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Journal of Pharmaceutical and Biomedical Analysis
32 (2003) 1055–1059
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Short communication

Enantiomeric resolution of some 2-arylpropionic acids using L-(−)-serine-impregnated silica as stationary phase by thin layer chromatography

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Received 17 May 2002; received in revised form 3 June 2002; accepted 4 June 2002

Abstract

The enantiomeric resolution of certain 2-arylpropionic acids was achieved on thin silica gel plates impregnated with optically pure L-(−)-serine as chiral selector. The mobile phase enabling successful resolution of (±)-ibuproxam and (±)-ketoprofen was acetonitrile–methanol–water (16:4:0.5, v/v/v) and (16:3:0.5, v/v/v) for (±)-tiaprofenic acid. The spots were detected with iodine vapors and the detection limits were found to be different for each of the 2-arylpropionic acid, ranging between 0.25 and 0.50 μg/ml. The effect of concentration of the impregnating chiral selector, temperature and pH on resolution has been studied. The procedure was applied successfully to resolve commercial ampoules of ketoprofen dosage formulation.
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Keywords: L-(−)-Serine; Enantiomeric resolution; Thin layer chromatography

1. Introduction

2-Arylpropionic acids are commonly prescribed non-steroidal agents with anti-inflammatory, analgesic and anti-pyretic properties used in the treatment of rheumatoid arthritis, osteoarthritis, and mild to moderate pain. Optical isomers of pharmaceutical drugs such as 2-arylpropionic acids can have differences in pharmacological activities, side effects and even toxic effects [1,2]. In this context, it is necessary to find reliable, sensitive and rapid methods for analysis and characterization of enantiomers of optically active compounds [3]. Analysis of enantiomeric purity of drugs is very important during production and storage of already existing drugs. In several countries, especially those with advanced pharmaceutical technologies and high production of drugs, the registration of a new chiral drug requires a full documentation of enantiodifferen-

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0731-7085/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0731-7085(03)00208-5

tiating procedures and detailed pharmacological activity of pure enantiomeric forms [4]. Racemic 2-arylpropionic acids have been resolved into their enantiomers either directly or indirectly using different chromatographic techniques. Resolution of enantiomers of some 2-arylpropionic acids by (−)-brucine, L-arginine as chiral selectors were achieved by Bhushan et al. [3–7]. TLC on silica gel impregnated with optically pure L-tartaric acid and L-histidine as chiral selectors were used for direct resolution of (±)-ephedrine and atropine into their enantiomers [7].

In this paper we examine the resolution of three non-steroidal anti-inflamatory agents (NSAIDs) on thin silica gel plates impregnated with optically pure L-(−)-serine.

2. Experimental

Racemic (±)-ibuproxam, (±)-ketoprofen, (±)-tiaprofenic acid (Fig. 1), and their respective S-(−)-isomers were obtained from Sigma–Aldrich Co. (St. Louis, MO, USA). Optically pure L-(−)-serine was purchased from Sigma–Aldrich Co. Ketoprofen ampoules were purchased from the local pharmacies. Silica gel G, with 13% calcium sulfate as binder having chloride, iron, and lead impurities up to 0.02% and with a pH 7.0 in a 10% aqueous suspension, was obtained from Fluka Chemika AG, (Buchs, Switzerland). The other reagents (ACS certified reagent grade) were used without further purification.

Impregnated thin layer plates (20 × 20 cm × 0.5 mm) were prepared using a Stahl type applicator (Camage Type 21603, Muttenz, Switzerland) by spreading a slurry of silica gel G (30 g) in ethanol–water (3+97 v/v, 75 ml) containing optically pure recrystallized L-(−)-serine (0.1 g). The slurry had a pH between 6 and 7. The plates were dried and activated overnight at 60 °C. The solutions of racemic 2-arylpropionic acids and their respective (−)-isomers (10^{-3} M) were prepared in 70% ethanol and were applied to the plates at a 10 μl level.

The sample solution of ketoprofen was prepared by weighting accurately a portion of the dry powder containing an equivalent of 10 mg of ketoprofen, transferring into a 100 ml calibrated flask, adding 70% ethanol to the mark and filtering after through shaking, dilute 0.5 ml of this solution to 100 ml with 70% ethanol to give a nominal concentration of 0.5 μg/ml w/v.

Cleaned, dried and paper-lined glass chambers (12 × 24 × 24 cm) were used for developing chromatograms. These were pre-equilibrated with developers for 15 min. The chromatograms were developed in solvent system acetonitrile–methanol–water (16:4:0.5, v/v/v) and (16:3:0.5, v/v/v) at 25±2 °C for 45 min. Solutions of racemic-2-arylpropionic acids and their S-forms (10^{-3} M) were applied side-by-side to the plates, with a 25-μl Hamilton syringe. Chromatograms dried at 40 °C and cooled to room temperature, and were placed in an iodine chamber.

3. Results and discussion

Several trial runs were systematically performed using different ratios of the solvent system acetonitrile–methanol–water for the plates impregnated with L-(−)-serine for the resolution of (±)-ibuproxam, (±)-ketoprofen, (±)-tiaprofenic acid, (Fig. 1). The successful solvent system were acetonitrile–methanol–water (16:4:0.5,v/v/v) for (±)-ibuproxam, (±)-ketoprofen, and (16:3:0.5,v/v/v) for (±)-tiaprofenic acid (Tables 1 and 2).

The results are average of at least five identical runs. Fig. 2 shows a schematic illustration of the chromatograms for resolution of racemic (±)-

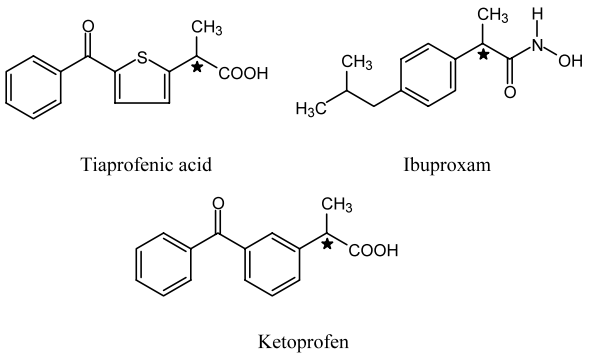

Fig. 1. The chemical structure of 2-arylpropionic acids used in this study. Asterisk denotes the position of the chiral carbon.

Table 1
hR_F ($R_F \times 100$) values of resolved (±)-arylpropionic acids on silica gel impregnated with L-(−)-serine

Sample number	2-Arylpropionic acid	hR_F values		
		Pure	Racemic mixture	
		S (−)	R (+)	S (−)
1	(±)-Ibuproxam	95	28	95
2	(±)-Ketoprofen	83	57	83
3	(±)-Tiaprofenic acid	93	53	93

Time: 45 min, for each run, solvent front, 15 cm; detection, iodine vapors; temperature, 25±2 °C; solvent system, acetonitrile–methanol–water (16:4:0.50 v/v/v), for (±)-ibuproxam and (±)-ketoprofen and (16:3:0.5 v/v/v) for (±)-tiaprofenic acid.

ibuproxam, (±)-ketoprofen and (±)-tiaprofenic acid.

Studies on effect of concentration of the impregnating reagent on (±)-ibuproxam, (±)-ketoprofen, and (±)-tiaprofenic showed that the best resolution was achieved at 0.1 g of L-(−)-serine in 30 g silica gel (0.33%). No resolution was observed for any of the racemic compounds when the proportion was decreased to 0.05 g (0.17%) or increased to 0.2 g (0.67%). Thus it seems serine is necessary to achieve enantioselectivity. Although reducing the amount of chiral selector by half would be expected to reduce or eliminate enantioselectivity, it is less clear as to why doubling it also failed to demonstrate enantioselectivity. It is possible that at the higher loading, serine was less well dispersed and impregnated into the silica particles. In order to achieve enantioselectivity interactions between the analyte and chiral selector, the process must take place within the confines of the pores of the silica gel. This indicates a steric parameter for the recognition process. The presence of excess chiral selector could give rise to interactions taking place outside of the silica pore and in the absence of the steric component, eliminate or prevent enantioselectivity.

Some alkaloids, such as (−)-quinine at 0.1% concentration, have been used as impregnating

Table 2
Effect of mobile phase system on enantiomeric resolution, sample concentration was 250 μg/ml

2-Arylpropionic compound	Mobile phase system [CH_3CN:MeOH:HO (v/v/v)]	hR_F Values			[hR_F (S)/hR_F (R)]
		Pure	Racemic mixture		
		S	R	S	
Ibuproxam	14:4:0.5	35	30	35	1.17
	16:4:0.5	95	28	95	3.39
	16:3:0.5	38	26	38	1.46
	17:3:0.5	29	25	29	1.16
Ketoprofen	14:4:0.5	37	32	37	1.16
	16:4:0.5	83	57	83	1.46
	16:3:0.5	47	43	47	1.09
	17:3:0.5	37	34	37	1.09
Tiaprofenic acid	14:4:0.5	39	35	39	1.11
	16:4:0.5	78	68	78	1.15
	16:3:0.5	93	53	93	1.76
	17:3:0.5	52	47	52	1.11

All other conditions as described previously (see Section 2).

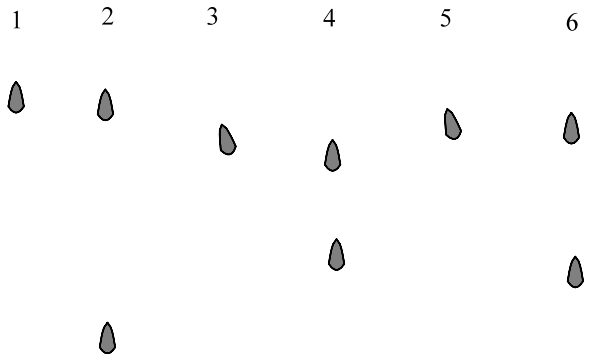

Fig. 2. Diagram of a chromatogram showing resolution of RS-arylpropionic acids. From left to right: spots 1, 3, and 5 for pure S-ibuproxam; S-ketoprofen; and S-tiaprofenic acids, respectively, and spots 2, 4, and 6, lower spots for R-isomers and upper spots for S-isomers resolved from the racemic mixtures of ibuproxam, ketoprofen and tiaprofenic acids, respectively, solvent front 15 cm; temperature, 25±2 °C; solvent system, acetonitrile–methanol–water (16:4:0.5 v/v/v) for ibuproxam and ketoprofen acid and (16:3:0.5 v/v/v) for tiaprofenic acid.

reagents for resolution of DL-amino acids [8], also (−)-brucine used as impregnating reagent for resolution of some 2-arylpropionic acids [5] and DL-amino acids [9]. They observed that the best resolution was at 0.1% of chiral selector, (−)-quinine and (−)-brucine for all racemic 2-arlypropionic acids and amino acids. The effect of pH on the resolution of (±)-ibuproxam, (±)-ketoprofen, and (±)-tiaprofenic acid was investigated in this work. The best resolution for the compounds standard was observed between pH 6 and 7 (Table 3). The pH was adjusted by addition of dilute phosphoric acid and/or dilute sodium hydroxide. The pH does play a significant role in chiral resolution and chiral discrimination [6].

According to the experimental conditions, a control experiment was performed by eluting an impregnated plate without spotting any of the test compounds when visualized there was uniform staining of the entire surface of the plate, indicating that L-(−)-serine impregnated on thin layer plates was immobilized uniformly on the stationary phase. A blank of non-impregnated stationary phase, spotted with all tested compounds was eluted, and resulted in no resolution of any compound.

Chiral interactions between the chiral selector and the analyte are known to be affected by temperature [5,10–13]. In our study the best resolution for all compounds was obtained at 25±2 °C. Increase in temperature showed a tendency for incomplete or partial separation of enantiomers, which may be explained by a swelling of the silica gel and increased in pore size leading to less than optimum steric effects; while a decrease in temperature eliminated enantioselectivity, probably due to shrinkage of the pores providing again less than optimum steric interaction.

Electrostatic interactions between the chiral selector and the racemic analytes on impregnated TLC plates, resulting in situ formation of transient diastereomers and thus resolution, has also been achieved [6,9].

Resolution in presence of water assumes the possibility of participation of some kind of hydrogen bonding. It is proposed that enantiomeric separation of 2-arylpropionic acids was due to electrostatic interactions between COO^- of the compounds and $-NH_3^+$ of serine and also hydrogen bonding [3]. The resolutions obtained for ketoprofen and tiaprofenic acid were both smaller than ibuproxam. It is possible that the ketone group between the two ring structures of ketoprofen and tiaprofenic acid interact with the terminal hydroxyl group of serine through hydrogen bonding, while the chiral centers of NSAID and serine formed the necessary transient diastereomeric pair. The formation of differential transient complexes may have been hindered within the sterically confined environment of the pore of the silica gel, resulting in relatively greater resolution for ibuproxam which does not possess the same ketone ring configuration.

The detection limits of the 2-arylpropionic acid compounds were 0.25, 0.5, 0.5 μg/ml for ibuproxam, ketoprofen and tiaprofenic acid, respectively. The method was successfully applied to commercial ampoules of ketoprofen dosage formulation, the results obtained for the resolution of ketoprofen formulation employed were similar to those of the authentic racemic ketoprofen.

The technique is versatile flexible, simple, direct and economical, and may become the method of

Table 3
Influence of temperature and pH on the enantiomeric resolution of 2-arylpropionic acids investigated on TLC impregnated with L-(−)-serine

Sample number	2-Arylpropionic compound	Mobile phase system	Temperature (°C), ±2	pH	hR_F values			$[hR_F (S)/hR_F (R)]$
					Pure	Racemic mixture		
					S	R	S	
1	Ibuproxam	16:4:0.5	15	4–5	25	25	25	1.00
			15	6–7	35	32	35	1.09
			15	8–9	18	16	18	1.13
			25	4–5	27	22	27	1.23
			25	6–7	95	28	95	3.39
			25	8–9	63	55	63	1.15
			35	4–5	22	22	22	1.00
			35	6–7	27	25	27	1.08
			35	8–9	14	14	14	1.00
2	Ketoprofen	16:4:0.5	15	4–5	29	25	29	1.16
			15	6–7	65	55	65	1.18
			15	8–9	28	24	28	1.17
			25	4–5	27	24	27	1.13
			25	6–7	83	57	83	1.46
			25	8–9	38	36	38	1.06
			35	4–5	22	20	22	1.10
			35	6–7	57	50	57	1.14
			35	8–9	19	16	19	1.19
3	Tiaprofenic acid	16:3:0.5	15	4–5	37	35	37	1.66
			15	6–7	43	36	43	1.19
			15	8–9	41	38	41	1.08
			25	4–5	66	55	66	1.20
			25	6–7	93	53	93	1.76
			25	8–9	52	48	52	1.08
			35	4–5	41	41	41	1.00
			35	6–7	54	43	54	1.26
			35	8–9	46	43	46	1.07

Mobile phase: acetonitrile–methanol–water (v/v/v), sample concentration 250 μg/ml.

choice, compared with other chromatographic techniques for fast routine enantiomeric purity analysis of these drugs.

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