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Stability-indicating HPTLC method for estimation of dexibuprofen in pharmaceutical dosage form

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ABSTRACT

The present work describes a stability-indicating HPTLC method for analysis of Dexibuprofen in bulk and pharmaceutical dosage form. Precoated silica gel 60 F_{254} plate was used as stationary phase. The separation was carried out using n-Hexane: ethylacetate: glacial acetic acid (7.5:2.5:0.2v/v/v) as mobile phase. The densitometric scanning was carried out at 225 nm. The Rf value for the drug was found to be 0.38. The linearity was obtained in the range 100-350ng /band with correlation coefficients (r^2 = 0.9973). The method was validated as per ICH guidelines. Dexibuprofen was subjected to forced degradation by acid, alkali, oxidation and dry heat. The degradation products were well resolved from the pure drug with significantly different Rf values.

Keywords: Dexibuprofen; HPTLC; Validation; Stability Studies.

INTRODUCTION

Dexibuprofen (DEXI), chemically know as (2S)-2-[4-(2-methylpropyl) phenyl] propanoic acid, [1,2,3]. is used therapeutically as an anti-inflammatory drug [4,5,6], which is also prescribed as anti- rheumatoid drug[7]. Some analytical methods have been reported for the analysis of Dexibuprofen in plasma like determination of Dexibuprofen in Ventricle Succus by RP-HPLC Method [8]. densitometric analysis of 2-arylpropionate derivatives in pharmaceutical preparations[9].

The present work describes a new method for determination of dexibuprofen in tablets using HPTLC-densitometry. The method is simple, requires less time for routine analysis of bulk and marketed formulation drugs.

MATERIALS AND METHODS

MATERIALS

Dexibuprofen was supplied as a gift sample by Emcure Ltd. All chemicals and reagents used were of HPLC/AR grade.

INSTRUMENTATION AND CHROMATOGRAPHIC CONDITIONS

The standard solution ranging from 100-350ng/band was applied on precoated silica gel 60 F_{254} plate in the form of bands with 100 µl sample syringe using automatic sample applicator LINOMAT V. It was developed in a twin trough glass chamber which was already saturated for 30 min. with the mobile phase. The mobile phase consisted of n-Hexane: ethyl acetate: glacial acetic acid: (7.5:2.5:0.2v/v/v). After development, plate was immediately dried with the help of dryer and was observed under UV chamber. The well resolved bands of drug were scanned at 225 nm with Camag TLC scanner III densitometer controlled by WINCAT's software version 4.

STANDARD SOLUTIONS AND CALIBRATION GRAPHS

Stock solution was prepared by dissolving 100 mg of DEXI in 100 ml methanol, from which 1 ml was further diluted to 100 ml with methanol to get stock solution of 10ng/µl. The standard solutions were applied to reach a concentration range 100-350ng/band for DEXI. The plate was developed on previously described mobile phase and well resolved band of drug were scanned at 225 nm with scanner. The peak areas were plotted against the corresponding concentrations to obtain the calibration curve.

ANALYSIS OF MARKETED FORMULATION:

Twenty tablets were weighed, finely powdered and powder equivalent to 100 mg DEXI was transferred into 100 ml volumetric flask, to this 30 ml of methanol was added and sonicated for 30 min. The volume was then made up to the mark using same solvent. The solution was filtered through Whatman paper No. 41. From the filtrate 1 ml was further diluted to 100 ml with methanol to get sample stock solution of DEXI 10 ng/µl. Sample solution were applied six times on TLC plate to give spot concentration 200 ng/band of DEXI. The plate was developed in the previously described chromatographic conditions. The peak area of the spots was measured at 225 nm and concentrations in the samples were determined using multilevel calibration.

METHOD VALIDATION

The method was validated in compliance with ICH guidelines. [10]

SPECIFICITY

The specificity of the method was ascertained by analyzing standard drug and sample. The spot for Dexibuprofen in sample was confirmed by comparing the *R*f and spectra of the spot with that of standard. The peak purity of DEXI was assessed by comparing the spectra at three different levels, i.e., peak start (*S*), peak apex (*M*) and peak end (*E*) positions of the spot.

PRECISION

The precision was determined at two levels, i.e. repeatability and intermediate precision. Repeatability was determined by six replicate applications and six times measurement of a sample solution at the

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Table 1: Standard calibration data for DEXI (n = 3)

Concentration (ng/band)	Mean Area ± SD
100	3053.50 ± 13.90
150	4618.91 ± 83.09
200	5767.31 ± 83.30
250	6989.98 ± 41.83
300	7858.77 ± 47.96
350	9336.48 ± 86.03

**Average of three determinations*

Table 2: Linear regression data for calibration curves

Detection Wavelength (nm)	225
Beer's Law Limit (ng/band)	100-350
Regression equation	y = 24.34x + 805.7
Correlation Coefficient (r^2)	0.9973
Intercept (c) ± SD	805.7 ± 35.43
Slope (m) ± SD	24.34 ± 0.33

Table 3: Results of marketed formulation analysis

Marketed formulation	Label claim (mg)	Area* of densitogram	Amt. of drug estimated (mg) ± S.D*	% Mean amount estimated*± S.D*
Brutek (Emcure Labs.)	300	5677.85	299.99 ± 0.10	99.99 ± 0.03

*Average of six determination

Table 4: Statistical evaluation of precision of developed method (n = 3)

Drug - DEXI	Repeatability*	Precision Intraday*	Interday*
Conc.(ng/band)	200	200	200
Mean area ± SD	5676.90 ± 3.07	5663.09 ± 1.78	5672.56 ± 0.45
% Content ± SD	99.99± 0.03	99.99± 0.03	99.99 ± 0.008
RSD (%)	0.05	0.03	0.18
S.E.	1.253	0.7273	0.1847

*Average of six determination

Table 5: Result from recovery studies (n = 3)

Level of recovery (%)	Amount) taken (ng/band	Amt of std added (ng/band)	Total amt recovered (ng/band)	% Recovery*	SD	S.E.	% COV
80	200	160	360.85	100.23	0.37	0.1525	0.10
100	200	200	401.07	100.26	0.32	0.1312	0.08
120	200	240	440.96	100.21	0.13	0.055	0.03

*Average of six determination

Table No.6: Results of robustness studies

A: Chromatographic Changes (% of n-hexane in mobile phase)

% change in mobile phase	Rf	Peak area
+2%	0.37	5689.00
0%	0.38	5678.70
-2%	0.39	5681.00
Mean*± S.D.	0.38 ± 0.02	5670.60 ± 7.6

*Average of six determination

B: Chromatographic Changes (chamber saturation)

Chamber saturation (Time in min.)	Rf	Peak area
33	0.41	5664.00
30	0.38	5679.00
27	0.37	5669.00
Mean*± S.D.	0.37 ± 0.02	5677.6 ± 4.04

C: Chromatographic Changes (development distance)

Development distance (mm)	Rf	Peak area
95	0.39	5682.00
90	0.38	5674.00
85	0.35	5677.30
Mean*± S.D.	0.39 ± 0.01	5671.25± 7.36

*Average of three determinations

D: Chromatographic Changes (Time from application to development)

Time from application to development	Rf	Peak area
0	0.38	5680.00
10min	0.38	5674.00
20min	0.40	5668.00
30min	0.40	5663.30
Mean*± S.D.	0.38 ± 0.01	5676.85 ± 0.10

*Average of three determinations

E: Chromatographic Changes (Time from development to scanning)

Time from development to scanning	Rf	Peak area
0	0.38	5677.0
10min	0.38	5676.8
20min	0.38	5676.8
30min	0.38	5676.8
Mean*± S.D.	0.38 ± 0.1	5676.85 ± 0.1

*Average of three determinations

Table No. 7: System suitability parameters

Parameter	DEXI
Retention time(min.)	0.38
Limit of detection (ng)	4.80
Limit of quantitation (ng)	14.55

Table 8: Results of forced degradation studies

Stress condition	Time hrs	% Assay of active substance	Mass balance (% assay + %degradation products)	R_f values of degradation products
Acid hydrolysis (0.1 M HCl)	24	21.22	100.00	0.01,0.30,0.85, 0.86,0.90
Base hydrolysis (0.1 NaOH)	24	31.70	100.01	0.01, 0.84
Oxidation (3% H_2O_2)	24	36.00	100.02	0.01,0.13,0.18, 0.44,0.85,0.92
Thermal degradation (50^{0}C)	24	28.50	100.01	0.42,0.85,0.90

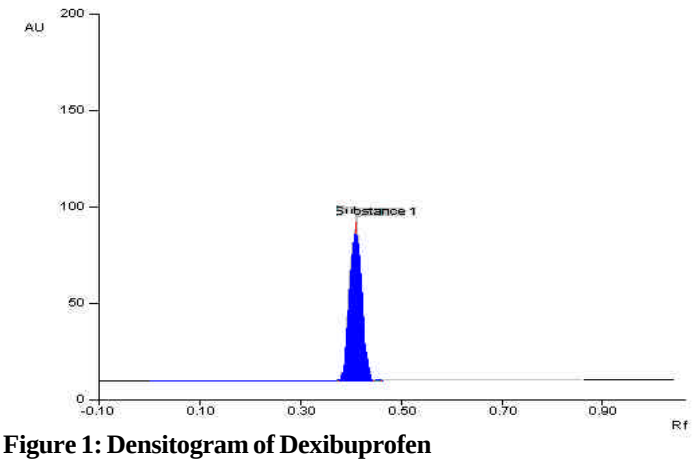

Figure 1: Densitogram of Dexibuprofen

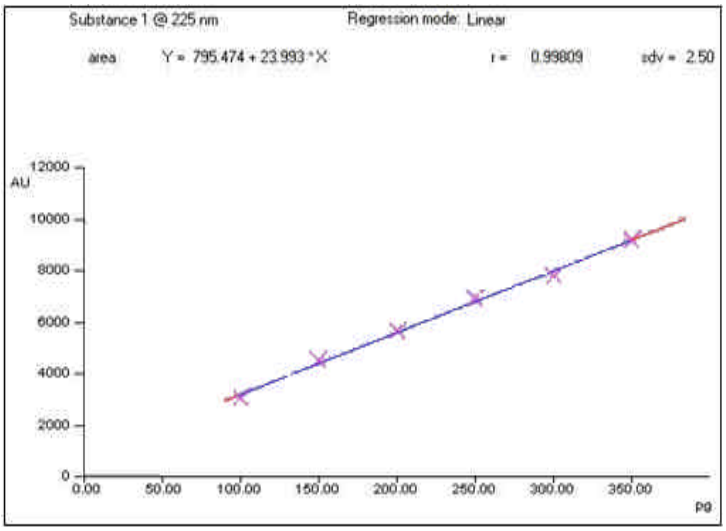

Figure 2: Calibration-curve of Dexibuprofen

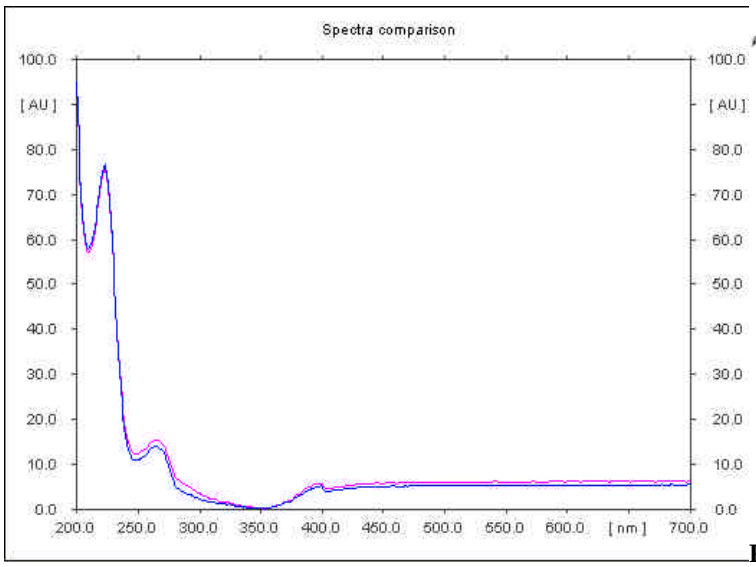

Figure 3: Spectrum of DEXI standard and sample measured from 200 to 400 nm.

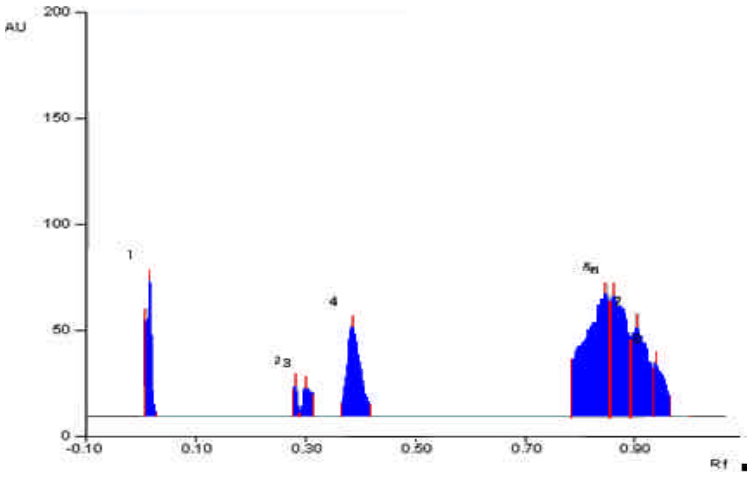

Figure4: Acid degradation

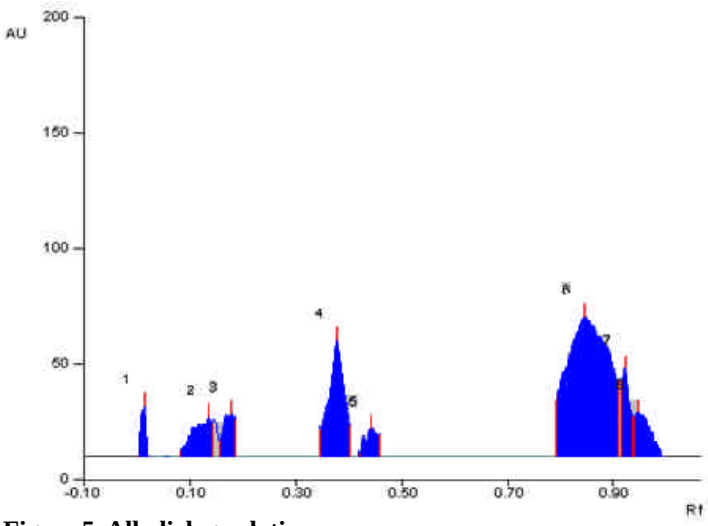

Figure 5: Alkali degradation

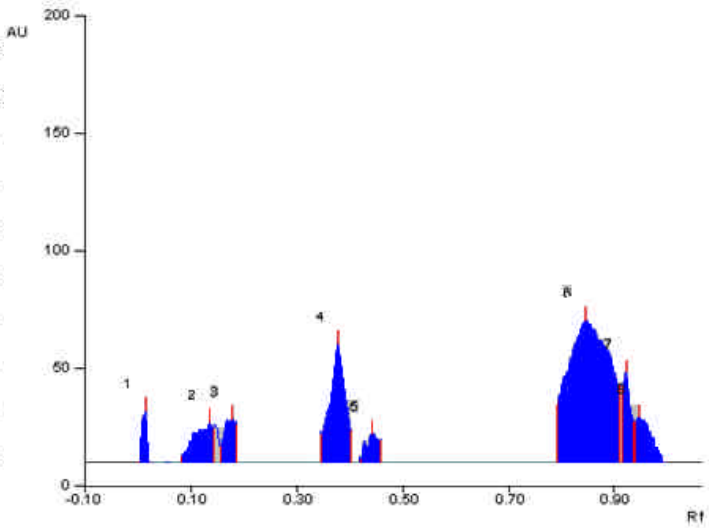

Figure 6: H_2O_2 degradation

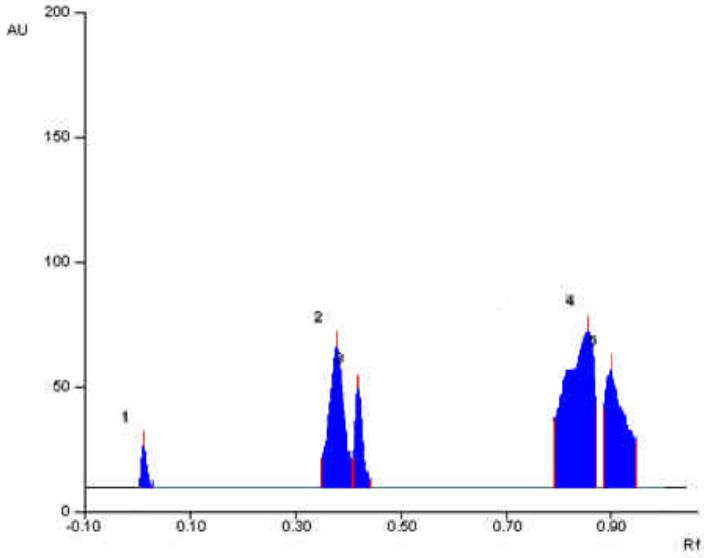

Figure 7: Heat degradation

analytical concentration. The intra and inter-day precision was determined by assay of the sample solution on the same day at different time intervals and on different days respectively.

RECOVERY STUDIES

A recovery study was carried out by standard addition method. DEXI corresponding to 80, 100 and 120% of label claim had been added to the preanalysed tablet sample solution. At each level of recovery three determinations were performed.

ROBUSTNESS

To study the robustness of the method, small but deliberate variations in mobile phase composition (± 2%), chamber saturation period (± 10%), development distance (± 10%), time from application to development (0, 10, 20, 30 min), time from development to scanning (0, 10, 20, 30 min) were carried out.

LIMIT OF DETECTION AND LIMIT OF QUANTITATION

The LOD and LOQ were separately determined based on the calibration curves. The standard deviation of the y- intercepts and slope of the regression lines were used.

FORCED DEGRADATION STUDIES:

In order to ensure that the analytical method was stability indicating, stress studies were performed.

ACID DEGRADATION STUDIES: 1 ml of 0.1N hydrochloric acid was added to 9 ml of drug solution to get the final concentration of 10µg/µl of drug. This solution was allowed to stand for 24 hrs.

ALKALI DEGRADATION STUDIES: 1 ml of 0.1N sodium hydroxide was added to 9 ml of drug solution to get the final concentration of 10µg/µl of drug. This solution was allowed to stand for 24 hrs.

OXIDATION STUDIES: 1 ml of a 3% hydrogen peroxide solution was added to 9 ml of drug solution to get the final concentration of 10µg/µl of drug. This solution was allowed to stand for 24 hrs.

TEMPERATURE STRESS STUDIES: A drug solution containing 10µg/ml of drug was exposed to 50°C for 24 hrs.

RESULT AND DISCUSSION

OPTIMIZATION OF PROCEDURES

Different proportions of n-Hexane: ethyl acetate: glacial acetic acid were tried while mobile phase selection. Ultimately n-Hexane: ethylacetate: glacial acetic acid (7.5:2.5:0.2v/v/v) was finalized as mobile phase. The spots developed were dense, compact and typical peak of DEXI was obtained as shown in fig 1. Peak was symmetrical in nature and no tailing was observed when plates were scanned at 225 nm.

LINEARITY

The analytical concentration ranges over which the drugs obeyed Beer Lambert's law was found to be 100-350ng /band. (r^2 = 0.9973). The standard calibration curve is given in fig 2 and standard calibration data for DEXI is given in Table No. 1 & 2.

ANALYSIS OF THE MARKETED FORMULATION

The spot at Rf 0.38 was observed in the densitogram of the drug samples extracted from tablets. There was no interference from the excipients commonly present in the tablets. The Dexibuprofen content was found to be close to 100% and the results are summarized in Table No 3. The low %RSD value indicated the suitability of this method for routine analysis.

PRECISION

Precision was evaluated by carrying out six independent sample preparation of a single lot of formulation. Percentage relative standard deviation (%RSD) was found to be less than 2% for within a day and day to day variations, which proves that method is precise. Results are shown in Table No 4.

RECOVERY STUDIES

To check the degree of accuracy of the method, recovery studies were performed in triplicate by standard addition method at 80%, 100% and 120%. Known amounts of standard DEXI was added to pre-analyzed samples and were subjected to the proposed HPTLC method. Results of recovery studies are shown in Table No 5.

ROBUSTNESS

The robustness of the method with determined by variations in mobile phase composition (± 2%), chamber saturation period (± 10%), development distance (± 10%), time from application to development (0, 10, 20, 30 min), time from development to scanning (0, 10, 20, 30 min). One factor at a time was changed at a concentration level of 10 µg/band of DEXI, to study the effect on the peak area of the drugs. The method was found to be unaffected by small changes with % RSD for all the parameters less than 2% indicating that method is robust.

STABILITY-INDICATING PROPERTY

HPTLC studies of the samples obtained during the stress testing of DEXI under different conditions using n-Hexane: ethylacetate: glacial acetic acid (7.5:2.5:0.2v/v/v) as the mobile phase shows different degradation peaks as shown in figures3-6. The amount of drug recovered after degradation studies and the Rf of degradation products are given in Table No 7.

ACID-INDUCED DEGRADATION

The drug was degraded in acidic condition and shows different degradation products at Rf 0.01, 0.30, 0.85, 0.86, 0.90, as shown in Figure 4.

BASE-INDUCED DEGRADATION

The drug was degraded in alkaline condition and shows different degradation products at Rf 0.01, 0.84 as shown in Figure 5.

HYDROGEN PEROXIDE-INDUCED DEGRADATION

The drug was degraded in hydrogen peroxide (3%) at room temperature and shows different degradation products at Rf 0.01, 0.13, 0.18, 0.44, 0.85, 0.92 as shown in Figure 6.

HEAT DEGRADATION

The drug when subjected to heat was degraded and degradation products appeared at Rf 0.42, 0.85, 0.90 as shown in Figure 7.

CONCLUSION

The proposed HPTLC method was validated as per ICH guidelines. The standard deviation, %RSD and standard error calculated for the method are low, indicating high degree of precision of the methods. The results of the recovery studies performed show the high degree of accuracy of the proposed methods. The results of the stress studies indicated the specificity of the method. Hence, it can be concluded that the developed HPTLC method is accurate, precise, selective and can be employed successfully for the estimation of Dexibuprofen in tablet formulation .

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