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HPTLC Method for Simultaneous Estimation of Nimesulide and Diclofenac Sodium in Capsule

SANJAY G. WALODE*, AMIT N. MALIYE, KRISHNA R. GUPTA, AVINASH V. KASTURE, SUDHIR G. WADODKAR and MADHUKAR R. TAJNE

Department of Pharmaceutical Sciences, Nagpur University Campus Amravati Road, Nagpur-440033, India

Tel: (91)(712)2500324; E-mail: sanjuwalode@rediffmail.com

A simple, rapid, sensitive high performance thin layer chromatographic (HPTLC) method has been developed and validated for simultaneous estimation of nimesulide and diclofenac sodium in capsule. It was performed on TLC plate precoated with silica gel 60 GF_{254} as a stationary phase using mobile phase comprising of *n*-hexane:ethylacetate:chloroform:glacial acetic acid (8 : 1 : 1.5 : 0.5) and the detection was carried out in absorbance/-reflectance mode at 256 nm showing R_f value 0.3 for nimesulide and 0.57 for diclofenac sodium. The per cent estimations of labelled claims of nimesulide and diclofenac sodium from marketed tablet were found to be 99.92 ± 0.2779, 100.65 ± 0.9630 by height and 99.93 ± 0.4562, 99.27 ± 0.5974 by area respectively. The method was validated in terms of accuracy, precision, specificity and ruggedness. Linearity was observed between 1.0–3.2 μg for nimesulide and 0.5–1.6 μg for diclofenac sodium. The recoveries of drugs by standard addition method were found in the range 99.14 and 100.99 for both the drugs. The proposed method is precise and accurate and can be used for routine analysis of nimesulide and diclofenac sodium in capsule formulation.

Key Words: Nimesulide, Diclofenac sodium, HPTLC, Validation.

INTRODUCTION

Nimesulide (NIM) and diclofenac sodium (DIC-Na) are non-steroidal anti-inflammatory drugs. Chemically nimesulide is N-(4-nitro-2-phenoxyphenyl) methane sulphonamide and official in B.P.[1] whereas diclofenac sodium is sodium [*o*-[(2,6-dichlorophenyl) amino] phenyl] acetate and official in B.P.[1] Literature suvey has revealed a few spectrophotometric[2–4] and HPLC[5–7] methods reported for estimation of NIM in combination with other drugs. The survey also revealed various spectroscopic[8–11] and HPLC[12–16] methods reported for determination of DIC-Na in combination with other drugs. However, spectrophotometric[17] and RP-HPLC[18] methods are also reported for simultaneous estimation of NIM and DIC-Na in combined dosage form.

The objective of the present work was to develop a sensitive and reproducible HPTLC method for the estimation of NIM and DIC-Na in fixed dose combination formulation.

EXPERIMENTAL

All chemicals and reagents used were of AR/HPLC grade. Silica gel 60 GF_{254} precoated aluminium plates with thickness 200 µm, E-Merck, Germany were used as stationary phase.

The instrument used was CAMAG-HPTLC system comprising of CAMAG LINOMAT IV automatic sample applicator, CAMAG TLC SCANNER III with CATS 4 software, CAMAG-UV cabinet and CAMAG twin trough glass chamber with stainless steel lids. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 190 and 400 nm.

Standard solution: Standard solutions of NIM (0.5 mg/mL) and DIC-Na (0.25 mg/mL) were prepared in methanol.

Mixed standard solution: Solution containing NIM (0.5 mg/mL) and DIC-Na (0.25 mg/ML) was prepared in methanol.

Experimental Chromatographic Conditions

Optimized standard experimental conditions were as follows:

Stationary phase: silica gel 60 GF_{254} TLC precoated aluminium foiled plates with thickness 200 µm. Mobile phase: *n*-hexane : ethyl acetate : chloroform : glacial acetic acid in the ratio of 8 : 1 : 1.5 : 0.5 Chamber saturation time: 10 min. Sample application: At a constant rate of 0.16 µL/s and scanning speed 10 min/s with 6 mm band. Separation technique: Ascending. Temperature: 20 ± 5°C. Relative humidity: 50–60%. Migration distance: *ca.* 70 mm. Scanning mode: Absorbance/reflectance. Slit dimension: 5 × 0.45 mm. Detection wavelength: 256 nm. The detection wavelength was selected from *in situ* overlain spectra of the drugs.

Calibration Curve Response

NIM and DIC-Na solutions ranging from 1.0–3.2 µg (0.5 µg/µL) and 0.5–1.6 µg (0.25 µg/µL) were applied on TLC plates by microlitre syringe with the help of automatic sample applicator. The plates were developed, dried and densitometrically scanned at 256 nm. Peak height and area were recorded for each concentration of drugs and curves (concentration *vs.* peak height/area) were constructed.

System Suitability Test

The system suitability test was performed by repeated application, each 4.0 µL of mixed standard solution and development of chromatograph. The mean value, standard deviation and coefficient of variance were calculated for peak height and peak area.

Standard Laboratory Mixtures

Different laboratory mixtures were prepared in the same manner as that of a standard solution to get the final concentration of about 0.5 mg/mL of NIM and

about 0.25 mg/mL of DIC-Na. 4.0 µL each of mixed standard solution (duplicate) and laboratory mixture (quadruplet) were applied on the TLC plates in the form of 6.0 mm band. The plates were then developed in presaturated twin trough chamber with mobile phase. After development the plates were dried with the help of hot air dryer and evaluated densitometrically at a wavelength of 256 nm.

Assay

Twenty capsules (Nicip-D labelled to contain NIM 100 mg and DIC-Na 50 mg per capsule) were weighed, emptied and mixed. An accurately weighed quantity of capsule powder equivalent to about 10 mg of NIM was shaken with 5.0 mL of methanol for about 15 min and the volume was made up to 10.0 mL. The solution was then filtered through Whatman No. 1 filter paper and 5.0 mL of filtrate was diluted to 10.0 mL, which was used as sample solution. After the preparation of sample the same procedure was followed as under laboratory mixture.

The contents of the drugs in average weight capsule were calculated as follows:

$$\% \text{ of labelled claim} = \frac{W_E}{W_A} \times 100$$

where W_E = wt. of drug estimated (µg).

W_A = wt. of drug applied (µg) on the basis of labelled claim.

Validation of Proposed Method

The proposed method was validated for the following parameters:

Accuracy: The accuracy of the proposed method was ascertained by carrying out recovery studies by standard addition method. Accurately known amounts of standard drugs were added to known amount of preanalysed capsule powder and it was analysed by the proposed method to ascertain if there are positive or negative interferences from excipients present in formulation.

The per cent recovery was calculated by using the following formula:

$$\% \text{ Recovery} = \frac{A - B}{C} \times 100$$

where A = Total drug estimated in mg

B = Amount of drug contributed by capsule powder (as per proposed method)

C = Amount of pure drug added

Precision: Replicate estimations of drugs in sample were carried out by proposed method and SD/RSD value was calculated as a measure of precision.

Specificity: Specificity is the ability of the method to access unequivocally the analyte of interest in the presence of components that may be expected to be present, such as impurities, degradation products and matrix components. Accurately weighed quantities of capsule powder, equivalent to about 10 mg of NIM, were exposed for 24 h to different stress conditions like room temperature (normal), at 50°C after addition of 1.0 mL of 0.1 N of HCl (acid), 1.0 mL of 0.1 N of NaOH (alkali), 1.0 mL of 3% H_2O_2 (oxidation), at 60°C (heat), in UV-cabinet

at 265 nm (UV). After 24 h the contents of flasks were shaken with methanol for 15 min and the volumes were made up to 10.0 mL, filtered, diluted and analyzed following the assay procedure.

Ruggedness: Ruggedness was tested under different conditions. *i.e.*, analyzing the sample on different days and by different analysts.

RESULTS AND DISCUSSION

The mobile phase, *n*-hexane : ethyl acetate : chloroform : glacial acetic acid in the ratio of 8 : 1 : 1.5 : 0.5 v/v yields good resolution of drugs under investigation on a silica gel 60 $GF_{254\ TLC}$ plate with R_f values 0.3 and 0.57 for NIM and DIC-Na respectively (Fig. 1). The other parameters as detailed under chromatographic

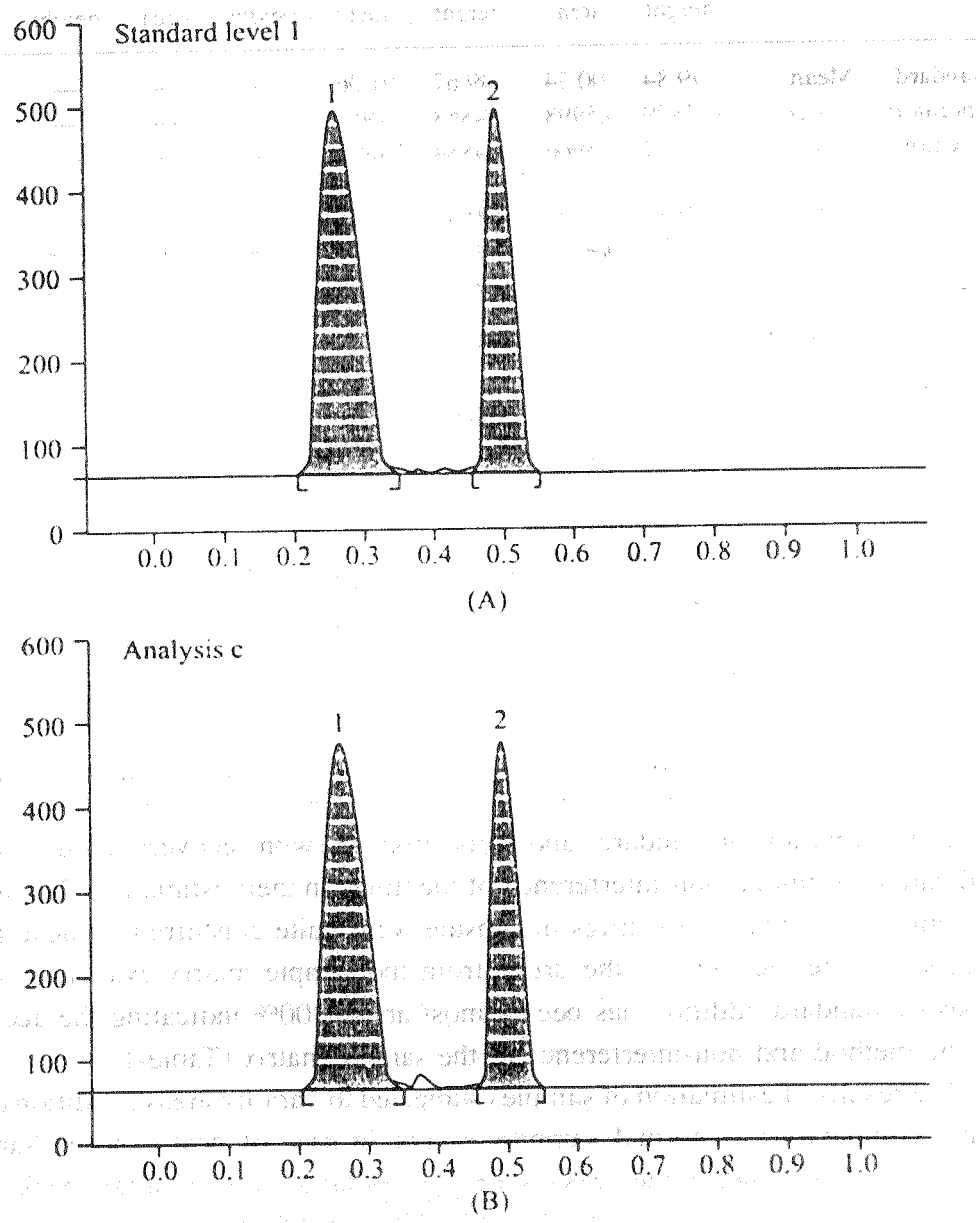

Fig. 1. Densitogram of nimesulide and diclofenac sodium at 256 nm: (A) standard, (B) sample

condition were optimized on the basis of exhaustive experimentation. Plots of concentration *vs.* peak height/peak area have been linear over concentration range 1.0–3.2 μg for NIM and 0.5–1.6 μg for DIC-Na with coefficient of correlation well above 0.99 (Table-2).

TABLE-1
ESTIMATION OF NIM AND DIC-Na AND RECOVERY STUDIES

Sample	Statistics	% Estimation of labelled claim*				% Recovery*			
		NIM		DIC-Na		NIM		DIC-Na	
		By height	By area	By height	By area	By height	By area	By height	By area
Standard laboratory Mixture	Mean	99.84	100.54	99.67	101.06	—	—	—	—
	± S.D.	0.7899	0.5998	0.4868	0.4950	—	—	—	—
	C.V.	0.7912	0.5966	0.4884	0.4898	—	—	—	—
Marketed preparation	Mean	99.92	99.93	100.65	99.27	99.14	100.99	100.51	99.60
	± S.D.	0.2779	0.4562	0.9630	0.5974	1.3900	1.5892	2.2961	2.1452
	C.V.	0.2781	0.4565	0.9568	0.6018	1.4021	1.5736	2.2844	2.1538

*Each reading is the mean of five observations.

TABLE-2
LINEARITY STUDIES OF CONCENTRATION *vs.* RESPONSE

Drug	Linearity range (μg)	Coefficient of correlation		Slope		Y-intercept	
		By height	By area	By height	By area	By height	By area
NIM	1.0–3.2	0.9902	0.9976	115.88	3268.02	185.46	3028.24
DIC-Na	0.5–1.6	0.9869	0.9959	211.70	3770.11	164.08	1842.30

The estimation of standard laboratory mixtures with recovery about 100% is indicative of mutual non-interference of the drugs in their estimation. The results of replicate estimation of drugs in capsule were quite concurrent indicating the precision. The recovery of the drugs from the sample matrix evaluated on the basis of standard addition has been almost about 100% indicating the accuracy of the method and non-interference of the sample matrix (Table-1).

The results of estimation of samples subjected to various stress conditions were quite comparable to normal samples except in case of heat-exposed samples (Table-3). The shapes of the peaks were also similar to normal sample peaks. This is indicative that either there is no degradation of the sample under stress conditions or the method is incapable of detecting it. However in case of

heat-exposed samples, although the results of DIC-Na by peak area are close to those of normal, peak broadening has been observed and it appears that peak height parameters be considered from specificity point of view.

TABLE-3
SPECIFICITY STUDY OF NIM AND DIC-Na

Sample	% Labelled claim*			
	NIM		DIC-Na	
	By height	By area	By height	By area
Normal	99.94	100.09	100.55	99.62
Acid	99.30	99.72	82.64	91.08
Alkali	99.73	99.96	102.64	97.05
Oxide	97.73	100.97	97.65	101.03
Heat	87.78	86.31	89.99	99.97
U.V.	98.05	100.28	97.18	101.10

*Each reading is the mean of five observations.

The estimation of samples on different days and by different analysts shows reproducibility of results indicating ruggedness of the method (Table-4).

TABLE-4
RUGGEDNESS STUDY OF NIM AND DIC-Na

Parameters	Statistics	% Labelled claim			
		NIM		DIC-Na	
		By height	By area	By height	By area
Different days	Mean	99.88	100.36	100.53	99.96
	± S.D.	0.4477	0.5384	1.0151	1.0329
	C.V.	0.4482	0.5364	1.0097	1.0333
Different analysts	Mean	99.14	101.02	99.05	100.96
	± S.D.	0.4050	0.2883	0.5493	0.6107
	C.V.	0.4085	0.2853	0.5546	0.6049

In general the method is simple, accurate, precise and reasonably specific and rugged and may be adopted for routine estimation of NIM and DIC-Na in their fixed dose capsule formulation.

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