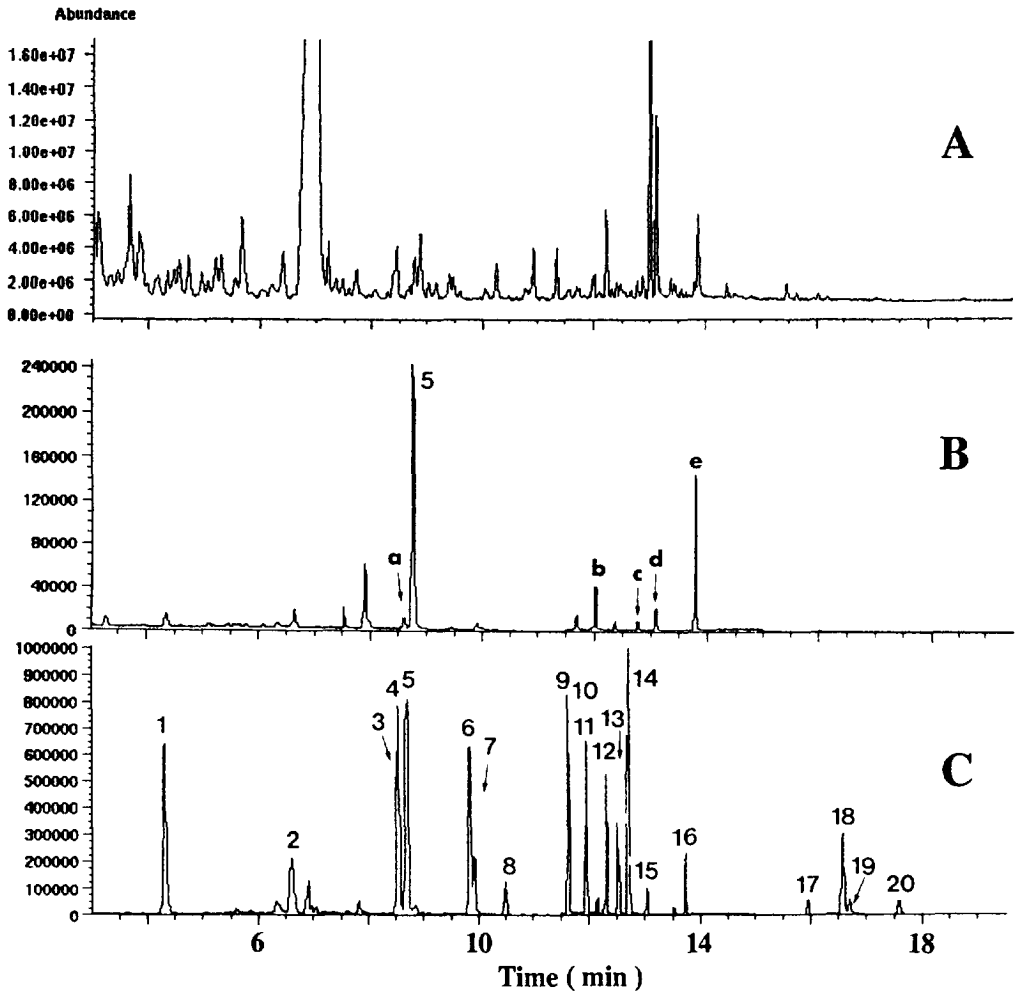

Fig. 2. Total ion chromatograms of blank urine extract in (A) scanning and (B) SIM modes, and (C) blank urine extract spiked with 240 ng each of NSAIDs in SIM mode. Peak numbers correspond to those in Table 1. GC–SIM–MS conditions are given in Section 2.5 and Section 2.6. Peaks: a=4-hydroxy-3-methoxyphenylacetic acid; b=*p*-hydroxyphenylacetic acid; c=*m*-hydroxy-phenylacetic acid; d=*o*-hydroxy-hippuric acid, e=docosahexanoic acid.

the third day, naproxen was decreased only slightly relative to the previous day.

4. Conclusion

A major advantage of the present rapid screening method is the selective detection of various NSAIDs by GC–SIM–MS as their TBDMS derivatives after SPE from urine. Characteristic $[M-57]^+$ high-mass ions permitted very selective monitoring of trace NSAIDs even in the presence of higher levels of coextracted urinary organic acids. The selective detection with high sensitivity will make this combination of SPE, TBDMS derivatization and GC–SIM–MS analyses suitable for the accurate screening of urine samples for various NSAIDs under double-blind conditions.

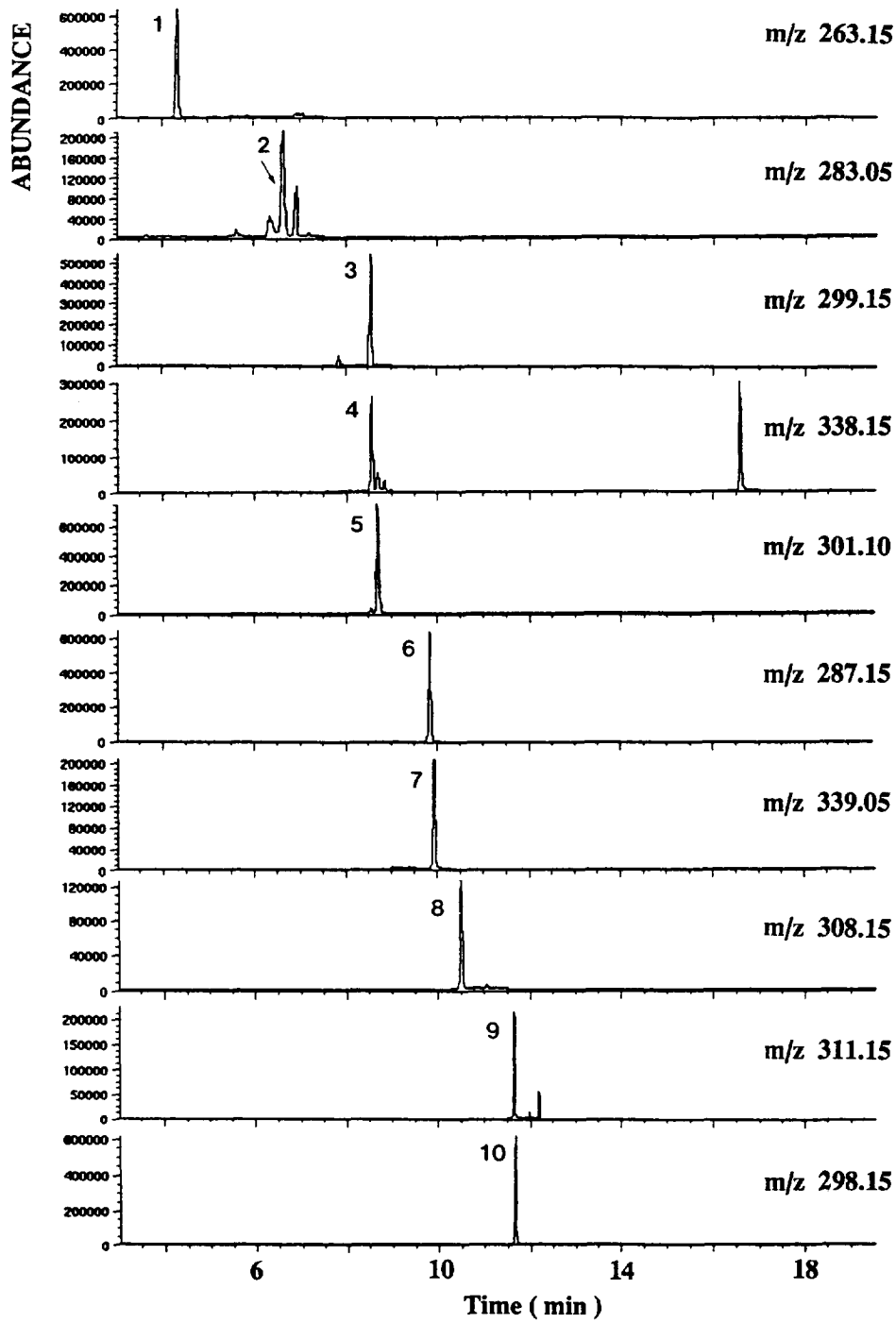

Fig. 3. Selected-ion chromatograms of individual NSAIDs (240 ng each) added into blank urine extract. The peak numbers correspond to those in Table 1. GC–SIM–MS conditions are given in Section 2.5 and Section 2.6.

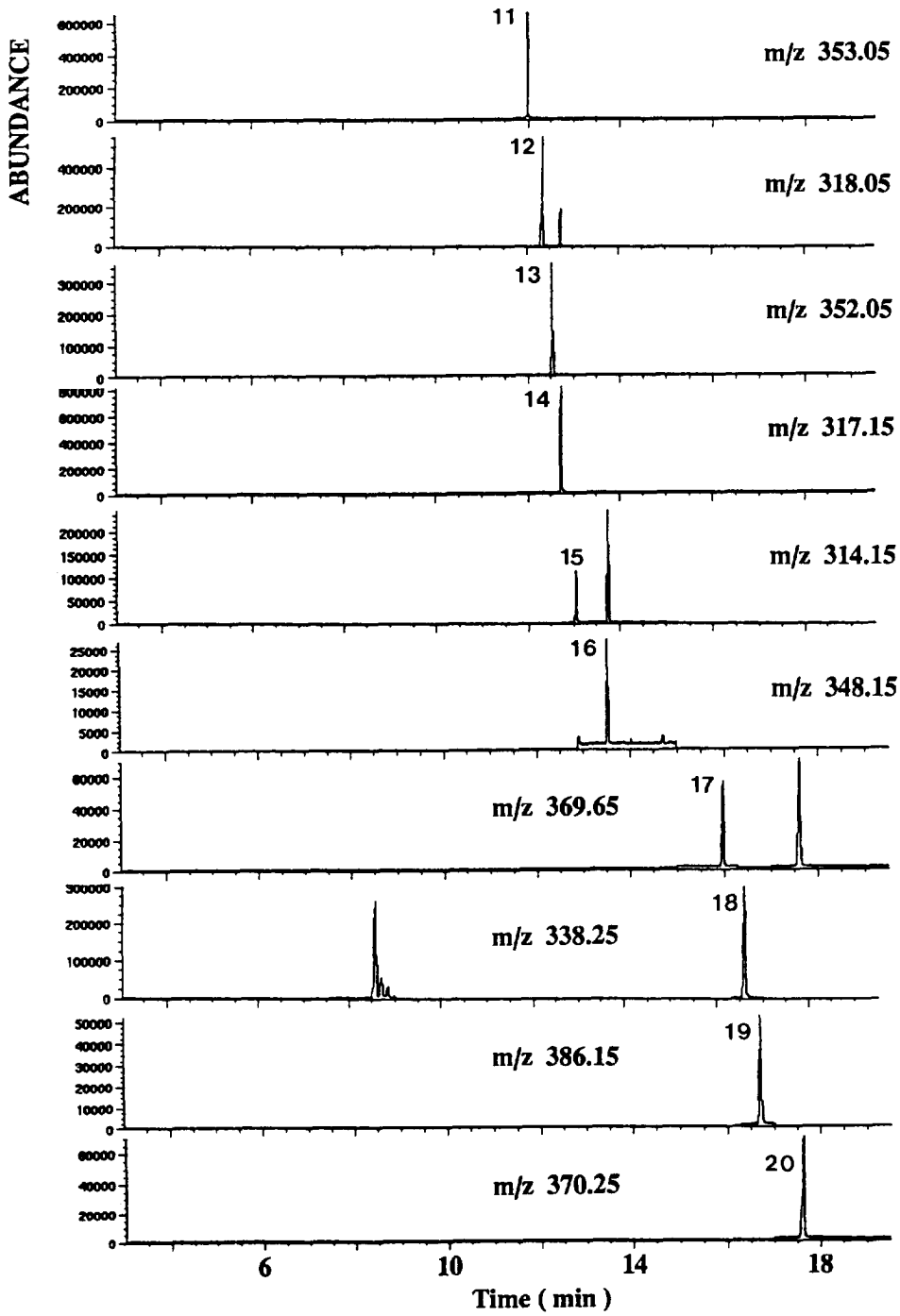

Fig. 3. (*continued*)

Table 3
Intra-day assay tests with blank urine extracts spiked with known amounts of NSAIDs

NSAID	AR (mean±S.D.)[a]				
	10 ng	20 ng	80 ng	160 ng	240 ng
Ibuprofen	0.55±0.08 (15.3)	0.99±0.13 (31.4)	3.52±0.12 (3.3)	7.86±0.70 (8.9)	12.18±0.56 (4.6)
Alclofenac	0.53±0.06 (11.8)	1.14±0.12 (0.7)	4.02±0.14 (3.5)	7.96±0.30 (3.7)	11.960±0.21 (6.7)
Fenoprofen	0.59±0.06 (10.0)	1.20±0.08 (6.6)	3.77±0.08 (2.1)	7.56±0.28 (3.6)	12.30±0.25 (2.1)
Flufenamic acid	0.53±0.09 (17.7)	1.11±0.24 (21.4)	3.78±0.12 (3.2)	7.81±0.05 (0.6)	12.11±0.11 (0.9)
Naproxen	0.61±0.01 (0.5)	1.21±0.14 (12.0)	3.96±0.34 (8.6)	7.39±0.29 (3.9)	12.37±0.17 (1.4)
Niflumic acid	0.48±0.08 (16.5)	1.18±0.15 (12.9)	3.96±0.35 (8.8)	8.22±0.91 (11.0)	11.90±0.76 (6.4)
Pirprofen	0.52±0.03 (6.4)	1.22±0.03 (2.8)	3.61±0.08 (2.3)	7.76±0.28 (3.6)	12.19±0.07 (0.6)
Ketoprofen	0.50±0.15 (29.2)	1.23±0.09 (7.2)	3.85±0.15 (3.9)	7.55±0.31 (4.0)	12.12±0.10 (0.9)
Mefenamic acid	0.52±0.02 (3.9)	1.17±0.06 (5.0)	3.79±0.13 (3.4)	7.43±0.17 (2.3)	12.35±0.14 (1.2)
Fenclofenac	0.57±0.07 (11.8)	1.25±0.02 (1.6)	3.77±0.13 (3.5)	7.62±0.24 (3.1)	12.28±0.16 (1.3)
Tolfenamic acid	0.49±0.08 (15.8)	1.26±0.05 (3.7)	3.58±0.10 (2.8)	7.78±0.14 (1.9)	12.19±0.01 (<0.1)
Diclofenac	0.53±0.03 (6.6)	1.19±0.08 (6.8)	3.73±0.16 (4.2)	7.63±0.24 (3.1)	12.21±0.07 (0.6)
Suprofen	0.49±0.01 (1.9)	1.16±0.13 (11.6)	3.82±0.28 (7.4)	7.92±0.86 (10.9)	12.26±0.15 (1.2)
Tolmetin	0.53±0.01 (2.0)	1.18±0.09 (7.4)	3.66±0.17 (4.5)	7.46±0.29 (3.9)	12.28±0.15 (1.2)
Zomepirac	0.53±0.01 (1.6)	1.24±0.01 (0.3)	3.57±0.23 (6.5)	7.78±0.08 (1.1)	12.48±0.17 (1.4)
Indoprofen	0.55±0.01 (0.6)	1.08±0.05 (4.7)	3.61±0.01 (0.2)	7.71±0.23 (3.0)	14.04±2.70 (19.2)
Fentiazac	0.53±0.01 (0.1)	1.22±0.01 (0.6)	3.56±0.34 (9.7)	7.19±0.53 (7.4)	12.60±0.34 (2.7)
Indomethacin	0.53±0.03 (5.3)	1.18±0.08 (7.0)	3.52±0.15 (4.4)	7.20±0.49 (6.8)	12.74±0.24 (1.9)

Analyzed on a Ultra-2 capillary column (25 m×0.20 mm I.D., 0.11 μm d_f), temperature programmed from 190°C (2 min) to 230° at 4°C/min, then to 290°C at 30°C/min, in SIM mode at dwell time of 80 ms and EM voltage of 2000 V.
[a]AR=Peak-area ratio relative to I.S., S.D.=standard deviation for $n=3$; Values in parentheses are relative standard deviations (%).

Table 4
Inter-day assay tests with blank urine extracts spiked with known amounts of NSAIDs

NSAID	AR (mean±S.D.)[a]				
	10 ng	20 ng	80 ng	160 ng	240 ng
Ibuprofen	0.53±0.12 (23.6)	1.11±0.11 (10.2)	3.85±0.33 (8.50)	7.70±0.32 (4.2)	12.21±0.25 (2.1)
Alclofenac	0.51±0.02 (3.9)	1.030±0.14 (13.9)	4.19±0.34 (8.2)	7.97±0.11 (1.4)	11.93±0.17 (1.4)
Fenoprofen	0.48±0.09 (19.5)	1.10±0.14 (12.3)	3.88±0.12 (3.0)	7.91±0.33 (4.1)	12.08±0.20 (1.7)
Flufenamic acid	0.52±0.05 (9.0)	1.05±0.10 (9.8)	4.07±0.25 (6.2)	8.05±0.23 (2.9)	11.83±0.23 (2.0)
Naproxen	0.47±0.13 (27.5)	1.10±0.12 (11.0)	3.99±0.06 (1.4)	7.88±0.43 (5.5)	12.06±0.28 (2.3)
Niflumic acid	0.46±0.04 (8.9)	0.95±0.21 (21.8)	4.47±0.44 (9.9)	8.43±0.47 (5.6)	11.60±0.41 (3.6)
Pirprofen	0.45±0.08 (17.2)	1.04±0.17 (16.7)	4.11±0.58 (14.2)	7.99±0.32 (4.0)	11.94±0.34 (2.8)
Ketoprofen	0.46±0.05 (10.5)	1.04±0.17 (6.4)	3.90±0.20 (5.2)	7.90±0.41 (5.2)	11.84±0.26 (2.2)
Mefenamic acid	0.46±0.05 (11.9)	1.13±0.17 (15.2)	3.94±0.13 (3.4)	7.95±0.49 (6.2)	12.03±0.32 (2.6)
Fenclofenac	0.52±0.07 (13.6)	1.13±0.22 (19.7)	3.89±0.11 (2.7)	8.00±0.44 (5.5)	11.91±0.32 (2.7)
Tolfenamic acid	0.44±0.06 (13.7)	1.13±0.16 (14.5)	3.95±0.34 (8.6)	8.14±0.46 (5.6)	11.89±0.36 (3.0)
Diclofenac	0.42±0.10 (22.6)	1.18±0.04 (3.7)	3.78±0.41 (10.9)	7.88±0.22 (2.7)	11.58±1.08 (9.3)
Suprofen	0.45±0.06 (12.6)	1.05±0.17 (16.4)	3.93±0.16 (4.0)	8.71±1.23 (14.1)	11.85±0.50 (4.2)
Tolmetin	0.52±0.04 (7.8)	1.04±0.14 (13.9)	3.87±0.20 (5.3)	8.59±1.47 (17.1)	11.43±1.26 (11.0)
Zomepirac	0.53±0.04 (8.2)	1.09±0.21 (18.9)	3.73±0.37 (9.9)	7.95±0.18 (2.2)	11.41±1.45 (12.7)
Indoprofen	0.54±0.05 (9.8)	0.96±0.15 (15.1)	3.75±0.39 (10.5)	7.80±0.46 (5.9)	12.76±1.14 (9.0)
Fentiazac	0.49±0.01 (2.5)	1.02±0.18 (17.4)	3.76±0.35 (9.2)	7.71±0.63 (8.2)	11.92±1.06 (8.9)
Indomethacin	0.52±0.09 (17.0)	0.93±0.23 (24.6)	3.73±0.45 (12.2)	7.84±0.59 (7.5)	11.80±1.23 (10.4)

Analyzed on a Ultra-2 capillary column (25 m×0.20 mm I.D., 0.11 μm d_f), temperature programmed from 190°C (2 min) to 230° at 4°C/min, then to 290°C at 30°C/min, in SIM mode at dwell time of 80 ms and EM voltage of 2000 V.
[a]AR=Peak-area ratio relative to I.S., S.D.=standard deviation for $n=3$; Values in parentheses are relative standard deviations (%).

Table 5
Overall efficiency of the combined solid-phase extraction, TBDMS derivatization and GC–SIM–MS measurement

NSAID	Recovery (mean±S.D.)[a]		
	20 ng	100 ng	200 ng
Ibuprofen	94.1±6.5 (6.9)	104.8±11.8 (11.3)	113.8±1.2 (1.1)
Alclofenac	97.6±5.6 (5.7)	114.9±2.5 (2.2)	113.8±5.5 (4.8)
Fenoprofen	82.0±0.6 (0.7)	91.3±4.8 (5.3)	97.5±3.0 (3.1)
Flufenamic acid	82.6±2.9 (3.5)	87.2±2.6 (3.0)	84.6±3.7 (4.3)
Naproxen	80.4±7.2 (9.0)	97.2±3.3 (3.4)	106.6±5.9 (5.6)
Niflumic acid	84.3±8.4 (10.0)	87.7±5.1 (5.8)	104.3±5.2 (5.0)
Pirprofen	83.6±3.0 (3.6)	109.4±10.0 (9.1)	90.2±9.6 (10.6)
Ketoprofen	105.1±5.2 (5.0)	101.5±7.7 (7.5)	113.9±1.2 (1.1)
Mefenamic acid	84.3±3.6 (4.2)	87.7±4.0 (4.5)	82.8±9.2 (11.1)
Fenclofenac	86.3±0.8 (0.9)	93.5±5.3 (5.7)	88.8±0.6 (0.7)
Tolfenamic acid	78.3±1.8 (2.3)	75.6±3.0 (4.0)	76.3±3.0 (3.9)
Diclofenac	78.7±6.4 (8.2)	107.4±6.8 (6.3)	102.0±9.0 (8.8)
Suprofen	109.2±9.9 (9.0)	106.7±8.0 (7.5)	104.4±11.4 (10.9)
Tolmetin	90.7±6.5 (7.1)	91.7±5.2 (5.6)	92.3±19.6 (21.2)
Zomepirac	86.2±6.6 (7.6)	94.3±2.8 (2.9)	90.3±2.2 (2.4)
Indoprofen	88.8±2.0 (2.2)	107.6±7.3 (6.8)	98.3±5.9 (6.0)
Fentiazac	77.5±0.8 (0.8)	103.4±4.5 (4.3)	95.7±4.4 (4.6)
Indomethacin	88.9±4.5 (5.8)	83.9±4.1 (4.9)	74.6±3.4 (4.6)

Analyzed on a Ultra-2 capillary column (25 m×0.20 mm I.D., 0.11 μm d_f), temperature programmed from 190°C (2 min) to 230° at 4°C/min, then to 290°C at 30°C/min, in SIM mode at dwell time of 80 ms and EM voltage of 2000 V.
[a] S.D.=Standard deviation for $n=3$; Values in parentheses are relative standard deviations (%).

Table 6
Amount of NSAIDs found in urine collected after oral administration

Subject	Amount found (ng)[a]			
	Ibuprofen[b]	Mefenamic acid[c]	Naproxen[d]	Naproxen[e]
A	324.93±38.00 (11.7)			
B	958.87±80.54 (8.4)			
C	751.00±53.21 (7.10)			
D		212.87±22.42 (10.5)		
E		344.00±17.12 (5.0)		
F		267.53±37.10 (13.9)		
G			63.87±13.12 (20.6)	
H			363.47± 7.34 (2.0)	
I			297.07± 6.66 (2.2)	
J				47.00± 1.74 (3.7)[1]
				12.30±1.42 (11.5)[2]
				11.00±1.15 (10.5)[3]

Analyzed on a Ultra-2 capillary column (25 m×0.20 mm I.D., 0.11 μm d_f), temperature programmed from 190°C (2 min) to 230° at 4°C/min, then to 290°C at 30°C/min, in SIM mode at dwell time of 80 ms and EM voltage of 2000 V.
[a] Amount found in 250 μl of urine.
[b] 1200 mg of ibuprofen was administrated A, B and C subjects.
[c] 1500 mg of mefenamic acid was administrated to D, E and F subjects.
[d] 500 mg of naproxen was administrated to G, H and I subjects.
[e] 250 mg of naproxen was administrated to J subject and urine was collected on [1] first, [2] second, and [3] third days.

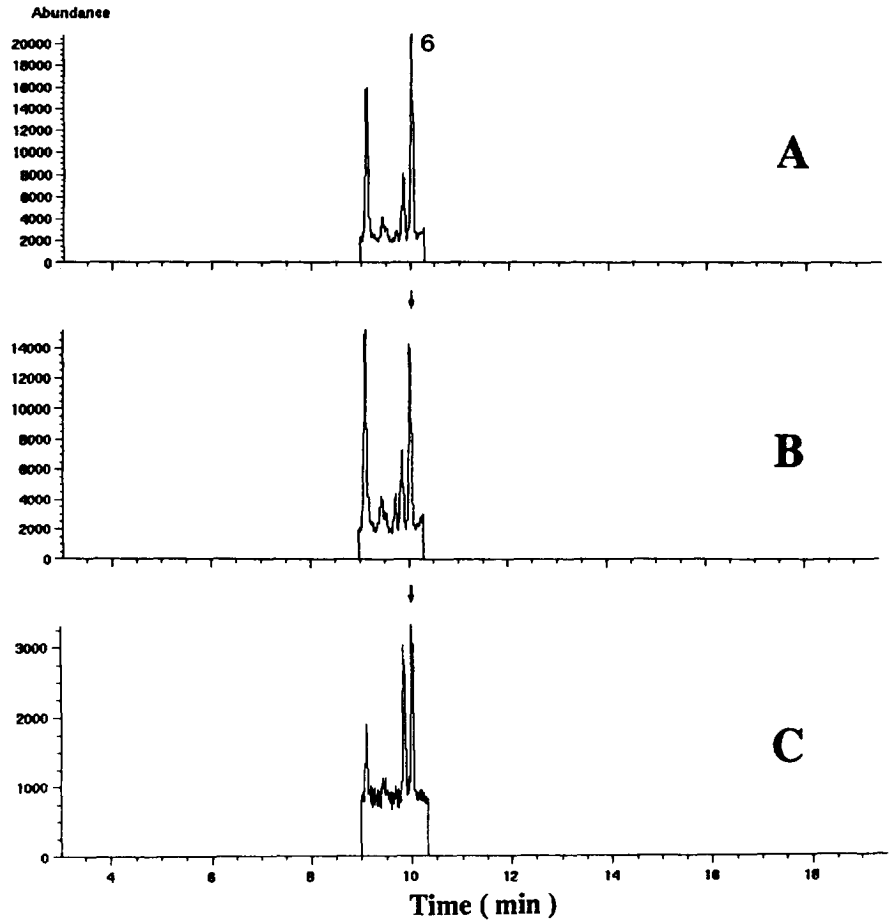

Fig. 4. Selected-ion chromatograms of naproxen from urine collected on the (A) first, (B) second, and (C) third days after oral administration of naproxen (250 mg). GC–SIM–MS conditions are given in Section 2.5 and Section 2.6. Peak: 6=naproxen.

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References

[1] A.K. Singh, Y. Jang, U. Mishra and K. Granley, J. Chromatogr., 568 (1991) 351.
[2] G.E. Harde, J.W. Lai and J.H. Morre, J. Liq. Chromatogr., 5 (1982) 1991.
[3] C. Giachetti, S. Canali and G. Zanolo, J. Chromatogr., 279 (1983) 387.
[4] H.J. Battista, G. Wehigher and R. Henn, J. Chromatogr., 345 (1985) 77.
[5] S.G. Owen, M.S. Roberts and W.T. Friesen, J. Chromatogr., 416 (1987) 293.
[6] F. Lapicque, P. Netter, B. Bannwarth, P. Trechot, P. Gillet, H. Lambert and R.J. Royer, J. Chromatogr., 496 (1989) 301.
[7] C. Giachetti, G. Zanolo, P. Poletti and F. Perovanni, J. High Resolut. Chromatogr. Commun., 13 (1990) 789.
[8] K.R. Kim, W.H. Shim, Y.J. Shin, J. Park, S. Myung and J. Hong, J. Chromatogr., 641 (1993) 319.
[9] C. Giachetti, A. Assandri, G. Zanolo and A. Tenconi, Chromatographia, 39 (1994) 162.
[10] K.R. Kim, Y.J. Shin, W.H. Shim and S. Myung, Arch. Pharm. Res., 17 (1994) 175.
[11] K.R. Kim, J.H. Kim, H.K. Park and C.H. Oh, Bull. Korean Chem. Soc., 14 (1993) 250.
[12] A.P.J.M. de Jong, J. Elema and B.J.T. van de Berg, Biom. Mass Spectrom., 7 (1980) 359.
[13] T.P. Mawhinney and M.A. Madson, J. Org. Chem., 47 (1982) 3336.