ELSEVIER

Journal of Chromatography B, 668 (1995) 175–181

JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS

Short communication

Stereospecific high-performance liquid chromatographic assay of ketoprofen in human plasma and urine

Robert A. Carr[a], Gilles Caillé[b], Anh Ho Ngoc[c], Robert T. Foster[a,*]

[a] *Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta T6G 2N8, Canada*
[b] *Department of Pharmacology, Faculty of Medicine, University of Montreal, Montreal, Quebec H3C 3J7, Canada*
[c] *Rhône-Poulenc Rorer Canada Inc., 8580 Esplanade, Montreal, Quebec H2P 2R9, Canada*

First received 5 September 1994; revised manuscript received 3 February 1995; accepted 3 February 1995

Abstract

A high-performance liquid chromatographic (HPLC) assay suitable for the analysis of the enantiomers of ketoprofen (KT), a 2-arylpropionic acid (2-APA) non-steroidal antiinflammatory drug (NSAID), in plasma and urine was developed. Following the addition of racemic fenoprofen as internal standard (I.S.), plasma containing the KT enantiomers and I.S. was extracted by liquid–liquid extraction at an acidic pH. After evaporation of the organic layer, the drug and I.S. were reconstituted in mobile phase and injected into the HPLC system. The enantiomers were separated at ambient temperature on a commercially available 250 × 4.6 mm amylose carbamate-packed chiral column (Chiralpak AD) column with hexane–isopropyl alcohol–trifluoroacetic acid (80:19.9:0.1, v/v/v) as the mobile phase pumped at 1.0 ml/min. The enantiomers of KT were quantified by ultraviolet detection with the wavelength set at 254 nm. The assay described allows for the direct quantitation of KT enantiomers without pre-column derivatization, and is suitable for clinical studies of KT enantiomers in human plasma and urine after administration of therapeutic doses.

1. Introduction

Ketoprofen (KT, (±)-2-(3-benzoylphenyl)-propionic acid, Fig. 1), is a 2-arylpropionic acid (2-APA) non-steroidal antiinflammatory drug (NSAID), which is marketed as the racemate. The *S*(+)-enantiomer has virtually all of the desired pharmacologic activity (eutomer), whereas the *R*(−)-enantiomer is regarded as being much less active (distomer) [1]. As the disposition of the enantiomers is stereoselective in man [2,3] and in some animal models [4], the measurement of KT in biological materials must be stereospecific.

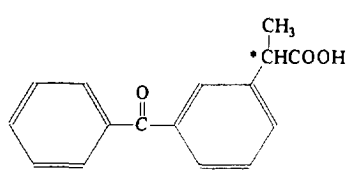

Fig. 1. Structure of ketoprofen (KT). The asterisk denotes the chiral center.

Over the past few years, there have been several analytical methods reported in the literature [5–9] which have utilized pre-column derivatization for the quantitation of KT enantiomers. These techniques have utilized gas chro-

* Corresponding author.

0378-4347/95/$09.50
SSDI 0378-4347(95)00070-4

matography (GC) [5] and high-performance liquid chromatography (HPLC) [6–9]. As these methods require pre-column derivatization of KT enantiomers using a homochiral reagent, sample preparation procedures often require more time involvement than those procedures devoid of any derivatization steps. The enantiomers of KT have also been resolved using chiral column technology which obviates the need for pre-column derivatization. These methods have reported the use of α_1-acid glycoprotein [10], ovomucoid [11], and bovine serum albumin [12] as the chiral stationary phases. Although Okamoto et al. [13] reported the use of amylose tris(3,5-dimethylphenylcarbamate) to separate KT enantiomers, their column was not commercially available and their method described qualitative, and not quantitative (i.e., not suitable for clinical analyses), separation of the enantiomers. More recently, KT enantiomers have been resolved using coupled achiral–chiral HPLC [14], although the HPLC system requirements are relatively complex.

In this paper, we report the separation of KT enantiomers using a commercially available chiral column. The method reported herein does not require any pre-treatment or achiral chromatography prior to chiral column chromatography.

2. Experimental

2.1. Chemicals

Racemic KT was a gift from Rhône-Poulenc Rorer Canada (Montreal, Canada). The internal standard (racemic fenoprofen calcium, I.S.) was obtained from Sigma (St. Louis, MO, USA). Sulfuric acid and analytical-grade 2,2,4-trimethylpentane were obtained from BDH (Toronto, Ontario, Canada). HPLC-grade hexane was obtained from Baxter Healthcare Corporation (Muskegon, MI, USA). Analytical-grade isopropanol, methanol, and HPLC-grade water were obtained from Mallinckrodt (Paris, KY, USA). Reagent-grade trifluoroacetic acid was obtained from Fisher Scientific (Fair Lawn, NJ, USA).

2.2. Chromatography

Enantiomer concentrations were determined using HPLC. The HPLC system consisted of a Model 590 pump, Model 712 Wisp autosampler, Model 486 ultraviolet detector, and Millennium computer data acquisition and integration software (Waters, Mississauga, Ontario, Canada).

The enantiomers of KT were resolved utilizing a 250 × 4.6 mm amylose carbamate-packed chiral column (Chiralpak AD, Chiral Technologies, Exton, PA, USA). The mobile phase was hexane–isopropyl alcohol–trifluoroacetic acid (80:19.9:0.1, v/v/v) pumped at a flow-rate of 1.0 ml/min. Ultraviolet detection was set at 254 nm.

2.3. Standard solutions

A 100 μg/ml stock solution of racemic KT was prepared in water–methanol (80:20, v/v). The I.S. stock solution consisted of 200 μg/ml (as base) of racemic fenoprofen in methanol. Another stock solution of racemic KT (used to determine extraction yield) was prepared as 100 μg/ml in methanol. The I.S. and KT stock solutions were stored at room temperature and 5°C, respectively.

2.4. Sample preparation of plasma

Drug-free human plasma samples (0.5 ml) were spiked with KT to give final concentrations of 50, 100, 200, 500, 1000, 2000, 5000, 7500 ng/ml of each enantiomer. To this was added 5 μg of each enantiomer of I.S., 100 μl of 0.6 *M* sulphuric acid, and 4 ml of 2,2,4-trimethylpentane–isopropanol (95:5, v/v). Samples were then vortexed for 10 s (Vortex Genie 2, Fisher Scientific, Edmonton, Alberta, Canada), and centrifuged at 1800 *g* for 5 min (Dynac II, Becton Dickinson, Parsipany, NJ, USA). The organic layer was transferred to a clean test tube, and evaporated to dryness (Savant Speed Vac concentrator-evaporator, Emerston Instruments, Scarborough, Ontario, Canada). The evaporated residue was then reconstituted with 180 μl of mobile phase, vortexed for 10 s, and an aliquot of 100 μl was injected into the HPLC.

2.5. Extraction efficiency

Utilizing methanolic stock solutions, amounts of either 100, 500, or 2500 ng of each KT enantiomer ($n = 3$) and 5 μg of each I.S. enantiomer were added to clean, dry glass tubes and evaporated to dryness. After addition of 0.5 ml drug-free plasma and 100 μl 0.6 *M* sulphuric acid to each tube, samples were extracted with 4 ml 2,2,4-trimethylpentane–isopropanol (95:5, v/v). The tubes were vortexed for 10 s and centrifuged at 1800 *g* for 5 min. An aliquot of 2 ml of the organic layer was then transferred to clean tubes, evaporated to dryness, and chromatographed. To compare these samples with those that were not extracted, another set of tubes containing the above amounts of drug were prepared and evaporated without the addition of plasma and subsequent extraction procedure. Peak areas of extracted drug versus one-half unextracted peak areas were compared under identical chromatographic conditions.

2.6. Sample preparation of urine

Applying the sample preparation technique described above for plasma samples to the analysis of urine samples proved unsuccessful as an interfering peak coeluted with the first KT peak. Therefore, urine sample preparation utilized a previously reported [6] extraction procedure. Briefly, to 0.5 ml urine containing KT enantiomers at concentrations of 200, 500, 1000, 2000, 5000, and 10,000 ng/ml was added 10 μg of each enantiomer of I.S. and 100 μl of 0.6 *M* sulphuric acid. The samples were then extracted with 4 ml 2,2,4-trimethylpentane–isopropanol (95:5, v/v). The tubes were vortexed for 10 s and centrifuged at 1800 *g* for 3 min, after which the organic phase was transferred to clean tubes and 3 ml water was added. Samples were again vortexed for 10 s and centrifuged for 3 min. The organic phase was discarded. To the remaining aqueous phase was added 200 μl of 0.6 *M* sulfuric acid and 3 ml chloroform. Samples were again vortexed for 10 s and centrifuged for 3 min. The aqueous layer was discarded and the remaining organic phase was evaporated to dryness. The resulting residue was reconstituted with 180 μl of mobile phase, vortexed for 10 s, and an aliquot of 100 μl was injected into the HPLC.

2.7. Quantitation

Weighted ($1/\times$) calibration curves for KT in plasma and urine were obtained by plotting the peak-area ratios (KT/I.S.) after extraction and analysis versus their corresponding added concentrations. For determinations in plasma, the first eluting I.S. peak was used as the I.S., whereas the second eluting peak was utilized for the I.S. in urine. Back-calculated concentrations of KT enantiomers were generated from the weighted ($1/\times$) regression curve. Results are reported as mean ± standard deviation (S.D.).

2.8. Accuracy and precision

Drug-free plasma was spiked with racemic KT at different concentrations corresponding to enantiomer concentrations ranging from 50 to 7500 ng/ml ($n = 9$). Accuracy, expressed as the percent error was measured by determining the concentration of drug measured in each sample relative to the known amount of each enantiomer added. Precision, expressed as percent coefficient of variation (%C.V.) was determined by back-calculation of concentrations from the respective calibration curves ($n = 9$). For urine, calibration curves were constructed over the enantiomer range of 200 to 10,000 ng/ml ($n = 9$).

3. Results and discussion

Separation of the enantiomers of KT was achieved using a commercially available Chiralpak AD column. Fig. 2 depicts representative chromatograms obtained from blank plasma, and a spiked plasma sample (100 ng/ml each KT enantiomer). Fig. 3 depicts representative chromatograms obtained from blank urine, and a urine sample spiked with 500 ng/ml of each KT enantiomer. The peaks corresponding to KT enantiomers eluted at approximately 7 and 8 min. The order of elution was determined using

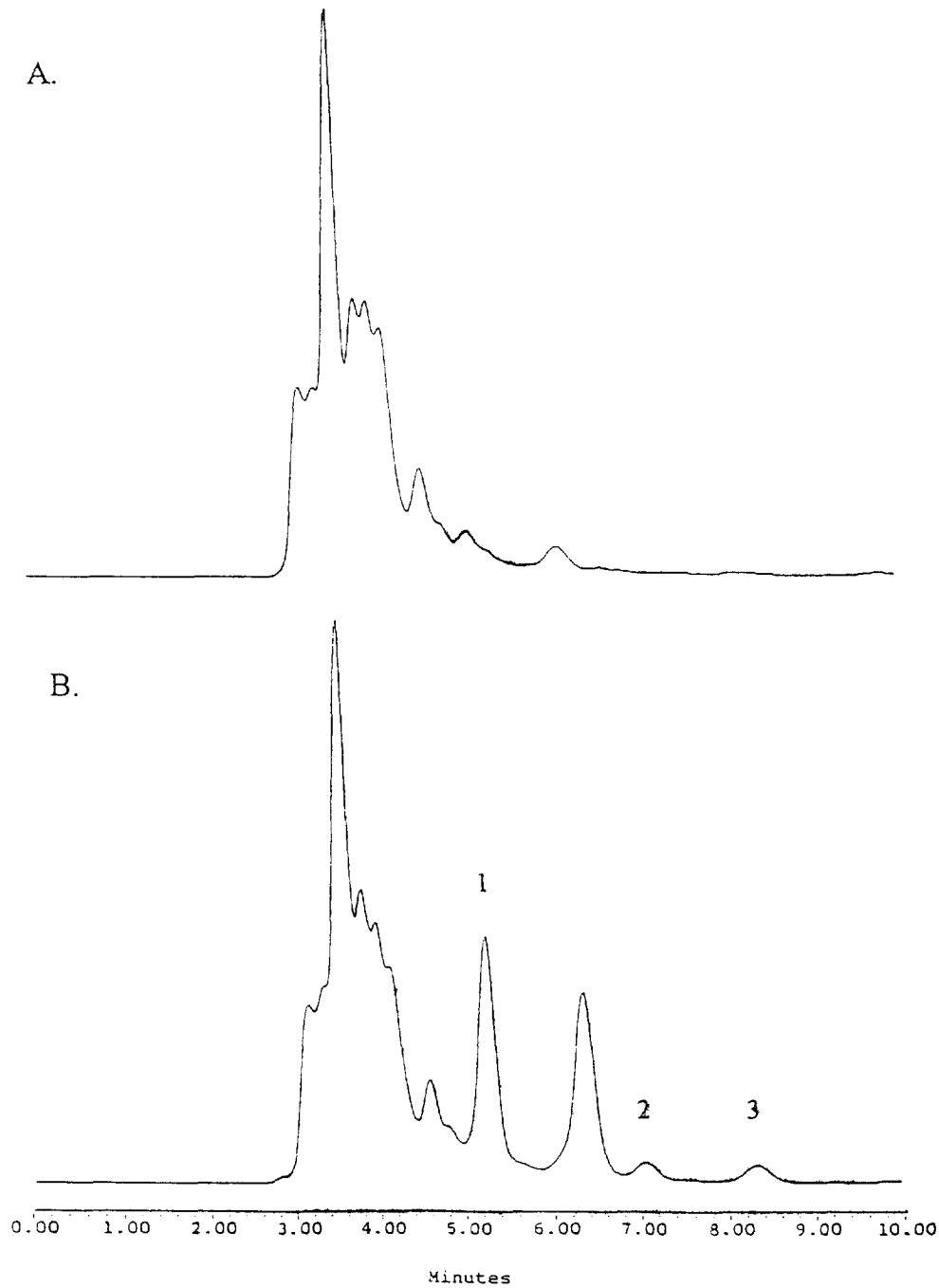

Fig. 2. Chromatograms of (A) blank plasma and (B) plasma spiked with 100 ng/ml of each KT enantiomer and 5 μg/ml of each I.S. enantiomer. Peaks: 1 = I.S.; 2 = *R*(−)-KT; 3 = *S*(+)-KT.

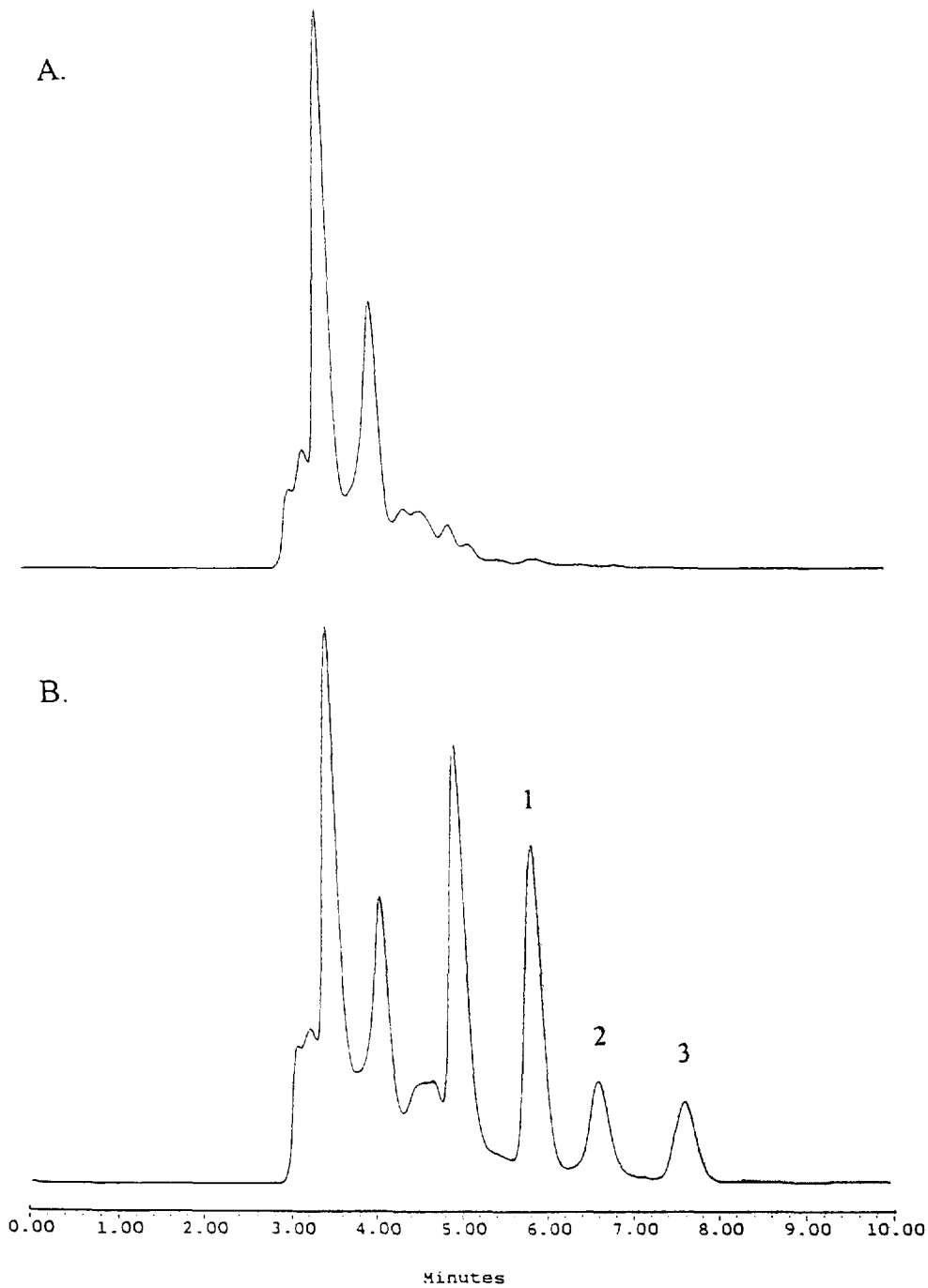

Fig. 3. Chromatograms of (A) blank urine and (B) urine spiked with 500 ng/ml of each KT enantiomer and 10 μg/ml of each I.S. enantiomer. Peaks: 1 = I.S.; 2 = *R*(−)-KT; 3 = *S*(+)-KT.

Table 1
Accuracy and precision of the method for plasma samples (mean ± S.D.)

Enantiomer concentration (ng/ml)			Accuracy (error%)		Precision (C.V.%)	
Added	Measured		*R*-KT	*S*-KT	*R*-KT	*S*-KT
	R-KT	*S*-KT				
50	50.8 ± 0.3	50.9 ± 1.8	1.7 ± 0.6	2.7 ± 2.8	0.6	3.5
100	98.1 ± 2.0	95.6 ± 5.5	2.4 ± 1.4	6.0 ± 3.0	2.0	5.8
200	196 ± 4.2	206 ± 5.4	2.3 ± 1.6	3.2 ± 2.6	2.1	2.6
500	501 ± 15	511 ± 14	2.3 ± 1.8	2.5 ± 2.1	3.0	2.7
1000	975 ± 23	976 ± 26	2.7 ± 2.0	2.6 ± 2.3	2.5	2.7
2000	2026 ± 58	2006 ± 61	2.5 ± 1.8	2.4 ± 1.7	2.9	3.0
5000	5111 ± 89	5004 ± 101	2.3 ± 1.7	1.5 ± 1.2	1.7	2.0
7500	7623 ± 195	7451 ± 177	2.6 ± 1.5	2.0 ± 1.3	2.6	2.4

an optical rotation detector (Shodex OR-1, Showa Denko) which indicated that the first and second eluting peaks corresponded to the *R*(−)- and *S*(+)-enantiomers, respectively. This elution order was the same as reported by Okamoto et al. [13] using their own amylose tris(3,5-dimethylphenylcarbamate) column. The resolution (R_s) and selectivity (α) for the peaks corresponding to the KT enantiomers were 2.35 and 1.17, respectively. The I.S. peaks eluted at approximately 5 and 6 min. The order of elution was not determined for the I.S., although the first eluting I.S. peak was consistently utilized as the I.S for plasma, whereas the second eluting peak was utilized as the I.S. for urine. Although the first eluting peak of the I.S. was used for quantitating KT enantiomer concentrations in plasma, either peaks corresponding to I.S. enantiomers would have yielded satisfactory results.

Tables 1 and 2 summarize the accuracy and precision of the method in plasma and urine, respectively. At the lowest calibration concentration of 50 ng/ml, the coefficient of variation (C.V.) was less than 4% in both plasma and urine samples. Over the entire concentration range examined, the C.V. of the method for plasma and urine never exceeded approximately 7.5%. In addition, accuracy was within approximately 6.5% of the expected value. The mean extraction yield was greater than 85% for plasma and was previously determined as approximating

Table 2
Accuracy and precision of the method for urine samples (mean ± S.D.)

Enantiomer concentration (ng/ml)			Accuracy (error%)		Precision (C.V.%)	
Added	Measured		*R*-KT	*S*-KT	*R*-KT	*S*-KT
	R-KT	*S*-KT				
200	204 ± 6	205 ± 5	2.2 ± 2.6	2.3 ± 2.5	2.9	2.4
500	487 ± 30	484 ± 26	4.6 ± 4.5	4.5 ± 4.0	6.2	5.4
1000	953 ± 62	957 ± 55	6.5 ± 3.9	5.7 ± 3.7	6.5	5.7
2000	1932 ± 143	1933 ± 137	5.6 ± 5.4	5.3 ± 5.2	7.4	7.1
5000	5278 ± 258	5272 ± 264	5.6 ± 5.2	5.5 ± 5.2	4.9	5.0
10000	10323 ± 396	10319 ± 396	3.5 ± 3.7	3.2 ± 3.6	3.8	3.8

75% for urine under the conditions stated for extraction [6]. The total time for the analysis of the KT enantiomers was within 10 min at ambient temperatures. No attempts were made to shorten the run-times by altering operating temperatures, as there was no need to speed up sample processing.

In conclusion, a sensitive, convenient stereospecific assay for KT was developed using a commercially available chiral column. The need for pre-column derivatization with a homochiral reagent was obviated, as was the need to couple achiral chromatography to chiral chromatography. The assay is valid for the determination of the enantiomers of KT with minimal sample preparation, thus enabling the use of this method for clinical studies.

Acknowledgements

This project was supported by Rhône-Poulenc Rorer Canada Inc. We would like to thank Mr. K. Tachibana for his invaluable assistance and expertise during column selection and use.

References

[1] A.J. Hutt and J. Caldwell, Clin. Pharmacokinet., 9 (1984) 371.
[2] R.T. Foster, F. Jamali, A.S. Russell and S.R. Alballa, J. Pharm. Sci., 77 (1988) 70.
[3] R.T. Foster, F. Jamali, A.S. Russell and S.R. Alballa, J. Pharm. Sci., 77 (1988) 191.
[4] R.T. Foster and F. Jamali, Drug Metab. Disp., 16 (1988) 623.
[5] N. Blazevic, M. Zinic, T. Kovac, V. Sunjic and F. Kajfez, Acta Pharm. Jugoslav., 25 (1975) 155.
[6] R.T. Foster and F. Jamali, J. Chromatogr., 416 (1987) 388.
[7] S. Bjorkman, J. Chromartogr., 339 (1985) 339.
[8] N.N. Singh, F.M. Pasutto, R.T. Coutts and F. Jamali, J. Chromatogr., 378 (1986) 125.
[9] J.H. Andrew, S. Fournel and J. Caldwell, J. Chromatogr., 378 (1986) 409.
[10] J. Hermansson and M. Eriksson, J. Liquid Chromatogr., 9 (1986) 621.
[11] T. Miwa, T. Miyakawa and M. Kayano, J. Chromatogr., 408 (1987) 216.
[12] S. Allenmark and S. Andersson, Chirality, 1 (1989) 154.
[13] Y. Okamoto, R. Aburatani, Y. Kaida, K. Hatada, N. Inotsume and M. Nakano, Chirality, 1 (1989) 239.
[14] Y. Oda, N. Asakawa, Y. Yoshida and T. Sato, J. Pharm. Biomed. Anal., 10 (1992) 81.