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Short communication

Rapid, sensitive and direct chiral high-performance liquid chromatographic method for ketoprofen enantiomers

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Abstract

The stereospecific HPLC assays reported for ketoprofen (KT) mainly utilize indirect approaches. These assays involve the formation of amide diastereomeric derivatives, which are then separated by chromatography. The advantages of indirect methods include versatility, good sensitivity and cost effectiveness; however, lengthy preparation time is often required. Therefore, we have developed a new direct stereospecific HPLC assay for KT enantiomers to improve preparation time and sensitivity. The KT enantiomers and indomethacin, internal standard (I.S.), were resolved using a Chiralpac AD column attached to 5 cm Supelcosil LC–SI at constant temperature (30°C). The mobile phase consisted of hexane–isopropanol–trifluoroacetic acid (90:10:0.1). Under chromatographic conditions employed *R*-KT, *S*-KT and I.S. were eluted at 12, 14 and 16 min, respectively. A linear concentration response relationship was found (0.05–5.0 μg/ml of enantiomers) which covered normally observed concentrations in plasma after conventional doses of KT. The minimum quantifiable concentration of the assay was found to be 0.025 or 0.25 μg/ml based on 1 ml of human or 0.1 ml of rat plasma samples, respectively. This direct HPLC method is suitable for pharmacokinetic studies of KT enantiomers and offers the advantages of shorter sample preparation and run time. This method is at least as sensitive as assays currently in use.

Keywords: Enantiomer separation; Ketoprofen

1. Introduction

There are two general approaches for HPLC analysis of enantiomers of chiral drugs. The first method requires covalent diastereoisomer formation via addition of a coupling reagent such as ethyl-chloroformate to produce a mixed anhydride. This is followed by derivatization with an optically pure reagent such as L-leucinamide to form diasterisomers [1,2]. The second method is based on transient formation of diastereoisomers through preferential interaction of the enantiomers with a chiral stationary phase resulting in the differential elution. The advantages of derivatization include versatility, good sensitivity and cost effectiveness; however, lengthy preparation time is often required. Furthermore, although rare, the optical purity of the derivatizing reagents may influence the enantiomeric composition leading to error in the quantification of individual enantiomers [3]. The possibility of racemization during the derivatization procedure is another problem associated with this technique [4]. Some 2-arylpropionic acid derivatives, depending on the molecular structure, the type and the concentration of the coupling reagent (e.g., thionyl chloride, phenyl-ethylamine, and ethylchloroformate), and/or pH of

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the medium may undergo partial enantiomeric racemization, e.g., 0.83% for ketoprofen [4]. In contrast to precolumn derivatization, use of chiral stationary phases (direct) minimizes the possibility of racemization. In addition, minimal sample preparation as well as sufficient sensitivity can be achieved with the direct method. Disadvantages of chiral methods include initial high cost and often, lack of durability of the column. Recent technological advances, however, have substantially improved the stability of the chiral columns. The objective of this work therefore, was to develop a direct enantiospecific HPLC assay for ketoprofen enantiomers.

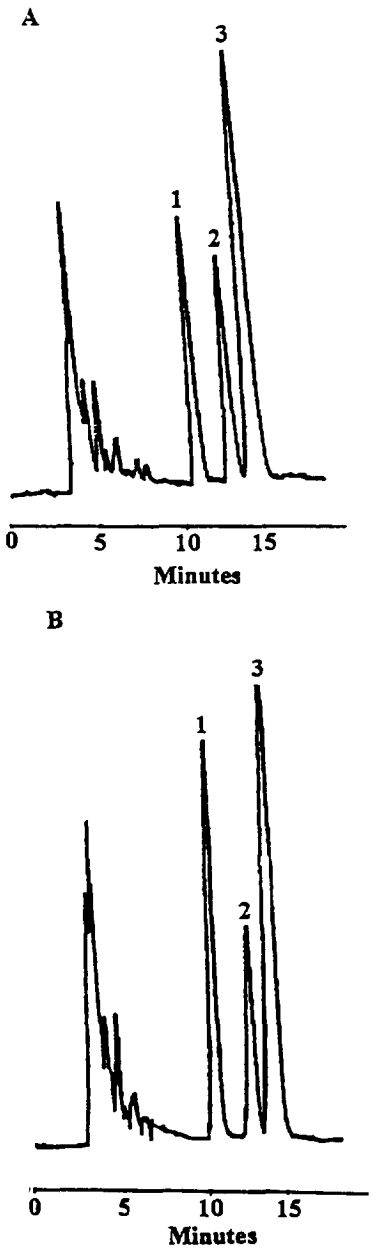

Fig. 1. HPLC chromatogram of a spiked sample of KT enantiomers (200 ng/ml racemate) in 100 μl of rat plasma (A); and a sample from rat plasma (1 h) following p.o. administration of 5 mg/kg R-KT (B). Peaks: 1=*R*-KT, 2=*S*-KT, 3=I.S.

2. Experimental

2.1. Apparatus

The organic layer was evaporated to dryness in a SCV 100H Savant Speed Vac concentrator with condensation trap (Emerson Instruments, Scarborough, Canada). The HPLC system (Waters Scientific, Mississauga, Canada) consisted of a Model M-45 pump, Model 481 UV spectrophotometer set at 254 nm, Model 712 WISP automatic sample processor and a Hewlett-Packard (Avondale, PA, USA) Model 3390 integrator–recorder. Enantiomers of KT were resolved on a Chiralpak AD (25 cm×4.6 mm I.D., Chiral Technologies, Exton, PA, USA) column attached to a 5 cm×4.6 mm I.D. Supelcosil LC–SI column (Oakville, Canada). The columns were kept at constant temperature (30°C) with a Waters column heating module and temperature controller, Model 1122/WTC-120 (Waters Scientific, Mississauga, Canada).

2.2. Chemicals

Racemic ketoprofen (KT) and indomethacin (I.S.) were purchased from Sigma (St. Louis, MO, USA). Stereochemically pure enantiomers were gifts from Sepracor Inc. (Marlborough, MA, USA). Stock solutions of racemic KT (10 mg/l) and I.S. (10 mg/l) were prepared in methanol and acetonitrile, respectively. All other solutions and reagents employed were analytical or HPLC grade.

2.3. Procedures

To 0.5 ml of human plasma were added 0.05 ml of I.S. Following acidification of the samples with 0.2

Table 1
Parameters indicating precision of the assay

Enantiomer	Coefficient of variation (%)					
	Slope		0.05 μg/ml		5.0 μg/ml	
	Intra-day	Inter-day	Intra-day	Inter-day	Intra-day	Inter-day
R	3.02	3.2	5.12	4.79	5.38	7.70
S	5.6	3.2	6.83	7.99	4.67	4.42

$n=3$ for each concentration.

ml of 0.6 *M* H_2SO_4, KT enantiomers and I.S. were extracted with 3 ml of isooctane–isopropanol (95:5) [extraction yields were 77.6% and 78.1% at 1.0 and 5 mg/l of KT ($n=3$), respectively], vortexed (30 s) and centrifuged for 5 min at 1800 *g*. The organic layer was transferred to a clean test tube and evaporated to dryness. The residue was then reconstituted in 0.2 ml of mobile phase (hexane–isopropanol–trifluoroacetic acid, 90:10:0.1) and aliquots of 0.02–0.1 ml injected onto the HPLC system with a flow of 1.0 ml/min.

3. Results and discussion

Under these chromatographic conditions *R*-KT, *S*-KT and I.S. were eluted at 12, 14 and 16 min, respectively (Fig. 1A and Fig. 1B). A linear concentration–response relationship was found (0.05–5.0 μg/ml of enantiomers with $r^2=0.998$ for both enantiomers) which covers normal plasma values (0.5 to 5 mg/l after 50 mg single oral dose) [5]. Precision of the assay is depicted in Table 1. The assay presented an acceptable precision (% errors ≤8.6 and ≤7.6 for 0.05 μg/ml, and ≤2.0 and ≤0.7 for 5.0 μg/ml of *R*- and *S*-enantiomers, respectively).

Our results indicate that this direct chiral method is a suitable assay to quantify KT enantiomers in both human and rat plasma. The minimum quantifiable concentration of the assay was found to be 0.025 or 0.25 μg/ml based on 1 ml of human or 0.1 ml of rat plasma samples, respectively. This method provides a similar sensitivity to stereospecific assays previously reported with the advantage of shorter sample preparation time, and the absence of derivatization induced stereochemical conversion [1,6]. In addition, we have analyzed over 500 plasma samples using this method indicating the durability of Chiralpak AD columns for enantiomeric resolution and quantification.

References

[1] R.T. Foster and F. Jamali, J. Chromatogr., 416 (1987) 388.
[2] E.L. Palylyk and F. Jamali, J. Chromatogr., 568 (1991) 187.
[3] A.M. Krstulovic, J. Chromatogr., 488 (1989) 53.
[4] M.R. Wright and F. Jamali, J. Chromatogr., 616 (1993) 59.
[5] F. Jamali and D.R. Brocks, Clin. Pharmacokin., 19 (1990) 197.
[6] B.C. Sallustio, A. Abas, P.J. Hayball, Y.J. Purdie and P.J. Meffin, J. Chromatogr., 374 (1986) 329.