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Development and validation of analytical method for naproxen and pantoprazole in capsule dosage form

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ABSTRACT

A simple sensitive and precise high performance liquid chromatographic method for the analysis of naproxen and pantoprazole has been validated and used for the developed, validated and used for the determination of compound in commercial pharmaceutical products. The compound were well separated on a on hypersil BDS C-18,250×4mm,5µg reversed phase column by use of a mobile phase consisting of mixed phosphate buffers (K_2HPO_4,KH_2PO_4)(PH:6.5) Acetonitrile (55:45 v/v) at a few rate of 1.0ml min^{-1} with detection wavelength at 290nm.the linearity range were 5 to 30µg/ml for naproxen and 0.4-2.4µg/ml for pantoprazole the recovery amount was more than 99%the high suitability of the method for determination of naproxen and pantaprozole in pharmaceutical dose form.

Keywords: Naproxen, pantoprozole, HPLC, Acetonitrile, Hypersil BDS.

INTRODUCTION

HPLC is one of the most widely used analytical techniques today, among the different chromatographic procedure, due to the significant evolution in liquid chromatographic instrument, providing superior qualitative and quantitative results [1]. Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use [2]. 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. 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. [3].

Pantaprazole is 5-Sodium-(Difluromethoxy)-[(3,4DiMethoxy-2-Pyridinyl)methyl] sulphinyl]-1-H-benzimidazloe.It is gastric proton inhibitor[4]. Literature survey reveals few analytical methods for estimation of naproxen sodium with other drug combinations like pseudoephedrine hydrochloride by HPLC [5]. The gastric proton inhibitors have structural resemblance H2 antagonst.They are the prodrugs and after absorption get converted to reactive thiophilic sulphonamide cations .Pantoprazole inhibits basal and stimulated gastric acid secerition.It is an irreversible inhibitor of the proton pump and it binds covalently to trhe enzyme H^+ / K+-ATPase, responsible for gastric acid production and is located in the secretary membranes of the parietal cell [6].There are different methods reported for quantitative determination of pantaprazole and naproxen bulk or pharmaceutical formulation include titrimetry, colorimetry and high performance liquid chromatography (HPLC)[7-21].The market survey that naproxen.It is indicated as antirheumatoid and NSAIDS. Literature survey revealed that naproxen and pantaprozole is official in IP, BP and USP. Although there are many methods reported for estimation of these drugs in combined dosage form.The present work was undertaken with an objective to develop an accurate, simple, precise and reliable method for simultaneous estimation of these two drugs in their combined dosage form by HPLC.

MATERIALS AND METHODS

Equipment and chromatographic conditions

The system used consist of SHIMADZU-SPD20AD detector the chromatographic separation was carried out at room temperature with hypersil BDSC-18 reversed phase column by use of a mobile phase consisting of mixed phosphate buffer (K2PO4,KH2po4) (Ph:6.5),acetonitrile (55:45V/V)at a flow rate of 10ml/min. The mobile was filtered through a 0.45µm membrane filter and degassed for 10mts.The injection volume for samples and standards were 20µg and eluted at a flow rate of 1ml/min at ambient temperature. The eluents were monitored at 290nm.

Materials and reagents

Naproxen and pantaprazole in combination ARTHOPAN 250(claimed labeled amount 250mg NAP and 20mg PAN per capsule) was procured from local pharmacies. HPLC grade acetonitrile was used and all other chemicals (Analytical grade) were used. NAP and PAN in pure form was denoted as a gift sample from Madras Pharmaceuticals, Chennai.

Preparation of Standard Solution

A working standard solution containing Naproxen 250 mg/100ml and Pantoprazole 20 mg/100ml was prepared by dissolving Naproxen and Pantoprazole sodium reference standard in mobile phase. The mixture was sonicated for 5 minutes or until the reference standard dissolved completely.

Preparation of sample Solution

Twenty capsules, each containing 250 mg Naproxen and 20 mg Pantoprazole were accurately weighed and finely powdered. A quantity of powder equivalent to 250 mg of Naproxen and 20 mg of Pantoprazole was weighted and transferred to a 100 ml volumetric flask. About 70 ml of mobile phase was added and shaken mechanically for 15 minutes. The mixture was then sonicated in ultrasonic bath for 5 minutes and made the volume up to 100 ml by the mobile phase. The solution was filtered with a Whatman filter paper no.1. Before injection, both

standard and sample solution was filtered through 0.45 µm syringe filter. Then 10 µl of standard and sample solutions were injected into column and chromatogram was recorded.

Validation of the HPLC method

Linearity

The stock solution of 5-30µg/ml, of NAP and 0.4-2.4 µg/ml of PAN were prepared and 20 µl fixed volume was injected. Linearity of the method was studied by injecting 6 concentrations of the drug prepared in the mobile phase in triplicate in to the HPLC system keeping the injection volume constant.

Accuracy

The accuracy of the analysis was evaluated by determined of recovery at three different concentrations equivalent to 80,100 and 120% of the amount preanalysed dosage form and average recoveries were calculated.

Precision

Five sample of 25mcg were prepared and analysed as per the sample preparation procedure. System precision and method precision were calculated.

Specificity

Specificity studies for method were performed for its ability to asses and unequivocally the NAP and PAN in the presence of capsule excipients. Chromatographic interferences from capsule excipients were examined. The average retention time for NAP and PAN were calculated.

Robustness

To evaluate robustness of a HPLC method, few parameters were deliberately varied. The parameters included variation of flow rate and the percentage of acetonitrile in the mobile phase.

System Suitability

System suitability parameters were evaluated from tailing factor, retention times and theoretical plates of standard chromatograms.

Limit of Detection and Quantitation

To determine the LOD and LOQ serial dilutions of mixed standard solutions of NAP and PAN were made from the standard stock solutions. The samples were injected in HPLC systems on the chromatograms were run and measured signal from the samples was compared with those of blank samples. The limit of detection (LOD) and limit of quantitation (LOQ) were calculated from the slope (s) of the calibration plot and the standard deviations of the response (SD).

RESULTS AND DISCUSSION

The conditions used for chromatography were optimized on the basis of experimentation. The method was validated in accordance with ICH guidelines for linearity, accuracy, precision, specificity and robustness. The mobile phase mixed phosphate buffer: acetonitrile (55:45 v/v) enables good resolution and separation using Hypersil BDS C-18 column (250× 4mm, 5.0µ). The retention time (Rt) values were 3.35min for NAP and 4.9min for PAN and detection

wavelength 290nm was selected from overlain spectra of the drugs acquired from UV spectrophotometer. [Figure 1]

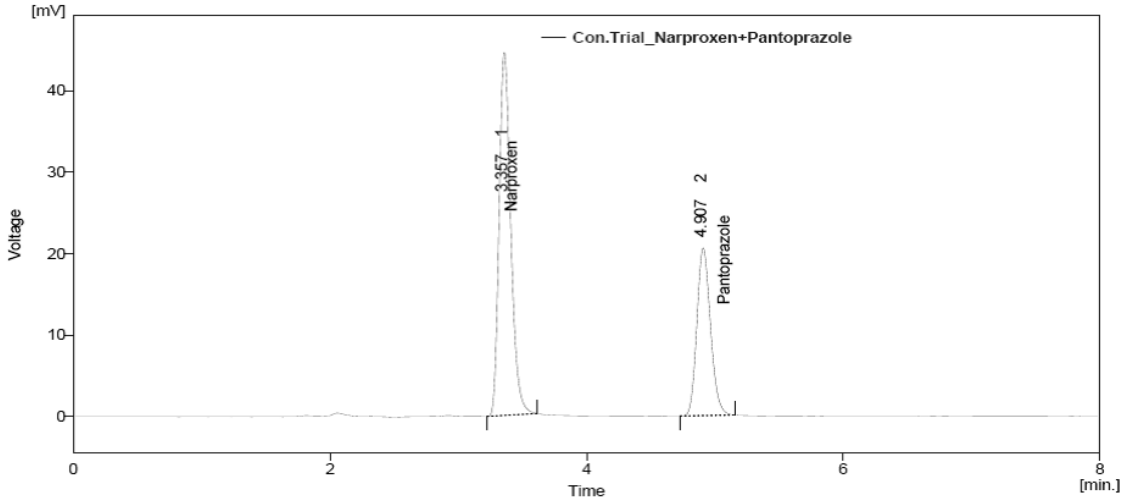

Figure No 1. Chromatogram Of conformation Trail

Linearity

Naproxen and pantoprazole showed good correlation coefficient (r^2= 0.9989 for NAP and r^2= 0.9987 for PAN) in given concentration range 5-30µg/ml for NAP and 0.4-2.4µg/ml for PAN and the UV absorbances was taken at 290nm (Table 1 a,b) (Figure 2 a,b)

Table 1 a. Linearity studies of Naproxen by RP-HPLC

Linearity Level	Concentration in µg/ml	Area	Statistical Analysis	
Level 1	5	63.43	Slope	10.17
Level 2	10	119.725		
Level 3	15	171.126	Correlation Coefficient	0.999
Level 4	20	220.036		
Level 5	25	272.036	r^2	0.9989
Level 6	30	318.277		

Table I b. Linearity studies of Pantoprazole and by RP-HPLC

Linearity Level	Concentration in µg/ml	Area	Statistical Analysis	
Level 1	0.4	29.027	Slope	73.40
Level 2	0.8	58.822		
Level 3	1.2	88.071	Correlation Coefficient	0.999
Level 4	1.6	117.261		
Level 5	2.0	148.078	r^2	0.9987
Level 6	2.4	175.180		

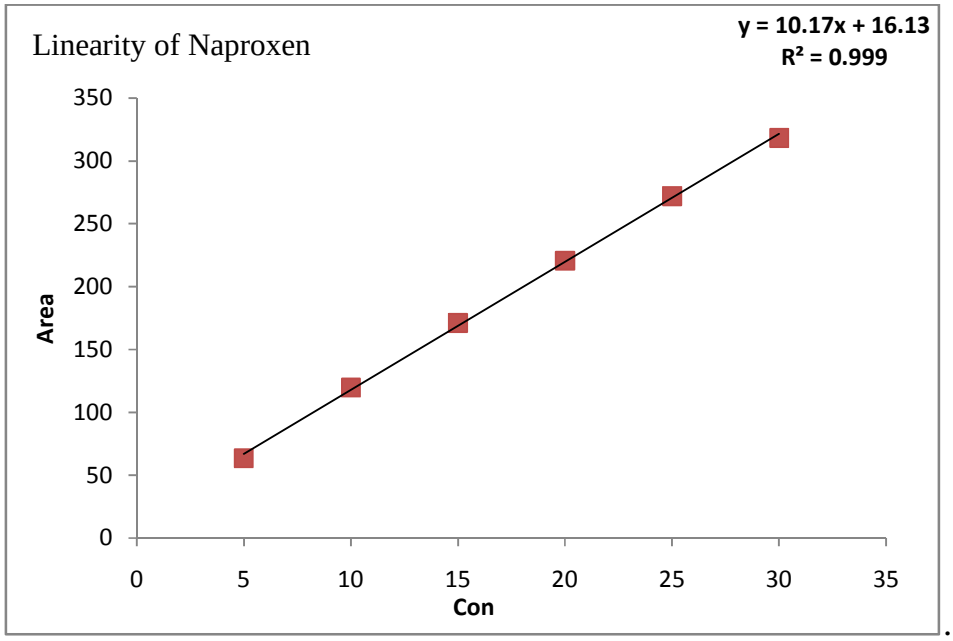

Figure-2 a. Linearity graph of Naproxen

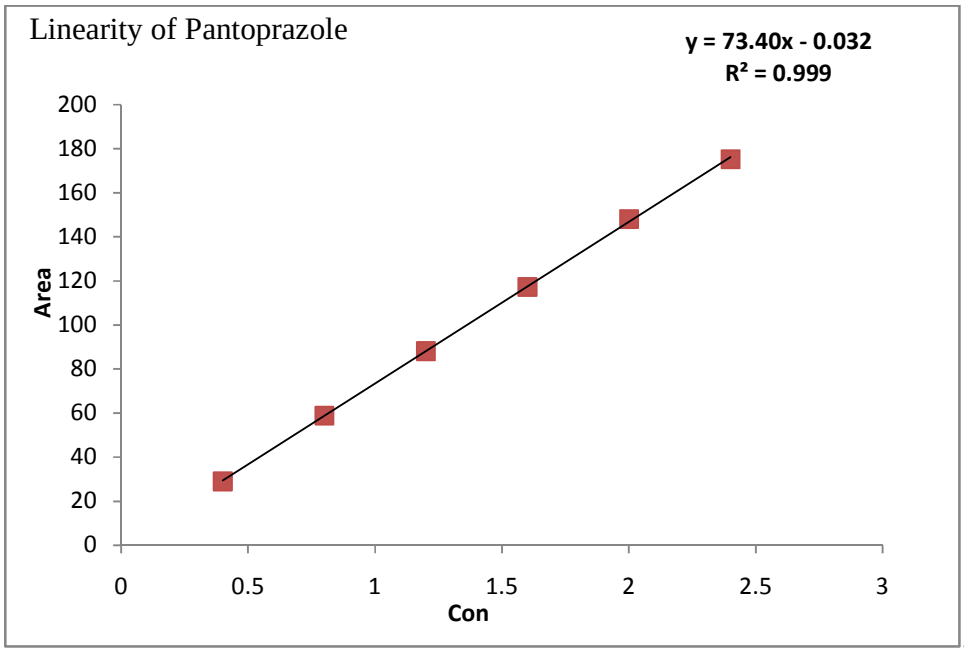

Figure-2 b .Linearity graph of Pantoprazole

Limit of Detection and Quantification

The limit of detection for NAP and PAN were found to be 0.158 and 0.026 respectively. The limit of quantitation was found to be 0.481 for NAP and 0.081 for PAN.

Precision

The results of the system precision and method precision experiments are shown in (table 2 a,b). The developed method was found to be precise as the %RSD values for system precision and method precision studies were <2%, respectively as recommended by ICH guidelines.

Table 2 a. System Precision of Naproxen and Pantoprazole

Injection	Peak Areas of Naproxen	Peak Areas of Pantoprazole
1	275.725	147.918
2	275.574	148.609
3	273.674	147.008
4	272.265	147.119
5	273.296	148.027
Mean	274.1068	147.7362
SD	1.342251	0.598432
%RSD	0.4896	0.4056

Table 2 b. Method Precision of Naproxen and Pantoprazole

Injection	Peak Areas of Naproxen	Peak Areas of Pantoprazole
1	273.895	146.511
2	272.604	146.723
3	273.245	147.182
4	274.805	147.763
5	272.914	146.985
Mean	273.410	147.0328
SD	0.802081	0.664472
%RSD	0.997417	0.179944

Specificity

The method was found to specific as complete separation of both NAP and PAN in presence of excipients was observed. The average retention time for NAP and PAN were found to be 3.35min and 4.9min, respectively.

Robustness

The robustness of the method was proved by varying the flow rate, mobile phase composition and temperature from the optimized chromatographic conditions and the tailing factors were found to be less than 2%.

Accuracy

The proposed method when used for extraction and subsequent estimation of NAP and PAN from pharmaceutical dosage form after spiking 80,100 and 120% of additional drug afforded average recoveries in between 99.16 to 99.59, for NAP and 99.86 to 99.89 for PAN. The results indicate that the method enables accurate estimation of the drugs in the capsule dosage form (Table 3 a, b).

Table 3 a. Accuracy of Naproxen

S.NO	Concentration % of spiked level	% Recovery	Mean % recovery	Standard deviation	Relative Standard Deviation
1	80%	99.32			
2	80%	99.28	99. 29	0.03464	0.03424
3	80%	99.28			
4	100 %	99.66			
5	100%	99.56	99.59	0.0577	0.0579
6	100%	99.56			
7	120%	99.24			0.067
8	120%	99.12	99.16	0.066	
9	120%	99.13			

Table 3 b. Accuracy of Pantoprazole

S.NO	Concentration % of spiked level	% Recovery	Mean % recovery	Standard deviation	% Relative Standard Deviation
1	80%	99.24			
2	80%	100.03	99.89	0.60119	0.60176
3	80%	100.42			
4	100%	99.45			
5	100%	98.93	99.86	1.18924	1.19090
6	100%	101.20			
7	120%	99.62			
8	120%	101.05	99. 89.	1.56694	1.57433
9	120%	97.92			

System Suitability

The system suitability parameters also reveal that the values were within the specified limits for the proposed method. The results are showed in (Table 4)

Table 4. System suitability of Naproxen and Pantoprazole

Parameters	Data of Naproxen	Data of Pantoprazole
No. of Theoretical plates	6237	9787
Tailing	1.133	1.102
Retention Time	3.357	4.907

CONCLUSION

The proposed HPLC method is simple,accurate and reproducible for simultaneous estimation of naproxen (NAP) and pantoprazole (PAN) in pharmaceutical dosage form, without interference from the excipients. The chromatographic method is validated according to ICH guidelines. Statistical test indicate that the method is suitable for the simultaneous estimation of the above drugs in pharmaceutical dosage form and for routine analysis of raw materials of above drugs in a quality control laboratories, where economy and time are essential.

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