International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 3, Suppl 2, 2011

Research Article

ENANTIOMERIC RESOLUTION OF (±)-FLURBIPROFEN USING L-(−)-SERINE-IMPREGNATED SILICA AS STATIONARY PHASE BY THIN LAYER CHROMATOGRAPHY

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Received: 20 Nov 2010, Revised and Accepted: 28 Dec 2010

ABSTRACT

The enantiomeric resolution of (±)-Flurbiprofen into its enantiomers was achieved using normal phase TLC on silica gel plates impregnated with L-serine as a chiral selector. Spots were located in iodine chamber. The detection limit was 0.369 µg for d(+)isomer and 0.413 µg for l(-). The effect of concentration of chiral selector on resolution has been studied and best results were obtained followed by frequent resolution of the enantiomers using this simplest technique. The procedure was applied successfully to resolve commercial formulation of flurbiprofen.

Keywords: Flurbiprofen, chiral separation, L-(−)-serine, thin layer chromatography

INTRODUCTION

Most non-steroidal anti-inflammatory drugs(NSAIDs),such as Flurbiprofen, (±)-2-(2-Flurbiphenyl-4-yl)propionic acid, are potnet used clinically as racemate. NSAIDs are non-selective inhibitor of prostaglandin biosynthesis in humans and indicated for the acute or long-term treatment of the signs and symptoms of rheumatoid arthritis and osteoarthritis[1]. Optical isomers of pharmaceutical drugs such as 2-arylpropionic acids can have differences in pharmacological activities, side effects and toxic effects[2,3]. Hence, it is necessary to find reliable, sensitive and rapid methods for analysis and characterization of enantiomers of opticall active compounds[4].

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 require a full documentation of enantiodifferentiating procedures and detailed pharmacological activity of pure enantiomeric forms[5].

Racemic 2-arylpropionic acids have been resolved into their enantiomers either directly or indirectly using different chromatographic techniques. Most of these methods involved direct separation using chiral high-performance liquid chromatographic (HPLC) columns and pre-column derivatization with optical pure chiral reagents. The direct separation of enantiomers has been achived on various types of columns, such as α-acid glycoprotein[6,7], vancomycin[8] and cellulose[9]. However, chiral HPLC columns are expensive and used for limited chiral drugs. The chiral derivatizing agents used in the separation of flurbiprofen enantiomers included L-leucinamide[10], S-(α)-methylbenzyamine[11], S-(-)-1-phenyletylamine[12], but the derivatization procedures were time-consuming. Silica gel impregnated with optically pure L-tartaric acid and L-histidine as chiral selectors by TLC were used for direct resolution of (±)-ephedrine and atropine into their enantiomers[13]. In this paper we examine the resolution of flurbiprofen on thin silica gel plates impregnated with optically pure L-(−)-serine.

MATERIALS AND METHODS

(±)-Flurbiprofen was obtained from FDC Limited (Mumbai,India). Market formulation, ARFLUR containing Flurbiprofen 50mg (F.D.C Ltd.) was obtained commercially. L-serine was used from Loba chemie. Silica Gel G was from S.d fine chem. Pvt Ltd.(Mumbai - India). Other reagents were used of analytical grade.

The Polarimeter, model Autopol IV automatic polarimeter, was used for the measurement of optical rotation of separated isomers. Spectrophotometric measurement was made on Shimadzu 1700 double beam UV Visible spectrophotometer.

Preparation of plates

Thin-layer plates (20×10cm×0.5mm) were prepared by spreading slurry of silica gel G (30gm) in distilled water (100ml) containing optically pure L-serine (0.1gm). The slurry had a pH between 6 and 7. The plates were dried and activated at 60°c for 1 hour.

Chromatographic method

The sample solution containing racemic flurbiprofen (1mg/ml) was prepared in methanol and applied to the plates at 10µl level. Chromatograms were developed at 25±2°c temperature for 20 min in toluene:ethylacetate:formic acid (5:5:0.05v/v/v), pre-equilibrated with the solvent system for 20-25 minutes. The developed plates were dried at room temperature. The same procedure described was followed for commercial formulation analysis.

Visualization

The detection of the test samples was carried out by Iodine chamber. The chiral separation factor (α) of the two separated spots was calculated as the ratio of the higher R_f-value and the lower R_f-value for the two enantiomers. The photograph of actual chromatogram was shown in following fig 1.

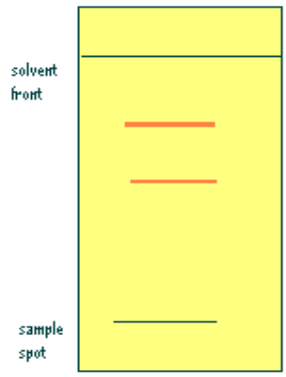

Fig. 1: Photograph of actual chromatogram

Optical rotation study of separated spots

The two separated spots were marked and scrapped off silica, dissolved in chloroform separately, centrifuge at 3000 rpm for 10 min. Supernant was collected separately, chloroform was evaporated and dry residues were collected. The dry residues of both separated enantiomers (100mg) dissolved in methanol and measure optical rotation using polarimeter.

Calibration plots of separated (+) and (-) flurbiprofen

The sample solutions were prepared by takining 10 mg of d-(+) and l-(-) flurbiprofen separately. The solutions were diluted to get final concentration of 100µg/ml of both (+) and (-) isomers of flurbiprofen. Using the stock solutions mentioned above, various aliquots were taken to get linear calibration plots for both the isomers.

RESULTS AND DISCUSSION

Several trial runs were performed using different ratios of the different solvent for the plates impregnated with L-(-)-serine for the resolution of (±)-flurbiprofen. The successful solvent system was toluene:ethylacetate:formic acid (5:5:0.05).

The effect of concentration of the impregnating reagent showed that the best resolution was achieved at 0.1gm of L-(-)-serine in 30gm silica gel (0.33%). No resolution was observed when the proportion was decreased to 0.05gm (0.17%) or increased to 0.2gm (0.67%). The lower concentration of chiral selector would be expected to reduce or eliminate enantioselectivity. It is possible that at higher concentration of chiral selector, serine was less disperesed and impregnated into the silica particles. The interaction between the analyte and chiral selector must take place within the confines of the pores of the silica gel. This indicates a stearic parameter for the recognition process. The presence of excess chiral selector could give rise to interaction taking place outside of the silica pore and in the absence of the stearic component, eliminate or prevent enantioselectivity. The best resolution of some 2-arylpropionic acids was at 0.1% of chiral selector[14].

The effect of pH on the resolution was investigated in this work. The best resolution was obsereved between pH 6 and 7. The pH was adjusted by addition of dilute phosphoric acid and/or dilute sodium hydroxide. The pH does play a significant role in chiral separation[15].

Chiral interaction between the chiral selector and the analyte are affected by temperature[14,16-19]. In this study the best resolution was obtained at 25±2°c. Increased in temperature showed 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 effect and decrease in temperature eliminate enantioselectivity due to shrinkage of the pores provide less than optimum steric interaction. The effect of mobile phase system on enantiomeric resolution was shown in Tabel 1.

Table 1: The effect of mobile phase system on enantiomeric resolution

Mobile phase system[toluene: ethylacetate:formic acid(v/v/v)]	hR_f values of separated isomers ($hR_f = R_f \times 100$)		Separation factor (α)
	1st isomer	2nd isomer	
7:3:0.1	71	77	1.08
6:4:0.1	75	82	1.09
5:5:0.1	70	82	1.15
5:5:0.05	67	81	1.21
4:6:0.05	73	81	1.10

Electostatic interaction between the chiral selector and analyte on impregnated TLC plates, resulting in formation of diastereomers and resolution has been achieves. The structure of flurbiprofen was shown in fig.2. Enantiomeric separation was due to electrostatic interaction between COO^- of the analyte and $-NH3^+$ of serine and also hydrogen bonding[4].

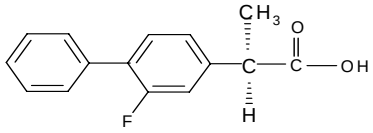

Fig. 2: structure of (±)-Flurbiprofen

The best separation was obtained using 0.33% of L-serine impregnated TLC plate in toluene:ethylacetate:formic acid solvent system at 6-7 pH and 25±2°c temperature. The hR_F ($R_F \times 100$) value for the resolved (+) isomer of flurbiprofen was 81 and (-) isomer of flurbiprofen was. The separation factor (α) was 1.21. The results were the average of five identical runs.

The specific optical rotations of two separated spots at hR_F-81and hR_F- 67were +0.27and -0.18 respectively

The calibration ranges for both the isomers were 2-14µg/ml. The recovery studies were carried out and the results were listed in table 2. The validation parameters were represented in table 3. The concentration of (+) and (-) flurbiprofen in market formulation were determined shown in table 4.

Table 2: Results of recovery study

Brand name	Isomers	Conc. of Form. (µg/ml)	Conc. of Std. added (µg/ml)	Conc. App. (µg)	Abs.	Conc. Found (µg)	% *recovery*
ARFLUR	d(+)	50	40	9	0.777	8.85	98.33%
	l(-)	50	40	9	0.699	8.92	99.11%
	d(+)	50	50	10	0.882	10.04	100.4%
	l(-)	50	50	10	0.773	9.89	98.90%
	d(+)	50	60	11	0.963	10.96	99.63%
	l(-)	50	60	11	0.861	11.02	100.18%
Mean recovery						d(+)	99.45%
						l(-)	99.39%
SD						d(+)	1.046
						l(-)	0.731

*Mean recovery and SD average of three determinations

Table 4: Results of marketed formulation analysis

Brand name	Conc./spot (µg)	Isomers	Absorbance	Conc. found (µg)	%label claim
ARFLUR	20 µg	d(+)	1.049	11.94	59.68%±0.425
		l(-)	0.648	7.97	39.83%±0.251

*Average of three determination

Table 3: validation parameters

Parameter	D(+) isomer	L(-) isomer
Wavelength	246.5 nm	246.5 nm
Range	2 -14 µg/ml	2 -14 µg/ml
Linearity	0.9991	0.9983
Intercept	0.0129	0.0024
Slope	0.0862	0.0791
Accuracy	99.45%±1.046	99.39%±0.731
Intra day precision	%RSD < 2	%RSD < 2
Inter day precision	%RSD < 2	%RSD < 2
LOD	0.369 µg/ml	0.413 µg/ml
LOQ	1.119 µg/ml	1.250 µg/ml

The method resolved d and l isomers clearly and they were found to be 60:40 (d: l) in pure as well as formulations. The techniques is versatile, flexible, simple, direct and economical compared to the other chromatographic techniques for routine enantiomeric purity analysis of flurbiprofen. TLC was found to be very simple, easy to carry out and less expensive compaired to other chromatographic methods. Finally the developed methods wassuccessfully utilized for enantiomeric resolution of Flurbiprofen in pure as well as commercial formulations.

ACKNOWLEDGEMENT

The authors would like to thank the FDC Limited (Mumbai,India) for providing gift sample of flurbiprofen.

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