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# DEVELOPMENT OF REVERSE PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR SIMULTANEOUS ESTIMATION OF MONTELUKAST SODIUM AND ACEBROPHYLLINE IN TABLET DOSAGE FORM

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## ABSTRACT

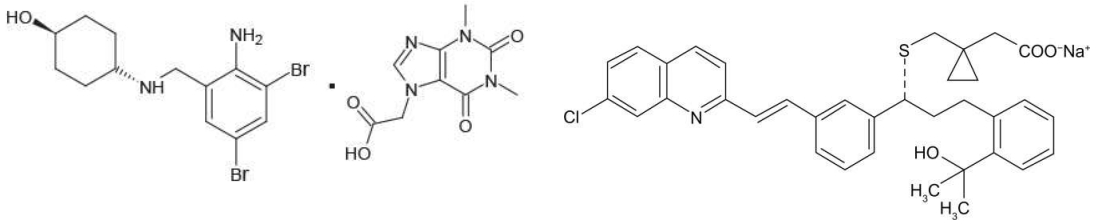
Montelukast sodium and Acebrophylline tablets, an oral combination, is used to treat asthma. The present work comprises developing and validating a RP-HPLC method for the estimation of Montelukast sodium and Acebrophylline simultaneously, in bulk and marketed dosage form**.** This simple method was found to be sensitive, accurate and reproducible as well. Drugs were separated using C-18 column (5.0 µ, 4.6 X 250 mm) and Methanol: Phosphate Buffer (85:15) + GAA5% ($P^H$3.5) was used as a mobile phase. Montelukast and Acebrophylline were eluted at respective retention times (RT) of 3.3 and 4.5 min and detected at 245 nm. For Montelukast and Acebrophylline, the linearity ranges were 4–20 µg/ml and 80–400 µg/ml respectively. The method was validated as per ICH guidelines and applied for estimation of Montelukast sodium/Acebrophylline in tablets with no interference of the excipient peaks. Thus, it can be successfully applied to the aforementioned drugs in bulk or combination dosage forms during quality control analysis.

**Keywords: Acebrophylline, Montelukast Sodium, RP-HPLC, Simultaneous estimation, Validation**

## INTRODUCTION

Asthma, in particular, among the pulmonary diseases, has emerged as a serious health concern in developing countries. Montelukast and Acebrophylline are some of the efficient drugs used for the treatment of asthma. Montelukast (MNLT), **(Figure 1 B)**, is an orally active drug, antagonizes cysteinyl leukotriene receptor (CysLT1), inhibits action of leukotriene D4 and in turn secondary ligands LTC4 and LTE4 present in the lungs and bronchial tubes. Thus, leukotriene mediated bronchoconstriction and inflammation is reduced. It gives relief in seasonal allergies and used as the maintenance treatment in asthma **[1, 2]**.

Acebrophylline (ACB), **(Figure 1 A)** is used as a bronchodilator to treat bronchial asthma and COPD in adults **[3, 4]**.




**A** **B**

**Figure 1: Chemical Structures of Acebrophylline (A) and Montelukast sodium (B)**

As per literature review, RP-HPLC, HPTLC and other spectrophotometric, spectrofluorometric are the available techniques for individual Montelukast sodium estimation **[5-12]** and Acebrophylline **[13-18]** in bulk and formulations. However, simultaneous detection for combination of these two medications is highly preferred over separate assays. Hence, RP-HPLC method was developed and validated for simultaneous estimation of MNLT and ACB.

## MATERIALS AND METHODS

### Materials

Acebrophylline and Montelukast (Pharmaceutical grade) were obtained from Lupin Pharmaceutical Pvt ltd (Pune) and Macleods Pharmaceutical Pvt ltd. (Mumbai) as a gift sample respectively. Merck and Qualigens Fine Chemicals provided the methanol (HPLC grade) for the experiment. Laboratory scale water (double distilled) was used.

### Instrumentation

The Waters HPLC system with 515 pump, 717 plus autosampler and 2998 photodiode array detector, operated by a software Empower-version 2 at a wavelength of 245 nm was used. The columns used were Symmetry C–18 and kromasil C–18.

Ideally, the solvent should completely solubilize the drug, so the solvent system consisting of Methanol: Phosphate Buffer

(85:15) + GAA (Glacial acetic acid) 5% with flow rate 0.8 ml/min. was selected.

**Selection of detection wavelength**

Drugs were dissolved in solvent system and PDA spectrum of drugs was taken. Drugs showed maximum absorbance at 245 nm.

**Optimization of HPLC method**

Different solvent systems were employed to inject and run pure drug substances. With acetonitrile and water at varying ratios, the peaks obtained were not sharp. As a result, methanol was used in place of acetonitrile, and various ratios were tested. Both drugs displayed development with this mobile phase. After experimentation with various methanol and phosphate buffer ratios, finally mobile phase consisting of Methanol: Phosphate Buffer (85:15) + GAA5% ($P^H$3.5) was selected. The flow rate was modified to 0.8 ml/min. The pH adjustment of mobile phase to 3.5 was done by glacial acetic acid. Tailing and capacity factors for both peaks were less than 2 and greater than 2 respectively with typical peak nature. Symmetry and the resolution of standards were found satisfactory. Ultimately, Methanol: Phosphate Buffer (85:15) + GAA5% was selected as a mobile phase for validation purpose.

| Chromatographic Parameters Selected | |
|---|---|
| **Parameter** | **Specification** |
| **Column** | **$C_{18}$ (250 mm × 4.6 mm, 5.0 µ).** |
| **Mobile phase** | **Methanol: Phosphate Buffer + GAA5%** |
| **Flow rate** | **0.8 ml/min** |
| **Wavelength** | **245 nm** |
| **Sample injector** | **Auto sampler** |
| **Temperature** | **40 °C** |

**Preparation of standard solutions**

Stock solutions were prepared using 25 mg of standard Montelukast and 25 mg of standard Acebrophylline in 25 ml mobile phase (1000 µg $ml^{-1}$). The stock solutions were used for preparation of standard solutions, which ranged in concentration from 4–20 µg /ml for Montelukast and 80–400 µg /ml for Acebrophylline, respectively. Under the aforementioned conditions, triplicate 10 µl volumes were injected twice for each concentration using an auto sampler. Calibration graphs were plotted for peak areas against drug concentrations.

**Formulation analysis**

To determine the quantity of Montelukast sodium and Acebrophylline in conventional tablets (TELEKAST-A: 10 mg of Montelukast + 200 mg of Acebrophylline), fine powder of tablets equivalent to 25 mg of Montelukast and 25 mg of Acebrophylline was weighed and dissolved in 50 ml mobile phase. Sample was sonicated for 40 min., centrifuged for 15 minutes at 8000 rpm and supernatant was

filtered using 0.45 µ nylon membrane filter and used as stock solution. Each sample solution (10 µl volume, six times) was injected into HPLC under the previously mentioned circumstances. The peak areas at 245 nm were measured and used for calculation of concentrations.

**Method Validation [19]**

The validation results (ICH guidelines) indicated that the developed HPLC method was reliable and suitable for the intended purpose. The method showed good linearity, precision, accuracy, and sensitivity with low LOD and LOQ values.

**Linearity**

Over the concentration range, eight calibration standard solutions were prepared. The above solutions were injected into the chromatograph. The peak responses for each level were recorded and slope, intercept, correlation coefficient, and regression coefficient ($R^2$) were calculated. For both drugs, calibration curves of area versus concentration were drawn.

**Precision**

The chromatographic system was injected with standard solution preparations six times, and the peak responses were noted. The measurement of the active compound's peak area and the repeatability of sample application were both expressed in terms of percent RSD.

**Accuracy**

For recovery studies, known amounts of mix standards of MNLT and ACB were added at 80%, 100 % and 120 % levels. The results obtained from the recovery studies showed that the developed method was found accurate for the analysis of MNLT and ACB in tablet dosage form.

**Limit of Detection (LOD) and Limit of Quantification (LOQ):**

LOD and LOQ are smallest concentrations of the analyte, which gives accurate detection and quantitation under given experimental conditions calculated using the standard formula.

**Robustness**

For robustness determination of the proposed method aliquots from homogenous lots were analyzed by changing physical parameters like composition and flow rate of mobile phase, pH of buffer, column temperature and wavelength, which may differ, but the responses were still within the specified limits of the assay. The standard and sample solutions were injected into the chromatograph at varied flow conditions ± 0.1 ml/min, mobile phase buffer pH ± 0.2 units, percentage of methanol ± 5 %, column temperature ± 2° C and wavelength by ± 1nm.

**RESULTS AND DISCUSSION**

**Method optimization**

The developed HPLC method for Montelukast and Acebrophylline was

optimized. For RP-HPLC method optimization, Kromasil $C_{18}$ (5.0 µ, 4.6×250 mm) and Symmetry $C_{18}$ (5.0 µ, 4.6×250 mm) columns were used. (**Table 1**: System suitability parameters).

**Method validation**

The developed RP-HPLC method was validated as per the ICH guidelines.

**Linearity and range**

The linearity range was found to be 4–20 µg/ml for MNLT and 80–400 µg/ml for ACB. The average peak area was plotted against the concentration in µg/ml, from which, correlation coefficient, slope of the regression line and the y-intercept were determined. **Table 2, Table 3,** and **Figure 2 and Figure 3** displays the calibration data and calibration curves.

**Precision**

Six replicate applications and six measurements of a sample solution at the analytical concentration were used to assess the precision of the method. Both the active compound's peak area measurement and the consistency of sample application were expressed in terms of percent RSD. **Table 4** presents the findings of the Precision studies.

**Accuracy**

A recovery study was performed on a drug sample by using the standard addition method in which MNLT and ACB corresponding to 80, 100, and 120% of label claim had been added. There were six determinations performed for each amount level. Results for the accuracy of Montelukast and Acebrophylline are shown in **Tables 5 and 6**, respectively.

**LOD and LOQ**

A known concentration of each drug was diluted to obtain approximate values 3 and 10 respectively in terms of signal to noise ratios. **Table** 7 displays the LOD and LOQ values. These results revealed that the method can detect and estimate low concentrations.

**Robustness**

The robustness of the RP-HPLC method was assessed using parameters such as mobile phase, flow rate, pH of buffer, column temperature, and wavelength. Results of the same are shown in **Table 8a,8b,8c,8d and 8e** respectively.

**Table 1: System Suitability Parameters**

| Parameter | Montelukast | Acebrophylline |
|---|---|---|
| Retention time | 3.5 | 4.3 |
| Tailing factor | 1.0 | 1.4 |
| Theoretical plates | 147340 | 552220 |
| Asymmetry | 1.1 | 1.4 |
| K prime | 2.4 | 3.2 |

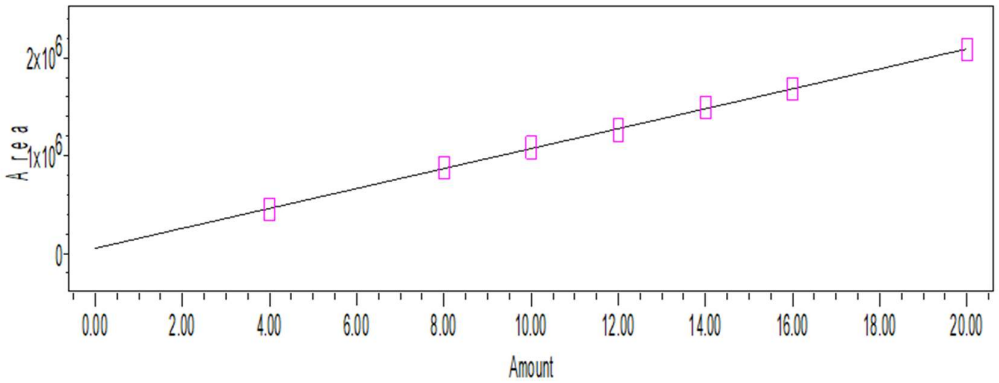



**Figure 2: Calibration curve for Montelukast**

**Table 2: Linearity data for Montelukast**

| Name | Time | $R^2$ | Equation | Fit Type | Curve Type | X-axis | Y-axis |
|---|---|---|---|---|---|---|---|
| MNLT | 3.525 | 0.999480 | Y = 1.02 X + 5.43 | Linear | LC | Amount | Area |

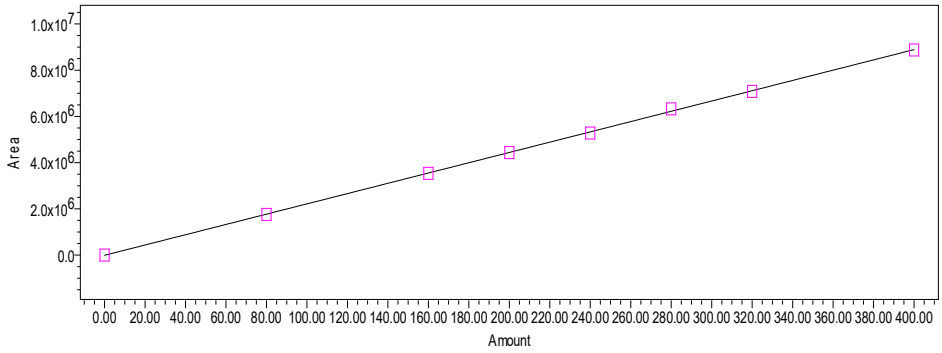



**Figure 3: Calibration curve for Acebrophylline**

**Table 3: Linearity data for Acebrophylline**

| Name | Time | $R^2$ | Equation | Fit Type | Curve Type | X-axis | Y-axis |
|---|---|---|---|---|---|---|---|
| ACB | 4.286 | 0.999751 | Y = 2.23 X - 9.05 | Linear | LC | Amount | Area |

**Table 4: Inter day precision**

| Conc. (µg/ml) | Montelukast | | | Conc. (µg/ml) | Acebrophylline | | |
|---|---|---|---|---|---|---|---|
| | Peak Area | Mean* ± S.D. | %RSD | | Peak Area | Mean* ± S.D. | %RSD |
| 12 | 1259947 | 1191558± 48358.33 | 4.058 | 240 | 5284493 | 5188040± 68202.33 | 1.314 |
| 12 | 1198254 | | | 240 | 5201348 | | |
| 12 | 1201457 | | | 240 | 5102497 | | |
| 12 | 1232540 | | | 240 | 5214781 | | |
| 12 | 1102478 | | | 240 | 5123658 | | |
| 12 | 1154672 | | | 240 | 5201465 | | |

*SD= Standard deviation, *RSD= Relative standard deviation

**Table 5: Accuracy data of Montelukast**

| Accuracy level | Amount of MNLT in Sample (µg/ml) | Amount of MNLT in Standard (µg/ml) | Total amount of MNLT (µg/ml) | Total amount of MNLT found (µg/ml) Mean (n=3) | % Mean Recovery (n=3) | %RSD |
|---|---|---|---|---|---|---|
| 80% | 10 | 8 | 18 | 18.26 | 98.13 | 1.98 |
| 100% | 10 | 10 | 20 | 20.36 | 99.5 | 1.26 |
| 120% | 10 | 12 | 22 | 22.36 | 99.83 | 0.89 |

*RSD= Relative standard deviation

Table 6: Accuracy data of Acebrophylline

| Accuracy level | Amount of ACB in Sample (µg/ml) | Amount of ACB in Standard (µg/ml) | Total amount of ACB (µg/ml) | Total amount of ACB found (µg/ml) Mean (n=3) | % Mean Recovery (n=3) | %RSD |
|---|---|---|---|---|---|---|
| 80% | 200 | 80 | 280 | 279.09 | 99.07 | 1.23 |
| 100% | 200 | 100 | 300 | 300.89 | 101.06 | 1.29 |
| 120% | 200 | 120 | 320 | 320.74 | 100.75 | 1.72 |

*RSD= Relative standard deviation

Table 7: LOD and LOQ

| Parameter | ACB (µg/ml) | MNLT ( µg /ml) |
|---|---|---|
| LOD (µg/ml) | 0.813ug/ml | 0.619ug/ml |
| LOQ (µg/ml) | 2.464ug/ml | 1.878ug/ml |

Table 8: Results of robustness study

a) Variable- Flow rate

| Flow Rate (ml/min) | Retention time | | Tailing factor | |
|---|---|---|---|---|
| | ACB | MNLT | ACB | MNLT |
| 0.6 | 4.6 | 3.7 | 1.2 | 1.0 |
| 0.8 | 4.3 | 3.5 | 1.4 | 1.0 |
| 1.0 | 3.9 | 3.0 | 1.3 | 1.1 |

b) Variable-pH of Buffer

| PH | Retention time | | Tailing factor | |
|---|---|---|---|---|
| | ACB | MNLT | ACB | MNLT |
| 3.2 | 4.2 | 3.4 | 1.4 | 1.0 |
| 3.5 | 4.3 | 3.5 | 1.4 | 1.0 |
| 3.7 | 4.3 | 3.4 | 1.4 | 1.2 |

c) Variable-Column Temperature

| Temp | Retention time | | Tailing factor | |
|---|---|---|---|---|
| | ACB | MNLT | ACB | MNLT |
| 38 | 4.1 | 3.2 | 1.5 | 1.2 |
| 40 | 4.3 | 3.5 | 1.4 | 1.0 |
| 42 | 4.3 | 3.2 | 1.3 | 1.0 |

d) Variable-Ratio of Mobile Phase

| Ratio | Retention time | | Tailing factor | |
|---|---|---|---|---|
| | ACB | MNLT | ACB | MNLT |
| 83:17 | 4.5 | 3.7 | 1.3 | 1.1 |
| 85:15 | 4.3 | 3.5 | 1.4 | 1.0 |
| 87:13 | 4.1 | 3.1 | 15 | 1.2 |

e) Variable-Wavelength

| nm | Retention time | | Tailing factor | |
|---|---|---|---|---|
| | ACB | MNLT | ACB | MNLT |
| 243 | 4.2 | 3.5 | 1.3 | 1.3 |
| 245 | 4.3 | 3.5 | 1.4 | 1.0 |
| 247 | 4.0 | 3.2 | 1.2 | 1.2 |

## CONCLUSION

For the determination of Montelukast and Acebrophylline, both in their pure form and in formulations, a simple, sensitive, accurate and specific RP-HPLC method was developed. The ICH guidelines were followed during method validation, and it was found to be suitable and can be applied for simultaneous determination of Montelukast sodium and Acebrophylline in bulk and in formulation.

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**REFERENCES**

**[1]** Nayak A. A review of Montelukast in the treatment of asthma and allergic rhinitis. Expert Opin Pharmacother. 2004;5(3):679.

**[2]** Nayak A, Langdon RB. Montelukast in the treatment of allergic rhinitis: an evidence-based review. Drugs. 2007;67(6):887.

**[3]** Giovanni A. Acebrophylline in the treatment of chronic obstructive pulmonary disease. Curr Ther Res. 1995; 56(2):169.

**[4]** Tapadar SR, Das M, Chaudhuri AD, Basak S, Mahapatra AB. The Effect of Acebrophylline vs Sustained Release Theophylline in Patients of COPD-A Comparative Study. J Clin Diagn Res. 2014;8(9):MC11.

**[5]** Pallavi K, Babu PS. Validated UV spectroscopic method for estimation of Montelukast sodium from bulk and tablet formulations. Int J Pharm Med Sci. 1(2), 2012:104.

**[6]** Patel PG, Vaghela VM, Rathi SG, Rajgor NB, Bhaskar VH. Derivative spectrophotometry method for simultaneous estimation of Rupatadine and Montelukast in their combined dosage form. Pharm Analysis. 2009;1(4):354.

**[7]** Pawar V, Pai S, Roa G. Development and validation of UV spectrophotometric method for simultaneous estimation of montelukast sodium and bambuterol hydrochloride in bulk and tablet dosage formulation. Jordan J Pharm Sci. 2008;1(2):152.

**[8]** Al-Rawithi S, Al-Gazlan S, Al-Ahmadi W, Alshowaier I, Yusuf A. Expedient liquid chromatographic method with fluorescence detection for Montelukast sodium in microsamples of plasma. J. Chromatogram B Biomed Sci Appl. 2001; 754:527.

**[9]** Radhakrishna T, Narasaraju A, Satyanarayana A. Simultaneous determination of Montelukast and Loratadine by HPLC and derivative Spectrophotometric methods. *J* Pharm Biomed Anal. 2003; 31:359.

**[10]** Kanakadurga DN, Rani AP, Madhavi BR, Mrudula. BS. New RP- HPLC method for the analysis of Montelukast sodium in pharmaceutical dosage forms. Int J Chemtech Res. 2010;2(1): 471.

**[11]** Patel SA, Patel SK, Patel DJ, Patel NJ. Analytical method

development and validationof Montelukast sodium and Bambuterol hydrochloride in combined dosage form by RP-HPLC. Int J Pharmtech Res. 2010;2(3):1767.

**[12]** Sane R, Menezes A, Mote M, Moghe A and Gundi G. HPTLC determination of Montelukast sodium in bulk drug and in pharmaceutical preparation. J. Planar Chromat. 2004; 17:75.

**[13]** Aligave AR, Dhamne HS, Gaikwad SS, Kondawar MS. Determination of Acebrophylline in bulk and pharmaceutical formulation by UV spectrophotometer. Curr Pharm Res. 2011; 3: 267.

**[14]** Saraswathi D, Priyadharisini J, Niraimathi V, Suresh AJ. Spectrophotometric Estimation of Acebrophylline in Bulk and Capsule Formulation. Int J Chem Sci. 2010;8(2):973.

**[15]** Dhaneshwar SR, Jagtap VN. Development and validation of stability indicating RP-HPLC-PDA method for determination of Acebrophylline and its application for formulation analysis and dissolution study. J Basic Appl Sci Res. 2011;1(11):1884.

**[16]** Saraswathi D, Gigi G, Niraimathi V, Jerad A. Estimation of Acebrophylline in pharmaceutical oral solid dosage form by RP-HPLC. J Pharm Res. 2010;9(3):1222.

**[17]** Solomon S, Manu S, Sivakumar R, Anand P, Venkatanarayanan R. Application of TLC-Densitometry method for estimation of Acebrophylline in pharmaceutical dosage forms. J Pharm Res. 2010; 3(11):2561.

**[18]** Beckett AH, Stenlake JB. Practical Pharmaceutical Chemistry, 4th ed. Part Two, CBS Publishers and Distributors, New Delhi; 2007.

**[19]** Validation of Analytical Procedures: Text and Methodology, Proceedings of International Conference on Harmonization (ICH). Geneva; 2005.