IJPSR (2013), Vol. 4, Issue 8 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 28 March, 2013; received in revised form, 04 May, 2013; accepted, 26 July, 2013; published, 01 August, 2013

SIMULTANEOUS QUANTITATION AND VALIDATION OF PHENYLEPHRINE HYDRO-CHLORIDE, AMBROXOL HYDROCHLORIDE AND LEVOCITRIZINE HYDROCHLORIDE IN SYRUP FORMULATION BY REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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Keywords:

Phenylephrine hydrochloride, Ambroxol hydrochloride, Levocetirizine hydrochloride, RP-HPLC, Simultaneous quantification, Validation

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ABSTRACT: A simple, precise and accurate RP-HPLC method was developed and validated for the estimation of Phenylephrine hydrochloride, Ambroxol hydrochloride and Levocetirizine hydrochloride in syrup formulation. This method is based on HPLC separation of the three drugs on Octadecyl Silane C18 column (250 mm × 4.6 mm, 5.0µ), with isocratic conditions and mobile phase containing 0.01M Sodium dihydrogen phosphate monohydrate buffer [pH 3.0, adjusted with Ortho Phosphoric Acid and 1.1 gm of Octane sulfonic acid sodium salt]: Acetonitrile: Methanol (60:30:10) at a flow rate of 1 ml/min, using UV detection at 230 nm. The retention time of Phenylephrine hydrochloride, Ambroxol hydrochloride and Levocetirizine hydrochloride in syrup formulation were found to be around 3.47 min, 11.8 min and 32.6 min respectively. The method was validated for linearity, accuracy, precision, ruggedness, robustness and solution stability as per ICH guidelines. The linearity of Phenylephrine hydrochloride, Ambroxol hydrochloride, Levocetirizine hydrochloride solutions ranged from 0.104 mg/ml to 0.303 mg/ml; 0.6 mg/ml to 1.804 mg/ml and 0.051 mg/ml to 0.150 mg/ml, respectively, equivalent to 50% to 150% of the working concentration. Statistical analysis showed that the proposed method is repeatable and selective for the estimation of Phenylephrine hydrochloride, Ambroxol hydrochloride and Levocitrizine hydrochloride in syrup formulation.

INTRODUCTION: The chemical name of Phenylephrine hydrochloride is (R)-(α)-m-Hydroxy-α-[(methyl amino) methyl] benzyl alcohol hydrochloride. The structure of Phenylephrine hydrochloride is given in the **Fig.1**. Phenylephrine acts predominantly by direct stimulation o alpha adrenergic receptors, which constrict resistance and capacitance of blood vessels, resulting in increased total peripheral resistance: increased systolic and diastolic blood pressure.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.4(8).3028-38

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.4(8).3028-38

Alpha-adrenergic effects include action on the dilator muscle of the pupil and local decongestant action in the arterioles.

The decongestant works by constricting blood vessels and reducing swelling in the nasal passages. Phenylephrine does not stimulate beta receptors except in large doses.

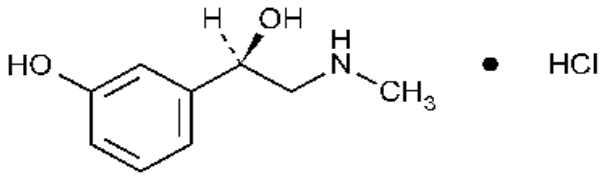


FIG.1: STRUCTURE OF PHENYLEPHRINE HYDROCHLORIDE

Chemically Ambroxol hydrochloride **(Fig. 2)** is *Trans*-4-(2-Amino-3, 5-dibrombenzylamino)-cyclo hexanol Hydrochloride. Ambroxol is an active N-desmethyl metabolite of the mucolytic bromhexine. Although its mechanism of action has not been fully defined, it may increase the quantity and decrease the viscosity of tracheobronchial secretions. It may also act as an expectorant, increasing mucociliary transport via stimulation of cilliary motility.

Ambroxol may stimulate the synthesis and secretion of pulmonary surfactant; the drug has been referred to as a "surfactant activator". It was postulated that ambroxol decreased airway hyper-reactivity by either increasing lysophosphatidyl-choline turnover and/or modifying epithelial secretions [1, 2, 3].

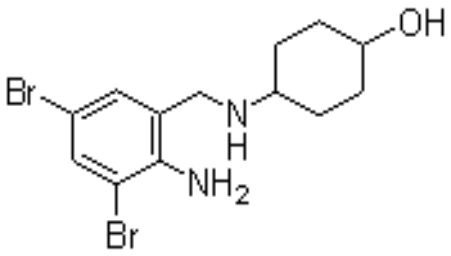


FIG. 2: STRUCTURE OF AMBROXOL

Levocetirizine, the (R) enantiomer of cetirizine **(Fig. 3)**, is a potent and selective antagonist of peripheral H1-receptors. Binding studies revealed that levocetirizine has high affinity for human H1-receptors (Ki = 3.2 nmol/l). Levocetirizine has an affinity 2-fold higher than that of cetirizine (Ki = 6.3 nmol/l). Levocetirizine dissociates from H1-receptors with a half-life of 115 ± 38 min. After single administration, levocetirizine shows receptor occupancy of 90% at 4 hours and 57% at 24 hours. The onset of action of levocetirizine 5 mg in controlling pollen-induced symptoms has been observed at 1 hour post drug intake in placebo controlled trials in the model of the allergen challenge chamber [3, 4, 5].

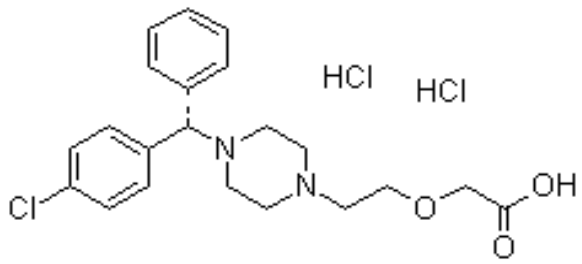


FIG. 3: STRUCTURE OF LEVOCETIRIZINE HYDROCHLORIDE

Literature review reveals that methods have been reported for analysis of phenylephrine hydrochloride, ambroxol hydrochloride and levocitrizine hydrochloride either alone or in combination with other drugs [6-18].

To date, there have been no published reports about the simultaneous quantitation of phenylephrine hydrochloride, ambroxol hydrochloride and levocitrizine hydrochloride by chromatographic method in syrup formulation.

This present study reports for the first time simultaneous quantitation of the same drugs by RPHPLC in syrup formulation dosage form. The proposed method is validated as per ICH guidelines [19].

MATERIALS AND METHODS: Working standards of pharmaceutical grade Phenylephrine hydrochloride, Ambroxol hydrochloride and Levocitrizine hydrochloride were obtained from Malladi Drugs, Alchymar ICM Sm Pvt. Ltd. and Auctus Pharma respectively. They were used without further purification and certified to contain 98.5-100.5%, 99.0-101.0% and 98.0-102.0% on dry weight basis for Phenylephrine hydrochloride, Ambroxol hydrochloride and Levocitrizine hydrochloride, respectively. All the chemicals were of HPLC grade. Water used was double distilled and filtered through 0.45µm filter.

Instrumentation: The HPLC system consisted of Intelligent HPLC pump model (Shimadzu Prominence) with sampler programmed at 10µl capacity per injection. The detector consisted of a UV/ VIS (SPD-20A/20AV Series). Data was integrated using LC Solution system. The column used was, Octadecyl Silane C18 column (250 mm × 4.6 mm, 5.0µ), with isocratic conditions.

Mobile phase containing 0.01M Sodium dihydrogen phosphate monohydrate buffer [pH 3.0, adjusted with Ortho Phosphoric Acid and 1 gm of Octane sulfonic acid sodium salt]: Acetonitrile: Methanol (60:30:10) at a flow rate of 1 ml/min, using UV detection at 230 nm. The mobile phase was filtered through a 0.45 micron membrane filter and degassed. The injection volume was 10µl and analysis was performed at ambient temperature.

Preparation of Standard Solution: About 20 mg of Phenylephrine hydrochloride, 120 mg of Ambroxol hydrochloride and 10 mg of Levocitrizine hydrochloride working standards were dissolved in 10ml of diluents in three separate 100ml volumetric flask and sonicated, then volume was made up to the mark with diluents.

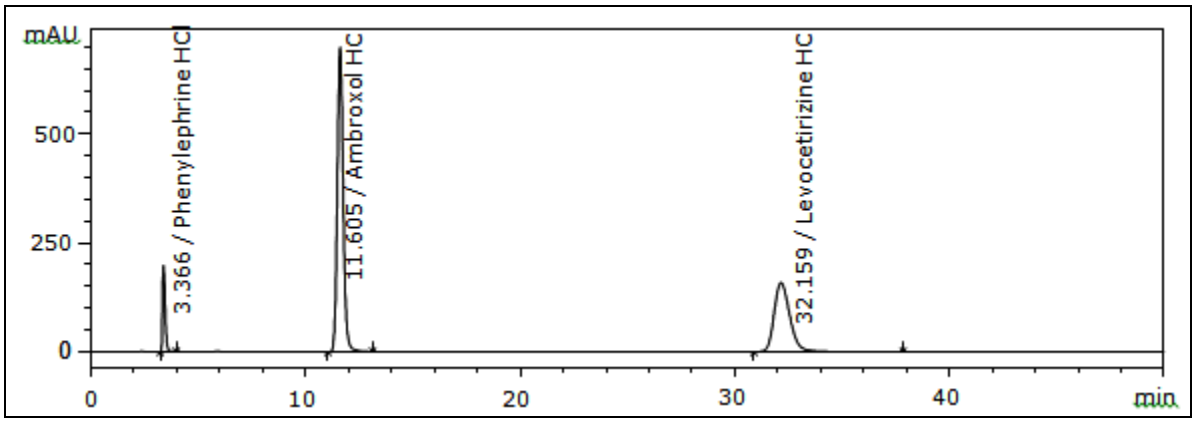
Preparation of Mixed Standard Solution: About 20 mg of Phenylephrine hydrochloride, 120 mg of Ambroxol hydrochloride and 10 mg of Levocitrizine hydrochloride working standards were dissolved in 10ml of diluents in a 100ml volumetric flask and sonicated, then volume was made up to the mark with diluents.

Preparation of Sample Solution: About 5 g of syrup was dissolved in 10ml of diluents in a 25ml volumetric flask and sonicated, then volume was made up to the mark with diluents.

TABLE 1: SUMMARY OF CHROMATOGRAPHIC CONDITION

Parameters	Condition
Column	Octadecyl Silane C18 (250 mm × 4.6 mm, 5.0µ)
Column temperature	30°C
Flow rate	1ml/min
Injection volume	10µl
Detection wavelength	230nm
Buffer	Phosphate buffer with pH 3.0
Mobile phase	Buffer: Acetonitrile: Methanol (60:30:10)
Diluents	Mobile phase

Optimization of HPLC Method: All drugs were subjected to chromatographic analysis using mobile phases of differing pH at mentioned chromatographic conditions **(Table 1)**. The changes in the retention time of all drugs were noted as a function of changing mobile phase, pH, strength and selectivity. After all the trials it was found that Sodium dihydrogen phosphate monohydrate buffer (pH 3 adjusted with orthophosphoric acid and 1.1g of octane sulfonic acid sodium salt) : Acetonitrile : Methanol in the ratio of (60:30:10) at flow rate of 10 µl/min gave acceptable retention time of 3.366, 11.605 and 32.159 with number of plates (N) 3058, 8502 and 9721 and good resolution (22.06 between Phenylephrine Hydrochloride and Ambroxol Hydrochloride, 22.74 between Ambroxol Hydrochloride and Levocetirizine Hydrochloride) for Phenylephrine hydrochloride, Ambroxol hydrochloride and Levocitrizine hydrochloride, respectively **(Fig. 4)**.



Name	Ret. Time	Area	Area %	Tailing	Resolution	Theoretical Plate#
Phenylephrine HCl	3.366	1730649	7.50	1.59	0.00	3058
Ambroxol HCl	11.605	13386263	57.99	1.13	22.06	8502
Levocetirizine HCl	32.159	7965633	34.51	1.26	22.74	9721

FIG. 4: CHROMATOGRAM OF PHENYLEPHRINE HCL (RT =3.366), AMBROXOL HCL (RT =11.605) AND LEVOCETIRIZINE HCL (RT =32.159)

Validation of the method: Validation of the optimized HPLC method was carried out with respect to the following parameters.

1. **Specificity:** Specificity was analyzed with the placebo (200mg/ml) and the drug substance [Phenylephrine hydrochloride (0.40mg/ml), Ambroxol hydrochloride (1.20mg/ml), Levocetirizine hydrochloride (0.40mg/ml)] separately at different concentrations.

2. **System Suitability:** A standard solution was prepared containing 0.2 mg/ml Phenylephrine hydrochloride, 1.2 mg/ml Ambroxol hydrochloride, 0.1 mg/ml Levocetirizine hydrochloride. Percentage relative standard deviation, tailing factor and theoretical plates of the Phenylephrine hydrochloride, Ambroxol hydrochloride, Levocetirizine hydrochloride peak in standard solution was calculated.

3. **Linearity and Range:** Linearity was performed by injecting the standard solution in duplicate ranging from 50% to 150% of working concentration of Phenylephrine hydrochloride, Ambroxol hydrochloride, and Levocetirizine hydrochloride by covering at least six points. The regression analysis was performed and the linearity range for determining assay was reported.

4. **Accuracy:** The accuracy of the method was determined by analyzing the solutions containing Phenylephrine hydrochloride, Ambroxol hydrochloride and Levocetirizine hydrochloride at approximately 50%, 100% and 150% of the working strengths spiked with placebo. Each level was analyzed in triplicate.

5. **System Precision:** System precision was performed by injecting the standard solution (0.2 mg/ml Phenylephrine hydrochloride, 1.2 mg/ml Ambroxol hydrochloride and 0.1 mg/ml Levocetirizine hydrochloride in diluents) in six times and the percentage relative standard deviation of six replicate injections of standards were determined.

6. **Method Precision:** The method precision was performed by analyzing a sample solution of Phenylephrine hydrochloride, Ambroxol hydrochloride, Levocetirizine hydrochloride at working concentration six times (six replicate sample preparation).

7. **Ruggedness (Intermediate Precision):** The ruggedness of the method was determined by analyzing a standard solution five times followed by a sample in duplicate with varying following parameters **(Table 2)**:

TABLE 2: PARAMETERS FOR OPTIMIZING INTERMEDIATE PRECISION

Parameter	1st Set	2nd Set
Analyst to analyst	Analyst-1	Analyst-2
Column to column	Column-1	Column-2
Reagent to reagent	Reagent-1	Reagent-1
Day to day	Day-1	Day-2
Instrument to instrument	Instrument-1	Instrument-2

8. **Robustness:** The robustness of the method was determined by analyzing a standard solution five times followed by a sample in duplicate with varying HPLC conditions as described below **(Table 3)**:

TABLE 3: HPLC CONDITIONS FOR DETERMINING THE ROBUSTNESS OF THE METHOD

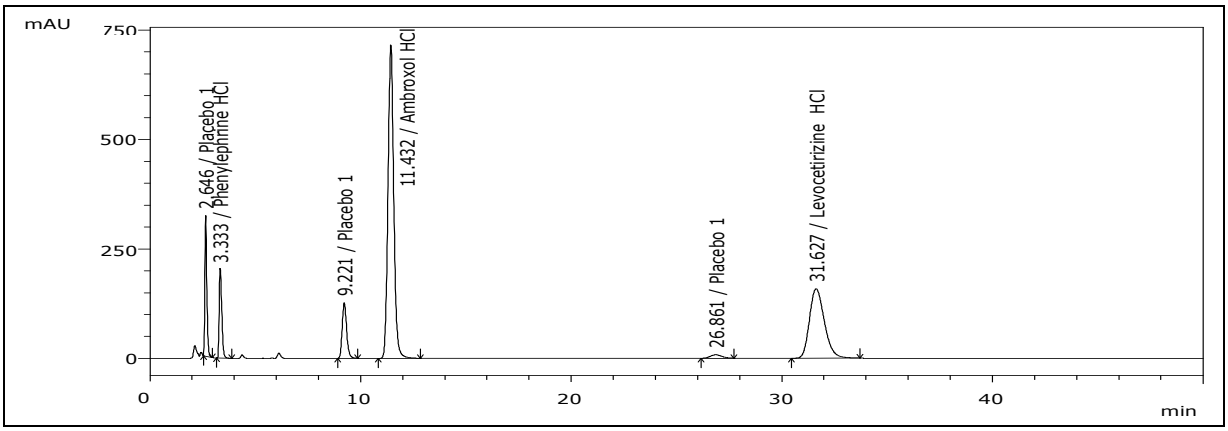
Property	Variation of flow rate	Variation of mobile phase composition		Variation of buffer pH
		Buffer	Methanol + Acetonitrile	
Actual	1ml/min	60%	40%	3.0
Low	0.9ml/min	58%	42%	2.9
High	1.1ml/min	62%	38%	3.1

9. **Solution Stability:** The stability of Phenylephrine hydrochloride, Ambroxol hydrochloride, Levocetirizine hydrochloride sample was measured at 100% of the target concentration by keeping the solution up to forty-eight hours at room temperature. The sample was analyzed at different intervals (12hrs, 24hrs, 48hrs), using freshly standards every time.

RESULTS AND DISCUSSION: The results of validation studies on simultaneous estimation method developed for Phenylephrine hydrochloride, Ambroxol hydrochloride and Levocitrizine hydrochloride in the current study

involving mobile phase Sodium dihydrogen phosphate monohydrate buffer (pH 3 adjusted with OPA) : Acetonitrile : Methanol in the ratio of (60:30:10) at flow rate of 10 µl/min are given below;

1. **Specificity:** Placebo solution was prepared separately at a concentration of 200mg/ml. A solution of placebo was spiked with Phenylephrine hydrochloride, Ambroxol hydrochloride, and Levocetirizine hydrochloride at working concentration. A mixed standard solution was prepared and these solutions were analyzed as per the HPLC method described in the protocol. The study shows that the placebo and API were accurately resolved **(Fig. 5)**.



Name	Retention time (minutes)	Resolution
Placebo1	2.646	0.00
Placebo2	9.221	18.81
Placebo3	26.861	20.42
Phenylephrine HCl	3.333	3.22
Ambroxol HCl	11.432	5.01
Levocetirizine HCl	31.627	4.15

FIG. 5: CHROMATOGRAM OF PLACEBO (200mg/ml) WITH PHENYLEPHRINE HCl (0.40mg/ml), AMBROXOL HCl (1.20mg/ml) AND LEVO-CETIRIZINE HCl (0.40mg/ml)

2. **System Suitability:** Phenylephrine hydrochloride, Ambroxol hydrochloride, Levocetirizine hydrochloride at working concentration was prepared and analyzed as per the HPLC method described in the protocol. **Table 4** shows the %RSD, tailing factor and theoretical plate of the peaks.

TABLE 4: SYSTEM SUITABILITY STUDIES

Retention time (min)			Peak area (mAu)			Tailing factor			Theoretical plate		
PEH	**AMBH**	**LCZH**	**PEH**	**AMBH**	**LCZH**	**PEH**	**AMBH**	**LCZH**	**PEH**	**AMBH**	**LCZH**
3.340	11.412	31.626	886524	13565354	2082682	1.485	1.117	1.187	3139	8484	9997
3.342	11.412	31.617	893134	13711577	2092803	182.4	1.114	1.175	3143	8554	10062
3.337	11.739	31.914	918252	13906971	2128239	1.539	1.111	1.189	3042	9180	10172
3.339	11.410	31.603	916542	14057769	2130409	1.495	1.107	1.166	3070	8577	10035
3.340	11.413	31.603	923201	14252676	2187226	1.493	1.105	1.190	3050	8595	9956
3.337	11.418	31.616	929227	14190768	2124022	1.502	1.088	1.135	3010	8614	10213
	Average		**911147**	**13947519**	**2124230**						
	%RSD		**1.89**	**1.945**	**1.728**						

3. **Linearity and Range:** The linearity of Phenylephrine hydrochloride, Ambroxol hydrochloride, Levocetirizine hydrochloride solutions were ranging from 0.104 mg/ml to 0.303 mg/ml; 0.6 mg/ml to 1.804 mg/ml and 0.051 mg/ml to 0.150 mg/ml, respectively which is equivalent to 50% to 150% of the working concentration. Five standard solutions at concentration within the mentioned range were prepared and analyzed as per method. The linearity results obtained are shown in the **Table 5 and Figure 6-8**, the line of best fit for

peak area versus weight of Phenylephrine hydrochloride, Ambroxol hydrochloride and Levocetirizine hydrochloride respectively. The HPLC method was shown to be linear of 50% to 150% of the working standards concentration with a regression coefficient 0.999. The range of the HPLC method for determining the assay is 50% to 150% of the working strength.

TABLE 5: LINEARITY STUDIES

%Level	Weight taken to working concentration			Concentration (mg/ml)			Peak area (mAu)		
	PHEN	AMBH	LCZH	PHEN	AMBH	LCZH	PHEN	AMBH	LCZH
50	10.40	60.00	5.10	0.104	0.600	0.051	427332	6391232	946894.5
75	15.30	90.30	7.50	0.153	0.903	0.075	623110.5	9482403	1339020
100	20.20	120.40	10.30	0.202	1.204	0.103	808016.5	12549979	1847244
125	25.50	150.30	12.70	0.255	1.503	0.127	1018744	15738131	2319743
150	30.30	180.40	15.00	0.303	1.804	0.150	1228502	19204839	2732228

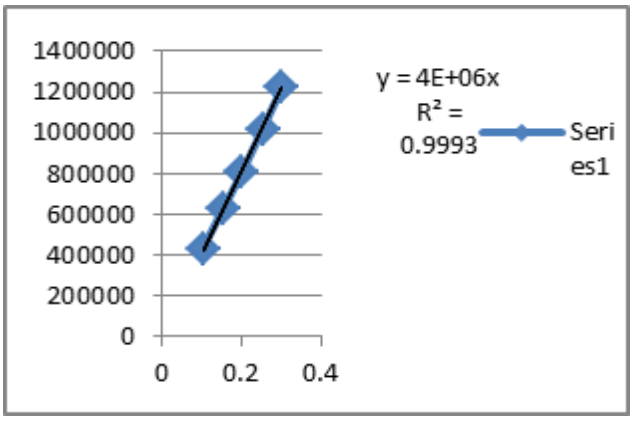


FIG. 6: LINEARITY GRAPH OF PHENYLEPHRINE

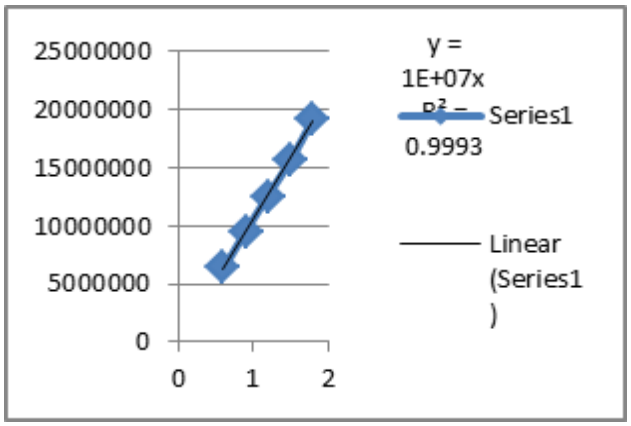


FIG. 7: LINEARITY GRAPH OF AMBROXOL

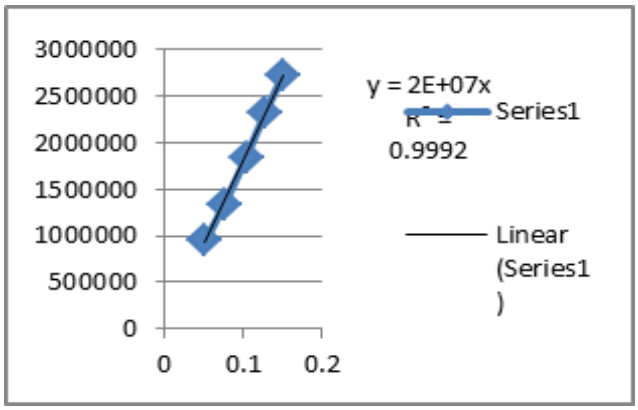


FIG. 8: LINEARITY GRAPH OF LEVOCETIRIZINE

4. **Accuracy:** The percentage recovery values were in the range of 98.0% to 102.0% which is within the acceptance criteria. The percentage recovery results obtained were listed in **Table 6**.

5. **Method Precision:** The method precision was performed by analyzing a sample solution at working concentration for six times. Results of percentage relative standard deviation of Phenylephrine hydrochloride, Ambroxol hydrochloride and Levocetirizine hydrochloride are summarized in **Table 7**.

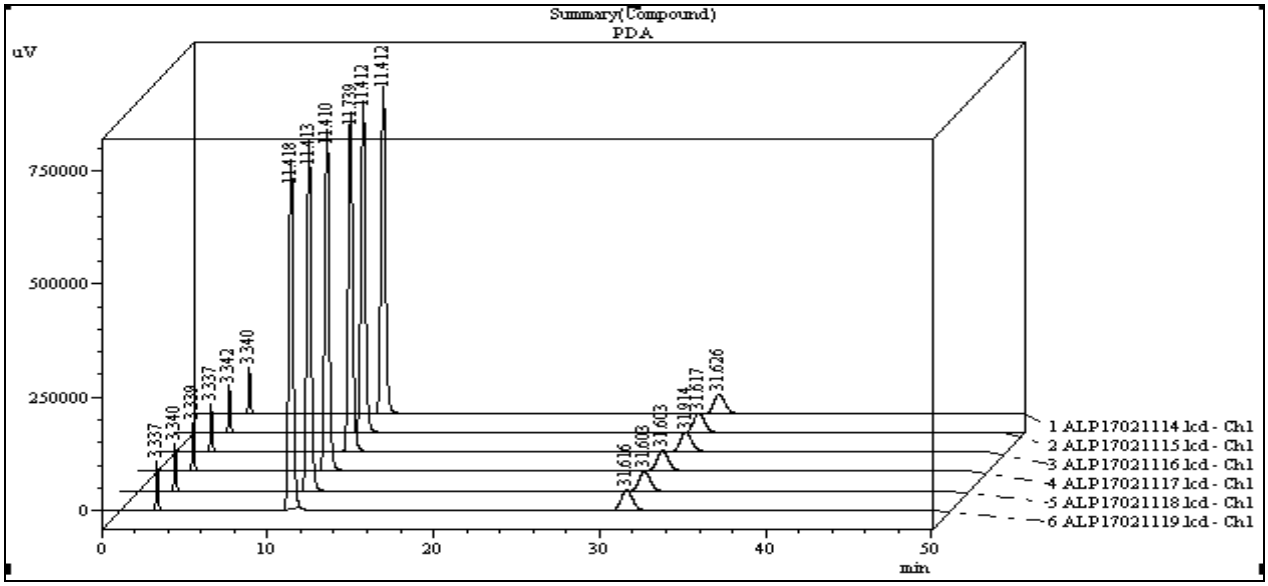
TABLE 6: PERCENTAGE RECOVERY STUDIES

% Level	Weight of working standards (mg)			Theoretical conc. (mg/ml)			Measured conc. (mg/ml)			% Recovery		
	PHEN	AMBH	LCZH	PHEN	AMBH	LCZH	PHEN	AMBH	LCZH	PHEN	AMBH	LCZH
50	9.8	59.5	5.1	0.098	0.595	0.051	0.0973	05993	0.0511	99.35	100.73	100.22
	9.8	60.3	5.2	0.098	0.603	0.052	0.0973	0.5946	0.0512	99.35	98.61	98.64
	9.9	59.8	5.1	0.099	0.598	0.051	0.0975	0.5958	0.0514	98.53	99.64	100.91
100	20.1	119.7	10.2	0.201	1.197	0.102	0.1974	1.1924	0.1025	98.25	99.62	100.49
	19.9	120.1	10.1	0.199	1.201	0.101	0.1962	1.2036	0.1021	98.63	100.22	101.13
	19.7	119.8	10.3	0.197	1.198	0.103	0.1959	1.1967	0.1019	99.48	99.89	98.96
150	29.8	179.8	14.9	0.298	1.798	0.149	0.2921	1.7875	0.1473	98.01	99.42	98.92
	29.8	180.3	14.7	0.298	1.803	0.147	0.2927	1.7992	0.1461	98.22	99.79	99.41
	29.6	180.1	14.8	0.296	1.801	0.148	0.2904	1.8039	0.1468	98.12	100.16	99.22

TABLE 7: PERCENTAGE RELATIVE STANDARD DEVIATION OF THREE DRUGS

Injection no	%Level	%Assay		
		PEH	AMBH	LCZH
1	100%	99.20	100.40	101.90
2	100%	99.62	100.62	100.05
3	100%	100.05	101.10	100.86
4	100%	98.53	99.47	100.76
5	100%	99.04	100.05	100.11
6	100%	99.82	101.28	100.98
	Mean % Area	**99.39**	**100.49**	**100.92**
	%RSD	**0.58**	**0.67**	**0.57**

6. **System Precision:** The %RSD for peak area variation of Phenylephrine hydrochloride, Ambroxol hydrochloride and Levocetirizine hydrochloride was 1.89, 1.91 and 1.73 respectively at the working concentration **(Figure 9)**.



Injection no	%Level	Peak area (mAu)		
		PEH	AMBH	LCZH
1	100%	886524	13565354	2082682
2	100%	893134	13711577	2092803
3	100%	918252	13906971	2128239
4	100%	916542	14057769	2130409
5	100%	923201	14229968	2187226
6	100%	929227	14190768	2124022
	Mean	**911146.67**	**13943734.50**	**2124230.17**
	%RSD	**1.89**	**1.91**	**1.73**

FIG. 9: CHROMATOGRAM FOR SYSTEM PRECISION

7. **Method Precision:** The method precision was performed by analyzing a sample solution at working concentration for six times. Results of percentage relative standard deviation of Phenylephrine hydrochloride, Ambroxol hydrochloride and Levocetirizine hydrochloride are summarized in **Table 8**. The percentage relative standard deviation for assay value of Phenylephrine hydrochloride, Ambroxol hydrochloride and Levocetirizine hydrochloride was 0.58, 0.67 and 0.57 respectively.

TABLE 8: PERCENTAGE RELATIVE STANDARD DEVIATION OF THREE DRUGS

Injection no	%Level	%Assay		
		PEH	AMBH	LCZH
1	100%	99.20	100.40	101.90
2	100%	99.62	100.62	100.05
3	100%	100.05	101.10	100.86
4	100%	98.53	99.47	100.76
5	100%	99.04	100.05	100.11
6	100%	99.82	101.28	100.98
	Mean % Area	**99.39**	**100.49**	**100.92**
	%RSD	**0.58**	**0.67**	**0.57**

8. **Ruggedness:** The ruggedness is assessed by evaluating the variability of the results obtained by the analysis of sample syrup formulation by different analyst on different days with different instruments, different reagents and different columns. The results are given in the **Table 9**.

TABLE 9: RUGGEDNESS STUDIES

Parameter	Details	% Assay			%RSD					
		PEH	AMBH	LCZH	PEH		AMBH		LCZH	
					Std	Spl	Std	Spl	Std	Spl
Analyst 1	Mahendren	100.18	100.33	101.36	0.25	0.62	0.01	0.47	0.43	1.46
Day 1	22-02-11	99.94	101.08	101.83	0.10	0.31	0.28	0.23	0.49	0.38
Column 1	Inertsil	99.68	100.52	101.71	0.21	0.27	0.19	0.26	0.62	0.31
Instrument 1	Shimadzu	99.98	101.63	101.30	0.77	0.71	0.35	0.41	0.19	0.31
Reagent 1	Merck	99.77	101.36	101.72	0.21	0.14	0.21	1.15	1.02	0.62
Analyst 2	Padmakana	98.96	98.41	101.16	0.02	0.06	0.07	0.11	0.12	0.17
Day 2	23-02-11	99.37	100.77	101.58	0.15	1.01	0.11	0.67	0.91	1.22
Column 2	Phenomenex	100.15	101.12	100.22	0.07	0.07	0.07	0.14	0.06	0.90
Instrument 2	Agilent	98.78	99.04	100.85	1.96	0.91	0.51	0.67	0.96	0.53
Reagent 2	Rankem	101.80	101.10	100.98	0.20	1.39	0.11	0.25	0.33	0.96

9. **Robustness:** Robustness of the method was tested by slightly changing the parameters like pH, flow rate and mobile phase ratio. The following **tables 10 (A), (B) and (C)** show the results for the variation of parameters that were altered to test the robustness of the method. System suitability was performed along with the sample analysis to assess if changes had a significant effect on the chromatography and assay values.

TABLE 10 (A): ROBUSTNESS TESTING

Sample ID	pH 2.9			pH 3.1		
	PEH	AMBH	LCZH	PEH	AMBH	LCZH
Retention time	3.496	12.120	35.987	3.342	10.262	26.17
Area	1611958	12837697	7274256	1611958	12837697	7274256
%Assay	100.69	99.45	101.10	100.95	100.65	100.94
%RSD	1.40	1.02	0.70	1.41	1.02	0.70
Theoretical plate	3589	8751	10044	32.70	7797	9945
Tailing factor	1.44	1.12	1.13	1.45	1.09	0.00

TABLE 10 (B): ROBUSTNESS TESTING

Sample ID	Buffer : solvent 58:42			Buffer : solvent 62:38		
	PEH	AMBH	LCZH	PEH	AMBH	LCZH
Retention time	3.024	10.054	27.911	3.668	12.376	33.992
Area	1472612	11612883	6795348	1799674	14217432	8290232
%Assay	98.96	101.24	100.63	101.65	100.97	99.07
%RSD	0.25	0.03	0.06	0.15	1.45	0.40
Theoretical plate	3491	8069	9384	3808	8895	9504
Tailing factor	1.45	1.07	1.12	1.42	1.07	1.10

TABLE 10 (C): ROBUSTNESS TESTING

Sample ID	Flow rate 0.9ml/min			Flow rate 1.1ml/min		
	PEH	AMBH	LCZH	PEH	AMBH	LCZH
Retention time	3.674	12.225	34.023	3.148	10.486	28.917
Area	1759543	13760583	8071399	1472399	11370620	6686562
%Assay	99.93	100.19	99.67	100.62	98.29	99.57
%RSD	0.10	0.07	0.07	0.53	1.80	0.28
Theoretical plate	3846	8653	9616	3416	8373	9905
Tailing factor	1.43	1.08	1.12	1.44	1.10	1.13

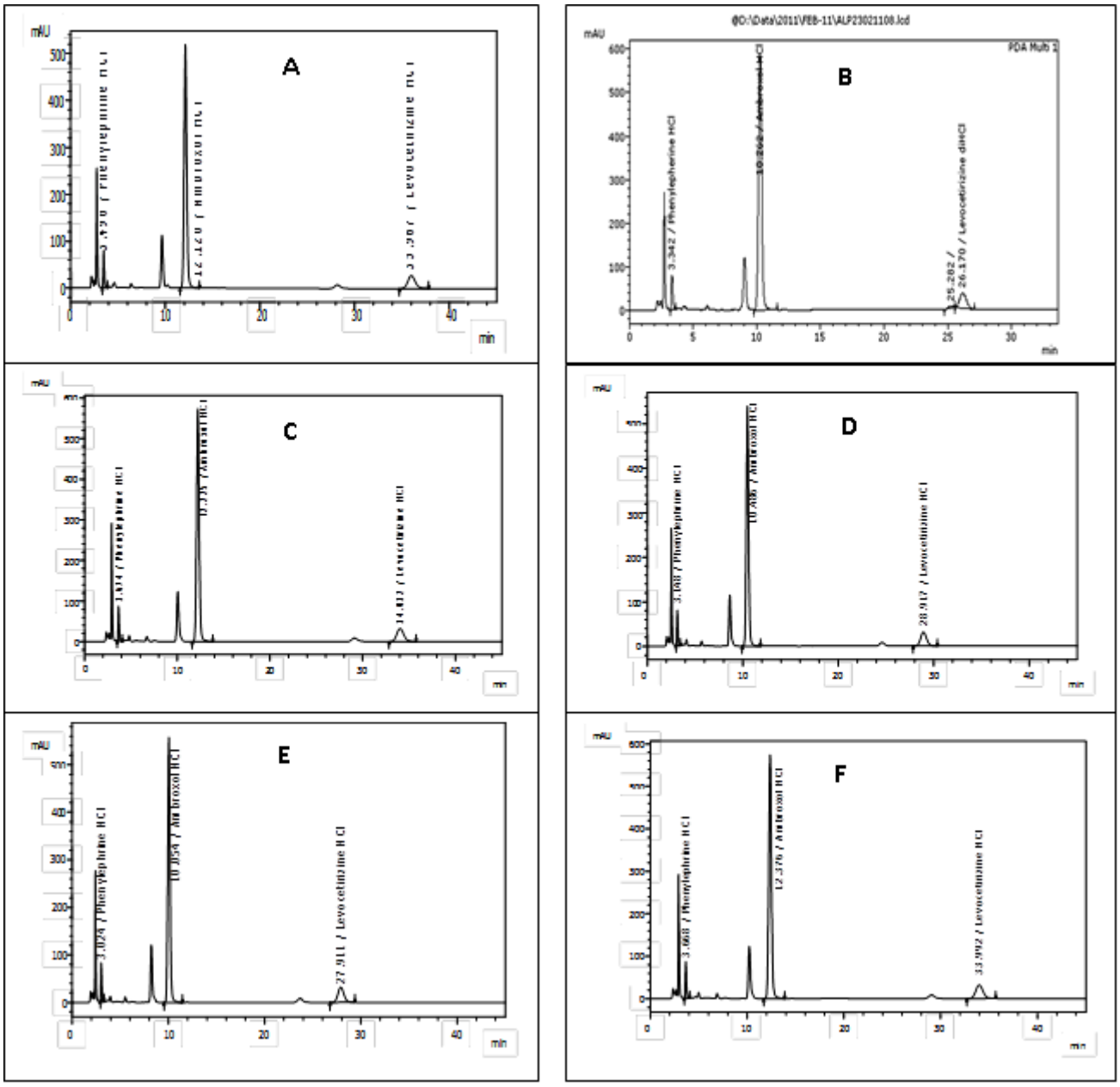


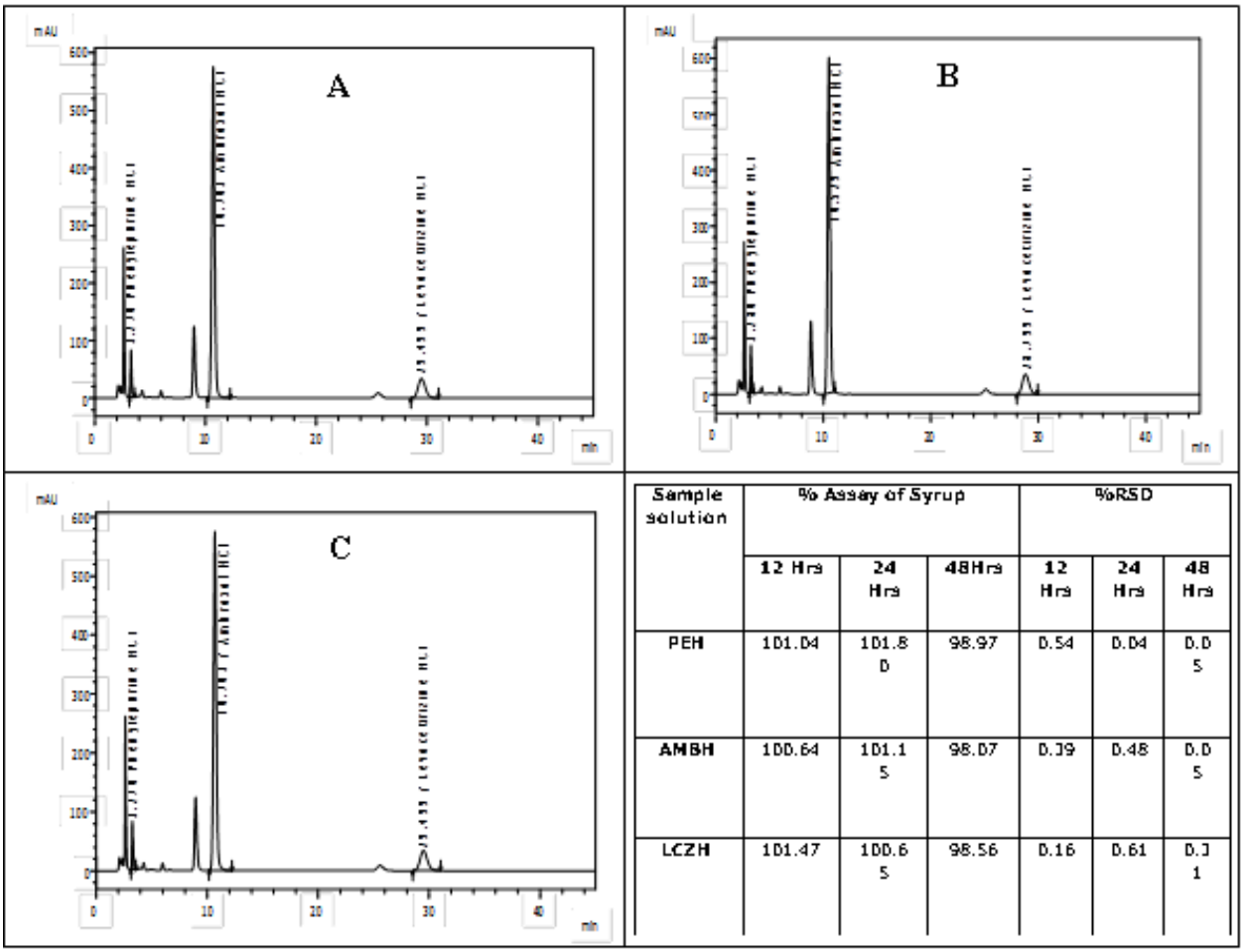
FIG. 10: CHROMATOGRAMS FOR DRUGS AT VARIOUS PARAMETERS (A) FOR PH 2.90, (B) FOR PH 3.10, (C) FOR FLOW RATE 0.9ML/MIN, (D) FOR FLOW RATE 1.1ML/MIN, (E) FOR MP 58:42 AND (F) FOR MP 62:38

The tailing factor was observed to be not more than 2.0 for Phenylephrine hydrochloride, Ambroxol hydrochloride and Levocetirizine hydrochloride and theoretical plates were within the acceptance criteria.

The retention time of Phenylephrine hydrochloride, Ambroxol hydrochloride and Levocetirizine hydrochloride were affected by the pH change and flow rate change and mixing of mobile phase composition **(Figure 10)**.

Hence, the system suitability criteria for the method were fulfilled and the assay values obtained were comparable.

10. **Solution Stability:** A solution of sample formulation at 100% of working concentration was kept at room temperature. The solutions were analyzed at different intervals (i.e. 12 hrs, 24 hrs and 48 hrs). The assay values obtained for sample solutions **(Figure 11)** were within the label claim and % RSD is within acceptance criteria i.e. not more than 2.0%. Therefore, sample solutions were stable up to 48 hours at room temperature.



Sample solution	% Assay of Syrup			%RSD		
	12 Hrs	24 Hrs	48Hrs	12 Hrs	24 Hrs	48 Hrs
PEH	101.04	101.80	98.97	0.54	0.04	0.05
AMBH	100.64	101.15	98.07	0.39	0.48	0.05
LCZH	101.47	100.65	98.56	0.16	0.61	0.31

FIG. 11: CHROMATOGRAMS OF SAMPLE SOLUTION AT DIFFERENT TIME INTERVAL (A) AT 12 HRS, (B) AT 24 HRS AND (C) AT 48 HRS INTERVAL

CONCLUSIONS: The combination syrup of Phenylephrine hydrochloride, Ambroxol hydrochloride and Levocetirizine hydrochloride was subjected to simultaneous determination by reverse phase HPLC method. Chromatographic figures, calibration data and recovery of the drug from the spiked concentrations were determined to assess the validity of the method. The results obtained by repeatability test, confirms the precision of the method. The proposed RP HPLC method gives good resolution between Phenylephrine hydrochloride, Ambroxol hydrochloride and Levocetirizine hydrochloride using a time 45 min and can be used for routine quality control analysis.

ACKNOWLEDGEMENT: The authors would like to thank Fourrts (India) Laboratories Pvt. Ltd. (Kelambakkam, Chennai, India) for providing gift samples of standard Phenylephrine hydrochloride, Ambroxol hydrochloride and Levocetirizine hydrochloride. The authors would like to thank, Mr. M. Ramalingam, Sr. General Manager (R&D), Fourrts (India) Laboratories Pvt. Ltd., Chennai, India for providing necessary facilities to carry out the work.

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How to cite this article:
Malakar P, Deb AR and Veliyath SK: Simultaneous Quantitation and Validation of Phenylephrine hydro-chloride, Ambroxol hydrochloride and Levocetrizine hydrochloride in Syrup formulation by Reverse Phase High Performance Liquid Chromatography. *Int J Pharm Sci Res* 2013: 4(8); 3028-3038. doi: 10.13040/IJPSR. 0975-8232.4(8).3028-38