Original Article

Simultaneous estimation of acebrophylline, montelukast, and levocetirizine dihydrochloride in marketed formulation by high-performance liquid chromatography method

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ABSTRACT

We have developed methods for simultaneous estimation of acebrophylline (ABP), montelukast (MLK), and levocetirizine dihydrochloride (levocetirizine [LCZ]) in pure and marketed formulation by reversed-phase high-performance liquid chromatography (HPLC). Method is simple, accurate, rapid, precise, economical, and reliable. The method was developed using waters HPLC an autosampler on column Macherey-Nagel C18 4.6 mm * 250 mm (5 µm) using a mixture of ammonium acetate buffer of pH 3.5 (pH adjusted with glacial acetic acid) and methanol in the ratio 15:85 v/v as mobile phase in an isocratic elution mode at a flow rate 0.6 ml/min, at 30°C with a load of 20 µl. Diluent used was water:methanol (50:50 v/v). The detection was carried out at 230 nm. Typical chromatogram with optimized condition gives sharp and symmetric peak with retention time of 5.287 min, 26.856 min, and 6.440 min for ABP, MLK, and LCZ, respectively. The method was validated with respect to linearity, robustness, precision, and accuracy and was successfully applied for the simultaneous quantitative determination of ABP, MLK, and LCZ in the solid dosage form and percentage purity were found to be 105%, 100%, and 96%, respectively.

Keywords: Acebrophylline, HPLC, levocetirizine HCl, montelukast

INTRODUCTION

For the discovery, development and manufacturing of pharmaceuticals, analytical method development plays an important role. Thus, to determine the amount or concentration of active pharmaceutical ingredient(s) in pure or pharmaceutical dosage form, analytical method development is a standardized laboratory procedure. Various analytical methods that are used by quality control laboratories to ensure the identity, purity, potency, and performance of drug products are high-performance liquid chromatography (HPLC), ultraviolet-spectrophotometry, high-performance thin-layer chromatography, titration, and fluorescence spectroscopy.[1]

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Nowadays, reversed-phase HPLC (RP-HPLC) chromatographic technique is the most commonly used separation technique due to its simplicity and versatility. It can handle compounds of a diverse polarity and molecular mass. Broad application range, reversed-phase chromatography is the most commonly used separation technique.[2]

Acebrophylline (ABP), 1,2,3,6-tetrahydro-1,3-dimethyl-2,6-dioxo-7H-Purine-7-acetic acid compound with trans-4-[[(2-amino-3,5-dibromophenyl)methyl]amino]cyclohexanol is the salt that is obtained when equimolar amounts of theophylline-7-acetic acid, a xanthine derivative reacts with specific bronchodilator activity and ambroxol, a mucolytic and expectorant. Due to inhibition of phospholipase A, and phosphatidylcholine, this novel drug has bronchodilating, anti-inflammatory, and mucoregulating effect.[3]

Montelukast sodium (MLK), [R-(E)]-1-[[[1-[3-[2-(7-chloro 2quinolinyl) ethenyl] phenyl]-3-[2-(1-hydroxy-1methylethyl) phenyl]

propyl] thio] methyl] cyclopropane acetic acid, monosodium salt is a selective. It is an orally active leukotriene receptor antagonist that inhibits the cysteinyl leukotriene CysLT1 receptor. Leukotrienes lead to narrowing and swelling of airways in lungs. It also causes allergy symptoms. By blocking leukotrienes, improves asthma symptoms, thus, control asthma and improves seasonal allergy symptoms.[4]

Levocetirizine (LCZ), chemically is [2-[4-[(r)-(4-chlorophenyl) phenylmethyl]-1-piperazinyl] ethoxy] acetic acid. It is a third-generation non-sedative antihistamine that is developed from the second generation antihistamine cetirizine.[5] It is the L-enantiomer of the cetirizine racemate. LCZ cause blockage of histamine receptors. It does not inhibit the actual release of histamine from mast cells but prevents it binding to its receptors. Thus, provides relief from the typical symptoms of hay fever by inhibiting the release of other allergy chemicals and increased blood supply to the area [Figure 1].[6]

As per literature survey, no analytical method has been developed for this particular combination (ABP, MLK, and LCZ) of drugs using RP-HPLC method. The aim of the present study is to develop sensitive, simple, rapid, economical, precise, and accurate RP-HPLC method to determine ABP, MLK, and LCZ in marketed tablet formulation [Table 1].

EXPERIMENTAL

Chemicals and reagents

Marketed formulation of Coekastle-A Manufactured Date: April 2015, Expiry Date: December 2016, Koye Pharmaceuticals Ltd. was procured from the local drug store of Faridkot, Punjab. Acetonitrile, methanol, and water, were purchased from Rankem (New Delhi, India). All these chemicals and solvents were of HPLC grade and were used without any further purification. All the required solutions were prepared in HPLC grade water. HPLC grade water was obtained from water purification systems ELIX 03 (MILLIPORE, USA). Unless otherwise specified, all solutions were filtered through a 0.2 µm Ultipor® N66® Nylon 6, 6 membrane filter (Pall Life Sciences, USA) before use.

Table 1: Physical properties of the standard drugs

Description	ABP	MLK	LCZ
Appearance	White to off-white crystal powder	White to pale yellow powder	White powder
Solubility	Methanol	Freely soluble in ethanol (95%), methanol and water	Freely soluble in ethanol (95%), methanol
Melting point	210–212°C	135.5°C	208–220°C

ABP: Acebrophylline, MLK: Montelukast, LCZ: Levocetirizine

Instrumentation

The analysis was performed on HPLC system of WATERS (Milford, USA) composed of 515 HPLC pump as a solvent delivery system equipped with Rheodyne injection valve with a 20 µl loop, WATERS 2998 photodiode array detector (PDA) detector set at wavelength range 190–400 nm. Separation was performed on Macherey-Nagel column 4.6 mm × 250 mm (5 µm). Chromatographic data were recorded and processed using EMPOWER-2 software.

Sample preparation

Stock solutions of ABP, MLK, and LCZ (1 mg/ml) were prepared in water:methanol (50:50) and stored at 2–8°C until used. Aliquots from each stock solution were diluted stepwise with mobile phase to obtain concentration 6–18 µg/ml for ABP and MLK and 3–12 µg/ml for LCZ. These concentration range was used for optimization of simultaneous estimation of drugs in the proposed method. Stock solution of marketed formulation was prepared in water:methanol (50:50) and diluted accordingly after proper separation of excipient.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

Chromatographic conditions were optimized for the proposed method. The optimized chromatographic conditions are depicted in Table 2.

Figure 2 shows graphs obtained from the optimization using following method: Mobile phase: 0.05 M potassium dihydrogen phosphate adjusted to pH: 6.0: Acetonitrile (60:40), diluent: Mobile phase;

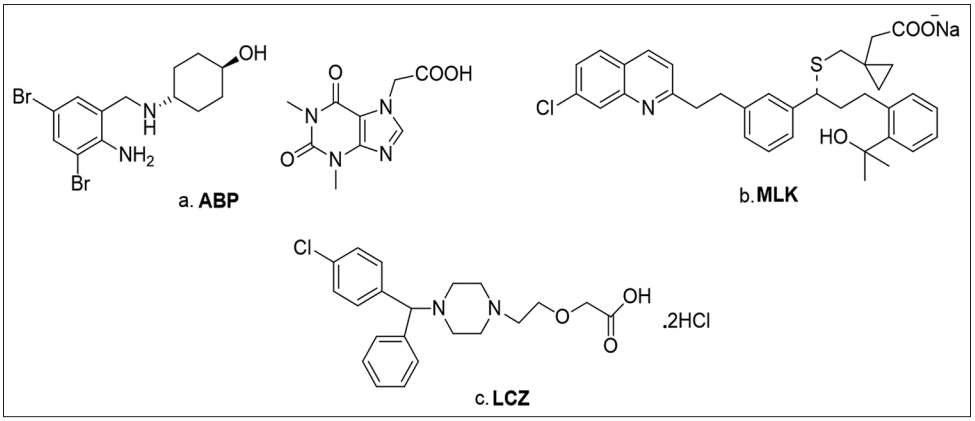

Figure 1: Chemical structure of acebrophylline, montelukast sodium, and levocetirizine dihydrochloride

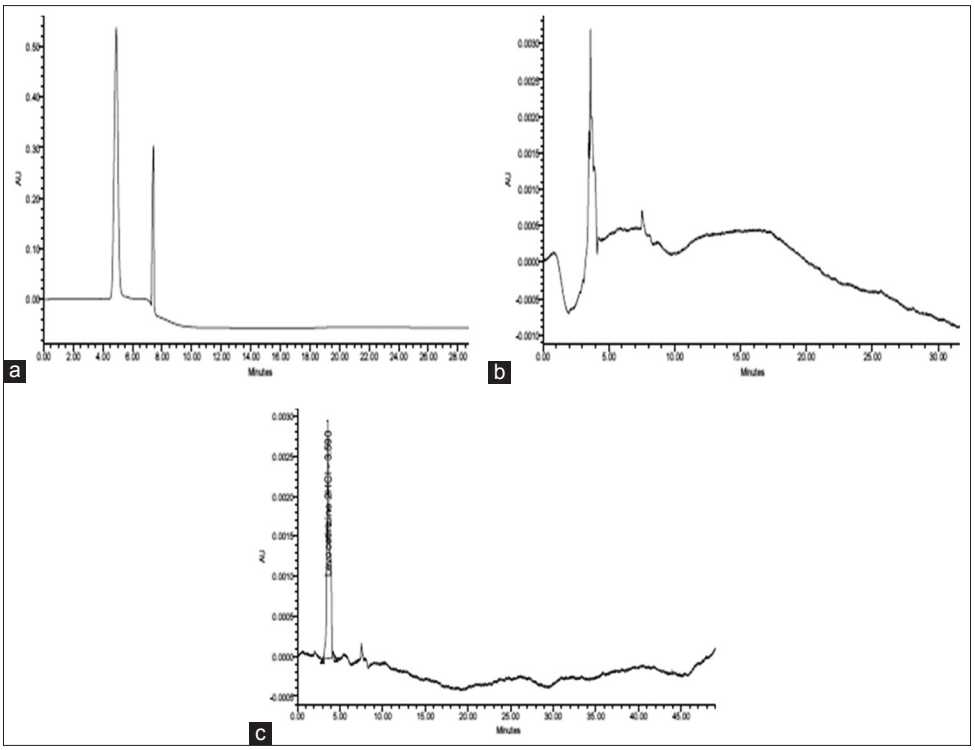

Figure 2: The optimize chromatograph in phosphate buffer and acetonitrile at 20 µL injection (a) acebrophylline (b) montelukast and (c) levocetirizine

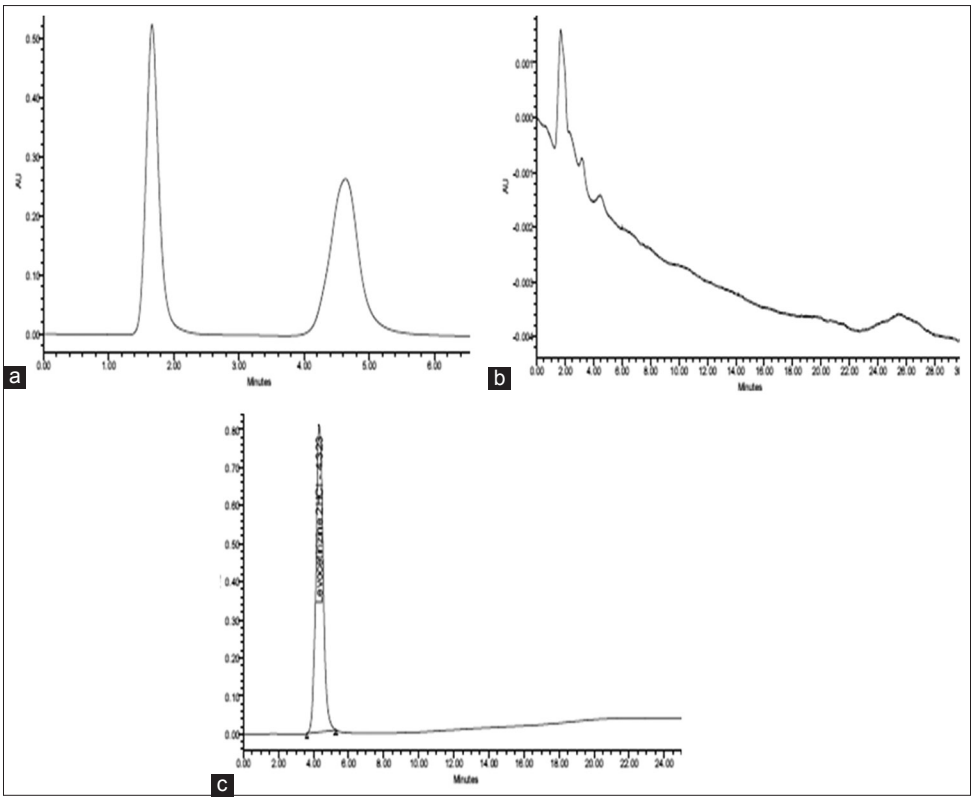

Figure 3: The optimize chromatograph in phosphate buffer and acetonitrile at 50 µL injection (a) acebrophylline (b) montelukast and (c) levocetirizine

column: C18, (150 mm * 4.6 mm); injection volume: 20 µL; flow rate: 0.7 mL/min at wavelength: 230 nm.

Figure 3 shows graphs obtained from the optimization using the same method but injection volume change from 20 to 50 µL.

Table 2: The list of chromatographic conditions used for RP-HPLC method optimization

Chromatographic conditions	Variations	Peaks
Mobile phase: Phosphate buffer (pH: 6): ACN [60:40], diluent: mobile phase, [Flow rate set at 0.7 mL/min]	Injection volume: 20 µL	No peak of montelukast and other 2 peaks not ideal
Mobile phase: Phosphate buffer (pH: 6): ACN [60:40] diluent: mobile phase flow rate set at 0.7 mL/min	Injection volume: 50 µL	No peak of montelukast and other 2 peaks not ideal
Mobile phase: Ammonium acetate buffer (pH: 3.5): MeOH, diluent: Water: MeOH (50:50), flow rate: 0.6 mL/min	Mobile phase: Ammonium acetate buffer (pH: 3.5): MeOH (20:80)	Separation of peaks but Rt for MLK is after 50 min
Mobile phase: Ammonium acetate buffer (pH: 3.5): MeOH, (15:85), diluent: Water: MeOH, diluent: (50:50), flow rate: 0.6 mL/min	Mobile phase: Ammonium acetate buffer (pH: 3.5): MeOH (15:85)	Good separation of peaks and sharp peaks appear

RP-HPLC: Reversed-phase high-performance liquid chromatography

Table 3: Validation parameters for RP-HPLC method of ABP, MLK, and LCZ

Validation parameter	ABP	MLK	LCZ
Absorption maxima, λ_{max} (nm)	275.15	296.26	246.61
Linearity range (µg/mL)	6–18	6–18	3–12
Coefficient of determination (r^2)	0.9996	0.9988	0.9999
Regression equation (Y^a)	Y=0.0005X+0.0001	Y=0.0019X−0.001	Y=0.001X−0.0004
Slope (b)	0.0005	0.0019	0.001
Intercept (a)	0.0001	0.001	0.0004
Limit of detection (µg/mL)	0.25	0.28	0.52
Limit of quantification (µg/mL)	0.8	0.9	1.6
Precision (%RSD)	Intraday=0.56 Interday=0.78	Intraday=0.72 Interday=0.84	Intraday=0.54 Interday=0.68

RP-HPLC: Reversed-phase high-performance liquid chromatography, ABP: Acebrophylline, MLK: Montelukast, LCZ: Levocetirizine, RSD: Relative standard deviation

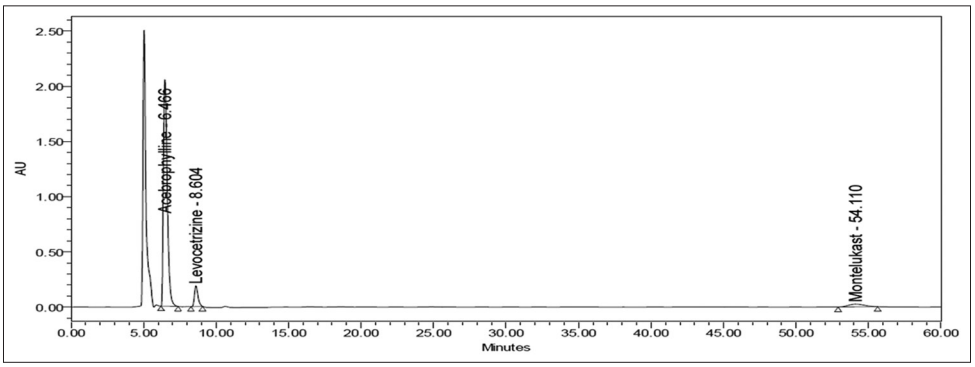

Figure 4: Separation of acebrophylline, montelukast, and levocetirizine

Figure 4 shows graphs obtained from the optimization using following method: Mobile phase: Ammonium acetate buffer (pH: 3.5): MeOH [20:80]; diluent: water: MeOH [50:50]; flow rate: 0.6 mL/min; column: Macherey-Nagel C18 4.6 mm * 250 mm (5 µm); column temperature: 30°C; wavelength: 230 nm and injection volume: 20 µL.

Separation was done for the combination but retention time was too far for MLK. Thus, another trial was done using following conditions: Buffer: Ammonium acetate adjusted to pH: 3.5 with glacial acetic acid, mobile phase: Buffer:methanol [15:85], diluent: Water:methanol [50:50] flow rate: 0.6 mL/min, column: Macherey-Nagel C18 4.6 mm * 250 mm (5 µm) column temperature: 30°C, and wavelength: 230 nm, injection volume: 20 µl. Typical chromatogram with optimized condition gives sharp and symmetric peak with retention time of 5.287 min, 26.856 min, and 6.440 min for ABP, MLK, and LCZ, respectively, as shown in Figure 5a-e.

Validation parameters

Calibration curve (linearity)

Calibration curve (ratio of peak area and concentration of ABP, MLK, and LCZ) was constructed by injecting five different concentrations of ABP, MLK, and LCZ. The regression equation was calculated in the form of Y = mX + C, where Y and X are the peak area and concentration of each standard drug, respectively. Results of the regression analysis and the coefficient of determination (r^2) are listed in Table 3. The high coefficient of determination values, i.e., 0.999 for ABP, and 0.999 for MLK, and 0.998 for LCZ, ($r^2 > 0.995$) indicated good linearity between their peak areas (Y) and standard drug concentrations (X µg/ml) in the range 6–18 µg/ml for ABP and MLK and 3–12 µg/ml for LCZ. Figure 5f explains the overlay spectra of ABP, MLK, and LCZ. Table 4 summarizes accuracy data of RP-HPLC method for ABP, MLK, and LCZ and Table 5 summarizes % purity of drugs. Figure 6 shows the linearity curve of ABP, MLK, and LCZ by RP-HPLC method.

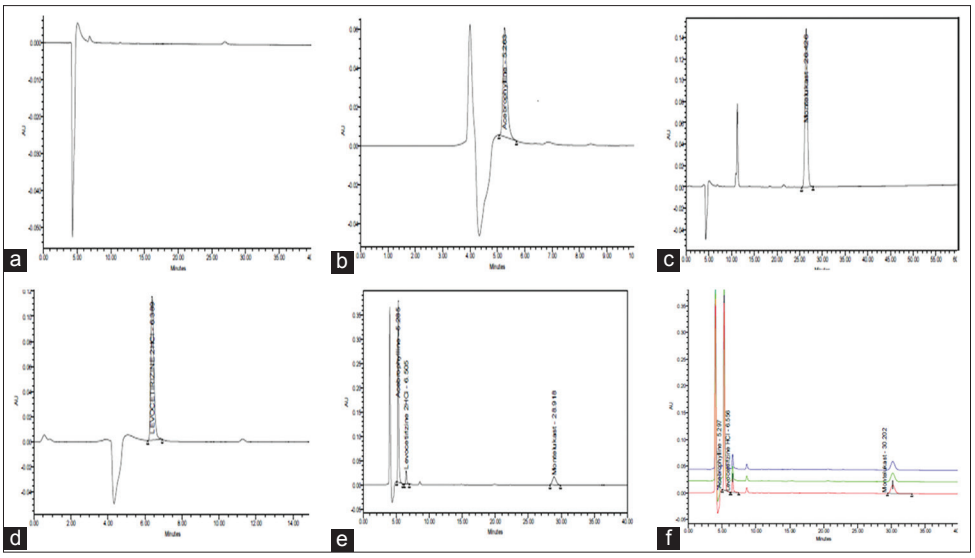

Figure 5: The optimized chromatograph (a) blank (b) acebrophylline (ABP) (c) montelukast (MLK) (d) levocetirizine (LCZ) (e) Separation of ABP, MLK, and LCZ (f) overlay spectra

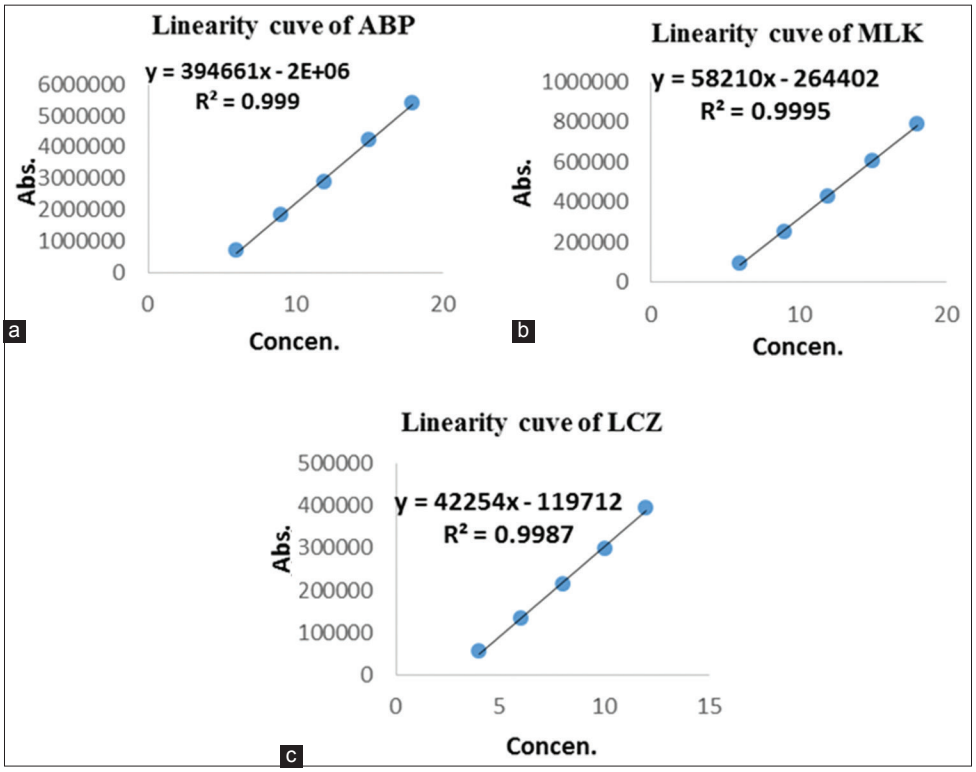

Figure 6: Linearity curve of (a) acebrophylline (b) montelukast (c) levocetirizine by reversed-phase high-performance liquid chromatography method

For marketed formulation, an assay was performed to check the purity of each drug in the formulation, and percentage purity of the drugs was calculated. Percentage estimation for ABP, MLK, and LCZ was found to be 105%, 100%, and 96%, respectively. The results were found to be complies with IP (2007). Thus, procured ABP, MLK, and LCZ were found to be in pure form.

CONCLUSION

For the analysis of drugs in multicomponent mixtures, RP-HPLC method is frequently used in today scenario. The validated developed HPLC methods used for simultaneous estimation of ABP, MLK, and LCZ were found simple, specific, accurate, rapid, precise, economical,

Table 4: Accuracy data of RP-HPLC method for ABP, MLK, and LCZ

Amount added (µg/ml)			% Recovery (w/w)		
ABP	MLK	LCZ	ABP	MLK	LCZ
8	8	8	97.8	98.6	96.4
10	10	10	99.7	99.6	99.1
12	12	12	100.2	100.2	100.6
Mean recovery			99.4	99.6	99.05
SD			1.11	0.71	1.88

RP-HPLC: Reversed-phase high-performance liquid chromatography, ABP: Acebrophylline, MLK: Montelukast, LCZ: Levocetirizine, SD: Standard deviation

Table 5: Percentage purities of ABP, MLK, and LCZ estimated by RP-HPLC method

Drug name	Drug claim (mg)	Amount found (mg)	%Estimation
ABP	200	210	105
MLK	10	10	100
LCZ	5	4.8	96

RP-HPLC: Reversed-phase high-performance liquid chromatography, ABP: Acebrophylline, MLK: Montelukast, LCZ: Levocetirizine

and reliable for contemporary analysis of drugs in a tablet. The purpose of proposed project was satisfied as tshe developed methods were meeting all the validation parameters requirements of the regulatory guidelines.

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