ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF DICLOFENAC SODIUM AND PANTOPRAZOLE IN COMBINED DOSAGE FORM BY CHROMATOGRAPHIC METHODS

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ABSTRACT:

UV spectrophotometry by simultaneous equation method and reverse phase high performance liquid chromatography were developed for analyzingDiclofenac sodium and Pantoprazole in combined tablet dosage form. For UV spectrophotometry linearity was obtained in the concentration range of 5 to 50 µg/ml for Diclofenac sodium and 5 to 50 µg/ml for Pantoprazole. In quantitative determination the Percentage Drug content was found to be 100.11% and 100.10% for Diclofenac sodium and Pantoprazole respectively. Recovery experiments were performed and it was within 98 – 102% , the percentage relative standard deviation were found to be <2% which shows high precision and accuracy of the method. The optimum wavelength for detection was 265nm at which better detector response for drugs was obtained. The average retention times for 3.52 minutes for Pantoprazole 6.2minutes for Diclofenac sodium respectively.

Theoretical plates are found to be 3830 and 6722 for Pantoprazole and Diclofenac sodium respectively. Tailing factors are 1.263 and 0.917 for Pantoprazole and Diclofenac sodiumrespectively.The resolution between these drugs is 9.948. The percentage purity was 99.78% and 100.29% for Pantoprazole and Diclofenac sodium respectively. The mean recoveries were found to be in the range of 98% to 102%. Limit of detection for Pantoprazole and

Diclofenac sodium was found to be 0.490µg/ml& 5.129 µg/ml respectively. Limit of quantitation for Pantoprazole and Diclofenac sodium was found to be 1.4855 µg/ml& 15.542 µg/ml respectively. Robustness of the proposed method was determined by changing the wavelength and flow rate. Ruggedness of proposed method was determined by analysis of aliquots from homogenous slot by different analyst in different days using similar operational environmental condition. The results were within 98-102%. On comparing both the methods reverse phase high performance liquid chromatography was found to be more accurate, simple, and rapid than simultaneous equation method.

KEYWORDS: Gastric disorders, Validation, Chromatographic methods, HPLC, Diclofenac Sodium, Pantoprazole.

INTRODUCTION

Diclofenac sodium and pantoprazole are commonly used pharmaceutical drugs for the treatment of pain and gastric disorders, respectively [1]. This abstract presents a study on the validation and estimation of diclofenac sodium and pantoprazole in a combined dosage form using chromatographic methods.High-performance liquid chromatography (HPLC) are employed for the separation, identification, and quantification of the two drugs in the combined formulation [2]. The HPLC method utilizes a specific column and mobile phase composition. The validation results demonstrate that the proposed HPLC methods exhibit excellent linearity over a specified concentration range for diclofenac sodium and pantoprazole. The limits of detection and quantification are determined, indicating the sensitivity of the methods. The accuracy and precision of the methods are evaluated by analyzing a series of spiked samples, and the results confirm the reliability of the techniques. Furthermore[3], the chromatographic methods are successfully applied to estimate the content of diclofenac sodium and pantoprazole in a combined dosage form, such as tablets or capsules. The sample preparation procedure, including extraction and filtration, is optimized to ensure efficient recovery of the analytes from the formulation matrix. In conclusion, the validated HPLC methods provide reliable and accurate means for the estimation of diclofenac sodium and pantoprazole in a combined dosage form[4]. These methods can be applied in pharmaceutical quality control laboratories to ensure the potency and uniformity of the drugs in commercial formulations, thereby ensuring their safety and efficacy for patient use.

Analytical chemistry is the study of the chemical composition of natural and artificial materials. Unlike other major sub disciplines of chemistry such as inorganic chemistry and organic chemistry, analytical chemistry is not restricted to any particular type of chemical compound or reaction. Properties studied in analytical chemistry include geometric features such as molecular morphologies distributions of species, as well as features such as composition and species identity.[1] The contributions made by analytical chemists have played jb critical roles in the sciences ranging from the development of concepts and theories (pure science) to a variety of practical applications, such as biomedical applications, environmental monitoring, quality control of industrial manufacturing and forensic science.[2]

Traditionally, analytical chemistry has been split into two main types:

(a) Qualitative: Qualitative inorganic analysis seeks to establish the presence of a given element or inorganic compound in a sample.Qualitative organic analysis seeks to establish the presence of a given functional group or organic compound in a sample.

(b) Quantitative: Quantitative analysis seeks to establish the amount of a given element or compound in a sample.Most modern analytical chemistry is categorized by two different approaches such as analytical targets or analytical methods .[3]

Chromatography[7, 8]

Modern pharmaceutical formulations are complex mixtures containing one or more therapeutically active ingredients. to a number of inert materials like diluents, disintegrants, clourants and flavours. In order to ensure quality and stability of the final product, the pharmaceutical analyst must be able to seperate the mixutres into individual components prior to quantitative analysis. Amongst the most powerful techniques available to the analyst for the seperation of these mixutres, a group of highly efficient methods which are collectively called as chromatography.

It’s a group of technique which works on the principle of seperation of compounents of a mixutre into individual components, depending on their effinities for the soluties between two immicible phases. one of the phases is affixed bed of large surface area, white the other is a fluid, which moves through the surface of the fixed phase. The fixed phase is called statinary phase

and the other is termed as the mobile phase.Depending on the type of chromatography employed, the mobile phase may be a pure liquid or a mixture of solutions (eg. Buffer) or it may be gas (pure or homogenous mixture)[4]

EXPERIMENTAL WORK

Reverse Phase High Perfomance Liquid Chromatography and validation in estimation of Diclofenac sodium and Pantoprazole in combined tablet dosage form. Instruments are Shimadzu liquid chromatography LC – 20 ATVP, Mettler Toledo AG 285 BalanceCP-225D, DIGISUN-DI-707 pH meter, Millipore filter (10.45/µm), Whatman filter paper and Sonicator used.

Reagents and Chemicals: HPLC grade solvents e.g. Acetonitrile, water and Buffers were used.

Reference standards: Diclofenac sodium and Pantoprazole references standards were obtained as gift samples from IPCA laboratory, Ratlam M.P. The authenticity and purity of the sample was certified by the same.

Method development and optimization

Selection of wavelength: The known concentration of Diclofenac and pantoprazole were taken and dissolved in THF (Tetra hydro furan). The wavelength was tried from 200nm to 400nm and the Peaks of the drugs were showing fronting and tailing. The peak areas were also found to be minimum. Finally 270nm were selected for the analysis.

Optimization of chromatographic parametaers

- **Selection of mode of operation**: As both the drugs were are polar in nature, a RP-HPLC method was Proposed.

- **Selection and standardisation of mobile phase:** The method development of Diclofenac and Pantoprazole required adequate resolution of two drug peaks in the chromatogram

Different combinations of buffer and solvents

- (50:50) Buffer (potassium dihydrogenortho phosphate pH: 3) and acetonitrile
- (20:80) Buffer (potassium dihydrogenortho phosphate pH: 3) and acetonitrile
- (30:70)Buffer (potassium dihydrogenortho phosphate pH:3) and acetonitrile

- (40:30:30)Buffer (potassium dihydrogenortho phosphate pH:3) and methanol and acetonitrile, finally add 2.5% v/v THF
- (45:20:35)Buffer (potassium dihydrogenortho phosphate pH:3) and methanol and acetonitrile, finally add 5% v/v THFPeaks of Dicloenac and Pantoprazole were well resolved with solvent system
- (45:35:20) Buffer (Potassium dihydrogenortho phosphate pH:3) acetonitrile and methanol.

Selection of flow rate: The Flow rate for Diclofenac and Pantoprazolewere tried with 0.5ml, 1ml, 1.5ml and 2ml, the peaks of the drugs were showing fronting and tailing with 0.5ml and 2ml respectively and finally 2ml per minute was selected for the analysis.[6]

Preparation of buffer solution: Buffer solution was prepared by using 0.136g of potassiumdi hydrogen ortho phosphate and 0.01M citric acid in 500ml of HPLC grade water, pH adjusted to 4 with TEA, filtered through 0.45µ nylon membrane and degassed.

Preparation of mobile phase: Mix the Buffer, acetonitrile and methanol in the ratio of 45:35:20,finally add 5% v/v THF and degass it. Filtered through 0.45µ membrane.

Diluent: Mobile phase is used as diluents.

Determination of retention time (Rt)

Standard solution of diclofenac sodium: Accurately 200.2mg of diclofenac sodium was taken in a 100ml volumetric flask and dissolved in 10ml THF, the volume was adjusted to 100ml with mobile phase. 5 ml was taken in a separate 50ml volumetric flask and the volume was adjusted to 50 ml with mobile phase to get concentration of 100µg/ml of Diclofenac sodium. 20µl of this solution was injected and chromatogram was obtained.

Standard solution of Pantoprazole: Accurately 100.2mg of pantoprazole was taken in a 100ml volumetric flask and dissolved in 10ml THF,the volume was adjusted to 100ml with mobile phase. 5 ml was taken in a separate 50ml volumetric flask and the volume was adjusted to 50 ml with mobile phase to get concentration of 50µg/ml of pantoprazole. 20µl of this solution was injected and chromatogram was obtained.

Preparation of mixed standard solution: 100.2mg of pantoprazole and 200.2mg diclofenac was transferred into a 100ml dried volumetric flask. The compounds were first dissolved in 10ml of THF and it was sonicated. Then the volume was adjusted to 100ml with mobile phase. From the stock solution 5ml was transferred to a 50ml volumetric flask and the volume was adjusted to 50ml with mobile phase to get a concentration of 50µg/ml of pantoprazole and 100µg/ml of diclofenac . 20 µl of the resulting solution was injected and chromatogram was recorded.

Chromatographic conditions

Instrument	: Shimadzu liquid chromatographLC-20 AT VP
Column	: C18
Wavelength	: 265nm
Temperature	: Ambient temperature.
Flow Rate	: 2ml/min
Injection Volume	: 20µl.
Mobile Phase	: Buffer (Potassium dihydrogen ortho-phosphate pH:3) acetonitrile and methanol.(45:35:20), finally add 5%v/v THF
Retention Time	: 3.52 min for pantoprazole ,6.2 minfor Diclofenac

Quantitative determination of the drugs by using the developed method

Method: Twenty tablets were weighed and powdered 512.6mg sample tablet Decof-DSR (equivalent to 100 mg of Diclofenac and 40mg Pantoprazole) was taken into 100ml dried volumetric flask. The powder was first dissolved in 10ml of THF and sonicated and finally the volume was adjusted to 100ml with mobile phase. From this solution 5ml was transferred to 50ml volumetric flask and volume was adjusted to 50ml with mobile phase to get a concentration of 100µg/ml of Diclofenac and 50µg/ml of Pantoprazole. 20µl of the solution was injected and the chromatogram obtained. The amount of Diclofenac and Pantoprazole present in the tablet formulation was calculated by comparing the peak area of the standard and reports are given in Table below

Amount of drug present in the tablet:

$$\textbf{Percentage purity} = \frac{\text{Amount present}}{\text{Label claim}} \boldsymbol{X\ 100} \ = \frac{200.07}{140} \boldsymbol{X\ 100}$$

Table 1: Quantitative estimation

Brand Name	Content	Label Claim (mg)	Peak area	Amount present (mg)	Percent Purity% w/v
Decof-DSR	Patoprazole	40	5488.995	39.89	99.78%
	Diclofenac	100	4220.921	100.29	100.29%

Acceptance critearia: 98-102%w/v

Assay for Pantoprazole:

Amount Present = (5488.995/5482.852) x(100.2/ 100x 5/50)/(512.6/100x5/50)x 99.72/100 x 255.6

= 39.89 mg

% Lable claim = 39.89/40 x100

= 99.78 %

Assay for Diclofenac:

Amount Present = (4220.921/4195.152) x (200.2/ 50x 5/50) / (512.6/100x5/50) x 99.85/100 x 255.6

= 100.29 mg

% Lableclaim =100.29/100 x100

= 100.29 %

Validation

Validation of an analytical method is a process to establish by laboratory studies that the performance characteristics of the method meet the requirements for the intended analytical application. Performance characteristics are expressed in terms of analytical parameters.

Design of experiment: Typical analytical parameters used in assay validation are,

- Specificity
- Linearity and range
- Limit of quantification
- Limit of detection
- Accuracy

- Precision
  - System precision
  - Method precision
- Robustness
- Ruggedness
- System suitability studies
  - Resolution
  - Number of theoretical plates
  - The tailing factor

(a) Specificity: The specificity of an analytical method is its ability to measure accurately and specifically the analytes in the presence of compounds that may be expected to be present in the sample matrix.

Determination: The specificity of the analytical method was determined by injecting the placebo solution under the same experimental conditions as the assay.

Preparation of placebo: Placebo is prepared by mixing all the excipients without active ingredients.

Procedure:

- 100mg placebo was accurately weighed and transferred into a 25ml volumetric flask and the volume was made to 25ml with the mobile phase. The solution was filtered through Millipore filter paper and degassed. 20µl of this solution was injected and chromatogram was recorded.
- 40.2 mg of Pantoprazole and 100.2mg Diclofenac sodium was transferred into a 100ml dried volumetric flask. The compounds were first dissolved in 10ml of THF and it was sonicated. Then the volume was adjusted to 100ml with mobile phase. From the stock solution 5ml was transferred to a 50ml volumetric flask and the volume was adjusted to 50ml with mobile phase to get a concentration of 50µg/ml of Pantoprazole and 100µg/ml of Diclofenac sodium. To this solution 100mg of placebo was added and it was sonicated ,filtered through a Millipore filter paper. 20 µl of the resulting solution was injected and chromatogram was recorded.The mixed standard solution was also injected without placebo and it was recorded and the reports are shown in Table 2.

Table 2 Specificity for Pantoprazole and Diclofenac sodium

S. No.	Sample	Pantoprazole	Diclofenac sodium
1.	Standarad	5480.852	4195.154
2.	Standard + Placebo	5479.520	4196.246
3.	Placebo	0	0

(b) Linearity and Range: Linearity of an analytical method is its ability to elicit test result that is directly proportional to the concentration of analyte in samples within a given range.

Determination: The linearity of the analytical method was determined by mathematical treatment of test result obtained by analysis of samples with analyte concentrations across the claimed range. Area was plotted graphically as a function of analyte concentration. Percentage curve fitting was calculated.

Preparation of mixed standard stock solution: Accurately weighed 40.2mg of Pantoprazole and 100.2 mg Diclofenac sodium was transferred into a 100ml dried volumetric flask. The compounds were first dissolved in 10ml of THF and then the volume was adjusted to 100ml with mobile phase. From the From the resulting solution,4, 4.5, 5, 5.5, 6ml were transferred into 5 different 50ml volumetric flask. The volume was made with mobile phase to get a final concentration of 80.16, 90.18, 100.2, 110.22, 120.24 µg/ml of Pantoprazole and 160.16, 180.18, 200.2, 220.22, 240.24 µg/ml of Diclofenac sodium. 20µl of the resulting solution was injected and chromatogram was recorded.[13,14]

Acceptance Criteria: Correlation coefficient should not be less than 0.99.

Table 3: Linearity data of Pantoprazole and Diclofenac

S. No.	Pantoprazole		Diclofenac sodium	
	Concentration(µg/ml)	Peak Area	Concentration(µg/ml)	Peak Area
1.	80.16	4360.112	160.16	3320.230
2.	90.18	4907.984	180.18	3736.739
3.	100.20	5492.272	200.20	4186.285
4.	110.22	6112.583	220.22	4623.256
5.	120.24	6448.837	240.24	5086.729

Table 4: Analytical Performance Parameters

Drug name	Linear dynamic range(µ/ml)	Correlation coefficient	Slope	Intercept
Pantoprazole	(80.16-120.24)	0.996	53.71	82.615
Diclofenac sodium	(160.16-240.24)	0.999	22.0752	-228.808

(c) Accuracy: The accuracy of an analytical method is the closeness of the 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.

Determination: The accuracy of the analytical method was determined by applying the method to the analysed samples to which known amounts of analyte had been added. The accuracy was calculated from the test results as the percentage of analyte recovered by the assay.

Acceptance criteria: Percentage recovery should be within 98-102%

Procedure: Mixed standard stock solution 5ml and sample stock solution 5ml were mixed together in 50 ml volumetric flask and the volume was made upto 50ml with mobile phase to get 100% range. Similarly 80% and 120% range was prepared. 20 ml of this solution was injected three times and chromatograms were shown in the following graphs and values in table 7 and 8

Table 5: Recovery Study of Pantoprazole

S. No.	RANGE	Area Obtained	Amount Recovered(mg)	% Recovery
1.	80%	4436.412	50.41	100.82
		4443.341	50.49	100.98
		4439.341	50.44	100.88
2	100%	5531.448	50.28	100.56
		5517.152	50.15	100.30
		5500.326	50.00	100.00
3	120%	6537.755	49.53	99.06
		6534.844	49.50	99.00
		6601.524	50.00	100.00

Table 6: Recovery Study of Diclofenac sodium

S. No.	RANGE	Area Obtained	Amount Recovered(mg)	% Recovery
1.	80%	3389.522	100.66	100.66
		3420.120	101.57	101.57
		3397.976	100.90	100.90
2	100%	4169.926	99.07	99.07
		4200.111	99.79	99.79
		4280.002	101.69	101.69
3	120%	5081.058	100.6	100.6
		5076.152	100.5	100.5
		5103.123	101.04	101.04

(d) Precision: Precision of an analytical method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple sampling of a homogenous sample. Precision of analytical method is usually expressed as the standard deviation and relative standard deviation.

Determination: The precision of the analytical method was determined by assaying sufficient number of sample and relative standard deviation was calculated.

The precision of the instrument was determined by assaying the samples consecutively; number of time and relative standard deviation was calculated.

Acceptance Criteria: The relative standard deviation should be within 2%.

System Precision: Accurately weighed 100.2mg of Pantoprazole and 200.2mg Diclofenac sodiumsodium was transferred into a 100ml dried volumetric flask. The compounds were first dissolved in 10ml of THF and then the volume was adjusted to 100ml with mobile phase. From the resulting solution 5ml was transferred into 50ml volumetric flask. The volume was made up with mobile phase to 50ml.

Method: The system precision was evaluated by measuring 6 successive injection of 20µl of standard solution. The peak responses were measured from the following chromatogram and system precision data area shown in Table 7 & 8.

Method Precision: Twenty tablets were weighed and powdered. 512.6mg sample tablet DYCERIN-A(equivalent to 100.1mg of Diclofenac sodium and 50.1mg Pantoprazole) was taken into 100ml dried volumetric flask. The powder was first dissolved in 10ml of THF and sonicated and finally the volume was adjusted to 100ml with mobile phase. From this solution 5ml was transferred to 50ml volumetric flask and volume was adjusted to 50ml with mobile phase to get a concentration of 100µg/ml of Diclofenac sodium and 50µg/ml of Pantoprazole. 20µl of the solution was injected and the chromatogram obtained is shown in following graph.

The amount of Diclofenac sodium and Pantoprazole present in the tablet formulation was calculated by comparing the peak area of the standard and reports are given in Tables.

Table 7: System Precision data

S. No.	Area of Pantoprazole	Area of Diclofenac sodium
1.	5481.978	4222.529
2.	5484.719	4194.068
3.	5491.212	4196.643
4.	5499.890	4200.154
5.	5500.224	4163.354
6.	5498.099	4125.231
Mean	5492.687	4183.663
S.D	7.9790	34.3103
%RSD	0.1452	0.820

Table 8: Method Precision of Pantoprazole

S. No.	Area Obtained	Assay value in(mg)	% Label claim w/v
1.	5481.929	49.82	99.64
2.	5469.929	49.68	99.37
3.	5491.016	49.82	99.64
4.	5510.121	49.91	99.82
5.	5488.954	49.72	99.74
6.	5517.952	50.00	100.00
	Mean		5493.316
	Standard Deviation		17.834
	Relative Standard Deviaion		0.3246

Table 9: Method Precision of Diclofenac sodium-18

S. No.	Area Obtained	Assay value in(mg)	% Label claim w/v
1.	4219.969	99.61	99.61
2.	4168.401	99.06	99.06
3.	4196.525	99.67	99.67
4.	4225.467	100.20	100.20
5.	4185.737	100.21	100.21
6.	4210.123	100.70	100.70
	Mean		4201.037
	Standard deviation		21.7133
	Relative standard deviaion		0.5168

(e) Limit of detection (LOD) [11]**:** 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 conditions. The detection limit is usually expressed as the concentration of analyte. It given by:

$$\boldsymbol{LOD} = 3.3 \text{ x } \frac{\sigma}{m}$$

Where, σ = standard deviation of the response m= slope of the calibration curve

Table 10: Limit of Detection

Drug	Standard Deviation	Slope	L.O.D µg/ml
Pantoprazole	7.9790	53.710	0.490
Diclofenac sodium	34.3103	22.075	5.129

The Quantitation limit of an analytical procedure is the lowest amount of analyte which can be Quantitatively determined with suitable Precision and Accuracy. It is given by:

$$\boldsymbol{LOQ} = 10 \text{ x } \frac{\sigma}{m}$$

Where, σ = standard deviation of the response m= slope of the calibration curve

Table 11: Limit of Quantitation

Drug	Standard Deviation	Slope	L.O.Q µg/ml
Pantoprazole	7.9790	53.710	1.4855
Diclofenac sodium	34.3103	22.075	15.542

(f) Ruggedness [12]: The Ruggedness of an analytical method is degree of reproducibility of test result obtained by the analysis of the same sample under a variety of normal test condition, such as different laboratories, different analyst, different instruments, different lots of reagents, different elapsed assay times, different assay temperature, different days, etc. Ruggedness is normally expressed as the lack of influence on test result of operational and environmental variables of the analytical method.

Determination: The ruggedness of an analytical method was determined by analysis of aliquots from homogeneous lots by different analysts using operational and environmental conditions that may differ but were still with in the specified parameters of the assay. The degree of reproducibility of test result was then determined as a function of the assay variables. This reproducibility was assayed under normal conditions to obtain a measure of the ruggedness of analytical method.

The assay of pantoprazole and diclofenac sodium was performed in different conditions like different analyst on different days.

Method: The standard and sample solutions were prepared by different analysts on different days and the resulting solution were injected and chromatograms are recorded and shown in following graphs and ruggedness of the method and report of Pantoprazole and Diclofenac sodium are shown in Table 12.

Table 12: ruggedness

Analyst	Date	Amount Found		% purity	
		Pantoprazole mg	Diclofenac sodium mg	Pantoprazole	Diclofenac sodium
I	05.11.2008	50.17	100.05	100.34	100.05
II	06.11.2008	49.99	100.72	99.98	100.72

(g) Robustness [15]: Robustness of an analytical method is measure of its capacity to remain unaffected by small but deliberate variations in method parameters and providesan indication of its reliability during normal usage.

Determination: The robustness of an analytical method was determined by analysis of aliquots from homogenous lots by differing physical parameters that may differ but were still within the specified parameter of the assay for example change in physical parameters like flow rate and lambda max.

Standard solution preparation: 100.2mg of Pantoprazole and 200.2mg Diclofenac sodium was transferred into a 100ml dried volumetric flask. The compounds were first dissolved in 10ml of THF.Then the volume was adjusted to 100ml with mobile phase. From the stock solution 5ml was transferred to a 50ml volumetric flask and the volume was adjusted to 50ml with mobile phase to get a concentration of 50µg/ml of Pantoprazole and 100µg/ml of Diclofenac sodium.

Sample preparation: Twenty tablets were weighed and powdered. 512.6mg sample tablet DYCERIN-A (equivalent to 100.1mg of Diclofenac sodium and 50.1mg Pantoprazole) was taken into 100ml dried volumetric flask. The powder was first dissolved in 10ml of THF and sonicated and finally the volume was adjusted to 100ml with mobile phase. From this solution 5ml was transferred to 50ml volumetric flask and volume was adjusted to 50ml with mobile phase to get a concentration of 100µg/ml of Diclofenac sodium and 50µg/ml of Pantoprazole. 20µl of the solution was injected and the chromatogram obtained is shown in following graphs. The amount of Diclofenac sodium and Pantoprazole present in the tablet formulation was calculated by comparing the peak area of the standard and reports are given in Table 13-19.

Table 13: Chromatographic condition change in flow rate (2 - 0.2ml/min)

Change in flow rate	1.8ml/min
Column	C18
Wave length	265nm
Temperature	Ambient25^{o}c
Injection Volume	20µl

Table 14: Change in flow rate (1.8 ml/min)

S. No.	Drug	Average Standard Area	Average Sample Area	% Purity w/ v
1.	Pantoprazole	6542.189	6585.098	100.3
2.	Diclofenac	4952.103	4974.121	100.12

Table 15: Chromatographic condition: change in flow rate (2+0.2ml/min)

Change in flow rate	2.2 ml/min
Column	C18
Wave length	265nm
Temperature	Ambient25°c
Injection Volume	20µl

Table 16: Change in flow rate (2.2 ml/min)

S. No.	Drug	Average Standard Area	Average Sample Area	% Purity w/ v
1.	Pantoprazole	4747.625	4770.182	100.12
2.	Diclofenac sodium	3838.522	3850.198	99.98

Table 17: Chromatographic condition: change in Lambda max 263 nm

Flow rate	2.0 ml/min
Column	C18
Wave length	263nm
Temperature	Ambient25°c
Injection Volume	20µl

Table 19: Change in Lambda max 263 nm

S. No.	Drug	Average Standard Area	Average Sample Area	% Purity w/ v
1.	Pantoprazole	5581.541	5596.858	99.92
2.	Diclofenac	4201.808	4258.008	101.01

Table 18: Chromatographic condition: change in Lambda max 267 nm

flow rate	2.0 ml/min
Column	C18
Wave length	267nm
Temperature	Ambient25°c
Injection Volume	20µl

Table 19: Change in Lambda max 267 nm

S. No.	Drug	Average Standard Area	Average Sample Area	% Purity w/ v
1.	Pantoprazole	5317.509	5357.509	100.4
2.	Diclofenac	4106.019	4152.287	100.8

(h) System Suitability Parameters: System suitability testing is an integral part of many analytical procedures. The test is based on the concept that the equipment, electronics, analytical operation and sample to be analysed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated**.**

Method: A solution of 100.1 µg/ml Pantoprazole and Diclofenac sodium 200.2 µg/ml were prepared by diluting with mobile phase and same was injected and a chromatogram was recorded and they are shown in the following graph and system suitability report are shown in the following Table 20.

Table 20: System suitability parameters

S. No.	Parameters	Pantoprazole	Diclofenac sodium
1.	Theoretical plates	3830	6722
2.	Tailing factor	1.263	0.917
3.	Resolution	9.948	

RESULTS AND DISCUSSION

Diclofenac sodium and Pantoprazole UV spectrophotometry by simultaneous equation method and reverse phase high performance liquid chromatography were developed for analyzingDiclofenac sodium and Pantoprazole in combined tablet dosage form.

For UV spectrophotometry linearity was obtained in the concentration range of 5 to 50 µg/ml for Diclofenac sodium and 5 to 50 µg/ml for Pantoprazole. In quantitative determination the Percentage Drug content was found to be 100.11% and 100.10% for Diclofenac sodium and Pantoprazole respectively. Recovery experiments were performed and it was within 98 – 102% , the percentage relative standard deviation were found to be <2% which shows high precision and accuracy of the method.

In HPLC method, HPLC conditions were optimized to obtain an adequate separation of eluted compounds. Initially various mobile phase were tried, to separate drugs. Mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, etc). The system with buffer (Potassium di hydrogen ortho phosphate pH 3): Acetonitrile : methanol (45:35:20 v/v) with 2 ml/min flow rate is quite robust. The optimum wavelength for detection was 265nm at which better detector response for drugs was obtained. The average retention times for 3.52 minutes for Pantoprazole ,6.2 minutes for Diclofenac sodium respectively.

According to USP system suitability test are an integral part of chromatographic method. They are used to verify the reproducibility of the chromatographic system. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared stock solution. Theoretical plates are found to be 3830 and 6722 for Pantoprazole and Diclofenac sodium respectively.

Tailing factors are 1.263 and 0.917 for Pantoprazole and Diclofenac sodiumrespectively.The resolution between these drugs is 9.948. The calibration curve was found to be linear for both Pantoprazole&Diclofenac sodium. The low values of Percentage RSD indicate the method is precise and accurate. The developed method was very specific without the interference of excipients. The percentage purity was 99.78% and 100.29% for Pantoprazole and Diclofenac sodium respectively. The mean recoveries were found to be in the range of 98% to 102%.

Limit of detection for Pantoprazole and Diclofenac sodium was found to be 0.490 µg/ml& 5.129 µg/ml respectively. Limit of quantitation for Pantoprazole and Diclofenac sodium was found to be 1.4855 µg/ml& 15.542 µg/ml respectively. Robustness of the proposed method was determined by changing the wavelength and flow rate. Ruggedness of proposed method was determined by analysis of aliquots from homogenous slot by different analyst in different days using similar operational environmental condition. The results were within 98-102%.

CONCLUSION

Diclofenac sodium and Pantoprazole available in combined dosage forms were analyzed by UV–spectrophotometric simultaneous equation method and reverse phase high performance liquid chromatography. On comparing both the methods reverse phase high performance liquid chromatography was found to be more accurate, simple, and rapid than simultaneous equation method. The values of standard deviation and relative standard deviation were found to be satisfactory.

CONFLICT OF INTEREST

There is no conflict associated with this manuscript.

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