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SEPARATION OF NON-STEROIDAL ANTI-INFLAMMATORY AGENTS BY HIGH-RESOLUTION GAS CHROMATOGRAPHY. PRELIMINARY TRIALS TO PERFORM PHARMACOKINETIC STUDIES

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SUMMARY

To assess the suitability of capillary gas chromatography for pharmacokinetic studies, a separation method was developed that is applicable to ten of the major organic acids widely used as analgesics and/or anti-inflammatory agents. The separation was performed by high-resolution gas chromatography on a 15-m SE-52 glass column before and after addition of the acids to plasma samples. The reliability of the method was analysed for each group of organic acids.

INTRODUCTION

Organic acids are the largest group of analgesic and anti-inflammatory drugs and methods such as packed column gas chromatography[1–4] and high-performance liquid chromatography[5–9] have been reported for their separation. This paper describes a separation method based on capillary column chromatography, which was applied to some of the most widely used organic acids. Although the separation of these compounds may not be a practical daily requirement, it is important to establish the chromatographic behaviour of different groups of organic acids on capillary columns in order to evaluate the feasibility of this technique in pharmacokinetic studies, especially where highly sensitive, specific determinations are required.

EXPERIMENTAL

Reference compounds and reagents

All compounds (Table I) were high-purity standards. Solvents were purchased from E. Merck (Darmstadt, F.R.G.). Chloroform was of analytical-reagent grade; *n*-hexane grade was used for residue analysis.

Diazomethane was prepared according to Vogel[10]. All solvents and reagents used in its preparation were purchased from Carlo Erba (Milan, Italy).

Chromatographic conditions

A Carlo Erba Model 2900 capillary gas chromatograph was used, equipped with an SE-52 column (MEGA, Milan, Italy) of 15 m × 0.32 mm I.D. with a film thickness of 0.4 to 0.45 μm and a flame-ionization detector. The oven temperature was initially

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TABLE I

COMPOUNDS STUDIES

Group	*No.*	*Trivial name*	*Systematic name*
1	1	Salicylic acid	2-Hydroxybenzoic acid
	2	Aspirin	2-(Acetyloxy)benzoic acid
	3	Ibuprofen	2-(4-Isobutylphenyl)propionic acid
	4	(Internal standard)	2,4-Dichlorophenoxyacetic acid
2	5	Flurbiprofen	2-(2-Fluoro-4-biphenyl)propionic acid
	6	Diflunisal	2-Hydroxy-5-(2,4-difluorophenyl)-benzoic acid
	7	(Internal standard)	2-(2-Chloro-4-biphenyl)propionic acid
	8	Ketoprofen	2-(3-Benzoylphenyl)propionic acid
3	9	Tolmetin	5-(p-Toluoyl)-1-methylpyrrole-2-acetic acid
	10	Zomepirac	5-(4-Chlorobenzoyl)-1,4-dimethyl-1H-pyrrolacetic acid
	11	(Internal standard)	5-(*p*-Carboxybenzoyl)-1-methylpyrrole-2-acetic acid
4	12	Fentiazac	2-Phenyl-4-*p*-chlorophenylthiazol-5-ylacetic acid
	13	Indomethacin	1-(*p*-Chlorobenzoyl)-5-methoxyl-2-methylindole-3-acetic acid
	14	(Internal standard)	2-Phenyl-4-*p*-chlorophenyl-thiazol-5-ylbutyl acetate

85°C, increased at 20°C/min to 180°C (held for 3 min), then at 3.5°C/min to 240°C (held for 30 min). The injector and detector temperatures were 250 and 275°C, respectively. Sample injection was splitless (15 sec) and with splitting ratio of 1:20. The carrier gas was helium at a flow-rate of 3 ml/min.

Esterification

All compounds, except No. 14, were esterified. Methyl esters were prepared by treatment with 0.2 ml of anhydrous diazomethane in diethyl ether for 5 min. After esterification, the excess of reagent was evaporated at room temperature under nitrogen.

Extraction

The reference compounds were extracted from plasma samples by the following procedure: the plasma sample was acidified with 30 μl of 6 *N* hydrochloric acid, 5 ml of chloroform were added, the tubes were shaken for 5 min and centrifuged, the aqueous phase was re-extracted with 5 ml of chloroform and re-centrifuged, and the organic phases were pooled and dried under nitrogen. The residue was esterified, as described above, and finally reconstituted with 0.5 ml of *n*-hexane. The amount injected was 0.5 μl.

Calibration graphs

Calibration graphs were constructed as follows. To each of several acidified human plasma samples (1 ml) a constant amount (2.5 μg) of internal standards and increasing amounts (0–10 μg) of reference compounds were added. Extraction was performed as described above.

Blank plasma samples were also extracted as above to ascertain that no interfering peaks were present.

RESULTS AND DISCUSSION

Fig. 1 shows the separation obtained after injection of a sample containing a mixture of all the reference compounds listed. The chromatographic conditions adopted appear to be appropriate for the analysis of both the salicylate group (compounds 1 and 2) and the propionic acid derivative group (compounds 3, 5 and 8). For these two organic acid groups, both 2,4-dichlorophenoxyacetic acid (compound 4) and 2-(2-chloro-4-biphenyl)propionic acid (compound 7) can be used as internal standards. The response factor was particularly high for ibuprofen (compound 3).

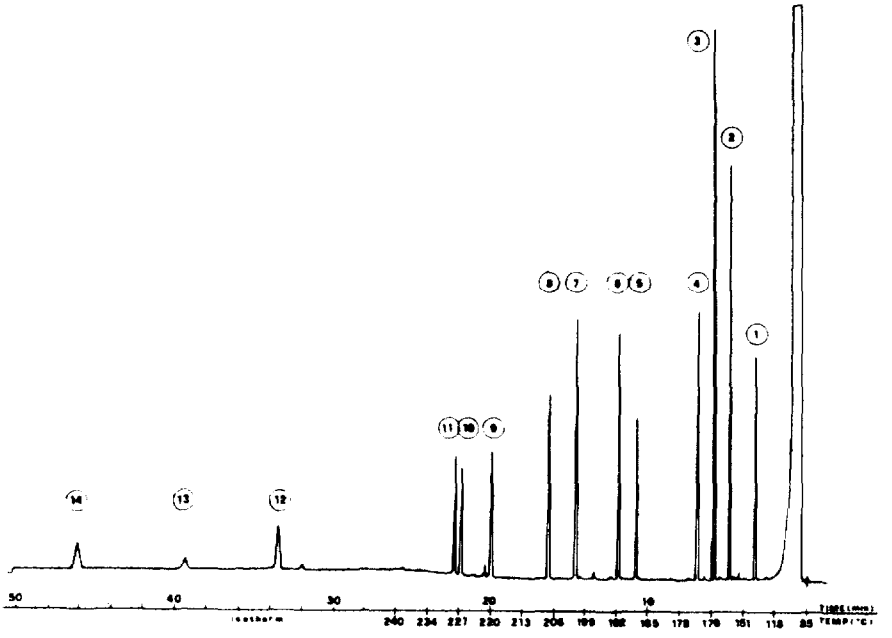

Fig. 1. Chromatogram of a mixture of methylated organic acids and internal standards. Amount injected: 0.5 μl, containing 2 ng of each compound. For identification of peaks, see Table I.

The same temperature programme appears adequate for the determination of the pyrrolacetic acid derivatives (compounds 9 and 10), even though the retention times of around 20 min would limit the number of analyses that could be run.

The temperature programme did not prove suitable for the heavier compounds, such as fentiazac (compound 12) and indomethacin (13).

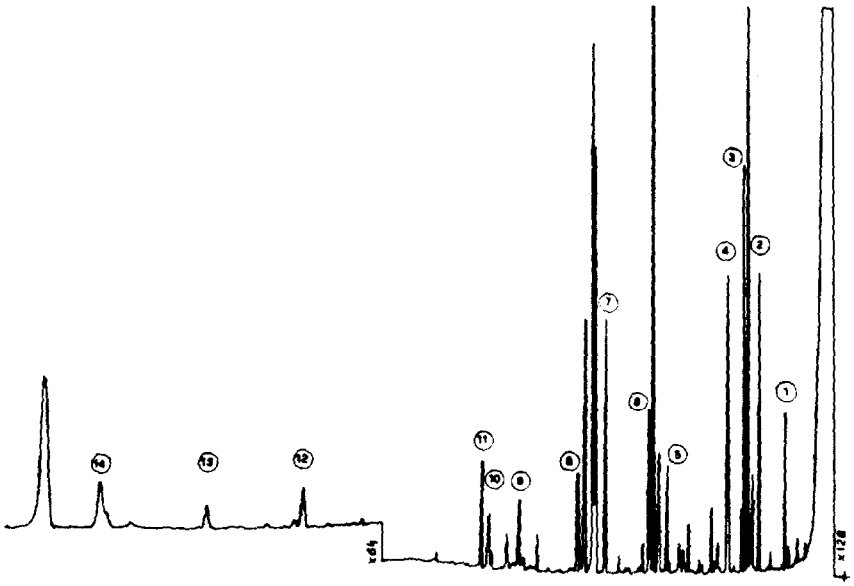

Fig. 2. Chromatogram of a blank plasma sample, supplemented with 2.5 μg of each organic acid and internal standard. Extraction and esterification as described in the text. For identification of peaks, see Table I.

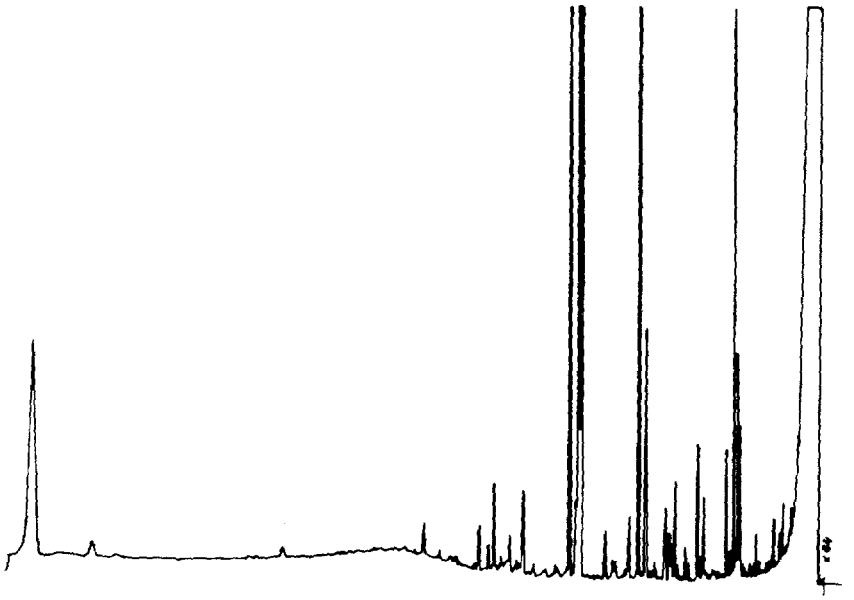

Fig. 3. Chromatogram of a blank plasma sample. Extraction and esterification as described in the text.

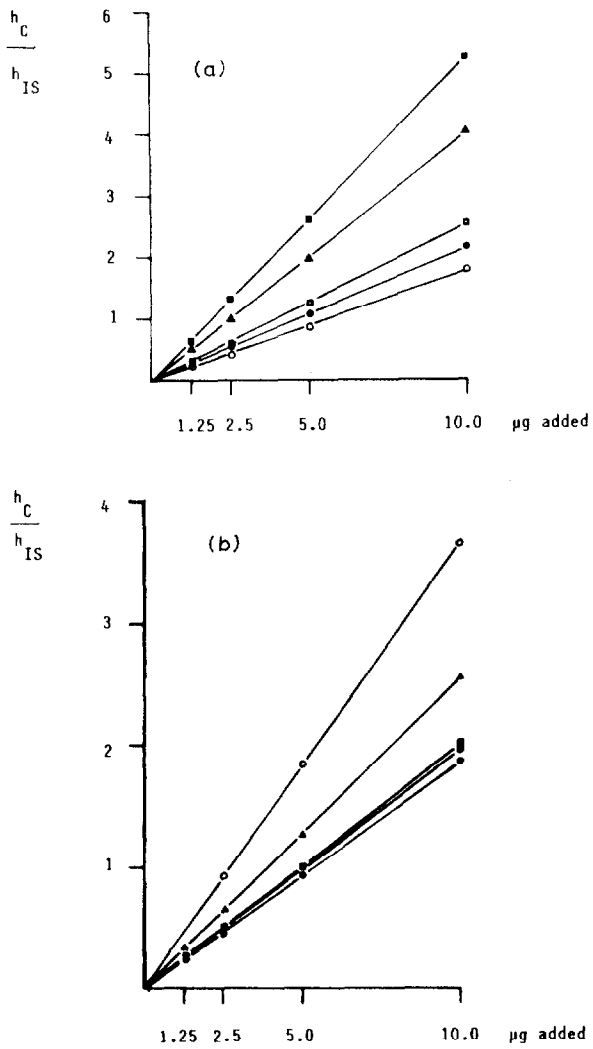

Fig. 4. Plot of the mean values at various concentration levels of the organic acids, showing strict linearity. (a) ●, Salicylic acid; ▲, aspirin; ■, ibuprofen; ○, flurbiprofen; □, diflunisal. (b) ●, Ketoprofen; ▲, tolmetin; ■, zomepirac; ○, fentiazac; □, indomethacin.

Chromatographic separation after the addition of the reference compounds to human plasma and subsequent extraction, is illustrated in Fig. 2.

Fig. 3 presents the chromatogram of a blank plasma sample, extracted and analysed under the same conditions (including esterification) as those used for Fig. 2. Although a large number of endogenous peaks are present, it is apparent where the reference compounds are eluted from the column without interference. This observation is confirmed by the reliability trials of the method, as summarized in Table II and Fig. 4, pertaining to linearity, and in Table III, pertaining to recovery. The results of this study indicate that capillary column chromatography is useful for the analysis of organic acids in biological samples.

TABLE II

EVALUATION OF QUANTITATIVE LINEARITY

Mean values (n = 3 for each point) of sample peak height/internal standard (I.S.) peak height (h_C/h_{IS})

Sample No.	*Compound*	*Amount added (μg)*					*Linear regression*	*r*
		0	*1.25*	*2.5*	*5*	*10*		
1	Salicylic acid	0	0.269	0.537	1.060	2.150	$y=0.2147x-0.0019$	0.9999
2	Aspirin	0	0.491	1.010	1.978	4.095	$y=0.4092x-0.0198$	0.9999
3	Ibuprofen	0	0.635	1.329	2.600	5.232	$y=0.5232x-0.0029$	0.9999
5	Flurbiprofen	0	0.213	0.422	0.864	1.764	$y=0.1767x-0.0101$	0.9999
6	Diflunisal	0	0.309	0.632	1.234	2.154	$y=0.2511x-0.0037$	0.9999
8	Ketoprofen	0	0.231	0.447	0.913	1.871	$y=0.1872x-0.0095$	0.9999
9	Tolmetin	0	0.319	0.646	1.276	2.577	$y=0.2575x-0.0022$	0.9999
10	Zomepirac	0	0.248	0.507	1.000	2.036	$y=0.2035x-0.0051$	0.9999
12	Fentiazac	0	-	0.908	1.855	3.865	$y=0.3691x-0.0028$	0.9999
13	Indomethacin	0	-	0.492	1.000	1.968	$y=0.1969x+0.0032$	0.9999

TABLE III

RECOVERY OF COMPOUNDS ADDED TO PLASMA

Mean values (n=4).

Sample No.	*Compound*	*Amount added (μg)*	*Amount recovered (μg)*	*S.D. (μg)**	*C.V. (%)***	*Amount added (μg)*	*Amount recovered (μg)*	*S.D. (μg)**	*C.V. (%)***
1	Salicylic acid	1.25	1.22	0.04	3.41	5.0	4.97	0.11	2.28
2	Aspirin	1.25	1.24	0.04	3.15	5.0	4.95	0.15	3.11
3	Ibuprofen	1.25	1.25	0.04	2.92	5.0	4.98	0.14	2.88
5	Flurbiprofen	1.25	1.23	0.04	3.40	5.0	4.96	0.16	3.25
6	Diflunisal	1.25	1.21	0.06	4.70	5.0	4.93	0.18	3.65
8	Ketoprofen	1.25	1.23	0.05	4.03	5.0	4.96	0.14	2.89
9	Tolmetin	1.25	1.23	0.04	3.18	5.0	4.95	0.14	2.78
10	Zomepirac	1.25	1.22	0.04	3.21	5.0	4.93	0.15	2.97
12	Fentiazac					5.0	4.94	0.15	3.11
13	Indomethacin					5.0	4.92	0.17	3.39

* S.D. = standard deviation.
** C.V. = coefficient of variation.

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