Online : ISSN 2349-669X
Print : ISSN 0973-9874

Validated UV-Spectrophotometric Method for Simultaneous Analysis of Aceclofenac and Pantoprazole in Bulk and Pharmaceutical Dosage Forms

SHAILENDRA SURYAWANSHI SANJAY[1*], ZARANAPPA[1], CHALUVARAJU K C[1], SARVESH[2], AND NAGESH K P[1]

[1]Department of Pharmaceutical Chemistry, [2]Department of Pharmaceutics, Government College of Pharmacy, Bengaluru-560027.

ABSTRACT

Current research work attempts to develop simple, precise, accurate, specific, cost effective, less time consuming validated UV-Spectrophotometric method for the simultaneous analysis of Aceclofenac (ACECLO) and Pantoprazole (PANTO) in bulk and pharmaceutical dosage forms by simultaneous equation method. Spectrophotometric simultaneous analysis of ACECLO and PANTO were carried out using phosphate buffer pH 6.8 as economical solvent. The analytical wavelengths for ACECLO and PANTO are 273 nm and 289 nm respectively. The developed UV-Spectrophotometric method was validated as per ICH guidelines in terms of linearity, precision, accuracy, specificity, sensitivity and ruggedness. Linearity was obtained in the concentration range of 5-30 µg/mL for ACECLO and 4-20 µg/mL for PANTO. The % RSD for intraday precision and interday precision of ACECLO was found to be 0.183 and 0.317 respectively. An intraday precision and interday precision of PANTO was found to be 0.194 and 0.894 respectively. In both cases values were within the acceptance limit of less than 2%. The mean percent recovery for ACECLO and PANTO were found to be 98.20 % and 100.56 % respectively. From the high recovery values it can be inferred that the method is free from the interference of excipients used in the formulation. Based on the results obtained the proposed method can be regarded as simple, precise, accurate, reliable and cost effective and can be used for routine quality control of ACECLO and PANTO in bulk and pharmaceutical dosage forms.

Key words: Aceclofenac, Pantoprazole, UV-spectrophotometry, ICH guidelines, Simultaneous analysis

Introduction

Aceclofenac, chemically, a phenyl acetic acid derivative, has anti-inflammatory and analgesic properties. It is a potent inhibitor of cyclo-oxygenase enzyme which involved in the production of prostaglandins[1]. Acecclofenac is practically insoluble in water and soluble in alcohol & methyl alcohol, freely soluble in acetone &dimethyl formamide. The chemical structure of Aceclofenac is (Fig.1).

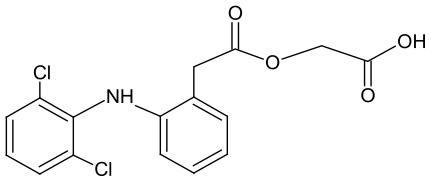

Fig. 1: Chemical structure of Aceclofenac

Pantoprazole, chemically, 5-Difluoromethoxyl Benzimidazole -2-yl-3,4-dimethoxy-2-pyridyl methyl sulfoxide . Pantoprazole, is a class of substituted Benzimidazole belongs to long acting proton pump inhibitor. It acts by suppressing gastric acid secretion through the inhibition of H^+ K^+ATPase at the secretory surface of the parietal cells and blocks the final step of gastric acid secretion. It is more acid stable proton pump inhibitor and has higher bioavailability than omeprazole. It is well absorbed from the Gastro Intestinal Tract (GIT). Its bioavailability is 77% and shows dose dependent response [3]. The chemical structure of Aceclofenac is (fig.2).

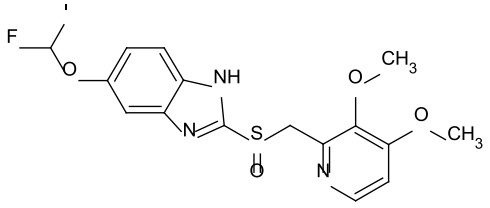

Fig 2: Chemical structure of Aceclofenac

*Address for correspondence: shailendrasss80@gmail.com

Literature survey revealed that various methods such as UV-spectrophotometry [3,4,5], visible spectrophotometry [6-8], RP-HPLC [9-12], HPTLC [13-16] for Aceclofenac and UV-spectrophotometry [17-20], RP- HPLC [21-25] for the estimation of Pantoprazole were reported individually and combination with other drugs. But no method was developed and validated for the simultaneous estimation of Aceclofenac and Pantoprazole in combination.

In view of the above facts in the present study, an attempt has been made to estimate the Aceclofenac and Pantoprazole simultaneously in bulk and pharmaceutical formulations using UV-Spectrophotometric method.

MATERIALS AND METHODS

Instruments used

Electronic analytical balance; UV-Spectrophotometer, Ultrasonic bath Sonicator were used in the study.

Reagents and chemicals

Aceclofenac and Pantoprazole standards were obtained as gift sample from Anglo French Drugs and Industries Ltd., Bengaluru. All the chemicals used were of AR grade and are obtained from the stores of Government College of Pharmacy, Bengaluru. ACECLO and PANTO tablet dosage form manufactured by Nov Nortis were procured from local pharmacy store.

Selection of solvent

By carrying out solubility profile study and literature survey it was found that ACECLOand PANTO are soluble in phosphate buffer with pH 6.8. Hence phosphate buffer having pH6.8 was chosen for the UV-Spectrophotometric analysis of ACECLO and PANTO.

Preparation of standard stock solutions

10 mg of each pure sample of ACECLO and PANTO were weighed separately, into two 10 mL volumetric flasks. Then small amount of phosphate buffer was added to dissolve the drugs and then the volume was made upto 10 mL to get a concentration of 1 mg/mL.

Selection of analytical wavelength

From the above standard stock solutions, 0.1 mL aliquots was taken separately into two 10 mL volumetric flask and diluted upto the mark with phosphate buffer and these solutions were scanned in the UV region of 200-400 nm. Maximum absorbance was seen at the wavelength of 273 nm for ACECLO and 289 nm for PANTO. Hence all absorbance measurements were made at 273 nm for ACECLO and 289 nm for PANTO.

Calibration Curve

A series of dilutions were prepared from the standard stock solutions of ACECLO and PANTO to obtain the concentration of 5-30 µg/mL of ACECLO and 4-20 µg/mL of PANTO. Absorbance of the above solutions was measured at 273 nm and 289 nm for ACECLO and PANTO respectively and a calibration curve of absorbance against concentration was plotted and the regression coefficient (R^2) was also determined.

Determination of absorptivity coefficient

The absorptivity coefficient of both drugs (ACECLO and PANTO) was determined at selected wavelengths by using the formula: **$A=A\ (^{1\%}_{1}\ cm)\ b\ x\ c$.**where, c = concentration of the absorbing species, in g/100 mL and b = path lengthin cm. The absorptivity values are then substituted in the following equations (1) and (2):

$$A_1 = ax1\ Cx + ay1\ Cy \quad \text{.....(1)}$$

$$A_2 = ax2\ Cx + ay2\ Cy \quad \text{.....(2)}$$

Where,

A_1 and A_2 are absorbances of sample at 273 nm and 289 nm respectively. ax1and ax2 are absorptivities of ACECLO at 273 nm and 289 nm respectively. ay1 and ay2 are absorptivities of ACECLO and PANTO at 273 nm and 289 nm respectively. Cx and Cyare concentrations of ACECLO and PANTO respectively.

Preparation of sample solutions

Average weight of twenty tablets containing 100 mg of ACECLO and 40 mg of PANTO (labeled claim) was calculated separately. The tablets were powdered well in glass morter with pestal. Quanity of powder equivalent to 100 mg of ACECLO and 40 mg of PANTO was weighed accurately and transferredseparately into 25 mL volumetric flasks. Then a small quantity of phosphate buffer was added and sonicated for 30 minutes to dissolve the drugs completely and then the volume was made upto the mark with phosphate buffer and filtered through 0.45 µm membrane filter. From this, 0.1 mL was taken and diluted with phosphate buffer. The absorbance of this solution was measured at 273 nm and 289 nm against phosphate buffer as a blank. The assay was performed in triplicate.

Analysis of tablet dosage form

Aliquots portion of the above sample stock solution was diluted with phosphate buffer and the absorbance was measured at appropriate wavelength and the concentration of the two drugs were determined using equations (3) and (4). Analysis was done in triplicate.

$$Cx = (A_2\ ay1 - A_1\ ay2) / (ax2\ ay1 - ax1\ ay2) \quad \text{..... (3)}$$

$$Cy = (A_1\ ax2 - A_2\ ax1) / (ax2\ ay1 - ax1\ ay2) \quad \text{..... (4)}$$

METHOD VALIDATION

The developed UV-Spectrophotometric method was validated as per ICH guidelines in terms of linearity, precision, accuracy, ruggedness, specificity, Limit of Quantification and Limit of Detection.

Linearity

A series of solutions were prepared using ACECLO and PANTO standard stock solutions at concentration range of 5-30 µg/mL and 4-20 µg/mL respectively. The absorbances of the resultant solutions were measured at 273 nm and 289 nm against phosphate buffer as blank. The calibration curves were constructed by plotting concentrations on x-axis and absorbance on y-axis. R^2 value not less than 0.999 was regarded as acceptance criteria.

Precision

Precision was studied to find out intra-day and inter-day variations in the test method of ACECLO and PANTO. Intra-day assay precision was found by analysis of standard drug thrice on the same day in different intervals of time. Inter-day assay precision was carried out at three different days and percentage relative standard deviation (% RSD) was calculated. The % RSD should not be more than 2.0%.

Accuracy

The accuracy of the developed method was determined by recovery studies at three different levels. The preanalyzed samples were spiked with 50, 100 and 150 % of mixed standard solution. The mixtures were analyzed and the recoveries were determined. The recovery study was carried out in triplicate. The mean % recovery of the ACECLO and PANTO at each levels should not be less than 98.0 % and not more than 102.0% was considered as the acceptance criteria.

Specificit

Specificity was performed to exclude the possibilities of interference of solvent in the region of maximum absorbance peaks of ACECLO and PANTO. The specificity of the method was tested under the normal conditions and results of the tests proved that the components other than ACECLO and PANTO did not produce the detectable peaks at the maximum absorbance peaks of both the drugs.

Sensitivity

Sensitivity of proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantification (LOQ). The LOD and LOQ of ACECLO and PANTO by proposed methods were determined using calibration standards. LOD and LOQ were calculated as 3.3s/S and 10s/S respectively, where S is the slope of the calibration curve and s is the standard deviation of response.

Ruggedness

The ruggedness expresses the variations within the laboratory conditions (different day, different analyst and different instrument.

RESULTS

The maximum absorbance of ACECLO and PANTO was found to be 273 nm and 289 nm in phosphate buffer with pH 6.8. The spectrum of ACECLO and PANTO were shown in the **Figure 3-4** respectively. The standard calibration curves, linearity range and absorptivity values of ACECLO and PANTO are presented in **Table 1-3**and **figure 5-6**. The results of recovery studies are depicted in **Table 3-4**. The precision results are presented in **Table 6**. Sensitivity data for ACECLO and PANTO are presented in **Table 7**. The assay results of ACECLO and PANTO are presented in **Table 8**. The summary of all validation parameters are presented in **Table 9.**

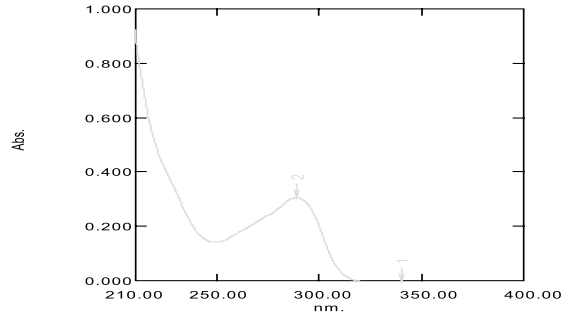

Fig. 3: UV Spectrum of Aceclofenac

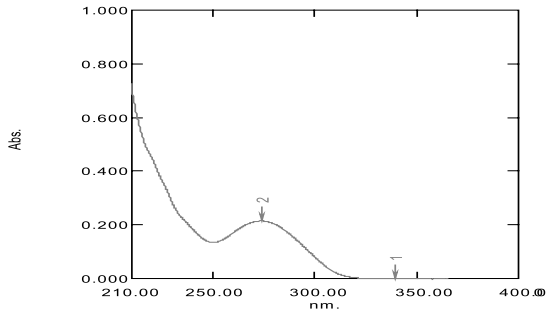

Fig. 4: UV Spectrum of Pantoprazole

Table - 1
Calibration curve Data of Aceclofenac and Pantoprazole

Conc of ACECLO	Absorbance at 273 nmµg/mL	Conc of PANTO	Absorbance at 289 nmµg/mL
5	0.153	0	0.00
10	0.306	4	0.184
15	0.463	8	0.369
20	0.612	12	0.556
25	0.748	16	0.736
30	0.873	20	0.896

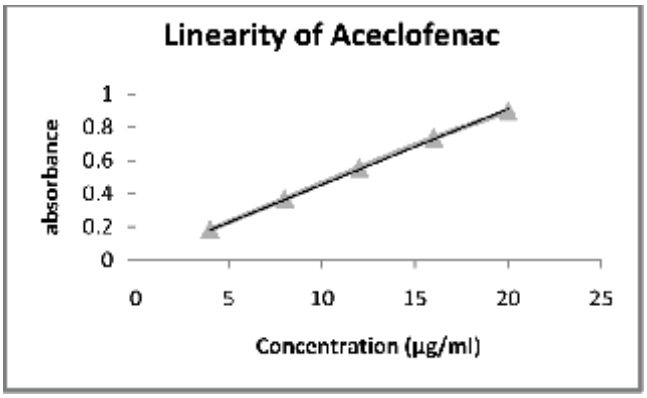

Fig. 5: Calibration Curve of Aceclofenac

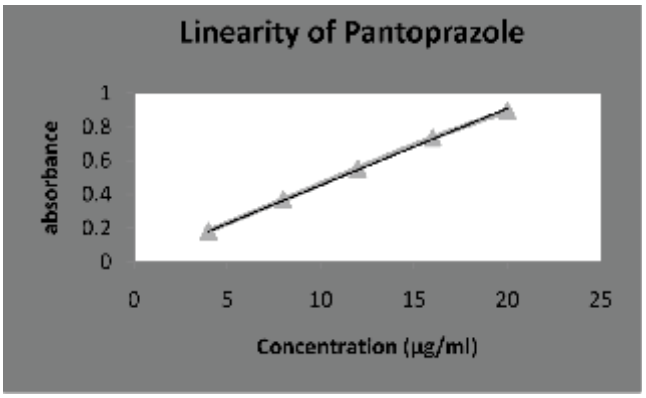

Fig.6: Calibration Curve of Pantoprazole

Table - 2
Linerity Data of Aceclofenac and Pantoprazole

Parameter	ACECLO	PANTO
Linearity Range	µg/mL 5-30	µg/mL 4-20
Rsed	0.01	0.01
R^2	0.9998	0.9998
P-value	0.73	0.73

Table - 3
Absortpivity values of Aceclofenac and Pantoprazole

Drugs	Absorptivity at 273 nm (ax_1)	Absorptivity at 289 nm (ax_2)
Aceclofenac	302.81	255.75
Pantoprazole	196.25	462.16

Table - 4
Accuracy study Data of Aceclofenac

Sample No.	Spike Levels	Amount Added (µg/mL)	Amount Found (µg/mL)	% Recovery	Statistical Analysis
1	50 50 50	5 5 5	4.981 4.979 4.989	99.62 99.58 99.58	**Mean** = 99.59 **SD** = 0.02 **%RSD** = 0.02
2	100 100 100	10 10 10	9.980 9.992 10.142	99.80 99.92 101.42	**Mean**= 100.38 **SD** = 0.90 **%RSD** = 0.90
3	150 150 150	15 15 15	15.150 14.986 14.992	101.00 99.90 99.946	**Mean**= 100.28 **SD**= 0.62 **%RSD** = 0.62

Table - 5
Accuracy study Data of Pantoprazole

Sample No.	Spike Levels	Amount Added (µg/mL)	Amount Found (µg/mL)	% Recovery	Statistical Analysis
1	50 50 50	4 4 4	3.027 2.973 2.973	100.90 99.11 99.11	**Mean**= 99.71 **SD** = 1.03 **%RSD** = 1.04
2	100 100 100	8 8 8	7.993 7.983 8.037	99.91 99.78 100.46	**Mean**= 100.05 **SD** = 0.36 **%RSD** = 0.36
3	150 150 150	12 12 12	11.270 11.293 11.023	102.45 102.66 100.190	**Mean**= 101.27 **SD** = 1.37 **%RSD** = 1.35

Table - 6
Precision study Data of Aceclofenac and Pantoprazole

Aceclofenac Concentration (µg/mL)	Intra day Precision (%RSD)	Inter day precision (%RSD)	pantoprazole Concentration (µg/mL)	Intra day Precision (%RSD))	Inter day precision (%RSD)
5	0.114	0.246	4	0.148	0.174
10	0.276	0.228	8	0.230	0.148
15	0.161	0.478	12	0.205	0.572

Table - 7
Sensitivity Data of Aceclofenac and Pantoprazole

Parameter	Aceclofenac	Pantoprazole
LOD (µg/mL)	1.48	0.73
LOQ (µg/mL)	4.49	2.20

Table - 8
Assay Results of Aceclofenac and Pantoprazole

Drug	Brand Name	Labeled amount	Amount found	% assay *
Aceclofenac	HIFENAC	100 mg	99.86	99.86%
Pantoprazole	PAN 40	40 mg	40.26	100.57%

Table 9
Summary of the validation parameters of the proposed method

Parameters	Aceclofenac	Pantoprazole
Maximum absorbance	273 nm	289 nm
Linearity	5-30 µg/mL	4-20 µg/mL
Correlation Coefficient	0.9998	0.9998
Precision (% RSD) (i) Intra day (ii) Inter day	 0.183 0.318	 0.194 0.298
Accuracy (% Recovery)	100.08	100.51
LOD	1.48	0.73
LOQ	4.49	2.20
Tablet Assay	99.86%	100.57%

DISCUSSION

The current research aims to develop a UV spectrophotometric method for the simultaneous analysis of ACECLO and PANTO in bulk and commercially available pharmaceutical dosage forms. The spectrum of ACECLO and PANTO in phosphate buffer solvent showed the absorptionmaximum at 273 nm and 289 nm respectively. The statistical analysis of data obtained for the calibration curve of ACECLO and PANTO in pure solution indicated a high level of precision for the proposed method, as evidenced by low value of coefficient of variation. The coefficient of correlation was highly significant. The linearity range was observed between 5-30 µg/mL for Aceclofenac and 4-20 µg/mL for Pantoprazole. The plot clearly showed a straight line passing through origin. The assay method was validated by low values of % RSD and standard error, indicating accuracy and precision of the methods. Excellent recovery studies further proves the accuracy of the method. The ruggedness of method was studied by using different instrument and different analyst. From the high values of recovery study it can be inferred that the method is free from excipients used in the formulation. Based on the results obtained the developed method can be regarded as simple, accurate, precise and reliable which can be employed for routine quality control of ACECLO and PANTO in bulk and pharmaceutical dosage forms.

CONCLUSION

The proposed UV-Spectrophotometric method was found to be simple, precise, sensitive, specific and

economic for simultaneous estimation of Aceclofenac and Pantoprazole in bulk and pharmaceutical dosage form with good accuracy and precision. The proposed method utilizes inexpensive solvents and the % recovery data shows that the method is free from interference of the excipients used in formulation and hence can be used for routine analysis in quality control laboratories.

ACKNOWLEDGEMENT

The authors are thankful to Principal Government College of Pharmacy, Bengaluru for his constant support and facilities provided to carry out the present work. Thanks are also due to Mr. Ganesh QC incharge Anglo French Drugs and Industries Limited, Bengaluru for providing the gift samples of ACECLO and PANTO.

REFERENCES

1. The United State Pharmacopoeia, USP 28/NF 23, Asian Edition, The United States Pharmacopoeial Convention, Inc., Rockville, MD. 2749-51.
2. http://en.wikipedia.org/wiki/Pantoprazole.
3. Nissankararao S, Anil KA, Bhimavarapa R, Renuka K, Anusha A.Unique UV spectrophotometric method for reckoning of Aceclofenac in bulk and pharmaceutical dosage forms using hydrotropic agents. Sch Acad J Pharm. 2013; 2(5):406-9.
4. Shah R, Magdum C, Patil K, Chougale DK, Naikwade N. Validated spectroscopic method for estimation of Aceclofenac from tablet formulation. Res J Pharm Tech 2008; 1(4):430-2.
5. Saravanan VS, Ware AL, Gopal N. UV-spectrophotometric determination of Aceclofenac in tablets. Asian J Chem, 2006; 18(4):3251-2.
6. Singhavi I, Goyal A. Visible spectrophotometric estimation of Aceclofenac and Indapamide from tablets using folin ciocateu reagent. Asian J Chem, 2007; 69(1):164-5.
7. Goyal A, Singhavi I. Visible spectrophotometric estimation of Aceclofenac in tablet formulation. Asian J Chem, 2006; 18(4):3157-9.
8. Aderibigbe SA, Adegoke OA, Idowu OS, Olaleye SO. Sensitive spectrophotometric determination of Aceclofenac following AZODYE formation with 4-carboxyll 2, 6-diNitrobenzene diazonium ion. Acta Poloniae Pharmaceutica-Drug Research 2012; 2(69): 203-11.
9. Paul K, Nagarajan JSK Chandan RS. Standardization an a RP-HPLC method for the estimation of Aceclofenac in dosage form .Int J Res Pharm Chem 2011; 1(4):853-9.
10. Sherikar OD, Puranik MP, Yeole PG. A validated reversed phase-high performance liquid chromatographic (RP-HPLC) method for simultaneous estimation of Aceclofenac drug substance and its related traces impurities in the solid dosage form. Int J Chem Tech Research 2011; 3(2):547-54.
11. Nyola N, Jayabalan GS, Kalra N, Praveen G Choudhary S. Development and validation of a RP-HPLC method for simultaneous estimation of Diacerine and Aceclofenac in pharmaceutical dosage form. J Pharmaceut Anal 2012; 1(1):1-8.
12. Manasa B, Ravikumar A. Development and validation of RP-HPLC method for the simultaneous estimation of Aceclofenac and Rabeprazole sodium in bulk and capsules. Int J Pharm Bio Sci 2012; 2(3):160-9.
13. Patel DR, Mehta FA, Shah DA, Chhalotiya UK. Development of HPTLC method for the estimation of Aceclofenac and Tolperisone hydrochloride in combination. Austin J Anal Pharm Chem 2015; 2(4):1-5.
14. William HD, Devi BP, Kurien J, Valsakumari PK, Mohandas CK. Validated high performance thin layer chromatographic estimation of Aceclofenac in bulk and pharmaceutical dosage forms. Int J Sci Res 2014; 3(6):2613-7.
15. Patil ST, Bhusari VK, Dhaneshwar SR. validated HPTLC method for simultaneous estimation of Thiocholchicoside and Aceclofenac in bulk and formulation. Int J Pharma and Bio Sci 2011; 2(2):482-90.
16. Yuvaraj G, Pavan KP, Elangovan N. The simultaneous estimation of Aceclofenac, Paracetamol and Chlorzozazone in pharmaceutical dosage forms by HPTLC. Int J Chem Pharm Sciences 2010; 1(2):24-7.
17. Madhukara BM, Babu CJG, Mani TT. Validated derivative spectrophotometric estimation of Pantoprazole in bulk and tablet dosage forms. Int J Adav Res 2015; 3(4):336-70.
18. Reddy MP, Reddy GR, Reddy NR. A simple spectrophotometric determination of Pantoprazole in pharmaceutical formulation. Chemical Science Transactions 2014; 3(1):203-12.
19. Pandya J, Solanki S, Patel M. Development and validation of differential spectrophotometric method for determination of Pantoprazole in tablet dosage form. Journal of Pharmaceutical Science & Bioscientific Research 2012; 2(1):2-4.
20. Kumar R, Singh H, Singh P. Development of UV spectrophotometric method for estimation of Pantoprazole in pharmaceutical dosage forms. J Chem Pharm Res 2011; 3(2):113-7.
21. Latha KP, Ramachandran D. Development and validation of RP-HPLC method for estimation of

Pantoprazole. Int J Curr Pharmaceut Res 2013; 5(2):119-21.

22. Siddartha B, Babu IS. Analytical method development and method validation for the estimation of Pantoprazole in tablet dosage form by RP-HPLC. Der Pharm Chemica 2013; 5(4):99-104.
23. Rao KS, Keshar NK, Choudhary PR, Pattnaik AK. RP-HPLC method for the estimation of Pantoprazole sodium. Int J Pharm Med Bio Sci 2012 July 1;1 (1): 48-53.
24. Binnor AK, Khagga M, Suryanarayana MV, Roy SB. A validated stability-indicating HPLC assay method to investigate stability of Pantoprazole injection with injectable solutions (5% Dextrose injection and 0.9% sodium chloride injection). Int J Res Pharm Sci 2012; 3(2):334-9.
25. Effat S, Shabani RN, Farhad A, Negahban AA, Barazandeh TM, Massoud A. Validated stability-indicating HPLC method for the determination of Pantoprazole in the presence of its degradation products. Asian J Res Chem 2010; 3(4): 879-84.