

DEVELOPMENT AND VALIDATION OF HPTLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF NAPROXEN AND PANTOPRAZOLE IN COMBINED DOSAGE FORM

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ABSTRACT

Objective: The simple, accurate and precise method for simultaneous determination of Naproxen and Pantoprazole in tablets by HPTLC methods is developed.

Methods: In this method, the chromatograms were developed using a mobile phase of ethyl acetate: glacial acetic acid (4.8:0.2). The method uses aluminum plates coated with silica gel 60 F₂₅₄ as stationary phase. Densitometric evaluation of the separated bands was performed at 310 nm.

Results: The R_F values for Pantoprazole and Naproxen were 0.3 and 0.65 respectively.

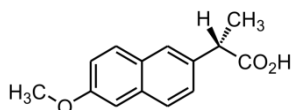
Conclusion: The proposed method HPTLC method is simple, economic, accurate & reproducible & can be used in routine analysis for simultaneous determination of Naproxen & Pantoprazole in combined dosage form.

Keywords: Naproxen, Pantoprazole, HPTLC, Validation.

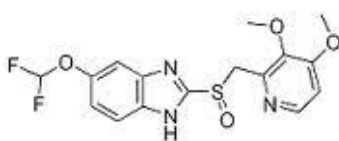
INTRODUCTION

This paper provides information regarding HPTLC –based analytical method development and evaluation of validation characteristics in accordance with best practice. HPTLC is superior to other analytical techniques in terms of total cost and time for analysis. It is an offline process in which various stages are carried out independently. Important features of HPTLC include the ability to analyze crude samples containing multi-components, application of large number of sample and a series of standards using the spray-on technique, choice of solvents for the HPTLC development is wide as the mobile phases are fully evaporated before the detection step, processing of standards and samples identically on the same plate leading to better accuracy and precision of quantification, different and universal selective detection methods, and in situ spectra recording in sequence to obtain positive identification of fractions, storage of total sample on layer without time constraints.

Naproxen is chemically (+)-(S)-2-(6-methoxynaphthalen-2-yl) propanoic acid. It is having molecular formula C₁₄H₁₄O₃ and its molecular weight is 230.259 g/mol. Naproxen is commonly used for the reduction of pain, fever, inflammation and stiffness caused by conditions including migraine, osteoarthritis, kidney stones, rheumatoid arthritis, psoriatic arthritis, gout, ankylosing spondylitis, menstrual cramps, tendinitis and bursitis. Literature survey reveals that several analytical methods have been reported for estimation of Naproxen and Pantoprazole by colorimetric method, HPLC. Pantoprazole is chemically (RS)-6-(Di fluoromethoxy)-2-[(3, 4-dimethoxy pyridin-2-yl) methylsulfanyl]-1H-benzo[d]imidazole. Pantoprazole is used for short-term treatment of erosion and ulceration of the esophagus caused by gastroesophageal reflux disease. It is having a molecular C₁₆H₁₅F₂N₃O₄S and its molecular weight is 383.371 g/mol.



1. Structure of naproxen



2. Structure of Pantoprazole

MATERIALS AND METHODS

Instrumentation

HPTLC system equipped with CAMAG LINOMAT V automatic sampler applicator; CAMAG TLC SCANNER; Integrator controlled by CATS4 software; CAMAG twin through glass chamber with stainless steel lid. Precoated silica gel F₂₅₄ on aluminum sheets (20 × 10 cm).

Chemicals and reagents

Standard sample of Naproxen and Pantoprazole were provided by central drug Testing Lab. Mumbai. Tablets of combined form were procured from the market. All other reagents were analytical grade. Ethyl acetate, Glacial acetic acid and methanol were obtained from science house (Mumbai, India). Stationary phase used is silica gel F₂₅₄ precoated aluminum plates.

Preparation of stock solution

Accurately 10mg of Naproxen and 10mg of Pantoprazole reference std is weighed and transfer in 10 ml volumetric flask 5ml methanol is added, sonicated for 15 minutes and diluted to prepare stock solution with methanol.

Sample preparation

To determine the content of Naproxen and Pantoprazole in tablet (label claim: 250mg of Naproxen and 20mg of pantoprazole). 5 tablets were weighed; average weight is taken and crushed to fine powder. 10 mg from it is transferred to 10 ml volumetric flask 5ml methanol is added and sonicated and diluted further with methanol.

Chromatography

Linear ascending development was carried out in a 20cm × 10cm twin through glass chamber (CAMAG) using the mobile phase ethyl acetate: glacial acetic acid (4.8:0.2) (v/v). The chamber is saturated for 20 minutes. Plates were dried in a current of air with the help of hair dryer. The source of radiation utilized in deuterium lamp emitting a continuous UV spectrum between 200nm to 400nm. Slit dimension were 5nm × 0.045nm and the scanning speed of 20mms⁻¹. For preparation of the calibration curve 1mg/ml of working standard of Naproxen and Pantoprazole in methanol is prepared. Calibration curve from 10-200 µg/ml for Naproxen is prepared and check for reproducibility, linearity and validating the proposed method. Same procedure is repeated for Pantoprazole using standard solution in the range of 50-250 µg/ml. Sample was spotted on precoated TLC plates by using Linomat 5 automatic sampler. TLC plates were developed up to 8cm contents of Naproxen and pantoprazole were determined by

comparing area of the chromatogram of sample with calibration curve

of working standard both.

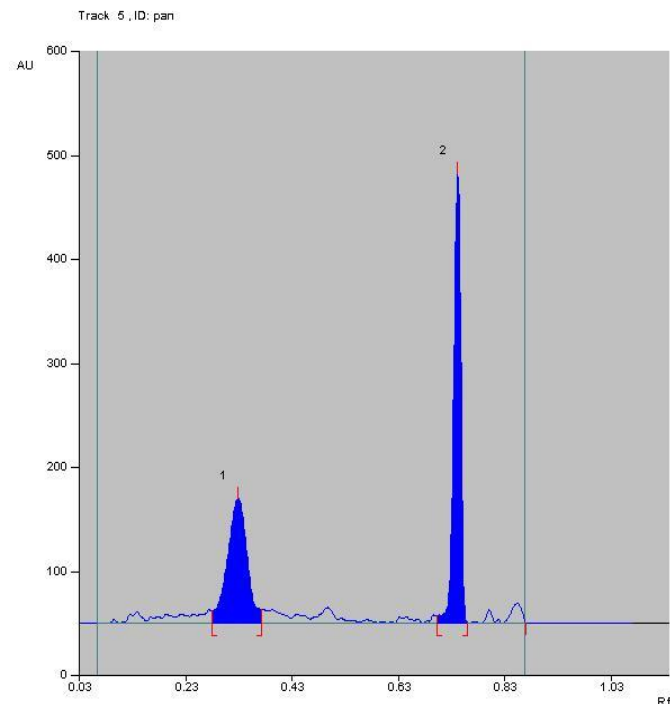


Fig. 1: Densitogram of naproxen and pantoprazole

RESULT AND DISCUSSION

Validation of method

Development method is validated in terms of linearity, accuracy, precision, LOD, LOQ, robustness.

Linearity

Appropriate volume of aliquots from standard Naproxen and Pantoprazole stock solutions were prepared and applied on the TLC plate in the range of 1.2-2.4 μ L to give a series of spots covering the range from 50 to 300 ng/spot and 250 to 1500 ng/spot respectively. The linear regression coefficient for Naproxen is 0.997 and for Pantoprazole is 0.995.

Precision

The Interday and intraday precision of the method were estimated by performing determination of drugs solution at three different concentrations, the results obtained in table.

Table 1: It shows precision of Naproxen.

Amount μ g/band	Intra-day precision		Inter-day Precision	
	Mean area (AU)	RSD(9%)	Mean area (AU)	RSD (%)
500	100.0694	1.476269	99.86532	1.666435
750	99.24021	1.157593	98.56001	0.899939
1000	100.1316	1.611476	100.4377	1.640885

Table 2: It shows precision of Pantoprazole.

Amount μ g/band	Intra-day precision		Inter-day Precision	
	Mean area (AU)	RSD(9%)	Mean area (AU)	RSD (%)
100	100.7743	1.476269	99.86532	1.666435
150	99.24021	1.157593	98.56001	0.899939
200	100.1316	1.611476	100.4377	1.640885

Robustness

Robustness was measured by analysis of the sample solution by making small changes to mobile phase composition. Ethyl acetate: Glacial acetic acid in the ratio 4.7:0.3 (v/v) and Ethyl acetate: Glacial acetic acid in the ratio of 4.9:0.1 (v/v) were selected.

Table 3: It shows robustness of Naproxen.

Saturation Time	Mean	RSD
15 min	99.943	1.020
25 min	100.356	1.661
Condition		
Ethyl acetate: Glacial acetic acid (4.7:0.3)	99.943	1.020
Ethyl acetate: Glacial acetic acid (4.9:0.1)	99.586	0.741

Table 4: It shows robustness of Pantoprazole.

Saturation Time	Mean	RSD
15 min	100.511	0.917
25 min	100.989	1.141
Condition		
Ethyl acetate: Glacial acetic acid (4.7:0.3)	100.800	1.183
Ethyl acetate: Glacial acetic acid (4.9:0.1)	100.275	0.995

Limit of detection & limit of quantization

The limits of detection (LOD) and limit of quantization (LOQ) were calculated from slopes of the calibration plots and the standard deviation (SD) of the response by the use of the equations $LOD = 3.3 \times SD/S$ and $LOQ = 10 \times SD/S$. The limit of detection and limit of quantization obtained by this method for Naproxen 13.52 & 40, Pantoprazole 4.2 & 12.82 respectively.

Specificity

Specificity of the method is ascertained by analyzing reference standard and samples. The bands for Naproxen and Pantoprazole formulations were confirmed by comparing R_f values and U.V spectra of these separated bands with those from standard the peak purity of Naproxen and Pantoprazole accessed by comparing the spectra acquired at the peak start(S) peak apex(N) and peak end(E) of a band.

Recovery

The analyzed samples was spiked with an additional 400,500,600 of Naproxen and 80,100,120 of Pantoprazole were analyzed again by proposed method. Recovery was 99.50- 101.15% which is shown in table.

Table 5: It shows recovery of Naproxen.

Amount of drug added (%)	Theoretical content (mcg)	Recovery (%)	RSD
400	500	100.2296	1.175355
500	500	99.8143	1.543492
600	500	99.28239	0.493749

Table 6: It shows recovery of Pantoprazole.

Amount of drug added (%)	Theoretical content (mcg)	Recovery (%)	RSD
80	100	99.94098	1.794321
100	100	101.152	1.457477
120	100	99.50031	0.774122

Assay

Tablet (labeled to contain 250 mg Naproxen and 20 mg Pantoprazole) sample was prepared by dispersing the equivalent amount of triturated powder in methanol. After appropriate dilution it was filtered through whatmann filter paper no. 41 and spotted on the TLC plate with standard solution of same concentration on adjoining track.

Table 7: It shows assay of Naproxen.

Concentration	Tablet assay		Bulk assay	
	Assay	RSD	Assay	RSD
750	102.390	0.813	100.743	1.501

Table 8: It shows assay of Pantoprazole:

Concentration	Tablet assay		Bulk assay	
	Assay	RSD	Assay	RSD
150	100.540	1.879	99.816	1.158

Repeatability

Repeatability of sample application was assessed by spotting 4 µl of drug solution 6 times on a TLC plate followed by development of plate and recording the peak area for 6 spots. The % RSD for peak area values of Naproxen and Pantoprazole were found to be 1.203 and 1.497.

Ruggedness

Ruggedness is measure of reproducibility of test results under normal, expected operating condition from instrument & from analyst, ruggedness was tested by analysis of 750, 150 mcg Naproxen and Pantoprazole for per band were listed in tables given below

Table 9: It shows ruggedness of Naproxen.

Variable	Recovery	RSD
Analyst I	99.329	1.310
Analyst II	100.417	1.266

Table 10: It shows ruggedness of Pantoprazole.

Variable	Recovery	RSD
Analyst I	99.596	1.688
Analyst II	100.068	1.236

CONCLUSION

The proposed method HPTLC method is simple, economic, accurate & reproducible & can be used in routine analysis for simultaneous determination of Naproxen & Pantoprazole in combined dosage form.

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