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DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF CETRIZINE DIHYDROCHLORIDE WITH ACECLOFENAC

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ABSTRACT

An HPLC method was developed and validated for the simultaneous determination of Cetirizine dihydrochlroride and aceclofenac. The chromatographic system was equipped with Phenomenex C_{18} column and PDA detector set at 230nm, in conjunction with a mobile phase of Acetonitrile and Heptane sulphonic acid in the ratio of 70:30 (pH 3.2, adjusted with 2M sulphuric acid) at a flow rate of 1.5ml/min. The retention time of Cetirizine dihydrochlroride and aceclofenac were found to be 1.70 ± 0.02 and 4.02 ± 0.02 minutes respectively. Linearity was observed in the concentration range of 1-10µg/ml for Cetirizine dihydrochlroride and 10-100µg/ml for aceclofenac, with good linearity response of 0.997 and 0.999 respectively. Percentage recoveries obtained for Cetirizine dihydrochlroride and aceclofenac were 100.42 % and 98.99%. The proposed method is precise, accurate, selective and rapid for the simultaneous determination of Cetirizine dihydrochlroride and aceclofenac.

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INTRODUCTION

Cetirizine dihydrochlroride is [2-[4-[4-Chlorophenyl] phenyl methyl]-1-piperazinyl] ethoxy] acetic acid dihydrochlroride, a piperazine derivative and active metabolite of hydroxyzine. It is a second generation antihistaminic drug used in treatment of allergic rhinitis, perinnial allergic rhinitis as well as chronic urticaria and purities. Literature survey reveals that several spectrophotometric, HPLC, HPLC coupled to Tandem mass spectrometry, capillary electrophoresis have been reported for determination of Cetirizine dihydrochlroride, alone and in combination with other drugs in formulation and biological fluids.

A combination of Cetirizine dihydrochlroride and anti-inflammatory/analgesic drugs are widely used in comprehensive management of allergic cold, allergic rhinitis, common cold, sinusitis, bronchitis, asthma urticaria and fever, inflammation with

analgesia. On survey of prescriptions we found that doctor prescribes Cetirizine dihydrochlroride with anti-inflammatory/analgesic drugs for synergistic action. The presence of these drugs in blood plasma is being estimated by HPLC method individually. Since the combination of Cetirizine dihydrochlroride and paracetamol is already available in market, we have decided to estimate simultaneously Cetirizine dihydrochlroride with aceclofenac by HPLC method using a physical mixture in the laboratory, as combination of these two drugs as formulations are unavailable in the market.

Aceclofenac (±)-2[[[2-[(2, 6-Dichlorophenyl) amino] phenyl] acetyl] oxy] acetic) is a non-steroidal anti-inflammatory drug (NSAID). It is used for the relief of pain and inflammation. It has higher anti-inflammatory action than conventional NSAIDs. It is a cytokine inhibitor. Aceclofenac works by blocking the action of a substance in the body called Cyclo-oxygenase. Cyclo-oxygenase is involved in the production of prostaglandins (chemicals in the body) which cause pain, swelling and inflammation. This paper presents simple, rapid, reproducible and an economical RP- HPLC method for simultaneous estimation of Cetirizine dihydrochlroride and aceclofenac in combination.

EXPERIMENTAL

Apparatus and Chromatographic conditions:

The analysis was performed by using Phenomenex C_{18} column, with a flow rate of 1.5ml/min. the mobile phase consists of Acetonitrile: Heptane sulphonic acid in the ratio of 70:30 (pH 3.2 adjusted with 2M sulphuric acid), the injection volume is 20µl and the photo diode array detection was carried out at 230nm.

Reagents and Solutions:

Acetonitrile and Heptane sulphonic acid used were of HPLC and AR grade respectively. Optimised chromatographic conditions are listed in [Table-1]

Table 1: System suitability parameters

Sl. No	Parameter	Cetirizine Dihydrochlroride	Aceclofenac
1	Retention Time (TR)	1.70	4.02
2	Theoretical Plates (N)	2850	5377
3	Tailing Factor (T)	1.28	0.98
4	Resolution (Rs)	0.000	5.66

Table 2: Validation Parameters

Sl. No	Parameter	Cetirizine Dihydrochlroride	Aceclofenac
1	Linearity Range	1-100µg/ml	4-100µg/ml
2	Correlation coefficient	0.999	0.999
3	LOD	1	2.25
4	LOQ	4	6
5	Recovery	100.42	98.99
	Precision (% RSD)		
6	Intra- Day	0.55	1.13
7	Inter- Day	0.64	0.95

Standard Solution Preparation (Mixture):

Preparation of Cetirizine dihydrochlroride and Aceclofenac Standard stock solution:

Accurately weighed 10mg of Cetirizine dihydrochlroride is transferred into 100ml volumetric flask, it is first dissolved in few ml of mobile phase (ACN: Heptane sulphonic acid, 70:30), then volume was made up to 100ml with mobile phase (Stock A). From stock A 10ml was pipetted out into a clean 100ml volumetric flask and 10mg

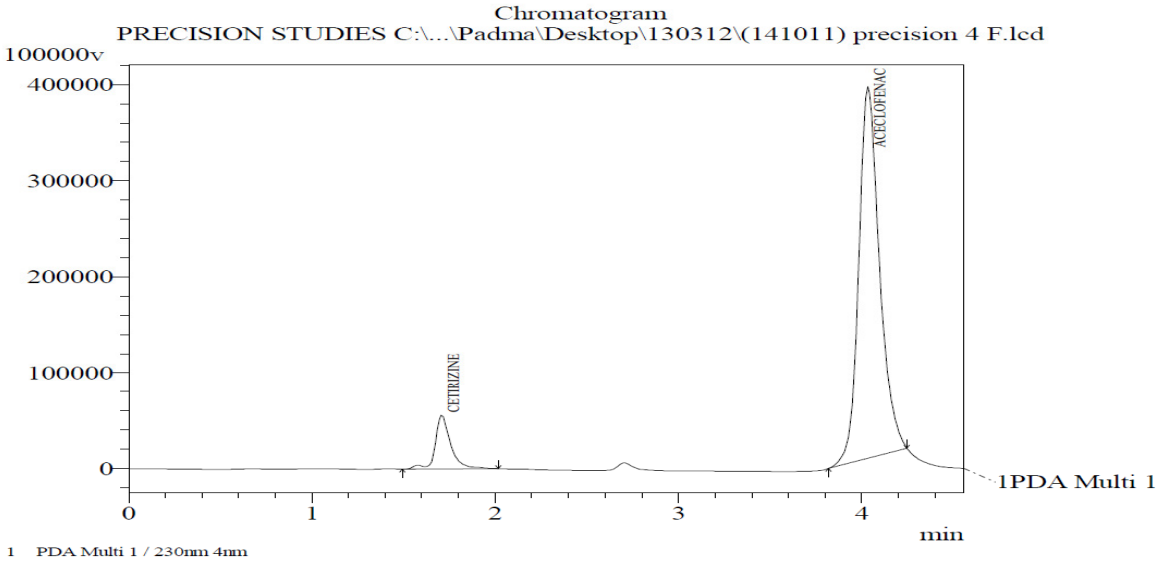
of Aceclofenac is transferred to it, volume is made with mobile phase to 100ml, to get a concentration of 10µg/ml of Cetirizine dihydrochlroride and 100µg/ml of Aceclofenac in the final solution.

METHOD VALIDATION

Linearity:

Several aliquots of standard solution (mixture) were taken into different 10ml volumetric flasks and diluted up to mark with mobile phase such that final concentration of Cetirizine dihydrochlroride and Aceclofenac is 1-10µg/ml and 10-100µg/ml respectively. Evaluations of two drugs were performed with PDA detector at 230nm, peak areas are recorded for all the peaks. The slope and intercept value for calibration curve was y =29799 + 1611 [R^2=0.997] for Cetirizine dihydrochlroride and y = 46373x +17281 [R^2=0.999] for aceclofenac. The results showed excellent correlation between peak area and concentration range indicated above regression graph are shown in the Figure 2 and 3 respectively.

Figure 1: Chromatogram of Standard Cetirizine dihydrochlroride and Aceclofenac



PeakTable

PDA Ch1 230nm 4nm

Peak#	Name	Ret. Time	Area	T Plate#	Tailing Factor	Resolution
1	CETIRIZINE	1.703	324162	2884.281	1.533	0.000
2	ACECLOFEN	4.028	3066949	5303.043	1.271	12.294
Total			3391111			

Figure 2: Linearity of Cetirizine

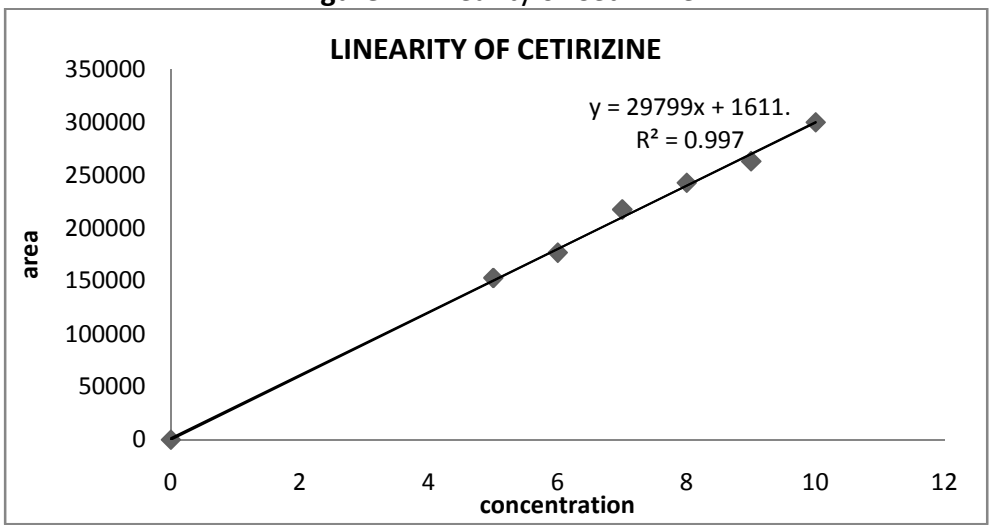
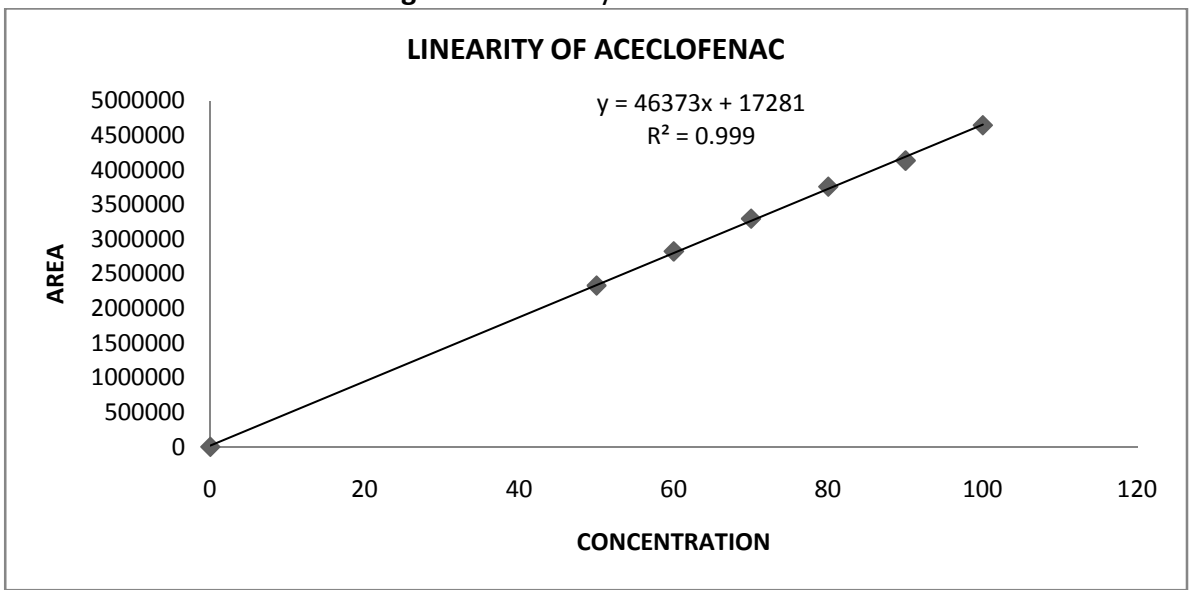


Figure 3: Linearity of Aceclofenac



Ruggedness and Robustness:

The ruggedness of the method was determined by carrying out the experiment on different instruments, days and columns. Robustness is determined by making slight changes in the chromatographic conditions like change in wave length, flow rate, mobile phase ratio, and pH. It was observed that there were no marked changes in chromatograms, which indicated that the developed RP-HPLC method is rugged and robust.

Recovery Studies:

The study of accuracy and reproducibility of the proposed method were followed by recovery studies. A fixed amount of sample mixture was taken and standard drugs were added at 80%, 100% and 120% levels. Each level was injected 9 times. The contents of Cetirizine dihydrochlroride and aceclofenac found by the proposed method are shown in the table 3. The lower values of RSD indicate that the method is accurate. The mean recoveries of Cetirizine dihydrochlroride and Aceclofenac were in the range of 100.42% - 98.66% and 98.99% -97.30% respectively.

Table 3: Data for simultaneous linearity of Cetirizine dihydrochlroride and Aceclofenac

	Concentration		Area	
Sl.No	**Cetirizine dihydrochlroride**	**Aceclofenac**	**Cetirizine dihydrochlroride**	**Aceclofenac**
1	10µg/ml	100µg/ml	299719.7	4645652
2	9µg/ml	90 µg/ml	262997.7	4134924
3	8µg/ml	80 µg/ml	242633	3757733
4	7µg/ml	70 µg/ml	217428.7	3296775
5	6µg/ml	60 µg/ml	176665.7	2823523
6	5µg/ml	50 µg/ml	152799.7	2330389

Limit of Detection and Limit of Quantification:

The limit of detection (LOD) and Limit of Quantification (LOQ) were determined individually by injecting progressively low concentrations of standard solution. The LOD is the smallest concentration of analyte that a measurable response of signal to noise ratio is of 3. The LOD for Cetirizine dihydrochlroride and aceclofenac were found to be 1 and 2.25µg/ml respectively. The LOQ is the smallest concentration of analyte that a measurable response of signal to noise ratio is of

10. The LOQ was 4 and 6 µg/ml for Cetirizine dihydrochlroride and aceclofenac respectively.

RESULTS:

Sl. No	Parameter	Acceptance Criteria		Cetirizine dihydrochlroride	Aceclofenac
1	Linearity	Percentage fitting curve should be more than 99.7 %.		Range (1 - 100µg/ml) % CF 99.7%	Range (4 - 100µg/ml) % CF 99.9%
2	LOD	S/N = 3		1µg/ml	2.25µg/ml
3	LOQ	S/N = 10		4µg/ml	6 µg/ml
4	Precision	Relative Standard Deviation Within 2%.	1.System Precision Area	0.77	0.79
			Retention time	0.30	0.10
			2.Method Precision	0.80	0.74
5	Accuracy	% recovery to be found within the range 0f 97-103%		98.66-100.42%	97.30-98.99%
6	Robustness	Change in Flow rate (Percentage found within 95-105%)	1.3 ml/min	103.03%	101.80%
			1.4 ml/min	99.30%	100.28%
			1.5 ml/min	100.88%	101.06%
			1.6 ml/min	97.97%	99.01%
			1.7 ml/min	99.66%	102.30%
		Change in Wave length (Percentage found within 95-105%)	220 nm	99.36%	102.50%
			225nm	99.55%	100.73%
			230 nm	101.02%	99.93%
			235 nm	97.38%	100.59%
			240 nm	99.68%	101.89%
		Change in Mobile phase ratio [ACN: Heptane sulpohinic Acid] (Percentage found within 95-105%)	65: 35	99.11%	100.46%
			60 : 40	100.54%	100.65%
			70 : 30	100.35%	99.90%
			75 : 25	102.33%	99.59%
			80 : 20	102.69%	99.83%
7	Ruggedness	Change in Instrument [Shimadzu Auto Sampler] (%RSD within 2%)		0.43	0.32
		Change in Column [Denail C_8 Column] (%RSD within 2%)		0.73	0.13
		Change in Day (%RSD within 2%)	Day 1	0.55	1.13
			Day 2	1.13	1.33
			Day 3	1.69	0.82
8	Specificity	No interference to Peak		Noise of Mobile phase didn't interfere with Cetirizine dihydrochlroride and Aceclofenac retention times	

CONCLUSION

Proposed study describes a new and simple RP-HPLC method for the simultaneous of Cetirizine dihydrochlroride and aceclofenac. The method validated was according to ICH guidelines, it is found to be simple, sensitive, accurate and precise. Therefore the proposed method can be used for quantification of Cetirizine dihydrochlroride and aceclofenac for a physical mixture of these drugs and dosage form can also be estimated in future as a combination is unavailable in the market. Further this method can be used for the estimation of Aceclofenac individually.

REFERENCES

1. Zhing-rong Tan, Dong-Sheng Ouyang, Gan Zhou, Lian-Sheng Wang, Zhi Li, Dan Wang, et al. Sensitive bioassay for simultaneous determination of Pseudoephedrine and Cetrizine in human plasma by Liquid-chromatography-ion trap spectrometry .J Pharm Biomed Anal.2006;42:207-212.
2. Walily A.F.M.El, Korany MA, Gindy AEl, Bedair MF. Spectrophotometric and high performance liquid chromatographic determination of Cetrizine dihydrochlroride in pharmaceutical tablets. J Pharm Biomed Anal.1998;17:435-42.
3. Jaber A M Y, Sherife H A A, Omari M M A, Badwan A.A.Determination of Cetrizine dihydrochlroride, related impurities and preservatives in oral solution and tablet dosage forms using HPLC. J Pharm Biomed Anal.2004;36:341-50.
4. Macek J, Ptacek P, Klima J. Determination of Cetrizine in human plasma by high – performaance liquid chromatography. J Chrom B. 1999;736:231-35.
5. Sevgi Karakus, I1kay Kucukguzel, S. Guniz Kucukguzel. Development and validation of rapid RP-HPLC method for the determination of Cetrizine or Fexofenadine with Pseudoephedrine in binary pharmaceutical dosage forms. J Pharm Biomed Anal.2008;46:295-302.
6. Zawalia N H, Abdul Azim Mohammad, E1 Kousy N M, E1-Moghazy Aly S M. Dtermination of Aceclofenac in bulk and pharmaceutical formulations. J pharm. Biomed Anal. 2001 Jul 16:243-51.
7. Vishnu Choudary, Kunal Ingale, Madhusmitha Sahoo, Pratima Syal, Snehal Ingale, Santosh Shinde, et al. Development and Validation of RP-HPLC-PDA method for simultaneous estimation of Drotaverine and Aceclofenac in combined dosag form.IJPRS.2010;1(30):253-58.
8. Santhosh Ghandhi, Padmanabh Deshpande, Vivek Rajmane, Tanmay Dodal, Jitesh Parab.Method development and validation for simultaneous estimation of Drotaverine hydrochloride and Aceclofenac in tablet dosage form by RP-HPLC.Int J Pharm Sci Review and Research.2010 Oct;4(3):49-52.
9. Hye Suk Lee, Chang Kyun Jeonga, Sung Jin Choia, Sang Beom Kima, Mi Hyun Leea, Geon Il Koa, Dong Hwan Sohna. Simultaneous determination of Aceclofenac and Diclofenac in human plasma by narrow bore HPLC using column switching. J Pharm Bio Anal. 2000 Oct;23(5):775-81.
10. Gopinath R, S Rajan, Meyyanathan SN, Krishnaveni N, Suresh B. A RP-HPLC method forsimultaneous estimation of Paracetamol and Aceclofenac in tablets.IJPS.2007;69(1):137-140.

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