http://jmscr.igmpublication.org/home/
ISSN (e)-2347-176x ISSN (p) 2455-0450
crossref DOI: https://dx.doi.org/10.18535/jmscr/v11i5.06

IGM Publication
Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

UFLC Simultaneous Determination of Paracetamol Pantaprazole and Aceclofenac in Bulk and by combining the Dosage Forms

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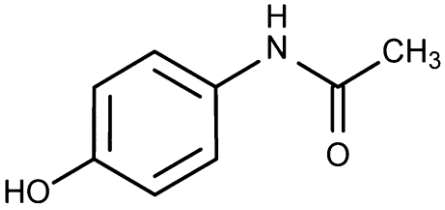
Abstract
An optimized UFLC method was successfully developed and validated for the detection and quantification of paracetamol, pantoprazole, and aceclofenac in both bulk and tablet formulations. the method is simple, accurate, rapid, and precise. reverse phase ODS column was utilized for separation, and a mixture of phosphate buffer (ph 4.5) and acetonitrile in the ratio of 40:60 was used as the eluent. the flow rate was maintained at 0.7 ml/min, and detection was performed using a uv spd20 detector at 284 nm, while the ambient temperature was kept constant. the proposed method was optimized and validated in accordance with the ich guidelines, making it suitable for routine quality control analysis. overall, this method provides a reliable and efficient means of analyzing these compounds in a variety of settings.
Keywords: *UFLC, Paracetamol, Pantaprazole, Aceclofenac, Method development.*

Introduction

Paracetamol

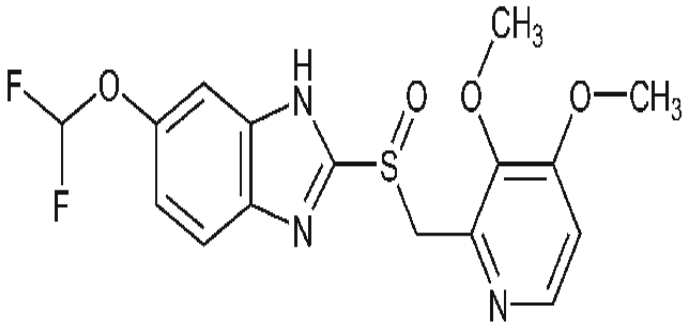
Chemically it is N-acetyl-Para-aminophenol and also known as Acetaminophen molecular formula $C_8H_9NO_2$ and Molecular weight is 151.163 g/mol soluble in water, density 1.263 g/cm^3, melating point 169^{o}c and boiling point 420^{o}c. It is metabolized primarily in the liver; by taking orally it is rapidly absorbed by the gastro intestinal tract. Paracetamol does not appear to inhibit the function of cyclooxygenase (COX) enzymes outside the CNS but selectively inhibit COX activities in the brain. It is mainly used to treat fever, pain, osteoarthritis, lower back pain, headache, postoperative pain, teeth pain and in combination with weak opioids such as codeine and steroid opioids such as morphine to improve analgesic effects.



Paracetamol *N*-(4-hydroxyphenyl) acetamide

Pantoprazole

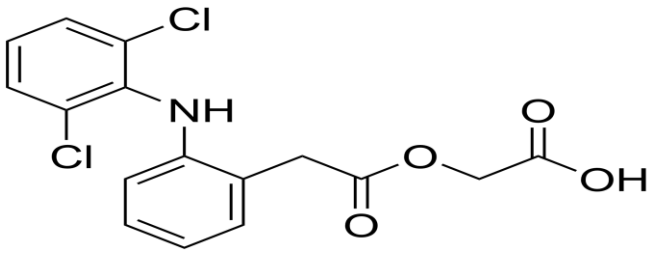
Chemically Pantoprazole is 6 difluromethoxy-2-((3,4dimethoxypyridin2-yl) methyl sulfinyl) – 1H-benzo(d)imidazole. It is a Proton pump inhibitor and suppresses gastric acid production by acting on the (H^+/ $K^{+)}$ ATPase enzyme system on the gastric parietal cells and there by decreases the amount of acid produced in the stomach, Molecules formula $C_{16}H_{15}F_2N_3O_4S$ and Molecular weight 383.371g/mol. it has a close related action combination of proton pump inhibitors with NSAIDS in plasma helps to improve the effectiveness of Therapy by minimizing the drug toxicity.It is used in the treatment of erosive esophagitis in childrens; Pantoprazole is metabolized in the liver by the cytochrome p450 system,



Pantoprazole [(2-{2, 6-dichlorophenyl) amino} phenylacetooxy acetic acid]

Aceclofenac

Chemically it is [2 {2,6dichlorophenyl) amino} Phenyl acetooxy acetic acid] and having the formula C_{16} H_{13} Cl_2NO_4 its molecular weight is 353.0216g/mol, it is a non-steroidal anti inflammatory drug and analogue of diclofenac. It is practically insoluble in water and mainly metabolized in the liver, it act by inhibiting cyclooxygenase enzymes which are involved in the production of prostaglandins. Prostaglandins are responsible for pain, swelling, inflammation and fever. The commonly used route of administration is oral and topical. it is also used in the treatment of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis.



Aceclofenac6-(difluoromethoxy)-2-[(3,4-dimethoxypyridin-2-yl)methylsulfinyl]-1*H*-benzimidazole

Materials and Method

Chemicals and the Reagents

working standards of Paracetamol pantaprazole and aceclofenac were donated by Greensmed lab Chennai the tablet dosage forms was procured from local market Paracetamol 500 mg from NOMARK, aceclofenac 100 mg from ICPA Laboratories and pantaprazole tablets 40 mg from FABISAN pharmaceuticals where used The weight equivalent of powder to be taken in calculation from labeled claim and average weight.

HPLC grade water and acetonitrile were supplied by Merck, Sodium dihydrogen orthophosphates

and orthophosphoric acid of analytical reagent (AR) grade were supplied by NICE are used for experimental studies

Buffer preparation

Accurately weighted 1.5 gm of sodium dihydrogenorthophosphate into a 1L volumetric flask add about 750 ml of HPLC grade water and sonicate for few minutes to degas and finally make up the volume with HPLC water and then the PH was adjusted to 4.5 with dilute orthophosphoric acid.

Standard preparation

Accurately weigh 100 mg of Paracetamol, Pantaprazole and Aceclofenac separately and were transferred into 100 ml clean dry separate standard flask, and was diluted with 75 ml of buffer and sonicate for 30 minutes and all the three standards flasks were made up to the final volume with buffer, from the above stock solutions 1 ml each was pipetted out into a 100 ml standard flask and then makeup to the final volume with buffer (to get 10 mcg/ ml solution)

Sample preparation

From the formulation procured from the local market the weight equivalent of powder (100mg) to be taken is calculated from labeled claim and average weight the weight equivalent of powder is weighted and transfer into clean dry separate standard flask of 100 ml capacity each. The Powder were dissolved in 75 ml butter solution and finally made up to 100 ml. The contents of all the three flasks are filtered, from the filtrate obtained 1 ml each of the filtrate is transferred into a clean dry 100 ml standard flask and suitably diluted to get a combination of 10 mcg /ml solution of each drugs.

Chromatographic Conditions

Chromatographic conditions were achieved by using C18 analytical phenomenal (Kinetex) column (5micron -C18, 150x4.6mm)the mobile phase consists of phosphate buffer (PH 4.5) and acetonitrile taken in the ratio of 40:60 the injection volume of the sample was 20 micro litter, the mobiles phase was degassed by Sonica ultrasonic sonicator before pumping into HPLC system the flow rate was set at 0.7 ml per minute and the wave length 284nm was selected for detection the column temperature was maintained at 30^{o}C

Method development

Several trials were performed for the method development and the best peak with least fronting factor was found in the fifth trials with reaction time of 3.541 for Paracetamol, 5.554 for pantaprazole and 8.142 for Aceclofenac.

Table Number 1 shows the best optimum separation conditions and the corresponding chromatography were shown in figure 1 to 5.

Table no 1

SL.No	Chromatographic Conditions	
1	Mode of separation	Isocratic Elution
2	Mobile phase	Buffer (PH 4.5) and Acetonitrile (40:60)
3	Column	Phenomenex (150 x 4.6mm, 5micron)
4	Flow rate	0.7ml/min
5	Detection wave length	284
6	Injection volume	20 micro liter
7	Column over temperature	30^{o}C
8	Run time	10 min

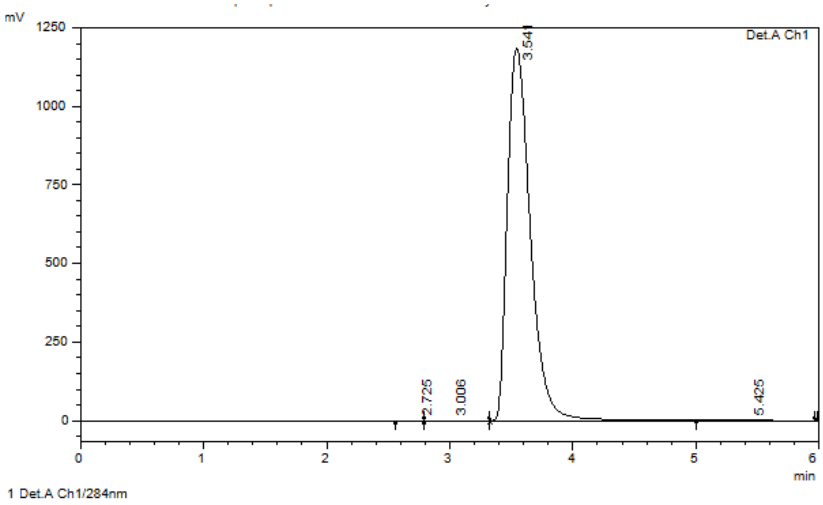


Fig.1 chromatogram showing the retention time for paracetamol standard

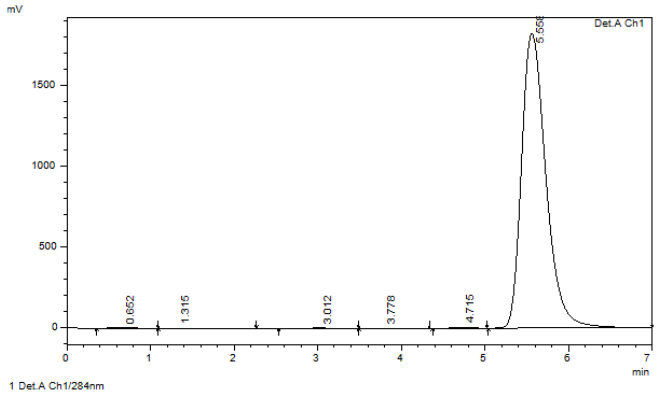


Fig 2 chromatogram showing the retention time for Pantoprazole standard

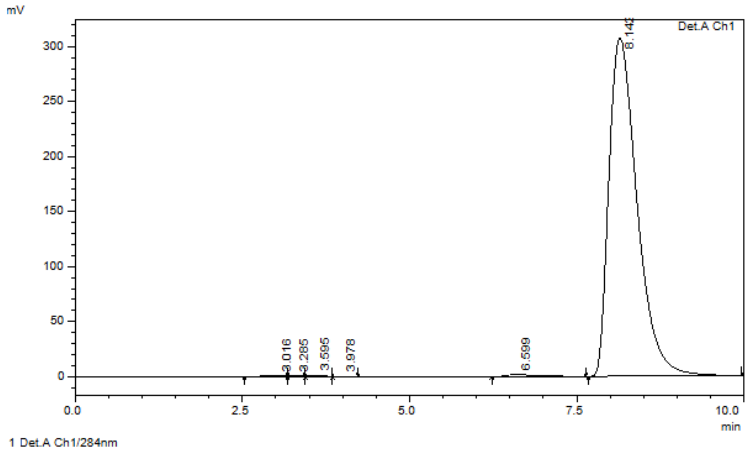


Fig 3 chromatogram showing the retention time for Aceclofenac standard

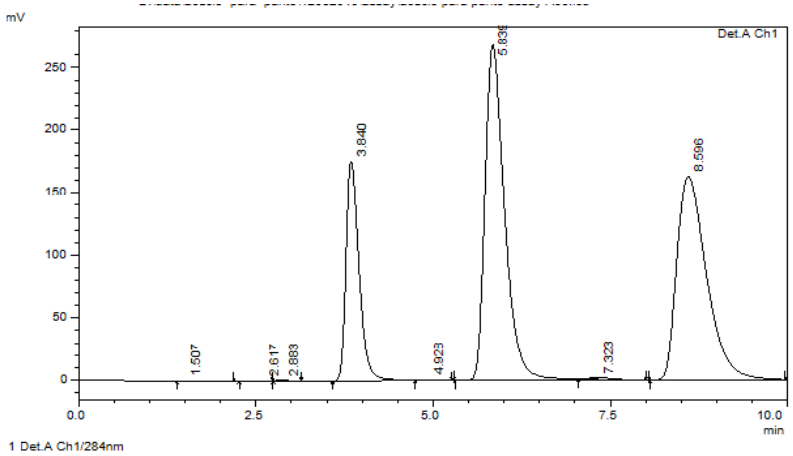


Fig 4 chromatogram showing the combined standards of Paracetamol, Pantoprazole and Aceclofenac

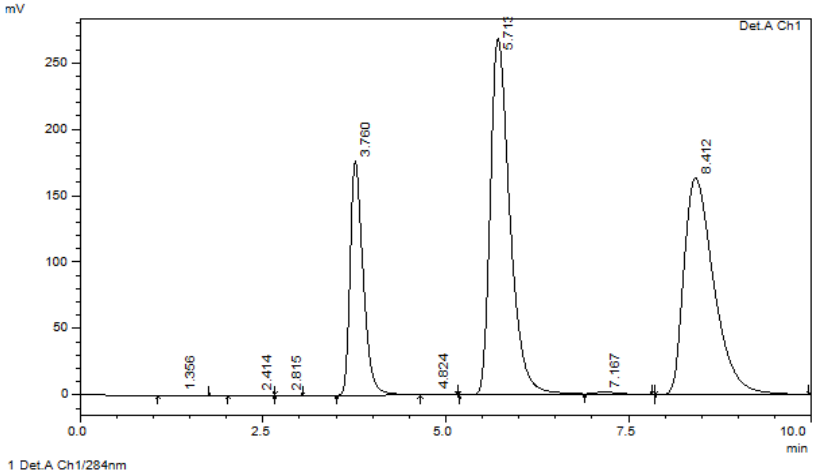


Fig 5 chromatogram showing the combined formulations of Paracetamol, Pantoprazole and Aceclofenac

Results and Discussion

Method validation

To validate the final assay conditions, the principle of validation parameters outlined in the ICH guidelines was followed. This involved evaluating various analytic parameters, including specificity, accuracy, precision, linearity, detection limit, and quantitation limit, in accordance with the ICH protocol. The aim of this validation was to ensure that the analytical method was reliable, accurate, and suitable for its intended use. Specificity was assessed to ensure that the analytical method could distinguish between the target compound and other interfering substances. Accuracy was evaluated to determine the closeness of the measured values to the true values of the target compound. Precision was assessed to determine the degree of variation between replicate measurements of the same sample. Linearity was evaluated to assess the ability of the method to produce results that are directly proportional to the concentration of the target compound. Detection limit and quantitation limit were assessed to determine the lowest concentration of the target compound that could be reliably detected and quantified, respectively. Overall, following the ICH guidelines and evaluating these parameters allowed for the validation of the final assay conditions, ensuring that the analytical method was suitable for its intended use and produced reliable and accurate results.

System suitability

As an essential part of HPLC procedures, system suitability tests were conducted to confirm that the chromatographic system was appropriate for its intended purpose. The purpose of these tests was

to ensure that the system could produce accurate and reliable results. In particular, the system suitability test focused on two specific parameters: theoretical plates and tailing factor. The theoretical plates are a measure of the separation efficiency of the chromatographic system, with a higher number indicating better separation. In this case, the target was to achieve more than 1500 theoretical plates. Meanwhile, the tailing factor is a measure of the symmetry of the peak, with a value of less than 2 indicating an acceptable peak shape. After conducting the system suitability tests, the results were evaluated and found to be within the acceptable limits. This data was summarized in a table for easy reference. By conducting these tests and ensuring that the results met the specified criteria, it was possible to confirm that the chromatographic system was suitable for the intended purpose and that the results obtained from the subsequent analysis were reliable and accurate.

Table no 2

Sl. No	Parameters	Paracetamol	Pantaprazole	Aceclofenac	Acceptable Criteria
1	Tailing Factor	1.395	1.489	1.554	Less than 2
2	Theoretical Plates	1841	2268	2231	Not less than 1500
3	Retention time	3.541	5.554	8.142	Less than 10
4	Area	10417819	5157188	4786061	
5	% RSD	0.35	0.65	0.72	Less than 2%
6	HETP	81.452	66.113	67.219	
7	Resolution	1.607	4.655	1.569	

Linearity

The linearity studies of the analytical measurements, which measures the proportion of absorbance to the concentration of the sample, were carried out for all the active ingredients. The purpose of these studies was to determine whether the response was linear across a specified range of concentrations. In this case, the linearity studies were performed for three active ingredients, namely Paracetamol, Pantoprazole, and Aceclofenac. The results showed that the response was linear across a range of 2-32 PPM for all three ingredients. This means that there was a direct proportionality between the concentration of the sample and the absorbance measured by the analytical instrument. By confirming the linearity of the analytical method for these active ingredients, it was possible to ensure that the method could provide accurate and reliable results over the specified range of concentrations. This information is critical for ensuring the quality of the analytical data produced by the method.

Specificity

Specificity refers to the ability of an analytical method to accurately and unequivocally detect a target compound in the presence of other components that may be present in the sample. To assess the specificity of the UFLC method, standard and sample preparations were studied and compared. The results showed that the UFLC method was specific, as the reaction times for both the standard and sample preparations were nearly identical, indicating that there was no interference from other components present in the sample. Moreover, no interference due to the diluents or mobile phase was observed during the analysis of the target compound, further confirming the specificity of the method. By demonstrating the specificity of the UFLC method, it was possible to ensure that the results obtained from the analysis were reliable and accurate, and that there was no interference from other components that may be present in the sample. This information is critical for ensuring the quality of the analytical data and for making informed decisions based on the results obtained from the analysis.

LOD and LOQ

Limit of detection is the lowest concentration which can be detected using a specific analytical procedure by instruments and limit of quantification is the lowest concentration which can be quantitatively analysed using a specific analytical procedure with an acceptable precision, accuracy and reliability. The signal to noise ratio are specifically monitored and validated for the analytical procedures and the instruments used for analysis.

The LOD value of Paracetamol, Pantaprazole and Aceclofenac were found to be0.65 mcg/ml, 0.92mcg/ml and0.89 mcg/mlrespectively and the LOQ values of AR grade Paracetamol, Pantaprazole and Aceclofenac were found to be 1.25, 1.47 and 1.45 mcg/mlrespectively.

Robustness

Deliberate alteration is method via flow rate, mobile phase ratio and temperature are made, but no recognized changes in the results were noted, and are within the range as per ICH guidelines. Robustness conditions like flow rate 0.6 ml/min and 0.8 ml/min, mobile phase concentration of 30:70 and 50:50 for buffer and acetonitrile and temperature changes at 25^{o}C and 35^{o}C was maintained and samples were injected in triplicate, system suitability parameters were not much affected and the RSD was within the limit - which denotes that the UFLC method development was robust.

Accuracy

The accuracy of the method were studies using recovery analysis and was determined by performing the recovery experiments at 75%, 100% and 125% of the target analyte concentration in the commercial formations selected. The percentage recovery of analyte at each concentration and mean percentage recovery for all the three analyte were studied, in this criteria of recovery of each concentration must be within the acceptable limit of 2%.

Accuracy Data of Paracetamol

SL.No.	Conc %	Peak Area	Amout Added mg	Amount Found mg	% Recovery	Mean Recovery %	SD	% RSD
1	75%	74521341	3.75	3.80	101.33		0.2	0.2
2	100%	10638020	5	4.98	99.6	100.47	0.6	0.2
3	125%	13046191	6.25	6.28	100.48		0.37	0.36

Accuracy Data of Pantoprazole

SL. No	Conc %	Peak Area	Amount Added mg	Amount Found mg	% Recovery	Mean Recovery %	SD	% RSD
1	75%	3653192	3.75	3.78	100.8		0.2	0.2
2	100%	5087421	5	4.98	99.6	100.18	0.6	0.09
3	125%	6298351	6.25	6.26	100.16		0.37	0.05

Accuracy Data of Aceclofenac

SL.No	Conc %	Peak Area	Amount Added mg	Amount Found mg	% Recovery	Mean Recovery %	SD	% RSD
1	75%	3699276	3.75	3.72	99.2		0.2	0.19
2	100%	4958763	5	4.96	99.2	99.63	0.6	0.58
3	125%	6148647	6.25	6.28	100.48		0.37	0.35

Precision

Precision in the measure of the degree of repeatability of procedures under normal operations and in normally expressed as the relative standard deviation (%RSD) and (%CV). Precision may be performed at different levels: Intraday and Inter day precisions. Precision data presenting the %RSD value for both intraday and inter day studies were less than 2% which indicates that the proposed method in Precise and consistent.

Intraday and Inter day data of Paracetamol, Pantaprazole and Aceclofenac

SL. No	Parameters	Inter Day			Intra Day		
		Paracetamol	Pantaprazole	Aceclofenac	Paracetamol	Pantaprazole	Aceclofenac
1	Retention Time	3.914	5.877	8.633	3.900	5.853	8.594
2	Avg. Peak Area	10649070	5187583	4850783	10630957	5164615	4839600
3	SD	113580	50766	50415	109918	52435	49355
4	% RSD	0.2	0.5	0.11	0.3	0.7	0.21

Conclusion

The stability indicating test method described in this study is a reliable, robust, and rapid method for estimating the concentration of paracetamol, pantaprazole, and aceclofenac in both bulk and formulated samples. During the analysis, no interfering peaks were observed at the elution time of the target compounds. The method was validated by assessing several system suitability parameters, including linearity, precision, accuracy, resolution, theoretical plate, and retention time. The results showed that the proposed method was appropriate for analyzing all three active ingredients. The linearity of the method was determined for the concentration range of 2-32 mcg/ml for all three active ingredients, and the LOD (limit of detection) values were found to be 0.65 mcg/ml, 0.92 mcg/ml, and 0.89 mcg/ml for paracetamol, pantaprazole, and aceclofenac, respectively. The LOQ (limit of quantification) values for AR grade paracetamol, pantaprazole, and aceclofenac were determined to be 1.25, 1.47, and 1.45 mcg/ml, respectively. To test the robustness of the method, several conditions were varied, including flow rate, mobile phase concentration, and temperature, and the samples were injected in triplicate. The system suitability parameters were not significantly affected by these variations, and the RSD (relative standard deviation) remained within acceptable limits, indicating that the UFLC method was robust and could be used for routine quality control analysis.In summary, the stability indicating test method described in this study is a reliable, accurate, and robust method for estimating the concentration of paracetamol, pantaprazole, and aceclofenac in both bulk and formulated samples. By using this method, it is possible to ensure the quality and consistency of these active ingredients in various formulations.

Acknowledgment

Author is thankful to Dr. Rakeshkumarjat, Principal and Professor, college of pharmacy SHRI Jagdishprasad Jhabarmal Tibrewala University, Jhunjhunu for his valuable advice.

Conflict of Interest

There is no conflict of interest in the work presented in this manuscript.

Reference

1. Joshi R and Sharma R: Development and validation of RP-HPLC method for simultaneous estimation of three-component tablet formulation containing acetaminophen, chlorzoxazone, and aceclofenac, Anal. Lett. 2008; 41(18): 3297-3308.
2. Ravi Kumar P, BhanuPrakash P, Murali Krishna M, Santha Yadav M and Asha Deepthi C: Simultaneous estimation of domperidone and pantaprazole in solid dosage form by UV Spectrophotometry. E. J. Chem. 2006; 3(12): 142-145.
3. Karthik A, Subramanian G, Ranjith kumar and UdupaN: Simultaneous estimation of paracetamol anddomperidone in tablets by reverse phase HPLC method, Indian J. Pharm Sci. 2007; 69(1): 142-144.
4. V.SreJanardhanan, R.Manavalan, K.Valliappan,HPLC Method For The Simultaneous Determination Of Proton-Pump Inhibitors With Domperidone In Human Plasma Employing Response Surface Methodology, International

Journal of Pharmacy and Pharmaceutical Sciences, (2011)Vol 4,309-317.

5. VenkatesanPalanivel,V.Sreejanardhanan,C handrasekaranMuralidharan, K.Valliappan, Improved HPLC method with the aid of chemometric strategy Determination of Loxoprofen in Pharmaceutical formulation, Actachimslov, 2012,59, 242-248.
6. V. Sree Janardhanan, R. Manavalan, K. Valliappan, Chemometric technique for the optimization of chromatographic system: Simultaneous HPLC determination of Rosuvastatin, Telmisartan, Ezetimibe and Atorvastatin used in combined cardiovascular therapy, Arabian Journal of Chemistry, King Saud University. (2011)
7. Bose A, Dash PP, Shahoo MK , "Simple Spectrophotometric methods for Estimation of Aceclofenac from bulk and formulation", Pharmaceutical Methods, 2010; 1(1), 57-60.
8. Mahaparale.PR ,Sangshetti JN , Kuchekar BS "Simultaneous Spectrophotometric Estimation of Aceclofenac and Paracetamol in Tablet Dosage Form", Indian Journal of Pharmaceutical Sciences, 2007;69(2), 289- 292,
9. Godse V P, Deodhar M N, Bhosale AV, Sonawane R A, Sakpal P S, Borkar DD and Bafana YS, "Reverse Phase HPLC Method for Determination of Aceclofenac and Paracetamol in tabletdosage form", AsianJ.Researchchem,2009;.Jan-Mar (2)1.
10. Kumar R.S, Pinderjit S.H, Development & validation of RP-HPLC method for simultaneous estimation of Naproxen & Pantaprazole in pharmaceutical dosage form, Indian J. of pharm. Research & development, 2011,Vol.2,227-232.
11. Basavaiah K, Anil Kumar U.R, Sensitive spectrophotometric methods for the Determination of pantaprazole sodium in pharmaceuticals using bromated bromide, Methyl orange and indigo carmine as reagents. Indian Journal of Chemical Technology, 2007;14, 611-615.
12. Sloka S.N, Gurupadayya B.M, Simultaneous Spectrophotometric determination of Naproxen and Pantoprazole in pharmaceutical dosage form, Journal of applied chemical research, 2011; Vol 17, 65-74.
13. Budavari S, Merck Index: An Encyclopedia of chemicals, drugs, &biological, 12th edition Merck research division.1966; 67.
14. Govt. of India, ministry of health and welfare, published by Indian pharmacopeia commission Ghaziabad, 2007; 1678.
15. Shaikh K.A, Devkhile A.B Simultaneous determination of Acelofenac, paracetamol and chlorzoxazone by RP-HPLC in pharmaceutical dosage form. Journal of chromatographic sci., (2008),46(7), 649-52.
16. Krishnan H.N, Simultaneous Estimation and Validation of Paracetamol, Aceclofenac and Chlorzoxazone by HPLC in Pure and Pharmaceutical Dosage Form, Asian J. of Chemistry, 2008; Vol. 20, 2557-2562.
17. Uttam D.P, Simultaneous Determination of Aceclofenac, Paracetamol and Chlorzoxazone by HPLC in Tablet Dose Form, E-Journal of Chemistry,2009;Vol.6, 289- 294.
18. Aaditya Singh, Shiv Bhadra Singh, simultaneous estimation of Sumatriptan succinate, Metachlopramide hydrochloride and Paracetamol by RPHPLC method, Journal of pharmaceutical science and research, (2012), Vol-4,1848-1851, ISSN:0975-1459