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Rapid Gas Chromatographic Profiling and Screening of Acidic Non-Steroidal Antiinflammatory Drugs in Biological Samples

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The solid-phase extraction (SPE) with subsequent *tert.*-butyldimethylsilyl (TBDMS) derivatization was investigated for the rapid profiling and screening of various carboxylated non-steroidal antiinflammatory drugs (NSAIDs) simultaneously in biological fluid samples. Compared to the conventional SPE in adsorption mode using Chromosorb 102, Chromosorb 107, Carbopak B and Thermosorb, the SPE in partition mode using Chromosorb P as the adsorbent, and ethyl acetate/methylene chloride as the eluting solvents provided highest overall recoveries of the NSAIDs from aqueous solutions with good precision. The solid-phase extracted NSAIDs were silylated with N-methyl-N-(*tert.*-butyldimethylsilyl)trifluoroacetamide to TBDMS derivatives and directly analyzed by capillary gas chromatography and gas chromatography-mass spectrometry. The usefulness of the present method was examined for the profiling and screening of saliva, serum and urine samples for various NSAIDs simultaneously.

Key words: NSAIDs, Solid-phase extraction, Chromosorb P, TBDMS derivatization, Screening, Biological sample

INTRODUCTION

The carboxylated acidic non-steroidal antiinflammatory drugs (NSAIDs) presently constitute the principal class of agents for controlling the pain and inflammation of the rheumatic diseases. Simultaneous detection and identification of these various NSAIDs is a commonly encountered problem for the clinical and toxicological screening especially in general-unknown cases (Battista *et al.*, 1985; Borra *et al.*, 1986; Dawson *et al.*, 1990; Giachetti *et al.*, 1983; Giachetti *et al.*, 1990; Lapicque *et al.*, 1989; Owen *et al.*, 1987; Singh *et al.*, 1991; Theis *et al.*, 1986).

The conventional clinical tests determine individual constituents which are known to be present. However, the profiling analysis by high resolution gas chromatography (GC), or high performance liquid chromatography (HPLC) offers the opportunity to detect and characterize multicomponents simultaneously in a single test. If simultaneous screening for the various NSAIDs is to be used routinely in the forensic and clinical chemistry laboratories, a simple separation scheme that permits to identify the most commonly used NSAIDs from complex biological matrices in a single analysis must be developed.

In the previous report (Kim *et al.*, 1993), we described the simultaneous GC analysis of 26 different NSAID standards as their *tert.*-butyldimethylsilyl (TBDMS) esters on a dual-capillary column GC system which provided positive confirmation of each NSAID based on the retention index (RI) matching. When these NSAIDs are present in the complex biological matrices, a suitable clean up is required prior to chromatographic analysis.

In the literature, classical liquid-liquid extraction methods (Battista *et al.*, 1985; Giachetti *et al.*, 1983; Giachetti *et al.*, 1990; Lapicque *et al.*, 1989; Owen *et al.*, 1987; Singh *et al.*, 1991) are exclusively used with a few solid-phase extraction (SPE) methods (Dawson *et al.*, 1990; Kim *et al.*, 1992; Theis *et al.*, 1986) for this purpose. However, SPE, because of its inherent advantages, has gained widespread acceptance as the sample method for HPLC and GC in major drug assays (Rood, 1990). Among the commonly used solid adsorbents, hydrophobic XAD-2 (Theis *et al.*, 1986), silica based bonded-phase C18 (Dawson *et al.*, 1990), and Chromosorb 107 (Kim and Shim,1992) have been used for the SPE of NSAIDs from biological specimens.

It is desirable that thorough studies on the sorption

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and desorption properties of the relevant solid adsorbents precede the proper choice of SPE conditions. Therefore, as the continuation of the NSAID screening works (Kim *et al.*, 1993), the present study was undertaken in order to examine the extraction efficiencies of Chromosorb 102, Chromosorb 107, Carbopak B, Thermosorb, and Chromosorb P for the simultaneous SPE of various carboxylated NSAIDs from aqueous samples. The optimal SPE conditions chosen were applied to the rapid qualitative screening of serum, saliva and urine samples for the spiked NSAIDs.

MATERIALS AND METHODS

Materials

Standards of the NSAIDs investigated were obtained from Sigma (St. Louis, MO, U.S.A.), and various pharmaceutical companies. The silylating reagent, N-methy-N-(*tert.*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) was obtained from Pierce (Rock-ford, IL, U.S.A.), triethylamine (TEA) from Aldrich (Milwaukee, WI, U.S.A.), and n-hydrocarbon standards (C_{16}-C_{36}, even numbers only) from Polyscience (Niles, IL, U.S.A.). All other chemicals were of analytical grade and used as received. Chromosorb P (acid -washed, 80-100 mesh),Chromosorb 102 and Chromosorb 107 (both 60-80 mesh), and Carbopak B (60-80 mesh) were purchased from Supelco (Bellefonte, PA, U.S.A.), and Thermosorb (80-100 mesh) from Chrompack (Bridgewater, NJ, U.S.A.).

NSAID and Internal Stock Solutions

The NSAID stock solutions containing various NSAIDs in their free acid forms were prepared each at a concentration of 10 μg/μl in methanol. Three internal standard (I.S) solutions were prepared by dissolving n-hexacosane, and n-octacosane in isooctane each at a concentration of 1 μg/μl, and zomepirac in methanol at a concentration of 0.2 μg/μl.

tert.-Butyldimethylsilylation

NSAID solutions containing an appropriate amount of I.S. were evaporated to dryness (N_2 , 50℃). To the residues were added TEA (10 μl), MTBSTFA (10 μl), and isooctane (30 μl), and the mixtures were vortex-mixed to form TBDMS derivatives at room temperature. The reaction mixtures were directly examined by GC and GC-MS. Calibration samples were prepared by derivatizing increasing amounts of NSAIDs (0.5～60 g) containing 1, 10, or 30 μg of internal standard. Calibration curves were constructed by plotting peak area ratios against weight ratios of each NSAID to internal standard.

Solid-Phase Extraction Tubes

For the SPE works in adsorption mode, individual SPE tubes of Chromosorb 102, Chromosorb 107, Carbopak B, and Thermosorb were prepared by packing 0.2 g each into 1 ml polypropylene empty tube secured with polypropylene frit (Supelco, Bellefonte , PA, U.S.A.). And they were washed sequentially with methanol, acetone, and methylene chloride followed by methanol, then equilibrated with pH 1.0 water before use. For the SPE works in partition mode, a male luer tipped glass tube (12 mm I.D.) of 5 ml was tightly packed with Chromosorb P (2.0 g) secured with glass wool plugs. It was then washed successively with pH 13 water, pH 1 water, methanol, acetone, methylene chloride, ethyl acetate followed by diethyl ether. It was activated under vacuo (150℃, 2 h) before use.

Solid-Phase Extraction

All the SPE works were performed with a Supelco visiprep solid vacuum manifold (Supelco Inc, Bellefante, PA, U.S.A.) equipped with a Eyela A-3S aspirator (Eyela, Japan). For the SPE in adsorption mode, a 1 ml of pH 1.0 water containing NSAIDs at 60 ppm was loaded onto a SPE tube followed by washing the packing with pH 1.0 water (3 ml). After the removal of moisture under full vacuum on the manifold , the SPE tube was eluted sequentially with methanol, acetone, methylene chloride (1 ml each). After addition of n-hexacosane or n-octacosane as I.S. at 30 ppm, the collected eluates were dried over anhyd. magnesium sulfate and subjected to the derivatization as described above. For the SPE in partition mode, a 1 ml of pH 1.0 water containing NSAIDs at 40 ppm and n-hexacosane or n-octacosane as I.S. at 10 ppm was saturated with sodium chloride and loaded onto an activated Chromosorb P tube, which was then wetted homogeneously down to 80% of the packing bed with the remaining part being dry. The Chromosorb P was eluted with a solvent (6 ml) such as diethyl ether, methylene chloride, isopropyl chloride, and ethyl acetate. The eluate was subjected to derivatization as described above. All samples were prepared in triplicate.

Sample Prepartion

A 1 ml of aqueous samples such as water, urine, saliva and diluted serum spiked with NSAIDs and zomepirac as I.S. each at 2 ppm was made basic (pH ~13.0) and extracted with diethyl ether (1 ml×3). The aqueous phase was acidified (pH ≤1.0) with sulfuric acid and saturated with sodium chloride. It was then subjected to the SPE in partition mode as before but using ethyl acetate (2 ml) and methylene chloride (3 ml) in sequence as the eluting solvents. The eluate was evaporated and the residue was reacted with TEA (5 μl) and MTBSTFA (10 μl) in the presence of n-de-

cane (15 μl). The diluted serum was prepared by adding 900 μl of water to 100 μl of serum.

Gas Chromatography

GC analyses for the SPE evaluation were conducted with a Pye Unicam GCV gas chromatograph (Pye Unicam Ltd. Cambridge, England), equipped with a flame ionization detector (FID) and interfaced to a Shimadzu C-R 2AX data processor (Shimadzu, Kyoto, Japan). A DB-1 (J&W Scientific, Rancho Cordova, CA, U.S.A.) fused-silica capillary column (13 m×0.25 mm I.D., 0.25 μm df) was used and 0.5 μl aliquots of samples were injected with a split ratio of 10 : 1. After an initial hold time of 3 min at 170℃, oven temperature was programmed to 280℃ at a rate 5℃/min. The injector and detector temperatures were set at 280℃, and 300℃, respectively. GC analyses for the retention index (RI) measurements were performed with a Hewlett Packard HP 5890A gas chromatograph, equipped with a split/splitless inlet system, and two FIDs, a HP 3392A integrator, and interfaced to a HP 5895A GC ChemStation (Hewlett Packard, Avondale, PA, U.S.A.) on dual DB-5 (30 m×0.25 mm I.D., 0.25 μm df) and DB-17 (30 m×0.25 mm I.D., 0.25 μm df) fused silica capillary columns (J&W Scientific, Rancho, Cordova, CA, U.S.A.). The injector and detector temperatures were maintained at 260℃, and 300℃, respectively. After an initial hold time of 2 min at 230℃, oven temperature was programmed to 280℃ at a rate of 4℃/min. A standard solution of n-hydrocarbons (C_{16}-C_{36}, even numbers only) in isooctane was co-injected with the samples in the split mode (30 : 1). For the screening analyses of water and biological fluids, a Shimadzu GC-9A gas chromatograph (Shimadzu, Kyoto, Japan), equipped with a FID and on-column injector was used with a HP-1 fused silica capillary column (12 m×0.20 mm I.D., 0.33 μm df). The detector temperature was 300℃ and helium was the carrier gas. Samples (ca 0.2 μl) were injected in the on-column injection mode, and the oven temperature was initially at 170℃ for 1 min, then programmed to 240℃ at a rate of 4℃ /min, and finally to 300℃ at a rate of 10℃/min. All samples were analyzed in triplicate.

Gas Chromatography-Mass Spectrometry

A Hewlett Packard HP 5890A gas chromatograph, interfaced to a HP 5970B MSD (70 eV, EI mode) which was on-line to a HP 59940A MS ChemStation system, was used with a HP-5 cross-linked capillary column (25 m×0.20 mm I.D., 0.33 μm df) to obtain mass spectra. Samples were introduced in the split injection mode (10 : 1) at 260℃, and the oven temperature was initially 230℃ then programmed to 280℃ at a rate of 5℃/min. The interface and ion source temperatures were 300 and ~250℃, respectively. The mass range scanned was 60 to 500 amu at a rate of 1.29 scan/sec.

RESULTS AND DISCUSSION

The NSAIDs studied are carboxylic acid derivatives (pKa=3.7-5.3) possessing relatively lipophilic and polarizable aromatic moieties. Prior to SPE, aqueous media were acidified to pH 1.0, thereby increasing the mole fractions of the unionized forms. Five adsorbents (Table I) which work well under the various pH conditions were examined for their sorption and desorption properties.

The SPE evaluation on the hydrophobic adsorbents and the moderately hydrophilic adsorbent were perfomed in the most widely used reversed-phase adsorption mode where the NSAIDs were simultaneously adsorbed onto the adsorbent surface, while water and water soluble interferences passed through unretained. The adsorbent was then washed with water and the NSAIDs were eluted in sequence with methanol, acetone, and methylene chloride.

Carbopak B, a non-polar inorganic graphitized car-

Table I. Characteristics of adsorbents studied

Adsorbent	Type of material	Surface character	Particle size (mesh)	Specific surface area (m^2/g)
Chromosorb 102	Organic styrene-divinyl benzene	Hydrophobic	60-80	300-500
Chromosorb 107	Organic acrylic ester	Moderately hydrophilic	60-80	400-500
Carbopak B	Inorganic graphitized carbon black	Hydrophobic	60-80	100
Thermosorb	Inorganic boron nitride	Hydrophobic	40-60	NA[a]
Chromosorb P	Inorganic calcined diatomaceous earth	Strongly Hydrophilic	80-100	4.0

[a] NA=not available

Table II. Effects of adsorbents on the simultaneous solid-phase extraction of the NSAIDs in adsorption mode

NSAIDs	Mean recovery ?SD of NSAID (%)[a]			
	Carbopak B	Thermosorb	Chromosorb 102	Chromosorb 107
Iburpofen	77.7± 1.7	49.0± 0.3	73.9± 0.9	80.6± 1.7
Alclofenac	75.1± 0.8	46.4± 0.3	67.0± 0.2	85.8± 1.4
Fenoprofen	97.7± 1.8	62.5± 1.1	81.9± 2.1	77.8± 6.6
Flufenamic acid	76.5± 3.1	85.6± 3.9	87.0± 3.9	82.3± 2.7
Flurbiprofen	66.8± 1.4	73.0± 2.0	83.6± 1.8	73.8± 2.0
Niflumic acid	79.3± 1.6	68.7± 1.2	80.5± 2.2	93.9± 2.4
Naproxen	trace	27.2± 0.6	88.9± 1.1	88.2± 9.2
Ketorpofen	48.1± 1.1	88.2± 0.3	81.4± 0.3	74.1± 4.3
Mefenamic acid	62.6± 1.2	79.9± 2.6	79.8± 0.3	73.9± 2.0
Tiaprofenic acid	58.0± 1.1	84.0± 3.6	83.1± 1.5	94.4± 1.3
Fenclofenac	80.1± 4.9	82.5± 3.1	82.8± 5.6	74.8± 4.5
Tolfenamic acid	65.4± 1.3	91.2± 2.4	76.1± 4.0	79.9± 3.0
Diclofenac	47.3± 2.4	71.5± 5.5	65.0± 6.6	26.4± 1.5
Suprofen	85.9± 2.0	79.6± 2.3	84.2± 6.5	54.5± 1.4
Tolmetin	74.3± 3.3	75.0± 3.8	17.9± 1.4	30.9± 3.8
Zomepirac	64.7± 3.8	30.2± 2.9	11.2± 0.4	82.7± 6.2
Lonazolac	trace	71.2± 2.1	96.1± 3.3	93.4± 1.7
Indoprofen	22.5± 2.6	83.0± 6.2	92.0± 8.5	76.8± 6.3
Fentiazac	trace	36.5± 3.2	69.5± 5.4	70.6± 5.8
Indomethacin	trace	46.9± 4.0	86.5± 0.9	85.4± 2.6

[a] 1 ml of pH 1.0 water containing NSAIDs at 60 ppm and n-hexacosane or n-octacosane as I.S. at 30 ppm loaded onto each SPE tube, washed with pH 1.0 water (3 ml) and eluted in sequence with 1 ml each of CH_3OH, CH_3COCH_3 and CH_2Cl_2, SD=Standard deviation for n=3.

Table III. Effects of eluting solvents on the simultaneous solid-phase extraction of NSAIDs using Chromosorb P in partition mode

NSAIDs	Mean recovery SD of NSAID (%)[a]			
	Isopropyl chloride	Diethyl ether	Methylene chloride	Ethyl acetate
Ibuprofen	65.7± 1.8	68.7± 2.2	86.9± 5.7	69.1± 2.1
Alclofenac	84.0± 1.9	98.9± 1.0	101.9± 0.9	98.9± 0.5
Fenoprofen	85.7± 2.2	90.8± 2.1	95.8± 1.8	88.1± 1.6
Flufenamic acid	90.8± 1.4	84.6± 2.1	96.8± 2.3	107.6± 2.1
Flurbiprofen	97.6± 2.7	97.2± 2.5	96.0± 0.6	95.4± 2.0
Naproxen	91.1± 4.1	80.6± 2.1	102.8± 1.2	101.5± 1.7
Niflumic acid	87.8± 0.7	101.6± 0.6	100.0± 0.8	102.8± 2.2
Pirprofen	trace	65.2± 0.2	73.8± 3.1	69.0± 0.4
Flunixin	trace	95.3± 1.7	95.9± 1.9	98.5± 1.6
Ketoprofen	trace	78.9± 1.4	100.2± 1.3	104.4± 1.8
Mefenamic acid	93.3± 0.4	92.4± 1.2	94.4± 1.3	98.2± 0.9
Fenclofenac	87.8± 0.5	82.3± 0.8	99.5± 0.3	100.0± 1.2
Tolfenamic acid	100.1± 0.7	100.0± 0.7	103.7± 2.2	110.3± 2.2
Diclofenac	32.9± 0.5	96.8± 0.3	121.6± 1.4	114.2± 1.1
Diflunisal	43.8± 1.3	85.3± 8.3	59.3± 0.9	79.4± 2.9
Suprofen	96.4± 4.6	93.6± 11.4	100.9± 3.4	101.0± 7.5
Zomepirac	trace	100.8± 4.8	89.6± 4.1	93.4± 1.5
Lonazolac	92.9± 0.7	96.6± 1.2	105.2± 3.9	101.7± 3.2
Indoprofen	trace	85.2± 2.8	89.6± 12.3	102.7± 5.1
Indomethacin	trace	101.7± 2.2	105.3± 2.9	105.1± 3.5

[a] 1 ml of pH 1.0 water containing NSAIDs at 40 ppm and n-hexacosane or n-octacosane 10 ppm loaded onto Chromosorb P tube and eluted with a 6 ml of solvent, SD=standard deviation for n=3.

bon black, gave the lowest overall recoveries (Table II). Because of its high affinity for the highly polarizable aromatic compounds (Bora *et al.*, 1986; Kim *et al.*, 1987), Carbopak B adsorbed the NSAIDs almost completely, but their desorption with solvents was not easily achieved. Moreover, it appears to cause alteration of naproxene, lonazolac, fentiazac, and indomethacin. With Thermosorb, a non-polar inorganic boron nitride, only the half of the NSAIDs were recovered in higher than 70%. Chromosorb 102, a non-polar organic styrene divinylbenzene copolymer, recovered most of the NSAIDs in good yields varying from 65.0 to 96.1%, except for tolmetin and zomepirac which were recovered exceptionally low. Chromosorb 107, an organic acrylic ester polymer of intermediate polarity, gave overall recoveries ranging from 70.6 to 94.4%, with the exception of diclofenac, tolmetin and suprofen.

The strongly hydrophilic inorganic diatomite, Chromosorb P, was evaluated in partition mode where water was preferrentially adsorbed onto about 80% of the adsorbent surface as a thin layer, while the NSAIDs were dissolved in the water layer. The NSAIDs were then eluted by water immiscible partitioning solvent, leaving behind water soluble interferences in the water layer held up strongly by the adsorbent. It has been established that carboxylic acids can be extracted efficiently from aqueous samples with this SPE method as reported earlier (Kim *et al.*, 1989).

Of the solvents tested in partition mode (Table III), isopropyl chloride though it is more polar than diethyl ether and mehylene chloride, showed a very erratic eluting behaviour. Diethyl ether was comparable to methylene chloride in the elution efficiency. Most polar ethyl acetate had uniformly high solvent strength except for ibuprofen and pirprofen. For the higher overall yields and faster solvent evaporation, ethyl acetate and methylene chloride in sequential elution mode were more suitable.

Compared to the three-step SPE in adsorption mode, the two-step SPE in partition mode yielded higher recoveries with good precision. In addition, it provided dry eluates, thus eliminating the moisture removing step. Because of these reasons, the SPE using Chromosorb P and ethyl acetate/methylene chloride was selected as the optimal SPE condition. When the solid-phase extracted NSAIDS were analyzed as their TBDMS derivatives by the dual-capillary column system, each NSAID was readily identified based on RI matching as reported earlier (Kim *et al.*, 1993).

When the present method was applied to biological samples containing NSAIDs at low concentrations, interfering neutral and basic compounds were removed in advance by ether extraction after basification, and the on-column injection mode was used for GC analysis. A typical GC profile of water sample (Fig. 1) shows that each NSAID was well resolved on a short HP-1 capillary column within 30 min. The NSAIDS added at 2 ppm were recovered from water in higher than 80% with good precision, except for the chemically

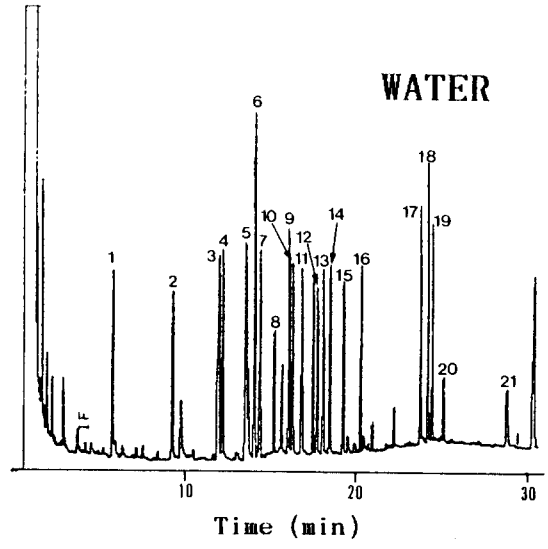

Fig. 1. GC profile of water sample spiked with NSAIDs at 2 ppm. GC conditions: HP-1 fused silica capillary column (12 m×0.20 mm I.D., 0.33 μm df), initially at 170℃ for 1 min, then programmed to 240℃ at a rate of 4℃/min, and finally to 300℃ at a rate of 10℃/min; 0.2 μl sample injected in the on-column injection mode; helium as the carrier gas; detector temperature at 300℃. Peak; 1=ibuprofen; 2=alclofenac; 3=fenoprofen; 4=flufenamic acid; 5=naproxen; 6=niflumic acid; 7=pirprofen; 8=flunixin; 9=ketoprofen; 10=mefenamic acid; 11=fenclofenac; 12=tolfenamic acid; 13=diclofenac; 14=suprofen; 15=tolmetin; 16=zomepirac (I.S.) 17=lonazolac; 18=indoprofen; 19=fentiazac; 20=indomethacin; 21=sulindac.

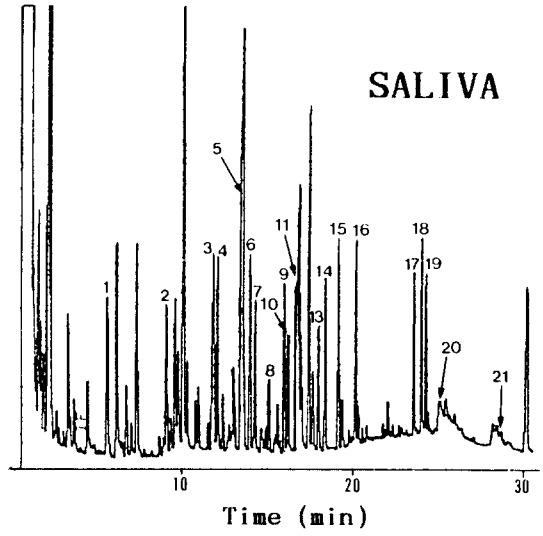

Fig. 2. GC profiles of saliva sample spiked with NSAIDs at 2 ppm. GC conditions and peak identities are the same as those in Fig. 1.

Table IV. Matrix effects on the simultaneous solid-phase extraction of NSAIDs in partition mode

NSAIDs	Mean recovery± SD of NSAIDs (%)[a]		
	Water	Saliva	Serum
Ibuprofen	81.4± 5.6	58.4± 12.0	85.2± 5.4
Alclofenac	107.7± 2.6	80.1± 3.9	97.7± 2.8
Fenoprofen	115.3± 0.6	86.4± 2.7	93.1± 4.3
Flufenamic acid	96.9± 1.3	62.8± 2.7	65.4± 7.2
Naproxen	115.5± 2.1	>100	>100
Niflumic acid	108.0± 4.3	55.6± 1.4	95.5± 4.2
Pirprofen	46.8± 4.1	39.2± 3.6	95.1± 8.7
Flunixin	101.2± 4.5	48.9± 0.6	86.2± 5.5
Ketoprofen	103.3± 2.0	66.4± 2.3	73.6± 5.0
Mefenamic acid	83.8± 4.8	42.4± 2.8	58.5± 6.8
Fenclofenac	92.1± 0.4	47.3± 8.3	>100
Tolfenamic acid	83.7± 2.9	33.1± 1.7	70.5± 8.5
Diclofenac	92.1± 2.5	50.4± 2.8	71.7± 13.2
Suprofen	104.1± 4.2	71.9± 2.0	82.7± 2.8
Tolmetin	89.6± 4.4	83.1± 8.8	73.3± 3.6
Lonazolac	87.0± 5.9	50.1± 3.4	62.7± 7.9
Indoprofen	92.0± 7.9	57.3± 1.3	76.2± 2.9
Fentiazac	96.1± 5.6	49.6± 5.1	73.2± 5.2
Indomethacin	78.8± 5.0	NC[b]	NC[b]
Sulindac	72.4± 15.6	NC[b]	NC[b]

[a] 1 ml of water,saliva or diluted serum (10 times) spiked with NSAIDs and zomepirac as I.S. at 2 ppm loaded onto Chromosorb P tube and eluted with ethyl acetate (2 ml) follow by methylene chloride (3 ml), SD=standard deviation for n=3.

[b] NC=not calculable due to overlapping.

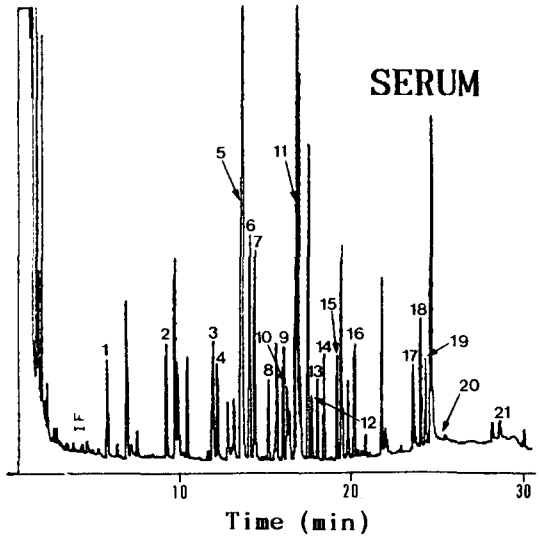

Fig. 3. GC profiles of serum sample spiked with NSAIDs at 2 ppm. GC conditions and peak identities are the same as those in Fig. 1.

unstable pirprofen, and the late eluting indomethacin and sulindac (Table IV).

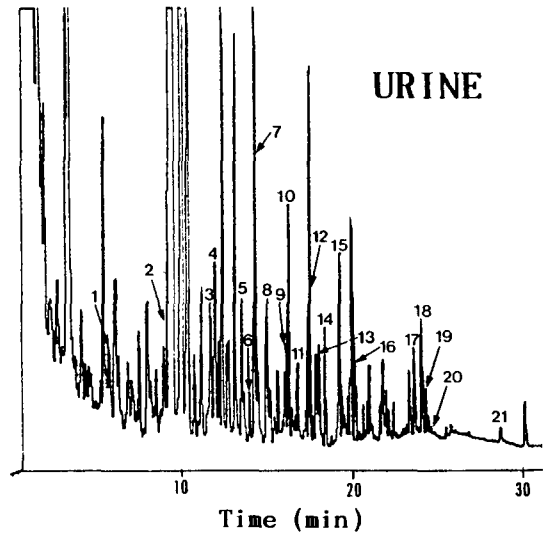

Fig. 4. GC profile of urine sample spiked with NSAIDs at 2 ppm. GC conditions and peak identities are the same as those in Fig. 1.

The present SPE method efficiently extracts organic acids as well (Kim *et al.*, 1989). Therefore, the matrix effects due to biogenically occurring organic acids were more enhanced as the concentrations of NSAIDs were lowered in the biological samples. Multiple endogenous organic acids coextracted from saliva and serum samples interfered with the resolution of several NSAIDs upon GC analyses (Fig. 2 and 3). Their recoveries were either lower or higher than those of water sample (Table IV) probably due to the incomplete peak resolution and some losses during the removal of interfering non-acidic compounds. For urine samples, it was not possible to separate most of the trace NSAIDs due to the presence of the urinary biogenic acids at much higher concentrations (Fig. 4). Most of the biogenic organic acids were identified and confirmed by GC-MS.

Though the resolution of each peak was not complete, the present SPE method with subsequent TBDMS derivatization followed by GC analyes provided rapid qualitative screening for various NSAIDs simultaneously in biological samples. To overcome these interference problem, GC-MS analyses in selected-ion monitoring (SIM) mode will be preferred.

CONCLUSION

A significant advantage of the present SPE using Chromosorb P and ethyl acetate/methylene chloride with subsequent silylation is that various carboxylated NSAIDs are simultaneously extracted in two-step and converted to the corresponding stable TBDMS derivatives in a single step. The rapid extraction and derivatization procedure will make this method suitable for

the rapid profiling and screening of NSAIDs in general-unknown cases. For the accurate quantitative screening of biological samples for the trace NSAIDs simultaneously, further investigation on the GC-MS-SIM analyses are in progress.

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