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Method Development and Validation for Simultaneous Estimation of Montelukast Sodium and Desloratadine by RP-HPLC

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Abstract

A novel, precise, accurate, rapid and cost effective isocratic reverse-phase high performance liquid chromatographic (RP-HPLC) method was developed, optimized and validated for the simultaneous estimation of Montelukast Sodium (MON) and Desloratadine (DES) in pharmaceutical dosage forms. The drugs were estimated using Hypersil BDS C18 (250 mm × 4.6 mm I.D., 5 µ particle size) column. The mobile phase composed of orthophosphoric acid and water in the ratio of 20:80 v/v, at a flow rate of 1.0 ml/min was used for the separation. Detection was carried out at 280 nm. The linearity range obtained was 10 - 30 µg/ml for MON and 5 - 15 µg/ml for DES with retention times of 2.929 min and 4.439 min for MON and DES respectively. The correlation coefficient values were found to be 0.999. Precision studies showed % RSD values less than 2% for both the drugs in all the selected concentrations. The percentage recoveries of MON and DES were in the range of 99.59% - 99.82% and 99.60% - 99.80% respectively. The limit of detection (LOD) and limit of quantification (LOQ) were 0.176 µg/ml, 0.587 µg/ml for MON and 0.087 µg/ml, 0.292 µg/ml for DES respectively. The method was validated as per the International Conference on Harmonization (ICH) guidelines. The proposed validated method was successfully used for the quantitative analysis of commercially available tablet dosage forms.

Keywords

Montelukast Sodium, Desloratadine, HPLC, Validation

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1. Introduction

Montelukast Sodium (MON) (**Figure 1**) is chemically 2-[1-({[(1R)-1-{3-[(E)-2-(7-chloroquinolin-2-yl)ethenyl] phenyl}-3-[2-(2-hydroxypropan-2-yl)phenyl]propyl]sulfanyl} methyl)cyclopropyl]acetic acid [1]. MON is a leukotriene receptor antagonist (LTRA) used for the maintenance treatment of asthma and to relieve symptoms of seasonal allergies [2] [3]. MON selectively antagonizes leukotriene D_4 (LTD_4) at the cysteinyl leukotriene receptor, $CysLT_1$, in the human airway. MON inhibits the actions of LTD_4 at the $CysLT_1$ receptor, preventing airway edema, smooth muscle contraction and enhanced secretion of thick, viscous mucus [4]-[7].

Desloratadine (DES) (**Figure 2**) is chemically 8-chloro-6, 11-dihydro-11-(4-deserdinylidene)-5*H*-benzo [5] [6] cyclohepta[1, 2-b]pyridine [3]. DES is a second generation, tricyclic antihistamine that which has a selective and peripheral H_1-antagonist action [4]. It is the active descarboethoxy metabolite of Loratidine. DES has a long-lasting effect and does not cause drowsiness because it does not readily enter the central nervous system [5]-[7].

MON with DES is used for the treatment of persistent allergic rhinitis [8]. Literature survey reveals that few analytical methods are available for the simultaneous estimation of MON and DES in pharmaceutical formulations by using UV [9]-[11] and HPLC [12]-[15]. Hence, we made an attempt to develop a simple method for the simultaneous estimation of MON and DES by RP-HPLC in pharmaceutical dosage forms. The proposed method was optimized and validated as per the International Conference on Harmonization (ICH) guidelines [16].

2. Experimental

2.1. Instrumentation

The analysis of the drug was carried out on a Waters HPLC system equipped with 2487 pump and UV/Visible dual absorbance detector was used. Hypersil BDS C18 column (250 mm × 4.6 mm I.D., 5 µ particle size) was used. The output of signal was monitored and integrated using Waters Empower 2 software.

2.2. Chemicals and Solvents

The working standards of Montelukast Sodium and Desloratadine were provided as gift samples by Spectrum Pharma Research Solutions, Hyderabad, India. Methanol (HPLC grade); Milli-Q water and orthophosphoric acid (AR grade) were obtained from Qualigens Ltd, Mumbai, India.

2.3. Mobile Phase Preparation

Mobile phase was prepared by mixing 20 ml orthophosphoric acid and 80 ml of Milli-Q water. Above mixture

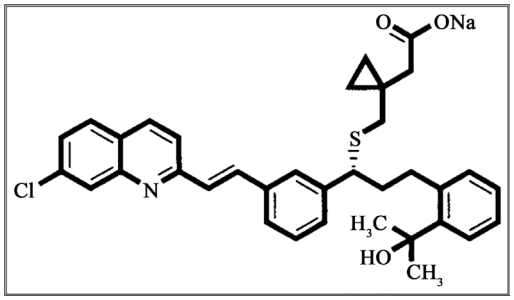

Figure 1. Chemical structure of MON.

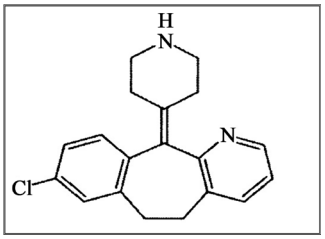

Figure 2. Chemical structure of DES.

was degassed in an ultrasonic water bath for 5 minutes and filtered through 0.45 µ filter under vacuum.

2.4. Diluent Preparation

Methanol was used as diluent.

2.5. Standard Solution Preparation for MON and DES

The standard solutions were prepared by weighing accurately 10 mg of MON and 5 mg of DES [working standards] and transferred into two 100 ml clean dry volumetric flasks. About 70 ml of diluent was added to each flask and sonicated to dissolve the powders completely. Final volumes were adjusted to the mark with diluent. From the stock solutions 5 ml was transferred into 25 ml volumetric flask and diluted up to the mark with same diluent to produce the concentrations of 20 µg/ml of MON and 10 µg/ml of DES.

2.6. Sample Solution Preparation for MON and DES

Twenty tablets of MON and DES were weighed and then finely powdered. An accurately weighed portion of the powder equivalent to 10 mg of MON and 5 mg of DES was transferred into 100 ml volumetric flask. About 70 ml of diluent was added and sonicated to dissolve the powder completely. From the above filtrate pip petted out 5 ml into a 25 ml volumetric flask and diluted with the diluent. Final volume was adjusted to the mark with diluent. From the stock solution 5 ml was transferred into 25 ml volumetric flask and diluted up to the mark with same diluent to produce the concentrations of 20 µg/ml of MON and 10 µg/ml of DES.

2.7. Chromatographic Conditions

A Hypersil BDS C18 (250 mm × 4.6 mm I.D., 5 µm particle size) column was used for analysis at ambient temperature. The mobile phase orthophosphoric acid and Milli-Q water (20:80 v/v) was pumped through the column at a flow rate of 1.0 ml/min. The sample injection volume was 10 µl. Detection was carried out at 280 nm and chromatographic run time was 6 min.

3. Method Development & Method Validation

3.1. Method Development

To develop a suitable and robust HPLC method for the simultaneous estimation of MON and DES, different mobile phases were employed to achieve the best separation and resolution. The method development was made with Hypersil BDS C18 column (250 mm × 4.6 mm I.D., 5 µ particle size) with the mobile phase composed of orthophosphoric acid and water in the ratio of 20:80 v/v, at a flow rate of 1.0 ml/min was used for the separation.

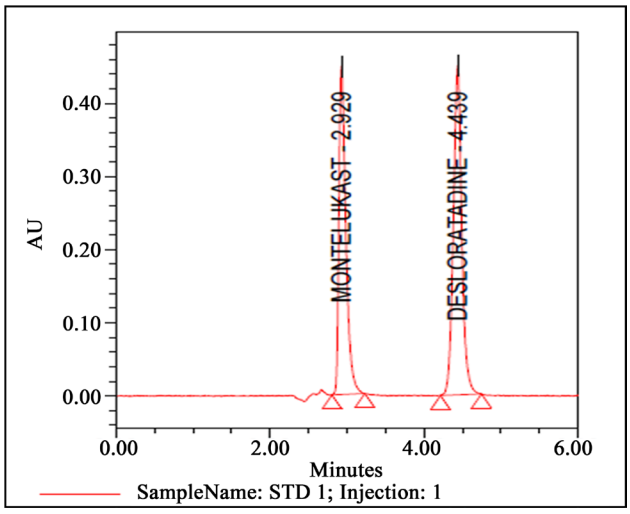

Figure 3. A typical HPLC chromatogram showing peaks of MON and DES.

Detection was carried out at 280 nm. The typical chromatogram of MON and DES was shown in **Figure 3**.

3.2. Method Validation

According to ICH guidelines, the developed method was validated to assure the reliability of the results of the analysis for different parameters like specificity, system suitability, precision, accuracy, linearity, LOD, LOQ, robustness and ruggedness.

3.2.1. Specificity

Specificity is the ability of a method to discriminate between the analytes of interest and other components that are present in the sample. Studies are designed to evaluate the degree of interference, if any, which can be attributed to other analyte, impurities, degradation products, reagent blanks and excipients. A study to establish the interference of blank and placebo were conducted. Diluent and placebo were injected into the chromatograph in the above chromatographic conditions and the blank and placebo chromatograms were recorded. Chromatograms of blank and placebo showed no peaks at the retention times of MON and DES peaks. This indicates that the diluent and placebo used in the sample preparation did not interfere in simultaneous estimation of MON and DES.

3.2.2. System Suitability

The system suitability parameters were determined by injecting the standard solutions of MON and DES. Parameters such as number of theoretical plates, tailing factor, retention time and resolution were determined. System suitability parameter values were shown in **Table 1**.

3.2.3. Precision

The precision of an analytical procedure expresses the closeness of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements. Precision of the method was performed as system precision and method precision.

To study the system precision, five replicate mixed standard solutions of MON and DES were injected. The percent relative standard deviation (% RSD) was calculated. The % RSD of peak areas of MON and DES should not be more than 2.0. Results of system precision studies are shown in **Table 2**.

To study the method precision, five replicate mixed sample solutions of MON and DES were injected. The percent relative standard deviation (% RSD) was calculated. The % RSD of peak areas of MON and DES should not be more than 2.0. Results of method precision studies were shown in **Table 3**.

3.2.4. Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and value found. The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed tablet solution. Percent recovery was calculated by comparing the area before and after the addition of the standard drug. The standard addition method was performed at three concentration levels of 50%, 100% and 150%. The solutions were analyzed in triplicate at each level as per the proposed method. The percent recovery and % RSD of peak areas for MON and DES at each level was calculated. The % Recovery for each level should be between 98.0% and 102.0%. Recovery studies for MON and DES results were shown in **Table 4**.

3.2.5. Linearity

The linearity of the analytical procedure is its ability (within a given range) to obtain the test results which are directly proportional to the concentration of analyte in the sample. Linearity was performed by preparing mixed standard solutions of MON and DES at different concentration levels including working concentration mentioned in experimental condition *i.e.*, 10 - 30 µg/ml for MON and 5 - 15 µg/ml for DES respectively. Ten micro litres of each concentration was injected in duplicate into the HPLC system. The response was read at 280 nm and the corresponding chromatograms were recorded. From these chromatograms, the mean peak areas were

calculated and linearity plots of concentration over the mean peak areas were constructed individually. The regressions of the plots were computed by least square regression method. The correlation coefficient should be not less than 0.99. Linearity plots are shown in **Figure 4** & **Figure 5**. Linearity results were shown in **Table 5** & **Table 6**.

Table 1. System suitability parameters for MON and DES.

Drug	Theoretical plates	Tailing factor	Retention time	Resolution
MON	5345	1.164	2.929 min.	-
DES	8501	1.056	4.439 min.	8.246

Table 2. System precision for MON and DES.

S. No.	MON Peak Area	DES Peak Area
1	2,810,887	3,426,286
2	2,812,494	3,424,376
3	2,810,808	3,420,684
4	2,815,063	3,422,650
5	2,811,717	3,421,187
Mean	2,812,194	3,423,037
SD	1744.845	2316.12
% RSD	0.06	0.06

Table 3. Method precision for MON and DES.

S. No.	MON Peak Area	DES Peak Area
1	2,812,954	3,420,257
2	2,813,565	3,422,883
3	2,817,759	3,428,553
4	2,813,565	3,422,883
5	2,812,954	3,423,898
Mean	2,814,159	3,423,695
SD	2035.29	3032.32
% RSD	0.07	0.08

Table 4. Recovery studies for MON and DES.

% Level	% Mean recovery of MON	% Mean recovery of DES
50	99.82	99.80
100	99.59	99.60
150	99.71	99.73

Table 5. Linearity of MON.

S. No.	Concentration (µg/ml)	Mean peak area
1	10	1,400,967
2	15	2,115,685
3	20	2,819,203
4	25	3,528,621
5	30	4,224,806

Table 6. Linearity of DES.

S. No.	Concentration (µg/ml)	Mean peak area
1	5	1,719,571
2	7.5	2,574,693
3	10	3,428,501
4	12.5	4,280,413
5	15	5,148,147

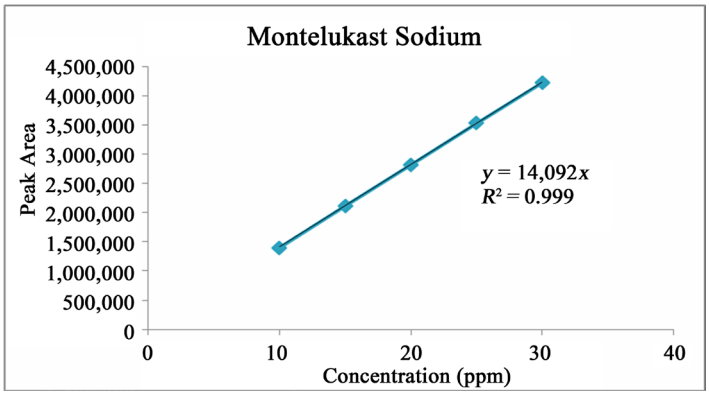

Figure 4. Linearity curve for MON.

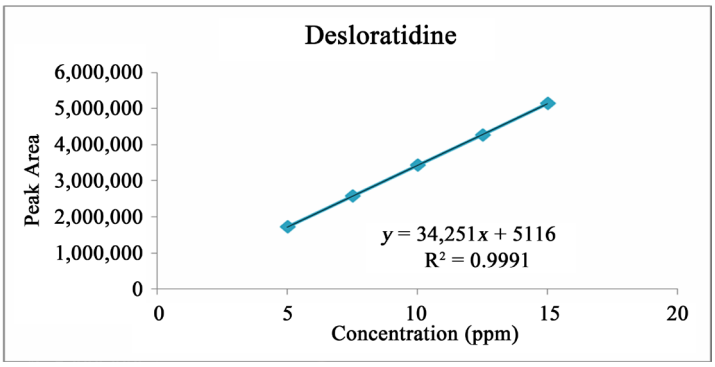

Figure 5. Linearity curve for DES.

3.2.6. Limit of Detection and Limit of Quantification

Limit of detection (LOD) is defined as the smallest concentration that can be detected but not necessarily quantified as an exact value.

LOD is calculated from the formula; LOD = $3.3\sigma/S$

where, σ = standard deviation of the response, S = slope of calibration curve.

Limit of quantification (LOQ) is defined as the lowest concentration that can be quantified reliably with a specified level of accuracy and precision.

LOQ is calculated from formula; LOQ = $10\sigma/S$

where, σ = standard deviation of the response, S = slope of calibration curve.

For this study six replicates of the analyte at lowest concentration were measured and quantified. The LOD and LOQ values of MON and DES were given in **Table 7**.

3.2.7. Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameter and provides an indication of its reliability during normal usage. As part of the robustness, deliberate change in the flow rate, mobile phase composition, temperature variation was made to

evaluate the impact on the method. The typical variations are variation in flow rate by ±0.2 ml/min and variation in temperature varied from ±10%. Mixed samples of both MON and DES were analyzed under these changed experimental conditions. The results of robustness study are shown in **Table 8**.

3.2.8. Ruggedness

The ruggedness of an analytical method is determined by analysis of aliquots from homogenous lots by different analysts using operational and environmental conditions that may differ but are still within the specified parameters of the assay. The assay was performed in different condition, different analyst and different dates. The results of ruggedness study are given in **Table 9**.

4. Results and Discussion

The HPLC procedure was optimized with a view to develop a simultaneous estimation method in tablet dosage form using Hypersil BDS C18 column (250 mm × 4.6 mm I.D., 5 µ particle size) in isocratic mode with mobile phase composed of orthophosphoric acid and water in the ratio of 20:80 v/v resulted in peak with good shape and resolution. The flow rate was 1.0 ml/min and both the components were measured with UV detector at 280 nm.

Typical chromatograms of the drugs MON and DES shown that no interfering peaks were found in the chromatogram of the formulation within the run time indicating that excipients used in tablet formulations did not interfere with the estimation of the drugs by the proposed HPLC method. The retention times for MON and DES was found to be 2.929 min and 4.439 min respectively and the peak shape was good. The number of theoretical plates calculated was 5345 for MON and 8501 for DES and tailing factor was 1.164 for MON and 1.056 for DES, which indicates efficient performance of the column. The results of system suitability parameters indicate good performance and hence the method is specific.

For system precision study, the % RSD was found to be 0.06 and 0.06 for MON and DES respectively, which are well within the acceptable criteria of not more than 2.0. For method precision study, the % RSD was found to be 0.07 and 0.08 for MON and DES respectively, which are well within the acceptable criteria of not more than 2.0. This reveals that the method is quite precise.

Table 7. LOD and LOQ for MON and DES.

S. No.	Parameter	MON	DES
1	LOD (µg/ml)	0.176	0.087
2	LOQ (µg/ml)	0.587	0.292

Table 8. Robustness for MON and DES.

S. No.	Parameter	MON		DES	
		Peak area	Tailing factor	Peak area	Tailing factor
1	Initial Sample	2,812,194	1.164	3,423,037	1.060
2	Flow rate (+0.2 ml/min)	3,192,458	1.163	3,970,475	1.056
3	Flow rate (−0.2 ml/min)	2,412,494	1.164	3,174,376	1.047
4	Temp. change (10% more)	2,983,302	1.163	3,879,560	1.059
5	Temp. change (10% less)	2,355,063	1.167	3,052,650	1.055
	% RSD		0.14		0.49

Table 9. Ruggedness for MON and DES.

S. No.	Parameter	MON	DES
		Peak area	Peak area
1	Analyst-1	2,812,954	3,420,257
2	Analyst-2	2,813,565	2,817,759
	% RSD	0.015	0.170

Satisfactory recoveries ranging from 99.59% - 99.82% for MON and 99.60% - 99.80% for DES respectively were obtained by the proposed method. This indicates that the proposed method was accurate.

The linearity was found satisfactory for the drugs in the range 10 - 30 µg/ml for MON and 5 - 15 µg/ml for DES. The correlation coefficient was found to be 0.999 for both MON and DES. This indicates that the method was linear.

The limit of detection and limit of quantification for MON were found to be 0.176 µg/ml and 0.587 µg/ml and for DES were found to be 0.087 µg/ml and 0.292 µg/ml respectively, which indicate the sensitivity of the method.

The deliberate changes in the method and operational conditions have not much affected the chromatograms. This indicates that the proposed method was robust and rugged.

5. Conclusion

The proposed RP-HPLC method was simple, specific, sensitive, precise and accurate and can be used for simultaneous estimation of MON and DES in bulk samples and its tablet dosage forms.

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