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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ACECLOFENC AND LANSOPRAZOLE

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ABSTRACT

A simple, sensitive, precise rapid and accurate reverse phase high performance liquid chromatography (RP- HPLC) method was developed and validated for simultaneous estimation of Aceclofenac and Lansoprazole. The Chromatographic separation was achieved by using Cosmosil C18(250 mm×4.6 mm, 5µ) as stationary phase and mobile phase consists of Acetonitrile: Ammonium acetate buffer with pH 5.0 (55:45 v/v) with a flow rate of 1ml/min. The analysis was performed at ambient temperature and the eluent was monitored at 280nm using UV detector. The retention time of Aceclofenac and Lansoprazole was found to be 3.3min and 4.2 min respectively and the calibration curves were linear (r^2 = 0.9995 and 0.9995) over a concentration range of 100-500µg/ml for Aceclofenac and Lansoprazole respectively. The Limit of detection (LOD) for Aceclofenac and Lansoprazole was observed to be 0.006µg/ml and 0.002µg/ml respectively, the limit of quantitation (LOQ) was found to be 0.2µg/ml and 0.4µg/ml respectively. The developed method was validated as per ICH guidelines using parameters like linearity, specificity, system suitability, precision, ruggedness, robustness, accuracy. All the validation parameters were found to be well within the acceptance criteria. Hence the proposed method can be used for the routine analysis of Aceclofenac and Lansoprazole in bulk and tablet dosage forms.

KEYWORDS: Aceclofenac, Lansoprazole, RP-HPLC, Simultaneous estimation. ICH guidelines.

INTRODUCTION

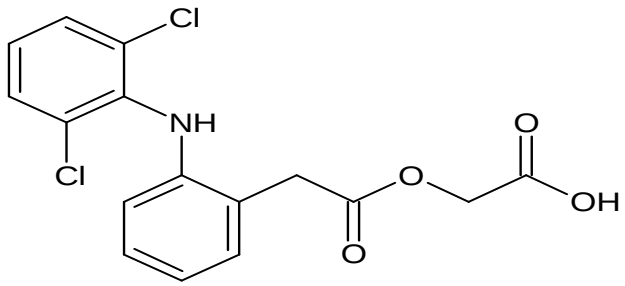
Aceclofenac (ACECLO) chemically, a phenyl acetic acid derivative, has anti-inflammatory and analgesic properties. It is a Non-steroidal anti-inflammatory drug (NSAIDs) used in various commercial pharmaceutical formulations for the treatment of fever, relief of pain and inflammation in rheumatoid arthritis, osteoarthritis and ankylosing spondylitis and reported to have good anti-rheumatic activity. ACECLO is the glycolic ester of Diclofenac. It is inhibitor of cytokine and works by blocking the action of a substance in the body called cyclooxygenase which involved in the production of prostaglandins and responsible for the generation of pain, swelling and their inflammatory conditions. ACECLO is practically insoluble in water and soluble in alcohol & methyl alcohol, freely soluble in acetone & dimethyl formamide.[1]

Lansoprazole (LANSO) chemically2-([3-methyl-4-(2,2,2-trifluoroethoxy) pyridine-2-yl] methyl sulfonyl)-1H-benzo[d] imidazole. belongs to the class of anti-secretory compounds, the substituted benzimidazoles, that do not exhibit Anti-cholinergic or Histamine H_2 -receptor antagonist properties, but rather suppress gastric acid secretion by specific inhibition of the (H^+, K^+)-ATPase enzyme system at the secretory surface of the gastric parietal cell. This enzyme system is regarded as the acid (proton) pump within the parietal cell; Lansoprazole has been characterized as a gastric acid-pump inhibitor, in that it blocks the final step of acid production. This effect is dose-related and leads to inhibition of both basal and stimulated gastric acid secretion irrespective of the stimulus. Lansoprazole does not exhibit anticholinergic or histamine type-2 antagonist activity.[3] Freely soluble in dimethyl formamide, soluble in ethyl acetate, Dichloromethane and acetonitrile.

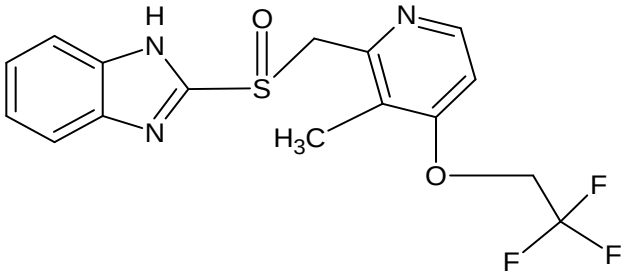
But main adverse effect of Aceclofenac (NSAID) is the direct and indirect irritation of the gastrointestinal tract, as the acidic molecule of drug directly irritates the gastric mucosa. As it act by inhibiting the enzymes cyclooxygenase-1 and cyclooxygenasae-2 (COX-1 and COX-2) which leads to the inhibition of prostaglandin synthesis in gastrointestinal tract and reduces the level of protective prostaglandins in the mucosal secretions and increased gastric irritation. These common gastric adverse effects can be reduced through suppressing acid production by concomitant use of proton pump inhibitors. Hence to minimize these side effects Aceclofenac is prescribed along with proton pump inhibitors like Lansoprazole as a therapeutic combination.

MATERIALS AND METHODS

Chemicals and Reagents: The reference samples of was purchased from SIGMA ALDRICH & was procured as a gift sample from DRUG TESTING LABORATORY, Acetonitrile, Methanol, and Water were of HPLC grade. Potassium dihydrogen phosphate & Disodium hydrogen phosphate used was of Analytical grade.



Structure of Aceclofenac



Structure of Lansoprazole

Instrument and Chromatography Condition

The High Performance Liquid Chromatography consisted of SHIMADZU-SPD-20A prominence auto sampler fitted with UV Visible detector (SPD-20A) with SHIMADZU-LC-20AT pump. The chromatogram was recorded using LC Solution software. The Chromatographic separation was achieved by using Cosmosil C18(250 mm×4.6 mm, 5µ) as stationary phase and mobile phase consists of Acetonitrile: Ammonium acetate buffer with pH 5.0 (55:45v/v) with a flow rate of 1ml/min. The analysis was performed at ambient temperature and the eluent was monitored at 280nm using UV detector.

Preparation of Mobile PHASE

Volume of 500 mL HPLC grade Acetonitrile and 500mL ammonium acetate buffer, prepared by dissolving 0.7708 gm of ammonium acetate crystal in 500mL of Millipore water. Both the solvent system was filtered with 0.45µ filter paper and sonicated for 20 mins. Mobile phase was used as diluent. Diluent Preparation: Mobile phase is used as diluent.

Preparation of Standard Solution: Accurately 10 mg of ACECLO & LANSO were weighed into a clean and dry 10mL volumetric flask separately dissolved with sufficient volume of diluent. The final volume was made up to 10mL with diluent to get the concentration of 1000µg/mL for ACECLO & LANSO.

Preparation of Working Standard Solution of Aceclo & Lanso

1 mL of standard stock solution was pipetted out into 10mL volumetric flask and further diluted with diluent to 10mL to get concentration of 100µg/mL.

Determination: Wavelength for detection was selected by examining the resulted solution that consists of ACECLO & LANSO in SHIMADZU UV- Spectrometer (UV- 1800) instrument. The maximum absorbance for ACECLO & LANSO was observed at 280nm and hence 280nm was selected as wavelength of detection.

Method Validation: The proposed method was validated in compliance with ICH guidelines for linearity, accuracy, precision, specificity, robustness, and system suitability parameters by the following procedures.

Linearity

Accurately 10 mg of ACECLO & LANSO was weighed into a clean and dry 10 mL volumetric flask, dissolved with sufficient volume of diluent. The volume was made up to 10 mL with diluent to get the concentration of 1000 µg/mL for ACECLO & LANSO.

Preparation of working standard solutions of ACECLO AND LANSO

The various concentration of working standard solutions of ACECLO & LANSO was made by pipetting 1.0mL, 2.0mL, 3.0mL, 4.0mL and 5.0 mL from stock (I) separately into a series of 10mL volumetric flask and diluted to 10mL to get the final concentration of 100µg/mL, 200µg/mL, 300µg/mL, 400µg/mL and 500µg/mL solutions respectively.

Determination

The working standard solutions of ACECLO & LANSO ranging from 100 µg/mL to 500 µg/mL were injected into a chromatograph at flow rate of 1 mL/min. Retention time and peak area obtained were recorded and standard calibration curve was plotted for ACECLO & LANSO and linearity equation was derived. The Correlation coefficient, % curve fitting were also calculated. The results obtained were shown in Table 1,2&3.

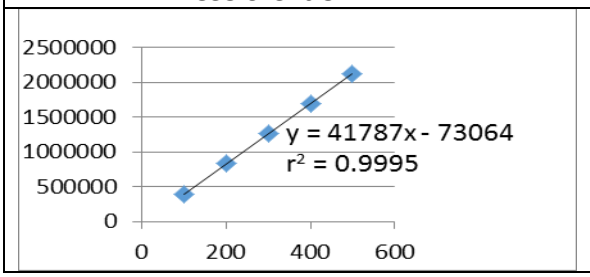
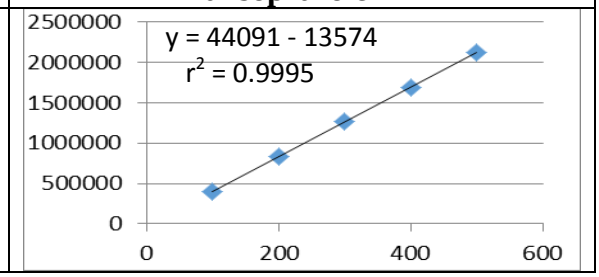
Table. 1: Linearity Data for Aceclofenc and Lansoprazole.

Sl. No.	Concentration (µg/mL)	Peak area for ACECLO	Peak area for LANSO
1	100 µg/mL	349257	396577
2	200 µg/mL	707790	829209
3	300µg/mL	1185860	1346814
4	400 µg/mL	1607151	1810123
5	500 µg/mL	2066068	2182955

Table. 2: Linearity Report of Aceclofenc and Lansoprazole.

Parameters	Aceclofenac	Lansoprazole	Acceptance Criteria
Linearity Range	100 to 500 µg/mL	100 to 500 µg/mL	-
Regression Equation	y = 41787x – 73064	y = 44091x – 13574	-
Correlation Coefficient	0.9995	0.9995	**More than 0.999**
Intercept	73064	13574	
Slope	41787	44091	

Table. 3: Standard Calibration Curves.

Aceclofenac	Lansoprazole
	

Accuracy

Preparation of sample stock solution: Twenty tablets each containing 100 mg of ACECLO and 100mg of LANSO was weighed and finely powered. Powder equivalent to 10 mg of ACECLO and 10mg of LANSO was taken and transferred into a clean, dry 10 mL volumetric flask. The powder was first dissolved in diluent and sonicated for 20 mins. The resulting mixture was then filtered through whatmann filter no 0.45 µ. The final volume of filtrate was made up to 10 mL with diluent.

Preparation of standard stock solution: Accurately weighed 10 mg of standard drug ACECLO and LANSO was transferred into a clean, dry 10 mL volumetric flask and the volume was made up to 10 mL with diluent to get the concentration of 1000 µg/mL of ACECLO and LANSO.

Preparation of standard and sample mixture: Level I (80%): Volume of 0.5 mL sample stock solution, 0.3 mL of standard solution was transferred to 10 mL volumetric flask and volume was made up to mark with diluent (three replicates).

Level II (100%): Volume of 0.5 mL sample stock solution, 0.5 mL working standard stock solution was transferred to 10 mL volumetric flask and volume was made up to mark with diluent (three replicates).

Level III (120%): Volume of 0.5 mL sample stock solution, 0.7 mL of working standard stock solution was transferred to 10 mL volumetric flask and volume was made up to mark with diluent (three replicates).

Determination: The resulting mixture was injected repeatedly into the chromatograph, the peak area and chromatogram obtained were recorded and the % recovery of standard ACECLO and LANSO was calculated. The results obtained are presented in Table 4,5&6.

Recovery study Data for ACECLO with marketed formulation

Table. 4: Recovery Study Data for Aceclofenac.

Level	Replicate	Std Conc. (µg/mL)	Sample Conc. (µg/mL)	Peak area	Total Conc found (µg/mL)	Amt of std. recovered (µg/mL)	% Recovery
80%	I	3	5	279405	7.99	2.99	99.99 %
	II	3	5	277350	7.94	2.94	98.03 %
	III	3	5	279219	7.99	2.99	99.99 %
100%	I	5	5	348250	9.97	4.97	99.42 %
	II	5	5	347012	9.93	4.93	98.71 %
	III	5	5	349270	10.00	5.00	100.00 %
120%	I	7	5	419108	12.00	7.00	100.00 %
	II	7	5	419108	11.85	6.85	97.99 %
	III	7	5	420359	12.03	7.03	100.51 %

Table. 5: Recovery Study Data for Lansoprazole.

Level	Replicate	Std Conc. (µg/mL)	Sample Conc. (µg/mL)	Peak area	Total Conc found (µg/mL)	Amt of std. recovered (µg/mL)	% Recovery
80%	I	3	5	317264	8.00	3.00	100.00
	II	3	5	314269	7.92	2.92	97.48
	III	3	5	318254	8.02	3.02	100.83
100%	I	5	5	395476	9.97	4.97	99.44
	II	5	5	398790	10.05	5.05	101.11
	III	5	5	394752	9.95	4.95	99.07
120%	I	7	5	474892	11.97	6.97	99.63
	II	7	5	479210	12.08	7.08	101.19
	III	7	5	479210	12.08	7.08	101.19

Table. 6: Summary of Recovery Studies for Aceclo and Lanso.

Level	Replicate	Std Conc. (µg/mL)	Sample Conc. (µg/mL)	Peak area	Total Conc found (µg/mL)	Amt of std. recovered (µg/mL)	% Recovery
80%	I	8	4	475892	11.99	7.99	99.99
	II	8	4	480253	12.10	8.10	101.37
	III	8	4	479824	12.09	8.09	101.23
100%	I	10	4	555207	13.99	9.99	99.99
	II	10	4	560291	14.12	10.12	101.28
	III	10	4	558753	14.08	10.08	100.89
120%	I	12	4	640914	16.16	12.16	101.34
	II	12	4	637683	16.07	12.07	100.66
	III	12	4	634523	15.99	11.99	99.91

Precision

1 mL of standard solution of ACECLO and LANSO was transferred into a 10 mL volumetric flask and final volume was then made up to 10 mL with diluent to get a concentration of 100µg/mL of ACECLO and LANSO.

Determination

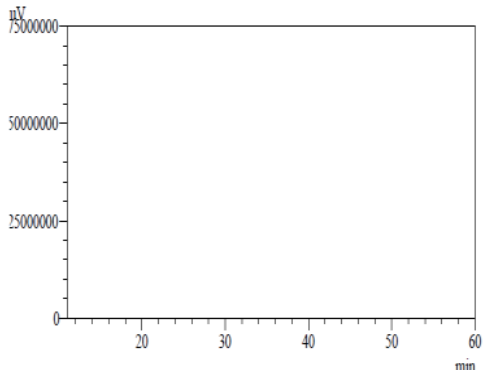
Successive six injections of working standard solution (six replicates) were injected into a HPLC chromatograph, the peak area and chromatograms obtained were recorded. The % relative standard deviation was calculated for peak areas and retention time of replicates. The results and chromatogram obtained were shown in Table 7.

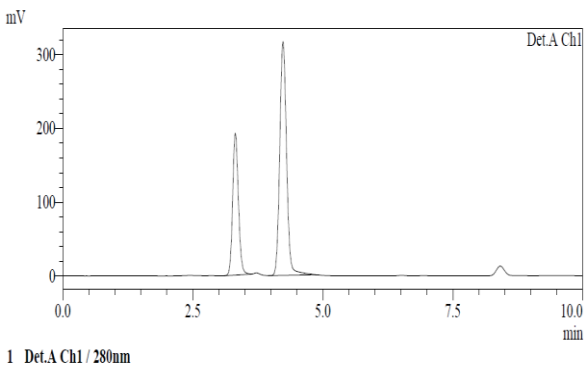
Table. 7: Report of Precision for Aceclofenac and Lansoprazole.

Precision Parameters	% Rsd of Aceclo	% Rsd of Lanso	Acceptance Criteria
System Precision	0.04	0.13	< 2.0%
Method Precision	0.17	0.41	< 2.0%
Intraday Precision	0.16	0.14	< 2.0%
Inter day Precision	0.06	0.06	< 2.0%

Specificity

The diluent, working standard of ACECLO and LANSO were injected separately into the chromatograph to examine that the ACECLO and LANSO peak is not affected by the mobile phase and diluent and the chromatogram was recorded and is presented in Fig 1-2.





Drugs	Retention Time	Area	Resolution	Tailing Factor	HETP	Theoretical Plate
Aceclo	3.319	14644062	0.000	1.109	37.607	3956.243
Lanso	4.231	2812212	4.05	1.167	30.853	4851.734

Chromatogram of only diluent was taken to check the interference of diluent with the peaks of ACECLO and LANSO at the retention time of respective drugs. There was no peak detected at retention time of ACECLO 3.2 min and LANSO 4.3 min. so, proposed method is specific in nature.

LOD and LOQ

LOD and LOQ for ACECLO and LANSO by this method were evaluated on the basis of signal-to-noise ratio method described in ICH guidelines. A signal-to noise ratio between 3 or 2:1 is generally considered acceptable for estimating the detection limit. A typical signal-to-noise ratio required for LOQ is 10:1. Using the proposed HPLC method, the LOD and LOQ values were calculated and are given in Table 8.

Table. 8: Data For lod and loq of Aceclofenac And Lansoprazole.

Parameter	ACECLO		LANSO	
	Peak Area	Concentration in µg/mL	Peak Area	Concentration in µg/mL
LOD	320	0.006	5296	0.2
LOQ	1800	0.002	2510	0.4

Robustness: To evaluate the robustness of the developed RP-HPLC method, small deliberate variations in the optimized parameters were made in chromatographic conditions like of flow rate, mobile phase ratio and wavelength. The effect of change in flow rate, mobile phase ratio and wavelength of detection on retention time and tailing factor were examined. The values obtained are mentioned in Table 9,10&11. The method was found to be unaffected by the small changes like ± 0.1 mL/min in flow-rate of mobile phase and change in mobile phase ratio from 55:45 to 50:50 & 60:40 and ± 5 nm in detection wavelength.

Table. 9: Robustness Data of Aceclofenac and Lansoprazole with Change in Flow Rate.

Change in Flow rate ml/min	Peak area* of Aceclo	%Assay	Peak area* of Lanso	% Assay
0.9	340950	97.60	381494	102.20
1.0	348243	99.70	393780	99.20
1.1	358024	102.50	404826	102.16

Table. 10: Robustness Data of Aceclofenac and Lansoprazole with Change in Mobile Phase.

Change in Mobile phase ratio v/v	Peak area* of ACECLO	% Assay	Peak area*of LANSO	% Assay
40:60	360250	103.10	380821	96.00
45:55	348243	99.70	393780	99.30
50:50	339034	97.00	383812	96.70

Table. 11: Robustness Data of Aceclofenac and Lansoprazole with Change in Wavelength.

Change in wavelength in nm	Peak area* of ACECLO	% Assay	Peak area* of LANSO	% Assay
275	332029	95.00	397902	100.30
280	348243	99.70	393780	99.20
285	334563	9.70	394747	99.50

System Suitability: Six replicate of sample containing ACECLO and LANSO were given to evaluate equipment, electronics, analytical operations and samples suitability. Parameters calculated for system suitability were %RSD of retention time and area, number of theoretical plates and Resolution. The results are given in Table 12.

Table. 12: Data for System Suitability Parameter for Aceclofenac and Lansoprazole.

Sr. No.	System Suitability Parameters	Aceclofenac	Lansoprazole	Acceptance Criteria
1.	Resolution	0.00	5.024	More than 2
2.	Tailing Factor	1.14	1.27	Less than 2
3.	HETP	47.265 mm	35.555 mm	-
4.	Theoretical Plates	3173.623	4218.788	More than 2000

Ruggedness: Intermediate precision expresses the variations within laboratories variations: (different days, different analysts, different equipment etc.). The Intermediate precision was performed for ACECLO and LANSO by different analyst on different instrument using different lot of column on different day. The % RSD for the same was calculated for Intermediate precision. The results are given in Table 13 & 14.

RESULT AND DISCUSSION

Table. 13: Intermediate Precision Data of Analyst 1.

	Aceclofenac		Lansoprazole	
Replicates	**Peak Area***	**%Assay**	**Peak Area***	**%Assay**
1	348316	99.70 **%**	396295	99.90 %
2	348612	99.80 **%**	396120	99.80 **%**
3	348923	99.90 **%**	396200	99.90 **%**
4	349216	99.90 **%**	396003	99.80 **%**
5	349312	100.00 **%**	396104	99.80 **%**
6	348965	100.00 **%**	396601	100.00 **%**
Mean	**348965**	**99.88 %**	**396220**	**99.86 %**

***Average of six determinations**

Table. 14: Intermediate Precision Data of Analyst 2.

	Aceclofenac		Lansoprazole	
Replicates	**Peak Area***	**%Assay**	**Peak Area***	**%Assay**
1	347956	99.60 %	396884	100.00 %
2	349216	99.90 %	398624	100.05 %
3	343306	98.20 %	388204	97.80 %
4	349401	100.00 %	383620	96.70 %
5	349106	99.90 %	398920	100.50 %
6	340602	97.50 %	399214	100.60 %
Mean	346602	99.18 %	394244	99.27

Average of six determinations

Optimized chromatography condition: Chromatographic conditions were screened for mobile phase composition, wavelength proportion and flow rate Finally, mobile phase of Acetonitrile: Ammonium acetate buffer with pH 5.0 (55:45v/v) was optimized to give symmetric peak with short runtime at UV detection wavelength of 280 nm and flow rate at 1mL/min was found to be appropriate with adequate separation between the two drugs. Chromatogram of ACECLO and LANSO at optimized chromatographic condition was recorded, the runtime was 10 min and the retention times of ACECLO and LANSO were found to be 3.2 and 4.3 min respectively.

CONCLUSION

The proposed HPLC method was found to be economical, simple, sensitive, accurate, precise, specific and robust and can be used for the routine quality control analysis of ACECLO and LANSO in bulk as well as in tablet formulation.

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REFERENCES

1. www.drugbank.ca/drugs/DB00586.
2. Aceclofenac [live search type of on internet] [Cited Sat, 25 Feb 2017, 01:05:55 + 05:30] Available from: https//www.drugbank.ca/drugs/DB06736.
3. Lansoprazole [Live search type of on internet] [Cited sun,05 mar 2017,09:35:30] Available form: https://www.drug bank.ca/drugs/DB00448.
4. Dashora K, Garg G, Saraf S. Spectrophotometric estimation of Aceclofenac in pharmaceutical dosage forms. Biosci. Biotechnol. Res. Asia, 2006; 3(1A): 277-8.
5. Saravanan VS, Ware AL, Gopal N. UV-spectrophotometric determination of Aceclofenac in tablets. Asian J Chem, 2006; 18(4): 3251-2.
6. Basavaiah K, Ramakrishna V, Kumar URA, Kumar U. Spectrophotometric and high performance liquid chromatographic determination n of Lansoprazole in pharmaceuticals Ind J Chem Tech, 2006; 13: 549-54.
7. 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.
8. Pradeep S, Javali C. Analytical method development and validation for simultaneous estimation of ivermectin and fenbendazole in bulk dosage form by RP-HPLC. World J Pharm Pharm Sci., 2016 Feb 4; 5(4).
9. Paul K, Nagarajan JSK Chandan RS. Standardization an RP-HPLC method for the estimation of Aceclofenac in dosage form. Int J Res Pharm Chem, 2011; 1(4): 853-9.
10. Ventrapragada BV, Kumar SA, Debnath M, Krishna NS, Saravanan J, Greeshma V. Method development and validation for the simultaneous estimation of Aceclofenac and Paracetamol in solid dosage form by using RP-HPLC. Int J Biopharm, 2014; 5(2): 109-18.
11. 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.

12. R Umadevi, Javali C, MD Karvekar, PL Somashekar development and validation of HPLC method for simultaneous estimation of Ciproflaxacin and Pantoprazole. UJP, 2014; 03(06): 34-38.
13. Rajaput S, Fanse S. RP-HPLC method for simultaneous estimation of Lansoprazole and Aspirin in bulk and laboratory mixture. J Adv Pharm Edu and Res., 2015; 5(2): 87-93.
14. Kachave RN, Kale M, Wagh RD. Development and validation of LC-MS/MS. The Open Analytical chemistry Journal, 2015; 8: 7-11.
15. ICH, Q2A Text on Validation of Analytical Procedures, 1994, Oct.
16. ICH, Q3B Validation of Analytical Procedures: Methodology, 1996, Nov.