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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF MONTELUKAST SODIUM, LEVOCETIRIZINE DIHYDROCHLORIDE AND ACEBROPHYLLINE IN FIXED-DOSE COMBINATION TABLETS

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Keywords:

Montelukast sodium, Levocetirizine dihydrochloride, Acebrophylline, RP-HPLC, Analytical method development, Validation

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ABSTRACT: A fixed-dose combination of montelukast sodium, levocetirizine dihydrochloride, and acebrophyl line is used in the treatment of asthma, COPD, allergic rhinitis and other conditions where patients feel difficulty in breathing. The present study was designed to develop and validate a simple, sensitive, precise and accurate isocratic reverse-phase high-performance liquid chromatography (HPLC) method for simultaneous estimation of montelukast sodium (MTKT), levocetrizine dihydrochloride (LTZ) and acebrophyl line (ABP) in fixed-dose combination tablets. Chromatographic separation of these drugs was achieved on Hypersil ODS C18 column (250 × 4.6 mm, 5µm) as stationary phase. A mixture of methanol, acetonitrile and 20 mM ammonium acetate buffer in the ratio of 60:30: 10v/v was used as the mobile phase at a flow rate of 0.8 ml/min with detection at 232 nm at a column temperature set at 35 ℃. The total elution time for the three drugs was about 10 min. The developed method was validated by conducting system suitability, selectivity, linearity, precision, accuracy, limits of detection, and limits of quantification as per ICH guidelines. The method was accurate with assay values of 99.89% w/w for LTZ, 100.30% w/w for ABP and 100.59%w/w for MTKT, precise with intra-day and inter-day % RSD less than 2% and robust with % RSD less than 2% for the three drugs. The developed method can be applied to simultaneously quantify fixed-dose combination products containing LTZ, ABP and MTKT.

INTRODUCTION: Montelukast (MTKT) is a leukotriene receptor antagonist (LTRA). It works by blocking the action of leukotriene D4 on the cysteinyl leukotriene the cysteinyl leukotriene receptor CysLT1 in the lungs and bronchial tubes by binding to it.

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This reduces bronchoconstriction and results in less inflammation. Because of this mechanism of action, it is used in the treatment of acute asthma attack. Levocetirizine (LTZ) is a third-generation non-sedative antihistamine indicated to relieve symptoms associated with seasonal and perennial allergic rhinitis [2].

Acebrophyl line (ABP), a mixture of theophylline-7-acetate and ambroxol is a mucolytic and bronchodilator used in the treatment of asthma and chronic obstructive pulmonary disease. It works by relaxing the airway muscles and also loosens mucus, making it easier to breathe [3, 4].

A fixed-dose combination of MTKT, ABP and LTZ, is available in the market by INTAS (LUKOTAS-tablet) to treat allergic asthma. An exhaustive literature survey indicated that many analytical methods were reported to estimate MTKT, ABP and LTZ individually and in combination in fixed-dose combination products [5, 31].

However, no HPLC method for the simultaneous estimation of the three drugs *i.e.*, MTKT, ABP and LTZ) has been reported till date. The present study aims to develop a quick, sensitive, accurate and reproducible HPLC method for the estimation of MTKT, ABP and LTZ in a fixed dose combination product that can be used in routine analysis in the pharmaceutical industry.

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 orthophosphoric acid 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 (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 is shown in **Fig. 1.**

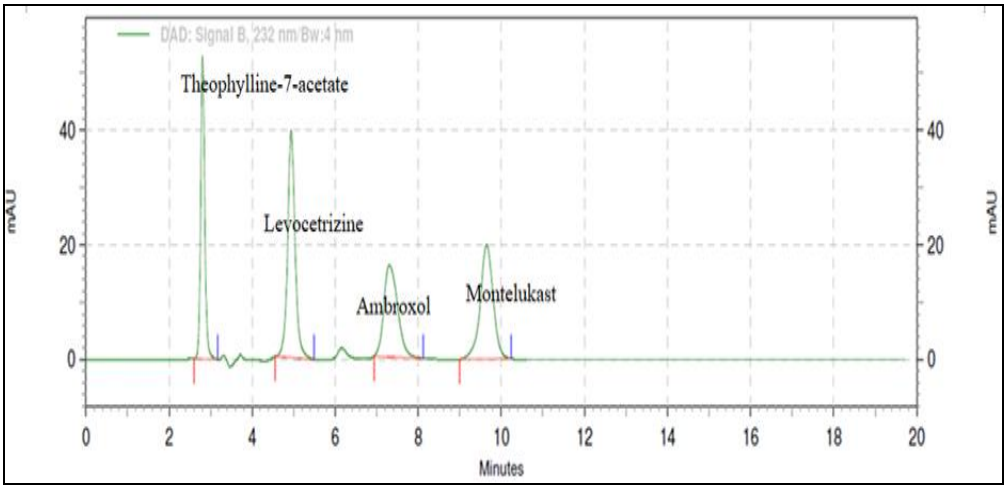

FIG. 1: CHROMATOGRAM OF ABP, LTZ AND MTKT IN STANDARD SOLUTION

RESULTS AND DISCUSSION:

Method Validation Studies of Optimized Analytical Method: The developed RP-HPLC method was validated for parameters like

System suitability, linearity, accuracy, precision, LOD, LOQ and robustness as per ICH guide [32].

System Suitability Studies: A standard solution was prepared as per the test method and injected into the chromatographic system. The system suitability parameters theoretical plates, resolution, and asymmetry factor were evaluated. The system suitability parameters are tabulated in **Table 1.** All parameters were found to be within limits.

TABLE 1: SYSTEM SUITABILITY STUDIES

Analytes	Mean retention time (min)	Mean tailing factor (T)	Mean theoretical plates (N)	Mean resolution (R)
Theophylline-7acetate	2.793	1.18105	4029	-
LTZ	4.913	1.08929	2830	8.58967
Ambroxol	7.267	1.26711	2336	5.12152
MTKT	9.547	0.97277	4654	3.92715

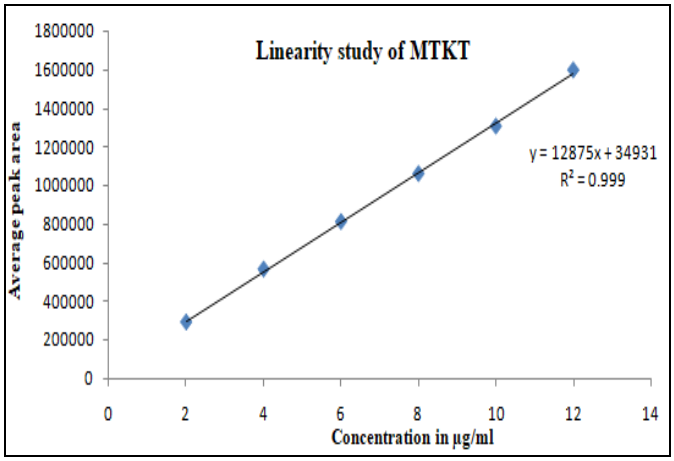

FIG. 2: LINEARITY STUDY OF MTKT

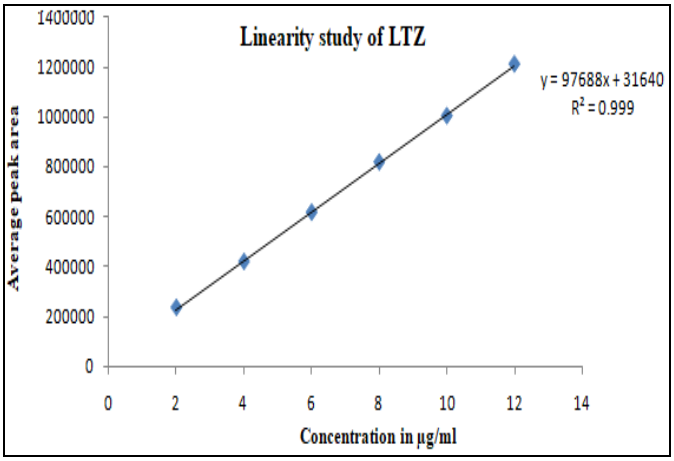

FIG. 3: LINEARITY STUDY OF LTZ

Specificity Studies: Specificity is the ability to assess the analyte unequivocally in the presence of components that may be expected to be present such as impurities, degradation product and excipient.

Specificity was determined by injecting blank and placebo samples and was analyzed for any interference. No peaks/interferences were observed at the retention time of the three drugs. The analytical method was found to comply with the acceptance criteria indicating that the developed method was specific.

Linearity Studies: A concentration range of 2-12 µg/ml for MTKT and LTZ and 20-120µg/ml for

ABP was selected for linearity studies. The linearity studies of MTKT, LTZ, ABP were performed in triplicate. A calibration curve was obtained by plotting the average peak area of each drug against each concentration level is shown in **Fig. 2**, **Fig. 3**, **Fig. 4** and **Fig. 5.**

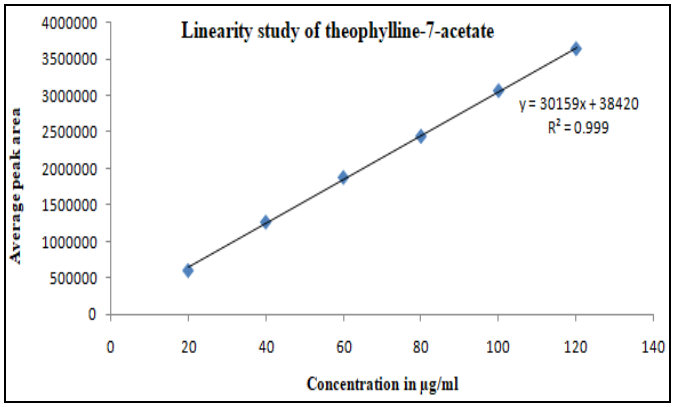

FIG 4: LINEARITY STUDY OF THEOPHYLLINE -7-ACETATE

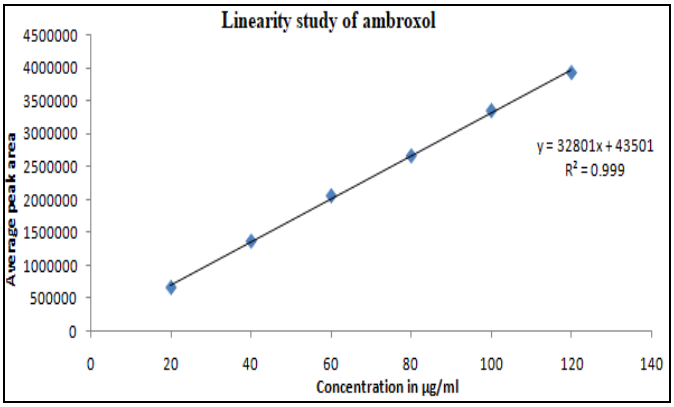

FIG 5: LINEARITY STUDY OF AMBROXOL

The linearity studies indicated in **Table 2.** exhibited an excellent correlation between average peak area and concentration for all the drugs.

The correlation coefficient for the three drugs was found to be greater than 0.999, which meets method validation acceptance criteria, and thus, the method was said to be linear for all three drugs.

TABLE 2: LINEARITY STUDIES

Analyte	Correlation coefficient
MTKT	0.9993
LTZ	0.9998
Theophylline-7-acetate	0.9995
Ambroxol	0.9991

Limit of Detection (LOD) and Limit of Quantitation (LOQ) Study: The LOD and LOQ were determined based on the standard deviation of y-intercept and slope of a regression line. LOD and LOQ were calculated by using the formulae given below

$$LOD = 3.3\ \sigma / S,\ LOQ = 10\ \sigma / S$$

Where, σ is the standard deviation of the response and S is the slope of the calibration curve.

Limit of Detection: The limit of detection of MTKT, LTZ, theophylline-7-acetate, ambroxol was found to be 0.35 µg/ml, 0.21 µg/ml, 3.24 µg/ml, 4.11 µg/ml respectively.

Limit of Quantification: The limit of quantification of MTKT, LTZ, theophylline-7-acetate, ambroxol was found to be 1.07 µg/ml, 0.63 µg/ml, 9.80 µg/ml, 12.45 µg/ml, respectively.

Precision Studies: Repeatability study, intraday precision study at different time points and interday precision study on three consecutive days were carried out for MTKT, LTZ and ABP at concentration of 10µg/ml, 10µg/ml and 20µg/ml respectively. % RSD was calculated from the average peak area obtained. The results obtained are presented in **Table 3.**

Accuracy Studies: To ensure the reliability and accuracy of the method, recovery studies were carried out for all the drugs at three different levels by using the standard addition method.

Standard known amount of LTZ (1.6 µg/ml for 80%, 2 µg/ml for 100% and 2.4 µg/ml for 120%), MTKT (3.2 µg/ml for 80%, 4 µg/ml for 100%, 4.8 µg/ml for 120 µg/ml), ABP (64 µg/ml for 80%, 80 µg/ml for 100% and 96 µg/ml for 120%) was added to sample at three different concentrations *i.e.* 80%, 100%, 120% of test concentration (n=3) and percent recovery were calculated. The result of percent recovery of MTKT, LTZ and ABP is represented in **Table 4-7.**

TABLE 3: PRECISION STUDIES OF LTZ, MTZ AND ABP

Studies conducted	% RSD of LTZ (10 µg/ml)	% RSD of MTZ (10 µg/ml)	% RSD of ABP (20 µg/ml)	
			% RSD of Theophylline-7-acetate (20 µg/ml)	% RSD of Ambroxol (20µg/ml)
Repeatability	0.18	0.06	0.55	0.57

Interday precision study carried out at 0, 4, 8 h	0.26	0.77	0.28	0.36
Intraday precision studies carried out on three consecutive days	0.18	0.45	0.28	0.36

The % RSD of LTZ, MTKT and ABP was found to be not more than 2% for all three studies conducted. Thus the method was found to comply with the acceptance criteria, indicating that the method was precise.

TABLE 4: ACCURACY STUDY OF MTKT

Level	Amount present (µg/ml)	Amount spiked (µg/ml)	Amount found (µg/ml)	% Recovery	Mean % recovery	SD	%RSD
80%	2	1.6	3.62	100.55	99.9	0.70	0.7
	2	1.6	3.57	99.16			
	2	1.6	3.6	100			
100%	2	2	4.04	101	100.25	0.75	0.75
	2	2	4.01	100.25			
	2	2	3.98	99.5			
120%	2	2.4	4.38	99.54	99.92	0.47	0.47
	2	2.4	4.42	100.45			
	2	2.4	4.39	99.77			

TABLE 5: ACCURACY STUDY OF LTZ

Level	Amount present (µg/ml)	Amount spiked (µg/ml)	Amount found (µg/ml)	%Recovery	Mean % recovery	SD	%RSD
80%	2	1.6	3.58	99.44	99.90	0.58	0.57
	2	1.6	3.62	100.55			
	2	1.6	3.59	99.72			
100%	2	2	3.96	99	99.66	0.76	0.76
	2	2	3.98	99.5			
	2	2	4.02	100.5			
120%	2	2.4	4.36	99.09	99.76	0.99	0.99
	2	2.4	4.44	100.9			
	2	2.4	4.37	99.31			

TABLE 6: ACCURACY STUDY OF THEOPHYLLINE-7-ACETATE

Level	Amount present (µg/ml)	Amount spiked (µg/ml)	Amount found (µg/ml)	% Recovery	Mean % recovery	SD	%RSD
80%	80	64	143.96	99.97	100.01	0.045	0.044
	80	64	144.1	100.06			
	80	64	144.04	100.02			
100%	80	80	160.08	100.05	100.01	0.035	0.034
	80	80	159.98	99.98			
	80	80	160.02	100.01			
120%	80	96	175.98	99.98	100	0.02	0.02
	80	96	176.05	100.02			
	80	96	176.02	100.01			

Robustness Study: The robustness of an analytical procedure measures its capacity to remain unaffected by small but deliberate variations in method parameters and indicates its reliability during normal usage. A deviation of ± 2 ℃ in column temperature, ± 0.2 ml/min in flow rate and ± 0.1 pH of the buffer, were tried individually. A standard solution mixture of MTKT, ABP and LTZ (10 µg/ml, 20 µg/ml, 10 µg/ml, respectively) was injected into the specified changes in operational conditions instrument in triplicate. % RSD was reported in **Table 8.**

TABLE 7: ACCURACY STUDIES OF AMBROXOL

Level	Amount present (µg/ml)	Amount spiked (µg/ml)	Amount found (µg/ml)	% Recovery	Mean % recovery	SD	%RSD
80%	80	64	143.95	99.96	100.01	0.05	0.05
	80	64	144.11	100.07			

	80	64	144.04	100.02			
100%	80	80	160.06	100.03	100.01	0.02	0.02
	80	80	159.99	99.99			
	80	80	160.04	100.02			
120%	80	96	175.96	99.97	100	0.03	0.03
	80	96	176.04	100.02			
	80	96	176.03	100.01			

TABLE 8: ROBUSTNESS STUDY

Analyte	Flow rate (ml/min)	% RSD	Column temperature (°C)	% RSD	pH of buffer	% RSD
Theophylline-7-acetate	0.6	0.0153	33	1.02187	5.4	0.2233
Levocetirizine		0.0128		1.03245		0.3258
Ambroxol		0.0143		1.03657		0.5773
Montelukast		0.1987		0.45342		0.5537
Theophylline-7-acetate	0.8	0.0578	35	1.20875	5.5	0.4333
Levocetirizine		0.1587		1.02245		0.5258
Ambroxol		0.1983		1.04657		0.5873
Montelukast		0.04873		0.85342		0.1537
Theophylline-7-acetate	1.0	0.0133	37	1.02875	5.6	0.0153
Levocetirizine		0.0158		1.04245		0.0128
Ambroxol		0.0173		1.05657		0.0143
Montelukast		0.1437		0.98342		0.1987

CONCLUSION: The proposed RP-HPLC method was found to be simple, accurate, precise, robust, and rapid the method was found to give good separation between the three compounds with a short analysis time. Hence the method can be used in quality control laboratories with respect to routine analysis for the assay of tablets containing MTKT, LTZ, and ABP.

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CONFLICTS OF INTEREST: Nil

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