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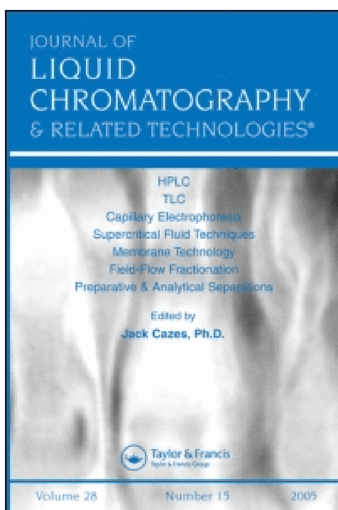

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Direct Separation and Quantitative Determination of Flurbiprofen Enantiomers in Pharmaceutical Formulations

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Direct Separation and Quantitative Determination of Flurbiprofen Enantiomers in Pharmaceutical Formulations

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ABSTRACT

A chiral liquid chromatographic (LC) method was developed and validated for the rapid quantitative determination of flurbiprofen (FLU) enantiomers (*R*-FLU and *S*-FLU) in pharmaceutical preparations. Baseline resolution of *R*- and *S*-FLU was achieved on a [(3*S*, 4*S*)-4-(3,5-dinitrobenzamido)-1,2,3,4-tetrahydro-phenentherene] coated on 5 μm silica gel column with a mobile phase of hexane–ethanol–acetic acid (950 : 50 : 2 v/v/v). The standard curve of *S*-FLU showed good linearity over the concentration range from 2.0 to 18.0 μg mL^{-1} with correlation coefficient of 0.9993. The intra-day precision (CV) of the sample, determined at two concentration levels (8.0 and 20.0 μg mL^{-1}

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of racemic FLU), was 0.16 and 0.23 for *R*-FLU and 0.14 and 0.46 for *S*-FLU. The intra-day accuracy of the sample expressed as percentage recovery was 100.1% and 100.4% for *R*- and *S*-FLU, respectively. The results showed precision, accuracy, and efficiency of the proposed method for the determination of *S*-FLU in pharmaceutical formulation.

Key Words: Enantiomeric separation; Chiral liquid chromatography; Method validation; Quality control; Flurbiprofen capsule.

INTRODUCTION

The flurbiprofen (FLU), chemically 2-(2-fluoro-4-biphenylyl)-propionic acid is a potent non-steroidal anti-inflammatory drug (NSAID). The prostaglandin synthetase inhibiting effect of FLU is attributable to the (*S*)-antipode; nevertheless, in therapeutics, it continues to be employed in the racemic form.[1–3] Therefore, the enantio-selective quantitation of *R*- and *S*-FLU in pharmaceutical formulations is a pharmacological need and is of potential clinical and pharmaceutical quality control importance. Few methods had been reported for the determination of FLU enantiomers. Most of them involved lengthy and time-consuming derivatization steps.[1,4]

Several liquid chromatographic (LC) procedures for the separation of *R*- and *S*-FLU, that make use of chiral stationary phases (CSPs), have recently been reported. Nevertheless, most of the studies are restricted to bio-analytical analysis, such as amylose *tris*-(dimethylphenylcarbamate),[5] human serum albumin coated silica,[6,7] bovine serum albumin coated silica (Resolvosil®),[8] or they endorse the separation mechanism involved, e.g., Pirkle concept Whelk-O 1®.[9,10] There are very few published reports on the quantitative determination of enantiomers in pharmaceutical preparations. Recently, Geisslinger and co-workers[11] reported a validated quantitative method for enantiomeric determination of FLU in human plasma. However, analysis time was over 15 min and the limit of quantitation was 50 ng mL^{-1}, with injection volume of 50 µL. Wilson[12] used packed column super critical fluid chromatography along with Chiralpak AD® to resolve the enantiomers of FLU.

The aim of the present study was to develop and validate an efficient enantio-selective method for quantitative determination of *R*- and *S*-FLU in order to be used in the quality control of pharmaceutical preparations.

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EXPERIMENTAL

Material

Sample, Reagents, and Solvents

The pharmaceutical sample was acquired from commercial sources. Each sustained release capsule contained 200.0 mg of racemic FLU in a granular form. The (*R*, *S*)-FLU (97%) and *S*-(+)-FLU (98%) (see Fig. 1) were acquired from Aldrich Chemical Company, Milwaukee, WI. All the solvents used were of HPLC grade, except acetic acid that was of analytical grade. Hexane, ethanol, and acetic acid were acquired from Merck (Darmstadt, Germany).

Instrumentation

A LC system consisted of a solvent delivery system, an auto injector fitted with 50 μL loop, an online degasification system, a column thermostat oven, and an UV/Vis photodiode array detector (Shimadzu Corporation, Japan), and a chiral stationary phase (*S*, *S*) Whelk-O 1® (250 × 4.6 mm i.d.) [(3*S*, 4*S*)-4-(3,5-dinitrobenzamido)-1,2,3,4-tetrahydro-phenentherene] (see Fig. 2) coated on 5 μm silica gel, 100 Å (Regis Technologies, Inc., USA), were used to perform the chiral separations.

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Methods

Analytical Conditions

Analytical conditions were standardized through the LC system using Whelk-O 1® as the CSP. The mobile phase constituted of 950 mL of hexane, 50 mL of ethanol, and 2 mL of acetic acid, with a flow rate of 0.9 mL min^{-1}. The detection was made at a wavelength of 246 nm. The volume of injection

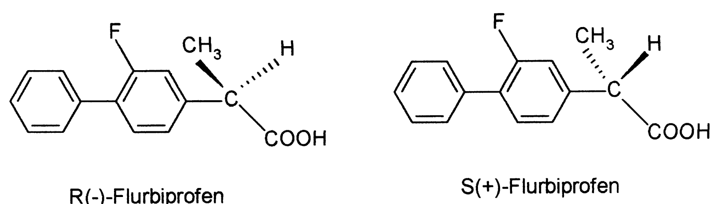

Figure 1. Structure of (*R*) and (*S*)-FLU.

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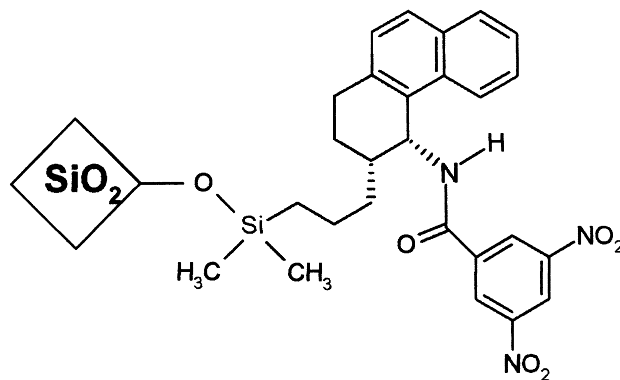

Figure 2. Structure of (*S*, *S*)-Whelk-O 1® CSP.

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was fixed at 20 μL. All analyses were done at room temperature and column temperature was controlled at 24°C ± 1. The mobile phase was prepared fresh each day, vacuum-filtered through a 0.22 μm Millipore® GVHP hydrophobic membrane. The LC system was washed for 60 min. in order to condition the system before injections were started.

Calibration Curve

The calibration curve for *S*-FLU was constructed by separate analyses, in triplicate, of nine different calibration standards of *S*-enantiomer from 2.0 to 18.0 $\mu g\,mL^{-1}$. The stock solution of *S*-FLU was prepared by weighing exactly 20.0 mg standard in a 50 mL volumetric flask. Approximately 45 mL of ethanol was added, the content of the flask was ultrasonicated for 10 min, and the volume was completed with the same solvent. Appropriate dilutions were made with mobile phase. The calibration curve was constructed by plotting mean peak area obtained vs. enantiomer concentration injected.

Preparation of Standard Solution for Determination of Intra-day Repeatability

Amount of racemic standard, equivalent to 20.0 mg was accurately weighed and transferred to a 50 mL volumetric flask. Approximately 45 mL of ethanol was added and the content of the flask was ultrasonicated for 10 min. The solution in the flask was diluted to volume with the same solvent. Appropriate dilutions were obtained by transferring aliquots of the above

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solution to volumetric flasks. The final dilution was made with mobile phase. Two final solutions were obtained containing 8.0 μg mL^{-1} and 20.0 μg mL^{-1} of racemic FLU, respectively.

Preparation of Sample Solution for Determination of Intra-day Repeatability

An amount of sample, in triturated form, equivalent to 200.0 mg of FLU was accurately weighed and transferred to a 100 mL volumetric flask. Approximately 95 mL of ethanol was added and the content of the flask was ultrasonicated for 10 min. The volume of the flask was completed with the same solvent and the final solution was filtered through Whatmann no. 1 paper filter. An aliquot of 10.0 mL was transferred to a 50 mL volumetric flask and the content was diluted to mark with mobile phase. The above solution was systematically diluted with mobile phase to obtain two final solutions containing 8.0 μg mL^{-1} and 20.0 μg mL^{-1} of racemic FLU, respectively.

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Sample Preparation for Recovery Test

Standard and sample solutions were prepared separately, as described above, to obtain racemic FLU at 40.0 μg mL^{-1}. Method accuracy was assessed by determining the agreement between the difference in the measured analyte concentrations of the fortified and unfortified sample and the known amount of analyte added to fortify the sample. Solutions of standard FLU at known concentrations were used to fortify solutions of the racemic sample.

All standard and sample solutions were filtered through 0.22 μm, Millipore® GVHP hydrophobic membrane, before injection into the system.

RESULTS AND DISCUSSION

Recent global advances in new regulatory guidelines for racemic or "pure" pharmaceuticals necessitate development of fast, sensitive, and reproducible methods for the quality control of optical antipodes present in commercial formulations.[13] The LC with CSPs has emerged as an efficient technique in the quantitative separation of optical isomers.

The objective of this work was to validate an analytical method that can be used for rapid, efficient, and routine analysis of FLU enantiomers in pharmaceutical preparations. The (*S*,*S*)-Whelk-O 1® is a "Pirkle concept"

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π-acceptor–π-donor CSP, specially developed for enantiomeric separation of NSAIDs.

The baseline separation of *R*-FLU ($k_1 = 0.77$) and *S*-FLU ($k_2 = 0.97$) enantiomers were obtained with run time of 9.4 min. The elution order was determined by injecting *S*-enantiomer separately, as shown in Fig. 3(a) and 3(b). The developed method was validated and applied for enantiomeric separation and quantitative determination of *S*-FLU in commercial samples and can be used in routine quality control.

System Suitability Test

The system suitability test was performed in order to evaluate the integrity of the proposed LC system. The test was performed by repeated analysis ($n = 10$) of sample solution (8.0 μg mL^{-1}). There was insignificant deviation in the values of relative retention (k_1 and k_2), selectivity (α), resolution (R_s), and in the number of theoretical plates (N) (Table 1).

Enantiomeric Ratio

The enantiomeric ratio of *R*- and *S*-isomer in pharmaceutical preparations is an important aspect from a therapeutic index and toxicological viewpoint. The ratio between *R*- and *S*-FLU enantiomers, in quality control samples, was determined at two concentration levels, i.e., at 8.0 and 20.0 μg mL^{-1}. The samples were prepared in a similar manner, as described for the repeatability test. The relevant details are presented in the Table 2. At each concentration level, the analyzed sample presented equal proportions of *R*- and *S*-FLU ($n = 10$).

Limit of Detection and Limit of Quantitation

The limit of detection (LD) and the limit of quantitation (LQ) were defined, based on the standard deviation and the slope of the standard curve.[14] The relevant data are presented in the Table 3. The low level of LD (4.7 pg) and LQ (20.0 pg) of *S*-FLU makes the method useful for quality control, as well as for bio-analytical applications.

Linearity

The linearity of the responses was verified at nine concentration levels, ranging from 2.0 to 18.0 μg mL^{-1} ($n = 3$). The calibration curve was con-

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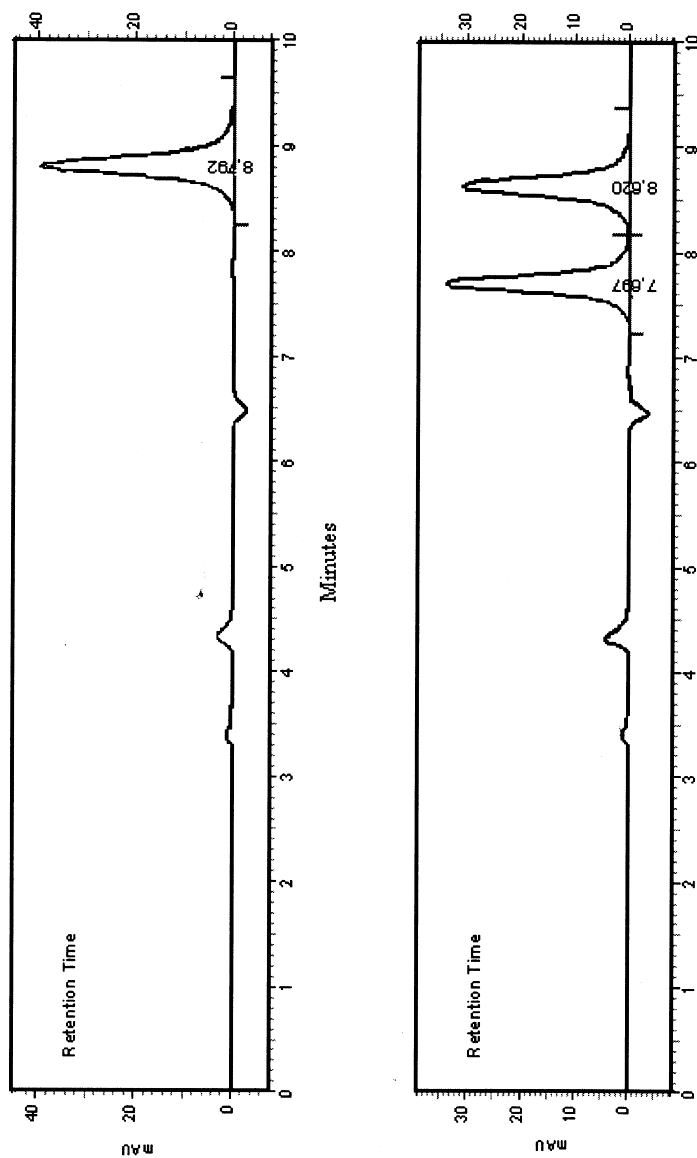

Figure 3. Enantiomeric separation of FLU in pharmaceutical formulation, (a) *S*-flurbiprofen standard (6.0 μg mL^{-1}); (b) (*R*/*S*)-FLU sample (12.0 μg mL^{-1}). Conditions: CSP Whelk-O 1®, mobile phase: hexane–ethanol–acetic acid (95 : 05 : 0.2 v/v/v), flow rate: 0.9 mL min^{-1}, UV detection: 246 nm, oven temperature 24°C ± 1, injection volume: 20 μL.

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Table 1. System suitability chromatographic data for flurbiprofen ($n = 10$).[a]

Sample	$k_1 = \frac{t_1 - t_0}{t_0}$	$k_2 = \frac{t_2 - t_0}{t_0}$	$\alpha = \frac{k_2}{k_1}$	$R_s = \frac{2(t_2 - t_1)}{w_1 + w_2}$	$N = 16\left(\frac{t_r}{w_b}\right)^2$	
A	0.77	0.97	1.26	2.54	$9683^{R\text{-FLU}}$	$8342^{S\text{-FLU}}$

[a]Determined as described in USP 24th ed.[18]

Table 2. Enantiomeric ratio of FLU isomers in commercial sample determined by proposed LC–CSP method.

Sample	Concentration level	R-isomer $(\%)^{n=10}$ (Confidence limit $\%)^{P=95\%}$	S-isomer $(\%)^{n=10}$ (Confidence limit $\%)^{P=95\%}$
A	$8.0\,\mu g\,mL^{-1}$	50.18 (105.32 ± 0.12)	49.82 (104.55 ± 0.11)
	$20.0\,\mu g\,mL^{-1}$	49.98 (104.35 ± 0.17)	50.02 (104.38 ± 0.34)

structed by plotting mean area response ($n = 3$) against concentration of S-FLU. The equation for calibration curve was represented $y = 96499x - 54820$. The correlation coefficient (r) of the plot was 0.9993, representing good linearity.

Precision

Precision of the method was studied by intra-day repeatability at two concentration levels i.e., 8.0 and $20.0\,\mu g\,mL^{-1}$. The variations were expressed

Table 3. Precision data for the analytical method through within day repetition of responses in the analysis of commercial sample.

Sample	Standard deviation	Coefficient of variation (%)[a]	Confidence limit $(\%)^{P=95\%}$	Limit of detection (pg)[a]	Limit of quantitation (pg)[a]
			$(8.0\,\mu g\,mL^{-1})$		
R-Isomer	0.17	0.16	105.32 ± 0.12	—	—
S-isomer	0.15	0.14	104.55 ± 0.11	4.7	20.0
			$(20.0\,\mu g\,mL^{-1})$		
R-Isomer	0.24	0.23	104.35 ± 0.17	—	—
S-Isomer	0.48	0.46	104.38 ± 0.34	14.9	49.7

[a]Mean of 10 determinations.

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Table 4. Accuracy data for the proposed LC–CSP method through recovery of *R*-FLU and *S*-FLU in commercial sample matrix.

Sample A	Added concentration ($\mu g\,mL^{-1}$)	Recovered concentration ± RSD ($\mu g\,mL^{-1})^{n=3}$	Recovery of standard (%)[a]
R-FLU	4.00	4.02 ± 0.1	100.5
	5.00	5.01 ± 0.1	100.2
	6.00	5.98 ± 0.1	99.7
S-FLU	4.00	4.03 ± 0.2	100.7
	5.00	5.02 ± 0.1	100.4
	6.00	6.01 ± 0.1	100.2

[a]Mean of three determinations.

in terms of coefficient of variation (%) values calculated from 10 replicate determinations of *R*- and *S*-FLU. A LC method is considered precise if the coefficient of variation is less than 2.0%.[15] The low level of CV with excellent confidence limits indicated a good repeatability (Table 3).

Accuracy

The accuracy of the method was determined by average recovery of analyte from sample matrix.[16] Accuracy of the method was determined at three concentration levels, 8.0, 9.0, and 10.0 $\mu g\,mL^{-1}$ of *R*- or *S*-FLU. The three concentrations were selected in such a way, so that, they fall within 80–120% of mean value and at the same time did not exceed the linear dynamic range of calibration curve.[14,15,17] The method shows good accuracy, as was evident from near 100% recovery of *R*-FLU, as well as *S*-FLU (Table 4).

CONCLUSION

The proposed method for enantiomeric separation and quantitative determination of FLU enantiomers in pharmaceutical formulation is efficient and sensitive. The method can be used for routine quality control of FLU enantiomers in commercial samples. The excipients of the commercial sample analyzed did not interfere in the analysis, which proved the enantio-specificity of the method. The ease of sample preparation permits fast and efficient application of the proposed method to the quantitation of *S*-FLU enantiomers, with precision and accuracy.

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