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Short Communication

The HPTLC resolution of the enantiomers of some 2-arylpropionic acid anti-inflammatory drugs

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Introduction

Recently the rapid and accurate determination of the stereoisomeric composition of optically active drugs and metabolites has become extremely important, because many drugs are stereoisomeric and the biological activity of their stereoisomers may differ.

Anti-inflammatory drugs derived from 2-arylpropionic acids have an asymmetric carbon atom and in this series the S(+) stereoisomers are reported to be consistently more active than the R(−) stereoisomers. Their pharmacological activity is influenced by stereoselective biotransformations [1]. For these reasons the separation and the selective dosage of the enantiomers of these drugs is of importance in biological studies.

Previously 2-arylpropionic acid isomers have been resolved indirectly by GLC and HPLC as diastereoisomeric R(+)-1-phenylethylamides [2, 3]. Recently the enantiomers have been separated directly by means of HPLC on a Pirkle-type chiral stationary phase [4, 5]. Nevertheless, in the latter case derivatization of the drugs is still necessary.

We here report a separation method of ketoprofen, indoprofen and suprofen enantiomers as their diastereoisomeric R(+) 1-phenylethylamides by means of HPTLC on silica-gel plates. This method, which has previously been applied in studies on ketoprofen (A. Lombard, L. Gabriel, V. Rossetti, M. Buffa, Atti VIII Convegno Nazionale Div. Chim. Farm. Soc. Chim. It. — Montecatini, 18–21 October 1982) is of interest as an easy and inexpensive routine test for the identification and spectroscopic evaluation of the chiral metabolic products of these drugs. As far as the authors are aware, a HPTLC method has not been reported previously for the compounds investigated in the present study.

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Materials and Methods

Materials

The following 2-arylpropionic acids have been analysed:

ketoprofen: RS, R(−) and S(+)-2-(3-benzoylphenyl)propionic acid;
suprofen: RS, R(−) and S(+) 2-(4-thiophenoylphenyl)propionic acid (R(+) and S(−) isomers were separated from the racemate in the authors' laboratory);
indoprofen: RS, R(−) and S(+) 2-[4-(2-isoindolinyl-1-one)phenyl] propionic acid.

Methods

Preparation of R(+) 1-phenylethylamides. The R(+)-1-phenylethylamides of the 2-arylpropionic acids were prepared by a modification of the method used by Tamura *et al.* for clidanac isomers [6].

To 0.4 μmol of the 2-arylpropionic acid (RS, R(−) or S(+)) was added 0.1 ml of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) solution (15.6 μmol ml^{-1} in CH_2Cl_2) with stirring at 0°C. After standing* at 0°C, 0.1 ml of a solution of R(+)-1-phenylethylamine hydrochloride (7.8 μmol ml^{-1} in CH_2Cl_2) was added and stirred for an appropriate time† at 28°C. To this solution 3 ml of CH_2Cl_2 was added and successively the solution was washed with 3 ml of water, 3 ml of 1.0 M hydrochloric acid and again with 3 ml of water. The organic layer was evaporated to dryness under reduced pressure and the residue dissolved in 0.1 ml of CH_3OH; 1 μl of the solution was spotted on the plate.

Thin-layer separation. The prepared R(+)-1-phenylethylamides were fractionated on precoated silica gel 60 F_{254} HPTLC plates (Merck) at 20°C with the two eluent systems: (A) benzene–methanol (93:7, v/v) and (B) chloroform–ethyl acetate (15:1, v/v).

In the case of the separation of indoprofen amides, the eluent B was modified‡ and a multiple ascent technique was used with eluent A for better resolution. The zones were visualized by means of UV radiation at 254 nm.

Results

Optimization of preparation of R(+)-1-phenylethylamides

The optimum reaction times with EDC at 0°C and with R(+)-1-phenylethylamine at 28°C were respectively for ketoprofen 1 and 24 h, for suprofen 30 min with both reagents, for indoprofen 24 h with both reagents. At shorter reaction times spots due to the underivatized drug and unidentified side reaction products were observed.

Thin-layer separation

All the diastereoisomeric R(+)-1-phenylethylamides examined were fully separated

*With EDC reaction times employed were 0.5, 1, 6 and 24 h.

†The reaction times with 1-phenylethylamine hydrochloride were 30 min, 1 h, 6 h and 24 h for each incubation time with EDC.

‡Chloroform–ethyl acetate (3:1).

Table 1
HPTLC retention data for derivatized arylpropionic acids

	Benzene–methanol (93:7)					Chloroform–ethyl acetate (15:1)				
	hR_f	c.v.%	(n)	w	R_s	hR_f	c.v.%	(n)	w	R_s
S(+) ketoprofen amide	48.1	4.1	(34)	0.28	2.30	51.0	3.5	(24)	0.26	3.10
R(−) ketoprofen amide	38.5	3.8	(34)	0.25		41.5	4.1	(24)	0.24	
S(+) suprofen amide	41.5	5.0	(30)	0.22	2.10	48.0	4.8	(26)	0.27	2.20
R(−) suprofen amide	36.5	3.6	(30)	0.20		40.0	4.6	(28)	0.24	
						Chloroform–ethyl acetate (3:1)				
S(+) indoprofen amide	38.8	5.4	(19)	0.20	1.06	51.0	1.9	(24)	0.27	2.80
R(−) indoprofen amide	35.5	4.9	(19)	0.18		42.0	2.9	(24)	0.24	

$$hR_f = R_f \times 100;$$

$$\text{c.v.\%} = \frac{\sigma}{\bar{x}} \cdot 100;$$

w = band width

$$R_s = \frac{d_B - d_A}{\frac{w_A + w_B}{2}}$$

where d_A, d_B are the migration distances and w_A, w_B are the band widths. The numbers in brackets indicate the number of data points upon which the evaluation is based.

by means of the chromatographic conditions described, as demonstrated by the hR_f and the resolution data given in Table 1.*

After a single ascent, for the amides of ketoprofen the resolution in eluents A and B was found to be 2.30 and 3.10 respectively. For the amides of suprofen the resolution was 2.10 and 2.20 and for the amides of indoprofen 1.06 and 2.80, for eluents A and B respectively.

The separation of the amides of indoprofen in the eluent benzene–methanol (93:7, v/v) is improved by multiple elution (three ascents), the resolution increasing from 1.06 to 2.17.

The sharp separation of the diastereoisomeric amides produced by HPTLC and the fact that they can subsequently be evaluated by reflectance spectroscopy indicate that this method is suitable for the analysis of the enantiomers of these chiral drugs and possibly their metabolites.

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*As is common practice in GLC and HPLC, the resolution on HPTLC plates is defined by means of the expression

$$R_s = \frac{d_B - d_A}{\frac{w_A + w_B}{2}},$$

in which d_A, d_B are the migration distances and w_A, w_B are the band widths.