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ISOCRATIC RP-HPLC METHOD FOR THE SIMULTANEOUS DETERMINATION OF PARACETAMOL AND ACECLOFENAC IN PURE AND IN COMBINED DOSAGE FORMS

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ABSTRACT

A simple, selective, accurate isocratic high Performance Liquid Chromatographic (HPLC) method was developed and validated for the analysis of Paracetamol and aceclofenac in pure and in combined dosage forms . Chromatographic separation achieved isocratically on a Thermo Scientific C_{18} column (250 mm x 4.6 mm I.D., 5 µm particle size) utilizing a mobile phase of phosphate buffer (pH 2.5) and acetonitrile in the ratio of 600:400v/v at a flow rate of 1.0ml/min with UV detection at 265nm.Aceclofenac was used as an internal standard. The retention time of paracetamol and aceclofenac was 2.989 and 3.631min respectively. The developed RP-HPLC method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation. This paper aimed at developing and validating an RP-HPLC method, being simple, accurate and selective, and the proposed method can be used for the estimation of these drugs in combined dosage forms.

KEY WORDS: Paracetamol, Aceclofenac, RP-HPLC, Validation

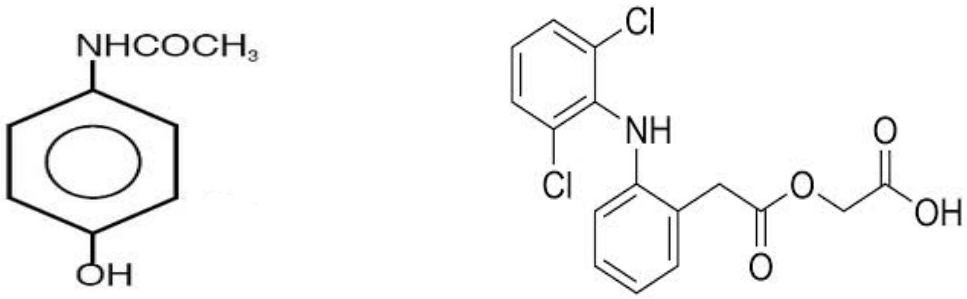
INTRODUCTION

Paracetamol [1,2] is a non-opiate, non-salicylate analgesic and antipyretic. Chemically, paracetamol is 4-hydroxyl acetanilide or N-(4-hydroxy phenyl) acetamide. Its empirical formula is $C_8H_9NO_2$ and molecular weight is 151.2.It acts by inhibiting prostaglandin synthetase centrally. Specifically, it is a potent inhibitor of cyclo-oxygenase in the CNS.

Aceclofenac [3,4] is an orally administered phenyl acetic acid derivative with effects on a variety of inflammatory mediators. It is from the class of non-steroidal anti-inflammatory drug. Chemically, it is 2-[(2,6-dichloro phenylamino) phenyl]acetoxy-acetic acid. Its empirical formula is $C_{16}H_{13}C_{l2}NO_4$ and molecular weight is 354.2. The mode of action of aceclofenac is largely based on the inhibition of prostaglandin synthesis.

Detailed survey of literature revealed that several RP-HPLC methods [5-14] for determination the of these drugs individually and few RP-HPLC methods[15-18] in other combinations in pharmaceuticals and biological preparations were reported. The objective of this present study were, therefore, to develop and validated a simpler, economic, rapid, precise, isocratic, and accurate RP-HPLC method with good sensitivity for quantitative analysis of paracetamol and aceclofenac in pure and in pharmaceutical dosage forms in accordance with International Conference on Harmonization (ICH) guidelines . The direct use of the mobile

phase for dilution of the formulations for quantitative analysis would minimize errors that might occur during tedious extraction procedures.



PARACETAMOL **ACECLOFENAC**

Figure.1. Molecular Structure Of Paracetamol And Aceclofenac

EXPERIMENTAL

INSTRUMENTATION: Chromatographic analysis in the present study was made by using Shimadzu HPLC class VP series, (Shimadzu Corporation, Kyoto, Japan) isocratic high pressure liquid chromatographic system equipped with an LC-10AT pump, a variable wavelength programmable UV/Visible detector SPD-10A, a CTO-10 AS column oven, an SCL-10A system controller. The chromatographic system utilizes a Shimadzu class VP series version 5.03 computer program to control hardware, and to acquire and store data. Thermo Scientific C_{18} column (250 mm x 4.6 mm I.D., 5 µm particle size) was used for the separation of BSN. Shimazdu (Tokyo, Japan) electronic weighing balance, model BL 220 H was used for weighing the samples. Elico pH meter (Hyderabad, India) LI 120 model was used for pH measurements.

CHEMICALS AND REAGENTS: Milli-Q water, Acetonitrile (HPLC Grade), Orthophosphoric acid (GR Grade), potassium dihydrogen orthophosphate monohydrate (GR Grade) was obtained from Qualigens Ltd., Mumbai. All other chemical of analytical grade were procured from local sources unless specified. All dilutions were performed in standard class-A, volumetric glassware.

PREPARATION OF PHOSPHATE BUFFER: The buffer solution was prepared by dissolving accurately weighed 6.8grams of potassium dihydrogen orthophosphate and transferred into a clean and dry 1000ml volumetric flask, dissolved and diluted with 1000ml water [HPLC Grade]. The final pH of the buffer was adjusted to 2.5 by using Orthophosporic acid

MOBILE PHASE PREPARATION: Prepare a filtered and degassed mixture of buffer and Acetonitrile in the ratio of 600:400 v/v respectively.

DILUENT PREPARATION: Mobile phase is used as diluent in the present assay.

PREPARATION OF STOCK & WORKING STANDARD SOLUTIONS: The stock solution was prepared by weighing accurately 100mg of Paracetamol and 100 mg of Aceclofenac and transferred into a clean and dry 100 ml volumetric flask. About 70 ml of diluent was added and sonicated. The volume was made unto the mark with the same diluent. From the above prepared stock solution pipette out suitable aliquots and transferred into a clean and dry 10ml volumetric flask, the diluent was added up to the mark to get final concentration of 200-1000µg/ml of Paracetamol and 50-250µg/ml of aceclofenac respectively.

PREPARATION OF SAMPLE SOLUTION: The sample solution was prepared by weighing equivalently 50 mg of paracetamol and aceclofenac and transferred into a 100 ml clean and dry volumetric flask and about 70ml of diluent was added and sonicated to dissolve it completely and the volume made up to the mark with the same solvent. From above prepared stock solution pipette out aliquots of the above solution and transferred into a clean and different dry 10 ml volumetric flasks, the diluent was added up to the mark 10ml to get final concentration of 200-1000µg/mL of paracetamol and 50-250µg/mL of aceclofenac respectively. 10µL volumes of these standard and sample solutions were injected five times and the peak areas were recorded. The mean and percentage relative standard deviation were calculated from the peak areas.

RESULTS AND DISCUSSION

HPLC METHOD DEVELOPMENT: The present selected drugs were subjected to chromatographic analysis using mobile phases of differing pH, flow rate using the under mentioned chromatographic conditions. The changes in the retention time of all drugs were noted as a function of changing mobile phase, pH, flow rate, strength and selectivity. Initially buffer and Acetonitrile in the ratio 70: 30 were tried but the peak eluted in the dead volume the two peaks were merged. Later buffer and Acetonitrile in the ratio of 65:35 v/v were tried. It was found that the retention time and resolution was increased but sharp peaks were not obtained. Finally buffer and Acetonitrile in the ratio of 600:400 v/v at flow rate of 1.0mL/min was tried. It was found that paracetamol and aceclofenac gave acceptable retention time, plates and good resolution at 265nm

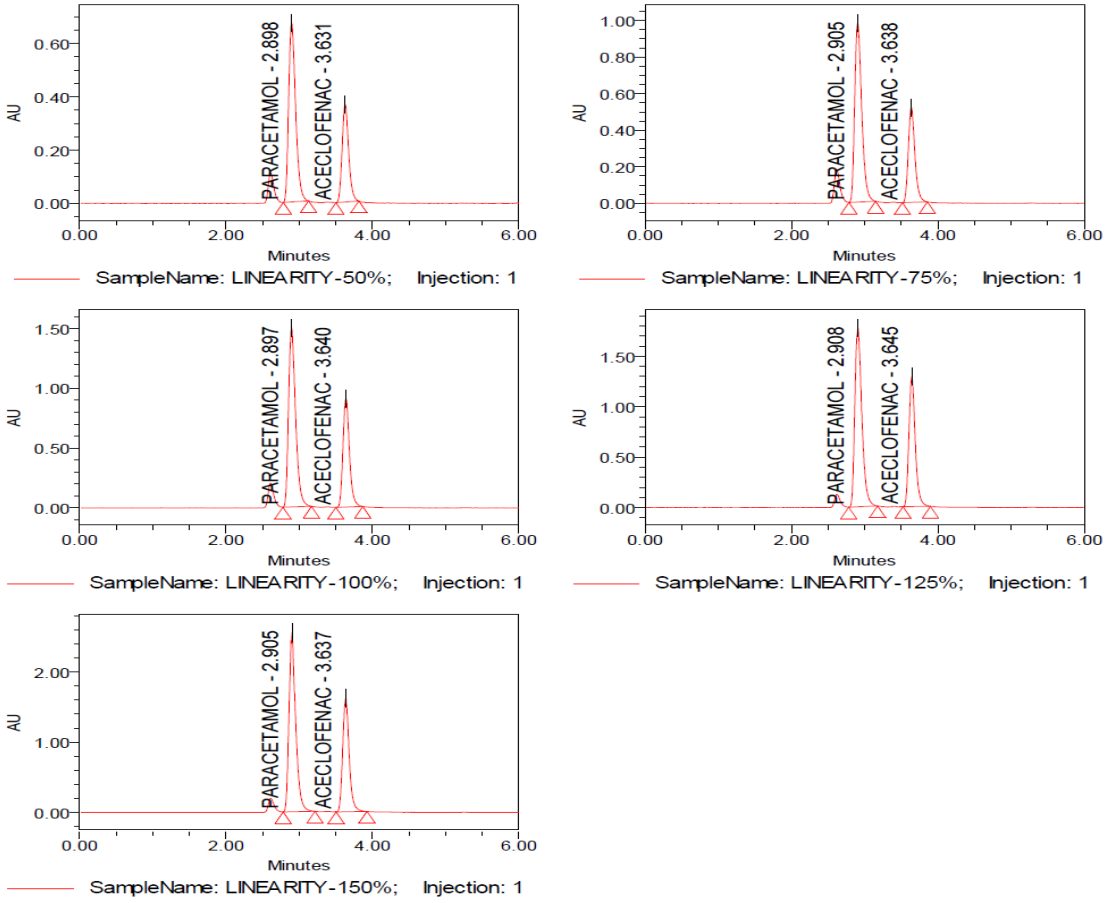


Figure.2. LINEARITY CHROMATOGRAMS OF PARACETAMOL AND ACECLOFENAC

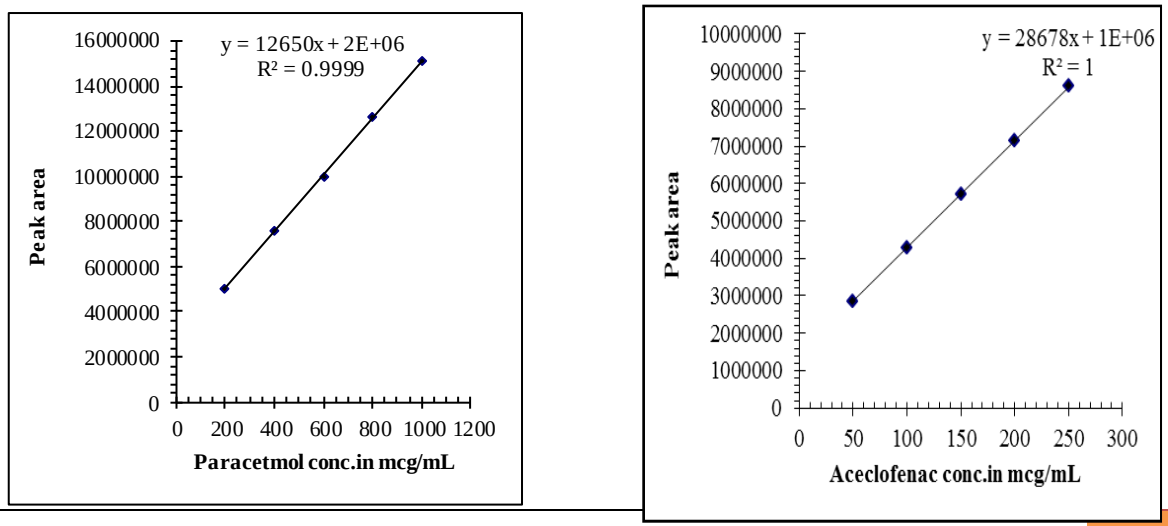


Figure.3.LINEARITY CURVE OF PARACETAMOL AND ACECLOFENAC BY THE PROPOSED METHOD

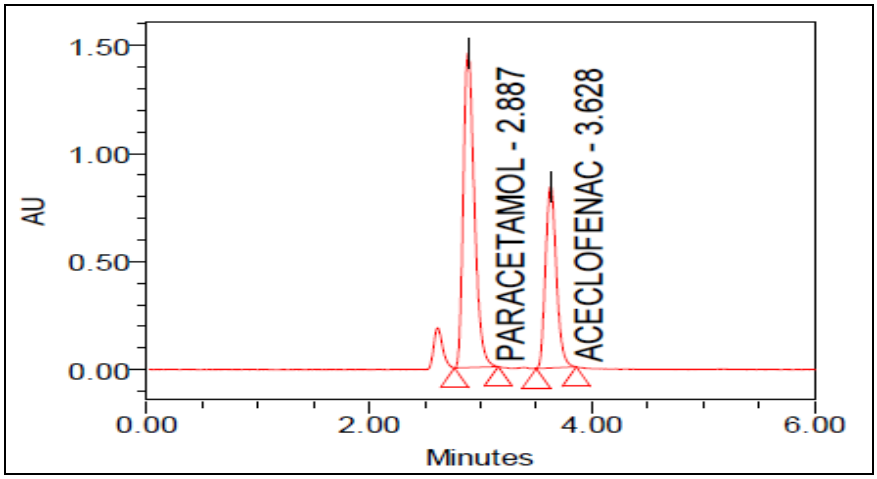


Figure.4. VALIDATED CHROMATOGRAM OF PARACETAMOL AND ACECLOFENAC FORMULATIONS

METHOD VALIDATION: The developed RP-HPLC method is validated in accordance with ICH guidelines for assay of paracetamol and aceclofenac using the following Parameters.

SPECIFICITY:

BLANK AND PLACEBO INTERFERENCE: A study to establish the interference of blank and placebo were conducted. Diluent and placebo was injected into the chromatograph in the defined above chromatographic conditions and the blank and placebo chromatograms were recorded. Chromatogram of blank solution showed no peaks at the retention time of paracetamol and aceclofenac peak. This indicates that the diluent solution used in sample preparation do not interfere in estimation of paracetamol and aceclofenac in tablets. Similarly chromatogram of placebo solution showed no peaks at the retention time of paracetamol and aceclofenac peak indicating that the placebo used in sample preparation do not interfere in estimation of paracetamol and aceclofenac in their formulations.

LINEARITY OF DETECTOR RESPONSE: The standard curve was obtained in the concentration range of 200-1000µg/ml for paracetamol and 50-250µg/mL for aceclofenac. Evaluation of two drugs were performed with PDA detector at 265 nm, peak area recorded for all the peaks and are given in Figure. 2. The linearity of this developed RP-HPLC method was evaluated by linear regression analysis. Slope, intercept and correlation coefficient [r2] of standard curve were plotted for paracetamol and aceclofenac Figure. 3 and calculated respectively. The slope and intercept value for calibration curve was y = 12650x + 2E+06 (R^2 = 0.999) for paracetamol and y = 28678x + 1E+06 (R^2 = 0.999) for aceclofenac. These results showed that an excellent correlation exists between peak area and concentration of drugs within the concentration range indicated above. The LOD value for paracetamol and aceclofenac were found to be 0.009µg/mL and 0.00145µg/mL, respectively and the LOQ value 0.0326µg/mL and 0.00484µg/mL and are reported in Table.1 respectively.

ACCURACY: The accuracy of the method was determined on three concentration levels by recovery experiments. The recovery studies were carried out in triplicate preparations on composite blend collected from 10 tablets of paracetamol and aceclofenac, analyzed as per the proposed method. The %RSD was ranged from 0.053-0.253 for paracetamol and 0.008-0.053 for aceclofenac with percentage recoveries ranged from 99.65-99.90% for paracetamol and 99.50-99.90% for aceclofenac respectively. From the data reported in Table.3, reported that the developed RP-HPLC method was found to be accurate for paracetamol and aceclofenac assay.

ROBUSTNESS STUDIES: The robustness study of the developed assay method for paracetamol and aceclofenac were established in all variance conditions. Assay value of the test preparation solution was not affected and it

was in accordance with that of actual. System suitability parameters were also found satisfactory; hence, the analytical method would be concluded as robust.

ANALYSIS OF MARKETED FORMULATION: Analysis of marketed tablets was carried out using the above said optimized mobile phase and HPLC conditions. The % drug content of tablets obtained by the proposed method for paracetamol and aceclofenac (Figure.4) was found to be 99.99 and 99.98, respectively. This showed that the estimation of dosage forms was accurate within the acceptance level of 95% to 100%. The results are given in Table.4.

TABLE:1: LINEARITY STUDIES OF PARACETAMOL AND ACECLOFENAC BY THE PROPOSED METHOD

LINEARITY STUDY FOR PARACETAMOL		
% LEVEL (APPROX.)	CONC. µg/ml	AREA
50	200	5042032
75	400	7571871
100	600	10014876
125	800	12615126
150	1000	15170798
Slope		12650
RSQ(r2)		0.9999
LOD		0.009
LOQ		0.0326

LINEARITY STUDY FOR ACECLOFENAC		
% LEVEL (APPROX.)	CONC. µg/mL	AREA
50	50	2861566
75	100	4290953
100	150	5727956
125	200	7156492
150	250	8598414
Slope		28678
RSQ(r2)		1.000
LOD (µg/ml)		0.00145
LOQ (µg/ml)		0.00484

TABLE:2:METHOD PRECISION (INTER AND INTRADAY) STUDIES FOR BY THE PROPOSED METHOD

SUMMARY SHOWING METHOD PRECISION BY PROPOSED METHOD

	FOR PARACETMOL		FOR ACECLOFENAC	
No. of samples	**Peak area**	**Rt**	**Peak area**	**Rt**
Set-1	5042313	2.887	2864210	3.633
Set-2	5048110	2.895	2863197	3.637
Set-3	5046169	2.889	2867886	3.632
Set-4	5040993	2.885	2861967	3.630
Set-5	5045501	2.889	2860920	3.628
Set-6	5046316	2.903	2869525	3.642
Over All Avg.	5044900	2.891	2864618	3.633
Over All Std Dev.	2962.134	0.0066	3395.502	0.0050
Over All %RSD	0.053	0.229	0.118	0.139

TABLE: 3:RECOVERY STUDIES FOR PARACETAMOL AND ACECLOFENAC BY THE PROPOSED METHOD

PARACETAMOL						
Spiked Level	**Sample Weight**	**Sample Area**	**µg/ml added**	**µg/ml found**	**% Recovery**	**% Mean**
50%	424.33	5042313	991.012	989.57	99.85	
50%	424.33	5048110	991.012	990.71	99.96	
50%	424.33	5046169	991.012	990.33	99.93	99.9
50%	424.33	5040993	991.012	989.31	99.82	[%RSD 0.053]
50%	424.33	5045501	991.012	990.20	99.91	
50%	424.33	5046316	991.012	990.36	99.93	
100%	848.65	10095670	1982.000	1981.31	99.96	
100%	848.65	10055441	1982.000	1973.42	99.56	99.67
100%	848.65	10048549	1982.000	1972.07	99.49	[%RSD 0.253]
150%	1273.00	15173218	2973.058	2977.80	100	99.95
150%	1273.00	15161657	2973.058	2975.53	100	[%RSD 0.118]

150%	1273.00	15166978	2973.058	2976.58	100	
150%	1273.00	15169405	2973.058	2977.05	100	
150%	1273.00	15188941	2973.058	2980.89	100	
150%	1273.00	15105620	2973.058	2964.54	99.71	

ACECLOFENAC

Spiked Level	Sample Weight	Sample Area	µg/ml added	µg/ml found	% Recovery	% Mean
50%	424.33	2864210	199.402	199.42	100	
50%	424.33	2863197	199.402	199.35	99.97	
50%	424.33	2867886	199.402	199.68	100	99.96
50%	424.33	2861967	199.402	199.27	99.93	[%RSD 0.049]
50%	424.33	2860920	199.402	199.19	99.89	
50%	424.33	2869525	199.402	199.79	100	
100%	848.65	5720880	398.800	398.32	99.87	
100%	848.65	5727224	398.800	398.76	99.98	99.9
100%	848.65	5724247	398.800	398.56	99.93	[%RSD 0.053]
150%	1273.00	8591523	598.212	598.19	99.99	
150%	1273.00	8593707	598.212	598.35	100	
150%	1273.00	8590578	598.212	598.13	99.98	99.95
150%	1273.00	8596122	598.212	598.51	100	[%RSD 0.008]
150%	1273.00	8594205	598.212	598.38	100	
150%	1273.00	8595611	598.212	598.48	100	

TABLE:4: ANALYSIS OF MARKETED TABLETS OF PARACETAMOL AND ACECLOFENAC BY THE PROPOSED METHOD

DRUG	LABALCLAIM	QUANTITYFOUND*	%ASSAY
PARACETAMOL	500mg	499.99	99.99
ACECLOFENAC	100mg	99.98	99.98

*Average of six determinations

CONCLUSIONS

It was concluded that the reproducibility, repeatability and accuracy of the proposed method for paracetamol and aceclofenac were found to be satisfactory which is evidenced by low values of standard deviation, percent relative standard deviation and standard error. The percent range of error (within 95% confidence limits) showed precision of the method. The accuracy and reproducibility of the proposed RP-HPLC method was confirmed by recovery experiments, performed by adding known amount of the drugs to the pre analyzed formulations and reanalyzing the mixture by proposed method. The percent recovery obtained indicates noninterference from the excipients used in the formulations. Therefore, it is concluded that the RP-HPLC method developed in the present investigation found to be simple, sensitive, accurate and precise and can be successfully applied for the simultaneous estimation of paracetamol and aceclofenac in tablets can be easily adopted as an alternative method to report routine determination of paracetamol and aceclofenac also finds its immense use in clinical, biological and pharmacokinetic studies.

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REFERENCES

[1]. The British Pharmacopoeia Commission. British Pharmacopoeia, Vol. II, London: the Stationery Office Books; 1998. 33.

[2]. Indian Pharmacopoeia, 4th edition. New Delhi: The Controller of Publications, Government of India; 1996. p. 347-348.

[3]. The British Pharmacopoeia Commission. British Pharmacopoeia, Vol. I, London: the Stationery Office Books; 2003. 1123-1124.

[4]. M.K. Chouksey, A. Khandpur, N.K. Tyagi and G.N. Singh, Indian Drugs, 43, 216 (2005).

[5]. Ravisankar S, Vasudevan M and Najan MJ. Reverse phase HPLC method for the estimation of Paracetamol, Chlorzoxazonze, and Diclofenac sodium in formulations. Indian Drugs.1997;34 (11):663-665.

[6]. Shenoy KRP, Krishnamurthy KS and Sumatheendra KS. Determination of Codeine phosphate, oxylamine succinate, Paracetamol and Caffeine in combined dosage formulation by reverse phase liquid chromatography. Indian Drugs. 2000;37(10): 486-488.

[7]. Shinde VM and Raman R. Simultaneous determination of paracetamol and chlormezanone in tablets by RP-HPLC. Indian Drugs.1998; 35 (8):521-523.

[8]. Yong Y F and Wang AJ. Determination of pseudo ephedrine and paracetamol in their tablets by RP-HPLC. Yaouw Fenxi Zazhi.1998; 16(4):216-218.

[9]. Vasudevan M, Ravisankar S, Ravibabu T and Nanjan MJ. Simultaneous estimation of Paracetamol, Methocarbamol and Ibuprofen by reversed phase HPLC method. IndianDrugs.2000;37(8):386-389.

[10]. Gao LQ., Liu WY and Xing JD. Determination of aceclofenac in plasma by RP-HPLC with solid phase back- flushing extraction method. Yaouw Fenxi Zazhi, 1998; 18(4):238-241.

[11]. Jin Y, Chen H and Zeng FD: Determination of aceclofenac in human plasma by RP-HPLC.Sepu.2004; 22(4):2-254.

[12]. Aloba OT, Adusumilli PS and Nigalaye AG. HPLC analysis of multicomponent product using a silica stationary phase and an aqueous organic phase. J. Pharm Biomed. Anal.1991;9(4):335-340.

[13]. Lee HS, Jeong CK, Choi SJ, Kim SB and Lee MH. Simultaneous determination of aceclofenac and diclofenac in human plasma by narrow bore HPLC using column switching. J.Pharm Biomed. Anal. 2000;23(5):775-781.

[14]. Kousey E, Zawilla NH and Abdul AM. Determination of aceclofenac in bulk and pharmaceutical formulation by HPLC. J. Pharm Biomed. Anal. 2002; 9(4):243-251.

[15]. Gopinath R, Rajan S, Meyyanathan SN, Krishnaveni N, Suresh BA, RP-HPLC method for simultaneous estimation of paracetamol and aceclofenac in tablets. Indian J Pharm Sci., 2007; 69:137-140.

[16]. Godse VP, Deodhar MN, Bhosale AV, Sonawane RA, Sakpal PS, Borkar DD and Bafana YSReverse Phase HPLC Method for Determination of Aceclofenac and Paracetamol in Tablet Dosage Form, Asian J. Research Chem.. 2(1): 2009,37-40

[17]. K.A. Shaikh and A.B. Devkhile, Simultaneous Determination of Aceclofenac, Paracetamol, and Chlorzoxazone by RP-HPLC in Pharmaceutical Dosage Form, Journal of Chromatographic Science, Vol. 46, 2008,649-652.

[18]. Rajan V. Rele and Rajesh N. Mali Advance simultaneous determination of paracetamol, thiocolchicoside and aceclofenac in tablets by reverse phase high performance liquid chromatography, Der Pharmacia Sinica, 2014, 5(1):34-39