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HPTLC determination of diclofenac sodium from serum

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Abstract

Diclofenac sodium is one of the potent Non Steroidal Anti-Inflammatory Drugs (NSAID) used in the treatment of inflammatory conditions. The present work deals with the estimation of diclofenac sodium from serum by a novel High Performance Thin Layer Chromatographic (HPTLC) method developed in our laboratory. Standard diclofenac sodium was spotted on Silica Gel 60 F_{254} precoated plates, which were developed using the mobile phase toluene:acetone:glacial acetic acid (80:30:1,v/v/v). Densitometric analysis of diclofenac sodium was carried out at 280 nm with diclofenac being detected at an R_f of 0.58. The method was subsequently developed to estimate diclofenac sodium from serum. Diclofenac sodium was extracted with ethyl acetate from serum samples, spotted on Silica Gel 60 F_{254} plates and the plates were developed using the above mentioned mobile phase. The method was validated for selectivity, extraction efficiency, sensitivity, accuracy, and intra and inter-day reproducibility studies. The extraction efficiency was found to range from 76 to 80%. The Limit of Detection (LOD) and Limit of Quantification (LOQ) of diclofenac sodium in serum were found to be 90 and 120 ng, respectively. The calibration curve of diclofenac sodium in serum was found to be linear in the range of 200–800 ng. The mean values ($\pm$S.D.) of correlation coefficient, slope and intercept were found to be 0.9876 ($\pm$0.0105), 0.0228 ($\pm$0.0036) and 6.15 ($\pm$1.4), respectively. The mean percentage coefficient of variation for accuracy, intra-day and inter-day analysis at 200–800 ng of diclofenac sodium were found to be 3.2, 6.35 and 8.025, respectively. The proposed method is a simple and sensitive method with good precision and reproducibility for the estimation of diclofenac sodium form serum samples.

Keywords: Diclofenac sodium; Densitometry; Serum

1. Introduction

Diclofenac sodium, [sodium(*o*-{(2,6-dichlorophenyl)amino}phenyl)acetate] is a synthetic Non Steroidal Anti-Inflammatory Drug (NSAID) widely used in clinical medicine for the treatment of inflammatory conditions such as rheumatoid

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arthritis, osteo arthritis and ankylosing spondilytis [1,2].

Various analytical methods to estimate diclofenac from biological fluids have been reported in the literature. These include High Pressure Liquid Chromatography (HPLC) [3–12], Gas Chromatography–Mass Spectroscopy (GC-MS) [13,14], TLC [15] etc. Most of these techniques have been found to be cumbersome and cost intensive.

Amongst the various chromatographic methods presently available, HPTLC, serves as an important tool in the assay of pharmaceuticals and phytopharmaceuticals. HPTLC enables qualitative, quantitative and preparative analysis with the same system, high-speed quantitative analysis, co-chromatography and minimal sample clean up. In the recent years, HPTLC has gained importance since it allows reliable quantitation of analytes at micro and even nanogram levels[16,17].

Apart from the various chromatographic methods available for the estimation of diclofenac sodium from the biological fluids and considering the importance of the sensitivity and reliability of HPTLC in the analysis of drugs and pharmaceuticals, an attempt was made to develop a simple, sensitive, accurate and a reproducible HPTLC method for the estimation of diclofenac sodium from serum.

2. Experimental

2.1. Materials

Diclofenac sodium was obtained from Mac Laboratories Ltd, Mumbai. Its identity was confirmed by Ultraviolet and Infra red spectra. All reagents and chemicals used were of chromatographic grade and procured from local sources.

2.2. Preparation of standard solution

A stock solution of diclofenac sodium (1 mg ml^{-1}) was prepared in methanol. The stock solution was further diluted with methanol to obtain a standard solution of diclofenac sodium (10 μg ml^{-1}).

2.3. Selection of mobile phase

Various solvent systems reported in the literature [18] for the TLC analysis of diclofenac sodium were tried and finally the system toluene:acetone:glacial acetic acid (80:30:1,v/v/v) was found to be appropriate with diclofenac being detected at an R_f of 0.58. A representative chromatogram depicting the R_f of diclofenac sodium in the above mentioned solvent system is shown in Fig. 1.

2.4. Extraction of diclofenac sodium from serum

To 0.5 ml of serum diclofenac sodium was spiked in the range of 50–2000 ng. An appropriate blank was prepared simultaneously. To each tube, 0.8 ml of acetonitrile was added as a protein precipitating agent, vortexed for 30 s and centrifuged at $1800 \times g$ for 15 min. The supernatent solution (0.8 ml) was transferred to another test tube to which 0.5 ml of 0.5 M Hydrochloric acid (HCl) was added and vortexed for 30 s. The drug was extracted using ethyl acetate (5 ml × 2) by vortexing for 5 min and centrifuging at $1800 \times g$ for 15 min. The organic layer (9 ml) containing diclofenac was separated and evaporated to dryness in a LV-Evaporator at 85 °C. The residue was reconstituted in 60 μl of methanol and 40 μl was spotted on precoated Silica Gel $60F_{254}$ plates (E. Merck) with the chromatographic conditions as mentioned in 2.5 to obtain diclofenac concentration in the range of 50–2000 ng.

2.5. Chromatographic conditions

The solutions of diclofenac sodium in different concentrations were spotted on HPTLC Aluminum plates (20 × 10) precoated with Silica Gel 60 F_{254} (layer thickness 0.2 mm) (E. Merck) using Camag LinomatIV and a 100 μl Hamilton syringe. The samples were streaked in the form narrow bands of length 5 mm, 10 mm from the bottom, 10 mm from margin and 5 mm apart at a constant flow rate of 10 s μl^{-1} using a nitrogen aspirator. Camag Twin Trough Chamber (20″ × 10″) was saturated for 20 min with the mobile phase toluene:acetone:glacial acetic acid

(80:30:1,v/v/v). After chamber saturation the plates were developed to a distance of 8 cm with the development time being 20 mins. Densitometric analysis was carried out using Camag TLC Scanner II (ver. 3.14) in the absorbance mode of 280 nm. Diclofenac sodium was detected at an R_f of 0.58. The chromatograms were integrated using CATS 3 software (ver 3.17).

2.6. Method validation

The HPTLC method developed for the estimation of diclofenac sodium in serum was validated for the following parameters.

2.6.1. Selectivity

The selectivity of the assay was determined in relation to interferences from endogenous substances in the drug–free serum. The chromatogram in Fig. 2 depicts the drug free serum and serum spiked with 400 ng of diclofenac sodium.

2.6.2. Extraction efficiency

To determine the extraction efficiency, diclofenac sodium was spiked in serum at concentrations of 200, 400, 600 and 800 ng and extracted using the stated extraction procedure. The peak height of the extracted drug was compared with that of the non-extracted drug and the percent extraction efficiency calculated as follows:

$$\%\text{Extraction Efficiency} = \frac{\text{Peak height mean of extracted drug}}{\text{Peak height mean of the non-extracted drug}} \times 100$$

2.6.3. Sensitivity

The sensitivity of the assay was determined in terms of LOD, LOQ, linearity range and correlation coefficient. Diclofenac sodium was extracted from serum and spotted in the range of 50–2000 ng. The LOD was calculated as three times the noise level and the LOQ was calculated as ten times the noise level. A graph of concentration versus response (height in mm) was plotted to determine the linearity range and correlation coefficient.

2.6.4. Accuracy

It was determined by repetitive spotting of diclofenac extracted from serum, (in the concentration range of 200–800 ng) averaging the peak height and determining the percentage coefficient of variance (% CV).

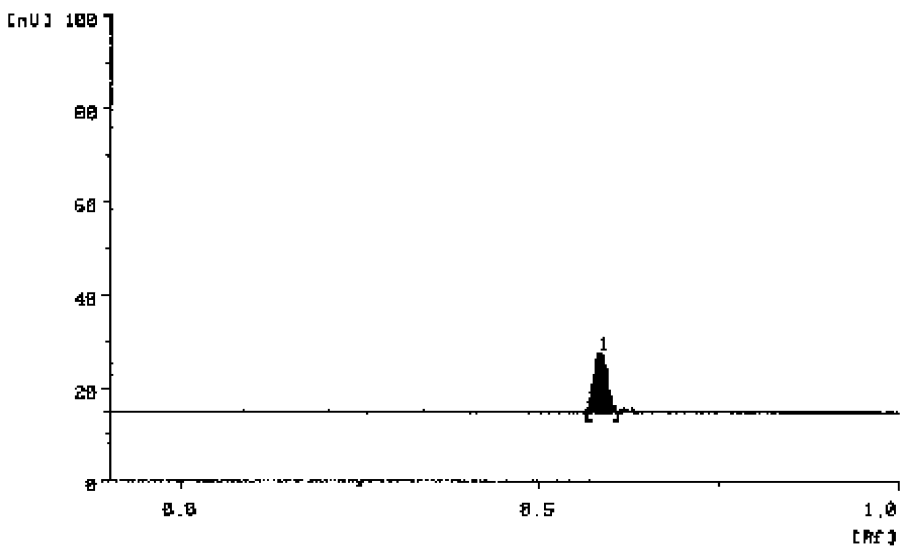

Fig. 1. A chromatogram of diclofenac sodium in the mobile phase toluene:acetone:glacial acetic acid (80:30:1, v/v/v) $R_f = 0.58$.

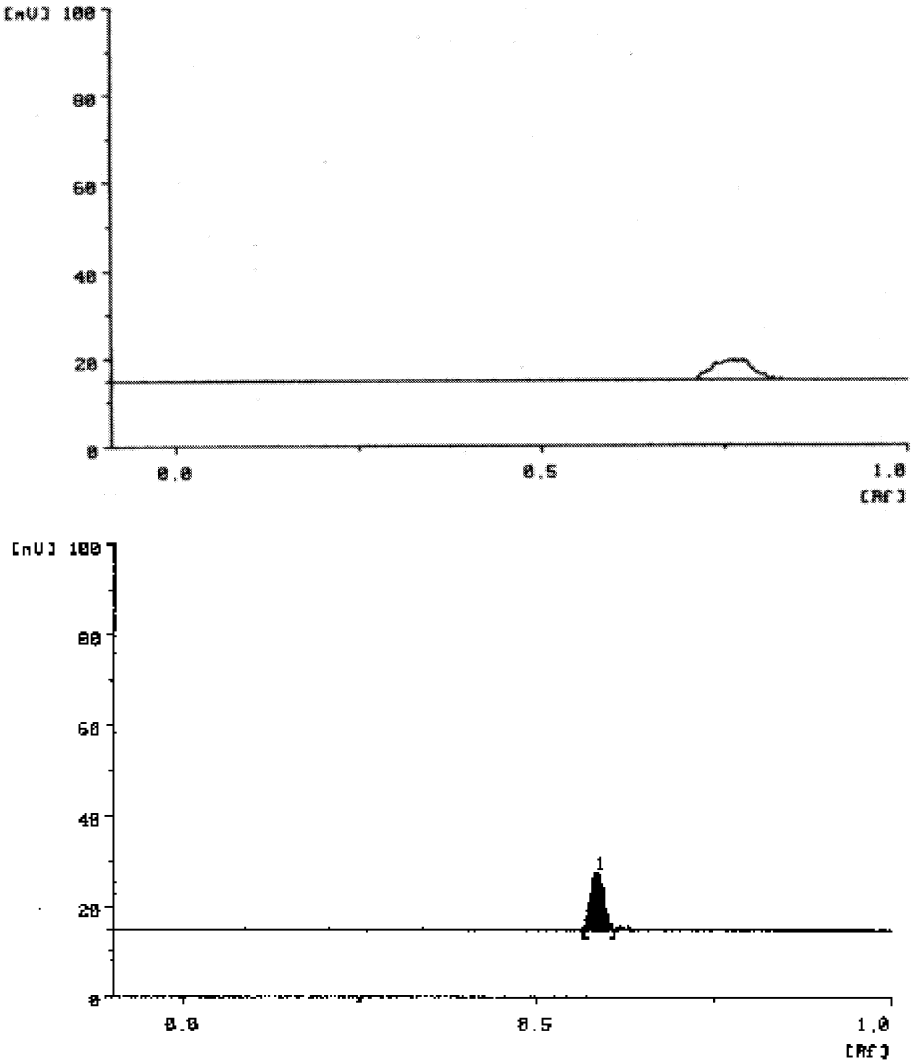

Fig. 2. Chromatograms of drug-free serum and serum spiked with 400 ng of diclofenac sodium.

2.6.5. Reproducibility studies

The intra-day reproducibility was evaluated by analyzing the samples repeatedly at concentrations of 200–800 ng of diclofenac sodium ($n = 5$).

The inter-day reproducibility was evaluated by analyzing the samples at concentrations of 200–800ng of diclofenac sodium over a period of 10 days ($n = 5$).

3. Results and discussion

3.1. Selectivity

Fig. 2 shows chromatograms of drug–free serum and serum spiked with 400 ng of diclofenac sodium. A comparison of both the chromatograms reveals that, at the R_f of diclofenac

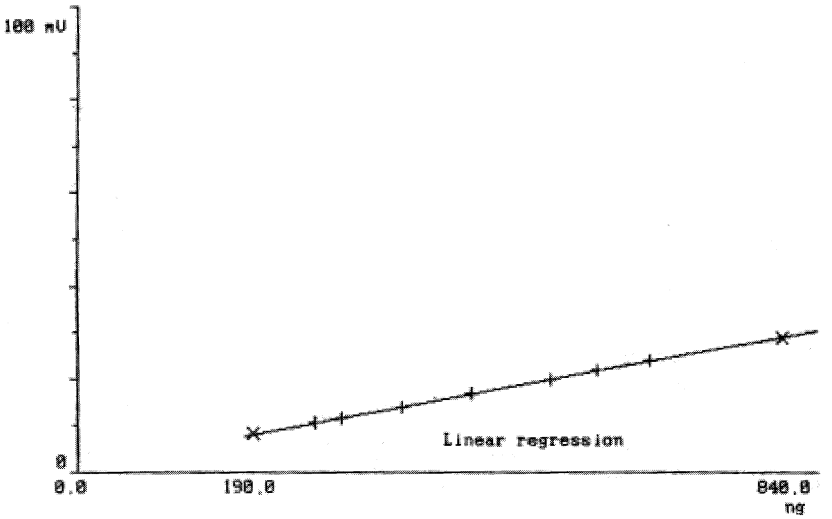

Fig. 3. Calibration curve of diclofenac sodium in serum.

sodium no interfering peaks are observed thereby confirming the selectivity of the method.

3.2. Extraction of diclofenac sodium from serum

The extraction of diclofenac sodium from serum was optimized by double extraction with 5 ml ethyl acetate. The extraction efficiency using the stated extraction procedure was found to range from 76 to 80%, averaging to 78%.

3.3. Sensitivity

After densitometric analysis of diclofenac sodium at 280 nm the lowest amount of drug which could be detected was found to be 90 ng and the lowest amount of drug which could be quantified was found to be 120 ng. The calibration curve of diclofenac sodium in serum was found to be linear in the range of 200–800 ng as shown in Fig. 3.

The mean values ($\pm$ S.D.) of correlation coefficient, slope and intercept were found to be 0.9876 ($\pm$ 0.0105), 0.0228 ($\pm$ 0.0036) and 6.15 ($\pm$ 1.4), respectively.

3.4. Accuracy

Table 1 depicts the accuracy of the quantitation of diclofenac sodium from serum. The percentage CV was found to range from 2.95 to 3.42%, averaging to 3.2%.

3.5. Reproducibility studies

Table 2 shows the intra-day and inter-day reproducibility studies of diclofenac sodium at different levels. It was observed that, the percentage CV of intra-day reproducibility and the inter-day reproducibility studies was almost comparable and within the stated limits.

4. Conclusion

As compared with the reported method [15], the above mentioned method developed in our labo-

Table 1
Recovery study of diclofenac sodium

Amount of diclofenac (ng)	% Accuracy ($\pm$ S.D.)	% CV	*n*
200	88.56 ($\pm$ 3.59)	3.25	5
400	87.74 ($\pm$ 2.79)	3.17	5
600	93.74 ($\pm$ 3.21)	3.42	5
800	92.85 ($\pm$ 2.57)	2.95	5
	Mean ($\pm$ S.D.) 90.72 $\pm$ 3.04	Mean 3.2	

Table 2
Intra-day and inter-day reproducibility studies of diclofenac sodium

Amount of diclofenac sodium (ng)	Mean height (± S.D.)	%CV	*n*
Intra–day reproducibility			
200	7.5 (± 1.5)	5.8	5
400	13.8 (± 0.9)	6.5	5
600	20.28 (± 1.39)	6.8	5
800	31.98 (± 1.12)	6.3	5
		Mean 6.35	
Inter–day reproducibility			
200	6.92 (± 1.05)	8.25	5
400	14.5 (± 1.1)	7.8	5
600	20.8 (± 1.69)	8.1	5
800	29.1 (± 1.59)	7.95	5
		Mean 8.025	

ratory is proposed to have advantages like, a higher extraction efficiency due to selective extraction of the drug from the serum, a higher sensitivity since the drug is scanned at its λ_{max} and chromatograms without any interferences due to appropriate selection of extraction solvents. The HPTLC method for the estimation of diclofenac sodium from serum developed and validated in our laboratory was found to be a simple, selective, sensitive, accurate and a reproducible method. Further, the above mentioned method being a simple method allows reliable quantitation of diclofenac sodium from serum. Furthermore the proposed method is less cumbersome and less cost intensive. This method after appropriate modifications (if required) can further be used for the estimation of diclofenac sodium in other biological fluids like plasma and urine.

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