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SIMULTANEOUS HPTLC ESTIMATION OF NAPROXEN AND PANTOPRAZOLE FROM CAPSULE DOSAGE FORMS.

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Abstract

The aim of the present study was to develop simple, accurate and precise method for simultaneous determination of Naproxen and Pantoprazole in capsules by HPTLC method. The proposed method shows that the chromatographic layer gives the best separation of the two component in the mobile phase consisting of Toluene: Methanol: Formic acid (8.5:1.3:0.2); other system like Chloroform: Ethyl acetate: Methanol: water (5.3:2.7:1.5:0.5); where the components move along with solvent front; chloroform: Methanol: Formic acid (10:2.5:0.5) where the component (Pantoprazole) not moved. Finally Toluene: Chloroform: Methanol: Formic acid (3.5:2.1:4.2:0.2) gave the complete separation with R_f values of Naproxen and Pantoprazole were 0.41 ± 0.02 and 0.51 ± 0.02 respectively. The method was validated with respect to accuracy, precision, limit of detection and limit of quantification. From the results it can be concluded that new HPTLC method could be used in routine analysis for simultaneous determination of Naproxen and Pantoprazole in combined dosage forms.

Keywords: Naproxen, Pantoprazole, HPTLC

INTRODUCTION

Naproxen is chemically (2s)-2-(6-methoxy-2-napthyl-1) Propanoic acid. It is used as analgesic anti-inflammatory (NSAID) commonly used for the reduction of moderate to severe pain, fever, inflammation and stiffness [1]. It works by inhibition both the COX-1 and COX- 2 enzymes. Like other NSAIDS naproxen is capable of producing disturbances in the gastro intestinal tract Naproxen is practically insoluble in water, soluble in ethanol (96%) and in methanol pka 4.2 [2,3]. Pantaprazole is 5-Sodium-(Difluromethoxy)-[(3,4DiMethoxy-2-Pyridinyl)methyl] sulphinyl]-1-H-benzimidazloe. It is gastric proton inhibitor [4]. The chemical structure of naproxen and pantoprazole was shown in **Figure 1.**

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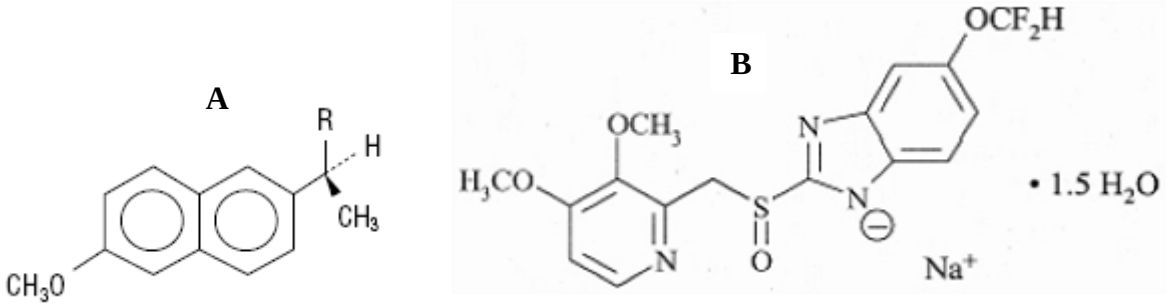

Figure 1: Chemical structure of a) naproxen and b) pantoprazole

A novel formulation in combination of NAP 250 mg and PAN 20 mg is commercially available in Indian market for treatment of osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, fever and prevent some of the gastrointestinal problems that NSAIDs can cause. Literature survey reveals few analytical methods for estimation of naproxen sodium with other drug combinations like pseudoephedrine hydrochloride by HPLC. [5,6] The present work demonstrates simple, rapid, accurate, reproducible and economical method for the simultaneous determination of NAP and PAN in pharmaceutical dosage form by HPTLC method which can be used for its routine analysis in laboratories.

MATERIALS AND METHODS

Materials

Fixed dose combination of capsule containing PAN 20 mg and NAP 250 mg was purchased from Local Indian market (Arthopan). All chemicals and reagents of analytical grade were purchased from Merck Chemicals, Mumbai, India. High purity deionized water was obtained from Millipore, Milli-Q (Bedford, MA, USA) water purification system.

METHODS

HPTLC analysis

Selection of stationary and mobile phase

A silica gel F_{254} was selected as the stationary phase and among the various mobile phase systems tried, a composition of Toluene: Chloroform: Methanol: Formic acid (3:5:2.1:4.2:0.2, v/v/v) was fixed. The R_f values of Naproxen and Pantoprazole were 0.41 ± 0.02 and 0.51 ± 0.02 respectively. Densitometric evaluation of the separated zones was performed at 241 nm.

Optimization of variants in HPTLC

Chamber saturation time

To achieve the reproducible R_f and peak areas of the two drugs, the chamber saturation time was optimized. The mobile phase was placed in the developing chamber and shaken well. During the trial, different equilibration times were maintained from 5 to 25 min. the plate spotted with the drug was kept for development after every trial equilibration time. The effect of chamber saturation time on peak shapes, development pattern and $R_f$value were studied. The chamber saturation time was fixed 15-25 min. as the R_f value and resolution were good for both Naproxen and Pantoprazole.

Plate equilibration (Pre-conditioning)

The plates were allowed to interact with the gas phase prior to chromatographic development i.e. without contacting with developing solvent (in a twin trough chamber). The plates were preconditioned for 5-30 min. and then the chromatograms were developed. The peak area, R_f value and peak shapes were noted and examined. The plates developed after 10min of equilibration was fixed.

OPTIMIZED PARAMETERS

Instrument	:	CAMAG Linomat V sample applicator, CAMAG HPTLC Scanner 3 with winCATS software.
Stationary phase	:	silica gel F 254
Mobile phase	:	Toluene: Chloroform: Methanol: Formic acid (3:5:2.1:4.2:0.2, v/v/v)
Diluent	:	96% Ethanol
Plate dimension	:	20 × 10 cm (width × Height)

Distance between tracks	:	10.0 mm
Band length	:	6.0mm
Volume applied	:	10µl
Scanning speed	:	20mm/s
Wavelength of detection	:	241nm
chamber	:	CAMAG Twin trough chamber

Preparation of stock solution

Stock solution-A

About 25 mg of Naproxen was accurately weighed and transferred to a 100ml volumetric flask dissolvedin 96% ethanol, and further made upto volume with same solvent to get the stock solution.

Stock solution-B

About 20 mg of Pantoprazole was accurately weighed and transferred to a 100ml volumetric flask dissolved in 96% ethanol, and further made upto volume with same solvent to get the stock solution.

Preparation of standard solution

Standard solution

Pipetted out 5ml (stock solution-A) and 1ml (stock solution-B) into 50ml volumetric flask and volume made up to the mark. From these suitable dilutions were made to obtain a final concentration of 25-125 µg/ml of Naproxen and 4-20 µg/ml of Pantoprazole.

Preparation of sample solution

The content of twenty capsules were accurately weighed and powered. The powder equivalent to average content of capsule was accurately weighed and transferred to 100 ml volumetric flask dissolved in 96% ethanol solution and made upto the volume with same solvent. From this suitable dilution are made to obtain a final concentration of 25 µg/ml of Naproxen and 2 µg/ml of Pantoprazole.

Procedure

Ten microliters of each of the standard and sample solution were spotted on the HPTLC plate using automatic application device. The chromatoplate was then developed in twin trough chamber containing the mobile phase. After development, it was scanned at 215nm and the peak areas were measured.

Procedure

Ten microliters of each of the standard and sample solution were spotted on the HPTLC plate using automatic application device. The chromatoplate was then developed in twin trough chamber containing the mobile phase. After development, it was scanned at 241 nm and the peak areas were measured

Linearity

A calibration curve was constructed for each of the drug independently by plotting the peak areas against concentrations. There exists a linear relationship in the two graphs showing concentrations ranging from 25-125 µg/ml of Naproxen and 4-20 µg/ml of Pantoprazole.

Precision

The precision of an analytical method were carried out by assaying a sufficient no. of aliquots of a homogeneous sample to be able to calculate statistically valid estimate of % RSD.

Recovery (accuracy)

To ensure the accuracy of the method, recovery studies were carried out by addition of a known quantity of the standard drug to the pre-analyzed samples (50,100 and 150%) of target level) and the whole content were reanalyzed by the proposed method.

RESULTS AND DISSCUSSION

The proposed method shows that the chromatographic layer gives **(Figure 2)** the best separation of the two component in the mobile phase consisting of Toluene: Methanol: Formic acid (8.5:1.3:0.2); other system like Chloroform: Ethyl acetate: Methanol: water (5.3:2.7:1.5:0.5); where the components move along with solvent front; chloroform: Methanol: Formic acid (10:2.5:0.5) where the component (Pantoprazole) not moved. Finally Toluene: Chloroform: Methanol: Formic acid (3.5:2.1:4.2:0.2) gave the complete separation with R_f values of Naproxen and Pantoprazole were 0.41 ± 0.02 and 0.51 ± 0.02 respectively. Total separation time for both components was reasonably short shown in **Figure 3 and 4.**

The linearity of the HPTLC method shown in **Figure 5 and 6** and the data used for assay was evaluated by spotting standard concentration of Naproxen and Pantoprazole ranging from 25 – 125 µg/ml and 4 – 20 µg/ml respectively. A summary of the data showing the slopes, y-intercept value, P-value are furnished in **Table 1 and 2**. The correlation coefficient all assay of Naproxen and Pantoprazole were all around 0.999. In addition, the analysis of residuals for the assay Naproxen and Pantoprazole shows that the values of randomly scattered around zero which show a good fit with the linear model. To evaluate whether the y-intercepts were significantly different than zero, the P- value was determined for each line. If P-value was >0.05 then the intercept was considered statistically equal to zero.

The precision for the Naproxen and Pantoprazole were evaluated by using homogeneous sample in six times determination (100% of target) the data are furnished in **Table 3**. Overall assay of Naproxen ranged from 99.47% to 100.29% with the mean value of 99.86%. Pantoprazole ranged from 99.49% to 100.34% with the mean value of 99.83%.

The accuracy of Naproxen and Pantoprazole was determined by fortifying sample and standard drug substances at concentration from 50 – 150% of target level. The data are furnished in **Table 4**. Overall recovery of Naproxen ranged from 99.54% to 101.01% with the mean value of 100.06%. Pantoprazole ranged from 99.83% to 100.51% with the mean value of 100.12%.

All the above parameters combined with the simplicity and care of operation ensure the use of proposed method in the assay of pharmaceutical dosage form containing this combination.

Table 1: Linearity data (HPTLC)

S. No	Naproxen			Pantoprazole		
	Conc.(µg/ml)	Peak area	R_f	Conc.(µg/ml)	Peak area	R_f
1	25	4582.98	0.39	4	1615.81	0.52
2	50	9052	0.42	8	3241.2	0.53
3	75	13759.07	0.41	12	4830.65	0.51
4	100	18178.79	0.41	16	6398.71	0.50
5	125	4582.98	0.40	20	8013.42	0.50

Table 2: Analytical performance parameter (HPTLC)

Parameter	Naproxen	Pantoprazole
Rf value	0.39	0.52
Slope	506.0 ± 8.860	398.8 ± 1.333
y-intercept	69.34 ± 58.77	34.14 ± 17.69
Correlation coefficient	0.9996	0.9999
p-value of intercept	0.24	0.25
Percentage of intercept at Quantification level	+4.19	+1.36
Limit of detection(ng/spot)	60	90
Limit of quantification (ng/spot)	80	130

Table 3: Precision data (HPTLC)

Naproxen	Pantoprazole
100.29	100.1
99.86	99.57
99.47	100.34

	99.93	99.57
	100.06	99.96
	99.57	99.49
Mean	**99.86**	**99.83**
% RSD	0.27	0.31

Table 4: Accuracy data (HPTLC)

% of target		Naproxen	Pantoprazole
50		101.01	100.36
		100.42	100.23
		99.87	99.90
	Mean	100.43	100.16
	%RSD	0.46	0.19
100		99.54	99.83
		99.97	100.21
		99.65	100.51
	Mean	99.72	100.18
	%RSD	0.18	0.27
150		100.06	100.32
		100.30	99.93
		99.75	99.87
	Mean	100.03	100.04
	%RSD	0.23	0.19
Grand mean		**100.06**	**100.12**
%RSD		0.29	0.06

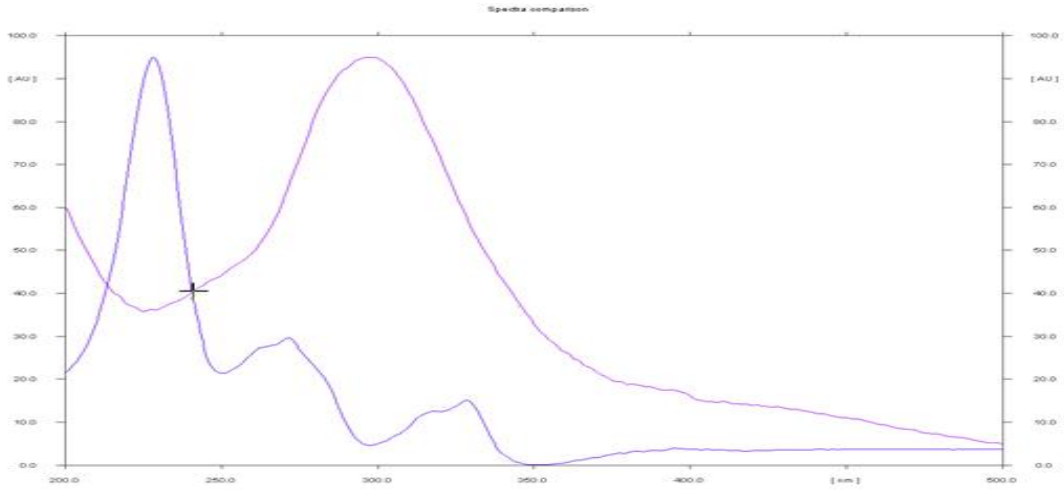

Figure 2: Comparative spectra of Naproxen and Pantoprazole (HPTLC)

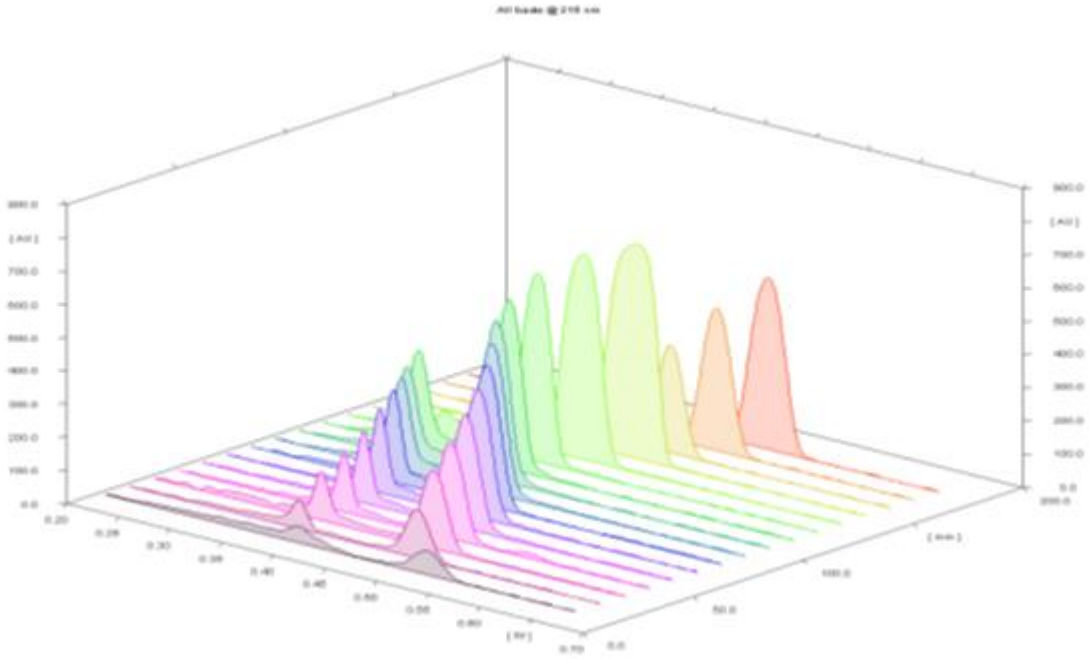

Figure 3: 3 Dimensional spectra of Naproxen and Pantoprazole (HPTLC)

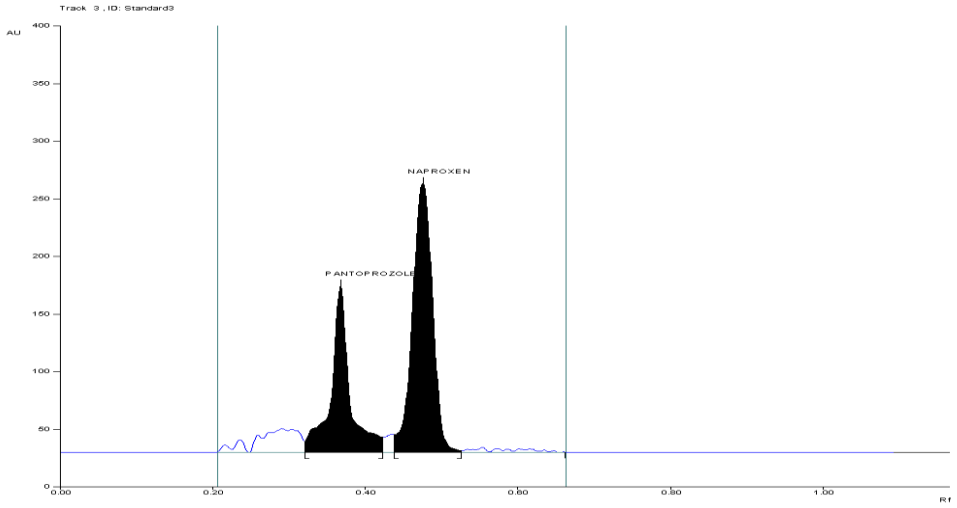

Figure 4: Densitogram of naproxen and pantoprazole (HPTLC)

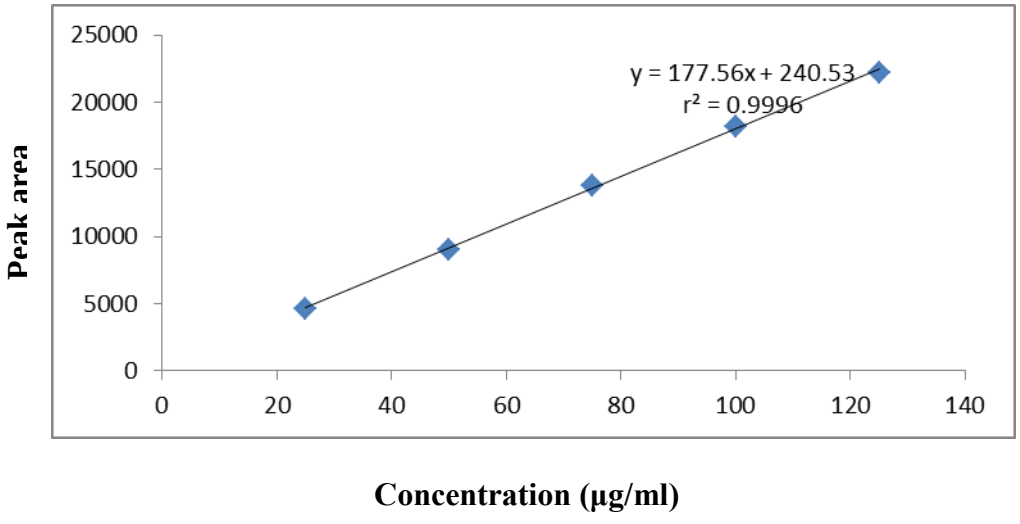

Figure 5: Linearity for Naproxen (HPTLC)

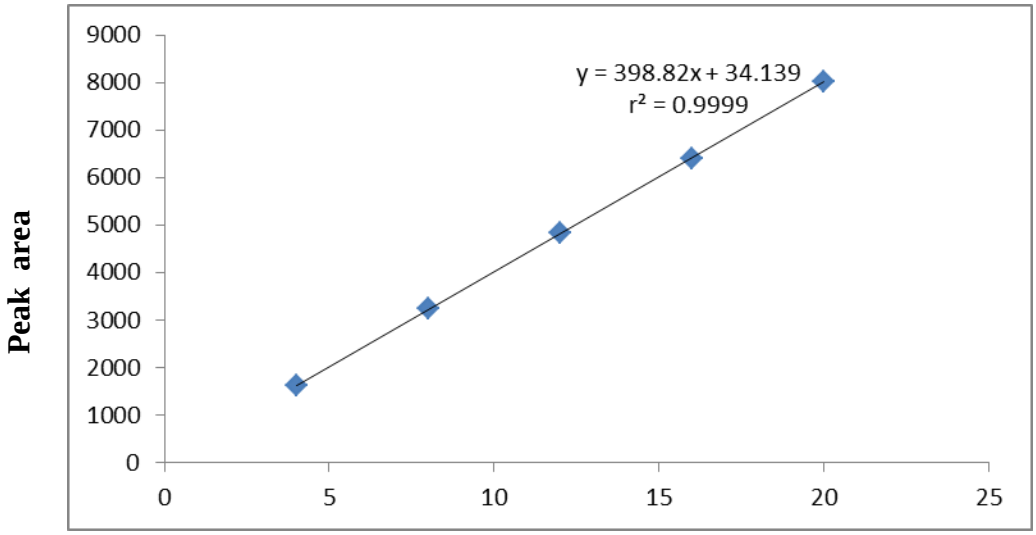

Figure 6: Linearity for Pantoprazole (HPTLC)

CONCLUSION

In conclusion, an economic, simple and rapid HPTLC method has been developed for simultaneous determination of naproxen and pantoprazole in capsule dosage form. The method was validated for linearity, precision, accuracy, LOD and LOQ as per ICH guidelines. Therefore, the proposed method could be applied for the routine analysis of pharmaceutical dosage forms containing naproxen and pantoprazole drug molecules.

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