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Simultaneous Estimation Of Montelukast Sodium And Desloratadine By RP-HPLC In Their Marketed Formulation

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Abstract: A reversed-phase-liquid chromatographic (RP-HPLC) method was developed for the determination of Montelukast Sodium (MTKT) and Desloratadine (DSL) in their marketed formulation. A reversed-phase C-18 column (250 mm × 4.8 mm i.d., particle size 5 µm) column with mobile phase consisting of methanol: water: Acetic acid (90:10:0.05 v/v/v) was used. The flow rate was 1.0 ml/ min and effluents were monitored at 280 nm. The retention times of Montelukast Sodium and Desloratadine were found to be 7.61±0.2 min and 2.23±0.3 min, respectively. The method was validated in terms of specificity, linearity, range, accuracy, precision, limit of detection (LOD) and limit of quantitation (LOQ). The method showed good linearity in the range of 40-140 µg/ml for Montelukast Sodium and 20-70 µg/ml for Desloratadine. The % recoveries of Montelukast Sodium and Desloratadine were found to be between 99.54-101.25 and 99.54-101.40. The percentage RSD for the method precision was found to be less than 2%. The proposed method was successfully applied to the estimation of Montelukast Sodium and Desloratadine in combined capsule dosage forms.
Keywords: Montelukast Sodium and Desloratadine, RP-HPLC.

INTRODUCTION[1-17]

Montelukast Sodium, a specific cystenyl leukotriene receptor antagonist belongs to a styryl quinolines with the chemical name [R-(E)]-1-[[[1-[3-[2-(7-chloro-2quinolinyl) ethenyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]thio] methyl]cyclopropane acetic acid sodium salt. It is official in IP-2010.

Desloratadine is a non sedating H1 anti- histamine with a chemical name 8-chloro-6,11-dihydro-11-(4-piperdinylidene)- 5*H*-benzo[5,6]cyclohepta[1,2-b]pyridine. It is not official in any pharmacopoeia. Fixed dose combination tablet containing MTKT and DSL used for the treatment of Asthma and Allergic rhinitis.

The literature review revealed that reveals methods are available for the determination of MTKT and DSL individually or in combination with the other drugs. But no method mentioned in the literature explained the simultaneous estimation of MTKT and DSL in bulk, dosage forms and in biological fluids. The present study describes a simple, rapid, accurate and economical method for simultaneous estimation of MTKT and DSL by RP-HPLC.

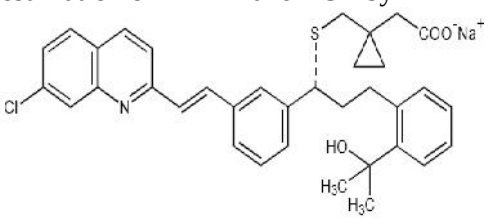

Fig-1 Structure ofMontelukast Sodium

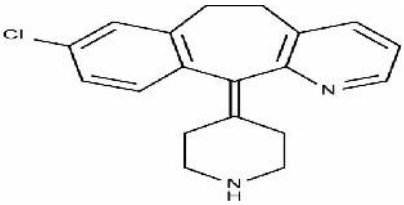

Fig-2 Structure of Desloratadine

EXPERIMENTAL[1-17]

CHEMICALS AND REAGENTS

Pure samples of MTKT and DSL were received from Biodeal Laboratories Ltd. Surendranagar, India. The marketed formulation studied was MONDESLOR tablet manufactured by Sun Pharmaceutical Baroda. Each tablet contains 10 mg MTKT and 5 mg DSL. HPLC grade methanol, water and acetic acid were obtained from Polychem Limited, Mumbai, India

INSTRUMENTATION

Separation was performed with a Shimadzu SPD-20 A, Equipped with a Rheodyne injector valve with a 20.0 µl loop and a UV/VIS detector.

CHROMATOGRAPHIC CONDITIONS

A Phenomenex C-18 column (250 mm * 4.8 mm, 5 µ) was used for the separation.The isocratic mobile phase was consisted of Methanol: Water: Acetic acid 90:10:0.01(v/v). The mobile phase was sonicated for 20 min and filtered through a 0.22 µ membrane filter. Flow rate of mobile phase was 1.0 ml/min. The UV–visible detector was set at 280nm.

PREPARATION OF STANDARD SOLUTION

52 mg MTKT (Equivalent to Montelukast 50 mg) and 25 mg DSL were accurately weighed and transferred to 100 ml volumetric flasks separately and dissolved in the methanol to give stock solution of 500 µg/ml and 250 µg/ml MTKT and DSL respectively. From this stock solution appropriate dilution in the range of 40-140 µg/ml and 20-70 µg/ml for MTKT and DSL respectively were prepared and analyzed. Mixed standard were prepared in the ratio of 1:2 as the formulation DSL and MTKT (5 mg, 10mg) respectively.

PREPARATION OF SAMPLE SOLUTION

Twenty tablets (Mondeslor) were accurately weighed a powdered finely. Powder equivalent to 100 mg of MTKT and 50 mg of DSL was transferred to a 100 ml volumetric flask. Add 60 ml methanol and this solution was ultrasonicated for 30 min with intermittent shaking and filtered through 0.45µ filter. The solution was filtered diluted to obtain the concentration of 100 µg/ml of MTKT and 50 µg/ml of DSL.

PREPARATION OF MOBILE PHASE

Mix Methanol, Water and Acetic acid in ratio of 90:10:0.05(v/v/v). Sonicate it for 30 minutes and filter through 0.2 µ size membrane filter.

METHOD VALIDATION

SPECIFICITY: Specificity was tested against standard compounds and against potential interferences in the presence of placebo. No interference was detected at the retention time of DSL and MTKT in sample solution.

LINEARITY AND RANGE: Linearity is studied to determine the range over which analyte response is a linear function of concentration. This study was performed by preparing standard solutions of six different concentrations in the range of 20-70 µg/ml and for DSL 40-140 µg/ml for MTKT. Each concentration was made in triplicate. The responses were measured as peak area. The calibration curves were obtained by plotting peak area against concentration.

ACCURACY: The accuracy of an analytical method is the closeness of results obtained by that method to the true value for the sample. It is expressed as recovery (%), which is determined by the standard addition method. Samples were spiked with 80, 100, and 120% of the standard and analyzed. The experiment was performed in triplicate. Recovery (%) and RSD (%) were calculated for each concentration.

PRECISION: Precision was considered at different levels, i.e. Method, System, Interday and Intraday.

Repeatability was studied by carrying out system precision And Method Precision. System Precision was determined from results for six replicate of synthetic mixture and Method Precision is for formulation Mixture.

Intraday precision of the developed method was evaluated by analyzing samples of three different concentration of MTKT (80,100,120 µg/ml) and DSL (40, 50, 60 µg/ml) in triplicates on same day. Interday was determined from the same concentration of three consecutive days. Results from determinations of precision were expressed as

LIMITS OF DETECTION AND LIMIT OF QUANTITATION: The LOD and LOQ were separately determined on the basis of standard calibration curve. The standard deviation of the

peak area of the standard solution (n = 3 determination) was used to calculate LOD and LOQ. Following formulae were used; LOD= 3.3×D/S and LOQ= 10×D/S, where, D is the standard deviation of the peak area of 3 determination and S is the slope of the calibration curve.

ROBUSTNESS: For demonstrating the robustness, some of experimental conditions were purposely altered and evaluated.

- Change mobile phase composition by ± 2 ml of (absolute) organic solvent.
- Change flow rate by ± 0.2 ml/minute (i.e. 1.2 ml/minute and 0.8 ml/minute)
- Change in detection wavelength ± 10 nm

% RSD should not greater than 2%.

ASSAY: To determine the content of MTKT and DSL in commercial tablets. (Each tablet containing 10 mg MTKT and 5 mg DSL) prepare the sample solutions to give concentration of 100 µg/ml and 50 µg/ml of MTKT and DSL respectively.

RESULTS AND DISCUSSION

METHOD DEVELOPMENT

Solvent selectivity (solvent type), solvent strength (volume fraction of organic solvent(s) in the mobile phase), additive strength, detection wavelength, and flow rate were varied to determine the chromatographic conditions giving the best separation. Several mobile phase compositions were tried to resolve the peaks of MTKT and DSL. The optimum mobile phase containing Methanol: Water: Acetic acid 90:10:0.05(v/v/v) was selected because it could resolve the peaks of DSL (RT = 2.23 ± 0.30 min) and MTKT (RT = 7.61 ± 0.20 min) with a resolution factor of 16.0 Quantification was achieved with UV detection at 280 nm on the basis of peak area at 1.0 ml/min flow rate. A typical HPLC chromatogram obtained during simultaneous determination of MTKT and DSL is given in (Figure 3).

LINEARITY AND RANGE: Different concentrations (20, 30, 40, 50, 60, 70 µg/ml of DSL and 40, 60, 80, 100, 120, 140 µg/ml of MTKT) of the mixture of two drugs were prepared for linearity studies. The calibration curves obtained by plotting peak area against concentration showed linear relationship over a concentration range of 20-70 µg/ml or DSL and 40-140 µg/ml for MTKT. The linear regression equations for DSL and MTKT were found to be y = 84.19x + 78.07 and y = 78.81x + 156.9 respectively. The regression coefficient values (r^2) were found to be 0.999 indicating a high degree of linearity. Calibration curves of DSL and MTKT are shown in (Figure 4 and 5 respectively). Regression characteristics of the proposed HPLC method are given in (Table 1).

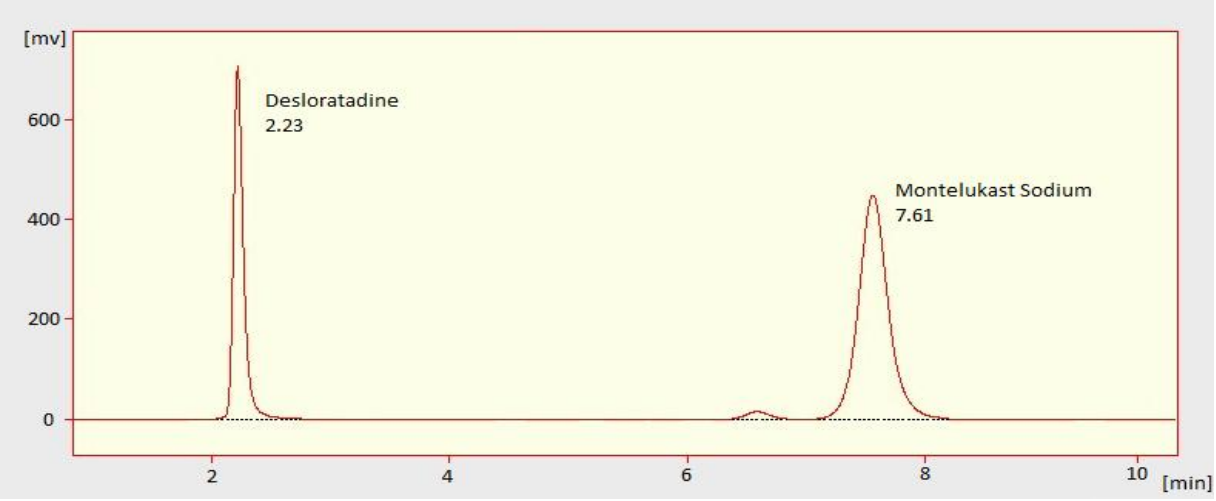

Fig-3. HPLC chromatogram obtained during simultaneous determination of DSL and MTKT

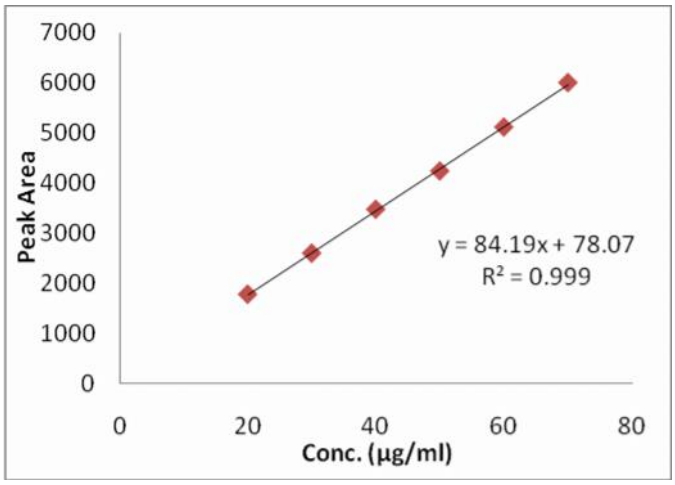

Fig-4 Calibration Curve of Desloratadine

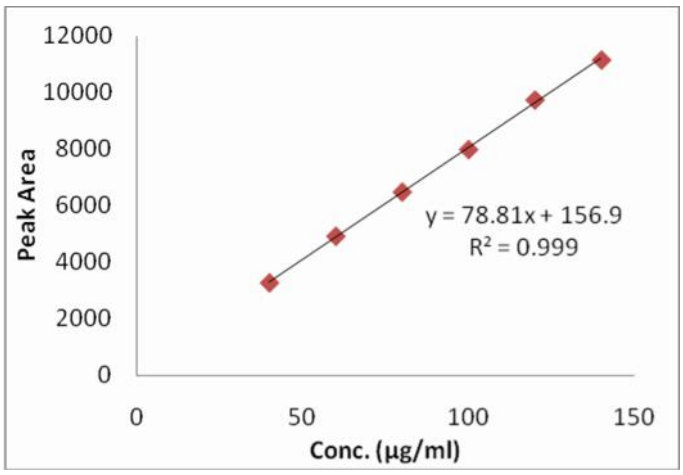

Fig-5 Calibration Curve of Montelukast Sodium

Table 1. Regression characteristics of the proposed HPLC method

Linearity Experiment	**DSL**	**MTKT**
Range (µg/ml)	**20-70**	**40-140**
Regression coefficient (r^2)	**0.999**	**0.999**
Slope	**84.19**	**78.81**
Intercept	**78.07**	**156.9**

PRECISION: The system and method precision showed a % RSD of 1.36 of DSL & 1.10 for MTKT and 1.81 of DSL & 1.52 for MTKT respectively. The intraday precision having %RSD of (0.55-1.18) of DSL and (0.44-0.86) for MTKT. Likewise, the interday precision showed a %RSD (0.63-1.54%) of DSL and (0.74-1.08%) for MTKT. All precision of method is illustrated in Table-2.

ACCURACY: Recovery studies were carried out by applying the standard addition method. Known amounts of standard DSL and MTKT corresponding to 80%, 100%, and 120% of the label claim were added to sample of capsule dosage form separately. The average % recoveries for DSL and MTKT in marketed formulation were found to be between 99.54-101.40% and 99.54-101.25% respectively. The results revealed that there was no interference of excipients. The results of accuracy are shown in Table-2.

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTITATION (LOQ): The limit of detection and limit of quantification were found to be 0.87 and 2.54 µg/ml for DSL and 0.69 and 2.09 µg/ml for MTKT.

ROBUSTNESS: The proposed method was found to be robust enough(% RSD < 2.) to withstand such slight changes and allow routine analysis of the sample. The result of robustness is shown in Table-2.

Table-2 Summary of Validation parameters for proposed method.

Parameters		**High Performance Liquid Chromatography**	
		DSL	**MTKT**
Cocn. Range	(µg/ml)	20-70	40-140
Precision	System	1.36%	1.10%
	Method	1.81%	1.52%
	Intraday	0.55-1.18%	0.44-0.86%
	Interday	0.63-1.54%	0.74-1.08%
Accuracy	80%	100.6% ±1.302	101.25% ±1.15
	100%	101.4% ±1.17	98.54% ±0.97
	120%	99.54% ±0.79	100.32% ±1.47
LOD	(µg/ml)	0.87	0.69
LOQ	(µg/ml)	2.54	2.09
Robustness	Change in the organic phase (88:12:0.05 v/v/v)	0.89	1.013
	Change in the organic phase (92:08:0.05 v/v/v)	0.69	1.22
	Change in the Wavelength (250 nm)	0.55	1.17
	Change in the Wavelength (270 nm)	0.88	0.96
	Change the flow rate (0.8 ml/min)	0.95	0.89
	Change the flow rate (1.2 ml/min)	0.86	0.821

ANALYSIS OF MARKETED FORMULATION:

The proposed method was applied for the determination of MTKT and DSL in their combined pharmaceutical formulation and the results are shown in table-3. The high % Recoveries (98.4-108%) confirms the suitability of the proposed method for routine determination of these components in combined formulation.

SYSTEM SUITABILITY PARAMETERS

For system suitability parameters, seven replicate injections of mixed standard solution were injected and parameters such as the resolution, theoretical plate and asymmetry factor of the peaks were calculated. The results are shown in Table 4.

CONCLUSION

A novel RP- HPLC method has been developed for the simultaneous estimation of MTKT and DSL in marketed formulations. The method gave good resolution for both the drugs with a short analysis time below 10 minutes. The developed method was validated. It was found to be novel, simple, precise, accurate, and sensitive. The good % recovery in tablet dosage form suggests that the excipients present in the dosage forms have no interference in the determination. The %RSD was also less than 2% showing high degree of precision of the proposed method. The proposed method can be used for routine analysis of MTKT and DSL in combined dosage form. It can be also used in the quality control in bulk manufacturing.

Table-3 Assay results for tablets using proposed method

Formulation	**Amount of drug taken(mg)**		**Amount of drug found(mg)**		**% Assay**	
Tablet (MONDESLOR)	MTKT	DSL	MTKT	DSL	MTKT	DSL
	10	5	10.14	4.95	101.42% ± 0.79	99.05% ± 0.51

Table 4. System suitability Parameter

Parameter	**DSL**	**MTKT**
Asymmetry Factor	1.142	1.007
Theoretical Plates	3902	4153
Resolution	11.5	

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