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Development and Validation of RP-HPLC Method for Simultaneous Estimation of Bepotastine Besilate and Montelukast Sodium in Tablet Dosage Form

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ABSTRACT

A combination of Bepotastine besilate and Montelukast sodium was approved by CDSCO as an anti-allergy drug. The objective was to develop a simple, accurate, and precise RP-HPLC method for simultaneous estimation of Bepotastine besilate and Montelukast sodium in a combined tablet dosage form. RP- HPLC method is developed and validated in their combined dosage form by using hypersil BDS C 18 (250mm x 4.6 mm, 5 µm) column and Buffer (Potassium Phosphate, pH 4.0): Methanol (70:30) as mobile phase, at a flow rate of 1 ml/min. Detection was carried out at 240 nm. HPLC method was developed and validated. The retention time of Bepotastine besilate and Montelukast sodium were found to be 6.81 min and 3.85 min respectively. The method has been validated for linearity, accuracy, and precision. Linearity was observed for was 0.127µg/ml and 0.097µg/ml for Bepotastine besilate and Montelukast sodium respectively. The LOQ were 0.385µg/ml and 0.293µg/ml for Bepotastine besilate and Montelukast sodium respectively. The proposed method was successfully applied for the simultaneous estimation of both the drugs in a commercial Combined dosage form. The RP-HPLC methods were found to be simple, accurate, robust, and reproducible.

Keyword: Bepotastine besilate, Montelukast sodium, RP- HPLC method, ICH Q2 (R1)

INTRODUCTION

The increasing incidence of allergic diseases, including allergic bronchial asthma, allergic rhinoconjunctivitis, and atopic dermatitis, is as yet unexplained. Path mechanistic studies have indicated that allergic inflammation contributes to the onset of acute or chronic symptoms of allergic diseases.The substance or medicine which may use to treat allergic reactions that substance called an ANTI-ALLERGIC drug. High-performance liquid chromatography(HPLC), formerly referred to as high-pressure liquid chromatography, is a technique in analytical chemistry used to separate, identify, and quantify each component in a mixture.

A sample is separated into its constituent components (or analytes)by distributing between the mobile phase and stationary phase.

Bepotastine besilate: Bepotastine besilate is an organosulfonate salt obtained by combining of bepotastine and benzenesulfonic acid. A topical, selective, and non-sedating histamine (H1) receptor antagonist is used for the treatment of itching associated with allergic conjunctivitis. It has a role as an H1-receptor antagonist and an anti-allergic agent.Its chemical formula is $C_{27}H_{31}ClN_2O_6S$, IUPAC name is 4-[4-[(S)-(4-chlorophenyl)-pyridin-2-ylmethoxy]piperidin-1-yl]butanoic acid.

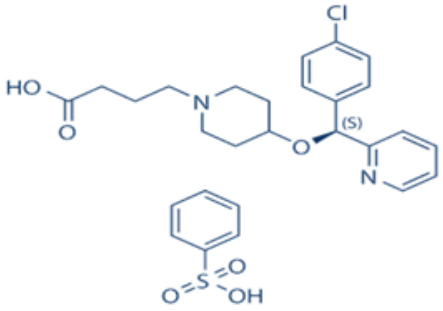

Fig. 1: Structure of Bepotastine besilate

Montelukast sodium: Montelukast sodium is in the leukotriene receptor antagonist family of medications. It works by blocking the reaction of leukotriene D4 in the lungs resulting in decreased inflammation and relaxation of smooth muscle. It's chemical formula is $C_{35}H_{35}ClNNaO_3S$, It's IUPAC name is 2-[1-[[(1*R*)-1-[3-[(*E*)-2-(7-chloroquinolin-2-yl)ethenyl]phenyl]-3-[2-(2-hydroxypropan-2-l)phenyl]propyl]sulfanylmethyl]cyclopropyl]acetate.

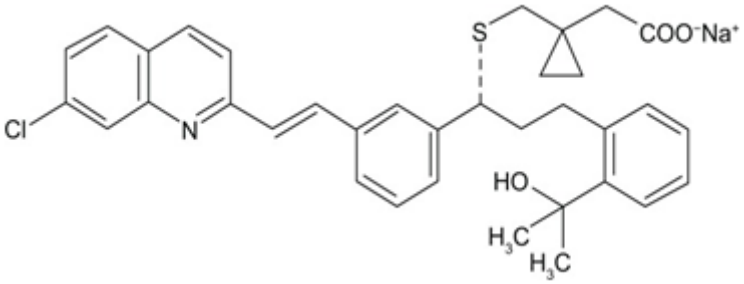

Fig. 2: Structure of Montelukast sodium

MATERIAL AND METHOD

Acetonitrile, Potassium Dihydrogen Phosphate, Orthophosphoric Acid, and Methanol were purchased from Merck Laboratory chemical, Rankem laboratory chemical, and industrial laboratory chemical retail trader from Anand, Gujarat. Bepotastine besilate And Montelukast sodium tablets with the strength of 10 mg were procured from a local medical store in the city. This formulation was used to recover the amount of bepotastine besilate and montelukast sodium from pharmaceutical tablet dosage form to ensure the applicability of the method for a custom analysis of the fixed-dose combination.

Instrumentation
The HPLC (CYBER LAB LC1000) instrument was equipped with column hypersil BDS C_{18} (250mm x 4.6 mm, 5 µm).

Method/ Experimental Work
Standard Stock Solution of Bepotastine besilate and Montelukast sodium Accurately weighed separately quantity of 10 mg Bepotastine besilate and 10 mg Montelukast sodium API were transferred into a 100 ml volumetric flask and dissolved in having a concentration of 100 µg/ml Bepotastine besilate and 100 µg/ml Montelukast sodium.

WorkingStandardSolutionofBepotastinebesilateandMontelukastsodium
From the above solution, 1 ml was taken into a 10 ml volumetric flask and was madeup to the mark with the mobile phase to get 10 µg/ml of Bepotastine besilate and 10µg/ml of Montelukast sodium.

Preparation of Mobile Phase
Prepare0.05MPotassiumDihydrogenPhosphatebydissolving6.8gmofPotassium Dihydrogen Phosphate in 1000 ml water, adjust pH 4 with o-Phosphoric acid (OPA).This solution was sonicated for 5 min for degassing and filtered through a 0.45µMilliporefilter.PreparetheratioofBuffer(pH4.0):Methanol(70:30).

Preparation of Sample Stock Solution
Bepotastine besilate and montelukast sodium were taken in equal quantities and mixed together to form of the tablet dosage form. The stock solution was prepared by dissolving a synthetic mixture equivalent to 10 mg of Bepotastine besilate or 10 mg of Montelukast sodium and was transferred to a 100ml volumetric flask. Then 60 ml methanol

was added and sonicated for 5 mins to ensure complete solubilization of the drug. After sonication, volume was made up to the mark with methanol. Filter the stock solution with Whatman filter paper no 42 and the final filtrate is collected as a sample stock solution.

Chromatographic Separation

Standard solutions of Bepotastine besilate and Montelukast sodium were injected incolumn with 20 µl micro-syringe. The chromatogram was run for appropriate minuteswith the mobile phase. The detection was carried out at a wavelength of 240 nm. Thechromatogram was stopped after separation was achieved completely. Data related topeaklikearea, height,retentiontime, resolution,etc.wererecordedusing thesoftware.

Chromatographic Trials

Table1: List of Chromatographic Trials

MobilePhase	Ratio (v/v)	Retention Time	Remarks
Bepotastine besilate and Montelukast sodium in Water: Methanol	50:50	3.67	One Peak Observed
Bepotastine besilatein Water: Methanol	50:50	-	Nopeak Observed by the injecting Bepotastine Besilate Solution
Montelukast sodium in Water: Methanol	50:50	3.69 (Montelukast sodium)	Montelukast sodium peak is observed
Bepotastine besilate and Montelukast sodium in Water:Methanol	30:70	2.96 (Montelukast sodium)	Montelukastsodiumpeak isobservedbutStill, Bepotastine besilate is not Observed
Bepotastine besilate and Montelukast sodium in Water : Methanol	15:85	2.57 (Montelukastsodium)	Montelukast sodium peak is observed and its retention time is reduced but Still, Bepotastine besilate is not observed
Bepotastine besilate and Montelukast sodium in Water: Acetonitrile	60:40	5.29 (Montelukastsodium)	Montelukast sodium peak is observed but Still, Bepotastine besilate is not observed
Bepotastine besilate and Montelukast sodium in Water: Acetonitrile	40:60	4.03 (Montelukastsodium)	Montelukast sodium peak is observed but Still, Bepotastine besilate peak is not observed
Bepotastine besilate and Montelukast sodium in Water: Acetonitrile	20:80	3.04 (Montelukastsodium)	Montelukast sodium peak is observed and its retention time is reduced but Still, Bepotastine besilate peak is not Observed
Bepotastine besilate and Montelukast sodium inwater (pH4.5): Methanol	50:50	3.96 (Montelukastsodium)11.87 (Bepotastinebesilate)	Montelukast sodium peakis observed and its peak shape is good. Bepotastine besilate peak is also bserved but its peak Shape is not good
Bepotastine besilate andwater (pH4.5): Methanol	50:50	11.87 (Bepotastinebesilate)	Bepotastine besilate peak is observed but its peak Shape is not good

Bepotastine besilate and Montelukast sodium in Buffer (pH4.0): Methanol	40:60	2.55 (Montelukast sodium) 2.80 (Bepotastine besilate)	Both the peak is observed but need separation between them
Bepotastine besilate and Montelukast sodium in Buffer (pH4.0):Methanol	50:50	2.70 (Montelukast sodium)3.11(Bepotastine besilate)	Slight separation is observed between both the peak
Bepotastine besilate and Montelukast sodium in Buffer (pH4.0): Methanol	60:40	3.18 (Montelukast sodium) 5.49 (Bepotastinebesilate)	Both the peak is observed and retention time is increased
Bepostatine besilate and Montelukast sodium in Buffer (pH4.0): Methanol	70:30	3.85 (Montelukast sodium)6.81(Bepostatine besilate)	Peak shape get sharper and it is well separated from each other

Chromatographic Conditions

Table2: Chromatographic Conditions of HPLC

Components	Description
Column	C_{18}(25 cm× 0.46cm)HypersilBDS
MobilePhase	Buffer (pH 4.0): Methanol(70:30)
FlowRate	1.0ml/min
DetectionWavelength	240nm
Runtime	10min

Injectionvolume	20.0µl

System Suitability Test

It is an integral part of the chromatographic method. These tests are used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed. System suitability tests are based on the concept that the equipment, electronics, analytical operations, and samples constitute an integral system that can be evaluated as a whole. System suitability testing provides assurance that the method will provide accurate and precise data for its intended use.

Acceptance criteria

- Theoretical Plates for the analyte peak should not be less than 2000.
- Tailing factor for the analyte peak should not be more than 2.0.

Linearity and Range

The linearity for Bepotastine besilate and Montelukast sodium were assessed by analysis of combined standard solutions in the range of 5-15 µg/ml and 5-15 µg/ml respectively, 0.5,7.5,1,1.25 and 1.5 ml solutions were pipettes out from the Stock solution of Bepotastine besilate (100 µg/ml) and Montelukast sodium (100 µg/ml) and transfer to 10ml volumetric flask and makeup with mobile phase to obtain 5,7.5,10,12.5, and 15µg/ml and 5,7.5,10,12.5, and 15 µg/ml for Bepotastine besilate and Montelukast sodium respectively. In terms of slope, intercept, and correlation coefficient value. The graph of peak area obtained versus respective concentration was plotted.

Acceptance criteria: The value of r 2 should be nearer to 1 or equal to 1.

Precision

Repeatability

A standard solution containing Bepotastine besilate (10 µg/ml) and Montelukast sodium (10 µg/ml) was injected six times and areas of peaks were measured and % R.S.D. was calculated. Acceptance criteria: % RSD of Area should not be more than 2.0% Intraday Precision A standard solution containing (5,10,15 µg/ml) of Bepotastine besilate and (5,10,15 µg/ml) of Montelukast sodium were analyzed three times on the same day and % R.S.D was calculated.

Acceptance criteria: % RSD of Area should not be more than 2.0%

Interday Precision

A standard solution containing (5,10,15µg/ml) of Bepotastine besilate (5,10,15µg/ml) of Montelukast sodium were analyzed three times on a different day and % R.S.D was calculated.

Acceptance criteria: % RSD of Area should not be more than 2.0%

Accuracy For Bepotastine besilate

5 µg/ml drug solutions were taken in three different flask labels A, B, and C. Spiked 80%, 100%, and 120% of the standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 240 nm. The amount of Bepotastine besilate was calculated at each level and % recoveries were computed.

For Montelukast sodium

5 µg/ml drug solutions were taken in three different flask labels A, B, and C. Spiked 80%, 100%, and 120% of the standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 240 nm. The amount of Montelukast sodium was calculated at each level and % recoveries were computed.

Acceptance criteria

% Recovery (individual) at each level should be between 98.00% and 102.00% 2.1.9. Limit of Detection and Limit of Quantitation The LOD was estimated from the set of 3 calibration curves used to determine method

linearity. The LOD may be calculated as,

LOD = 3.3 × (SD/Slope)

Where SD = Standard deviation of Y-intercepts of 3 calibration curves.

Slope = Mean slope of the 3 calibration curves.

The LOQ was estimated from the set of 3 calibration curves used to determine method

linearity. The LOQ may be calculated as,

LOQ = 10 × (SD/Slope)

Where SD = Standard deviation of Y-intercepts of 3 calibration curves.

Slope = Mean slope of the 3 calibration curves.

Robustness

Following parameters were changed one by one and their effect was observed on

system suitability for standard preparation.

1. Flow rate of the mobile phase was changed (± 0.2 ml/min) 0.8 ml/min and 1.2

ml/min.

2. pH of the Mobile phase was changed (± 0.2) to 3.8 and 4.2

3. Ratio of the Mobile phase was changed (±2) Buffer: Methanol (68:32) and Buffer:

Methanol (72:28).

Acceptance criteria

- Number of theoretical plates for the analyte peak should not be less than2000.
- Asymmetry value for the analyte peak should not be more than 2.0
- % RSD for the analyte peak should not be more than 2.0%.

Analysis of Market Formulation

Take synthetic mixture equivalent to 10 mg Bepotastine besilate and 10 mg of Montelukast sodium was transferred to a 100 ml volumetric flask, shake for 15 minutes, and made up volume up to the mark with the mobile phase. The solution was filtered through Whatman filter paper no. 42 and the first few drops of the filtrate were discarded. 1 ml of this solution was diluted to 10 ml with the mobile phase. The solution was injected at 10 µl. The areas of the resulting peak were measured at 240 nm.

RESULT AND DISCUTION

Identification by IR Spectroscopy

Bepotastine besilate

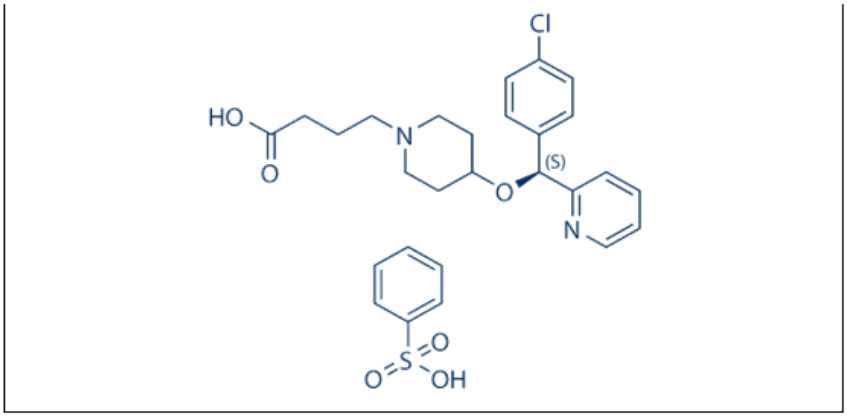

Fig3: Structure of Bepotastine besilate

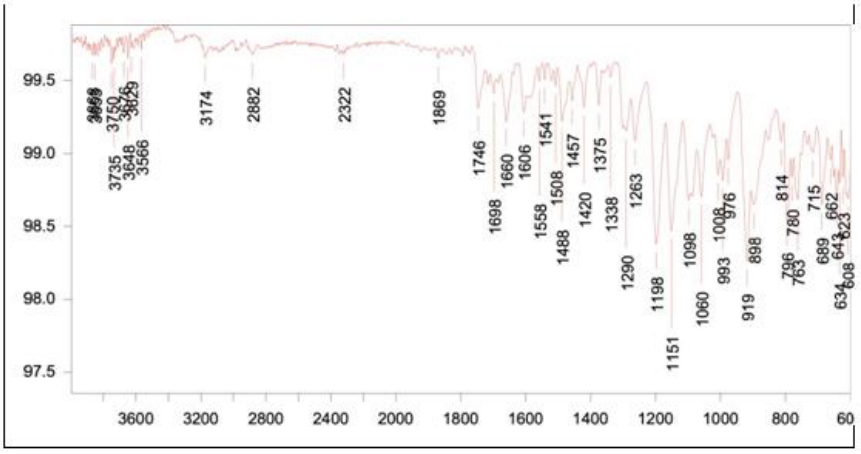

Fig4: IR Spectra of Sample Bepotastine besilate

Table 3: IR Interpretation of Bepotastine besilate

FunctionalGroup	Frequency(cm^{-1})
C-Nstretching	1008and 1198
C=Cstretching	1420-1606
C=Ostretching	1660-1746
O-Hstretching	3174

Montelukast sodium

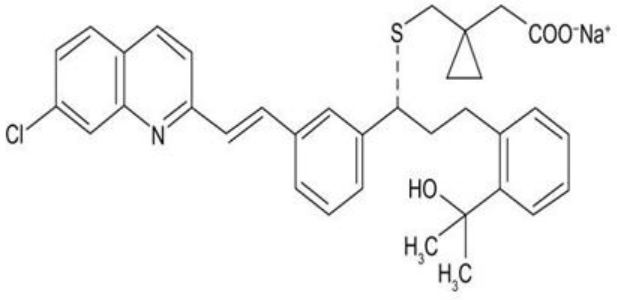

Fig5: Structure of Montelukast sodium

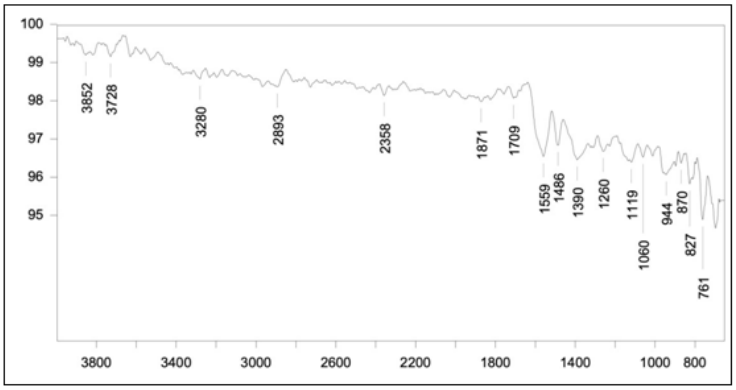

Fig 6: IR Spectra of Sample Montelukast sodium

Table 4: IR Interpretation of Montelukast sodium

FunctionalGroup	Frequency(cm^{-1})
C-NStretching	1060
C-Ostretching	1709
C=C stretching	1486-1559
C-Ostretching	1119

METHOD DEVELOPMENT
Wavelength Determination

UV spectra of Bepotastine besilate and Montelukast sodium were taken in Methanol and λmax was observed using Systronic 119

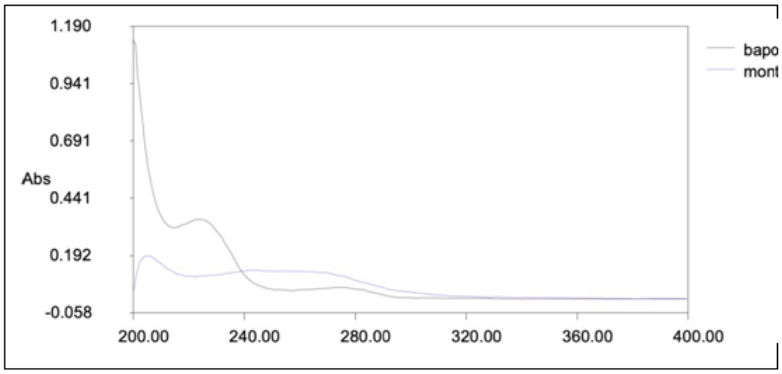

Fig 7: Overlay UV Spectrum of Bepotastine besilate and Montelukast sodium showing Selection of Wavelength Detection.

Observation
Bepostatine besilate and Montelukast sodium are both drugs that give higher absorbance at 240 nm.
So 240 nm has been selected as the detection wavelength.
Note: All the chromatograms are shown at the wavelength of 240 nm. So, 240 nm is shown in the final optimized method.

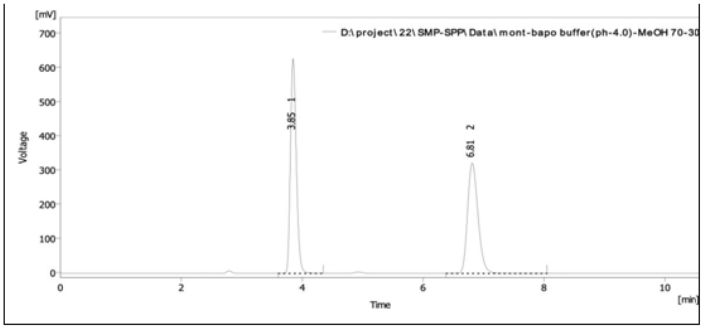

Fig 8: Chromatogram of Bepotastine besilate and Montelukast sodium in Buffer (pH4.0): Methanol (70:30 v/v) (Final)

Observed values for System Suitability Test

1. Resolution (Rs): Resolution was observed at 11.787, depicted in Table 6.6.

2. Column efficiency (N): The number of plates observed for Bepotastine besilate and Montelukast sodium were 7373 and 6775, respectively, depicted in Table 6.6.

3. Symmetry factor (S): Tailing factors observed for Bepotastine besilate and Montelukast sodium were 1.419 and 1.360, respectively, depicted in Table 6.6.

Table5: Results for System Suitability Test.

Parameters	Bepostatine besilate	Montelukast sodium
Theoreticalplatesper column	7373	6775
Symmetry factor/Tailing factor	1.419	1.360
Resolution	11.787	

Method validation

System Suitability Parameters

System suitability tests are used to verify that the resolution and repeatability of thesystem were adequate for the analysis intended. The parameters used in this test were the chromatographic peak, retention time, resolution, theoretical plate number, capacity factor, and tailing factor.

Table6: System Suitability Parameters

Parameter	Bepotastine besilate	Montelukastsodium
Retentiontime (min)	6.810	3.847
Theoreticalplatesper column	7373	6775
Tailingfactor/Symmetryfactor	1.419	1.360
Resolution	11.787	

Specificity

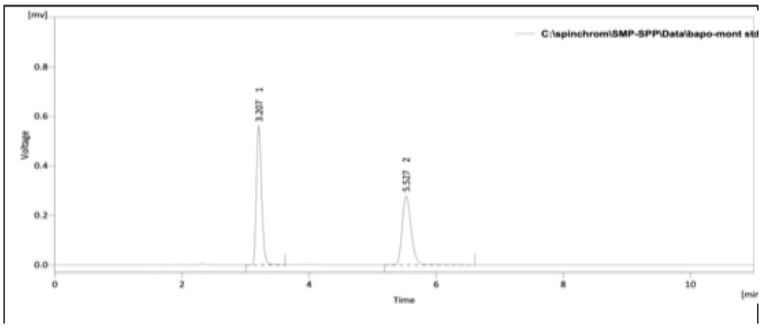

Fig 9: Chromatogram of Bepotastine besilate and Montelukast sodium Standard

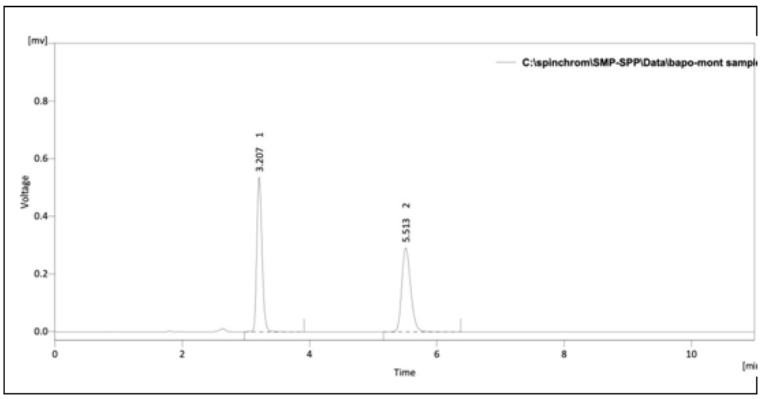

Fig. 10: Chromatogram of Bepotastine besilate and Montelukast sodium Sample

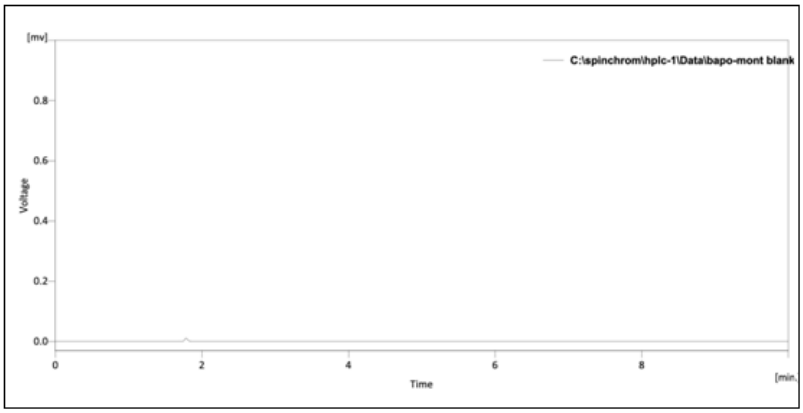

Fig. 11: Chromatogram of Bepotastine besilate and Montelukast sodiumBlank

The Chromatograms of Bepotastine besilate and Montelukast sodium standards and Bepotastine besilate and Montelukast sodium sample show no interference with the Chromatogram of Bepotastine besilate and Montelukast sodium Blank, so the Developed method is Specific.

LinearityandRange

ThelinearityforBepotastinebesilateandMontelukastsodiumwereassessedbyanalysisof combined standard solution in a range of5-15 µg/ml and 5-15 µg/ml respectively.The correlation coefficient for calibration curve Bepostatine besilate and Montelukastsodiumwasfoundto0.999 be0.998 respectively.

The regression line equation for Bepotastine besilate and Montelukast sodium are asfollowing:

For Bepotastine besilate: **y = 276.4x - 20.185** andForMontelukast sodium: **y = 80.48x -19.12**

Table7:Linearity Data for Bepotastine besilate.

Sr.No	Concentration(µg/ml)	Area
1	5	1363.447
2	7.5	2044.602
3	10	2746.26
4	12.5	3448.236
5	15	4116.664

Table8: Linearity Data for Montelukast sodium

Sr.No	Concentration(µg/ml)	Area
1	5	1592.772
2	7.5	2386.066
3	10	3204.900
4	12.5	4015.007
5	15	4802.524

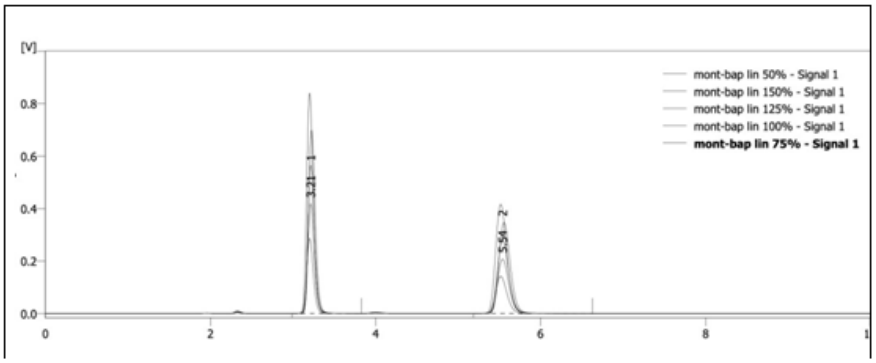

Fig. 12: Overlay chromatogram of different concentrations of binary mixtures of Bepotastinebesilate and Montelukast sodium

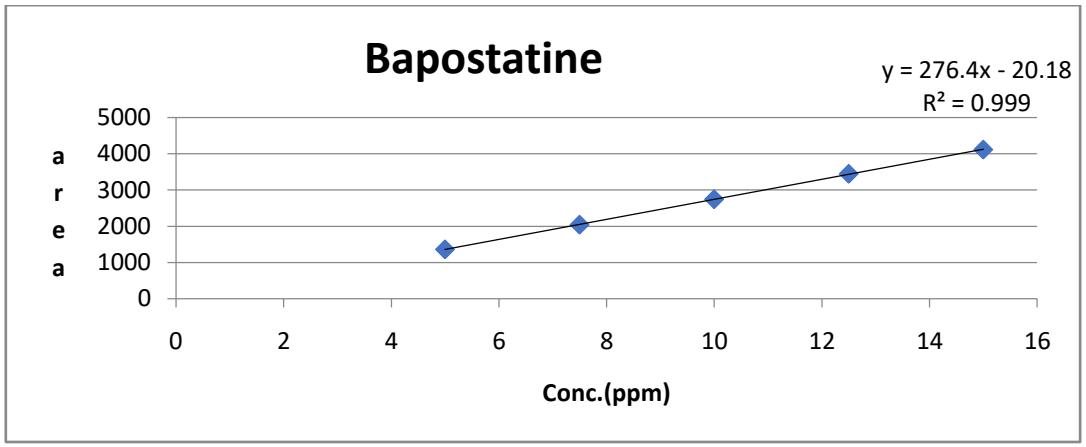

Fig. 13: Calibration Curve of Bepotastine besilate (5-15µg/ml).

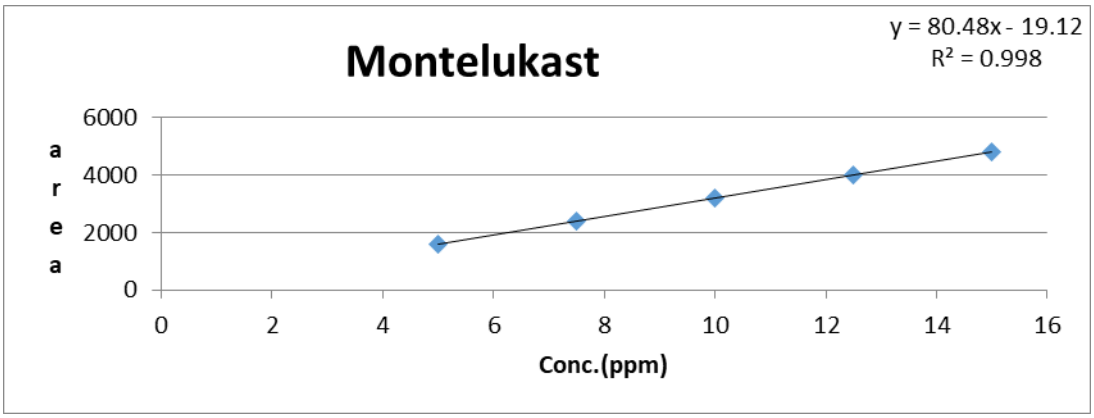

Fig. 14: Calibration Curve of Montelukast sodium (5-15µg/ml).

Precision

Repeatability

The data for repeatability of peak area measurement for Bepotastine besilate and Montelukast sodium, based on six measurements of the same solution of Bepotastine besilate and Montelukast sodium are depicted in table 6.10 and 6.11. The % RSD for Bepotastine besilate and Montelukast sodium was found to be1.121and 0.916 respectively.

Table9: Repeatability Data for Bepotastine besilate.

Bepotastine besilate				
Sr.No.	**Conc(µg/ml)**	**Area**	**Mean±S.D(n=6)**	**%R.S.D**
1.	10	2759.922 2765.588 2768.121 2826.946 2821.006 2815.324	2792.818±31.303	1.121

Table10: Repeatability data for Montelukast sodium

Montelukast sodium				
SrNo.	**Conc(µg/ml)**	**Area**	**Mean± S.D(n=6)**	**%R.S.D**
1.	10	3220.823	3256.062 ±29.833	0.916
		3240.183		
		3230.473		
		3267.063		
		3292.208		
		3285.62		

Intraday precision

The data for intraday precision for Bepotastine besilate and Montelukast sodium isshownin table 6.12. The% R.S.D. forIntraday precision was found tobe1.259-1.431for Bepotastine besilate and 0.638-0.957 for Montelukast sodium.

Table 11: Intraday precision data for Estimation of Bepotastine besilate and Montelukast sodium.

Sr. No.	**Bepotastine besilate**			**Montelukast sodium**		
	Conc.(µg/ml)	**AreaMean±S.D. (n=3)**	**%R.S.D**	**Conc.(µg/ml)**	**AreaMean±S.D. (n=3)**	**%R.S.D**
1	5	1371.184 ±18.361	1.339	5	1608.353±15.389	0.957
2	10	2712.681±38.830	1.431	10	3182.929±29.887	0.939
3	15	4062.773±51.142	1.259	15	4757.115±30.372	0.638

Intraday precision

The data for interday precision for Bepotastine besilate and Montelukast sodium isshown in table 6.13. The % R.S.D. for interday precision was found to be 0.621-1.009 for Bepostatine besilate and 0.731-1.071 for Montelukast sodium.

Table 12: Interday Precision data for Estimation of Bepotastine besilate and Montelukast sodium.

Sr. No.	**Bepotastine besilate**			**Montelukast sodium**		
	Conc.(µg/ml)	**AreaMean±S.D. (n=3)**	**%R.S.D**	**Conc.(µg/ml)**	**Area Mean±S.D.(n=3)**	**%R.S.D**
1	5	1356.380±8.419	0.621	5	1573.415±16.048	1.02
2	10	2734.584±21.094	0.771	10	3205.297±34.341	1.07
3	15	4079.893±41.164	1.009	15	4768.029±34.853	0.73

Accuracy

The accuracyofthe methodwasconfirmed bya recovery studyfrommarketedformulation at three levels of standard addition.The results are shown in table 6.14and 6.15. Percentage recovery for Bepostatine besilate was 100.148-100.346 %, whileforMontelukast sodium, itwas foundtobe in therangeof100.373-100.970 %.

Table13: Recovery Data for Bepotastine besilate.

Sr.No.	**Conc.Level(%)**	**Sampleamount(µg/ml)**	**AmountAdded(µg/ml)**	**Amountrecovered(µg/ml)**	**% Recovery**	**%MeanRecovery±S.D**

1	80%	5	2.5	2.518	100.718	100.244 ± 1.267
2		5	2.5	2.530	101.205	
3		5	2.5	2.470	98.808	
4	100%	5	5	5.031	100.623	100.148± 0.512
5		5	5	4.980	99.606	
6		5	5	5.011	100.216	
7	120%	5	7.5	7.461	99.474	100.346 ± 0.798
8		5	7.5	7.539	100.524	
9		5	7.5	7.578	101.040	

Table14: Recovery Data for Montelukast sodium.

Sr.No.	Conc.Level (%)	SampleAmount	AmountAdded	Amountrecovered(µg/ml)	% Recovery	%MeanRecovery±S.D
1	80%	5	2.5	2.541	101.655	100.970 ± 1.058
2		5	2.5	2.494	99.751	
3		5	2.5	2.538	101.503	
4	100%	5	5	5.037	100.748	100.373± 1.741
5		5	5	5.095	101.897	
6		5	5	4.924	98.475	
7	120%	5	7.5	7.528	100.376	100.499 ± 0.622
8		5	7.5	7.588	101.173	
9		5	7.5	7.496	99.947	

LOD and LOQ

The calibration curve was repeated for five times and the standard deviation (SD) of the intercepts was calculated. Then LOD and LOQ were calculated as follows:

LOD = 3.3 * SD/slope of calibration curve
LOQ=10 *SD/slope of calibration curve

Limit of Detection

Table 15: Limit of Detection Data for Bepotastine besilate and Montelukast sodium.

Bepotastinebesilate	Montelukastsodium
LOD=3.3 x (SD / Slope) =3.3 x (10.645/276.403) =0.127 µg/ml	LOD=3.3 x (SD / Slope) =3.3 x (9.434/321.938) =0.097 µg/ml

Limit of Quantitation

Table 16: Limit of Quantitation Data for Bepotastine besilate and Montelukast sodium.

Bepotastine besilate	Montelukast sodium
LOQ=10 x(SD / Slope) =10 x (10.645/276.403) =0.385 µg/ml	LOQ=10 x (SD / Slope) =10 x (9.434/321.938) =0.293 µg/ml

Robustness

The effect of changes was found to be with in the acceptance criteria as shown in table 17and table18 The % RSD should be less than 2%.

Table17: Robustness data for Bepotastine besilate.

SrNo.	Area atFlow rate(-0.2 ml/min)	Area atFlow rate(+0.2 ml/min)	Area atpH(-0.2)	Area atpH(+0.2)	Area atMobilephase(-2)	Area atMobilephase(+2)
1	2726.17	2678.018	2757.329	2600.421	2757.152	2657.87
2	2774.305	2623.731	2794.795	2643.22	2776.511	2718.89
3	2803.915	2670.73	2808.844	2651.05	2784.962	2710.618
% R.S.D	1.418	1.109	0.956	1.036	0.514	1.228

Table18: Robustness data for Montelukast sodium

SrNo.	Area atFlow rate(-0.2 ml/min)	Area atFlow rate(+0.2 ml/min)	Area atpH(-0.2)	Area atpH(+0.2)	Area atMobilephase(-2)	Area atMobilephase(+2)
1	3210.671	3125.226	3235.645	3062.547	3217.571	3117.657
2	3232.399	3086.282	3284.602	3109.38	3240.139	3157.468
3	3272.25	3140.691	3278.011	3093.793	3259.627	3163.273
% R.S.D	0.964	0.899	0.813	0.772	0.650	0.789

Analysis of marketed formulation by developed method.

Applicability of the proposed method was tested by analyzing the commercially available in synthetic mixture. The results are shown in table 6.20.

Table19 : Analysis of Marketed Formulation.

Syntheticmixture	Labelclaim		Assay(% oflabel claim*) Mean± S.D.	
	Bepotastine besilate	Montelukast sodium	% Bepotastine besilate	% Montelukast sodium
	10mg	10mg	102.457 ± 0.809	97.886 ± 0.465

DISCUSSION

A New RP-HPLC method has been developed for estimation of Bepotastine besilate and Montelukast sodium in tablet dosage form was rapid, accurate, precise, economic, and easy to perform.

The linearity was investigated in the range of 5-15µg/mL (r^2 = 0.998) for Bepotastine besilate and 5-15 µg/ml (r^2 = 0.999) for Montelukast sodium. The LOD were 0.127 µg/ml and 0.097µg/ml for Bepotastine besilate and Montelukast sodium, respectively. The LOQ were 0.385µg/mL and 0.127µg/mL for Bepotastine besilate and Montelukast sodium, respectively.

This method was found to be simple, accurate, robust, and reproducible.

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