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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF DESLORATIDINE AND MONTELUKAST HCL IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A selective, precise, isocratic and accurate Reverse Phase High Performance Liquid Chromatography method was developed for the simultaneous determination of Desloratidine and Montelukast HCl in reverse phase X-terra C8 column (250 x 4.6mm, 5µ). The mobile phase consists of mixture of 0.1% Orthophosphoric acid and Acetonitrile (60:40). The pH of 0.1% Orthophosphoric acid was adjusted to 3 using 0.1% v/v Triethylamine. The flow rate was 0.7ml/min and the effluents were monitored at the detection wavelength of 283nm using UV detector. The retention times of Desloratidine and Montelukast HCl were found to be 1.7 and 4.8min respectively. The method was validated for the linearity, accuracy, precision and robustness as per ICH guidelines. Desloratidine and Montelukast HCl were found to be linear in the range of 5-25µg/ml and 10-30 µg/ml respectively.

KEYWORDS: *Desloratidine, Montelukast HCl, RP-HPLC, Analytical Method Development, Validation.*

INTRODUCTION

Desloratadine [1] (8-chloro-6,11-dihydro-11-(4-piperdinyli dene)-5*H*-benzo[5,6]cyclohepta[1,2-b]pyridine) is a non-sedative metabolite of Loratadine, a second generation long acting antihistaminic drug with selective peripheral H_1 receptor antagonistic activity (Fig 1). It has demonstrated anti allergic properties by inhibiting the release of pro-inflammatory cytokines such as IL-4, IL-6, IL-8 and IL-13 from human mast cells/ basophilic as well as inhibition of the expression of the adhesion molecule P-selection on Endothelial cells.

Montelukast [2] (2-[1-({[(1R)-1-{03-[(E)-2-(7-chloroquinolin-2-yl)ethenyl]phenyl}-3-[2-(2-hydroxypropan-2-yl)phenyl]propyl]sulfan yl}methyl)cyclopropyl]acetic acid)is a leukotriene receptor antagonist (LTRA) used for the maintenance treatment of asthma and to relieve symptoms of seasonal allergies (Fig 2). Montelukast is a $CysLT_1$ antagonist; that it blocks the action of leukotriene D4 (and secondary ligands LTC4 and LTE4) on the cysteinyl leukotriene receptor $CysLT_1$ in the lungs and bronchial tubes by binding to it. This reduces the bronchoconstriction otherwise caused by the leukotriene and results in less inflammation.

The early asthmatic response (EAR) to inhaled allergen results from IgE-mediated release of multiple mast-cell mediators, including leukotrienes and histamine, both of which cause bronchoconstriction. Combination therapy directed at blocking the effects of both mediators might protect against the EAR better than either therapy alone. Combination of Desloratadine (DST) and Montelukast (MNK) provided superior efficacy to either blocker administered. Combination of Desloratadine plus Montelukast is also effective in the treatment of Chronic Urtcaria. It may be a valid alternative in patients with relatively mild Chronic Urtcaria, in view of its efficacy and the lack of adverse events.

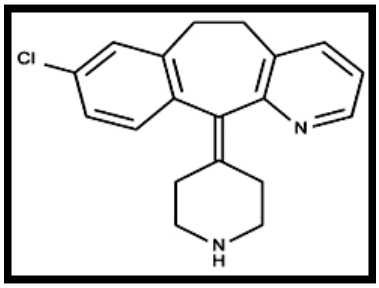

Fig. 1: Structure of Desloratadine

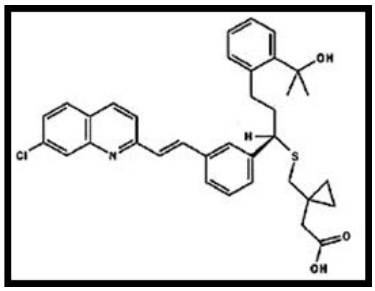

Fig. 2: Structure of Montelukast

Literature survey [3-12] revealed that there were methods reported for estimation of DST and MNK in combined dosage forms and

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for individual drugs using UV and HPLC. Here is an attempt made to develop RP-HPLC method for simultaneous estimation of RST and EZE.

MATERIALS AND METHODS

Experimental:

Instrumentation and apparatus:

The analysis was carried out by using Waters HPLC with UV detector. Column used was Waters x-terra C8, 5µm (4.6 x 150mm). All materials were weighed on SHIMADZU Electronic balance model AX 200, to dissolve the drug completely without leaving any particles Ultra Sonicator (Fast clean) was used at room temperature and vaccum filter pump (PCI analytics) was also used during the analysis for degassing the mobile phase.

Materials used:

DST and MNK APIs were procured from Aurbindho laboratories, Hyderabad. The tablet formulation was purchased from local market (MONDESLOR containing 5mg of DST and 10mg of MNK). All the reagents used in this method were of analytical grade.

Selection of detection wavelength:

The working standard solutions of DST and MNK were scanned separately in UV range. From the spectra, the detection wavelength selected was 283nm, as both the drugs are having considerable absorption (Fig.3).

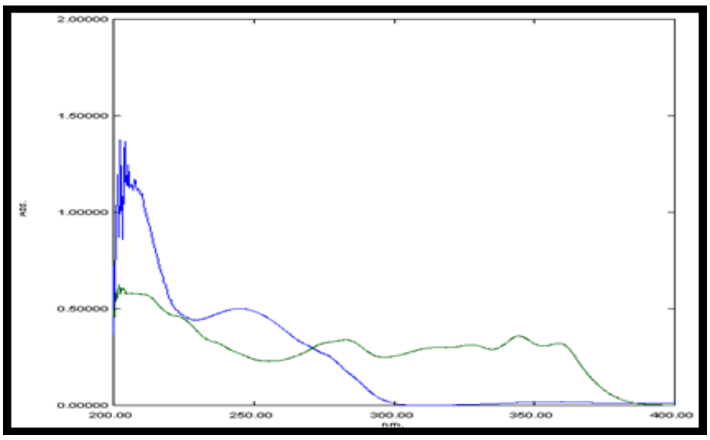

Fig. 3: Overlain Normal spectra of Desloratidine and Montelukast standards

Method Development

Preparation of Standard stock solutions:

Accurately weigh and transfer 10 mg of RST and EZE into a two separate 10ml clean dry volumetric flasks, add few ml of the mobile phase and shake till the drug dissolves completely. Then make up to the volume with mobile phase to get 1000µg/ml of standard stock solution of both DST and MNK.

Preparation of working standard solutions:

Transfer separately 1ml of standard stock solution of both DST and MNK into two separate 10ml volumetric flasks, make upto the volume to obtain the concentration of 100µg/ml of working standard solutions for both the drugs.

Sample analysis:

10 tablets were weighed and finely powdered. The tablet powder equivalent to 10mg of MNK was transferred into 10ml volumetric flask. About 5ml of mobile phase was added to the flask and sonicated for about 5mins and made up to the volume with mobile phase.

The contents were filtered through Whatmann filter paper. From this sample stock solution, working sample solution containing 15 µg/ml of DST and 30 µg/ml of MNK was prepared. The solution prepared was injected into the HPLC to obtain the %content of DST and MNK in the tablets (Fig. 4).

Validation of the method:

Linearity and Range:

Linearity was performed for both DST and MNK. Solutions of concentrations of 5-25µg/ml for DST and 10-50 µg/ml for MNK were prepared using working standard solutions. Chromatograms of both the drugs concentrations were taken using RP-HPLC. A calibration graph was plotted, peak area versus concentration for both the drugs (Fig. 5, 6 and 7).

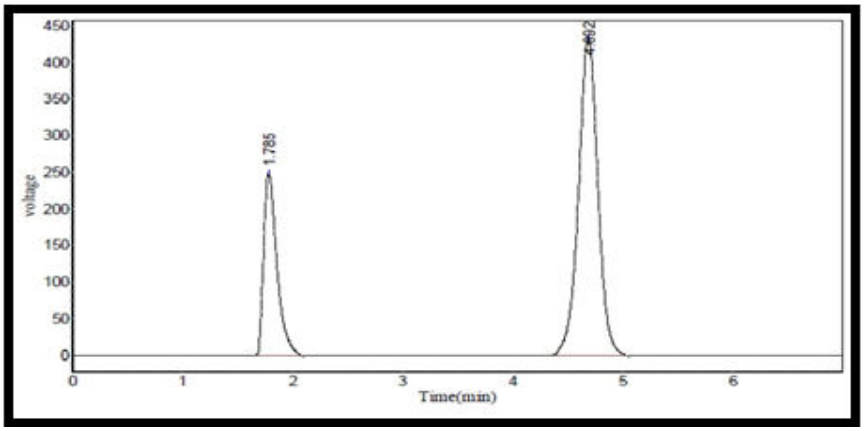

Fig. 4: Chromatogram of Formulation

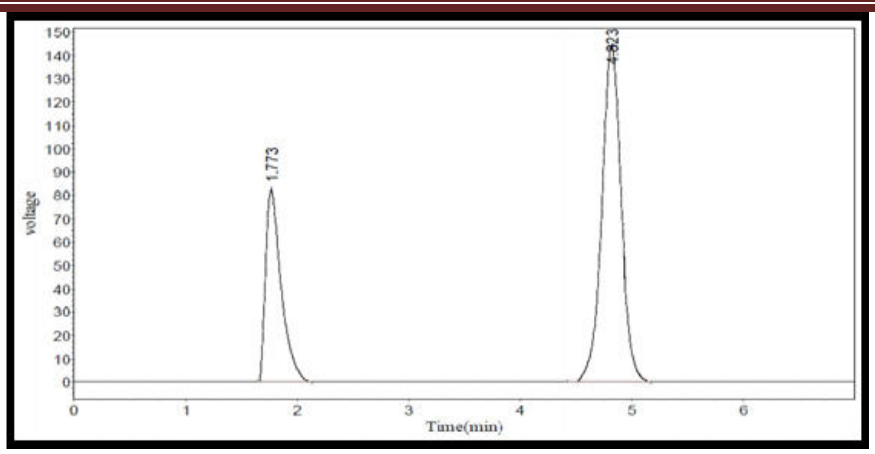

Fig. 5: Chromatogram of DST and MNK

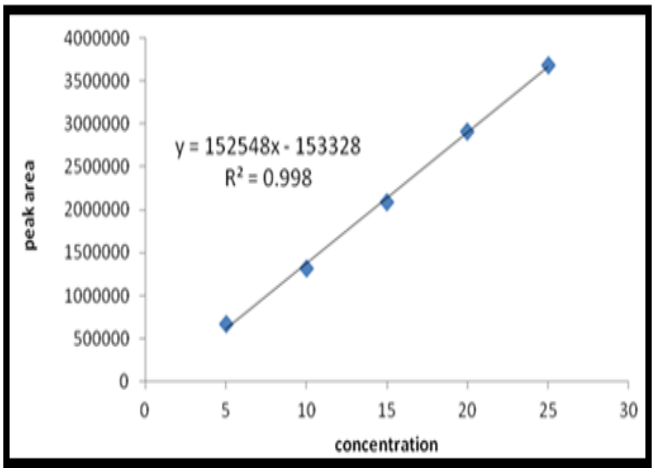

Fig. 6: Linearity graph of DST

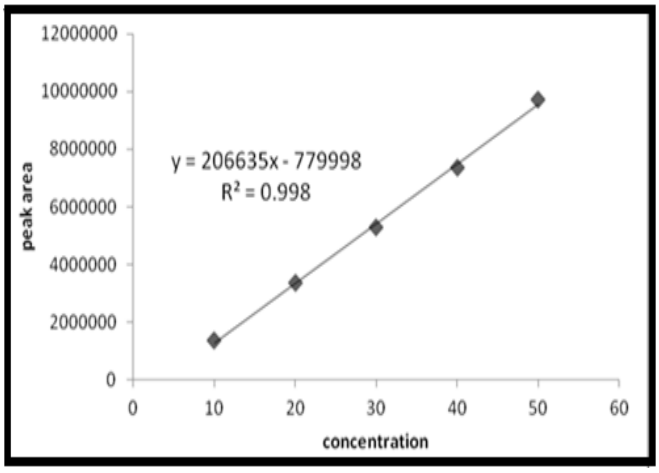

Fig. 7: Linearity graph of MNK

Precision:

Intraday precision was done by carrying out analysis of standard drug solutions at two different concentrations in the linearity range for three times on the same day and %RSD was calculated. The results are shown in Table 1.

Inter day precision was done by carrying out the analysis of standard drug solutions at two different concentrations in the linearity range for three days over a period of one week and %RSD was calculated. The results are shown in Table 2.

Accuracy:

Recovery studies of the drug were carried out for by mixing known quantity of standard drugs with the analyzed sample formulation and the contents were reanalyzed by the proposed method. This was carried out at 50, 100 levels. Results of recovery are shown in Table 3.

LOD and LOQ:

LOD and LOQ were calculated from linearity data. The LOD of DST and MNK were found to be 0.67µg/ml and 1.11µg/ml respectively. The LOQ of DST and MNK were found to be 2.1µg/ml and 3.1µg/ml respectively.

Table No. 1: Intraday Precision

Level	Concentration (mcg/ml)		Peak Area		%RSD	
	DES	MONT	DES	MONT	DES	MONT
1	5	10	669132	1382689	0.8	**0.5**
			680251	1374649		
			671549	1368652		
2	10	20	1297726	3411368	0.9	**0.7**
			1301894	3363037		
			1322110	3303251		

Table No. 2: Interday Precision

Level	Concentration (mcg/ml)		Peak Area		%RSD	
	DES	MONT	DES	MONT	DES	MONT
1	5	10	669041	1382689	1.48	**0.91**
			681251	1364659		
			661519	1358655		
2	10	20	1297726	3251358	0.92	**1.69**
			1321894	3363037		
			1312110	3313231		

Table No. 3: Accuracy

Drug	Amount added (µg/ml)	Amount recovered (µg/ml)	% Recovery	*% RSD
DES	2.5	2.46	98.6	0.555
	10	10.13	101.3	0.657
	12.5	12.33	98.7	0.825
MON	5	4.96	99.2	1.07
	10	9.97	99.7	0.293
	15	14.79	98.6	0.846

**mean of three observations*

RESULTS AND DISCUSSION

A simple, accurate reverse phase high performance liquid chromatographic method for simultaneous determination of DST and MNK has been developed. The method development was performed on Waters HPLC with reciprocating pump using N2000 Chromatographic system software; Waters x-terra C8, 5µm (4.6 x 150mm) column, using isocratic mode of elution. The detector used was UV detector to detect variable wavelengths. A mixture of 0.1% Orthophosphoric acid and Acetonitrile (60:40) was used as mobile phase. The pH of 0.1% Orthophosphoric acid was adjusted to 3 using 0.1% v/v Triethylamine. The flow rate was 0.7ml/min and the effluents were monitored at the detection wavelength of 283nm using UV detector. The retention times of DST and MNK were found to be 1.7 and 4.8min respectively. The run time of the method was set for 7min. The separation of DST and MNK was good with good peak shape and resolution (Table 4).

The linearity was found to be 2-25µg/ml for DST and 10-50 µg/ml for MNK with the regression coefficient of 0.998 for both the drugs. The method was found to be accurate as as the % recovery is within the acceptable range of 98%-102%. Method was found to be precise based on the %RSD values of DST and MNK. The %RSD was calculated for peak areas and was found to be within the limits (NMT 2%). The assay performed on formulation shown the % content of 98.6 and 99.6 for DST and MNK respectively (Table 5).

Table No. 4: Chromatographic Conditions

HPLC System	Waters
Column	Waters x-terra C8, 5µm (4.6 x 150mm)
Mobile Phase	0.1% Orthophosphoric acid and Acetonitrile (60:40)
Flow rate	0.7ml/min
Injection Volume	20µl/min
Total run time	7.0 mins
Mode of separation	Isocratic
Detector	UV Detector,283nm

Table No. 5: Assay of formulation

Drug	Amount labelled (mg)	Amount found (mg)	% Assay
Desloratidine	5.0	4.93	98.6
Montelukast	10.0	9.96	99.6

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