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Development of RP-HPLC method for the simultaneous estimation of ambroxol hydrochloride, cetirizine hydrochloride and antimicrobial preservatives in combined dosage form

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ABSTRACT

A simple, efficient and reproducible Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) method for simultaneous estimation of Ambroxol hydrochloride, Cetirizine hydrochloride, Methylparaben and Propylparaben in combined liquid pharmaceutical formulation has been developed. The separation was carried out on Hypersil BDS C_{18} (200x 4.6mm i.d., 5µm) column using acetonitrile: 0.05 M potassium dihydrogen orthophosphate (adjusted to pH 3.5 with ortho phosphoric acid) in the ratio of 33:67 v/v as eluent. The flow rate was 1 ml/min and effluent was detected at 230 nm. The retention times of ambroxol hydrochloride, cetirizine hydrochloride, methylparaben and propylparaben were 3.97 min, 15.30 min, 5.74 min, and 17.54 min, respectively. The percentage recovery was within the range between 98.36% and 99.96% for ambroxol hydrochloride, 100.00% and 101.49% for cetirizine hydrochloride, 99.58% and 100.40% for methylparaben and 100.00% and 101.82% for propylparaben. The linear ranges were found to be 192-288µg/ml (r^2 = 0.9970) for ambroxol hydrochloride, 16-24µg/ml (r^2 = 0.9957) for cetirizine hydrochloride, 64-96µg/ml (r^2 = 0.9961) for methylparaben and 6.4-9.6µg/ml (r^2 = 0.9915) for propylparaben. The percentage relative standard deviation for accuracy and precision were found to be less than 2%. Hence, the method could be successfully applied for routine analysis of ambroxol hydrochloride, cetirizine hydrochloride, methylparaben and propylparaben in combined liquid dosage form.

Keywords: Ambroxol Hydrochloride, Cetirizine Hydrochloride, Antimicrobial Preservatives, RP-HPLC, Syrup, Estimation

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INTRODUCTION

Ambroxol hydrochloride (Fig. 1) chemically, trans-4-[(2-amino-3, 5-dibromobenzyl) amino] cyclohexanol hydrochloride, is semi-synthetic derivative of vasicine obtained from Indian shrub "*Adhatoda vasica*". It is a metabolic product of bromhexine. It is used as broncho secretolytic and an expectorant drug [1]. Cetirizine hydrochloride (Fig. 2) chemically, (2-{4-[(4-Chlorophenyl) (phenyl) methyl] piperazin-1-yl} ethoxy) acetic acid hydrochloride, is an orally active and selective H_1-receptor antagonist. It is piperazine derivative and metabolite of hydroxyzine [2]. Combinations of Ambroxol hydrochloride with Cetirizine hydrochloride in drug formulation used as antihistaminic H_1 blockers. Methylparaben (Fig. 3) and Propylparaben (Fig. 4) are used as either single or in combinations in drug products as antimicrobial preservatives to prevent alteration of product preparations. Methylparaben is the methyl ester of p-hydroxybenzoic acid and propylparaben is the propyl ester of p-hydroxybenzoic acid.

Liquid preparations are particularly susceptible to microbial growth because of the nature of their ingredients. Such preparations are protected by the addition of preservatives that prevent the alteration and degradation of the product formulation [3]. The finished product release specifications should include an identification test and a content determination test with acceptance criteria and limits for each antimicrobial preservative present in the formulation.

Hence their (methylparaben and propylparaben) antimicrobial and antifungal properties make them an integral part of the product formulation. This encourages the development of new method for the simultaneous estimation of ambroxol hydrochloride, cetirizine hydrochloride, methylparaben and propylparaben in combined liquid dosage form, to provide driving force in today's pharmaceutical industry.

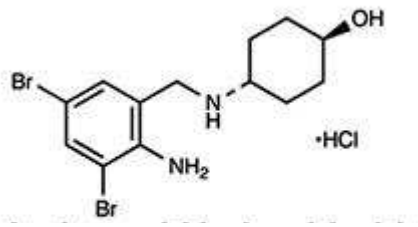

Figure 1: Structure of Ambroxol hydrochloride

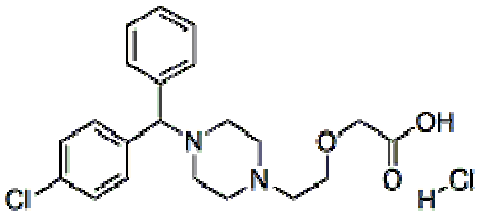

Figure 2: Structure of Cetirizine hydrochloride

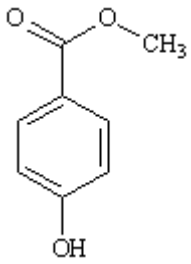

Figure 3: Structure of Methylparaben

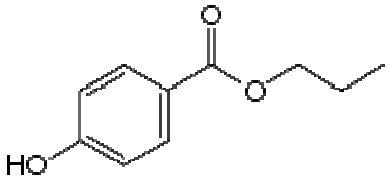

Figure 4: Structure of Propylparaben

The literature survey reveals that a few analytical methods have been reported for the estimation of these drugs individually or in combination with other drugs by spectrophotometry [4], high performance liquid chromatography [5-6], gas liquid chromatography [7] and capillary electrophoresis [8] however, no method has been reported for the simultaneous estimation of ambroxol hydrochloride, cetirizine hydrochloride , antimicrobial preservatives such as methylparaben and propylparaben in combined liquid dosage form. Hence, a sample, rapid, precise, accurate RP-HPLC method for the simultaneous estimation of ambroxol hydrochloride, cetirizine Hydrochloride methylparaben and propylparaben in combined liquid dosage form is developed and validated.

MATERIALS AND METHODS

Chemicals and reagents

Acetonitrile of HPLC grade was purchased from E.Merck (India) Ltd., Mumbai. Potassium dihydrogen orthophosphate and orthophosphoric acid of AR grade were obtained from Qualigens Fine Chemicals Ltd., Mumbai. ambroxol hydrochloride, cetirizine hydrochloride, methylparaben and propylparaben were a gift sample by Sai Mirra Innopharm Pvt. Ltd., Chennai – 600 098, Tamil Nadu, India. The commercially available syrup with ambroxol hydrochloride, cetirizine hydrochloride, methylparaben and propylparaben, was procured from the local market.

Instrumentation and chromatographic conditions

The chromatographic separation was carried out on HPLC system (Agilent 1100 Series, Germany) with UV- Visible dual absorbance detector (PDA), Hypersil BDS C_{18} column (200 x 4.6mn; 5µm). The mobile phase consisting of 0.05M of Potassium dihydrogen ortho phosphate buffer (pH 3.5 adjusted with orthophosphoric acid) and acetonitrile were filtered through 0.45µ membrane filter before use, degassed and were pumped from the solvent reservoir in the ratio of 67:33 v/v was pumped into the column at a flow rate of 1.0 ml/min. The detection was monitored at 230 nm. The volume of injection loop was 20 µl prior to the injection of the drug solution; the column was equilibrated for at least 30 min. with the mobile phase following through the system. The column and the HPLC system were kept in ambient temperature (25º C).

Preparation of Standard solutions

Ambroxol Hydrochloride Working standard solution:

30 mg of Ambroxol Hydrochloride Working standard was weighed and transferred carefully in 50 ml volumetric flask. About 20 ml of mobile phase was added, sonicated to dissolve the drug completely and the volume was made up with mobile phase. 20 ml of above solution was diluted to 50 ml with mobile phase (240µg/ml).

Cetirizine Hydrochloride Working standard solution:

20 mg of Cetirizine Hydrochloride Working standard was weighed and transferred carefully in 100 ml volumetric flask. About 20 ml of mobile phase was added, sonicated to dissolve the drug completely and the volume was made up with mobile phase. 5 ml of above solution was diluted to 50 ml with mobile phase (20µg/ml).

Methylparaben Working standard solution:

20 mg of Methylparaben Working standard was weighed and transferred carefully in 25 ml volumetric flask. About 10 ml of mobile phase was added, sonicated to dissolve the drug completely and the volume was made up with mobile phase. 5 ml of above solution was diluted to 50 ml with mobile phase (80µg/ml).

Propylparaben Working standard solution:

20 mg of Propylparaben Working standard was weighed and transferred carefully in 25 ml volumetric flask. About 10 ml of mobile phase was added, sonicated to dissolve the drug completely and the volume was made up with mobile phase. 10 ml of above solution was diluted to 100 ml with mobile phase and 5 ml of the resulting solution was further diluted to 50 ml with mobile phase (8µg/ml).

Analysis of Sample Preparation

12.5 gm of the sample syrup was weighed and transferred carefully in a clean and dry 100 ml volumetric flask and make up the volume to 100 ml of mobile phase. 20 ml of above solution was diluted to 50 ml with mobile phase.

Amount of Ambroxol Hydrochloride / Cetirizine Hydrochloride / Methylparaben / Propylparaben present in each 5 ml of the Syrup

$$= \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Standard dilution}}{\text{Sample dilution}} \times \frac{\text{Potency}}{100} \times \text{Wt/ml} \times 5$$

RESULTS AND DISCUSSION

All of the analytical validation parameters for the proposed method were determined according to International Conference on Harmonization (ICH) guidelines [9].

System Suitability

It is essential for the assurance of the quality performance of chromatographic system. Five injections of standard drug solutions, Ambroxol hydrochloride, Cetirizine hydrochloride, Methylparaben and Propylparaben were given separately to the system. The mean area. Standard deviation and % RSD were calculated for the standard drug solutions and mentioned in Table 1, 3, 5 and 7. The system suitability parameters such as retention time, number of theoretical plate and peak area response were also be calculated for the standard drug solutions and mentioned in Table 2, 4, 6 and 8. It was observed that all the values are with in the limits.

Table 1: System suitability for Ambroxol hydrochloride

S.No.	Standard	Concentration (µg/ml)	Area
1.	Standard -1	240	4596.8
2.	Standard -2	240	4599.4
3.	Standard -3	240	4603.0
4.	Standard -4	240	4606.3
5.	Standard -5	240	4607.5
		Mean	**4602.6**
		Standard deviation	**4.505**
		RSD in %	**0.10**

Table 2: System suitability parameters for Ambroxol hydrochloride

S.No.	System suitability parameters	Ambroxol hydrochloride
1.	Retention time	3.97 min
2.	Number of theoretical plate	2504
3.	Peak area response	4602.6

Table 3: System suitability for Cetirizine hydrochloride

S.No.	Standard	Concentration (µg/ml)	Area
1.	Standard -1	20	685.3
2.	Standard -2	20	686.0
3.	Standard -3	20	686.2
4.	Standard -4	20	680.7
5.	Standard -5	20	687.0
		Mean	**685.04**
		Standard deviation	**2.5005**
		RSD in %	**0.37**

Table 4: System suitability parameters for Cetirizine hydrochloride

S.No.	System suitability parameters	Cetirizine hydrochloride
1.	Retention time	15.30 min
2.	Number of theoretical plate	6656
3.	Peak area response	685.04

Table 5: System suitability for Methylparaben

S.No.	Standard	Concentration (µg/ml)	Area
1.	Standard -1	80	2223.5
2.	Standard -2	80	2224.7
3.	Standard -3	80	2225.2
4.	Standard -4	80	2228.2
5.	Standard -5	80	2229.8
		Mean	**2226.2**
		Standard deviation	**2.6204**
		RSD in %	**0.12**

Table 6: System suitability parameters for Methylparaben

S.No.	System suitability parameters	Methylparaben
1.	Retention time	5.74 min
2.	Number of theoretical plate	6161
3.	Peak area response	2226.28

Table 7: System suitability for Propylparaben

S.No.	Standard	Concentration (µg/ml)	Area
1.	Standard -1	8	179.9
2.	Standard -2	8	179.5
3.	Standard -3	8	187.6
4.	Standard -4	8	180.7
5.	Standard -5	8	181.4
		Mean	**181.8**
		Standard deviation	**3.3131**
		RSD in %	**1.82**

Table 8: System suitability parameters for Propylparaben

S.No.	System suitability parameters	Propylparaben
1.	Retention time	17.54 min
2.	Number of theoretical plate	12704
3.	Peak area response	181.82

Specificity

The specificity of the HPLC method is illustrated in Fig. 5, where complete separation of Ambroxol hydrochloride, Cetirizine hydrochloride, Methylparaben and Propylparaben were noticed in presence of other inactive excipients used in liquid formulation. In addition, there was no any interference at the retention time of in the chromatogram

of placebo solution. In peak purity analysis with PDA, purity angle was always less than purity threshold for the analyte. This shows that the peaks of analyte were pure and excipients in the formulation does not interfere the analyte.

Table 9: Specificity for Ambroxol hydrochloride

S.No.	Name	No. of Injections	Area
1.	Blank	1	Nil
2.	Placebo	1	Nil
3.	Standard	1	2714.98
4.	Sample	1	2904.93

Table 10: Specificity for Cetirizine hydrochloride

S.No.	Name	No. of Injections	Area
1.	Blank	1	Nil
2.	Placebo	1	Nil
3.	Standard	1	390.13
4.	Sample	1	389.63

Table 11: Specificity for Methylparaben

S.No.	Name	No. of Injections	Area
1.	Blank	1	Nil
2.	Placebo	1	Nil
3.	Standard	1	1044.04
4.	Sample	1	1114.87

Table 12: Specificity for Propylparaben

S.No.	Name	No. of Injections	Area
1.	Blank	1	Nil
2.	Placebo	1	Nil
3.	Standard	1	426.27
4.	Sample	1	484.57

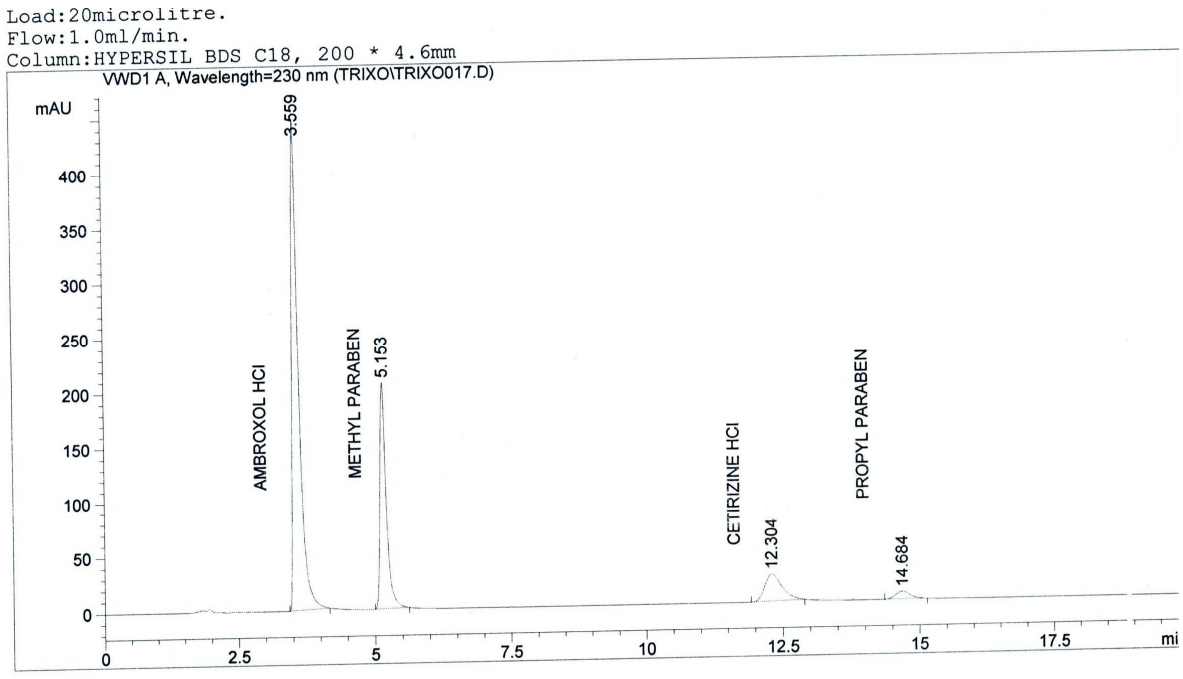

Figure 5: Typical HPLC chromatogram of Sample Syrup
(ambroxol hydrochloride, cetirizine hydrochloride, methylparaben and propylparaben)

Linearity and Range

The Linearity of this method was determined at five levels from 80%– 120% of operating concentrations for Ambroxol hydrochloride, Cetirizine hydrochloride, Methylparaben and Propylparaben. The plot of peak area of each sample against respective concentration of Ambroxol hydrochloride, Cetirizine hydrochloride, Methylparaben and Propylparaben were found to be linear (Fig. 6, 7, 8 and 9) in the range of 80%– 120% of operating

concentrations. Beer's law was found to be obeyed over this concentration range. The linearity was evaluated by linear regression analysis using least square method. The regression equations were found to be Y= 0.071x – 43.2 , Y= 34.965 x – 31.5, Y= 25.176 x +21.5 and Y= 19.425 x + 2.48 for Ambroxol hydrochloride Cetirizine hydrochloride, Methylparaben and Propylparaben respectively and correlation coefficient of the standard curves were found to be 0.9970, 0.9957, 0.9961 and 0.9915 for Ambroxol hydrochloride Cetirizine hydrochloride, Methylparaben and Propylparaben respectively . It observed that correlation coefficient and regression analysis are with in the limits.

Figure 6: Linearity of response for Ambroxol hydrochloride

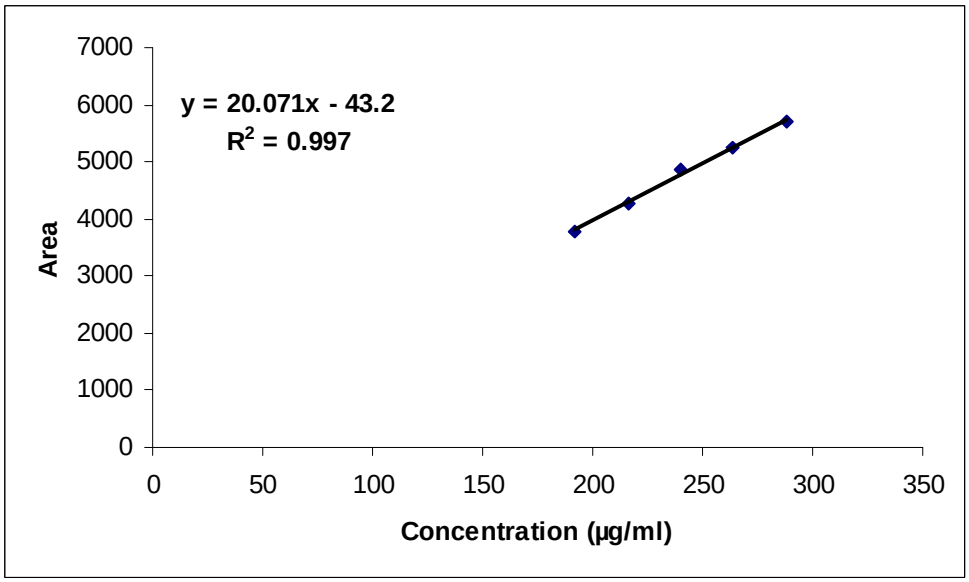

Figure 7: Linearity of response for Cetirizine hydrochloride

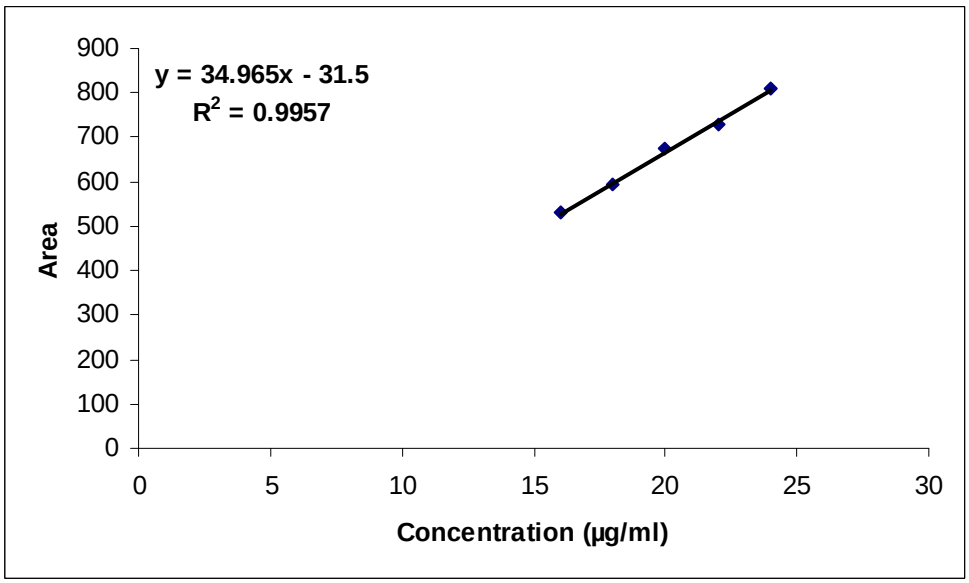

Figure 8: Linearity of response for Methylparaben

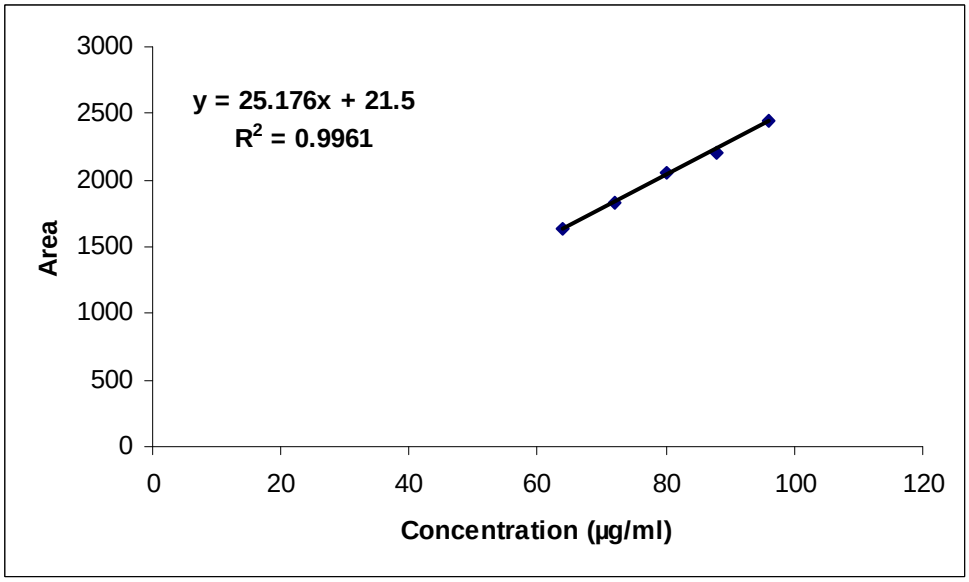

Figure 9: Linearity of response for Propylparaben

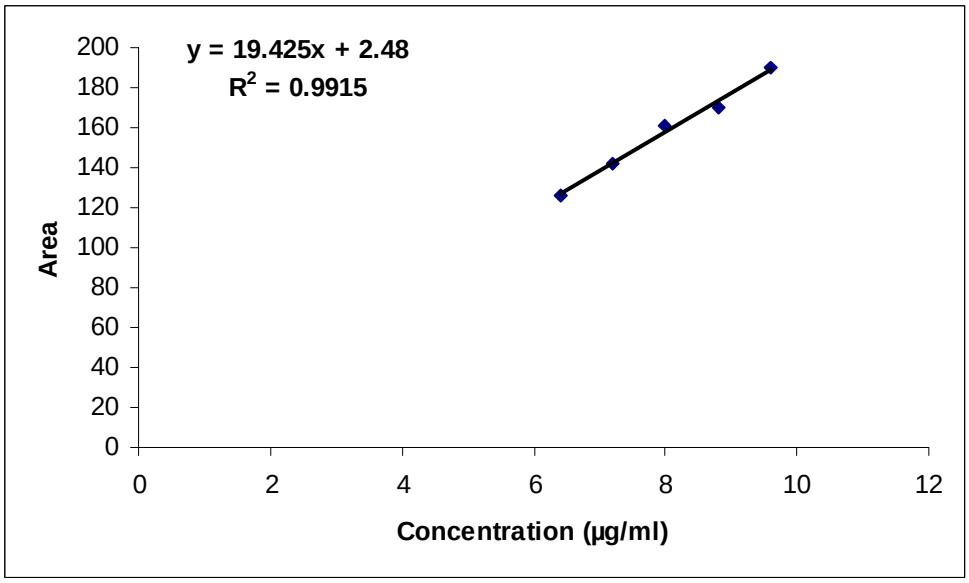

Table13: Accuracy for Ambroxol hydrochloride

S.No.	Sample Weight (g)	Area	Amount recovered (mg/5ml)	Recovery (%)
1.	12.6015	3815.8	23.96	99.79
2.	12.6037	3821.8	23.99	99.92
3.	12.6104	3824.6	24.00	99.96
4.	12.3115	4606.3	29.61	99.16
5.	12.3246	4609.2	29.59	99.10
6.	12.3514	4612.8	29.55	98.96
7.	12.3541	5515.2	35.32	98.36
8.	12.3604	5542.4	35.47	98.77
9.	12.3717	5534.5	35.39	98.55
			Mean	**99.17**
			Standard deviation	**0.5937**
			RSD in %	**0.60**

Accuracy

Accuracy of the method was found out by recovery study by standard addition method. The known amounts of standards, Ambroxol hydrochloride, Cetirizine hydrochloride, Methylparaben and Propylparaben were added to pre-

analysed samples at a level from 80% up to 120% and then subjected to the proposed HPLC method individually. The results of recovery studies were shown in Table 13, 14, 15 and 16. It was observed that the mean percentage recoveries were found to be for Ambroxol hydrochloride, Cetirizine hydrochloride, Methylparaben and Propylparaben, respectively which demonstrated that the method was highly accurate.

Table 14: Accuracy for Cetirizine hydrochloride

S.No.	Sample Weight (g)	Area	Amount recovered (mg/5ml)	Recovery (%)
1.	12.6015	596.4	2.04	100.99
2.	12.6037	596.5	2.04	100.99
3.	12.6104	600.2	2.05	101.49
4.	12.3115	729.4	2.55	100.79
5.	12.3246	730.5	2.55	100.79
6.	12.3514	733.2	2.56	101.19
7.	12.3541	874.3	3.04	100.00
8.	12.3604	871.1	3.05	100.33
9.	12.3717	880.9	3.07	100.99
			Mean	**100.84**
			Standard deviation	**0.4448**
			RSD in %	**0.44**

Table 15: Accuracy for Methylparaben

S.No.	Sample Weight (g)	Area	Amount recovered (mg/5ml)	Recovery (%)
1.	12.6015	1887.9	8.13	100.37
2.	12.6037	1887.4	8.12	100.25
3.	12.6104	1890.5	8.13	100.37
4.	12.3115	2277.0	10.04	100.40
5.	12.3246	2276.4	10.02	100.20
6.	12.3514	2276.4	10.00	100.00
7.	12.3541	2719.0	11.94	99.67
8.	12.3604	2717.9	11.93	99.58
9.	12.3717	2725.0	11.95	99.75
			Mean	**100.06**
			Standard deviation	**0.3250**
			RSD in %	**0.32**

Table 16: Accuracy for Propylparaben

S.No.	Sample Weight (g)	Area	Amount recovered (mg/5ml)	Recovery (%)
1.	12.6015	161.9	0.83	100.61
2.	12.6037	162.9	0.84	101.82
3.	12.6104	161.8	0.83	100.61
4.	12.3115	199.0	1.05	100.96
5.	12.3246	198.7	1.04	100.00
6.	12.3514	200.4	1.05	100.96
7.	12.3541	238.4	1.25	100.81
8.	12.3604	238.1	1.25	100.81
9.	12.3717	238.8	1.25	100.81
			Mean	**100.82**
			Standard deviation	**0.4743**
			RSD in %	**0.47**

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the homogenous sample under the prescribed conditions.

Reproducibility

It examines the precision between laboratories and is often determined in collaborative studies. Reproducibility data for Ambroxol hydrochloride, Cetirizine hydrochloride, Methylparaben and Propylparaben were shown in Table 17, 18, 19 and 20. This indicated that method was highly precise.

Table 17: Precision - Reproducibility for Ambroxol hydrochloride

S.No.	Sample Name	Concentration (µg/ml)	Area
1.	Standard -1	240	4740.4
2.	Standard -2	240	4766.2
3.	Standard -3	240	4735.7
4.	Standard -4	240	4734.5
5.	Standard -5	240	4733.7
6.	Standard -6	240	4732.4
		Mean	**4740.48**
		Standard deviation	**12.8948**
		RSD in %	**0.27**

Table 18: Precision - Reproducibility for Cetirizine hydrochloride

S.No.	Sample Name	Concentration (µg/ml)	Area
1.	Standard -1	20	618.8
2.	Standard -2	20	623.2
3.	Standard -3	20	619.4
4.	Standard -4	20	618.7
5.	Standard -5	20	621.3
6.	Standard -6	20	617.6
		Mean	**619.83**
		Standard deviation	**2.0500**
		RSD in %	**0.33**

Table 19: Precision - Reproducibility for Methylparaben

S.No.	Sample Name	Concentration (µg/ml)	Area
1.	Standard -1	80	2019.9
2.	Standard -2	80	2029.1
3.	Standard -3	80	2017.0
4.	Standard -4	80	2014.4
5.	Standard -5	80	2015.6
6.	Standard -6	80	2014.0
		Mean	**2018.33**
		Standard deviation	**5.6898**
		RSD in %	**0.28**

Table 20: Precision - Reproducibility for Propylparaben

S.No.	Sample Name	Concentration (µg/ml)	Area
1.	Standard -1	8	155.8
2.	Standard -2	8	155.5
3.	Standard -3	8	153.3
4.	Standard -4	8	154.4
5.	Standard -5	8	155.4
6.	Standard -6	8	154.8
		Mean	**154.86**
		Standard deviation	**0.9201**
		RSD in %	**0.59**

Repeatability

Repeatability is the precision of a method under the same operating conditions over a short period of time. One aspect of this is instrumental precision. A second aspect is sometimes termed intra-assay precision and involves multiple measurements of the same sample by the same analyst under the same conditions. Repeatability data for Ambroxol hydrochloride, Cetirizine hydrochloride, Methylparaben and Propylparaben were shown in Table 21, 22, 23 and 24. This indicated that method was highly precise.

Table 21: Precision - Repeatability for Ambroxol hydrochloride

S.No.	Sample Name	Wt.taken (g)	No. of reading	Area	Amount (mg/ 5ml)
1.	Sample -1	12.7011	1	5102.4	31.39
2.	Sample -2	12.6946	1	5101.2	31.39
3.	Sample -3	12.7034	1	5109.1	31.42
4.	Sample -4	12.6846	1	5068.8	31.22
5.	Sample -5	12.6804	1	5043.5	31.07
6.	Sample -6	12.7287	1	5119.5	31.42
				Mean	**31.31**
				Standard deviation	**0.1430**
				RSD in %	**0.46**

Table 22: Precision - Repeatability for Cetirizine hydrochloride

S.No.	Sample Name	Wt.taken (g)	No. of reading	Area	Amount (mg/ 5ml)
1.	Sample -1	12.7011	1	688.6	2.62
2.	Sample -2	12.6946	1	684.2	2.60
3.	Sample -3	12.7034	1	682.1	2.59
4.	Sample -4	12.6846	1	680.4	2.59
5.	Sample -5	12.6804	1	682.4	2.60
6.	Sample -6	12.7287	1	686.4	2.60
				Mean	**2.60**
				Standard deviation	**0.0109**
				RSD in %	**0.42**

Table 23: Precision - Repeatability for Methylparaben

S.No.	Sample Name	Wt.taken (g)	No. of reading	Area	Amount (mg/ 5ml)
1.	Sample -1	12.7011	1	2197.9	10.09
2.	Sample -2	12.6946	1	2201.5	10.11
3.	Sample -3	12.7034	1	2196.3	10.08
4.	Sample -4	12.6846	1	2195.5	10.09
5.	Sample -5	12.6804	1	2183.8	10.04
6.	Sample -6	12.7287	1	2211.6	10.13
				Mean	**10.09**
				Standard deviation	**0.0303**
				RSD in %	**0.30**

Table 24: Precision - Repeatability for Propylparaben

S.No.	Sample Name	Wt.taken (g)	No. of reading	Area	Amount (mg/ 5ml)
1.	Sample -1	12.7011	1	164.9	1.01
2.	Sample -2	12.6946	1	165.0	1.01
3.	Sample -3	12.7034	1	166.0	1.02
4.	Sample -4	12.6846	1	163.2	1.00
5.	Sample -5	12.6804	1	164.4	1.01
6.	Sample -6	12.7287	1	166.0	1.01
				Mean	**1.01**
				Standard deviation	**0.0663**
				RSD in %	**0.63**

Robustness

Measure of method's capacity to remain unaffected by small, but deliberate variations in method.

Change in the ratio of solvents in the mobile phase (± 2.0 %)

Two sample preparations were analyzed as per the methodology by changing the ratio of solvents in the mobile phase by means of ± 2.0 %. The robustness data Ambroxol hydrochloride, Cetirizine hydrochloride, Methylparaben and Propylparaben by changing the ratio of solvents in the mobile phase by ± 2.0 % was shown in Table 25, 26, 27 and 28. It was observed that there were no marked changes in the chromatograms, which demonstrated that the proposed method was robust.

Table 25: Robustness - Change in the ratio of solvents in the mobile phase (± 2%) for Ambroxol hydrochloride

S.No.	Sample Name	Wt. taken (g)	Area (Buffer: Acetonitrile)		Amount (mg/5ml) Buffer: Acetonitrile)	
			69:31	65:35	69:31	65:35
1.	Sample -1	12.4014	4767.8	4771.8	31.22	31.21
2.	Sample -2	12.7135	4901.6	4776.1	31.31	31.25
				Mean	**31.26**	**31.23**
				Standard deviation	**0.0636**	**0.0282**
				RSD in %	**0.20**	**0.09**

Table 26: Robustness -Change in the ratio of solvents in the mobile phase (± 2%) for Cetirizine hydrochloride

S.No.	Sample Name	Wt. taken (g)	Area (Buffer: Acetonitrile)		Amount (mg/5ml) Buffer: Acetonitrile)	
			69:31	65:35	69:31	65:35
1.	Sample -1	12.4014	682.5	787.0	2.60	2.64
2.	Sample -2	12.7135	701.5	785.6	2.60	2.63
				Mean	**2.60**	**2.63**
				Standard deviation	**0.0**	**0.0070**
				RSD in %	**0.0**	**0.27**

Table 27: Robustness - Change in the ratio of solvents in the mobile phase (±2%) for Methylparaben

S.No.	Sample Name	Wt. taken (g)	Area (Buffer: Acetonitrile)		Amount (mg/5ml) Buffer: Acetonitrile)	
			69:31	65:35	69:31	65:35
1.	Sample -1	12.4014	2341.9	2385.3	10.06	9.98
2.	Sample -2	12.7135	2409.2	2385.4	10.09	9.99
				Mean	**10.07**	**9.98**
				Standard deviation	**0.0212**	**0.0070**
				RSD in %	**0.21**	**0.07**

Table 28: Robustness -Change in the ratio of solvents in the mobile phase (±2%) for Propylparaben

S.No.	Sample Name	Wt. taken (g)	Area (Buffer: Acetonitrile)		Amount (mg/5ml) (Buffer: Acetonitrile)	
			69:31	65:35	69:31	65:35
1.	Sample -1	12.4014	189.4	207.6	1.00	1.01
2.	Sample -2	12.7135	196.0	207.2	1.01	1.01
				Mean	**1.00**	**1.01**
				Standard deviation	**0.0070**	**0.0**
				RSD in %	**0.70**	**0.0**

Ruggedness

Six sample preparations were analyzed as per the methodology by a different analyst on a different instrument on a different day. The robustness data Ambroxol hydrochloride, Cetirizine hydrochloride, Methylparaben and Propylparaben were shown in Table 29, 30, 31 and 32. It was observed that there were no marked changes in the chromatograms, which demonstrated that the proposed method was rugged.

Table 29: Ruggedness data for Ambroxol hydrochloride -Change of analyst

S.No.	Sample Name	Wt. taken (g)	Area	Amount (mg/5ml)
1.	Sample -1	12.7015	4685.2	31.24
2.	Sample -2	12.7048	4690.2	31.27
3.	Sample -3	12.7331	4702.3	31.28
4.	Sample -4	12.7240	4725.0	31.45
5.	Sample -5	12.7181	4707.3	31.35
6.	Sample -6	12.7145	4713.3	31.40
			Mean	**31.33**
			Standard deviation	**0.0823**
			RSD in %	**0.26**

Table 30: Ruggedness data for Cetirizine hydrochloride -Change of analyst

S.No.	Sample Name	Wt. taken (g)	Area	Amount (mg/5ml)
1.	Sample -1	12.7015	782.5	2.63
2.	Sample -2	12.7048	773.6	2.60
3.	Sample -3	12.7331	779.8	2.62
4.	Sample -4	12.7240	787.8	2.64
5.	Sample -5	12.7181	779.9	2.62
6.	Sample -6	12.7145	779.8	2.62
			Mean	**2.62**
			Standard deviation	**0.0132**
			RSD in %	**0.51**

Table 31: Ruggedness data for Methylparaben -Change of analyst

S.No.	Sample Name	Wt. taken (g)	Area	Amount (mg/5ml)
1.	Sample -1	12.7015	2358.2	10.03
2.	Sample -2	12.7048	2360.3	10.04
3.	Sample -3	12.7331	2365.2	10.04
4.	Sample -4	12.7240	2376.2	10.09
5.	Sample -5	12.7181	2366.6	10.05
6.	Sample -6	12.7145	2367.1	10.06
			Mean	**10.05**
			Standard deviation	**0.0213**
			RSD in %	**0.21**

Table 32: Ruggedness data for Propylparaben -Change of analyst

S.No.	Sample Name	Wt. taken (g)	Area	Amount (mg/5ml)
1.	Sample -1	12.7015	206.5	1.01
2.	Sample -2	12.7048	206.8	1.01
3.	Sample -3	12.7331	204.7	1.00
4.	Sample -4	12.7240	205.1	1.00
5.	Sample -5	12.7181	206.2	1.01
6.	Sample -6	12.7145	206.2	1.00
			Mean	**1.00**
			Standard deviation	**0.0054**
			RSD in %	**0.54**

CONCLUSION

The Proposed study describes new and simple RP-HPLC method for the simultaneous estimation of Ambroxol hydrochloride, Cetirizine hydrochloride, Methylparaben and Propylparaben in combined liquid dosage form. The method was validated as per ICH guidelines and found to be simple, sensitive, accurate and precise. Therefore the proposed method can be successfully used for the routine analysis of simultaneous estimation of ambroxol hydrochloride, cetirizine hydrochloride, methylparaben and propylparaben in combined liquid dosage form without interference.

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