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High-performance thin-layer chromatographic determination of flurbiprofen in plasma

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Abstract

A high-performance thin-layer chromatographic (HPTLC) method for the assay of flurbiprofen in plasma is reported. The drug was extracted from acidified plasma with hexane–diethyl ether (80:20). The mobile phase composition was *n*-hexane–ethyl acetate–glacial acetic acid (60:30:10). Densitometric analysis of flurbiprofen was carried out at 247 nm. The calibration curves of flurbiprofen in methanol and in plasma were linear in the range 40–400 ng. The mean values of correlation coefficient, slope and intercept were 0.995±0.003, 0.075±0.002 and 4.39±0.05 for standard curves in methanol and 0.992±0.002, 0.066±0.007 and 3.40±0.72 for standard curves in plasma, respectively. The limit of quantitation for flurbiprofen in human plasma was 40 ng, and no interference was found from endogenous compounds. The recovery of flurbiprofen from human plasma using the described extraction procedure was about 87%. The coefficient of variation for within-day and between-day analyses was 2.53% and 3.96% for 200 ng and 1.76% and 2.30% for 400 ng flurbiprofen concentration, respectively. The method was utilized to monitor plasma concentration of flurbiprofen post administration of sustained release capsules in human patient volunteers.

Keywords: Flurbiprofen

1. Introduction

Flurbiprofen, (*RS*)-2-(2-fluorobiphenyl-4-yl) propionic acid, is a non-steroidal anti-inflammatory, antipyretic and analgesic drug used in the treatment of rheumatoid arthritis, osteoarthritis, ankylosing spondylitis and mild to moderate pain [1,2]. Various analytical methods have been developed to study the absorption, metabolism and excretion of flurbiprofen in animals and man. Several GLC procedures for the quantitative determination of flurbiprofen are reported [3,4] but these require either derivatization or electron capture detection. HPLC procedures with varying sensitivities and utilizing small plasma samples have also been reported [5–15]. These methods involve procedures which are often time consuming and cumbersome.

HPTLC facilitates automatic application and scanning in situ. Several samples can be run simultaneously using a small quantity of mobile phase

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unlike HPLC and GLC. This lowers analysis time and cost per analysis. Substances are permanently stored on the plate. This makes it possible to repeat detection (scanning) of one or several fractions of the chromatogram with the same or different parameters. Hence a HPTLC procedure for determination of flurbiprofen from plasma has been developed.

This paper describes a rapid, specific, sensitive and reproducible method for measuring flurbiprofen in human plasma.

2. Experimental

2.1. Reagents and chemicals

Flurbiprofen was supplied by Boots India Ltd. and FDC Ltd., Mumbai, India. Analar grade solvents were purchased from Ranbaxy Chemicals Ltd., Delhi, India.

A stock solution of flurbiprofen (20 μg/ml) was prepared in methanol.

2.2. Instrumentation

A Remi cyclomixer was used for mixing and vortexing the samples. The samples were spotted on Camag HPTLC aluminium plates with silica gel 60F 254 (layer thickness 0.2 mm) using a Camag Linomat Model IV. The samples were streaked in the form of narrow bands of 4 mm length at a constant rate of 5 μl/s using a nitrogen aspirator. The length of the chromatogram run was 7 cm and the time required for each run was approximately 10 min. The separation was visualized by irradiation of the plates with a short wavelength (254 nm) ultraviolet lamp. Densitometric analysis of the separated components was carried out using the Camag TLC scanner II in the absorbance mode at 247 nm. Scanning speed was kept at 1 mm/s. Integration of chromatogram was performed using the Camag TLC scanner/integrator system LCI-100.

2.3. Extraction of flurbiprofen from human plasma

Aliquots of stock solution of flurbiprofen in methanol equivalent to 1–20 μg of the drug were pipetted in glass stoppered centrifuge tubes. Methanol was evaporated at 45°C under a gentle stream of nitrogen gas. Control plasma (1 ml, drug free plasma) was added to each centrifuge tube and the tubes were vortexed on a cyclomixer for 5 min. Appropriate blank was prepared simultaneously. Hydrochloric acid 3 *M* (1 ml) was added to each of the centrifuge tubes and the tubes were vortexed for 5 min. For increased recovery the compounds were extracted twice using 5 ml hexane–diethyl ether (80:20) and gentle mixing for 15 min. Between extractions, the samples were centrifuged for 15 min at 900 *g*. The combined extracts were evaporated at 45°C under a gentle stream of nitrogen gas. The sample residue was reconstituted with 500 μl methanol and 20 μl of these solutions were spotted to obtain flurbiprofen concentration in the range of 40–800 ng.

2.4. Selection of mobile phase

Various solvent systems reported in literature for TLC analysis of flurbiprofen were tried and an appropriate system consisting of *n*-hexane–ethyl acetate–glacial acetic acid (60:30:10) was selected.

2.5. Standard curve of flurbiprofen in methanol

Stock solution of flurbiprofen (20 μg/ml) was prepared in methanol. Appropriate quantities of this stock solution were spotted to obtain flurbiprofen concentration in the range of 40–800 ng.

2.6. Standard curve of flurbiprofen in plasma

Standard curve of flurbiprofen in plasma was prepared in the concentration range of 40–800 ng following the same procedure as described in Section 2.3

2.7. Recovery studies

Recovery of flurbiprofen from the sample by the isolation procedure was demonstrated by external standardization. To six of twelve sample tubes 200 ng flurbiprofen (stock solution equivalent to 200 ng flurbiprofen) was added. The contents were evaporated to dryness. To each of the twelve tubes, 1 ml of drug free plasma was added. Further processing was

done as described before in Section 2.3. The supernatant from each of the tubes one to six was poured into empty 10×75 mm glass tubes, while contents from tubes seven to twelve were poured into analogous tubes containing 200 ng flurbiprofen in methanol. The contents of all the twelve tubes were evaporated to dryness at 45°C under a gentle stream of nitrogen gas. The residue was reconstituted in 500 μl methanol, 20 μl of which was spotted and the area under the curve determined. The ratio of the mean area under the curves from tubes one to six divided by the mean of the area under the curves of tubes seven to twelve multiplied by 100 expressed the percentage recovery of flurbiprofen. A similar experiment was carried out at flurbiprofen concentration of 400 ng. Here the residue obtained after removal of the solvent was reconstituted in 1 ml of methanol.

2.8. Accuracy, precision and variation of the assay

Accuracy and precision of the assay was tested at 200 ng and 400 ng level of flurbiprofen. A comparison of the concentration of flurbiprofen in the experimental samples ($n=6$) was done with the theoretical concentration.

The within day variation of the assay was assessed by assaying six independently prepared plasma samples corresponding to concentrations of 200 ng and 400 ng hourly for a 10-h period. The between day variation of frozen aliquots of human plasma containing 200 ng and 400 ng of flurbiprofen was examined by analysis of extracted samples ($n=6$) of each concentration for six days. This study also indicated stability of flurbiprofen in plasma.

2.9. Drug administration to patient volunteers

A marketed sustained release capsule formulation of flurbiprofen was evaluated in a single dose bioavailability study in six patient volunteers suffering from mild osteoarthritis. Written consent was obtained from the patients prior to the trial which was approved by the local ethical committee of the hospital. All the subjects were in the age group 25–40 years, 55–75 kg in weight and 1.6–1.8 m in height. All subjects fasted for 12 h prior to drug administration. Each then received a 200-mg sustained release capsule of flurbiprofen. Food was withheld for an additional 2 h. Blood specimens (5 ml) were serially drawn from the cubital vein immediately before capsule administration (0 h) and at 2, 4, 8, 12 and 24 h post administration. The blood samples were collected in heparinized tubes and centrifuged immediately. Plasma was separated and stored at −20°C until analysis.

3. Results and discussion

3.1. Extraction of flurbiprofen from human plasma

The method described herein for analysis of flurbiprofen from human plasma required small amounts of plasma (1 ml). The extraction procedure developed was rapid. The methods reported in literature [3–15] employ extraction, solid-phase extraction and precipitation. Although perhaps slightly more time consuming than simple precipitation, this procedure was considerably more rapid than normal liquid–liquid extractions and more thorough than simple precipitation.

3.2. Standard curve of flurbiprofen in methanol and plasma

A series of standard curves ($n=6$) of flurbiprofen were prepared both in methanol and in plasma over a concentration range of 40–800 ng. All standard curves were linear over the range 40–400 ng. No significant difference was observed in the slopes of the standard curves in methanol (ANOVA; $p>0.05$) and in plasma (ANOVA; $p>0.05$). Beyond 400 ng, the standard curves deviated from linearity. The mean values of correlation coefficient, slope and intercept were 0.995±0.003 (S.D.), 0.075±0.002 (S.D.) and 4.39±0.05 (S.D.) for standard curves in methanol, while those in plasma showed mean values of 0.992±0.002 (S.D.), 0.066±0.007 (S.D.) and 3.40±0.72 (S.D.) respectively. The coefficient of variation for the area under the curve obtained for standard curve low points, i.e. 40 ng, were 0.84% for the standard curve in methanol and 1.52% for the standard curve in plasma. A R_F value of 0.56 was obtained using the solvent system *n*-hexane–ethyl acetate–glacial acetic acid (60:30:10). No metabo-

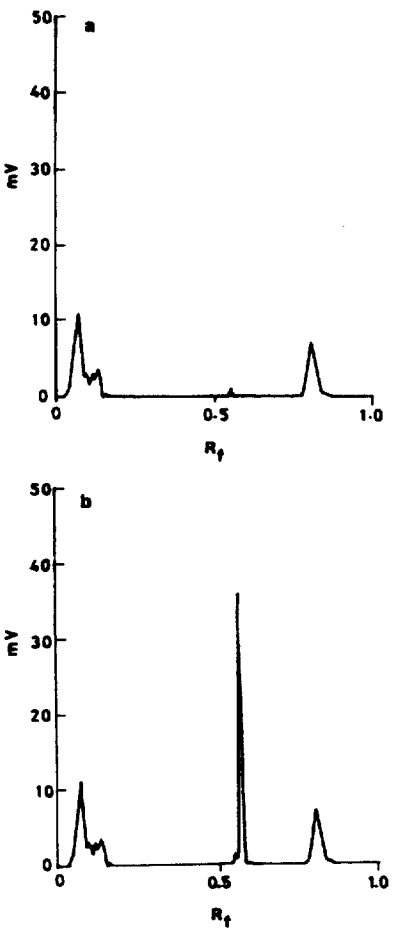

Fig. 1. Typical chromatogram of (a) blank human plasma, (b) flurbiprofen extracted from human plasma.

lites of flurbiprofen were detected in human plasma as has been earlier reported [16,17]. However there are reports of metabolites in urine [8,12,14,17–19]. Fig. 1 shows a typical chromatogram of (a) blank human plasma and (b) flurbiprofen extracted from human plasma.

The limit of detection for flurbiprofen in plasma was found to be 20 ng. Below this concentration, the spot for flurbiprofen was not clearly visible. The limit of quantitation was 40 ng. This was the lowest concentration of flurbiprofen in plasma that was accurately detected and integrated by the instrument used. The coefficient of variation was 1.52% ($n=6$). No interference from endogenous compounds in plasma was observed (Fig. 1).

3.3. Recovery studies

This study was undertaken to document the extraction efficiency of the method. The results indicated that the recovery of flurbiprofen from human plasma using the described extraction procedure was 87.86±3.72% at the 200 ng level (low flurbiprofen concentration) and 87.24±2.84% at the 400 ng level (high flurbiprofen concentration). The results also confirmed reproducibility of the method.

3.4. Accuracy and precision

The results in Table 1 revealed excellent accuracy and high precision of the assay method.

The low coefficient of variation was indicative of acceptable within-day and between-day variation of the assay. No additional spots were evident in the chromatogram, moreover the low coefficient of variation is indicative of the stability of the samples to freeze–thaw cycles.

Table 1
Accuracy, precision and variation of the assay

	Accuracy and precision		Within-day variation		Between-day variation	
	Theor. conc. (ng/spot)		Theor. conc. (ng/spot)		Theor. conc. (ng/spot)	
	200	400	200	400	200	400
Mean found±S.D. (ng/spot)	198±3	396±5	198±5	398±7	202±8	391±9
Coefficient of variation (%)	1.52	1.26	2.53	1.76	3.96	2.30

$n=6$

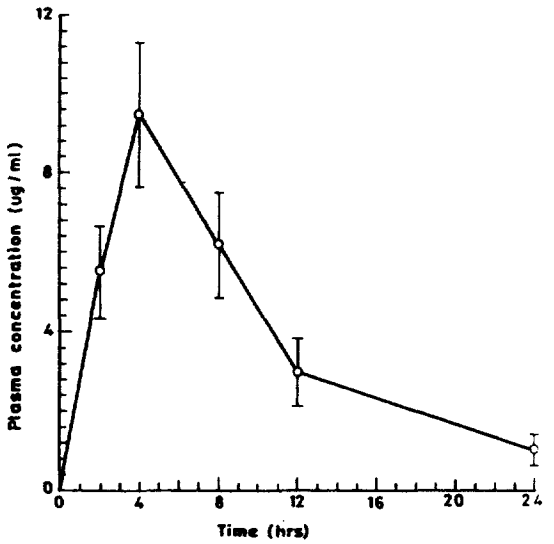

Fig. 2. Average flurbiprofen plasma levels after the administration of a single oral sustained release marketed capsule of flurbiprofen.

3.5. Plasma levels of flurbiprofen in arthritic patients

The utility of the analytical method was assessed by determining the plasma concentration of flurbiprofen following oral administration of a sustained release capsule of flurbiprofen (200 mg) in six patient volunteers suffering from mild osteoarthritis of the knees. Fig. 2 shows the mean plasma concentration–time curve. A peak mean plasma level of 9.47±1.84 μg/ml (S.D.) was observed at 4 h post administration. The mean elimination rate constant was found to be 0.110±0.02 h (S.D.). Plasma drug disappearance half life was 6.46±1.25 h (S.D.). The mean area under the plasma concentration time curve was 94.11±20.81 μg·h/ml (S.D.).

4. Conclusion

The method described herein is rapid, selective, sensitive and reproducible. It allows rapid analysis of flurbiprofen in plasma at a sensitivity which is suitable for the plasma levels of the drug reported in biopharmaceutical and therapeutic analysis.

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