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## Liquid Chromatographic Estimation of Cyclobenzaprine Hydrochloride and Aceclofenac in Pharmaceutical Formulation.

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### Research Article

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#### ABSTRACT

A sensitive, selective and precise high performance liquid chromatographic method has been developed and validated for the simultaneous determination of Cyclobenzaprine Hydrochloride and Aceclofenac both as a bulk drug and in formulation. The method employed Luna C18 column (250 x 4.6 mm id, 5 µm particle size) as the stationary phase while Methanol and 0.02 M KH<sub>2</sub>PO<sub>4</sub> (75: 25 v/v, pH 3) was used as mobile phase. The R<sub>t</sub> of Cyclobenzaprine Hydrochloride and Aceclofenac were observed to be 4.2 and 9 minutes, respectively. Analysis was carried out in absorbance mode at 280 nm. The linear regression analysis data for the calibration plots showed a good linear relationship for Cyclobenzaprine Hydrochloride and Aceclofenac over a concentration range of 0.01-20 µg/ml and 0.1-40 µg/ml respectively with correlation co-efficient of 0.9990 for Cyclobenzaprine Hydrochloride and 0.9990 for Aceclofenac. The LOQ was found to be 0.01 and 0.1 µg/ml respectively for Cyclobenzaprine Hydrochloride and Aceclofenac. The method was validated as per ICH guideline and it was found to be accurate, precise and robust. Marketed formulation was analyzed successfully.

#### INTRODUCTION

Cyclobenzaprine Hydrochloride is chemically known as 3-(5H-dibenzo[a,d]cyclohepten-5-ylidene)-N,N-dimethyl-1-propanamine<sup>[2,3]</sup>. CBP is used in treatment of as an adjunct to rest and physical therapy for relief of muscle spasm associated with acute, painful musculoskeletal conditions<sup>[4]</sup>. Aceclofenac; 2-[2-[(2,6-dichlorophenyl)amino]phenyl]acetyl]oxyacetic acid <sup>[1]</sup>. It is used for the relief of pain and inflammation in rheumatoid arthritis, osteoarthritis <sup>[5,6]</sup>. Literature survey revealed there is no published RP-HPLC method for this combination of drug. The present paper describes a simple, accurate and precise method for simultaneous estimation of CBP and ACF in combined tablet dosage form. The proposed method is optimized and validated as per the International Conference on Harmonization (ICH) guidelines <sup>[7]</sup>. In the present work, a successful attempt has been made to estimate both these drugs simultaneously by RP-HPLC method.

#### MATERIALS AND METHODS

##### Instruments

Instrument Perkin Elmer USA, Series 200, Phenomenex Luna C18 column (250 x 4.6 mm id, 5µm particle size) was used for analytical method development. The chromatographic data were processed by Totalchrom navigator HPLC version 6.3.1 Software.

## Materials

FLEXABENZE PLUS (15 mg CBP and 200 mg ACF) manufactured by Macleods pharmaceuticals LTD, Mumbai. All chemicals and reagents used were of AR grade.

## Reagents

All the chemicals used were of AR grade.

## Selection of Analytical wavelength

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is the one that gives good response for the drugs that are to be detected. Overlay UV spectra of both the drugs showed that CBP and ACF absorbed appreciably at 280 nm, so detection was carried at this wavelength (Figure 1).

## Preparation of Mobile Phase

2.72 gm of  $\text{KH}_2\text{PO}_4$  was weighed and dissolved in 1000 ml of HPLC water to prepare 0.02 M  $\text{KH}_2\text{PO}_4$  buffer. Mobile phase was prepared by mixing 750 ml of Methanol and 250 ml 0.02 M  $\text{KH}_2\text{PO}_4$ . The pH was adjusted to 3 using o-phosphoric acid (1%). Solution was filtered through Whatman filter paper (No. 41) and sonicate for 10 min and this solution was used as a mobile phase.

## Preparation of Standard Stock Solutions

CBP (10 mg) and ACF (10 mg) were accurately weighed and transferred to two separate 10 ml volumetric flask and dissolved in few ml of methanol. Volumes were made up to the mark with methanol to yield a solution containing 1000  $\mu\text{g}/\text{ml}$  of CBP and 1000  $\mu\text{g}/\text{ml}$  of ACF, respectively. Appropriate aliquots from above solution were taken and diluted with mobile phase to obtain 100  $\mu\text{g}/\text{ml}$  of CBP and 100  $\mu\text{g}/\text{ml}$  of ACF, respectively.

## Chromatographic conditions

A Phenomenex® C-18 (250 x 4.6 mm id) chromatographic column equilibrated with mobile phase Methanol: 0.02 M  $\text{KH}_2\text{PO}_4$  (75:25, % v/v). pH 3 was adjusted using o-phosphoric acid (1%). Mobile phase flow rate was maintained at 1 ml/min and effluents were monitored at 280 nm. The sample was injected using a 20  $\mu\text{L}$  fixed loop, and the total run time was 12 min.

## Calibration curve for CBP and ACF

Appropriate aliquot of stock solution of CBP and ACF was taken in same 10 ml volumetric flasks. The volume was made up to the mark with mobile phase to obtain final concentration of 0.01, 0.1, 1, 5, 10, 20  $\mu\text{g}/\text{ml}$  of CBP and 0.1, 0.5, 2, 10, 20, 40  $\mu\text{g}/\text{ml}$  of ACF, respectively.

## VALIDATION PARAMETER

### Linearity and Range

The calibration curve for CBP was found to be linear in the range of 0.01-20  $\mu\text{g}/\text{ml}$  with a correlation coefficient of 0.9990. The calibration curve for ACF was found to be linear in the range of 0.1-40  $\mu\text{g}/\text{ml}$  with a regression coefficient of 0.9990. The regression analysis of calibration curves are reported in table 2 and 3.

### Precision

#### Intraday precision

The intraday studies were carried out by measuring response for 3 concentrations for 3 times a day. The % RSD values for CBP & ACF were found to be 0.49 – 1.02 and 0.51- 0.93 for intraday respectively. These %RSD value was found to be less than  $\pm 2.0$  indicated that the method is precise (Table 4 & 5).

### **Interday precision**

The interday studies were carried out by measuring response for 3 concentrations for 3 times at 3 different days. The % RSD values for CBP & ACF were found to be 0.50 -1.13 and 0.58 - 1.08 for interday respectively. These %RSD value was found to be less than  $\pm 2.0$  indicated that the method is precise (Table 4 & 5).

### **Repeatability**

The repeatability studies were carried out by measuring response for a single concentration for 6 times a day. The % RSD values of repeatability for CBP and ACF were found to be 0.54 and 0.80 (Table 6). These %RSD value was found to be less than  $\pm 1.0$  indicated that the method is precise.

### **Accuracy**

The accuracy of the method was determined by calculating recoveries of CBP & ACF by method of standard addition. The recoveries found to be 99.57 - 101.10 % and 99.78 -101.81 % for CBP & ACF respectively (Table 7).

### **Limit of detection and limit of quantification**

LOD is the lowest amount of the analyte that can be detected but not quantified. From the visual observation of chromatogram, the detection limits for CBP & ACF were 0.003  $\mu\text{g/ml}$  and 0.03  $\mu\text{g/ml}$ , respectively. LOQ is the lowest amount of the analyte that can be detected and quantified. The quantitation limits were 0.01 and 0.1  $\mu\text{g/ml}$  respectively. The above data shows that a microgram quantity of both the drugs can be accurately and precisely determined.

### **Robustness**

Robustness study was carried out by changing the mobile phase composition, pH and flow rate (Table 8). There is no significant change in the results so the proposed method is robust.

### **Specificity**

The specificity study was carried out to check the interference from the excipients used like Micro crystalline cellulose, Lactose and magnesium stearate in the formulations by preparing synthetic mixture containing both the drugs and excipients. The specificity study was carried out to check the interference from the excipients. Recoveries of both the drugs were above 98%. The results of the specificity study are shown in the Table 9.

### **Solution stability**

Stability of standard and sample solution of CBP and ACF were evaluated at room temperature for 24 hr. Both the drugs were found to be stable with a recovery of more than 98% (Table 10).

### **System suitability parameters**

System suitability test was carried out and the results are summarized in Table 11.

The validation parameters are summarized in Table 12.

### **Analysis of Marketed Formulation**

Marketed formulation was analyzed using proposed method which gave percentage recovery for CBP & ACF were 99.49% and 110.01% respectively. Rt of 4.2 min and 9.0 min were observed in the chromatogram for CBP and ACF, and no interference from the excipients present in the marketed tablet formulation was observed (Table 13).

**Table 1: Optimized chromatographic conditions for HPLC**

Parameter	Conditions
Mobile phase	Methanol : 0.02 M KH <sub>2</sub> PO <sub>4</sub> Buffer (75:25 v/v) , pH 3
Column	Phenomenex ODS C-18 column, 250 x 4.6 mm i.d. (5 µm particle size)
Detection wavelength	280 nm
Flow rate	1ml/min
Temperature	Ambient condition
Retention time	4.2 min and 9.0 min
Run time	12min
Syringe size	20 µl

**Table 2: Result of calibration readings for CBP by HPLC method**

Concentrations (µg/ml)	Area	% RSD
	Mean ± S.D. (n=5)	
0.01	4756.63± 49.80	1.05
0.1	26489.67± 440.00	1.66
1	75465.76± 483.27	0.64
5	235406.7± 1473.40	0.62
10	455476.1± 3101.54	0.68
20	896231.00± 4958.97	0.55
Slope	22012.40 ± 98.86	
Intercept	16560.40± 1106.78	
Regression co-efficient (r <sup>2</sup> )	0.9990	

**Table 3: Result of calibration readings for ACF by HPLC method**

Concentrations (µg/ml)	Area	% RSD
	Mean ± S.D. (n=5)	
0.1	4541.99± 46.53	1.02
0.5	32510.73± 277.41	0.85
2	80459.85 ± 490.98	0.61
10	298180.30 ± 2450.21	0.82
20	554208.60 ± 3892.63	0.70
40	1073425.00 ± 9497.82	0.88
Slope	52764.60± 432.60	
Intercept	22999.00± 1136.82	
Regression co-efficient (r <sup>2</sup> )	0.999	

**Table 4: Precision data for estimation of CBP (n=3)**

Conc. µg/ml	Intraday (area) ± S.D	% RSD	Interday (area) ± S.D	% RSD
0.05	4745.22±49.23	1.03	4738.28±53.58	1.13
1	75503.20±401.79	0.53	75518.58±450.46	0.59
20	897072.30±4435.22	0.49	895039.00±4505.12	0.50

**Table 5: Precision data for estimation of ACF (n=3)**

Conc. µg/ml	Intraday (area) ± S.D	% RSD	Interday (area) ± S.D	% RSD
0.1	4557.87± 42.80	0.93	4536.62± 49.04	1.08
1	80532.46± 418.69	0.51	80421.48± 474.47	0.58
20	1082813± 5940.64	0.54	1088943± 10049.05	0.92

Table 6: Repeatability data for CBP and ACF

Concentration	CBP	CBP	ACF	ACF
	5 µg/ml	Rt (min)	10 µg/ml	Rt (min)
Area	236221.3	4.24	299698.2	9.12
	237941.3	4.14	298987.1	9.18
	235098.5	4.23	296103.6	9.12
	236995.4	4.19	298949.6	9.14
	236547.3	4.13	299856.4	9.2
	237032.9	4.21	299874.3	9.02
Mean.	236639.4	4.20	298911.5	9.13
Std. Dev.	952.287	0.03	1437.373	0.06
% RSD	0.40	0.93	0.48	0.68

Table 7: Accuracy data of CBP &amp; ACF by the proposed HPLC method (n=3)

Conc. of drug from formulation (µg/ml)		Amount of standard drug spike (µg/ml)		% recovery Mean ± SD		% RSD	
CBP	ACF	CBP	ACF	CBP	ACF	CBP	ACF
5	10	0	0	99.98±0.64	99.98±0.46	0.64	0.46
5	10	2.5	5	99.85±0.32	99.78±0.50	0.33	0.50
5	10	5	10	101.10±0.56	101.81±0.26	0.56	0.25
5	10	7.5	15	99.57±0.45	100.59±0.51	0.45	0.51

Table 8: Robustness results of CBP &amp; ACF in given formulations

Parameter	Method condition	Rt		% RSD of peak area	
		CBP 1 µg/ml	ACF 2µg/ml	CBP 1µg/ml	ACF 2µg/ml
pH	Actual (3)	4.21	9.10	0.82	0.94
	2.5	4.10	9.25	0.77	0.67
	3.5	4.	1.89	0.58	0.74
Mobile phase Ratio (Methanol: 0.02 M KH <sub>2</sub> PO <sub>4</sub> )	Actual (75:25)	4.21	9.10	0.89	0.92
	70: 30	4.32	9.21	0.74	0.83
	80: 20	4.18	8.91	0.78	0.71
Flow rate	Actual (1ml/min)	4.21	9.10	0.86	0.91
	0.9 ml/min	4.36	9.36	0.80	0.87
	1.1 ml/min	3.89	8.87	0.76	0.79

Table 9: Specificity data of CBP &amp; ACF by the proposed HPLC method

Amount of drug taken	Amount of drug found	% drug found ±SD
CBP (5µg/ml)	4.94	98.95±0.24
ACF (10µg/ml)	9.97	99.75±0.42

Table 10: Solution stability study of CBP &amp; ACF by the proposed HPLC method

Time (Hrs.)	Area (n=3)		% drug found	
	CBP 1 (µg/ml)	ACF 2 (µg/ml)	CBP	ACF
0	75890.26	80548.23	100	100
3	75466.36	80320.68	99.44	99.71
6	75018.65	79626.95	98.85	98.86
24	74759.69	78949.21	98.51	98.01

**Table 11: System suitability data of CBP & ACF by the proposed HPLC method**

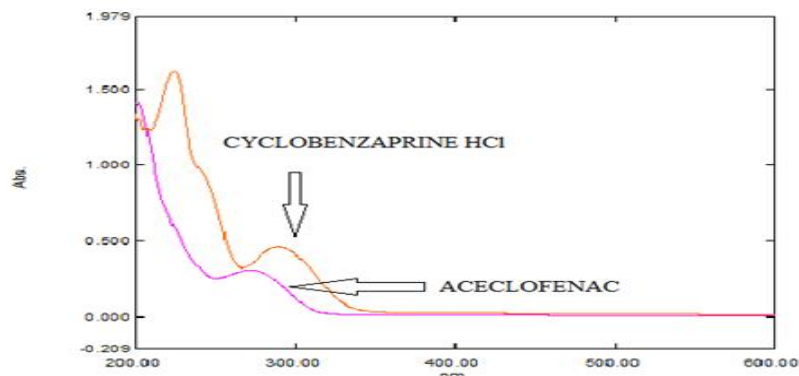
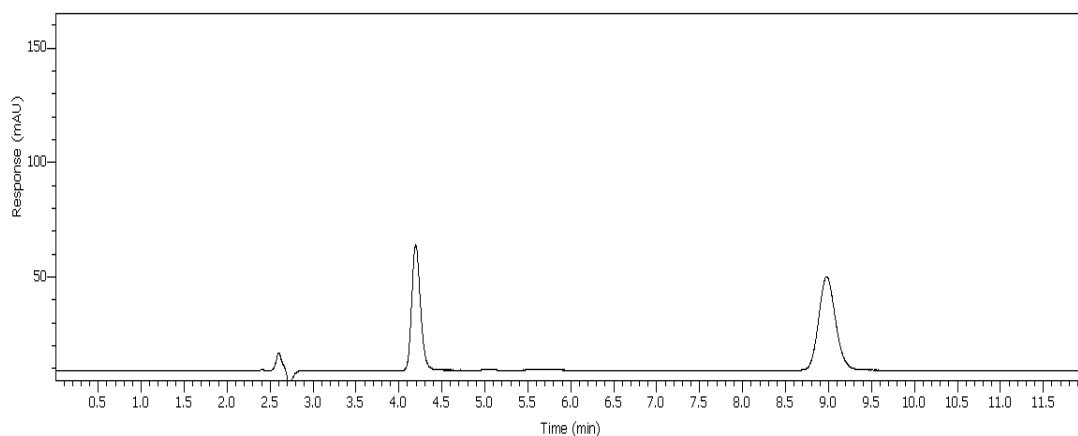
Parameters	CBP	ACF
Assymmetric factor	2.00	1.50
Resolution	13.71	
Theoretical Plates	3541.55	4472.49

**Table 12: Summary of Validation Parameters of HPLC**

Parameters	CBP	ACF
Range( $\mu\text{g/ml}$ )	0.01-20	0.1- 40
Retention time (min)	4.2	9.0
Assymmetric factor	2.00	1.5
Resolution	13.71	
Theoretical Plates	3541.55	4472.49
Intra-day Precision (n=3) (%RSD)	0.49- 1.03	0.51- 0.93
Inter-day Precision (n=3) (%RSD)	0.50-1.13	0.58- 1.08
Repeatability (%RSD)	0.40	0.48
Accuracy (%Recovery)	99.57 % -101.10%	99.78 % - 101.81 %
Detection limit ( $\mu\text{g/ml}$ )	0.003	0.03
Quantitation limit ( $\mu\text{g/ml}$ )	0.01	0.1
Robustness	Robust	Robust
Specificity	Specific	Specific

**Table 13: Analysis of marketed formulation (n=3)**

Formulation	Amount of drug taken ( $\mu\text{g/ml}$ )		Amount found ( $\mu\text{g/ml}$ )		% drug found $\pm$ SD	
	CBP	ACF	CBP	ACF	CBP	ACF
Flexabenze Plus	1.5	20	1.49	20.20	99.33 $\pm$ 0.67	101.01 $\pm$ 0.67

**Figure 1: Overlain Spectra of CBP (10  $\mu\text{g/ml}$ ) and ACF (10  $\mu\text{g/ml}$ ) in mobile phase.****Figure 2: Chromatogram showing peaks of CBP (10  $\mu\text{g/ml}$ ) and ACF (10  $\mu\text{g/ml}$ ) using mobile phase methanol: 0.02 M  $\text{KH}_2\text{PO}_4$  (75: 25 v/v, pH 3) in mix standard.**

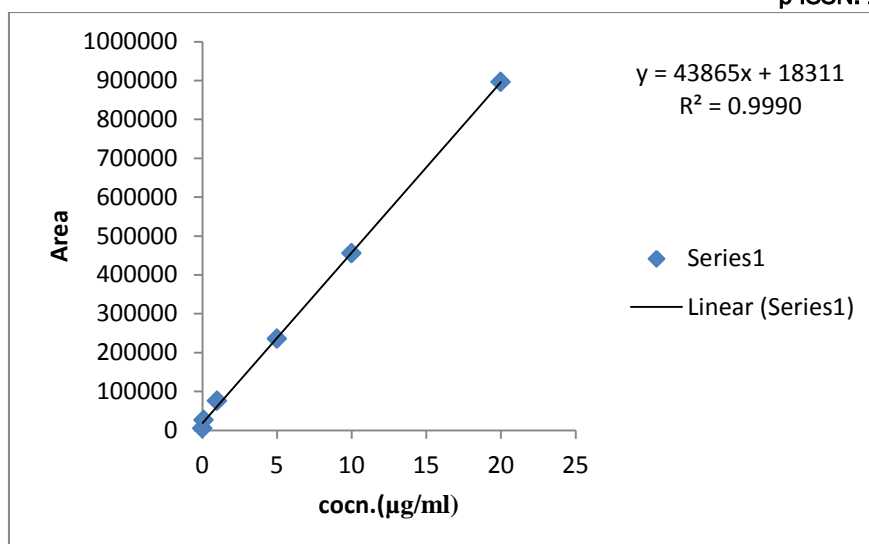


Figure 3: Calibration Curve of CBP by HPLC method

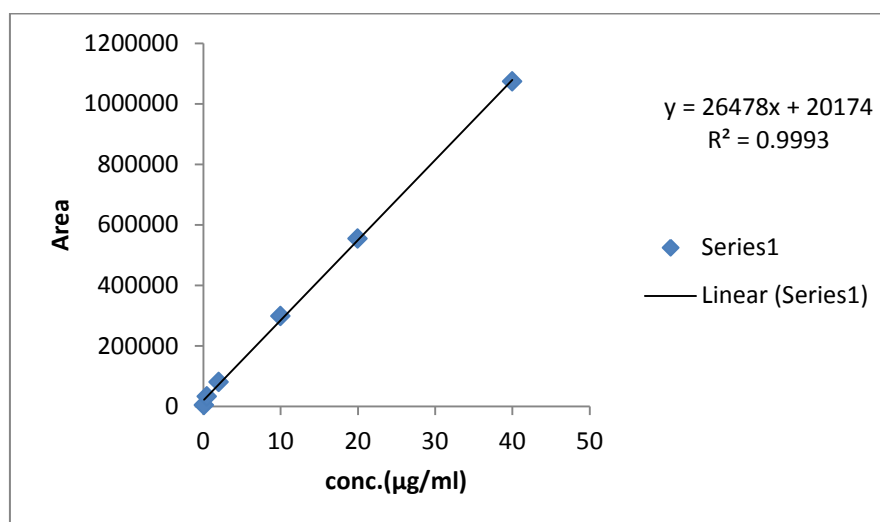


Figure 4: Calibration Curve of ACF by HPLC method

### CONCLUSION

The HPLC method for the estimation of CBP and ACF has been developed. The proposed method was validated as per ICH Q2 (R1) guideline for accuracy, precision, linearity, specificity and robustness. The developed method was successfully applied to marketed formulation.

### REFERENCES

1. Indian Pharmacopoeia; Indian Pharmacopoeia Commission, Ghaziabad, 2010 Volume1, pp. 770.
2. British Pharmacopoeia; volume I , London: HMSO Publication; 2007,vol 1, pp 41,42
3. United State Pharmacopoeia, The Official Compendia of Standards;United States Pharmacopoeial Convention Inc; Rockville, MD, USP30-NF25, 2004, pp.52
4. The Merck Index- An Encyclopedea of Chemicals, Drugs and Biological; 14th Edn; Merk Co. Inc. White House, 2001, pp 2319.
5. Martindale - The Extra Pharmacopoeia; 31st Edn; The Royal Pharmaceutical Society,London, 1996, pp 1519
6. Tripathi KD. Essential of Medical Pharmacology; 6th Edn; Jaypee Brothers Medical Publishers Pvt. Ltd, 2008, pp-315.
7. ICH guidelines, Validation of Analytical Procedures, Text and Methodology Q2 (R1); I.C.H. Tripartite Guidelines, 2005, pp 5-17.

8. Heinitz ML. Determination of cyclobenzaprine in tablet by HPLC. *J Pharm Sci.* 1982;71(6):656-658.
9. Constanzer M, Chavez C, Maluszeuski B. Development and comparison of HPLC method with tandem mass spectrometric and ultraviolet absorbance detection for the determination of cyclobenzaprine in human plasma and urine. *J Chromatogr B Biomed Appl.* 1995;666(1):117-126.
10. Poklis JL, Wolf CE, Goldstein A, Wolfe MC, Poklis SA. Detection and quantification of tricyclic antidepressant and other psychoactive drug in urine by HPLC-MS/MS for pain management compliance testing. *J Clin Lab Anal.* 2012;26(4):286-294.
11. Coulter C, Taruc M, Tuyay J, Moore C. Antidepressant drugs in oral fluid by liquid chromatography-tandem mass spectrometry. *J Anal Toxicol.* 2010;34(2):64-72.
12. Xiang Y, Zhou QZ, Peng K, Li D, Chen X, Jiang H, Zheng H. Determination of cyclobenzaprine in human plasma by liquid chromatography – electrospray ionization tandem mass spectrometry and its application in a pharmacokinetic study. *Biomed Chromatogr.* 2012;26(9):1053-1058.
13. Paul K, Nagarajan JSK, Chandan RS. Standardization and RP-HPLC method for the estimation of aceclofenac in dosage form. *Int J Res Pharm Chem.* 2011;1(4):853-859.
14. Bhinge JR, Kumar RV, Sinha VR. A simple and sensitive stability indicating RP-HPLC assay method for determination of aceclofenac. *J Chromatogr Sci.* 2008;46(5):440-444.
15. Raja RK, Sankar GG, Rao AL. Development and validation of RP-HPLC method for the estimation of aceclofenac in tablet dosage form. *Indian Drugs.* 2005;42(10):693-696.
16. Kim E, Ahn B, Noh K, Kang, Gwak HS. Quantitative determination of aceclofenac and its three major metabolites in rat plasma by HPLC-MS/MS. *J Sep Sci.* 2012;35(17):2219-2222.
17. Gopinath R, Rajan S, Meyyanathan SN. A reverse phase high performance liquid chromatographic method for simultaneous estimation of paracetamol and aceclofenac in tablet. *Int J Pharm Sci.* 2007;69(1):137-140.
18. Deodhar MN, Godse VP, Bhosale AV, Sonawane RA, Sakpal PS, Borkar DD, Bafana YS. Reverse phase high performance liquid chromatographic method for determination of aceclofenac and paracetamol in tablet dosage form. *Asian J Res Chem.* 2009;2(1):37-40.
19. Dhaneshwa SR, Raut KO, Bhusari VK. Validated HPLC method for simultaneous estimation of paracetamol, aceclofenac and thiocolchicoside in bulk drug & formulation. *Res J Pharm Biol Chem Sci.* 2011;2(2):435-438.
20. Avanthi K, Tejaswi P, Vivek SP, Sudheer, Vamshikrishna G. Simple RP-HPLC for simultaneous determination of diacerein and aceclofenac in tablet dosage form. *Indo American Jf Pharma Res.* 2011;3(5):3606-3616.
21. Manasa B, Ravikumar A. Development and validation of reverse phase high performance liquid chromatographic method for the simultaneous estimation of aceclofenac and rabeprazole sodium in bulk & capsule. *Int J Pharm Biol Sci.* 2012;2(3):160-169.
22. Gupta KR, Samrit VE, Mahapatra AH, Wadokar SG. Stability indicating high performance liquid chromatographic assay of diacerein and aceclofenac in tablets. *Am J Pharm Res.* 2011;1(3):202-218.
23. <http://www.inventi.in/Article/ppaqa/260/11.aspx>.
24. Chandra P, Rathod AS, Lohidasan S, Mahadik KR. Application of HPLC for simultaneous determination of aceclofenac, paracetamol and tramadol HCl in pharmaceutical dosage form. *Sci Pharm.* 2012;80:337-351.
25. Sharma MC, Sharma S. Application to dissolution study and HPLC assay method for determination of diacerein and aceclofenac in tablet dosage form. *Am – Eurasian J Sci Res.* 2011;6(3):149-154.
26. Gandhi S, Deshpande P, Rajmane V, Dodal T, Parab J. Method development and validation for simultaneous estimation of drotaverin HCl and aceclofenac in tablet dosage form by RP-HPLC. *Int J Pharm Sci.* 2010;4(3):49-52.
27. Jain JR, Shah SA, Shah DR, Chauhan RS. RP-HPLC method for simultaneous estimation of drotaverine HCl and aceclofenac in their combined tablet dosage form. *Der Pharma Chemia.* 2011;3(4):245-252.
28. Shaikh KA, Devkhile AB. Simultaneous determination of aceclofenac, paracetamol and chlorzoxazone by RP-HPLC in pharmaceutical dosage form. *J Chromatogr Sci.* 2008;46:649-652.
29. Ganthi HR., Bapatu HR, Maram RK and Mallu UR. Single RP-HPLC method for quantification of aceclofenac, paracetamol and chlorzoxazone in formulation. *Int J Sci Inn Discov.* 2012;2(4):471-490.
30. Shaikh KA, Patil AT, Ingol AB. Sensitive liquid chromatographic method for simultaneous estimation of diacerein and aceclofenac in tablets dosage form. *Int J Ind Chem.* 2012;3(3):2-7.