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

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Abstract

A high-performance thin-layer chromatographic (HPTLC) method for quantitation of ibuprofen from plasma is described. The drug was extracted from acidified plasma with hexane–isopropanol (85:15). The mobile phase composition was *n*-hexane–ethyl acetate–anhydrous acetic acid (75:25:2). Densitometric analysis of ibuprofen was carried out at 222 nm. The calibration curves of ibuprofen in chloroform and in plasma were linear over the range 2–20 μg. The mean values of intercept, slope and correlation coefficient were 0.0422±0.0018, 1.0356±0.0213 and 0.9976±0.0013 for standard curves in chloroform and 0.1044±0.003, 0.8759±0.0213 and 0.9939±0.001 for standard curves in plasma, respectively. The limit of detection of ibuprofen from human plasma (assay sensitivity) was 50 ng and no interference was found from endogenous compounds. The recovery of ibuprofen from human plasma using the described extraction procedure was about 85%. The mean relative standard deviations for within-day and between-day analyses were 2.24 and 2.6% for 5 μg and 3.67 and 3.2% for 15 μg ibuprofen concentration, respectively. The method was utilized to monitor the plasma concentration of ibuprofen post administration of sustained release capsules in human patient volunteers.

Keywords: Ibuprofen

1. Introduction

Ibuprofen, D,L-2(4-isobutylphenyl) propionic acid, is an anti-inflammatory, antipyretic, analgesic drug widely used in the treatment of arthritis. To study the absorption metabolism and excretion of ibuprofen in animals and man, various analytical methods have been developed. Several GLC procedures for the quantitative determination of ibuprofen are reported [1–7], but majority of them require either derivatization or an electron capture detector. Numerous HPLC procedures with varying sensitivities, utilizing small plasma samples and simple procedures have also been reported [8–19]. These methods involve procedures which are often time-consuming and cumbersome.

Over the past decade, high-performance thin-layer chromatographic (HPTLC) has been successfully used in the analysis of pharmaceuticals, plant constituents and biomacromolecules [20–24]. HPTLC facilitates automatic application and scanning in situ. Moreover a large number of samples can be run simultaneously using a small quantity of solvent unlike HPLC and GLC. Hence a HPTLC procedure

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for the determination of ibuprofen from plasma has been developed.

This paper describes a rapid, simple, economical, specific and sensitive HPTLC method for measuring ibuprofen in plasma or serum. Application of this method to human plasma is described.

2. Experimental

2.1. Reagents and chemicals

Ibuprofen was supplied by Sekhsaria Chemical (Mumbai, India). Chromatographic grade solvents were purchased from Ranbaxy Chemicals (Delhi, India). All other reagents were of analytical grade and were used without further purification.

A stock solution of ibuprofen (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 TLC aluminium plates, precoated with silica gel 60F 254 (layer thickness 250 μm) using a Camag Linomat Model IV. The samples were streaked in the form of narrow bands of 4 mm width at a constant rate of 10 μl/s under a nitrogen atmosphere. The length of chromatographic run was 9 cm and the time required for each run was approximately 15 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 a Camag TLC scanner Model II 1988 in the absorbance mode at 222 nm. Scanning speed was kept at 4 mm/s. Integration of the chromatograms was performed using the Camag TLC scanner/integrator system LCI-100.

2.3. Assay procedure

2.3.1. Extraction of ibuprofen and its metabolites from blood

Aliquots of stock solution of ibuprofen in methanol (4–80 μg) were pipetted in glass stoppered centrifuge tubes. Methanol was evaporated at 45°C under a gentle steam 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 (1 *M*, 0.25 ml) was added to each of the centrifuge tubes and the tubes were vortexed for 5 min. The drug was then extracted in 5 ml hexane–isopropanol (85:15) by centrifuging at 900 *g* for 20 min. Solvent was evaporated at 45°C under a gentle stream of nitrogen gas.

The residue obtained was reconstituted with 0.1 ml chloroform and 50 μl of these solutions were spotted to obtain ibuprofen concentration in the range of 2–40 μg.

2.3.2. Selection of mobile phase

Various solvent systems reported in the literature [25–27] for TLC analysis of ibuprofen were tried and finally the system *n*-hexane–ethyl acetate–anhydrous acetic acid (75:25:2) was found to be appropriate.

2.3.3. Standard curve of ibuprofen in chloroform

Stock solution of ibuprofen (1 μg/μl) was prepared in chloroform. Appropriate quantities of this stock solution were spotted to obtain ibuprofen concentration in the range of 2–40 μg.

2.3.4. Standard curve of ibuprofen in plasma

Standard curve of ibuprofen in plasma was prepared in the concentration range 2–40 μg following the same procedure as described in Section 2.3.1.

2.3.5. Recovery and reproducibility studies

Recovery of ibuprofen from the sample by the isolation procedure was demonstrated by external standardisation. To six of twelve sample tubes 5 μg ibuprofen (stock solution equivalent to 5 μg ibuprofen) 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 above in Section 2.3.1. The supernatant from each of 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 5 μg ibuprofen in methanol. The contents

of all the twelve tubes were evaporated to dryness under a stream of nitrogen gas at 45°C. The residues were reconstituted in 100 μl chloroform, 50 μl of which were 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 ibuprofen. A similar experiment was carried out at ibuprofen concentration of 15 μg.

2.3.6. Drug administration to patient volunteers

A marketed sustained-release capsule formulation of ibuprofen was evaluated in a single-dose bioavailability study in four patient volunteers suffering form 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 25–35 years, 60–80 kg in weight and 1.62–1.80 m in height.

All subjects were fasted for 12 h prior to drug administration. Each then received a 300-mg sustained-release capsule of ibuprofen. Food was withheld for an additional 2 h. Blood specimens (5 ml) were serially drawn from the cubital vein using heparinised syringes at 0, 2, 4, 6, 8, 10 and 12 h post administration. Plasma was separated and stored at −20°C until analysis.

3. Results and discussion

3.1. Extraction of ibuprofen from human plasma

The method described herein for analysis of ibuprofen from human plasma required small amounts of plasma (1 ml). The extraction procedure developed was rapid. The methods reported in the literature [1–19] employ extraction, solid-phase extraction and precipitation. Our method combines precipitation and extraction. 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. Selection of mobile phase

Reports in the literature [28] state that a system optimized for TLC can be adapted for HPTLC by carrying out modifications in the solvent strength and selectivity. The selection of mobile phase was therefore attempted by modification of TLC systems reported for identification and purity testing of ibuprofen from bulk and pharmaceuticals. The solvent system consisting of *n*-hexane–ethyl acetate–anhydrous acetic acid (75:25:2), which gave dense and compact spots with sufficient separation in R_f values of ibuprofen and its metabolites (Fig. 1), was selected.

3.3. Standard curve of ibuprofen in chloroform and plasma

A series of standard curves (six) of ibuprofen was prepared both in chloroform and in plasma over a concentration range of 2–40 μg. All standard curves were linear over the range 2–20 μg. The mean values of intercept, slope and correlation coefficient were 0.0422±0.0018, 1.0356±0.0213 and

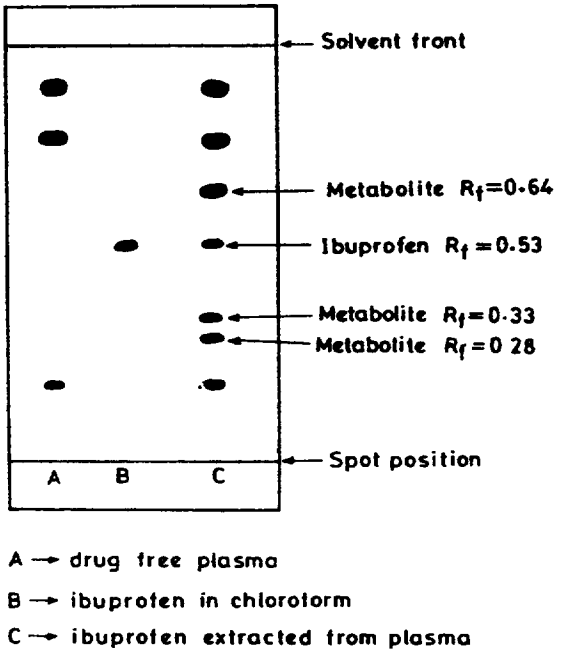

Fig. 1. Schematic representation of the TLC plate after the development of spots.

0.9976±0.0013 for standard curves in chloroform, while those in plasma showed mean values of 0.1044±0.0032, 0.8759±0.0213 and 0.9939±0.001, respectively. The intercept values were 1.98% (for standard curve in chloroform) and 5.57% (for standard curve in plasma) of the area under the curve obtained for the standard curve low points, i.e. 2 μg. The limit of reliable quantification was set at 2–20 μg as no significant difference was observed in the slopes of the standard curves in this range (ANOVA; $p>0.05$). Beyond 20 μg, the standard curves deviated from linearity.

The detection limit for ibuprofen in plasma was found to be 50 ng. This was the lowest concentration of ibuprofen in plasma that was accurately detected and integrated by the instrumentation used. The coefficient of variation was 1.33% ($n=6$) with a signal-to-noise ratio grater than 10:1. Below this concentration, the spot for ibuprofen was not clearly visible. No interference from endogenous compounds in plasma was observed.

3.4. Recovery and reproducibility studies

This study was undertaken to document the extraction efficiency of the method. Fig. 2 shows a typical chromatogram of ibuprofen extracted from plasma. The results indicated that the recovery of ibuprofen from human plasma using the described extraction procedure was 85.99±4.32% at the 5 μg level (low ibuprofen concentration) and 85.01±3.46 at the 15 μg level (high ibuprofen concentration). The results also confirmed the reproducibility of the method.

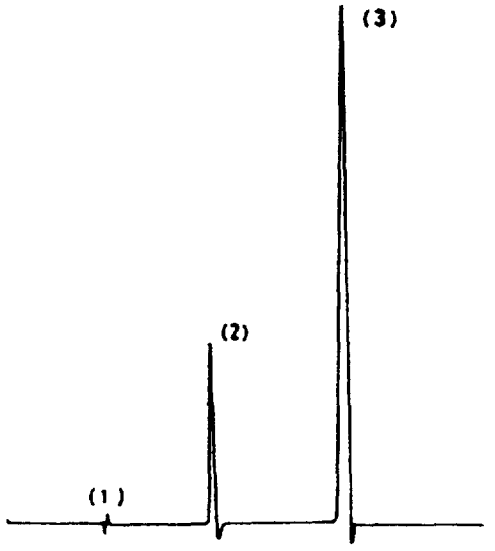

Fig. 2. Typical chromatogram of (1) normal human plasma, (2) plasma spiked with 5 μg ibuprofen and (3) 15 μg ibuprofen.

Accuracy and precision of the assay were tested at the 5 μg and 15 μg level of ibuprofen. The results shown in Table 1 revealed excellent accuracy and high precision of the assay method.

The within-day reproducibility of assay was assessed by assaying ten independently prepared plasma samples corresponding to ibuprofen concentrations of 5 μg and 15 μg hourly for a 10-h period. The stability of frozen aliquots of human plasma containing 5 and 15 μg of ibuprofen was examined by analysis of extracted samples ($n=6$) of each concentration for ten days. The results are presented in Table 2. The low coefficient of variation indicated good within-day and between-day reproducibility of assay. There was no indication of compound instability as a result of freezing and thawing over a period of ten days.

3.4.1. Plasma levels of ibuprofen in arthritic patients

The utility of the analytical method was assessed by determining the plasma concentration of ibuprofen following single oral administration of a sustained-release capsule of ibuprofen (300 mg) in four patient volunteers suffering from mild osteoarthritis of the knees. Fig. 3 shows the mean plasma concentration time curve. A peak mean plasma level (±S.D.) of 20.69 (±1.82) μg/ml was observed at 4 h post administration. The mean elimination rate constant was found to be 0.62 (±0.03) h^{-1}. Plasma drug disappearance half life was 4.20 (±0.86) h. The

Table 1
Accuracy and precision of ibuprofen HPTLC assay

Ibuprofen concentration (μg)		Difference	R.S.D.
Experimental[a]	Theoretical	(%)	(%)
5.013	5.0	0.26	1.39
14.970	15.0	0.2	1.87

[a] $n=6$.

Table 2
Within-day and between-day reproducibility of the assay

	Within-day reproducibility		Between-day reproducibility	
Theoretical concentration (μg)	5.00	15.00	5.00	15.00
Actual				
Mean (μg)	5.16	14.97	5.08	15.01
Range (μg)	4.21–5.92	13.85–16.25	4.09–5.75	13.67–17.01
C.V. (%)	4.23	6.01	5.45	6.27

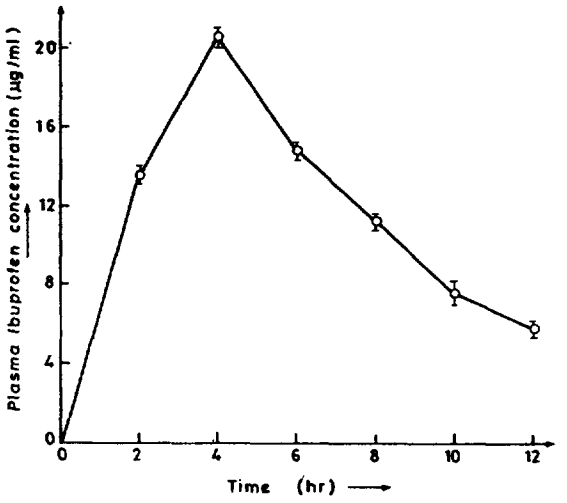

Fig. 3. Average ibuprofen plasma levels after the administration of a single oral sustained-release marketed capsule of ibuprofen.

mean area under the plasma concentration time curve was 176.64 (±25.76) μg h/ml.

4. Conclusion

The method described herein is rapid, selective, sensitive and economical. It allows rapid analysis of ibuprofen 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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