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Method Development and Validation for the Simultaneous Estimation of Montelukast Sodium and Rupatadine Fumerate in Tablet Dosage form By RP-HPLC Method

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Abstract: *A simple, precise, cost effective stability indicating RP-HPLC method has been developed and validated for the determination of Rupatadine fumerate and Montelukast sodium in pharmaceutical compositions. Montelukast sodium was highly susceptible to acidic condition and photo degradation; while Rupatadine fumerate was moderately degrade under alkaline condition. Methods: The chromatographic separation was achieved on hibar R 250-4, C-18 columns (250mm ×4.6mm,5um) using a mobile phase consisting of Methanol:Water (90:10v/v) with ortho phosphoric acid at a flow rate of 1ml/min. Detection wavelength was found 252 nm. Results: The Retention times of Rupatadine and Montelukast were found 4.31 and 11.59 minute respectively. The method was found to be linear over the range of 15-40 µg/ml for both the drugs with correlation co-efficient (r2) 0.996 & 0.999 for Rupatadine and Montelukast respectively. Percentage recoveries obtained for both the drugs were 99.49-100.25% and 99.52-100.53% for Rupatadine and Montelukast respectively. The %RSD for precision and accuracy of the method was found to be less than 2%. Conclusion: The method was validated according to the ICH guidelines with respect to specificity, linearity, accuracy, precision and robustness. Developed HPLC method can resolve all decrement peak of both drug. So this method is stability indicating in nature. The method developed can be used for the routine analysis of Rupatadine and Montelukast from dosage form.*

Keywords: Montelukast Sodium, Rupatadinefumerate HPLC Analytical Method Develop Mentation etc.

I. INTRODUCTION

Analytical chemistry is a branch of chemistry that deals with the separation, identification and determination of components in a sample. It is the science of making quantitative measurements, which requires background knowledge of chemical and physical concepts.

Pharmaceutical analysis plays a major role today, and it can be considered as an interdisciplinary subject. Pharmaceutical analysis principles from various branches of science like physics, chemistry, microbiology, nuclear science, electronics etc. Analytical instrumentation plays an important role in the production and evaluation of new products and in the protection of consumers and the environment. This instrumentation provides the lower detection limits required to assure safe foods, drugs, water, and air.

Specific technologies and instrumentation:

1. Spectrometric techniques
2. Electrochemical techniques
3. Chromatographic techniques
4. Miscellaneous techniques

High Performance Liquid Chromatography

HPLC are the most widely used of all of the analytical separation techniques, The HPLC equipment approaching the quantitative determinations and above all its wide spread applications and to the public.

Examples of such materials include amino acids, nucleic acids, hydrocarbons, carbohydrates, drugs, terpenoids,

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1.1 Objectives of the Work

1. To develop method for Montelukast sodium and Rupatadine fumerate.
2. To validate the developed method of Montelukast sodium and Rupatadinefumerate.

II. Instruments and Chemicals

The instruments and chemicals which were required to perform this study are as shown in table:

2.1 List of Instrument

SR. NO.	TOOL	MANUFACTURER
1.	HPLC	Shimadzu (Prominence-I LC-2030C 3D)
2.	UV	Shimadzu (UV- Pharmaspec 1700)
3.	pH Meter	Lab india
4.	Analytical Balance	Sartoriuscubis
5.	Milli-Q Water	Milli-Q (Millipore)

2.2 List of Device

SR. NO.	APPARATUS	MANUFACTURER
1.	Volumetric flask	Rankem
2.	Beaker	Borosilicate Glass
3.	Pippete	Borosilicate Glass
4.	Measuring cylinder	Tarson

List of Reagents

SR.NO	REAGENT	MANUFACTURER
1	Acetonitrile	Merck life science, Mumbai

III. Research Methodology Results and Discussion

3.1 Materials and Methods

- **Materials:** MTKT, ABP, and LTZ were obtained from Morepen, Ami Lifesciences Pvt. Ltd and Chandra life sciences respectively. Lukotas tablets containing 5 mg of LTZ, 10 mg MTKT, and 200 mg ABP were taken for this study. The reagents used were of analytical grade. Distilled and de-ionized HPLC-grade water, HPLC grade methanol, HPLC grade acetonitrile, ammonium acetate and orthophosphoricacid were purchased from S.D. Fine Chem Ltd.
- **Preparation of Diluent:** A mixture of methanol: 20 mM ammonium acetate buffer pH 5.5: acetone trile was prepared in the ratio of 1:1:1 to be used as a diluent.
- **Preparation of Standard Stock Solution:** 10 mg each of MTKT, ABP, and LTZ standards were accurately weighed and transferred to respective 100 ml clean, dry volumetric flask to which 80 ml of methanol was added and sonicated for 5 min and made up to the volume with methanol to obtain a concentration of 100 µg/ml of each drug.
- **Preparation of Working Standard Solution of MTKT, LTZ and ABP:** A working standard solution was prepared by pipetting 1 ml each from a stock solution of 100 µg/ml of MTKT and LTZ and transferred to 10 ml clean, dry volumetric flask to which 2 ml aliquot from a stock solution of 100 µg/ml of ABP was added and volume was made up to the mark with the diluent to produce 10 µg/ml of MTKT and LTZ each and 20 µg/ml of ABP.
- **Preparation of Sample Solution:** 20 tablets were weighed and crushed. A powder equivalent to 2 mg of LTZ, 80 mg ABP, and 4 mg MTKT was weighed and transferred to 100 ml volumetric flask to which an additional 4 mg standard of LTZ and MTKT was added. 75 ml of methanol was added to the flask and was sonicated for 15 min. Later volume was made up to the mark using methanol. The sample solution was then filtered through 0.45 µm Whatman filter paper. 1 ml of this filtrate was then transferred to a clean dry 10 ml volumetric flask and made up to the mark using a diluent to produce 6 µg/ml of LTZ, 8 µg/ml of MTKT, and 80 µg/ml of ABP.
- **Chromatographic Conditions:** Chromatographic conditions were selected to better separate drugs with the minimum time required for analysis. Chromatographic separation was achieved on the Hypersil ODS C18 column

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(250 × 4.6 mm, 5 µm) as stationary phase. The mixture of methanol, acetonitrile, and 20 mM ammonium acetate buffer in the ratio of 60:30: 10v/v was used as a mobile phase at a flow rate of 0.8 ml/min. The UV detection was carried out at 232 nm with column temperature set at 35 ℃.

- **RP-HPLC Method Development and Optimization Studies:** Preliminary trials using different columns, varying mobile phase compositions, varying flow rates at several wavelengths were employed for simultaneous estimation of MTKT, LTZ, and ABP in a fixed dose combination product. The wavelength of 232 nm was selected as it was found to produce less noise, gave good resolution, peak purity, peak symmetry. Out of the several methods tried a method giving better separation of drugs was finally obtained. This method was further optimized to curtail the tailing effect to obtain better peak shape with a good number of theoretical plates. System suitability study was then conducted using standard preparation and evaluated after every 6 injections. Several validation parameters such as specificity, linearity, LOD and LOQ determination, precision, accuracy, and robustness were then performed to validate the developed method for its intended use A typical RP-HPLC chromatogram for simultaneous determination of ABP, LTZ, and MTKT from standard preparation.

As part of the presence, a high performance liquid chromatography analytical method using a photodiode detection system was used to determine rosuvastatin as a percentage by weight of calcium and amlodipine benzylate, for validation and validation. The experimental conditions were chosen taking into account the chemical nature, molecular weight and solubility of amlodipine besylate. Amlodipine besylate were dissolved in a polar solvent and RP-HPLC was selected for evaluation. The column was chosen between back pressure, solution, peak shape, theoretical plates, and daily repeatability and dissolution time between the amlodipine besylate peaks. Taking all these factors into account, the YMC Pro C18 column (ID 150 mm x 4.6 mm, particle size 5 µm) was found to give satisfactory results. The choice of buffer is based on the chemical structure of both drugs. To select the mobile phase, preliminary experiments with the mobile phases of the various compositions showed a poor water form adjusted to acidic pH by the addition of orthophosphoric acid and methanol. When methanol and water were replaced with acetitrol and phosphate buffer (adjusted to pH 3.5 with dilute orthophosphoric acid) (45:55, v / v), a better peak shape was obtained. The proportion of components in the mobile phase is optimized to shorten the retention time and allow the two molecules to separate well. After scanning the standard resolution with a PDA detector between 190 and 370 nm, the detection wavelength was selected to be 242 nm. Detection at 242 nm gave good response and good linearity.

3.2 Linearity

For linearity, seven points calibration curve were obtained in a concentration range from 20-80 µg/ml for Amlodipine besylate. The response of the drug was found to be linear in the investigation concentration range and the linear regression equation for Amlodipine besylate was y = 22772x + 38920 with correlation coefficient 0.9987. where x is the concentration in µg/ml and y is the peak area in absorbance unit.

3.2 LOD and LOQ

The limit of detection and limit of quantification were estimated by sequential dilution of amlodipine besylate solution to obtain a signal-to-noise ratio of 3:1 LOD and 10:1 LOQ. The LOD values for amlodipine besylate were 0.1 ppm and 0.1 ppm, respectively, and the LOQ values were 0.5 ppm and 0.4 ppm, respectively.

3.3 Precision

These studies are determined by assessing method accuracy and intermediate accuracy. The accuracy of the system is assessed by an analysis five times that of the standard solution. The accuracy of the analytical method is determined by analysis of six sample groups.

All six sample replicates were examined and the mean% analysis, standard deviation % relative standard deviation were calculated. The reciprocal accuracy of the diagnostic method was determined by performing the accuracy of the procedure on the second day under the same experimental conditions. All six sample replicates were tested and mean test scores, standard deviation % relative standard deviation were calculated. The accuracy test data are shown in Table 6.1 for the daily and daily accuracy of amlodipine besylate. The RSD values for daily accuracy tests and daily accuracy were <2.0% for amlodipine besylate, which confirms the accuracy of the procedure.

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3.4 Accuracy

Accuracy was assessed by determining the recovery procedure at three different concentrations (corresponding to the concentrations of test solution 50, 100 and 150%) with known amounts of amlodipine besylate (25, 50 and 75 µg / ml) solvent and the amount of amlodipine besylate. Three layers were made for each concentration and injected in duplicate. The% yield was calculated and recorded for each phase according to Tables 6.2 and 6.3. The mean recovery of amlodipine besylate ranged from 98.37% to 101.79%, which is satisfactory.

Accuracy Level	Amount Added	Amount Found	% Recovery	Mean	Std dev.	% RSD
50%	24.92	24.9377	100.07	101.17%	0.95	0.94
	25.04	25.4542	101.65			
	25.08	25.5287	101.79			
100%	49.84	49.0252	98.37	98.5%	0.11	0.11
	49.92	49.1868	98.53			
	49.96	49.2613	98.60			
150%	74.8	73.6240	98.43	98.77%	0.3	0.3
	74.88	74.1461	99.02			
	74.84	73.9902	98.86			

Table: For Amlodipine Besylate

3.5 Robustness

The strength of the method is assessed by testing the test solution after less conscious changes in diagnostic conditions. Factors selected in this study were flow rate (± 0.1 ml / min), pH of the pulse buffer solution (± 0.2), and cell phone composition (acetonitrile water, 50:50 and 40:60, volume ratio). The strength of developmental test methods is shown in Table 6.4 and Table 6.5. The results showed that the analytical value of the test preparation did not change in all conditions of variability and was consistent with the true value. The system benefit variables were found to be appropriate. Therefore, the method of analysis would be considered reliable.

Robust Conditions	% Assay	System Suitability Parameters	
		Asymmetry	% RSD
Flow 0.9 ml/min	99.82	1.14	0.27
Flow 1.1 ml/min	100.09	1.12	0.28
Buffer-CAN (50:50,v/v)	100.04	1.09	0.11
Buffer-CAN (60:40,v/v)	99.93	1.11	0.26
Buffer pH 3.3	99.94	1.06	0.21
Buffer pH 3.7	100.01	1.08	0.15

Table: Evaluation data of robustness study of Amlodipine Besylate

IV. SOLUTION STABILITY STUDY

The stability of the test solution is evaluated in two ways: the short-term stability of the stock solution (STSSS) for 12 hours and the long-term stability of the stock solution (LTSSS) for 12 days and the solutions are stored at room temperature at 2-5 ° C and tested for 12 hours And 12 days. The aging reaction is evaluated with a freshly prepared standard solution.
Table: Stability data of Amlodipine Besylate

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Replicates	STSSS(At RT)		LTSSS(At 3-5ºC)	
	0.0 hr(area)	12.0 hr(area)	0 Day(area)	12 Day(area)
2	2252800	2251870	2260560	2261981
3	2252671	2253436	2268351	2264002
4	2256089	2252256	2273154	2267412
5	2258158	2252998	2267149	2257010
Mean	2255099	2252120	2267191.8	2262545
Mean Area Ratio	1.0013		1.0020	

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