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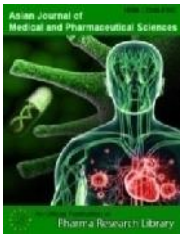

**Research Article** **Open Access**

# RP- HPLC Method Development and Validation for Simultaneous Estimation of Olopatadine and Montelukast

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**A B S T R A C T**

The aim of this work was focused on to develop and validate accurate, simple reverse phase high-performance liquid chromatography method for simultaneous estimation of olopatadine and montelukast in pharmaceutical dosage form. The chromatographic separation was performed on Agilent Eclipse XDB-C18 (4.6 x 150mm, 5.0µm), with a mobile phase comprising of a mixture phosphate buffer and Acetonitrile (20:80) at flow rate of 1mL/min with detection wavelength at 270 nm. Retention times of olopatadine and montelukast were found to be 2.7 minutes and 3.5 minutes respectively. The developed method was validated according to ICH guidelines, linearity of olopatadine was found to be in the range of 20-100 µg/mL and that of montelukast was found to be in the range of 40-200 µg/mL. The percentage recoveries for both drugs were found in the range of 100-101%. The limit of detection and the limit of quantification values for olopatadine were found to be 2.98 and 9.97 and that for montelukast were found to be 2.97 and 9.98 respectively.

**Keywords:** Olopatadine, Montelukast, Reverse phase high performance liquid chromatography, Validation, Degaradation.

**A R T I C L E I N F O**

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## 1. Introduction

Montelukast sodium is described chemically as [R-(E)]-1-[[[1-[3-[2-(7chloro-2-quinolinyl) ethenyl] phenyl]-3-[2-(1hydroxy-1-methylethyl) phenyl] propyl] thio] methyl] cyclopropane acetic acid, monosodium salt. Montelukast is

a leukotriene receptor antagonist (LTRA) used for the maintenance treatment of asthma and to relieve symptoms of seasonal allergies. It is usually administered orally. Montelukast is a CysLT1 antagonist and it blocks the action of leukotriene D4 on the cysteinyl leukotriene receptor CysLT1 in the lungs and bronchial tubes by binding to it. This reduces the bronchoconstriction otherwise caused by the leukotriene and results in less inflammation [1]. The chemical structure of Montelukast is shown in Figure1. [2]
Olopatadine hydrochloride, chemically, 11[(Z)-3-(Dimethyl amino) propylidene] – 6 - 11dihydrodibenz [b, e] oxepin-2-acetic acid hydrochloride is a dibenzoxipine derivative used for systemic treatment of allergic rhinitis, urticaria, and bronchial asthma. It is a histamine H1 receptor antagonist and is used as an antiallergic and anti-inflammatory agent [2]. The chemical structure of Olopatadine is shown in Fig.2. [3]

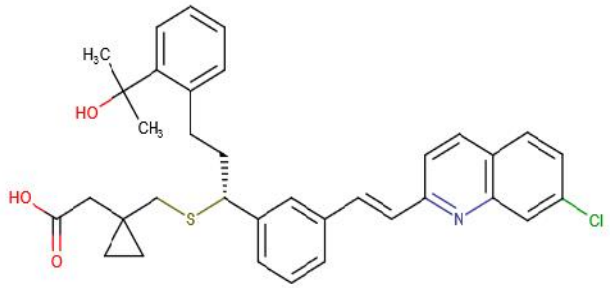


**Figure 1:** Structure of Montelukast

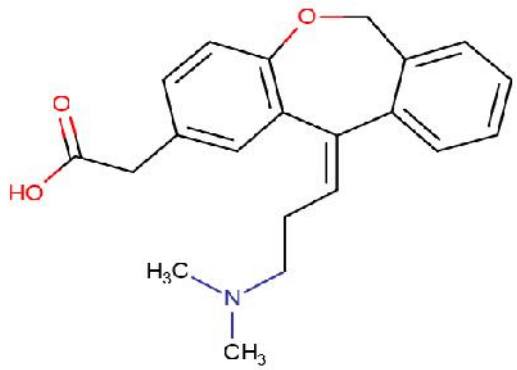


**Figure 2:** Structure of Olopatadine

Literature survey of Olopatadine and Montelukast revealed few methods based on UV Spectrophotometry [**4-5**] , and Chromatography [**6-8**] have been reported for determination of both drugs in single and combined dosage forms. The present work describes the development and validation as per ICH guidelines[**9**] of reverse phase high performance liquid chromatographic (RP-HPLC) method, which can quantify these components simultaneously.

## 2. Experimental

Materials and Methods: Reagents required Acetonitrile: HPLC grade, Water: HPLC grade, Potassium dihydrogen phosphate: AR grade. Drugs used The gift samples of Olopatadine and Montelukast were kindly provided by Reddy's laboratories and the marketed generic formulation containing Olopatadine (5 mg) and Montelukast (10 mg) were procured from local pharmacy.

**Instrumentation and Chromatographic Conditions:**

The developed method HPLC system with UV detector data were acquired and processed by Empower software. The separation was carried out at ambient temperature by using a Agilent Eclipse XDB (4.6 x 250mm, 5µm). The mobile phase consisting of Phosphate buffer: Acetonitrile (20:80v/v). The flow rate was 1 ml/min. The injection volume was 20 µL and detection at a wavelength of 270 nm. Mix a mixture of above buffer 200 ml (20%) and 800ml of Acetonitrile HPLC (80%) degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

**Standard Solution Preparation:**

Accurately weigh and transfer 10mg and 20mg of Olopatadine and Montelukast working standard into a 10ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 2.0 ml of Olopatadine and Montelukast of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

**Sample Solution Preparation:**

Accurately weigh and transfer equivalent 10 and 20mg of Olopatadine and Montelukast working standard into a 10ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 2.0 ml of Olopatadine and Montelukast of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

**Linearity of pure standard solution:**

The linearity of the samples of Olopatadine and Montelukast was prepared by suitably diluting working solution and found to be linear response of drug over a range of 20-100 µg/ml concentration for the Olopatadine and 40-200 µg/ml for Montelukast respectively. The three such linearities of Olopatadine and Montelukast were taken for correlation co-efficient and standard deviation calculation.

**Table 1:** Linearity of Olopatadine

| S.No | Olopatadine (µg/mL) | Area (mV.s) |
|---|---|---|
| 1 | 20 | 76231 |
| 2 | 40 | 158515 |
| 3 | 60 | 226559 |
| 4 | 80 | 304841 |
| 5 | 100 | 379205 |

**Table 2:** Linearity of Montelukast

| S.No | Montelukast (µg/mL) | Area (mV.s) |
|---|---|---|
| 1 | 40 | 598232 |
| 2 | 80 | 1195329 |
| 3 | 120 | 1782729 |
| 4 | 160 | 2322400 |
| 5 | 200 | 2991309 |

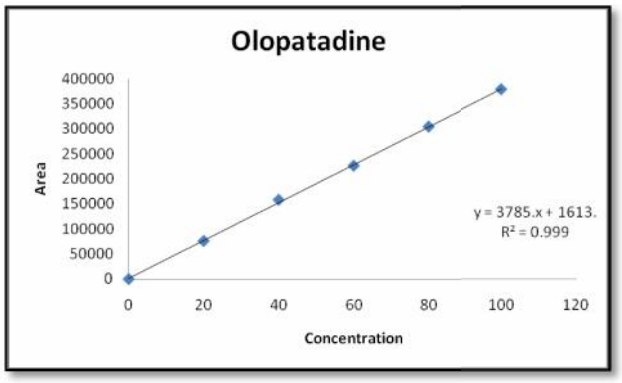


**Figure 3:** Linearity of Olopatadine

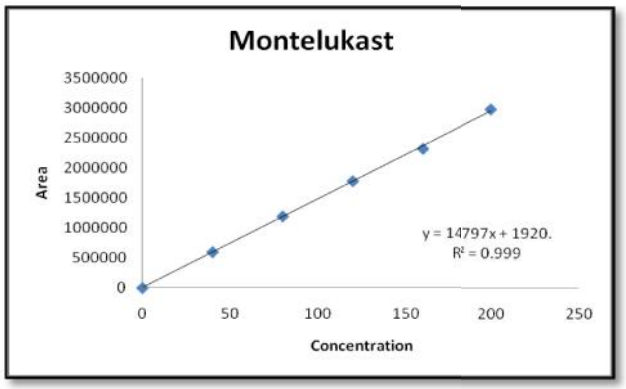


**Figure 4:** Linearity of Montelukast

**Analysis of Formulation**

The developed procedure was extended to formulation of Olopatadine and Montelukast, the combination was available in the market as generic drug of strength 5 mg of Olopatadine and 10 mg of Montelukast respectively. Average weight of twenty tablets were taken and crushed to make powder, weighed powder containing 100 mg Olopatadine was transferred to 100ml of volumetric flask and volume was made up to the mark with diluent (Acetonitrile: Buffer) (80:20) and filtered through whatmann filter paper in to another 100ml volumetric flask and make up to mark with same diluent. The same procedure as mentioned for the pure drug was followed for the formulation. The concentrations of both Olopatadine and Montelukast were determined by measuring peak area at 270 nm.

**Table 2:** Assay Results of Olopatadine

| S. No. | **Olopatadine** | |
|---|---|---|
| 1 | Labeled Amount (mg ) | 5 mg |
| 2 | Amount Found mg/tab | 5.05 |
| 3 | % Recovery | 101.00 |
| 4 | %RSD (n=5) | 0.02 |

**Table 2:** Assay Results of Montelukast

| **S. No.** | **Montelukast** | |
|---|---|---|
| 1 | Labeled Amount (mg ) | 10 mg |
| 2 | Amount Found mg/tab | 10.01 |
| 3 | % Recovery | 100.08 |
| 4 | %RSD (n=5) | 0.04 |

## 3. Results and Discussion

**Method Validation Summary:**

**Precision:**

**Preparation of stock solution:**

Accurately weigh and transfer 10mg and 20mg of Olopatadine and Montelukast working standard into a 10ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)**.** Further pipette 2.0 ml of Olopatadine and Montelukast of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

**Procedure:**

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

**Table 3:** Precision results of Olopatadine

| **Injection** | **Area** |
|---|---|
| Injection-1 | 224888 |
| Injection-2 | 226588 |
| Injection-3 | 222742 |
| Injection-4 | 226484 |
| Injection-5 | 221184 |
| **Average** | 224377.2 |
| **Standard Deviation** | 2369.5 |
| **%RSD** | 1.1 |

**Table 4:** Precision results of Montelukast

| **Injection** | **Area** |
|---|---|
| Injection-1 | 1764636 |
| Injection-2 | 1775828 |
| Injection-3 | 1755281 |
| Injection-4 | 1788337 |
| Injection-5 | 1751775 |
| **Average** | 1767171 |
| **Standard Deviation** | 15064.7 |
| **%RSD** | 0.9 |

**Acceptance Criteria:**

The % RSD for the area of five standard injections results should not be more than 2%.

**Intermediate Precision/Ruggedness:**

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions.

**Preparation of stock solution:**

Accurately weigh and transfer 10mg and 20mg of Olopatadine and Montelukast working standard into a 10ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)**.** Further pipette 2.0 ml of Olopatadine and Montelukast of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

**Procedure:**

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

**Table 5:** Ruggedness of Olopatadine

| Injection | Area |
|---|---|
| Injection-1 | 231244 |
| Injection-2 | 224759 |
| Injection-3 | 228244 |
| Injection-4 | 230601 |
| Injection-5 | 231879 |
| Injection-6 | 233017 |
| **Average** | 229957.3 |
| **Standard Deviation** | 3002.7 |
| **%RSD** | 1.3 |

**Table 6:** Ruggedness of Montelukast

| Injection | Area |
|---|---|
| Injection-1 | 1840886 |
| Injection-2 | 1854238 |
| Injection-3 | 1856862 |
| Injection-4 | 1871402 |
| Injection-5 | 1882182 |
| Injection-6 | 1850620 |
| **Average** | 1859365 |
| **Standard Deviation** | 14939.9 |
| **%RSD** | 0.8 |

**Acceptance Criteria:**

The % RSD for the area of six standard injections results should not be more than 2%

**Accuracy:**

**Preparation of Standard stock solution:**

Accurately weigh and transfer 10 and 20mg of Olopatadine and Montelukast working standard into a 10ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 2.0 ml of Olopatadine and Montelukast of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

**Preparation Sample solutions:**

**For preparation of 50% solution (With respect to target Assay concentration):**

Accurately weigh and transfer 5 and 10mg of Olopatadine and Montelukast working standard into a 10ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 2.0 ml of Olopatadine and Montelukast of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

**For preparation of 100% solution (With respect to target Assay concentration):** Accurately weigh and transfer 10 and 20mg of Olopatadine and Montelukast working standard into a 10ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)**.** Further pipette 2.0 ml of Olopatadine and Montelukast of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

**For preparation of 150% solution (With respect to target Assay concentration):**

Accurately weigh and transfer 15 and 30mg of Olopatadine and Montelukast working standard into a 10ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 2.0 ml of Olopatadine and Montelukast of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

**Procedure:**

Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions. Calculate the Amount found and Amount added for Olopatadine and Montelukast and calculate the individual recovery and mean recovery values.

**Acceptance Criteria:** The % Recovery for each level should be between 98.0 to 102.0%

**Linearity:**

**Preparation of stock solution:**

Accurately weigh and transfer 10 and 20mg of Olopatadine and Montelukast working standard into a 10ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 2.0 ml of Olopatadine and Montelukast of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. From the above stock solution further dilutions were made to get the concentrations equivalent to 20, 40, 60.80 & 100 ppm and 40, 80, 120,160,200 ppm of Olopatadine and Montelukast respectively.

**Procedure:** Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

**Acceptance Criteria:**

Correlation coefficient should be not less than 0.999.

**Limit of Detection: (for Olopatadine)**

Accurately weigh and transfer 10mg of Olopatadine working standard into a 10ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Futher dilutions were made to get the concentration equivalent to 60µg/ml from the above stock solution. Futher dilutions were made to get the concentration equivalent to 0.48µg/ml from the above stock solution.

S/N Ratio = 176/59 = 2.98

**Limit of Quantification:**

Futher dilutions were made to get the concentration equivalent to 1.62 µg/ml from the above stock solution.

S/N Ratio = 588/59 = 9.97

**Limit of Detection: (for Montelukast)**

**Preparation of 120µg/ml solution:**

Accurately weigh and transfer 20mg of Montelukast working standard into a 10ml clean dry volumetric flask

add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Futher dilutions were made to get the concentration equivalent to 120 µg/ml from the above stock solution.

**Preparation 0.120µg/ml solution:**

Futher dilutions were made to get the concentration equivalent to 0.120 µg/ml from the above stock solution.

S/N Ratio = 175/59 = 2.97

**Limit of Quantification:**

**Preparation of 0.396µg/ml solution:**

Futher dilutions were made to get the concentration equivalent to 0.396 µg/ml from the above stock solution.

S/N Ratio = 589/59 = 9.98%

**Robustness:**

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 flow rate was varied at 0.9 ml/min to 1.1ml/min. Standard solution 60 ppm & 120 ppm of Olopatadine & Montelukast prepared and analysed using the varied flow rate along with method flow rate. On evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10%. The method is robust only in less flow condition. The Organic composition in the Mobile phase was varied from 72% to 88%. Standard solution 60 µg/ml and 120 µg/ml of Olopatadine and Montelukast was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method.

**The results are summarized**

On evaluation of the above results, it can be concluded that the variation in 10% Organic composition in the mobile phase affected the method significantly. Hence it indicates that the method is robust even by change in the Mobile phase ±10.

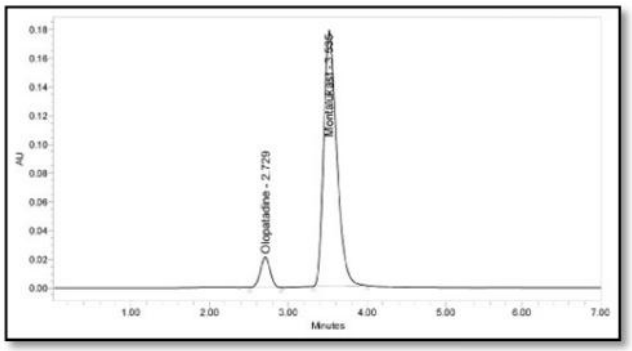


**Figure 5:** System suitability

**Degradation studies:** The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies on the Olopatadine and Montelukast using the proposed method.

**Preparation of Stock solution**

Accurately weigh and transfer 10mg and 20mg of Olopatadine and Montelukast working standard into a 10ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 2.0 ml of Olopatadine and Montelukast of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

**Acid Degradation**

Pipette 3.0 ml of above solution into a 10ml volumetric flask and 3 ml of 0.1N HCl was added. Then, the volumetric flask was kept at 60ºC for 6 hours and then neutralized with 0.1 N NaOH and make up to 10ml with diluent. Filter the solution with 0.22 microns syringe filters and place in vials.

**Alkaline Degaradation**

Pipette 3.0 ml of above solution into a 10ml volumetric flask and add 3 ml of 0.1N NaOH was added. Then, the volumetric flask was kept at 60ºC for 6 hours and then neutralized with 0.1N HCl and make up to 10ml with diluent. Filter the solution with 0.22 microns syringe filters and place in vials.

**Thermal degradation**

Olopatadine and Montelukast sample was taken in petridish and kept in Hot air oven at $110^0$ C for 24 hours. Then the sample was taken and diluted with diluents and injected into HPLC and analysed.

**Oxidative degradation:**

Pipette 3.0 ml above stock solution-2 into a 10ml volumetric flask solution, 1 ml of 3% w/v of hydrogen peroxide was added and the volume was made up to the mark with diluent . The volumetric flask was then kept at room temperature for 15 min. Filter the solution with 0.45 microns syringe filters and place in vials.

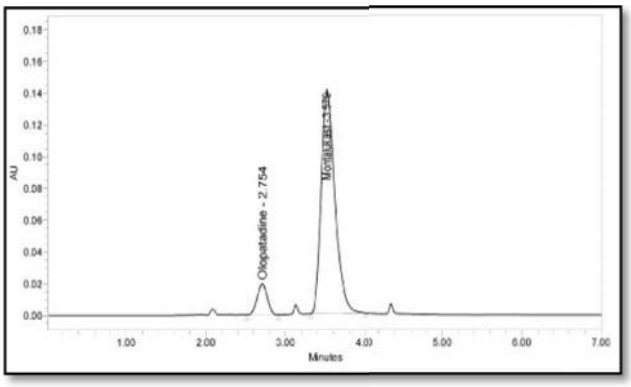


**Figure 6:** Degaradation studies

**Table 7:** Accuracy results for Olopatadine

| %Concentration (at specification Level) | Area | Amount Added (mg) | Amount Found (mg) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|
| 50% | 116307 | 5 | 5.02 | 99.56 | 99.99 |
| 100% | 229450 | 10 | 9.91 | 99.10 | |
| 150% | 351909 | 15 | 15.20 | 101.32 | |

**Table 8:** Accuracy results for Montelukast

| %Concentration (at specification Level) | Area | Amount Added (mg) | Amount Found (mg) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|
| 50% | 931709 | 10 | 10.08 | 100.84 | 100.23 |
| 100% | 1831455 | 20 | 19.82 | 99.11 | |
| 150% | 2792357 | 30 | 30.22 | 100.74 | |

**Table 9:** System suitability results for Olopatadine

| S.No | Flow Rate (ml/min) | System Suitability Results | |
|---|---|---|---|
| | | USP Plate Count | USP Tailing |
| 1 | 0.9 | 3216.39 | 1.13 |
| 2 | 1.0 | 3295.07 | 1.14 |
| 3 | 1.1 | 3187.92 | 1.09 |

**Table 10:** System suitability results for Montelukast

| S.No | Flow Rate (ml/min) | System Suitability Results | |
|---|---|---|---|
| | | USP Plate Count | USP Tailing |
| 1 | 0.9 | 4289.49 | 1.29 |
| 2 | 1.0 | 4249.73 | 1.32 |
| 3 | 1.1 | 4264.95 | 1.27 |

*****Results for actual flow (1 ml/min) have been considered from Assay standard.

**Table 11:** System suitability results for Olopatadine

| S.No | Change in Organic Composition in the Mobile Phase | System Suitability Results | |
|---|---|---|---|
| | | USP Plate Count | USP Tailing |
| 1 | 10% less | 3214.67 | 1.17 |
| 2 | *Actual | 3295.07 | 1.14 |
| 3 | 10% more | 3193.78 | 1.10 |

**Table 12:** System suitability results for Montelukast

| S.No | Change in Organic Composition in the Mobile Phase | System Suitability Results | |
|---|---|---|---|
| | | USP Plate Count | USP Tailing |
| 1 | 10% less | 4187.64 | 1.35 |
| 2 | *Actual | 4249.73 | 1.32 |
| 3 | 10% more | 4159.61 | 1.25 |

***** Results for actual Mobile phase composition (20:80 Phosphate buffer: Acetonitrile) have been considered from standard.

**Table 13:** Results of Degaradation studies:

| | Olopatadine | %degraded | Montelukast | %degraded |
|---|---|---|---|---|
| Standard | 230733 | | 1842958 | |
| Acid | 207653 | 10 | 1595191 | 13.44 |
| Base | 196243 | 14.95 | 1661172 | 9.86 |
| Peroxide | 215615 | 6.55 | 1534811 | 16.72 |
| Thermal | 165438 | 28.3 | 1568944 | 14.87 |

## 4. Conclusion

The proposed RP-HPLC method for estimation of Olopatadine and Montelukast is simple, rapid, specific, accurate, isocratic and precise with simple mobile phase. The method gives good resolution between the compounds with a short analysis time.

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