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Analytical method development and validation for simultaneous estimation of naproxen and pantoprazole in capsule dosage form by RP-HPLC

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ABSTRACT

A simple, rapid, precise, accurate RP-HPLC method has been developed and validated for the simultaneous estimation of Naproxen and Pantoprazole in combined dosage forms. Chromatographic separation was achieved with mobile phase consisting of Methanol: Phosphate Buffer P^H 5.4in the ratio of 70:30 v/v with Hypercil C18 (250 × 4.6 mm × 5 µm),column at a flow rate of 1 mL/minand detection wavelength was 259 nm. The retention times of Naproxen and Pantoprazole was found to be 3.33 minand 1.90 minrespectively. The method was validated in terms of Linearity, Range, Accuracy, Precision, Specificity, LOD, LOQ, Robustness and system suitability according to ICH guidelines. Commercial Capsule formulation was successfully analyzed using the developed method and the proposed method is applicable to routine analysis for determination of Naproxen and Pantoprazole in Capsule dosage form.

Keywords: Naproxen, Pantoprazole, RP-HPLC, development, validation.

INTRODUCTION

Naproxenis a non-steroidal anti-inflammatory drug (NSAID) with analgesic and antipyretic properties. Both the acid and its sodium salt are used in the treatment of rheumatoid arthritis and other rheumatic or musculoskeletal disorders, dysmenorrhea and acute gout. Naproxen IUPAC Name (+)-(*S*)-2-(6-methoxy naphthalen-2-yl) propanoicacid. Chemical formula $C_{14}H_{14}O_3$. The inducible Cyclooxygenase, COX-2, generates prostaglandins involved in inflammation. Inhibition of COX-1 is thought to be associated with gastrointestinal and renal toxicity while inhibition of COX-2 provides anti-inflammatory activity.

Naproxen itself is rapidly and completely absorbed from the GI tract with an in vivo bioavailability of 95%. At therapeutic levels naproxen is greater than 99% albumin-boundNaproxen is extensively metabolized to 6-0-desmethyl naproxen and both Parent and metabolites do not induce metabolizing enzymes.The observed terminal elimination half-life is approximately 15 hours. 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

Pantoprazole is used for short-term treatment of erosion and ulceration of the esophagus caused by gastroesophageal reflux disease. Initial treatment is generally of eight weeks' duration, after which another eight week course of treatment may be considered if necessary. It can be used as a maintenance therapy for long term use after initial response is obtained.

Pantoprazole IUPAC name

(*RS*)-6-(Difluoromethoxy)-2-[(3,4-dimethoxypyridin-2-yl)methyl ulfinyl]-1*H*-benzo[*d*] imidazole. Chemical Formula**:** $C_{16}H_{15}F_2N_3O_4S$ Bioavailability: (oral, delayed release tablets), approximately 77%, Protein binding: about 98% to primarily albumin**.** Major metabolized Hepatic; cytochrome P450 CYP2C19; minor metabolism from CYP3A4, 2D6, and 2C9Hepatic; cytochrome P450 CYP2C19; minor metabolism from CYP3A4, 2D6, and 2C9. Excreted Fecal: (oral or IV, normal metabolizers), 18%Renal: (oral or IV, normal metabolizers), approximately 71%, none as unchanged Dialyzable: no (hemodialysis).Total body clearance: (IV) 7.6 to 14 L/hour. Total body clearance: (oral, pediatrics) 0.18 to 2.08 L/hr/kg.

Literature survey revealed a few analytical methods like UV[6], HPTLC[11] and HPLC for analysis of naproxen, pantoprazole as single drug and in combination with other drugs like Rizatriptan[9], domperidon[8] etc.

MATERIALS AND METHOD

INSTRUMENTATION

Instruments

Liquid chromatography consists of the following component: HPLC – WATERS Model NO.2690/5 series Compact System Consisting of Hypersil-C18 BDS (250x4.6 mm.5µ) Column with auto sampler. Mobile phase is a mixture of methanol and phosphate buffer P^H 5.4 (70:30v/v) with flow rate of 1.0ml/min,injection volume of 20µl. Detected with PDF detector at wave length of 259 mn. The mobile phase was filter with What man filter No.1 and degasser for 10min.Electronic balance (SARTORIOUS) Digital P^H meter (POLOMAN) Sonicator (FAST CLEAN).

Chemicals

HPLC water (HPLC grade) – Merck Speciality Pvt., Mumbai.

Methanol (HPLC grade) – Merck Speciality Pvt., Mumbai.

Phosphate Buffer P^H 5.4

% purity of Naproxen 99.5%. } Madras Pharmaceuticals, Chennai

%purityofPantoprazole is 99.63%. } Madras Pharmaceuticals, Chennai

Sample-commercial tablet ARTHOPAN 250

CRESECNT THERAPEUTICS

(claimed labeled amount 250 mg NAP and 20mg PAN per capsule) was procured from local pharmacy.

Preparation of standard stock solution

Weigh down 10 mg's of Naproxen and Pantoprazole drugs and dissolved in 10ml of Mobile phase taken in two 10 ml of volumetric flasks separately and sonicated for 20 minutes to get 1000ppms and 1 ml was taken from each solution and diluted to 10 ml with mobile phase.

Preparation of working standard solution

Reference solution (a): The solution was prepared by dissolving 10.0 mg of accurately weighed Naproxen RS and 10.0 mg Pantoprazole RS in methanol, in a 100.0 mL volumetric flask. Reference solution (b): The solution was prepared by diluting 10.0 mL of reference solution (a) with methanol into a 50.0 mL volumetric flask.

Preparation of sample drug solution for pharmaceutical formulations

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 hat man 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.

RESULT AND DISCUSION

METHOD VALIDATION [10]

The method was validated with reference to ICH guidelines i.e. Linearity, Accuracy, Precision, Specificity, Ruggedness respectively.

System suitability

System suitability testing is an integral part of many analytical procedures. The tests are based on the equipment, electronics, analytical operation and sample to be analyzed.

A Standard solution was prepared by using Naproxen and Pantoprazole working standard as per test method and was injected Five times into the HPLC system.

The system suitability parameters were evaluated from standard chromatograms by calculating the % RSD from five replicate injections for Naproxen and Pantoprazole combination, retention times and peak area was recorded in the table-1and 2.

ACCEPTANCE CRITERIA

- The % RSD for the retention times of principal peak from 5 replicate injections of each Standard solution should be not more than 2.0 %.
- The % RSD for the peak area responses of principal peak from 5 replicate injections of each standard Solution should be not more than 2.0%.
- The number of theoretical plates (N) for the Naproxen and Pantoprazole peaks is NLT 3000.
- The Tailing factor (T) for the Naproxen and Pantoprazole peaks is NMT 2.0.

TABLE: 1 Data of System Suitability for Naproxen

Injection	RT	Peak Area	USP Plate count	USP Tailing
1	3.332	820383	11125	1.192208
2	3.327	820863	11006	1.220526
3	3.308	820757	11983	1.113553
4	3.323	820083	11005	1.160980
5	3.304	820008	11998	1.205406
Mean	3.318	820418	11424	1.190535`
SD	0.0121	385.5168	-------	-------
% RSD	0.36	0.047	-------	-------

TABLE: 2 Data of System Suitability for Pantoprazole

Injection	RT	Peak Area	USP Plate count	USP Tailing
1	1.912	284946	9670	0.930582
2	1.917	284666	9745	0.927512
3	1.897	284307	9742	0.934847
4	1.906	284841	9774	0.932795
5	1.906	284222	9807	0.920719
Mean	1.907	284596	9747	0.929291
SD	0.0075	320.4767	-------	-------
% RSD	0.39	0.11	-------	-------

SPECIFICITY

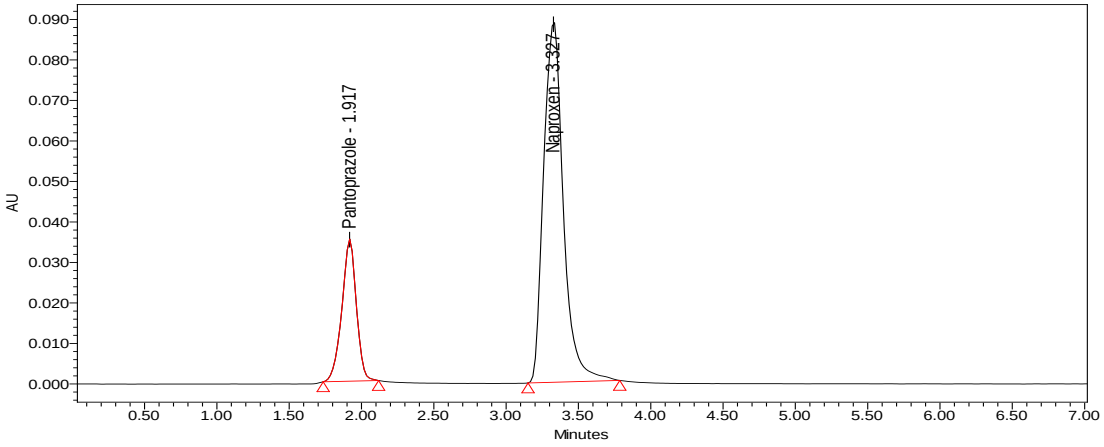
Naproxen and Pantoprazole identification:

Solutions of standard and sample were prepared as per the test method are injected into chromatographic system and chromatograms wasrecorded in the figures 1and 2.

ACCEPTENCE CRITERIA

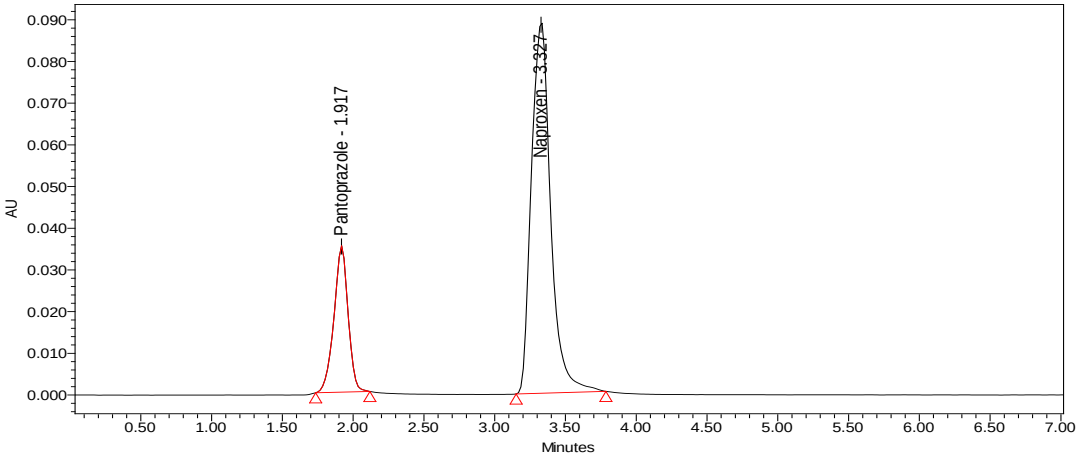
- Chromatograms of standard and sample should be identical with near Retention time.

FIG: 1 Chromatogram of standard solution.



Inference: Got a peak for standard at an Rt of 3.3 for Naproxen and 1.9 Pantoprazole

FIG: 2 Chromatogram of sample solution.



Inference: Got a peak for sample at an R_t 3.3 for Naproxen and 1.9 for Pantoprazole

OBSERVATION

The chromatograms of Standard and Sample were same identical with same retention time. As shown in fig 1and 2.

ACCURACY

The accuracy of an analytical method is the closeness of that results obtained by that method to the true value.

Accuracy may often be expressed as percent recovery by the assay of known added amount of analyte.

A study of Accuracy was conducted. Drug Assay was performed in triplicate as per test method with equivalent amount of Naproxen and Pantoprazole into each volumetric flask for each spike level to get the concentration of Naproxen and Pantoprazole equivalent to 50%, 100%, and 150% of the labeled amount as per the test method. The average % recovery of Naproxen and Pantoprazole was calculated and recorded in table 3and 4.

ACCEPTANCE CRITERIA

- The mean % recovery of the Naproxen and Pantoprazole at each spike level should be not less than 98.0% and not more than 102.0% for both the drugs separately.

$$\%\text{Recovery} = \frac{\text{Amount found}}{\text{Amount added}} \times 100$$

TABLE: 3 Data of Accuracy for Naproxen

Concentration % of spiked level	Amount added (ppm)	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery	
50% Injection 1	20	19.83	99.15	**MEAN**	99.98
50% Injection 2	20	20.18	100.9		
50% Injection 3	20	19.98	99.9		
100% Injection 1	40	39.98	99.95	**MEAN**	99.9
100% Injection 2	40	40.01	100.02		
100% Injection 3	40	39.89	99.7		
150% Injection 1	60	60.2	100.33	**MEAN**	100.06
150% Injection 2	60	59.94	99.9		
150% Injection 3	60	59.98	99.96		

TABLE: 4 Data of Accuracy for Pantoprazole

Concentration % of spiked level	Amount added (ppm)	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery	
50% Injection 1	20	19.82	99.10	**MEAN**	99.70
50% Injection 2	20	19.98	99.95		
50% Injection 3	20	20.01	100.05		
100% Injection 1	40	39.80	99.50	**MEAN**	99.80
100% Injection 2	40	40.10	100.25		
100% Injection 3	40	39.94	99.85		
150% Injection 1	60	60.13	100.21	**MEAN**	100.04
150% Injection 2	60	59.97	99.95		
150% Injection 3	60	59.99	99.98		

PRECISION

The precision of the analytical method was determined by assaying sufficient number of sample and relative standard deviation was calculated and result value was recorded in table 5,6,7 and 8.

Repeatability

a. System precision: Standard solution prepared as per test method and injected five times.

b. Method precision: Prepared six sample preparations individually using single as per test method and injected each solution.

ACCEPTANCE CRITERIA

- The % relative standard deviation of individual Naproxen and Pantoprazole, from the six units should be not more than 2.0%.
- The individual assays of Naproxen and Pantoprazole should be not less than 98% and not more than 102.0%.

(a) System precision

TABLE: 5 Data of Repeatability (System precision) for Naproxen

	Injection	Peak Areas of Naproxen
Concentration	1	820383
40ppm	2	820433
	3	820994
	4	820083
	5	820863
Statistical	**Mean**	820551
Analysis	**SD**	372.412
	% RSD	0.045

TABLE: 6 Data of Repeatability (System precision) for Pantoprazole

	Injection	Peak Areas of Pantoprazole
Concentration	1	284946
40ppm	2	284272
	3	284577
	4	284841
	5	284666
Statistical	**Mean**	2846660
Analysis	**SD**	260.702
	% RSD	0.91

(b) Method precision

TABLE: 7 Data of Repeatability (Method precision) for Naproxen

	Injection	Peak Areas of Naproxen
Concentration	1	820383
40ppm	2	820863
	3	820757
	4	820083
	5	820008
	6	820994
Statistical	**Mean**	820514.7
Analysis	**SD**	417.18
	% RSD	0.05

TABLE: 8 Data of Repeatability (Method precision) for Pantoprazole

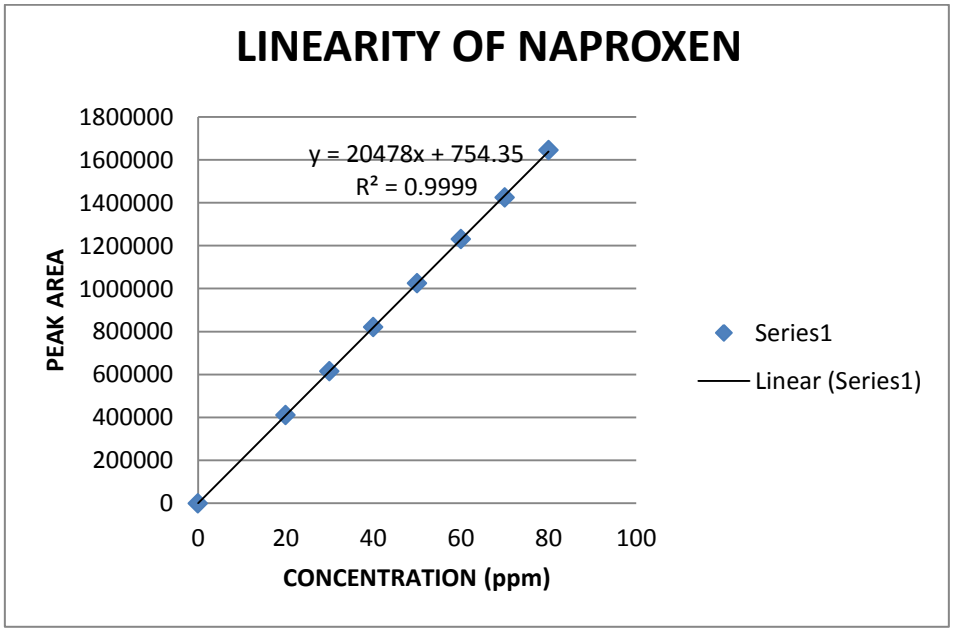
	Injection	Peak Areas of Pantoprazole
Concentration	1	284946
40ppm	2	284666
	3	284307
	4	284841
	5	284222
	6	284577
Statistical	**Mean**	284593
Analysis	**SD**	286.75
	% RSD	0.1

Linearity

A Series of solutions are prepared using Naproxen and Pantoprazole working standards at concentration levels from 20ppm to 80 ppm of target concentration.Measure the peak area response of solution at Level 1 and Level 6 six times. A graph was plotted taking concentration on the x-axis and peak area on y-axis. Recorded was shown in tables 9and 10.

ACCEPTANCE CRITERIA

- Correlation Coefficient should be not less than 0.9990.
- % of RSD for level 1 and Level 6 should be not more than 2.0%.



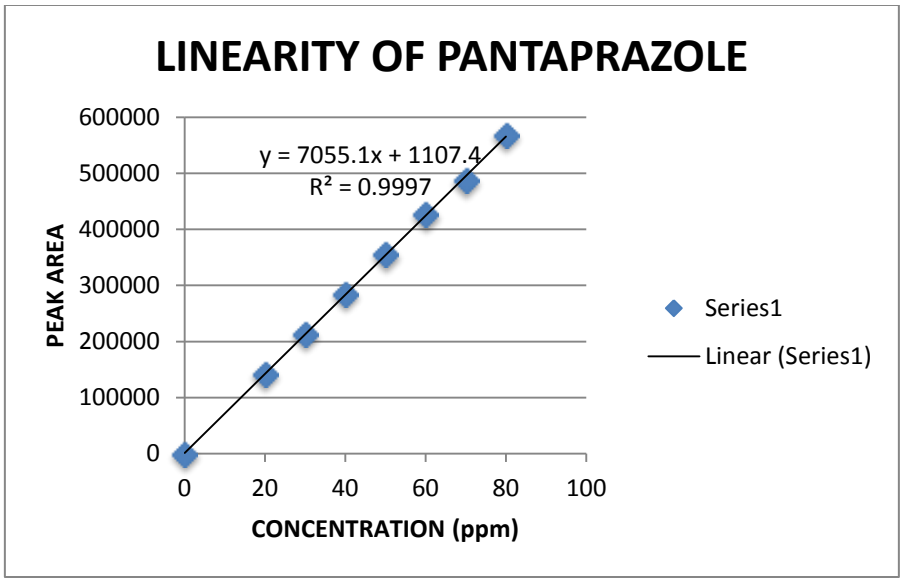


TABLE: 9 Data of Linearity (Naproxen]

Concentration (ppm)	Average Area	Statistical Analysis	
0	0	Slope	20478
20	410805	y-Intercept	754.35
30	615822	Correlation Coefficient	0.9999
40	820623		
50	1025773		
60	1230546		
70	1435164		
80	1644634		

TABLE: 10 Data of Linearity (Pantoprazole)

Concentration (ppm)	Average Area	Statistical Analysis	
0	0	Slope	7055.1
20	142055	y-Intercept	1107.4
30	213367	Correlation Coefficient	0.9997
40	284806		
50	355281		
60	426547		
70	487252		
80	568853		

OBSERVATION

The linear fit of the system was illustrated graphically and recorded result was tables 9and 10.

RUGGEDNESS

a) System to system variability

System to system variability study was conducted on different HPLC systems, under similar conditions at different times. Six samples were prepared and each was analyzed as per test method.

Comparison of both the results obtained on two different HPLC systems, shows that the assay test method are rugged for System to system variability and result was shown in table 11and 12.

ACCEPTANCE CRITERIA

- The % relative standard deviation of Naproxen and Pantoprazole from the six sample preparations should be not more than 2.0%.
- The % assay of Naproxen and Pantoprazole should be between 98.0%-102.0%.

OBSERVATION

The % RSD was found within the limit.

TABLE : 11 Data of system to system variability (Naproxen)

S.NO:	Peak area
1	820383
2	820863
3	820757
4	820083
5	820994
6	820545
Mean	820604.16
%RSD	0.04

TABLE: 12 Data of system to system variability (Pantoprazole)

S.NO:	Peak area
1	284946
2	284666
3	284307
4	284222
5	284577
6	284873
Mean	284598
%RSD	0.1

ROBUSTNESS

a) Effect of variation of flow rate

A study was conducted to determine the effect of variation in flow rate. Standard solution prepared as per the test method was injected into the HPLC system using flow rates 0.8ml/minand 1.2ml/min. The

ACCEPTANCE CRITERIA

The Tailing Factor of Naproxen and Pantoprazole standards should be NMT 2.0 for Variation in Flow.

System suitability parameters were evaluated and found to be within the limits for 0.8ml/min, 1.2ml/min flow.

Naproxen and Pantoprazole was resolved from all other peaks and the retention times were comparable with those obtained for mobile phase having flow rates 1.0ml/min and recorded results was shown in tables 13 and 14.

TABLE: 13 Data for Effect of variation in flow rate (Naproxen)

Flow 0.8 ml	Std Area	Tailing factor	Flow 1.0 ml	Std Area	Tailing factor	Flow 1.2 ml	Std Area	Tailing factor
	1046031	1.207463		820383	1.192208		683632	1.306329
	1046426	1.145488		820863	1.220506		683323	1.207373
	1046645	1.138795		820757	1.173553		683585	1.243551
	1046031	1.207463		820083	1.16098		683632	1.306329
	1046426	1.145488		820008	1.205406		683323	1.207373
Avg	1046311	_	Avg	820418	_	Avg	683499	_
SD	271.47	_	SD	385.5	_	SD	161.8	_
%RSD	0.025	_	%RSD	0.046	_	%RSD	0.023	_

TABLE: 14 Data for Effect of variation in flow rate (Pantoprazole)

Flow 0.8 ml	Std Area	Tailing factor	Flow 1.0 ml	Std Area	Tailing factor	Flow 1.2 ml	Std Area	Tailing factor
	280718	0.895639		284946	0.930582		187326	0.928898
	280317	0.903769		284666	0.927512		187302	0.928628
	280159	0.909391		284307	0.934847		187713	0.925901
	280718	0.895639		284841	0.932795		187326	0.928898
	280317	0.903769		284222	0.920719		187302	0.928628
Avg	280445	_	Avg	284596	_	Avg	187393	_
SD	256.7	_	SD	320.4	_	SD	178.8	_
%RSD	0.09	_	%RSD	0.11	_	%RSD	0.095	_

b) Effect of variation of temperature

A study was conducted to determine the effect of variation in temperature. Standard solution prepared as per the test method was injected into the HPLC system at 20ºC temperature. The system suitability parameters were evaluated and found to be within the limits for a temperature change of 20ºc.

Similarly sample solution was chromatographed at 25ºC temperature.Naproxen and Pantoprazole were resolved from all other peaks and the retention times were comparable with those result are shown in tables 15and 16.

ACCEPTANCE CRITERIA

The Tailing Factor of Naproxen and Pantoprazole standard and sample solutions should be NMT 2.0 for Variation in temperature.

TABLE: 15 Data for Effect of variation in Temperature (Naproxen)

Temp20ºc	Std Area	Tailing factor	Temp27ºc	Std Area	Tailing factor	Temp25ºc	Std Area	Tailing factor
	1046031	1.207463		820383	1.192208		683632	1.306329
	1046426	1.145488		820863	1.220506		683323	1.207373
	1046645	1.138795		820757	1.173553		683585	1.243551
	1046031	1.207463		820083	1.16098		683632	1.306329
	1046426	1.145488		820008	1.205406		683323	1.207373
Avg	1046311	_	Avg	820418	_	Avg	683499	_
SD	271.47	_	SD	385.5	_	SD	161.8	_
%RSD	0.025	_	%RSD	0.046	_	%RSD	0.023	_

TABLE: 16 Data for Effect of variation in Temperature (Pantoprazole)

Temp20ºc	Std Area	Tailing factor	Temp27ºc	Std Area	Tailing factor	Temp25ºc	Std Area	Tailing factor
	280718	0.895639		284946	0.930582		187326	0.928898
	280317	0.903769		284666	0.927512		187302	0.928628
	280159	0.909391		284307	0.934847		187713	0.925901
	280718	0.895639		284841	0.932795		187326	0.928898
	280317	0.903769		284222	0.920719		187302	0.928628
Avg	280445	_	Avg	284596	_	Avg	187393	_
SD	256.7	_	SD	320.4	_	SD	178.8	_
%RSD	0.09	_	%RSD	0.11	_	%RSD	0.095	_

LIMIT OF DETECTION (LOD)

It is the lowest amount of analyte in a sample that can be detected but not necessarily quantities as an exact value under the stated, experimental conclusions. The detection limit is usually expressed as the concentration of analyte.

From the linearity data calculate the limit of detection and quantitation, using the following formula.

$$LOD = \frac{3.3\ \sigma}{S}$$

σ = standard deviation of the response

S = slope of the calibration curve of the analyte.

$$LOQ = \frac{10\ \sigma}{S}$$

σ = standard deviation of the response

S = slope of the calibration curve of the analyte.

Naproxen

From the linearity plot the LOD

$$LOD = 3.3\ \sigma / S$$

$$= \frac{3.3 \times 260.702}{20478} = 0.04$$

PANTOPROZOLE

$$LOD = \frac{3.3\ \sigma}{S}$$

$$= \frac{3.3 \times 320.47}{7055} = 0.14$$

LIMIT OF QUANTIFICATION (LOQ)

The quantitation limit of an analytical procedure is the lowest amount of analyte in a sample which can be

quantitatively determined with suitable precision and accuracy.

Pantoprazole

$$LOQ = \frac{10\,\sigma}{S}$$

$$= \frac{10 \times 320.476}{7055} = 0.45$$

NAPROXEN

$$LOQ = \frac{10\,\sigma}{S}$$

$$= \frac{10 \times 260.702}{20478} = 0.126$$

SUMMARY AND CONCLUSION

A simple, rapid, precise, accurate RP-HPLC method has developed and validated for the simultaneous estimation of Naproxen and Pantoprazole in combined dosage forms. Chromatographic separation was achieved with mobile phase consisting of Methanol:Phosphate Buffer P^H 5.4 in the ratio of 70:30 v/v with Hypercil C18(250 × 4.6 mm × 5 µm),column at a flow rate of 1 mL/minand detection wavelength was 259 nm. The retention times of Naproxen and Pantoprazole was found to be 3.33 min and 1.90 minrespectively. The excipients in the formulation dose not interfere in the estimation of active drug. The determination of the Naproxen and Pantoprazole by RP-HPLC method analysis yielded well resolved peaks with in short time <10min. the studies was conducted at three different concentrate of about 50%, 100%, 150% the valve of standard deviation was satisfactorily low and recovery was close to 100%, the correlation co-coefficient of linearity studies was found to be 0.9999for Naproxen and 0.9997 for pantoprazole.

The method was validated in terms of Linearity, Range, Accuracy, Precision, Specificity, LOD, LOQ, Robustness and system suitability according to ICH guidelines. Commercial Capsule formulation was successfully analyzed using the developed method and the proposed method is applicable to routine analysis for determination of Naproxen and Pantoprazole in Capsule dosage form.

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