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Assay of tolfenamic acid and its metabolites by liquid chromatography on dynamically modified silica: application in pharmacokinetics

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Abstract: An LC method able to separate all known metabolites of tolfenamic acid was developed. It was applied to characterize the metabolic profiles after single and multiple peroral doses of tolfenamic acid to volunteers. Up to 80% of the dose, mostly conjugated metabolites, could be recovered in the urine by this method. Two of the metabolites showed plasma half-lives of several days and peculiar conjugation properties.

Keywords: *Tolfenamic acid; metabolism; pharmacokinetics; anti-inflammatory drugs; liquid chromatography; dynamically modified silica.*

Introduction

Tolfenamic acid, *N*-(2-methyl-3-chlorophenyl)-anthranilic acid, the active substance of Clotam®, is a potent anti-inflammatory drug [1, 2]. Tolfenamic acid inhibits the biosynthesis of prostaglandins as effectively as indomethacin, and at markedly lower concentrations than acetylsalicylic acid. Like other fenamates, it has an inhibitory action on prostaglandin receptors, too [3]. Tolfenamic acid is remarkably non-irritative towards the gastric mucosa [4], is well tolerated by patients [5], and shows good results in clinical studies, including long-term treatment of rheumatoid arthritis [6–10]. Clinical efficacy in dysmenorrhoea [11] and migraine [12] has also been documented.

The present studies concern the metabolism and pharmacokinetics of tolfenamic acid [13, 14]. In this paper a chromatographic method is described that separates tolfenamic acid, all metabolites known at present and, in addition, two unknown metabolites, i.e. eight compounds in all (Scheme 1). The method has been applied to determine the metabolic profile in humans after peroral single and multiple doses of tolfenamic acid in two volunteers.

The choice of a chromatographic system using dynamically modified silica was made after the failure of several attempts to separate tolfenamic acid and its metabolites in a reversed-phase system on chemically bonded phases. The use of dynamically modified

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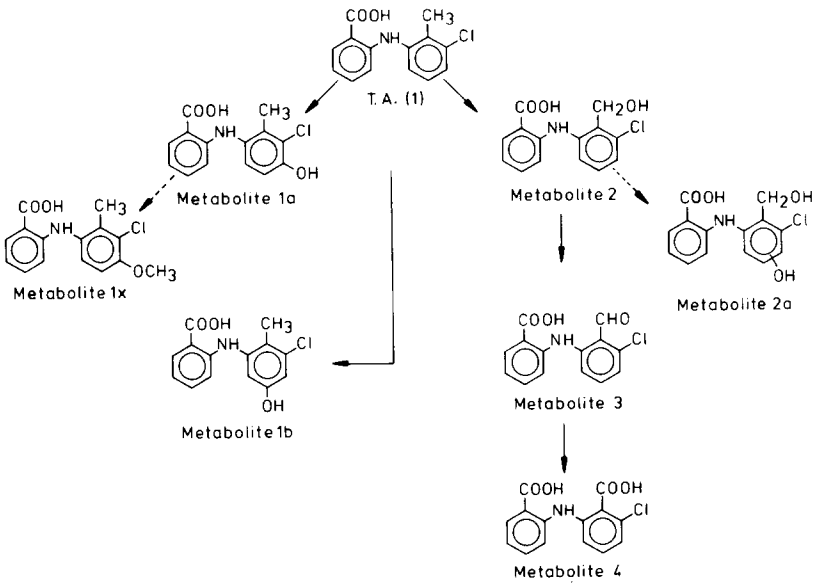

Scheme 1
Metabolism of tolfenamic acid. Glucuronic acid conjugates are omitted (see text for details).

silica in high-performance liquid chromatography (HPLC) has proved to be a powerful tool when a certain selectivity has to be achieved and when it is important to be able to reproduce this selectivity over a period of time [15–19]. The authors have developed a method where tolfenamic acid and all its known metabolites are rapidly separated on such a system.

Experimental

Protocol

Two healthy volunteers participated in the study: one female aged 49 and 60 kg body weight, and one male aged 46 and 64 kg body weight. They were considered healthy by history and by physical examination. Written consent was obtained from the volunteers before the study.

Clotan® capsules, GEA batch 92085, containing 100 mg tolfenamic acid were used. The volunteers received first a single dose (200 mg) and after a clearance period of one week they received 200 mg t.i.d. for 5 days at 7 a.m., 12 o'clock, and 6 p.m.

Blood samples. Blood samples were drawn by venipuncture into heparinized tubes prior to dosing and at ½, 1, 2, 3, 4, 6, 10, 25, 33, 49 and 72 h after administration of a single dose; for the multiple dose study samples were taken on day 3 and 4 at 7 a.m., 9 a.m., 12 o'clock, 2 p.m. and 6 p.m.; on day 5 at 7 a.m., 9 a.m., 12 o'clock, 2 p.m., 4 p.m., 6 p.m. and 8 p.m.; and on days 6 and 7 at 7 a.m. and 4 p.m. Plasma was separated and stored at −18°C until analysis. Eleven days after the last dose an additional sample was drawn.

Urine samples. Total urine was collected at the following intervals: 0–2, 2–4, 4–6, 6–8, 8–12, 12–24, 24–48 and 48–72 h after administration of a single dose. In the multiple

dose study, the urine samples were collected at the dosing intervals up to the 5th day and thereafter at 6–10 p.m., 10 p.m.–7 a.m., and on the 6th and 7th day from 7 a.m. to 4 p.m. and from 4 p.m. to 7 a.m. Eleven days after the last dose a single urine sample was collected. Diuresis was measured and all aliquots stored at −18°C until analysis.

Samples of faeces. Faeces were collected for 0–24 and 24–48 h after administration of a single dose. The faeces were dried *in vacuo* at 37°C, weighed, powdered and aliquots stored at −18°C until analysis.

Apparatus

A Waters liquid chromatograph consisting of a Model 6000A pump, a Model 710B WISP autoinjector, a Model 440 ultraviolet (UV) absorbance detector (280 nm), a Model 730 data module and a Model 720 system controller was used. The columns were thermostated in a model LC Kratos oven 250/3.

Chromatography

The column set-up employed was as previously described [15, 16]. The analytical column was a Knauer column, 120 × 4.6 mm i.d., packed with either LiChrosorb Si 60 (5 μm) or Polygosil 60 (5 μm). The guard column (150 × 4.6 mm i.d.), situated between the pump and the autoinjector, was dry packed with LiChroprep Si 60 (15–25 μm). Both columns were operated at 40°C. The mobile phase for the final analytical system was acetonitrile–methanol–0.2 M potassium phosphate (pH 7.5)–water (25:20:5:50 v/v) containing 1.25 mM *N,N,N*-trimethylhexadecyl-ammonium bromide (CTMA). The flow-rate was 1.5 ml min^{-1}.

Extraction procedures

Plasma. (a/b). An aliquot of 1.0 ml plus 2.5 ml phosphate buffer pH 6.5 with or without adding β-glucuronidase is taken following incubation at 37°C for 3 h. Then add 0.5 ml 3 N H_2SO_4 and 6.5 ml dichloromethane. Mix in a rotary mixer for 10 min and centrifuge at 5°C for 10 min (2500 rpm). Aspirate the aqueous phase and withdraw 5.0 ml of the organic phase. Evaporate with a gentle stream of N_2 at 35°C and redissolve in 96% ethanol (100 μl) and inject in liquid chromatograph (20–40 μl).

(c). As above, but with sulfatase instead of β-glucuronidase incubation.

(d). Plasma (1.0 ml) plus 0.5 ml 0.1 M NaOH refluxed for 1 h. Then proceed as above, from the point of adding 0.5 ml 3 N H_2SO_4.

Urine. (a/b). To urine (1.0 ml) is added 0.5 ml phosphate buffer pH 6.5, with or without adding β-glucuronidase and following incubation at 37°C for 3 h. Then add 200 μl 3 N H_2SO_4 and 1.5 ml methanol–acetonitrile mixture (1:1 v/v). Mix on a rotary mixer and centrifuge for 10 min at 10°C (2500 rpm). Inject 15 μl of the centrifugate in the chromatograph.

(c). As above, but substituting sulfatase incubation for β-glucuronidase.

(d). Urine (1 ml) plus 0.5 ml 0.1 M NaOH is refluxed for 1 h. Then proceed as above from the point of adding 200 μl 3 N H_2SO_4.

Faeces. (a/b). To faeces (100 mg dry weight) is added 2.5 ml phosphate buffer pH 6.5 with or without adding β-glucuronidase and following incubation for 3 h at 37°C. Then add 0.5 ml 3 N H_2SO_4 and 6.5 ml dichloromethane. Mix in a rotary mixer and centrifuge

at 5°C for 10 min (2500 rpm). Aspirate the aqueous phase and withdraw 5.0 ml of the organic phase. Evaporate with a gentle stream of N_2 at 35°C, redissolve in 96% v/v ethanol (500 μl) and inject in the liquid chromatograph (20 μl).

Standardization

Control plasma, urine or faeces, spiked with tolfenamic acid and metabolites (1a, 1b, 2, 3, 4), were analysed every day concurrently with the samples in order to construct standard curves and check the methodology.

Chemicals

Metabolite 1a, metabolite 2, metabolite 3 and metabolite 4 were synthetic products from the Synthetic Laboratories GEA Ltd. (Denmark). Tolfenamic acid and Clotam® capsules were also obtained from GEA Ltd. Metabolite 1b was obtained from Medica Ltd (Finland) in a slightly impure state isolated from human urine.

β-glucuronidase (105-8, bacterial type 1) and sulfatase (S-9751 Type H-2) were obtained from Sigma. Acetonitrile HPLC S-grade was obtained from Rathburn Chemicals (Walkerburn, Great Britain). Polygosil 60 was obtained from Macherey-Nagel & Co. (Düren, FRG). All other chemicals were of analytical reagent grade and obtained from E. Merck (Darmstadt, FRG).

Results and Discussion

The chromatographic system

As a basis for the development of the separation method a system previously described [16] was used. It was found that in order to elute tolfenamic acid within a reasonable time it was necessary to use a higher concentration of organic modifier and a lower amount of cetyltrimethylammonium bromide (CTMA) than in the standard system, due to the lipophilicity of tolfenamic acid and its ability to form ion pairs with CTMA. When using 60% v/v methanol as the organic modifier it was not possible to separate the two metabolites 1a and 3, and when using 40% v/v acetonitrile separation of 3 and 2 could not be performed (Table 1). The final eluent containing 25% v/v acetonitrile and 20% v/v methanol was able to separate all the compounds under consideration. In order to achieve the lowest detection limits shorter retention times were desirable. In dynamically modified silica this may be accomplished without changing the selectivity or the column length and without increasing the flow rate, by using another type of silica with a smaller surface area [18]. Using Polygosil 60 instead of LiChrosorb Si 60 the k'-values were halved without any change in selectivity (Table 1).

Chromatograms of a spiked plasma sample and of a urine sample from the multiple dose study are shown in Fig. 1.

Detection limits, reproducibility and recovery

The detection limits, i.e. the sensitivity of the method, defined as a signal-to-noise ratio of three, were determined using standard solutions of each compound. The minimum detectable quantities, expressed in ng injected on-column, were found to be in the range of 10–20 ng for all the known metabolites and tolfenamic acid.

The detection limits of metabolites 1a, 2 and 3 and of tolfenamic acid in plasma are therefore about 50 ng ml^{-1}. The detection limits for metabolite 1b and 4 in plasma are 70 and 170 ng ml^{-1}, respectively, taking the recoveries (Table 2) into consideration.

Table 1
k'-values for tolfenamic acid and some of its metabolites when changing the organic modifier in the eluent containing 0.01 M potassium phosphate pH 7.5 and 1.25 mM CTMA. Flow-rate: 1.5 ml min^{-1}

Organic modifier	% v/v in eluent	Column packing material	k' Metabolite 1a	Metabolite 3	Metabolite 2	Tolfenamic acid
Methanol	60	LiChrosorb Si 60	4.8	5.0	6.5	14.4
Acetonitrile	40		5.9	8.7	9.0	19.8
Acetonitrile + methanol	25 + 20		7.0	8.0	9.9	23.0
Acetonitrile + methanol	25 + 20	Polygosil 60	3.4	4.1	5.0	11.1

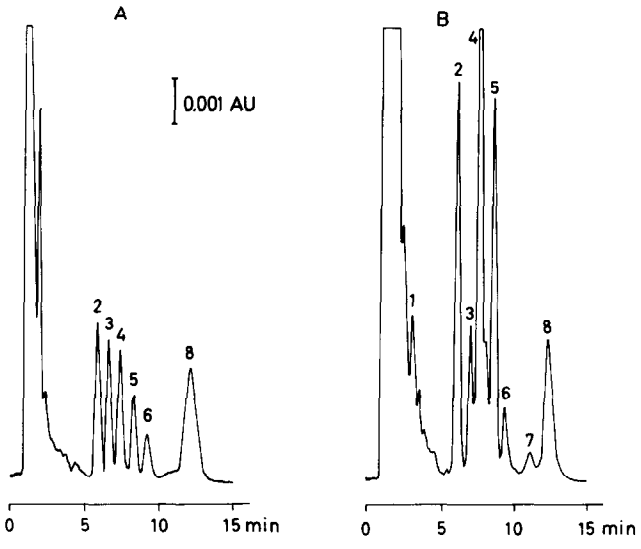

Figure 1
Chromatograms of: A, a plasma sample spiked with tolfenamic acid and its metabolites (10 μg of each per ml plasma); and B, a urine sample from volunteer 1 at day 5 at 12 h in the multiple dose study. Peak identification: 1: Metabolite 2a; 2: Metabolite 1a; 3: Metabolite 3; 4: Metabolite 2; 5: Metabolite 1b; 6: Metabolite 4; 7: Metabolite 1x; and 8: Tolfenamic acid (1).

Table 2
Analysis of plasma samples spiked with tolfenamic acid and five of its metabolites

Sample	Concentration (ng ml^{-1})	Mean concentration found (n = 4, ng ml^{-1})	Precision* RSD (%)	Recovery (%)
Tolfenamic acid	2500	2502	2.5	100
	1000	934	4.4	93
	250	210	3.9	84
Metabolite 1a	2500	2549	1.0	102
	1000	1026	3.6	103
	250	250	9.0	100
Metabolite 1b	2500	1950	†	78
	1000	790	†	79
	250	176	5.0	71
Metabolite 2	2500	2432	4.1	97
	1000	959	5.6	96
	250	257	4.4	103
Metabolite 3	2500	2655	2.6	106
	1000	1010	1.7	101
	250	244	4.0	98
Metabolite 4	2500	725	18.1	29
	1000	320	24.1	32
	250	68	8.6	27

* Relative standard deviation.
† Only a single determination was carried out due to limited amount of standard.

The reproducibility and precision of the method were determined by adding standard solutions to plasma samples. The results are shown in Table 2. For metabolite 1a, 2 and 3 and for tolfenamic acid the recoveries were *ca* 100%; the precision was also good, but the recovery and the precision of metabolite 4 were poor.

When analysing the urine samples, recovery and precision were excellent for all the substances, due to the simple sample preparation.

Pharmacokinetics and metabolism of tolfenamic acid

The pharmacokinetics of tolfenamic acid in man have been described by a two-compartment open model, with a central compartment volume of about 6 1, and a β-phase volume of about 25 1, with a total elimination rate constant of 1.6 h^{-1} and a β-phase half-life of about 2 h. The total plasma clearance was around 150–200 ml min^{-1}, and complex absorption was observed, with an average half-life of about 2 h [14, 20].

As seen before, very different tolfenamic acid plasma profiles are observed for the two subjects in this study after *p.o.* administration, mainly due to differences in the absorption phases (Figs 2 and 4). The excretion in the urine and faeces of tolfenamic acid are similar to earlier results (Tables 3–5).

The elimination of tolfenamic acid occurs principally by extrarenal mechanisms, i.e. by hepatic metabolic clearance. The metabolism of tolfenamic acid in man is shown in Scheme 1. Metabolite 1a, 1b, 2 and 3 have been identified previously [13, 21–23]. Metabolite 4 has been synthesized and identified in biological fluids by chromatography. The structures of metabolite 1x and 2a are speculative only. They appear as small chromatographic peaks in subjects treated with Clotam® (Fig. 1). They have profiles corresponding to metabolites 1a and 2, respectively, and finally their polarities in the chromatographic system are consistent with the structures proposed.

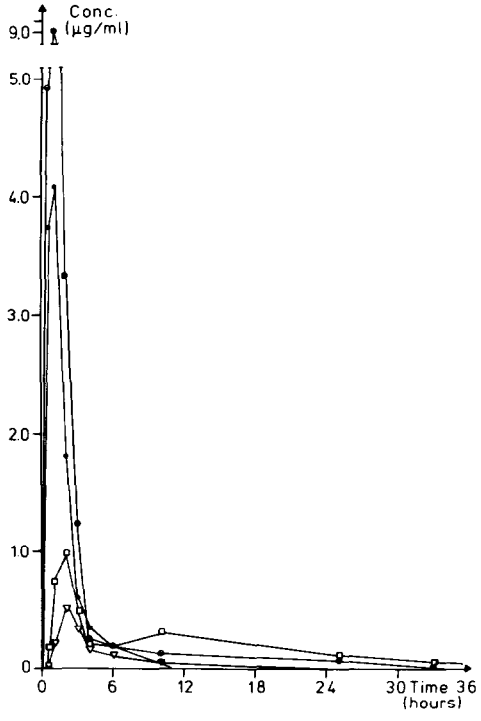

Figure 2
Plasma concentrations after single peroral dose of 200 mg tolfenamic acid for volunteer 1, ●: T.A.; ⊙: T.A. as conjugate; □: Metabolite 1a as conjugate; ▽: Metabolite 1b as conjugate.

Table 3
Cumulative urinary excretion of tolfenamic acid (TA) and metabolites after a single peroral dose of 200 mg to two volunteers

	Urinary excretion (% of dose)																	
Time			Metabolites															
after	T.A.		1a		1b		2		3		4		"1x" ‡		"2a"		"Total"	
admin.	S.1	S.2	S.1	S.2	S.1	S.2	S.1	S.2	S.1	S.2	S.1	S.2	S.1	S.2	S.1	S.2	S.1	S.2
2	3.8	0.4	1.6	0.1	N.D.*	0	4.1	0.5	1.2	0	0	0	0.3	0	N.D.†	N.D.†	11.0	1.0
4	5.7	1.6	3.7	1.6	—	0	9.2	4.0	3.2	1.1	0	0	0.4	0	—	—	22.3	8.3
6	—	3.4	—	4.6	—	0.2	—	11.4	—	3.2	—	0.1	—	0.1	—	—	—	23.0
8	6.6	3.7	5.0	5.6	—	0.3	12.9	13.2	4.3	4.0	0.2	0.2	0.6	0.2	—	—	29.6	27.2
12	7.2	4.0	6.8	6.6	—	0.4	15.0	14.9	5.5	4.7	0.3	0.3	0.7	0.2	—	—	35.5	31.1
24	8.1	4.5	10.6	9.7	—	1.4	17.6	17.8	6.8	6.3	0.7	0.7	0.9	0.4	—	—	44.7	40.8
48	8.1	4.5	—	10.9	—	1.9	17.6	18.7	6.8	6.6	1.4	1.3	0.9	0.4	—	—	45.4	44.3
72	8.1	4.5	11.5	10.9	—	1.9	17.6	18.7	6.8	6.6	1.7	1.9	0.9	0.4	—	—	46.6	44.9

*N.D.: The peak was badly resolved from the larger metabolite 2 peak in this run.
†N.D.: The peak was either not resolved from background peaks or was too low in its concentration.
‡Calculated with T.A. as reference standard.
S. = subject.

Table 4
Excretion of tolfenamic acid (TA) and its metabolites in the faeces after a single per oral dose of 200 mg to two volunteers

Subject	Excretion in the faeces (0–48 h) (% of dose) Tolfenamic acid	Metabolite 1a	Metabolite 1b	Metabolite 3	"Total"
1	1.0	0.2	0.2	0.4	1.8
2	5.9	N.D.*	0.6	0	6.5

* Not determined: obscured by a larger background peak.

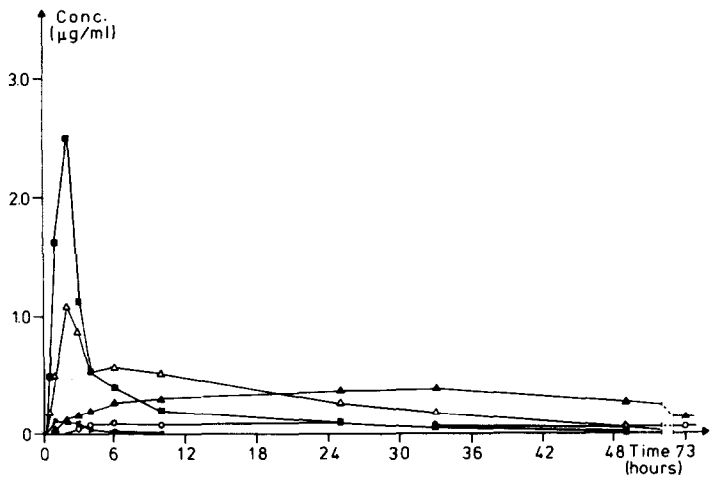

Figure 3
Plasma concentrations after single peroral dose of 200 mg tolfenamic acid for volunteer 1. ■: Metabolite 2 as conjugate; △: Metabolite 3 as conjugate; ▲: Metabolite 3; ○: Metabolite 4 as conjugate; *: Metabolite 2a as conjugate.

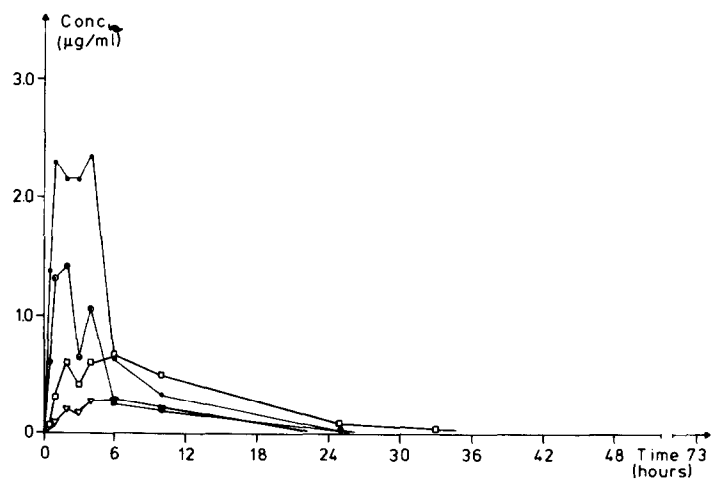

Figure 4
Plasma concentrations for volunteer 2 after single dose T.A. (200 mg) *p.o.* Symbols as in Fig. 2.

Table 5
Daily urinary excretion of tolfenamic acid and its metabolites after multiple peroral doses of 200 mg to two volunteers

Daily urinary excretion (% of dose)																		
			Metabolites															
	T.A.		1a		1b		2		3		4		"1x"		2a†		"Total"	
Day/dose	S.1	S.2	S.1	S.2	S.1	S.2	S.1	S.2	S.1	S.2	S.1	S.2	S.1	S.2	S.1	S.2	S.1	S.2
1/600 mg	10.1	5.3	9.8	8.3	1.1	1.5	22.2	14.9	4.2	5.2	0.4	0.4	0.7	0.6	1.2	2.2	49.7	38.4
2/600 mg	7.9	6.3	12.9	13.4	5.2	6.4	22.8	21.0	6.8	8.2	1.3	1.3	0.8	1.2	1.7	3.5	59.4	61.3
3/600 mg	8.6	5.9	12.2	12.9	10.2	8.9	23.6	20.6	4.9	6.3	1.3	1.8	0.7	1.0	2.5	3.4	64.0	60.8
4/600 mg	9.9	6.0	16.2	14.2	14.5	12.0	29.4	20.3	6.9	6.5	1.2	2.1	1.1	1.1	3.8	4.0	83.0	66.2
5/600 mg	8.7	6.7	15.3	15.0	15.8	11.8	28.3	21.1	4.5	8.5	1.4	2.1	0.9	1.2	3.3	2.6	78.2	69.0
6/0 mg	0.2	0	4.5	6.3	10.3	10.7	3.6	2.0	0.6	1.8	1.1	2.0	0.1	0	0.5	0	20.9	22.8
7/0 mg	0	0	2.2	3.0	7.4	8.1	1.0	0.4	0	0	1.0	1.5	0	0	0	0	11.6	13.0
Overall recovery in 7 days																	73.4	66.3

* Calculated as T.A. with T.A. as reference standard.
† Calculated as metabolite 1a with metabolite 1a as reference standard.
T.A. = tolfenamic acid.
S.1. = subject 1.
S.2. = subject 2.

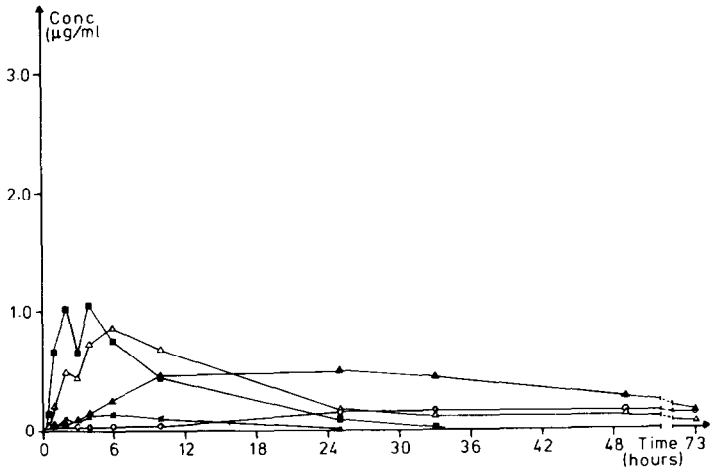

Figure 5
Plasma concentrations for volunteer 2 after single dose T.A. (200 mg) *p.o.* Symbols as in Fig. 3.

Excretion

The total drug excretion is mainly by renal excretion of the metabolites (Tables 3–5) as conjugates of tolfenamic acid; the metabolites of Scheme 1 account for up to about 80% of the dose determined by the present chromatographic method. The advantage of the multiple dose study with respect to estimating the amounts excreted is clear from the tables. Practically no tolfenamic acid or metabolites are excreted in unconjugated forms in the urine. β-Glucuronidase treatment yielded the reported amounts. Sulfatase treatment (from *Helix pomatia* with glucuronidase activity) and hydrolysis in hot alkali yielded the same results, except that metabolite 3 was totally decomposed by base. Earlier, it was found that acid hydrolysis did not release any drug compounds in the unconjugated form [24]. These findings clearly indicate that tolfenamic acid and its first-order metabolites are all excreted as glucuronic acid conjugates, more specifically as ester glucuronides.

Metabolite pharmacokinetics

The present study revealed several interesting features. The tendency to give, or the appearance of explicit double peaks in the plasma profiles of tolfenamic acid and some of the metabolites strongly suggest enterohepatic recirculation of these compounds (Figs 2–5). The recovery of metabolites in the faeces supports the idea of biliary excretion. Enterohepatic recirculation has been suggested as a typical feature of fenamates [25].

Metabolites 3 and 4 exist both in the unconjugated and in the conjugated forms in plasma. The profiles are non-parallel, with the conjugated form reaching the maximum first and having the shortest half-life. Thus, the conjugated form could not be associated with the plasma-circulating metabolite as its only precursor. This may be due to the possibility that metabolite 3 conjugate is formed directly from metabolite 2 conjugate, or formed *in situ* from metabolite 3 just after its formation in the liver. Another possibility is that the conjugate is formed from intestinal enzymes during enterohepatic recirculation [26].

Because of these interesting but complex terms, the present authors have refrained from performing speculative pharmacokinetic calculations.

The apparent pharmacokinetic parameters are as follows.

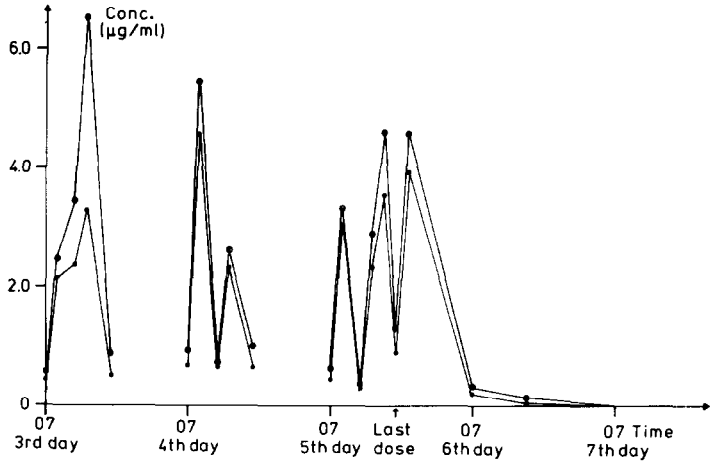

Figure 6
Plasma concentrations for volunteer 1 after multiple dose T.A. (200 mg t.i.d.) *p.o.* ●: T.A., ⊙: T.A. plus T.A. conjugate.

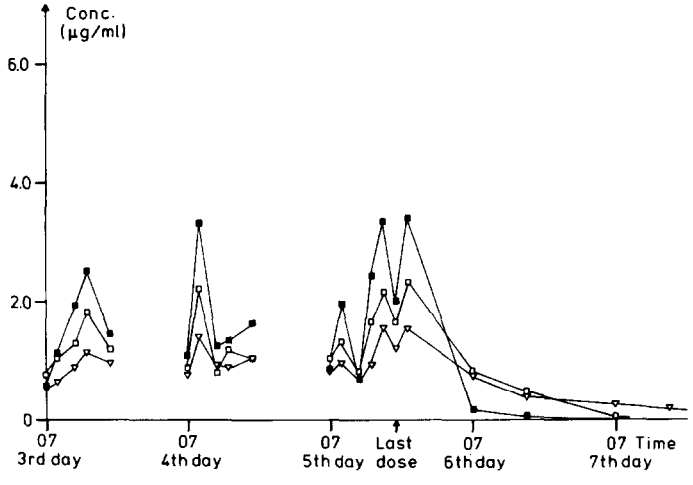

Figure 7
Plasma concentrations for volunteer 1 after multiple dose T.A. (200 mg t.i.d.) *p.o.* ■: Metabolite 2 as conjugate; □: Metabolite 1a as conjugate; ▽: Metabolite 1b as conjugate.

Tolfenamic acid glucuronide: Plasma half-life around 4 h, renal clearance about 60 ml min^{-1} and volume of distribution of the order of 10–40 l.

Metabolite 1a glucuronide: Plasma half-life around 9 h, renal clearance about 50 ml min^{-1} and volume of distribution of the order of 30–40 l.

Metabolite 1b glucuronide: Plasma half-life around 16 h, renal clearance about 75 ml min^{-1} and volume of distribution around 100 l.

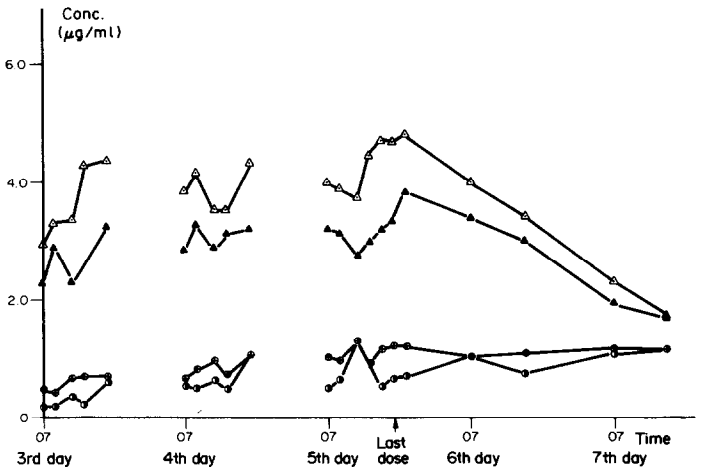

Figure 8
Plasma concentrations for volunteer 1 after multiple dose T.A. (200 mg t.i.d.) *p.o.* ▲: Metabolite 3; △: Metabolite 3 plus its conjugate; ◑: Metabolite 4; ⊕: Metabolite 4 plus its conjugate.

Metabolite 2 glucuronide: Plasma half-life around 6 h, renal clearance about 70 ml min^{-1} and volume of distribution about 17 l.

Metabolite 3 (unconjugated + glucuronide): Plasma half-life around 20 h, renal clearance about 10 ml min^{-1} and volume of distribution of the order of 10–20 l.

Metabolite 4 (unconjugated + glucuronide): Plasma half-life around 120 h, renal clearance about 10 ml min^{-1} and volume of distribution about 100 l.

Metabolites 1x and 2a: These were only observed in the urine samples and in a few plasma samples (Table 5).

Pentikäinen *et al.* [22] have calculated the half-lives of some of the metabolites from a single-dose human experiment. Their figures were obtained from thin-layer chromatographic analysis of the urine. The half-lives for tolfenamic acid, and for metabolites 1a, 1b and 2 were of the same order of magnitude as reported in the present paper, whereas metabolite 3 was not reported by Pentikäinen *et al.* For metabolite 4, however, there is a discrepancy as the present authors found a plasma half-life around 120 h, whereas Pentikäinen found an average excretion half-life of *ca* 11 h. The reason for this discrepancy seems to be a big difference in the half-lives of the glucuronide (about 9 h) and the unconjugated metabolite (about 120 h) found in this experiment. Pentikäinen *et al.* measured a hybrid half-life of the glucuronide and other polar, non-resolved metabolites at the start of the TLC plate.

Futher study in the fields of biliary excretion, individual single metabolite dosing and on the structure elucidation of 1x, 2a and perhaps other minor metabolites could be valuable.

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References

[1] B. Alhede, G. H. Nielsen, K. Neuholdt and D. Zekiros, Pharmacological properties of tolfenamic acid, (*N*-(2-methyl-3-chlorophenyl)anthranilic acid) (6414) Report of Research Dept. GEA (1974).
[2] I.-B. Lindén, J. Parantainen and H. Vapaatalo, *Scand. J. Rheumatol.* **5**, 129 (1976).
[3] H. Vapaatalo, J. Parantainen, I.-B. Lindén and H. Hakkarainen, "Prostaglandins and vascular headache." Italian and Scandinavian Migraine Societies: *Headache, New Vistas*, pp. 287–300. Biomedical Press, Florence (1977).
[4] C. K. Axelson, L. V. Christiansen, Aa. Johansen and P. Ejby Poulsen, *Scand. J. Rheumatol.* **6**, 23 (1977).
[5] V. Rejholec and B. Alhede, A study of the tolerability of tolfenamic acid in patients with osteoarthrosis during one year's treatment (1982).
[6] E. Zachariae and J. Sylvest, *Scand. J. Rheumatol.* **1**, 97 (1972).
[7] L. Nyfos, *Scand. J. Rheumatol.* suppl. **24**, 5 (1979).
[8] V. Rejholec, H. Vapaatalo, O. Tokola and G. Gothoni, *Scand. J. Rheumatol.* suppl. **24**, 8 (1979).
[9] V. Rejholec, H. Vapaatalo, O. Tokola and G. Gothoni, *Scand. J. Rheumatol.* suppl. **24**, 14 (1979).
[10] K. Sørensen and L. V. Christiansen, *Scand. J. Rheumatol.* suppl. **20**, 1 (1977).
[11] A. Kauppila and O. Ylikorkala, *Eur. J. Obstet. Gynecol. Reprod. Biol.* **7**, 59 (1977).
[12] H. Hakkarainen, H. Vapaatalo, G. Gothoni and J. Parantainen, *Lancet* **ii**, 326 (1979).
[13] S. Bols Pedersen, B. Alhede, O. Buchardt, J. Møller and K. Bock, *Arzneim. Forsch.* **31**, 1944 (1981).
[14] S. Bols Pedersen, Pharmacokinetics of Tolfenamic acid in man. Report of Biochemical Dept. GEA (1981).
[15] S. H. Hansen, *J. Chromatogr.* **209**, 203 (1981).
[16] S. H. Hansen, P. Helboe, M. Thomsen and U. Lund, *J. Chromatogr.* **210**, 453 (1981).
[17] S. H. Hansen, P. Helboe and U. Lund, *J. Chromatogr.* **240**, 319 (1982).
[18] S. H. Hansen, P. Helboe and U. Lund, *J. Chromatogr.* **260**, 156 (1983).
[19] S. H. Hansen, P. Helboe and U. Lund, *J. Chromatogr.* **270**, 77 (1983).
[20] P. J. Pentikäinen, P.J. Neuvonen and C. Backman, *Eur. J. Clin. Pharmacol.* **19**, 359 (1981).
[21] A. Kuninaka, K. Sugai, R. Kimura and R. Murata, *J. Pharmacol. Japan* **101**, 1 (1981).
[22] P. J. Pentikäinen, A. Penttilä, P. J. Neuvonen, R. G. Khalifah and C. E. Hignite, *Eur. J. Drug Metab. Pharmacokinet.* **7**, 259 (1982).
[23] R. G. Khalifah, C. E. Hignite, P. J. Pentikäinen, A. Penttilä and P. J. Neuvonen, *Eur. J. Drug Metab. Pharmacokinet.* **7**, 269 (1982).
[24] B. Alhede, The transformation of GEA 6414 (tolfenamic acid) in the organism. GEA report (1972).
[25] A. J. Glazko, *Ann. Phys. Med.* **9**, 23 (1967).
[26] M. K. Cassidy and J. B. Houston, *J. Pharm. Pharmacol.* **32**, 57 (1980).

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