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Simultaneous estimation of Cetrizine Hydrochloride and Montelukast Sodium: RP-HPLC

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Abstract: This present work is concerned with the application of simple, accurate, precise and highly selective reverse phase high performance liquid chromatographic (RP-HPLC) method for simultaneous estimation of Cetrizine HCl and Montelukast Sodium in Bulk drugs. Chromatographic separation was achieved isocratically at 25°C ± 0.5°C with a Inertsil, C_{18} (250 x 4.6 mm i.d., 5µ) as stationary phase and Phoshpate buffer (pH adjusted to 3.5 ± 0.02 with dil. Ortho phosphoric acid) Acetonitrile (35:65, v/v) as eluent, at a flow rate of 1.2 ml/min. Detection is carried out using a UV detector at 234 nm. The developed method was validated for linearity, accuracy, precision, limit of detection, limit of quantification, robustness parameters and found to be in good accordance with the prescribed values. The retention time of Cetrizine HCl and Montelukast Sodium was found to be 2.45 and 4.2 min respectively. The method was found to be linear in the range of 0.5-50µg/ml and 1-100µg/ml of target concentration for Cetrizine HCl and Montelukast Sodium standards respectively. The correlation coefficients were found to be 0.9994 and 0.9996 respectively. Thus the proposed method can be successfully applied for simultaneous determination of Cetrizine HCl and Montelukast Sodium in routine bulk drug analysis.
Key words: Cetrizine HCl, Montelukast Sodium, RP-HPLC, Validation, Bulk drugs.

Introduction

Montelukast sodium is a fast acting and potent cysteinyl leukotriene receptor antagonist, used in the treatment of asthma[1]. Chemically it is 2- [1-[(R)-[3-[2(E)-(7-chloroquinolin-2-yl) vinyl] phenyl] - 3-[2- (1-hydroxy-1-methylethyl) phenyl] propyl -sulfanylmethyl] cyclopropyl] acetic acid sodium salt (Fig. 1). The only leukotriene modifier approved by the US Food and Drug Administration for use by children[2] from 2 to 12 years of age. A rapid onset of action is seen after the administration of Montelukast sodium, with improvement seen on the first day of treatment[3] and these positive effects may be additive to those of inhaled corticosteroids[4]. While inhaled beta-agonists are still considered the first-line therapy for treatment of asthma, Montelukast sodium may be given due consideration for use as first line therapy in patients with mild persistent asthma, for

additional control in those who remain symptomatic during treatment with inhaled corticosteroids, for patients that are steroid phobic, or for those who have difficulties with compliance[5,6].

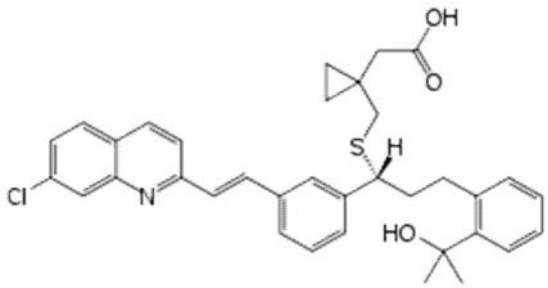

Fig. 1: Sructure of Montelukast

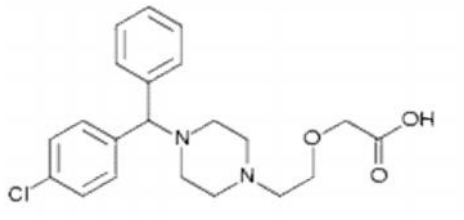

Fig. 2: Sructure of Cetirizine

Cetrizine hydrochloride (CTZ) is a major metabolite of hydroxyzine and a racemic selective histamine receptor (H1) antagonist used in the treatment of urticaria, angioedema, allergies and hay fever[7]. Chemically 2 - [4 - [(4 - chlorophenyl) phenylmethyl] - 1 - piperazinyl] ethoxy] acetic acid (Fig. 2) a second-generation antihistamine. It is a white or almost white powder, freely soluble in water, practically insoluble in acetone and in methylene chloride. Following oral administration of tablet or syrup, CTZ is rapidly absorbed within 1 h to maximum concentration. The mean elimination half-life of CTZ is 8.3 h and the apparent total body clearance for CTZ is approximately 53 ml min[-18]. Numerous authors have reported CTZ detection methods in biological fluids and pharmaceutical formulations[9-13]. Recently different HPLC methods have been reported for the estimation of Montelukast sodium both individually and also in combination with other drugs in pharmaceutical dosage forms, which are either tedious or expensive methods[14-17].

Hence, the aim of this work is to develop accurate, specific, cost effective, repeatable and validated HPLC method for the simultaneous estimation of Cetrizine hydrochloride and montelukast sodium in the bulk drug samples. The proposed method was validated as per the International Conference on Harmonization (ICH) guidelines.

Experimental

Apparatus: Younglin High Performance Liquid Chromatograph (YL9100 HPLC, Anyang, Korea) with UV-detector was used with LC solutions software.

Chromatographic Conditions:

Chromatographic separations were achieved by using Inertsil, C_{18} column (250 x 4.6 mm, 5µ) with a flow rate of 1.2ml/min (Isocratic). The mobile phase consisting of the mixture of Methanol and the Acetonitrile: Phosphate Buffer pH (3.5) at different proportions are degassed in a sonicator for about 10minutes. The injection volume is 20µl and the ultra violet detection was at 234nm.

Reagents and solutions:

Pure samples of Cetrizine HCl and Montelukast Sodium USP of 100mg and other ingredients such as Acetonitrile and water used were of HPLC grade. All other chemicals like Ortho phosphoric acid, Potassium Di-hydrogen phosphate used were of AR grade. Optimized chromatographic conditions are listed in Table. 1.

Preparation of standard solution:

Accurately weighed 10mg of the Cetrizine HCl and Montelukast Sodium reference standard was transferred to 10ml clean and dry volumetric flask. Then the volume were made up to the mark with the diluent (Mobile Phase) and mixed well. This yielded standard stock solution with concentration (1000µg/ml) of Cetrizine HCl and Montelukast Sodium. From the stock solution, 0.25ml of Cetrizine HCl and 0.5ml Montelukast Sodium were taken and transferred to the 10ml clean and dry volumetric flask respectively. Then the volumes were made up to the mark with the diluent (Mobile Phase) and mixed well. These yielded a standard solution with concentration 25µg/ml and 50µg/ml of Cetrizine HCl and Montelukast Sodium and were injected respectively Fig. 3.

Table No. I: Optimized chromatographic conditions

Parameters	Method
Stationary phase (column)	Inertsil-Extend C_{18} (250 × 4.6 mm, packed with 5 µm)
Mobile Phase	65:35 (Acetonitrile : Phosphate Buffer)
pH	3.5 ± 0.02
Flow rate (ml/min)	1.2
Run time (minutes)	8.0
Column temperature (°C)	35 °C
Volume of injection loop («l)	20
Detection wavelength (nm)	234
Drugs RT (min)	2.45 & 4.2

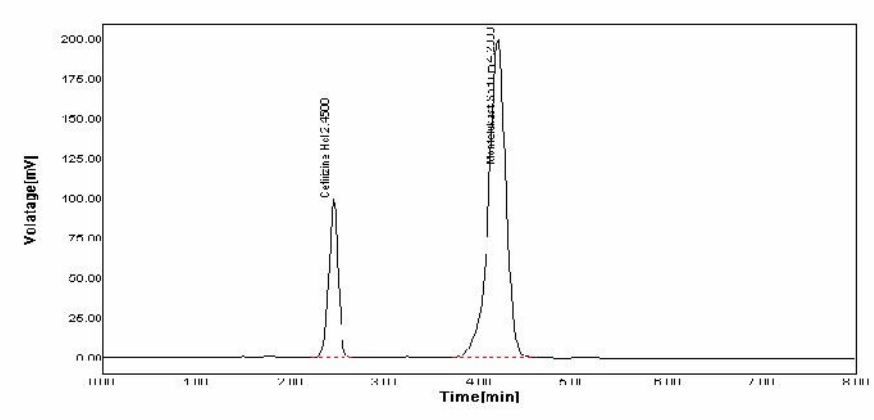

Fig. 3: Standard Chromatogram of Cetrizine HCl and Montelukast Sodium

Validation experiments were performed to demonstrate System suitability, precision, linearity, Accuracy study of analytical solution and robustness.

Linearity & Range: The Linearity of detector response is established by plotting a graph to concentration versus area of Cetrizine HCl and Montelukast Sodium standards and determining the correlation coefficient. A series of solution of Cetrizine HCl and Montelukast Sodium standard solutions in the concentration ranging from about 0.5-50µg/ml and 1-100µg/ml of Cetrizine HCl and Montelukast Sodium levels of the target concentrations were prepared and injected into the HPLC system Fig. 4 & 5.

Accuracy: Accuracy for the assay of Cetrizine HCl and Montelukast Sodium is determined by applying the method in triplicate samples to which known amount of Cetrizine HCl and Montelukast Sodium standards are added at different levels (50%, 100%, and 150%).

Precision: The precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample.

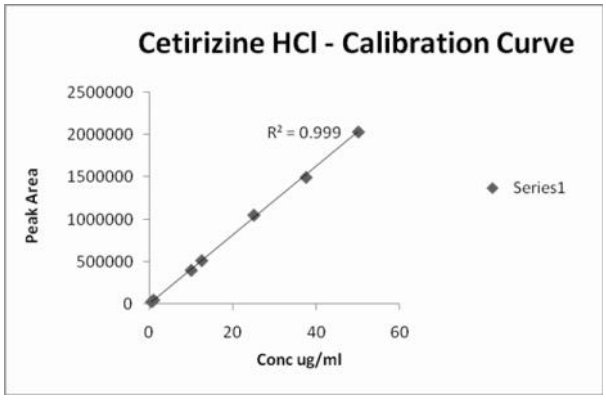

Fig. 4: Linearity Curve of Cetrizine Hcl

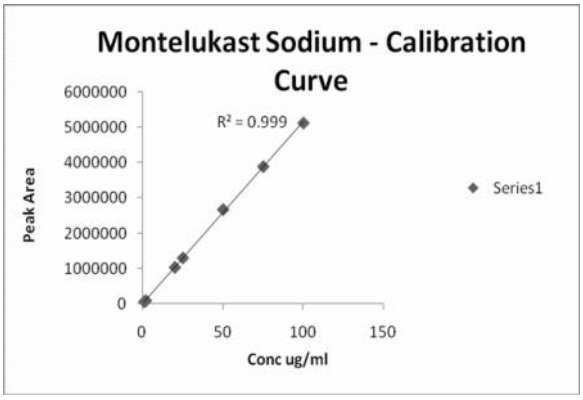

Fig. 5: Linearity Curve of Montelukast Sodium

Results And Discussion

Cetrizine HCl and Montelukast Sodium standards having concentrations 25µg/ml and 50µg/ml were scanned in UV- region between 200-400nm.λ_{max} of Cetrizine HCl and Montelukast Sodium were found to be at 234nm. Cetrizine HCl and Montelukast Sodium Retention times were found to be 2.45 and 4.2 min respectively.

The estimation of Cetrizine HCl and Montelukast Sodium were carried out by RP-HPLC using Mobile phase having a composition of 350 volumes of phosphate buffer, 650 volumes of Acetonitrile. The pH was adjusted to be 3.5 ± 0.02. Then finally filtered using 0.45µ nylon membrane filter and degassed in sonicator for 10minutes. The column used was Inertsil, C_{18} (250 x 4.6 mm, 5µ is suitable). Flow rate of Mobile phase was 1.2ml/min and all the Optimized chromatographic conditions are listed in Table I.

System suitability parameters such as RSD for six replicate injections was found to be less than 2%, theoretical plates -7445.4 and 9765.8, and tailing factor -1.093 and 1.028 for Cetrizine HCl and Montelukast Sodium respectively. The acceptance criteria of System Suitability is RSD should be not more than 2.0% and the method show System Suitability (0.121%, 0.13%) for Cetrizine HCl and Montelukast Sodium respectively, which shows that the method is repeatable and they are listed in Table II.

The acceptance criteria of Method Precision and injection Precision %RSD should be not more than 2.0% and the method show Method Precision (0.208%, 0.137%) and injection Precision (0.078%, 0.075) for Cetrizine HCl and Montelukast Sodium respectively, which shows that the method is precise and they are listed in Tables III, IV.

The validation of developed method shows that the drug stability is well within the limits. The linearity of the detector response were found to be linear from 0.5-50µg/ml and 1-100µg/ml of target concentration for Cetrizine HCl and Montelukast Sodium standards respectively with a correlation coefficient value greater than 0.999. The correlation coefficient values of 0.9994, 0.9996 of Cetrizine HCl and Montelukast Sodium respectively, shows that the method is capable of producing good response in UV-detector and they are listed in Table VI.

The Accuracy limit is the % recovery, which were found be in the range of 98.41-101.28% and 98.33-100.97% of Cetrizine HCl and Montelukast Sodium respectively. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and the results of all System suitability parameters are listed in Table VI.

The values of LOD and LOQ are given in Table VII. The low levels of LOD and LOQ indicate that the method is quite sensitive for the determination of LOD (0.1µg/ml, 0.2µg/ml) & LOQ (0.35µg/ml, 0.7µg/ml) for Cetrizine HCl and Montelukast Sodium respectively in the presence of each other.

Table No. II: System Suitability for Cetrizine HCl and Montelukast Sodium

Conc. of Cetrizine & Montelukast	Injection	Area of Cetrizine	RT	Area of Montelukast	RT
25 & 50ppm	**Inj-1**	1049765	2.43	2657926	4.04
	Inj-2	1050674	2.39	2652798	4.1
	Inj-3	1048927	2.40	2658245	3.97
	Inj-4	1049284	2.45	2653846	4.02
	Inj-5	1049705	2.42	2661947	3.99
	Inj-6	1046894	2.43	2659542	3.98
Statistical Analysis	**Mean**	1049208	2.42	2657384	4.016667
	SD	1276.381	0.021909	3465.887	0.048442
	% RSD	0.121652	0.905327	0.130425	1.206035
	Tailing Factor	1.0935		1.0289	
	Plate Count	7445.4		9765.8	

Table No.III: Summary of results of Method Precision parameter for Cetrizine HCl and Montelukast Sodium

	Inj-1	Inj-2	Avg.	MEAN	SD	% RSD
CETRIZINE HCl						
MP-1	1051036	1056748	1053892	1050820.9	2186.7288	0.20809719
MP-2	1049216	1049465	1049340.5			
MP-3	1051835	1049652	1050743.5			
MP-4	1052007	1051737	1051872			
MP-5	1048574	1050059	1049316.5			
MP-6	1049265	1050257	1049761			
MONTELUKAST SODIUM						
MP-1	2653865	2659825	2656845	2656766.5	3646.1481	0.13724007
MP-2	2659244	2661947	2660595.5			
MP-3	2649837	2658245	2654041			
MP-4	2654634	2659542	2657088			
MP-5	2659489	2653846	2656667.5			
MP-6	2652798	2657926	2655362			

Table No.IV: Summary of results of Injection Precision parameter for Cetrizine HCl and Montelukast Sodium

No. of Injection	Cetrizine HCl	Montelukast Sodium
I.P-1	1048573	2657614
I.P-2	1049735	2658634
I.P-3	1049824	2655374
I.P-4	1048263	2654362
I.P-5	1050453	2653286
I.P-6	1049624	2656455
Mean	1049412	2655954
SD	827.8671	2008.885
% RSD	0.078889	0.075637

Table No. V: Summary of results of Linearity parameter for Cetrizine HCl and Montelukast Sodium

Cetrizine Hcl Conc. (ppm)	Average	Montelukast Sodium Conc. (ppm)	Average
0.5	25928	**1**	53387
1	45494	**2**	83479
10	397790	**20**	1026327
12.5	512480	**25**	1294197
25	1050059	**50**	2666178
37.5	1496121	**75**	3885428
50	2033843	**100**	5122408

Table No. VI: Summary of results of Accuracy parameter for Cetrizine HCl and Montelukast Sodium

CETRIZINE HCl								
Conc.		**inj-1**	**inj-2**	**inj-3**	**Mean**	**% Recovery**	**STD**	**% RSD**
12.5pm	50%	516174	515315	518653	516714	98.41618	1733.28	0.33544
25ppm	100%	1048416	1046246	1045682	1046781	99.68786	1443.47	0.13789
37.5pm	150%	1598459	1588259	1599259	1595326	101.2848	6132.91	0.38443
MONTELUKAST SODIUM								
25ppm	50%	1307462	1316543	1308643	1310883	98.33422	4937.43	0.37664
50ppm	100%	2663574	2654278	2656174	2658009	99.69359	4912.06	0.18480
75ppm	150%	4045734	4025573	4043463	4038257	100.9749	11042.9	0.27345

Table No.VII: Summary of results of LOQ for Cetrizine HCl and Montelukast Sodium

Injection	Area of Ceti.	Area of Monte.
Inj-1	18894	38986
Inj-2	18497	38976
Inj-3	18671	38985
Inj-4	18598	38754
Inj-5	18783	39387
Inj-6	18687	38983
Mean	18688.33	39011.83
SD	138.803	205.2953
% RSD	0.742725	0.526239

Conclusion

The developed RP-HPLC method was developed and validated for the simultaneous determination of Cetrizine HCl and Montelukast Sodium in bulk drugs. The validation data indicate good precision, accuracy and reliability of the method. The developed method offers several advantages in terms of simplicity in mobile phase, isocratic mode of elution, easy sample preparation steps and comparative short run time which makes the method specific and reliable for its intended use in simultaneous determination of Montelukast and Cetrizine in Bulk drugs.

The assay result obtained by this method is in fair agreement. This method can be use for the determination of Cetrizine HCl and Montelukast Sodium in commercial formulations.

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