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Short communication

Direct high-performance liquid chromatography resolution on chiral columns of tiaprofenic acid and related compounds in bulk powder and pharmaceutical formulations

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Abstract

High-performance liquid chromatography (HPLC) was employed for the separation and determination of impurities [5-benzoyl-2-acetylthiophene (BAT), 5-benzoyl-2-ethylthiophene (BET) and (*RS*)-5-benzoyl-α-methyl-3-thiopheneacetic acid (3-isomer of tiaprofenic acid) contained in bulk racemic tiaprofenic acid and pharmaceutical formulations. Chiral columns containing the 3,5-dimethylphenylcarbamate of cellulose and amylose were used. The effect of the organic modifier, 2-propanol, in the mobile phase was studied. The HPLC method gave good performances from qualitative and a quantitative standpoints, allowing the enantiomeric ratios of tiaprofenic acid and its 3-isomer to be determined together with the related impurities BAT and BET.

1. Introduction

Stereochemistry is a major determinant of both pharmacodynamic and pharmacokinetic properties of non-steroidal anti-inflammatory drug derivatives of arylpropionic acids [1,2]. In fact the (*S*)-enantiomers of this class have a consistently higher pharmacological activity than the (*R*)-enantiomers; further, the (*R*)-(−)-enantiomers of ibuprofen and naproxen were found to be converted in vivo into the corresponding (*S*)-(+)-enantiomers [3–6]. However, only naproxen and flunaxaprofen are administered as the pure (*S*)-(+)-enantiomer.

Two methods are currently used to achieve the chiral chromatographic separation of racemic mixtures: (i) formation of diastereoisomeric derivatives before HPLC separation or addition of a chiral selector to the mobile phase and (ii) use of chemically bonded chiral stationary phases (CSPs). The second approach seems to be more promising, as the drawbacks arising from optically impure reagents or different rates of formation of the diastereoisomers are avoided.

Racemic mixtures of amides or anilides of arylpropionic acids have been resolved by means of HPLC on a CSP composed of (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine or a variant of this chiral selector covalently bonded to silica [7–10]. In another study, a series of 1-naphthalenemethylamides derivatives of anti-inflammatory agents was separated either on microbore or

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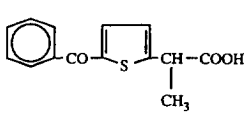
normal columns using as chiral selector the N,N′-3,5-dinitrobenzoyl derivative of (*R*,*R*)-diaminocyclohexane bonded to a siliceous matrix [11,12].

Underivatized anti-inflammatory agents have been separated on a chiral α_1-acid glycoprotein column [13]; the influence of N,N′-dimethyloctylamine added to the mobile phase was studied. With the same CSP, ketoprofen, ibuprofen and fenoprofen were determined in plasma [14] and the effects of the mobile phase composition, pH and temperature on the chiral resolution were studied.

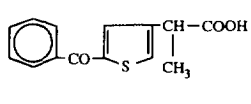
Furthermore, racemic tiaprofenic acid was resolved using immobilized human serum albumin as the CSP [15]. Recently, the enantiomers of ibuprofen, ketoprofen and naproxen were separated using α-chymotrypsin absorbed or covalently bonded on silica as the CSP [16].

Pirkle and co-workers [17,18] utilized the principle of reciprocity for preparing a new promising CSP able to separate underivatized non-steroidal anti-inflammatory drug enantiomers.

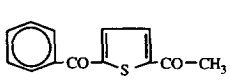
Currently, tiaprofenic acid is dispensed and administered as a racemic mixture for the treatment of acute and chronic arthritis and osteoarthritis. In view of the increasing legislative concern regarding the development and use of chiral drugs, enantioselective analytical methods are required to study the pharmacokinetics and pharmacodynamics of each of the enantiomers.



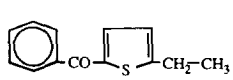
Tiaprofenic acid (1)



3-isomer of tiaprofenic acid (2)



BAT (3)



BET (4)

Fig. 1. Structures of tiaprofenic acid and its impurities.

In this paper, we describe a simple, isocratic method for the simultaneous separation, identification and measurement of enantiomers of *rac*-tiaprofenic acid and its *rac*-3-isomer, together with the achiral impurities 5-benzoyl-2-acetylthiophene (BAT) and 5-benzoyl-2-ethylthiophene (BET) (Fig. 1) in bulk powder and pharmaceutical formulations. Chiral columns containing cellulose and amylose derivatives adsorbed on the silica surface were used.

2. Experimental

2.1. Materials

Stainless-steel Chiralcel OD and Chiralpak AD columns (250 × 4.6 mm I.D.) (Daicel Chemical Industries, Tokyo, Japan) were used. HPLC-grade solvents were purchased from Carlo Erba (Milan, Italy). Trifluoroacetic acid (TFA) of Uvasol grade was obtained from Merck (Darmstadt, Germany). *rac*-Tiaprofenic acid was kindly supplied by Roussel-Pharma (Milan, Italy). The impurities (BAT, BET and the *rac*-3-isomer of tiaprofenic acid) were obtained from Roussel-Uclaf (Paris, France).

2.2. Apparatus

Chromatography was performed using a Waters (Milford, MA, USA) M 510 HPLC pump, a Rheodyne (Cotati, CA, USA) Model 7125 injector with a 20-μl sample loop, and a Model 991 programmable multi-wavelength diode-array detector (Waters).

2.3. Operating conditions

The following chromatographic conditions were used: with Chiralcel OD mobile phase, *n*-hexane–2-propanol–TFA (98.5:1.5:0.1) degassed in an ultrasonic bath before use; flow-rate, 1 ml/min; with Chiralpak AD, mobile phase, *n*-hexane–2-propanol–TFA (94.0:6.0:0.1)

degassed in an ultrasonic bath before use; flow-rate, 0.5 ml/min; column temperature, 20°C volume injected, 20 μl; and detection wavelength 296 nm. The columns were carefully washed every day with 60 ml of *n*-hexane. In this way, no decrease in efficiency was observed throughout the work.

2.4. Linearity of detector response

Calibration graphs were obtained by injecting the impurities BET, BAT and 3-isomer in the concentration range 0.2–4.0 μg/ml and tiaprofenic acid in the range of 50.0–2000.0 μg/ml.

2.5. Samples preparation

Tablets

In a mortar, ten tablets (previously weighed) were pulverized and, taking into account the average mass of the same, an amount of powder corresponding to 300 mg of tiaprofenic acid was weighed exactly. The powder was suspended in methanol (3 × 20 ml) and stirred for 30 min. The methanol was then filtered through a filter-paper and dried with a gentle stream of nitrogen and the residue was dissolved in 20 ml of *n*-hexane–2-propanol (9:1). A 1-ml volume of this solution was diluted to 5 ml before injection.

Injectable ampoules

The lyophilized content of a single ampoule (200 mg of tiaprofenic acid) was dissolved in 5 ml of deionized water and the solution was acidified to pH 3 with 0.1 *M* HCl. The aqueous solution was then extracted with diethyl ether (4 × 15 ml) in a separating funnel and the organic layer was washed with water (2 × 15 ml). The organic phase was dried over anhydrous Na_2SO_4, then concentrated to dryness with a gentle stream of nitrogen. The residue was dissolved in 20 ml of *n*-hexane–2-propanol (9:1). A 1-ml volume of this solution was diluted to 5 ml before injection.

Following the extraction procedures described above, we obtained recoveries of 91% for tablets and 98% for ampoules.

3. Results and discussion

Currently HPLC methods are employed for the determination of tiaprofenic acid and its impurities in bulk products and pharmaceutical formulations. These methods, however, do not permit the evaluation of the enantiomeric ratio of the compounds containing a stereogenic centre (tiaprofenic acid and its 3-isomer) together with the achiral impurities BAT and BET. The aim of this work was the determination of the enantiomeric ratio of tiaprofenic acid and its 3-isomer and, at the same time, the determination of the related compounds BAT and BET in bulk products and pharmaceutical formulations.

Cellulose- and amylose-based CSPs (Chiralcel OD and Chiralpak AD) have been successfully employed to separate directly the enantiomers of ibuprofen [19], ketoprofen and tiaprofenic acid [20]; nevertheless, the enantiomeric resolution of the *rac*-3-isomer of tiaprofenic acid, as far as we know, has never been attempted. We adapted the method described by Okamoto et al. [20] to obtain the separation of the enantiomers of tiaprofenic acid and its 3-isomer from the remaining impurities BAT and BET.

Both racemic mixtures of tiaprofenic acid and its 3-isomer were efficiently resolved on Chiralcel OD and Chiralpak AD columns (Figs. 2 and 3); however, the order of elution of the enantiomers of tiaprofenic acid and the 3-isomer were not determined owing to the lack of a single enantiomer.

The enantioselectivity factors (α), obtained with the Chiralcel OD column, showed a dependence on eluent composition; higher values of α were observed with the decreasing 2-propanol content in the mobile phase (Table 1). Small amounts of 2-propanol (less than 1.5%) in the mobile phase resulted in too long elution times of tiaprofenic acid and its isomer, whereas poor enantioseparation of the 3-isomer of tiaprofenic

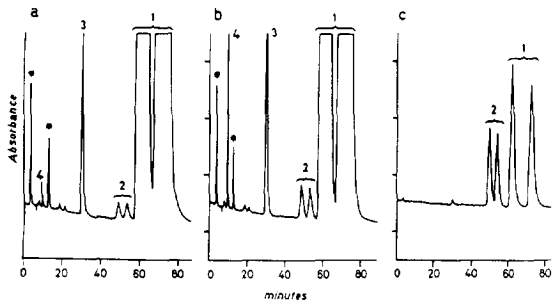


Fig. 2. HPLC of the compounds in Fig. 1. Eluent, *n*-hexane–2-propanol–TFA (95.0:5.0:0.1); column, Chiralcel OD; detection wavelength, 296 nm. (A) Overloaded tiaprofenic acid (10 μg); (B) overloaded tiaprofenic acid (10 μg) spiked with BAT (42 ng), BET (42 ng) and 3-isomer (42 ng); (C) resolution of the enantiomers of tiaprofenic acid and 3-isomer. Asterisks indicate unknown impurities.

acid was obtained with 2-propanol content in the mobile phase higher than 5%.

The amylose-based column was more effective and the separation (α) and resolution factors (R) were better than those obtained with the cellulose-based column (Tables 1 and 2); in addition, the shorter retention times and slower flow-rates obtained with the Chiralpak AD column gave savings in time and solvents. Surprisingly, good values of the resolution of the enantiomers of tiaprofenic acid and its 3-isomer were obtained with the Chiralpak AD column all the whole range of 2-propanol concentrations used

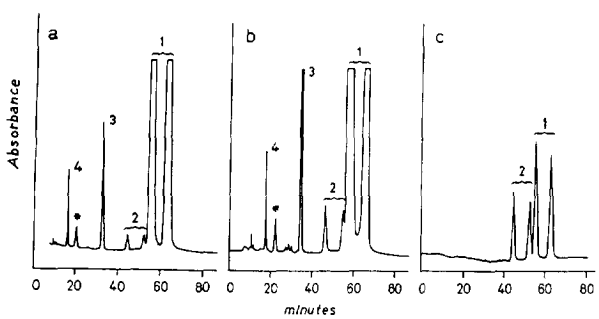


Fig. 3. HPLC of the compounds in Fig. 1. Eluent, *n*-hexane–2-propanol–TFA (94.0:6.0:0.1); column, Chiralpak AD; detection wavelength, 296 nm. (A) Overloaded tiaprofenic acid (10 μg); (B) overloaded tiaprofenic acid (10 μg) spiked with BAT (42 ng), BET (42 ng) and 3-isomer (42 ng); (C) resolution of the enantiomers of tiaprofenic acid and 3-isomer. Asterisks indicate unknown impurities.

Table 1
Chromatographic data for tiaprofenic acid and the 3-isomer on a Chiralcel OD column

Compound	k_1'[a]	α[b]	R_s[c]	Eluent[d]
Tiaprofenic acid	1.97	1.12	1.38	A
	4.17	1.13	1.72	B
	7.52	1.13	1.91	C
	19.93	1.18	2.60	D
3-Isomer	1.77	1.00	No resolution	A
	3.53	1.05	0.62	B
	7.88	1.06	1.00	C
	15.62	1.09	1.51	D

[a] Retention factor of the first-eluted enantiomer.
[b] Enantioselectivity factor.
[c] Resolution factor.
[d] Eluents: (A) *n*-hexane–2-propanol–TFA (90.0:10.0:0.1); (B) *n*-hexane–2-propanol–TFA (95.0:5.0:0.1); (C) *n*-hexane–2-propanol–TFA (97.0:3.0:0.1); (D) *n*-hexane–2-propanol-TFA (98.5:1.5:0.1).

in the mobile phase (Table 2). Nevertheless, the 2-propanol content in the mobile phase was optimized to improve the regioselectivity between the more retained enantiomer of the 3-

Table 2
Chromatographic data for tiaprofenic acid and the 3-isomer on a Chiralpak AD column

Compound	k_1'[a]	α[b]	R_s[c]	α[d]	Eluent[e]
Tiaprofenic acid	2.47	1.21	3.02	1.02	A
	3.59	1.19	2.62	1.05	B
	5.13	1.17	3.17	1.05	C
	6.99	1.16	3.07	1.13	D
	8.05	1.16	3.23	1.06	E
3-Isomer	1.89	1.28	3.90		A
	2.76	1.24	3.69		B
	3.99	1.22	3.70		C
	5.30	1.16	4.17		D
	6.23	1.21	3.93		E

[a] Retention factor of the first-eluted enantiomer.
[b] Enantioselectivity factor.
[c] Resolution factor.
[d] Regioselectivity factor calculated as k_1' (tiaprofenic acid)/k_2' (3-isomer).
[e] Eluents: (A) *n*-hexane–2-propanol–TFA (85.0:15.0:0.1); (B) *n*-hexane–2-propanol–TFA (90.0:10.0:0.1); (C) *n*-hexane–2-propanol–TFA (92.5:7,5:0.1); (D) *n*-hexane–2-propanol-TFA (94,0:6.0:0.1); (E) *n*-hexane–2-propanol–TFA (95.0:5.0:0.1).

isomer and the less retained isomer of tiaprofenic acid (Table 2); this was of great importance in obtaining the separation of the 3-isomer impurity from tiaprofenic acid, mainly in pharmaceutical formulations (Fig. 4).

From this point of view, the Chiralcel OD column showed a higher regioselectivity than the amylose-based column (Tables 1 and 2); in fact, no resolution was obtained between the less retained enantiomer of tiaprofenic acid and the more retained 3-isomer when the Chiralpak AD column was overloaded to detect the 3-isomer impurity in commercial samples (Fig. 3). For this reason, we deemed the Chiralcel OD column more appropriate than the Chiralpak AD for the present problem.

Addition of a small amount of TFA to the mobile phase had a beneficial effect on the separation and the resolution, as it minimizes the ionization of the carboxylic groups of the analytes (tiaprofenic acid and its 3-isomer) and reduces their interaction with the polar sites of the CSPs.

The detection limits obtained with this chromatographic system, using the Chiralcel OD column, were ca. of 5 ng for the 3-isomer of tiaprofenic acid and 1 ng for BAT and BET, calculated on a response of twice the noise level,

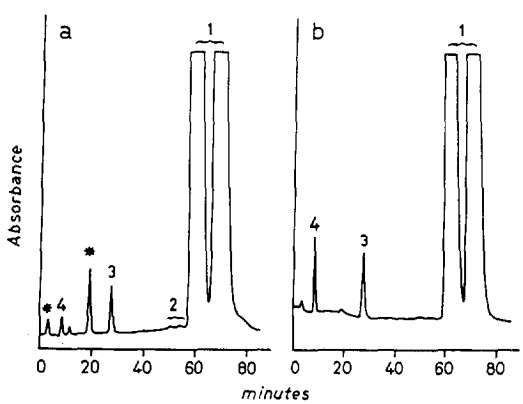


Fig. 4. HPLC of pharmaceutical formulations: (a) tablets and (b) injectable ampoules. Sample injected 10 μg of tiaprofenic acid; eluent *n*-hexane–2-propanol–TFA (95.0:5.0:0.1); column, Chiralcel OD; detection wavelength, 296 nm. Asterisks indicate unknown impurities. The recoveries are 91% for tablets and 98% for ampoules.

and they appeared adequate for application to industrial products.

The calibration graphs (seven points for the impurities and tiaprofenic acid) showed good linearity with a correlation coefficient of 0.9986 for BAT ($c = 0.010A + 0.850$), 0.9992 for the BET ($c = 0.012A + 0.661$), 0.9987 for the 3-isomer of tiaprofenic acid ($c = 0.017A - 0.940$) and 0.9990 for the tiaprofenic acid ($c = 1.651 \cdot 10^{-5}A + 0.192$) ($c$ = sample concentration in μg/ml, A = area counts). Table 3 shows the accuracy and precision data at each individual standard concentration.

The use of a linear photodiode-array detector turned out to be useful in confirming that the chiral separation of a pure racemic compound had, in fact, taken place. As enantiomers behave identically in symmetrical environments, they will absorb non-polarized light in exactly the same way, giving identical spectra (Fig. 5).

4. Conclusions

The HPLC method described for the determination of impurities BAT, BET and the 3-isomer of tiaprofenic acid in bulk product and pharmaceutical formulations appears to be easy to use, reproducible and sensitive. The main advantage is that a single chromatographic run allows the simultaneous determination of the impurities in tiaprofenic acid together with the enantiomeric ratio of tiaprofenic acid and its 3-isomer.

The Chiralcel OD column was preferred to the Chiralpak AD because of the higher regioselectivity shown towards *rac*-tiaprofenic acid and its 3-isomer.

No derivatization of the compounds containing a stereogenic centre (tiaprofenic acid and the 3-isomer) is required for the separation, and therefore the drawbacks due to racemization or different rates of reaction of the single enantiomer or side-reactions of the impurities, which do not contain a stereogenic centre (BAT and BET), with the derivatizing agent are avoided.

In conclusion, with the increasing interest in enantiomerically pure drug formulations in both the pharmaceutical industry [21] and regulatory

Table 3
Inter-day precision and accuracy for BET, BAT, 3-isomer and tiaprofenic acid standards

Compound	Parameter[a]	Amount of samples injected (ng per 20 μl)						
		84	63	42	31.5	21	10.5	5.25
BET	*M*	83.5	62.9	41.3	30.9	21.4	9.8	6.1
	R.S.D. (%)	1.2	0.9	3.5	5.6	2.0	11.7	0.2
	R.E. (%)	−0.6	−0.2	−1.7	−1.9	+1.9	−6.7	+16.0
BAT	*M*	83.7	63.6	42.2	30.9	21.8	10.0	6.3
	R.S.D. (%)	0.6	1.1	1.8	7.0	4.7	11.8	4.9
	R.E. (%)	−0.3	+0.9	+0.5	−1.9	+3.8	−4.7	+20.0
3-Isomer	*M*	83.3	63.9	46.9	31.9	22.5	10.2	6.5
	R.S.D. (%)	1.5	1.4	2.4	3.5	11.6	11.5	19.9
	R.E. (%)	−0.8	+1.4	+11.7	+1.3	+7.1	−2.8	+23.8
		Amount of samples injected (μg per 20 μl)						
		40	20	10	8	4	2	1
Tiaprofenic acid	*M*	40.0	19.9	10.5	7.2	3.8	2.2	1.3
	R.S.D. (%)	3.7	4.8	2.4	6.6	10.7	3.9	9.9
	R.E. (%)	+0.07	−0.2	+5.4	−10.2	−6.0	+9.0	+35.0

[a] *M* = mean value; R.S.D. = relative standard deviation; R.E. = relative error; $n = 3$ in each instance.

authorities [22–25], a chromatographic method able to determine simultaneous chiral and achiral impurities is suitable for quality control of pharmaceutical formulations and for investigations of stereoselective pharmacokinetics and biotransformations.

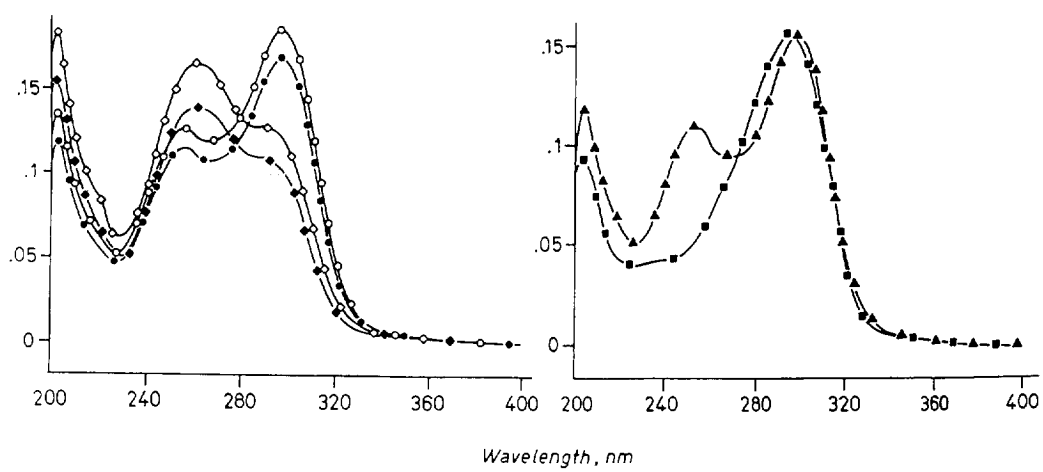


Fig. 5. UV spectra of the compounds in Fig. 1. ○, ● = Tiaprofenic acid; ◇, ◆ = 3-isomer; ▲ = BAT; ■ = BET. Left, open and filled symbols: 1st and 2nd eluted enantiomers, respectively.

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