ABSTRACT

A novel, precise, accurate and rapid isocratic reversed-phase high performance liquid chromatographic/ultraviolet (RP-HPLC/UV) method was developed, optimized and validated for simultaneous determination of Montelukast Sodium and Desloratadine. The method showed adequate separation for Montelukast Sodium and Desloratadine and best resolution was achieved with ACE 5 C18 column (150 mm × 4.6 mm i.d, 5 µm particle size) using Acetonitrile: Methanol: Water (15:80:5, v/v) as a mobile phase at a flow rate of 1.0 ml/min and wavelength of 283 nm. The calibration curves were linear over the concentration ranges of 5-50 µg/ml for Montelukast Sodium and Desloratadine. The limit of detection (LOD) and limit of quantification (LOQ) for Montelukast Sodium were 0.09 and 0.27 µg/ml while for Desloratadine were 0.11 and 0.34 µg/ml, respectively. All the analytes were separated in less than 6.0 min. The proposed method could be applied for routine laboratory analysis of Montelukast Sodium and Desloratadine in pharmaceutical dosage form. Methods were validated statistically and recovery studies were carried out. The proposed methods have been applied successfully to the analysis of cited drug either in pure form or in synthetic mixture of both drugs with good accuracy and precision. The method herein described can be employed for quality control and routine analysis of drugs in pharmaceutical formulations.
INTRODUCTION
Montelukast sodium (MTKT), 1-[(R)-m-[(E)-2-(7-chloro-2-quinolyl) vinyl]-α-[(1-hydroxy-1-methylethyl)phenethyl]benzyl]thiomethyl] cyclopropaneacetate sodium is a leukotriene receptor antagonist, used in the treatment of asthma. It is not official in IP, BP and USP. Various analytical methods, such as liquid chromatography with fluorescence detection, stereo selective HPLC for MTKT and its enantiomer, simultaneous HPLC and derivative spectroscopic method with loratadine, stability indicating HPLC method for Montelukast sodium in tablets and human plasma have been reported. Desloratadine (DESLO), 13-chloro-2-(piperidin-4-ylidene)-4-azatricyclo[9.4.0.0^(3,8)]pentadeca-1(11),3,5,7,12,14 hexaene. Desloratadine is a drug used to treat allergies. Various analytical methods, such as liquid chromatography, Spectrophotometric, spectrofluorometric and HPLC determination of desloratadine in dosage forms and human plasma, Stability-Indicating RP-UPLC with Sodium Benzoate. The combined dosage forms of MTKT and DESLO are available in the market for the rhinitis and treatment of allergies and chronic urticaria. Present study involves development and validation of Q-Absorbance Ratio Method and Dual Wavelength Spectrophotometry method for the estimation of MTKT and DESLO in combination dosage form. The proposed methods were optimized and validated as per International Conference on Harmonization (ICH) guidelines.

The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for the simultaneous estimation of MTKT and DESLO in their combined dosage forms. The present communication describes simple, sensitive, rapid, accurate, precise and cost effective chromatographic method.

EXPERIMENTAL
Apparatus

- RP-HPLC instrument equipped with a UV-Visible detector and a photodiode array detector, (Shimadzu, LC-2010C_H, Japan,), auto sampler, ACE 5 C18 column (150 mm x 4.6 mm i.d, 5 µm particle size) and LC-solution software were used.

- Analytical balance (Sartorius CP224S, Germany)
· Triple distillation unit consisting of borosilicate glass

· Digital pH meter (LI 712 pH analyzer, Elico Ltd., Ahmedabad)

Ultra sonic cleaner (Frontline FS 4, Mumbai, India)

Reagents and materials
MTKT and DESLO bulk powder was kindly gifted by Acme Pharmaceuticals Ltd., Mehsana, Gujarat, India. Methanol (AR Grade, S. D. Fine Chemicals Ltd., Mumbai, India), Acetonitrile (AR Grade, S. D. Fine Chemicals Ltd., Mumbai, India) and Whatman filter paper no. 41 (Millipore, USA) were used in the study.

Preparation of standard stock solutions
An accurately weighed standard MTKT and DESLO powder (10 mg) were weighed and transferred to 100 ml separate volumetric flasks and dissolved in methanol. The flasks were shaken and volumes were made up to mark with methanol to give a solution containing 100 µg/ml of each MTKT and DESLO.

Preparation of Working Standard Solutions
An aliquot of stock solution 1 ml of standard stock solution was transferred in 10ml volumetric flask and adjusted up to mark with methanol having concentration (10µg/ml).

Preparation of Sample Solution
MTKT and DESLO in commercial tablets (each tablet containing 10 mg MTKT and 5 mg DESLO), 20 tablets were weighed and finely powdered. A quantity of powder equivalent to 10 mg of MTKT and 5 mg of DESLO was weighed accurately and transferred to a 50 ml volumetric flask and the volume was made up with the solvent. It was sonicated for 30 minutes and then filtered through a nylon 0.45 µm – 47 mm membrane filter. From the above prepared solution, 0.5 ml dilute in 10 ml volumetric flask and the volume was made up with the solvent.

Chromatographic Condition

* Stationary phase: ACE 5 C18 column (150 mm x 4.6 mm i.d., 5 µm particle size) was used at ambient temperature.


* Flow rate: 1.0 ml/min

* Injection volume: 20 µL

* Detection: The elution was monitored at 283 nm using PDA detector.

Method development
A satisfactory separation and good peak symmetry for MTKT and DESLO was obtained with a mobile phase Acetonitrile: Methanol :Water [15:80:5, v/v/v] at a flow rate of 1.0 ml/min to get better reproducibility
and repeatability (Figure 3). Overlaid UV spectrum showed that both drugs showed good absorbance at 283 nm, hence the wavelength of 283 nm was selected for quantification of MTKT and DESLO (Figure 4).

Method Validation

Calibration Curve (Linearity)
Calibration curves were constructed by plotting peak areas Vs concentrations of MTKT and DESLO and the regression equations were calculated. The calibration curves were plotted over the concentration range 5-50 µg/ml for MTKT and 5-50µg/ml for DESLO. Accurately measured standard working solutions of MTKT (0.5, 1, 1.5, 2, 3, 4 and 5 ml) and DESLO (0.5, 1, 1.5, 2, 3, 4 and 5 ml) from 100 µg/ml of stock solution were transferred to a series of 10ml of volumetric flasks and diluted to the mark with methanol. 20 µL of each solution were injected under the operating chromatographic conditions described above.

Accuracy (% Recovery)
The accuracy of the method was determined by calculating recovery of MTKT and DESLO by the standard addition method. Known amounts of standard solutions of MTKT were added to pre quantified sample solutions of DESLO. Known amounts of standard solutions of DESLO were added to pre quantified sample solutions of MTKT. The amounts of MTKT and DESLO were estimated by applying obtained values to the regression equation of the calibration curve.

Method Precision (% Repeatability)
The precision of the method was checked by repeatedly injecting six sample solutions of MTKT (30 µg/ml) and DESLO (30 µg/ml) under the same chromatographic condition and measurements of peak area, retention time and tailing factor. Percentage relative standard deviation (RSD) or % coefficient of variation (CV) should not be more than 2.

Intermediate Precision (Reproducibility)
The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of sample solutions of MTKT (10, 20, 30 µg/ml) and DESLO (10, 20, 30 µg/ml). The results were reported in terms of relative standard deviation (RSD).

Limit of Detection and Limit of Quantification
LOD and the LOQ of the drug were calculated using the following equations as per International Conference on Harmonization (ICH) guidelines.

\[
\text{LOD} = 3.3 \times \frac{\sigma}{S}
\]
\[
\text{LOQ} = 10 \times \frac{\sigma}{s}
\]
Where \( \sigma \) = the standard deviation of the response

\[ S = \text{Slope of calibration curve.} \]

**ANALYSIS OF MTKT AND DESLO IN SYNTHETIC MIXTURE:**

The response of the sample solution was measured at 283 nm under the chromatographic condition mentioned above for the quantification of MTKT and DESLO. The amounts of MTKT and DESLO present in sample solution were determined by applying values of the peak area to the regression equations of the calibration curve.

**RESULTS AND DISCUSSION:**

**Linearity**

Linear correlation was obtained between peak area Vs concentrations of MTKT and DESLO in the concentration of 5-50 \( \mu \)g/ml for MTKT and 5-50 \( \mu \)g/ml for DESLO. Regression parameters are mentioned in Table and the calibration curves of these two drugs at 283 nm are shown in Fig 5 and 6.

**Method Precision (% Repeatability)**

The %RSD values for MTKT and DESLO were found to be 0.10 and 0.13 %, respectively. The RSD values were found to be <2 %, which indicates that the proposed method is repeatable.

**Intermediate Precision (Reproducibility)**

The low RSD values of interday and intraday for MTKT and DESLO, respectively, reveal that the proposed method is precise.

**LOD and LOQ**

LOD values for MTKT and DESLO were found to be 0.09 \( \mu \)g/ml and 0.11 \( \mu \)g/ml, respectively and LOQ values for MTKT and DESLO were found to be 0.27 \( \mu \)g/ml and 0.34 \( \mu \)g/ml respectively. These data show that the proposed method is sensitive for the determination of MTKT and DESLO.

**Accuracy**

The recovery experiment was performed by the standard addition method. The recoveries obtained were 100.45 ± 1.07 % and 101.58 ± 0.56 % for MTKT and DESLO, respectively (Table 3). The low value of standard deviation indicates that the proposed method is accurate. Results of recovery studies are shown in Table 3.

**Assay of the Combined dosage form**

The proposed validated method was successfully applied to determine MTKT and DESLO in their synthetic mixture. The result obtained for MTKT and DESLO was comparable with the corresponding labeled amounts (Figure 7) (Table 4).

**CONCLUSION:**

In this proposed method the linearity
is observed in the concentration range of 5-50 µg/ml and 5-50 µg/ml with co-efficient of correlation, $(r^2) = 0.998$ and $(r^2) = 0.997$ for MTKT and DESLO, respectively at 283 nm. The result of the analysis of pharmaceutical formulation by the proposed method is highly reproducible and reliable and it is in good agreement with the label claim of the drug. The method can be used for the routine analysis of the MTKT and DESLO in synthetic mixture without any interference of excipients.

ACKNOWLEDGEMENT
The authors are thankful to Acme Pharmaceutical Ltd., Mehsana, India for providing gift sample of MTKT and DESLO for carry out the research work.

Fig. 1: Chemical structure of Montelukast Sodium

Fig. 2: Chemical structure of Desloratadine.
Fig 3 Chromatogram of Standard Solution of MTKT and DESLO at 283 nm
Figure 4: U.V. Spectrum of MTKT and DESLO.

Fig 5 Calibration Curve of MTKT at 283 nm
Fig 6 Calibration Curve of DESLO at 283 nm

![Calibration Curve of DESLO at 283 nm](image)

\[ y = 4941x - 278.5 \]
\[ R^2 = 0.997 \]

Figure 7: Chromatogram of sample solution of MTKT (10 µg/ml) and DESLO (5 µg/ml) at 283 nm

![Chromatogram of sample solution of MTKT and DESLO at 283 nm](image)
Table 1 System Suitability Parameters of Chromatogram for MTKT and DESLO

<table>
<thead>
<tr>
<th>Parameters</th>
<th>MTKT ± RSD (n = 6)</th>
<th>DESLO ± RSD (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention time (min)</td>
<td>2.205±0.095</td>
<td>5.7± 0.030</td>
</tr>
<tr>
<td>Tailing factor</td>
<td>0.943± 0.4519</td>
<td>1.080± 0.5194</td>
</tr>
<tr>
<td>Theoretical plates</td>
<td>2119.62± 1.45</td>
<td>2438.39± 1.29</td>
</tr>
<tr>
<td>Resolution</td>
<td>6.082 ± 0.24</td>
<td></td>
</tr>
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</table>

Table 2 Recovery Data for the proposed Method

<table>
<thead>
<tr>
<th>Drug</th>
<th>Level</th>
<th>Amount of sample taken (µg/ml)</th>
<th>Amount of standard spiked (%)</th>
<th>Mean % Recovery ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTKT</td>
<td>I</td>
<td>10</td>
<td>50 %</td>
<td>99.71 ± 1.22</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>10</td>
<td>100 %</td>
<td>101.68 ± 1.61</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>10</td>
<td>150 %</td>
<td>99.97 ± 0.69</td>
</tr>
<tr>
<td>DESLO</td>
<td>I</td>
<td>5</td>
<td>50 %</td>
<td>102.04 ± 0.85</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>5</td>
<td>100 %</td>
<td>101.75 ± 0.74</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>5</td>
<td>150 %</td>
<td>100.95 ± 1.86</td>
</tr>
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</table>
### Table 3 Analysis of MTKT and DESLO (n = 6)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Level</th>
<th>Amount of sample taken (µg/ml)</th>
<th>Amount of standard spiked (%)</th>
<th>Mean % Recovery ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTKT</td>
<td>I</td>
<td>10</td>
<td>50 %</td>
<td>99.71 ± 1.22</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>10</td>
<td>100 %</td>
<td>101.68 ± 1.61</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>10</td>
<td>150 %</td>
<td>98.97 ± 0.69</td>
</tr>
<tr>
<td>DESLO</td>
<td>I</td>
<td>5</td>
<td>50 %</td>
<td>102.04 ± 0.85</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>5</td>
<td>100 %</td>
<td>98.75 ± 0.74</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>5</td>
<td>150 %</td>
<td>99.26 ± 1.86</td>
</tr>
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### Table 4 Regression Analysis Data and Summary of Validation Parameter for the proposed Method

<table>
<thead>
<tr>
<th>Parameters</th>
<th>RP-HPLC method</th>
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<tr>
<td></td>
<td>MTKT</td>
</tr>
<tr>
<td>Concentration range (µg/ml)</td>
<td>5-50</td>
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<tr>
<td>Slope</td>
<td>38297</td>
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<tr>
<td>Intercept</td>
<td>31907</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.998</td>
</tr>
<tr>
<td>LOD (µg/ml)</td>
<td>0.09</td>
</tr>
<tr>
<td>LOQ (µg/ml)</td>
<td>0.27</td>
</tr>
<tr>
<td>% Recovery (Accuracy, n = 6)</td>
<td>100.45 ± 1.07</td>
</tr>
<tr>
<td>Repetability (% RSD, n = 6)</td>
<td>0.10</td>
</tr>
<tr>
<td>Precision (%RSD)</td>
<td></td>
</tr>
<tr>
<td>Interday (N = 3)</td>
<td>0.37-0.75</td>
</tr>
<tr>
<td>Intraday (N = 3)</td>
<td>0.12-0.33</td>
</tr>
</tbody>
</table>
REFERENCES

14. Salinas F., Nevado J.J. and Espinosa M.A., A new spectrophotometric method for quantitative multicomponent analysis resolution of mixtures of Salicylic and


