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Capillary gas chromatography of acidic non-steroidal antiinflammatory drugs as *tert.*-butyldimethylsilyl derivatives

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ABSTRACT

The quantitative conversion of 26 acidic non-steroidal anti-inflammatory drugs (NSAIDs) simultaneously to their corresponding *tert.*-butyldimethylsilyl (TBDMS) derivatives in a single step was examined. The NSAIDs dissolved in triethylamine were silylated with N-methyl-N-(*tert.*-butyldimethylsilyl)trifluoroacetamide in isooctane at room temperature for 30 min and subsequently analysed by capillary gas chromatography and gas chromatography–mass spectrometry. The TBDMS derivatives were eluted as untailed sharp peaks and the characteristic $[M-57]^+$ ions in the mass spectra permitted their rapid confirmation. The temperature-programmed retention index (I) sets measured on a DB-5 and DB-17 dual-capillary column system were characteristic of each NSAID to be used for the rapid identification by computer I matching. The derivatization yields of the NSAIDs studied were linear in the range 10–120 μg with high overall precisions. The method provided simultaneous screening and accurate confirmation of each drug when applied to serum samples spiked with NSAIDs.

INTRODUCTION

The many acidic non-steroidal anti-inflammatory drugs (NSAIDs), which are monocarboxylic aromatic acids, constitute the principal class of agents for controlling the pain and inflammation of rheumatic diseases. The carboxylated NSAIDs include salicylates, acetic acids, propionic acids and fenamates.

The simultaneous detection and identification of these NSAIDs is a commonly encountered problem for the systematic screening especially in general unknown cases [1–6]. In the literature, high-performance liquid chromatography (HPLC) [1,3,4,6] has been extensively employed with a few gas chromatographic (GC) methods [2,5,6] for this purpose. However, it is well known that GC is more suitable for qualitative and quantitative multi-component analyses because of its inherent high resolution, sensitivity and precision compared with HPLC. In the analyses of essential oils, drugs and organic acids, GC peaks are routinely identified by comparing their characteristic retention index (I) sets with reference values measured on columns of different polarity [7–11].

GC analyses of the carboxylated NSAIDs require an appropriate derivatization procedure and mainly methylation with hazardous diazomethane has been used to convert the carboxyl functions into the corresponding methyl

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esters [2,5]. In recent years, *tert.*-butyldimethylsilyl (TBDMS) derivatization of carboxyl functions has been widely used [10–16], principally because of the high hydrolytic stability and superior GC and mass spectrometric (MS) properties of the TBDMS derivatives [12,17]. However, no report on the application of TBDMS derivatization to the NSAIDs has been published. Previously, we reported the simultaneous TBDMS derivatization of volatile and non-volatile organic acids [16].

If systematic screening for NSAIDs is to be used routinely in forensic and clinical chemistry laboratories, a simple and rapid GC profiling method that can identify the most commonly used NSAIDs in a single analysis must be developed.

As the first step towards that goal, this study was undertaken to investigate the optimum conditions for converting 26 carboxylated NSAID mixture simultaneously into their corresponding TBDMS derivatives in a single step, rapidly and quantitatively. The structures of the TBDMS derivatives which are new to the literature were confirmed by GC–MS.

EXPERIMENTAL

Materials

Standards of the NSAIDs studied were obtained from Sigma (St. Louis, MO, USA) and various pharmaceutical companies. The silylating reagent, N-methyl-N-(*tert.*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) was obtained from Pierce (Rockford, IL, USA), triethylamine (TEA) from Aldrich (Milwaukee, WI, USA) and *n*-alkane standards (C_{16}–C_{36}, even numbers only) from Polyscience (Niles, IL, USA). All other chemicals were of analytical-reagent grade and used as received.

NSAID solutions and internal standard solutions

NSAID solutions containing NSAIDs in their free acid forms, 10 μg/μl in methanol or acetone, were used as stock solutions. *n*-Hexacosane or *n*-octacosane used as the internal standard (I.S.) was dissolved in isooctane at a concentration of 1 μg/μl.

tert.-Butyldimethylsilylation

An NSAID mixed solution containing 20 μg of each NSAID was evaporated to dryness under a gentle stream of nitrogen at 50°C after adding 10 μl of I.S. solution. To the residue were added 10 μl of TEA, 10 μl of MTBSTFA and 30 μl of isooctane, and the mixture was vortex mixed to form TBDMS derivatives. The reaction mixture was directly examined by GC and GC–MS. The effects of TEA addition and heating at 60°C on the derivative yields were investigated. The derivative yield curves were prepared by plotting the heating time against the peak-area ratio of NSAID to the internal standard. The standard samples for constructing the calibration graphs were prepared with four NSAID standard solutions containing 10, 20, 60 and 120 μg of each NSAID and 40 μg of internal standard.

TABLE I

ACIDIC NON-STEROIDAL ANTI-INFLAMMATORY DRUGS STUDIED

Group	NSAID (abbreviation)
Salicylates	Acetylsalicylic acid (ASA)
	Diflunisal (DFN)
	Salicylic acid (SCA)
Acetic acids	Alclofenac (ACF)
	Diclofenac (DCF)
	Fenclofenac (FCF)
	Fentiazac (FTZ)
	Indomethacin (IMC)
	Lonazolac (LNZ)
	Sulindac (SLD)
	Tolmetin (TMT)
	Zomepirac (ZPR)
Propionic acids	Fenoprofen (FPE)
	Flurbiprofen (FBF)
	Ibuprofen (IBF)
	Indoprofen (IPF)
	Ketoprofen (KPF)
	Naproxen (NPX)
	Pirprofen (PPF)
	Suprofen (SPF)
	Tiaprofenic acid (TPA)
Fenamates	Flufenamic acid (FFA)
	Flunixin (FNX)
	Mefenamic acid (MFA)
	Niflumic acid (NFA)
	Tolfenamic acid (TFA)

Sample preparation

A 200-μl volume of serum spiked with NSAIDs at 100 ppm was acidified to pH 1.0 with sulphuric acid and saturated with sodium chloride. It was then extracted with diethyl ether (4 × 1 ml). The collected ether layers were evaporated to dryness at 50°C under a stream of nitrogen. The residue was subjected to derivatization, as described above.

Gas chromatography

GC analyses were conducted with a Pye Unicam (Cambridge, UK) GCV gas chromatograph equipped with a flame ionization detector and interfaced to a Shimadzu (Kyoto, Japan) C-R 2AX data processor. A DB-1 (J & W Scientific, Rancho Cordova, CA, USA) fused-silica capillary column (13 m × 0.25 mm I.D.; 0.25 μm film thickness) was used. Nitrogen at a flow-rate of

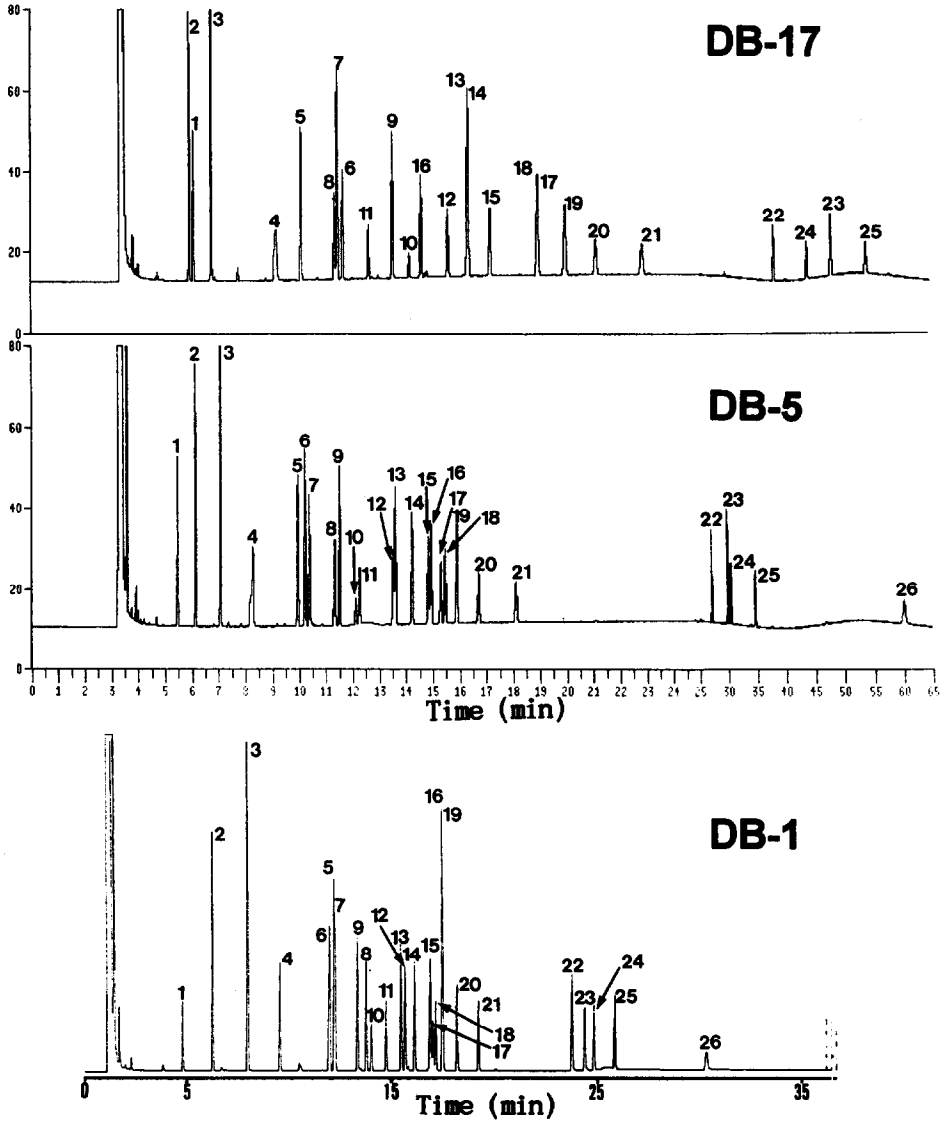

Fig. 1. Chromatograms of the mixture of 26 NSAIDs as their TBDMS derivatives separated on DB-1 (13 m × 0.25 mm I.D.), DB-5 (30 m × 0.25 mm I.D.) and DB-17 (30 m × 0.25 mm I.D.) fused-silica capillary columns. Peak numbers correspond to the numbers in Table II. GC conditions are given in the text.

0.7 ml/min was used as the carrier gas, and 0.5-μl aliquots of samples were injected with a splitting ratio of 10:1. After an initial hold time of 3 min at 170°C, the oven temperature was programmed to 280°C at a rate 5°C/min. The injector and detector temperatures were 280 and 300°C, respectively.

GC analyses for the retention index (I) measurements and dual-capillary profiling analysis were performed with a Hewlett-Packard HP 5890A gas chromatograph, equipped with a split/splitless inlet system, two flame ionization detectors, and an HP 3392A integrator and interfaced to an HP 5895A GC ChemStation (Hewlett-Packard, Avondale, PA, USA) on dual DB-5 (30 m × 0.25 mm I.D.; 0.25 μm film thickness) and DB-17 (30 m × 0.25 mm I.D.; 0.25 μm film thickness) fused-silica capillary columns (J & W Scientific). After an initial hold time of 2 min at 230°C, the oven temperature was programmed to 280°C at a rate of 4°C/min. A standard solution of n-alkanes (C_{16}–C_{36}, even numbers only) in

TABLE II

GAS CHROMATOGRAPHIC AND MASS SPECTRAL DATA FOR TBDMS DERIVATIVES OF NSAIDS

No.	NSAID	GC I^a data set		Mass spectral data set[b]						
		DB-5	DB-17	$[M]^+$	$[M-57]^+$	$[M-15]^+$	$[M-131]^+$	$[M-159]^+$	Other ions	
1	ASP	1790.8	2058.6	294(0)	237(44)	279(1)	163(2)	135(17)	195(100)	121(8)
2	IBU	1876.5	2039.8	320(0)	263(100)	305(2)	189(0)	161(2)	75(11)	73(7)
3	SCA	1993.5	2149.1	366(0)	309(100)	351(6)	235(1)	207(0)	195(5)	73(21)
4	ACF	2103.0	2388.1	340(0)	283(100)	325(1)	209(0)	181(1)	75(21)	242(12)
5	FFA	2258.4	2587.4	395(35)	338(100)	380(3)	264(23)	236(1)	244(27)	222(16)
6	FPF	2239.9	2466.7	356(0)	299(100)	341(1)	225(0)	197(2)	75(24)	73(10)
7	FBF	2269.2	2571.0	358(0)	301(100)	343(1)	227(0)	199(2)	75(9)	179(8)
8	NFA	2335.6	2571.0	396(19)	339(100)	381(4)	265(7)	237(1)	245(30)	218(5)
9	NPX	2349.4	2717.3	344(10)	287(100)	329(1)	213(1)	185(11)	75(7)	73(7)
10	PPF	2392.1	2759.3	365(30)	308(100)	350(2)	234(3)	206(36)	306(58)	75(14)
11	FNX	2400.0	2655.4	410(41)	353(73)	395(100)	279(9)	251(8)	239(31)	263(19)
12	MFA	2485.3	2890.1	355(59)	298(100)	340(4)	224(42)	196(1)	223(12)	209(8)
13	KPF	2479.8	2847.9	368(0)	311(100)	353(2)	237(0)	209(0)	295(19)	75(7)
14	FCF	2523.9	2890.1	410(0)	353(100)	395(2)	279(1)	251(1)	75(9)	215(7)
15	TFA	2561.4	2936.1	375(32)	318(100)	360(3)	244(29)	216(1)	209(7)	208(7)
16	DFN	2567.9	2789.7	478(0)	421(100)	463(3)	347(1)	319(0)	73(24)	307(7)
17	TPA	2590.7	3027.5	374(0)	317(100)	359(3)	243(1)	215(2)	73(11)	105(7)
18	DCF	2600.0	3026.4	409(19)	352(100)	394(2)	278(2)	250(1)	75(30)	214(23)
19	SPF	2621.5	3069.1	374(0)	317(100)	359(1)	243(0)	215(1)	75(11)	111(7)
20	TMT	2661.4	3116.2	371(25)	314(92)	356(5)	240(1)	212(31)	119(100)	73(29)
21	ZPR	2729.1	3187.1	405(40)	348(100)	390(3)	274(3)	246(36)	139(43)	73(20)
22	LNZ	3045.1	3559.1	426(4)	369(100)	411(2)	295(5)	267(6)	75(8)	73(5)
23	IPF	3110.3	>3600.0	395(1)	338(100)	380(2)	264(1)	236(15)	73(6)	75(4)
24	FTZ	3123.9	>3600.0	443(7)	386(100)	428(1)	312(4)	284(5)	73(33)	239(13)
25	IMC	3212.0	>3600.0	471(31)	414(38)	456(3)	340(1)	312(21)	139(100)	111(21)
26	SLD	3532.0	nd[c]	470(7)	413(72)	455(20)	339(1)	311(2)	397(100)	73(61)

[a] Retention index (I) values on DB-5 and DB-17 (30 m × 0.25 mm I.D., 0.25 μm film thickness) capillary columns programmed from 230°C (held for 2 min) to 280°C at 4°C/min. Relative standard deviations ranged from 0.01 to 0.05% for three measurements.

[b] m/z Values with relative abundances of ions (%) in parentheses.

[c] Not detected.

isooctane was co-injected with the samples in the split mode (30:1). All samples were analysed in triplicate.

Gas chromatography–mass spectrometry

A Hewlett-Packard HP 5890A gas chromatograph, interfaced to an HP 5970B mass-selective detector (70 eV, EI mode) which was coupled on-line to a HP 59940A MS ChemStation system, was used with an HP-1 cross-linked capillary column (12 m × 0.20 mm I.D.; 0.33 μm film thickness) to obtain mass spectra. Samples were introduced in the split injection mode (30:1) at 260°C, and the oven temperature was initially 180°C and then programmed to 280°C at 20°C/min. The interface and ion source temperatures were 300 and *ca.* 250°C, respectively. The

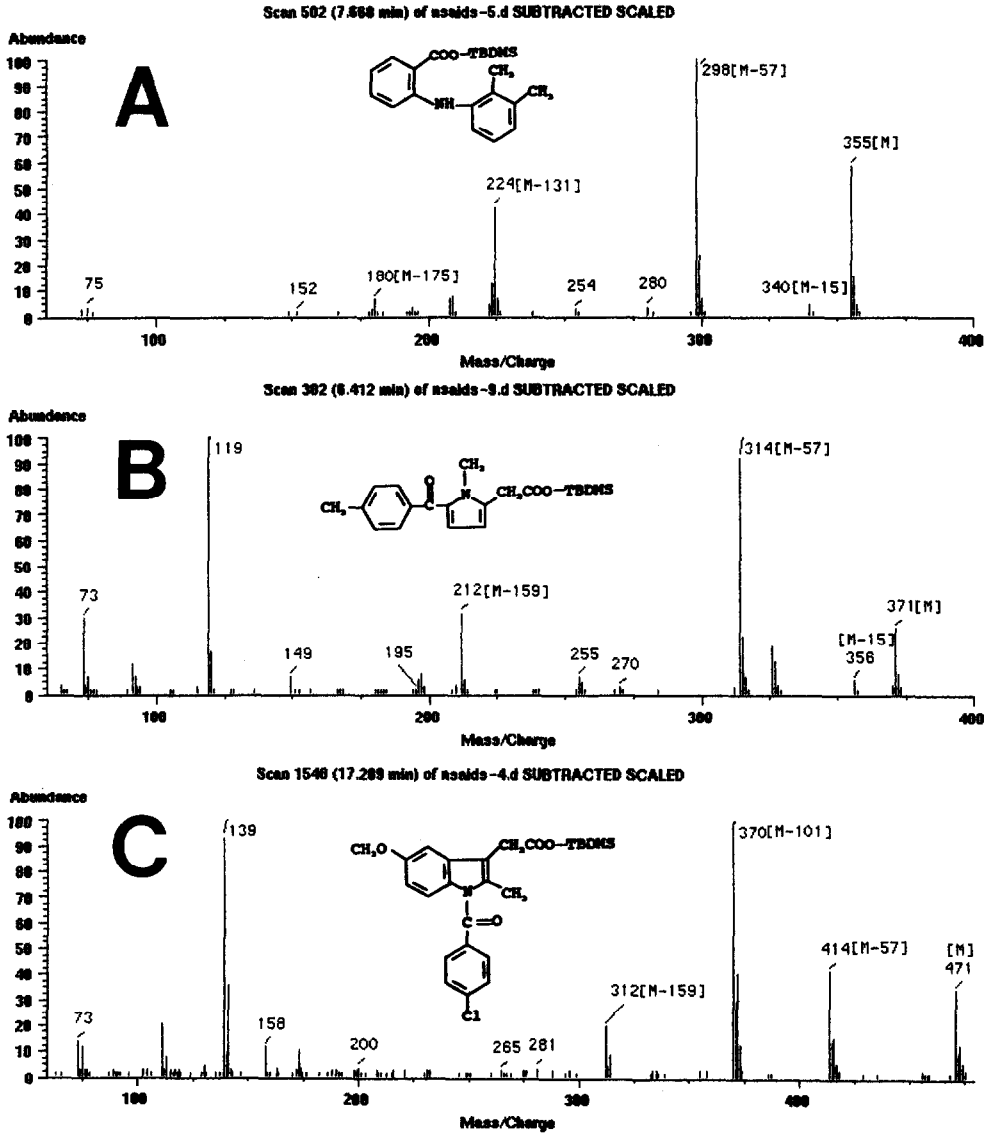

Fig. 2. Electron impact mass spectra of TBDMS derivatives of (A) mefenamic acid, (B) tolmetin and (C) indomethacin.

mass range scanned was from 60 to 500 u at a rate of 1.29 scans/s.

RESULTS AND DISCUSSION

The TBDMS derivatization of the NSAIDs and their metabolites offer advantages over methylation, as the carboxyl groups and any protic polar groups if present are all converted into TBDMS derivatives which generate diagnostically useful $[M-57]^+$ ions in their mass spectra. The 26 NSAIDs examined in this study are listed in Table I.

On reaction with the silylating reagent, MTBSTFA, in isooctane, all the compounds converted into single TBDMS derivatives. The separation of the 26 NSAIDs as TBDMS derivatives on three different fused-silica capillary columns is presented in Fig. 1. Each NSAID derivative displayed a single symmetrical peak. On the short non-polar DB-1 column, no resolutions were achieved between flufenamic acid

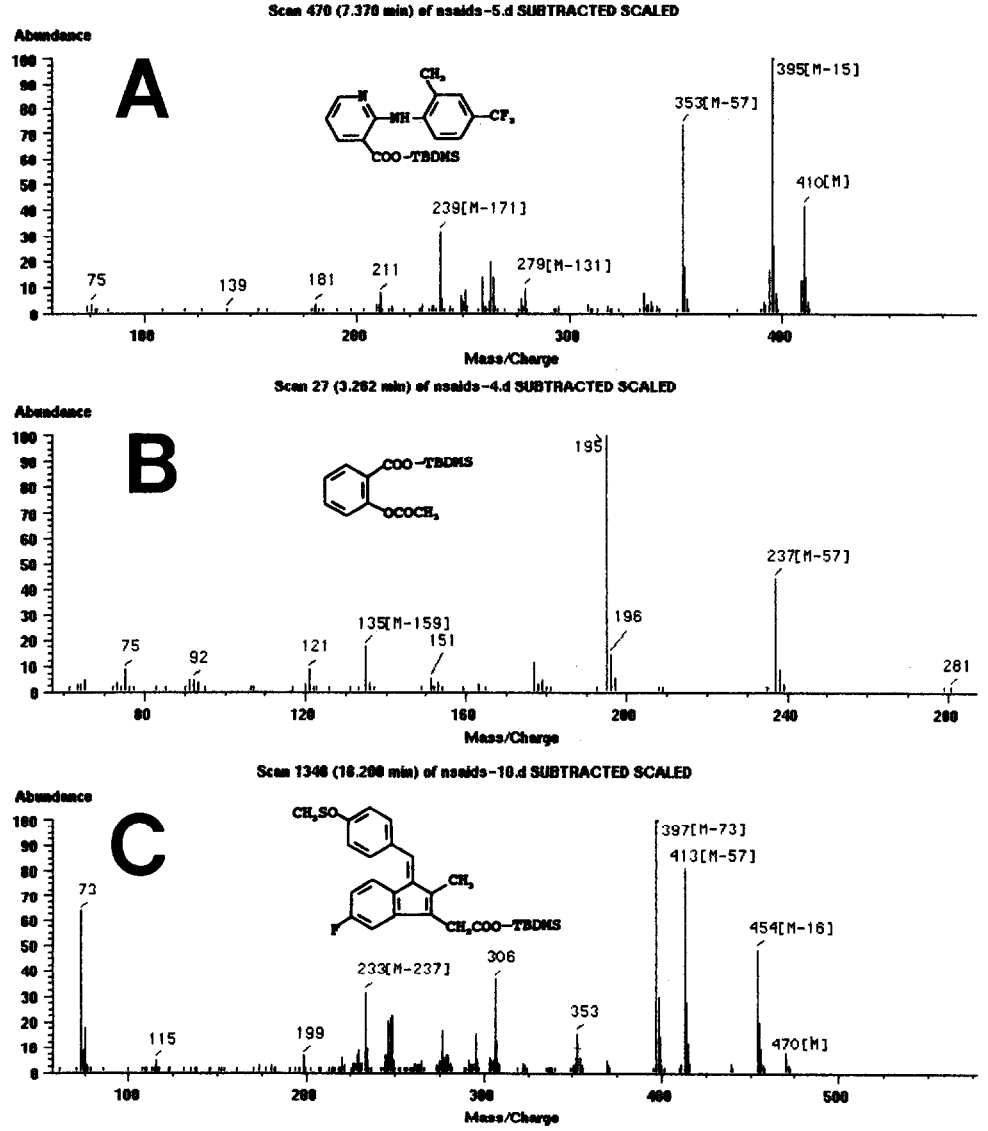

Fig. 3. Electron impact mass spectra of TBDMS derivatives of (A) flunixin, (B) aspirin and (C) sulindac.

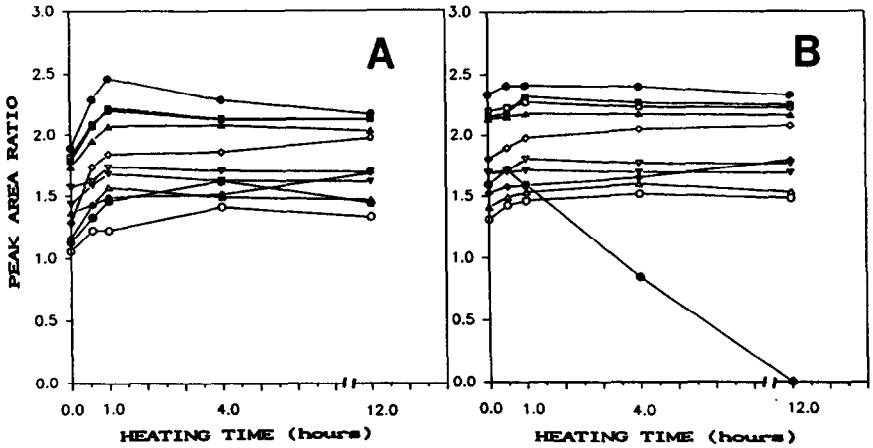

Fig. 4. Effect of heating at 60°C (A) without and (B) with addition of triethylamine on the derivative yields for (●) ibuprofen, (▽) alclofenac, (▼) fenoprofen, (□) flubiprofen, (■) naproxen, (△) niflumic acid, (▲) ketoprofen, (◇) mefenamic acid, (◆) lonazolac, (○) indomethacin and (*) tiaprofenic acid. The peak-area ratio of each NSAID to the internal standard is plotted against heating time.

and flurbiprofen, between diflunisal and suprofen or between tolfenamic acid, tiaprofenic acid and diclofenac, whereas they were well resolved on the longer DB-5 column. On the non-polar DB-5 column, no resolutions between mefenamic acid and ketoprofen or between tolfenamic acid and diflunisal were observed, but they were well separated on the medium-polarity DB-17 column. Ketoprofen and tiaprofenic acid co-eluted with fenclofenac and diclofenac, respectively, on DB-17, but were well resolved on the DB-5 column. Sulindac was not eluted from the DB-17 column.

Temperature-programmed retention index (I) sets for each derivative measured on both columns are given in Table II. The I sets were characteristic of each NSAID, thus being useful for the rapid identification by the computer I library matching as described in our organic acid profiling work [10].

The derivatives were subjected to GC–MS for the confirmation of their structures, which are new to the literature. All the monocarboxylic NSAIDs were converted into monoTBDMS derivatives with the exception of salicylic acid and diflunisal, which possess one phenolic hydroxyl group and formed bisTBDMS derivatives. The electron-impact MS data are summarized in Table II.

As is characteristic of the TBDMS derivatives [12–17], the intense $[M-57]^+$ ions formed by the loss of the labile *tert.*-butyl function, together with weak $[M-15]^+$, $[M-131]^+$ and $[M-159]^+$ ions generated by the losses of CH_3, OTBDMS and COOTBDMS from the molecular ions, respectively, were useful for the structural confirmation of each NSAID.

$[M-57]^+$ ions constitute the base peaks for most NSAIDs and the molecular ions are intense for the NSAIDs having two aromatic rings with a few exceptions, as exemplified by the mass spectrum of mefenamic acid (Fig. 2A). The base peaks at m/z 119 of tolmetin (Fig. 2B) and at m/z 139 of indomethacin (Fig. 2C) are formed by the preferential cleavage of the bond between CO and the pyrrole ring and the bond between CO and the indole ring, respectively.

For flunixin the ion at m/z 395 formed by the loss CH_3 from the molecular ion constitutes the base peak while the base peaks at m/z 195 of aspirin and at m/z 397 of sulindac appear to represent the losses of CH_2CO and CH_4 from the $[M-57]^+$ ions, respectively, as shown in Fig. 3.

In a previous study [16], we established that the addition of triethylamine (TEA) improves the overall precision of the simultaneous TBDMS derivatization of volatile and non-vol-

TABLE III

LINEAR REGRESSION ANALYSIS FOR THE CALIBRATION GRAPHS OF NSAIDS AS THEIR TBDMS DERIVATIVES

NSAID	Regression line[a]		Correlation coefficient,
	m	*b*	*r*
ASA	0.009	−0.020	0.9999
IBU	0.035	0.048	0.9997
ACF	0.026	−0.051	0.9995
FPF	0.024	0.030	0.9999
FFA	0.030	−0.060	0.9993
FBF	0.029	0.018	0.9999
NPX	0.034	−0.065	0.9994
NFA	0.020	0.004	0.9999
PPF	0.026	−0.057	0.9993
FNX	0.013	0.009	0.9999
KPF	0.034	−0.054	0.9996
MFA	0.028	0.009	0.9998
FCF	0.027	−0.042	0.9997
DCF	0.022	−0.045	0.9992
SPF	0.029	−0.061	0.9995
TFA	0.024	0.022	0.9998
IPF	0.024	−0.002	0.9999
DFN	0.028	0.014	0.9999
ZPR	0.015	0.012	0.9999
LNZ	0.020	0.028	0.9993
IMC	0.016	0.028	0.9996
TPA	0.028	0.008	0.9999
TMT	0.026	−0.019	0.9999
FTZ	0.024	0.018	0.9998
SLD	0.016	−0.010	0.9999

[a] m = Slope = relative mass response = mean peak-area ratio of NSAID × mass of I.S./mass of NSAID; b = y-intercept.

atile organic acids. When the effects of TEA and heating at 60°C on the silylation of NSAIDs were tested, similar trends were obtained as shown by the derivative yield curves for eleven NSAIDs in Fig. 4.

Without TEA addition, the derivatization of most NSAIDs went to completion on heating for 4 or 12 h (Fig. 4A). For ibuprofen, its response began to reduce when heated over 1 h. When TEA was added (Fig. 4B), the reaction was nearly complete (>95%) on mixing at room temperature, with the exception of NSAIDs containing carboxyl functions which are sterically hindered or near to an electron-withdrawing heterocyclic ring, such as mefenamic acid, niflumic acid, lonazolac and indomethacin. They required heating for more than 1 h to obtain the higher derivative yields. Tiaprofenic acid, however, started to give an abruptly reduced response when heated for longer than 30 min.

When reacted at room temperature for 30 min without heating in the presence of TEA, the overall derivative yields were satisfactory. Under this condition, the TBDMS derivatization of the 26 NSAID mixtures was examined for the precision and linearity of the calibration graphs. Linear responses in the range 10–20 μg were obtained with correlation coefficients varying from 0.9992 to 0.9999 (Table III). The relative standard deviations ($n = 3$) ranged from 0.1 to 10.0%, but in most instances they were lower than 5%, except for the inherently unstable aspirin. The overall reproducibility appears to be satisfactory for the quantification of NSAIDs. The TBDMS derivatives were stable for at least 2 months when stored at 4°C, with the exception of aspirin, which was gradually converted into stable salicylic acid.

We added the data sets of the NSAIDs listed in the Table II to our own TBDMS reference libraries [10] built in the GC and MS ChemStations for the rapid peak identification of unknown NSAIDs in screening work, and the libraries will continue to be expanded to include the major metabolites and other acidic NSAIDs.

The present derivatization and dual-capillary profiling analysis were applied to serum samples spiked with various NSAIDs. Simultaneous screening and accurate confirmation of the 26 NSAIDs could be achieved, as exemplified in Fig. 5.

CONCLUSIONS

A significant advantage of the present silylation with MTBSTFA in isooctane after TEA addition over methylation is that the carboxylated NSAIDs are simultaneously converted in a single step into the corresponding stable TBDMS derivatives, which generate diagnostically useful $[M - 57]^+$ ions in their mass spectra. The rapid derivatization process will make this method suitable for routine NSAID screening work. For the selective isolation of trace NSAIDs from biological samples prior to TBDMS derivatiza-

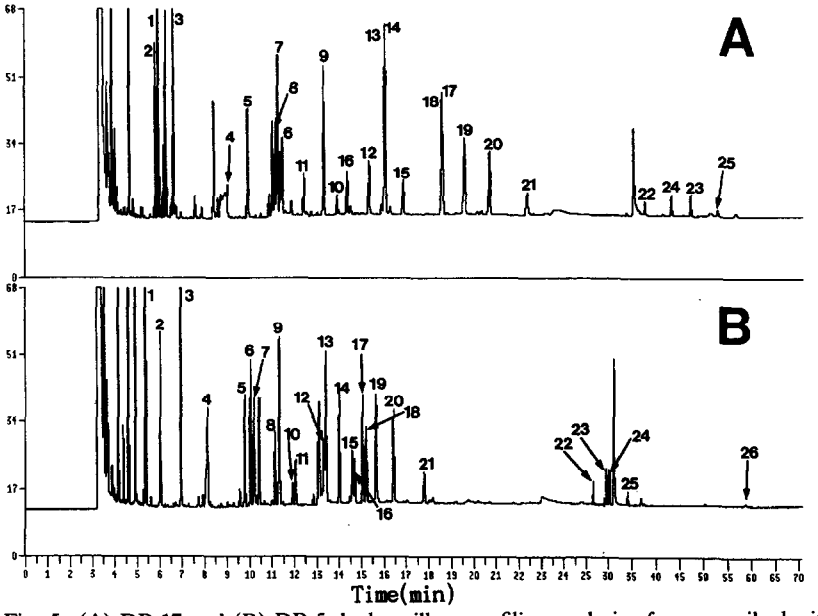

Fig. 5. (A) DB-17 and (B) DB-5 dual-capillary profiling analysis of serum spiked with 26 NSAIDs at 100 ppm. Peak numbers correspond to the numbers in Table II. GC conditions are given in the text.

tion, further investigation of efficient solid-phase extraction is in progress. The extension of the present method to the rapid profiling and screening of NSAIDs at the low concentrations present in biological samples is under way.

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