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Method Development and Validation for the Simultaneous Determination of Paracetamol, Peudoephedrine Hydrochloride, and Levocetirizine Hydrochloride in Tablet Dosage Form.

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ABSTRACT

The objective of the current study was to develop a simple, accurate, precise and rapid reversed-phase HPLC method and subsequent validation using ICH suggested approach for the determination of anticold pharmaceutical dosage forms containing tertiary mixtures of paracetamol, pseudoephedrine hydrochloride, and levocetirizine hydrochloride. The chromatographic separation of paracetamol, pseudoephedrine hydrochloride, and levocetirizine hydrochloride was achieved on a Zorbax C18 (150mm×4.6 mm; 5µm particle size) column using UV detection at 210. The optimized mobile phase was consisted of TEA solution (pH 3)–acetonitrile (85:15, v/v). The retention times were 4.428, 6.262 and 13.284 min for paracetamol, pseudoephedrine hydrochloride, and levocetirizine hydrochloride, respectively. The proposed method provided linear responses within the concentration ranges 60.8-179.2 µg/ml, 106.0-285.5 µg/ml, and 10.4-29.7µg/ml. Correlation coefficients (*r*) of the regression equations were greater than 0.999 in all cases. The precision of the method was demonstrated using intra- and inter-day assay R.S.D. values which were less than 2% in all instances. No interference from any components of pharmaceutical dosage forms or degradation products was observed. According to the validation results, the proposed method was found to be specific, accurate, precise and could be applied to the quantitative analysis of these drugs in tablet containing paracetamol, pseudoephedrine hydrochloride, and levocetirizine hydrochloride tertiary mixtures.

Keywords: Paracetamol; Pseudoephedrine hydrochloride; Levocetirizine hydrochloride; Reverse-phase HPLC; Validation.

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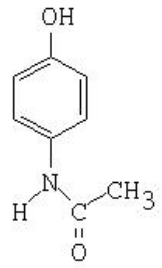
INTRODUCTION

Most cold medicines contain multiple active ingredients that include antipyretics, analgesics, antitussive agents, mucolytic agents, bronchodilators, antihistamines, and several vitamins. Combinations of these compounds were analyzed using RP-HPLC procedures. However, as it is difficult to analyze simultaneously many different kinds of ingredients using a single method, ingredients were often divided into several groups based on their chemical properties, i.e. cationic compounds in one run and anionic or neutral compounds in another run. Gradient elution was also required for simultaneous analysis [1].

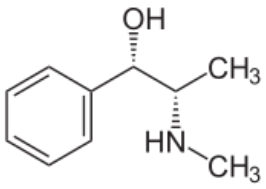
Pseudoephedrine, (1*S*,2*S*)-2-methylamino-1-phenylpropan- 1-ol , is formulated with several antihistaminic active substances including cetirizine [2] and fexofenadine [3,4] as antihistaminic-decongestant combination in capsule or coated tablet forms for the treatment of seasonal allergic rhinitis. A derivative spectrophotometric method have been reported for the determination of pseudoephedrine in binary mixtures with antihistamines including cetirizine, fexofenadine and loratadine [5]. Pseudophedrine acts as a decongestant by stimulating alpha-adrenergic receptors of vascular smooth muscle, thus constricting dilated arterioles within the nasal mucosa and reducing the blood flow to the engorged area [6].

Analgesics such as paracetamol are widely used drugs, not only as pain relievers but also in several diseases (musculoskeletal and joint disorders, rheumatic disorders,arthritis, and rheumatism) [7]. Their determination in pharmaceutical dosage forms (quality control) remains of great interest. Among the various analytical techniques, high- performance liquid chromatography (HPLC) constitutes the most popular chromatographic method for separating mixtures of analgesic drugs and related compounds. Paracetamol (PR), a para-aminophenol derivative, has analgesic and antipyretic properties and weak anti-inflammatory activity [7].

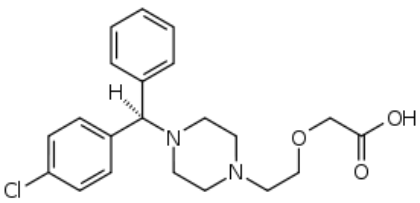
Levocetirizine dihydrochloride (LEVC) is chemically, (RS)-2-{4-[(R)-p-chloro-α-phenylbenzyl]-1-piperazinyl} ethoxyacetic acid dihydrochloride [7]. LEVC is usually used in allergic conditions including rhinitis [8].



Paracetamol



Pseudoephedrine HCl



Levocetirizine HCL

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EXPERIMENTAL

Materials and reagents

The working standards of Paracetamol, Pseudoephedrine Hydrochloride and Levocetirizine Hydrochloride were collected from Okasa pharma Pvt. Ltd, Satara, India.

Methanol , Acetonitrile and Water were purchased from Cipla Ltd. Triethylamine and Ortho-Phosphoric acid were purchased from R.F.C.L. Ltd. and Merck companies, respectively. Pharmaceutical finished dosage forms utilized in the present work include Actiflu tablet. Claimed to contain Paracetamol (500 mg), Pseudoephedrine Hydrochloride (30 mg) and Levocetirizine Hydrochloride (5 mg).

Instrumentation

The HPLC system (Make-Shimadzu LC 2010A HT) consisted of a quaternary gradient pump with autosampler facility. The detector consisted of a UV-Vis model operated at a wavelength 210 nm. The software used was Chromeleon 6.2 version . The column used was Inertsil ODS 3V (250mm×4.6mm, 5µm). Absorbance measurements were made on UV-Visible spectrophotometer (Shimadzu UV-Visible Spectrophotometer, Model-1800). The pH meter used was of systroics model EQMK VI. Mettler Toledo AG135, Mettler Toledo AB204-S weighing balance were used and for sonication of mobile phase (Trans-o-sonic) sonicator was used.

Chromatographic conditions

Different solvent systems were tried in order to find the best condition for separation of Paracetamol, Pseudoephedrine Hydrochloride and Levocetirizine Hydrochloride in presence of other active ingredients and minerals. The optimal composition of mobile phase was optimized to be Mobile Phase A: Buffer **(**Buffer Preparation: 1ml of Triethylamine dissolved in 1000 ml of purified water, pH adjusted to 3.0 by Orthophosphoric acid) and Mobile Phase B: Acetonitrile. The flow rate were set at 1 ml/min and UV detection was carried out at 210 nm. The mobile phase and samples were filtered using 0.45 µm membrane filter before injecting in HPLC system. The Injection Volume was 20µl was used. Mobile phase was degassed by Ultrasonicator (Tran-sonic). All determinations were performed at column oven temperature 25˚C. Run Time**:** 25 Min with Gradient Programme as given as follows:

Table 1: Gradient Programme

Time(min)	Mobile Phase A	Mobile Phase B
0.01	85	15
7.00	85	15
10.00	40	60
16.00	40	60
18.00	85	15
25.00	85	15

Preparation of Stock solution

Preparation of Pseudoephedrine Hydrochloride (stock solution-1)

60 mg of Pseudoephedrine Hydrochloride was weighed and transferred into 25ml volumetric flask. Added 15 ml of methanol and sonicated for 5 minutes. Cooled and made volume up to 25 ml with methanol.

Preparation of Levocetirizine Hydrochloride (stock solution-2)

20 mg of Levocetirizine Hydrochloride was weighed and transferred into 50 ml volumetric flask. Added 35 ml of Methanol and sonicated for 5 minutes. Cooled and made volume up to 50 ml with methanol.

Standard preparation for Paracetamol

20 mg of Paracetamol was weighed and transferred into 100 ml volumetric flask Added sufficient quantity of methanol to dissolved and sonicated for 5 minutes. Pipetted out 5ml from stock solution-1 and 5ml from stock solution-2 and transferred into 100 ml volumetric flask.

Preparation of sample solution (Actiflu Tablet)

Weighed accurately about 0.6576 mg of sample of Actiflu Tablet and transferred into 250 ml volumetric flask. Added sufficient quantity of methanol sonicated for 30 min. Cooled and made volume up-to with 250ml with methanol. (for Pseudoephedrine Hydrochloride and Levocetirizine Hydrochloride) Further, diluted 5 ml of this solution to 50 ml with methanol filter through 0.45µ syringe filter. (For Paracetamol).

RESULT AND DISCUSSION

Optimization of the chromatographic conditions

During the optimization of the separation method, three columns (Kromasil C18 5 µm, 250mm×4.6 mm; Zorbax C8 5µm, 150mm×4.6 mm; Symmetry C18 5 µm,150mm×4.6 mm), two organic solvents (methanol and acetonitrile) and different pH values (2.0–5.0) with and without ion pairing agent (hexane sulphonate, heptane sulphonate, octane sulphonate) were tested. The concomitant effects of optimum eluent composition , and pH for the determination of Paracetamol, Pseudoephedrine Hydrochloride and Levocetirizine Hydrochloride in the presence of their degradants by HPLC were studied. The preliminary studies were carried out by the injection of a sample solution containing Paracetamol, Pseudoephedrine Hydrochloride and Levocetirizine Hydrochloride. Paracetamol in the sample is present in higher amounts than the other two components and in addition has a higher absorption coefficient compared to pseudoephedrine hydrochloride [9].In the HPLC system, mobile phase consisted of two different solvents Triehylamine buffer pH 3 (A) and acetonitrile (B), . For these preliminary experiments, a Triehylamine buffer pH 3 and acetonitrile (75:25) was employed. Flow rate of mobile phase was 1.5mLmin−1. Of the

stationary phases experienced, Kromasil C18 gave the best results in terms of peak shape, resolution and analysis time. To overcome the weak retention of Pseudoephedrine Hydrochloride, formation of its ion pair with hexane sulphonate was tried; but this resulted in very late elution or no peaks for Pseudoephedrine Hydrochloride and Levocetirizine Hydrochloride. After trying several mobile phases containing acetonitrile and methanol with various buffers, the one consisting of Triehylamine buffer pH 3 and acetonitrile in gradient programme proved to be useful for better resolution and peak symmetry. The optimal composition of mobile phase was optimized to be Mobile Phase A: Buffer **(**Buffer Preparation: 1ml of Triethylamine dissolved in 1000 ml of purified water, pH adjusted to 3.0 by Orthophosphoric acid) and Mobile Phase B: acetonitrile. The flow rate were set at 1 ml/min and UV detection was carried out at 210 nm. The mobile phase and samples were filtered using 0.45 µm membrane filter before injecting in HPLC system. The Injection Volume was 20µl was used. All determinations were performed at column oven temperature 25˚C. Run Time**:** 25 Min with gradient programme .TEA not only provided the desired pH together with orthophosphoric acid, but also prevented peak tailing of our basic analytes due to its silanol masking feature [10].

Validation of the method

The aim of method validation was to confirm that the present method was suitable for its intended purpose as described in ICH guidelines Q2A and Q2B [11]. The described method has been extensively validated in terms of specifity and repeatability, linearity, accuracy, precision and intermediate precision, robustness, solution stability,limits of detection (LOD) and quantification (LOQ) and system suitability. The precision (% relative standard deviation) was expressed with respect to the intra- and inter-day variation in the expected drug concentrations. The accuracy was expressed in terms of percent recovery of the known amount of the active pharmaceutical ingredients (APIs) added to the known amount of the pharmaceutical dosage forms. After validation, the developed method have been applied to pharmaceutical dosage forms containing Paracetamol, Pseudoephedrine Hydrochloride and Levocetirizine Hydrochloride.

Specificity and Repeatability

Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present. The specificity of the given HPLC method was determined by the complete separation of Pseudoephedrine Hydrochloride, Levocetirizine Hydrochloride and Paracetamol in presence of its degradation products along with other parameters like retention time (tR), capacity factor (k), tailing or asymmetrical factor (T) etc.

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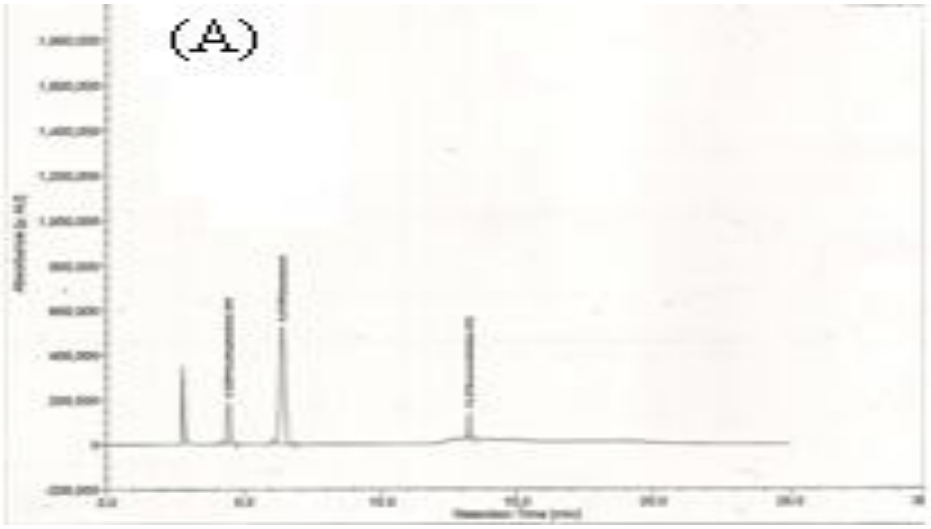
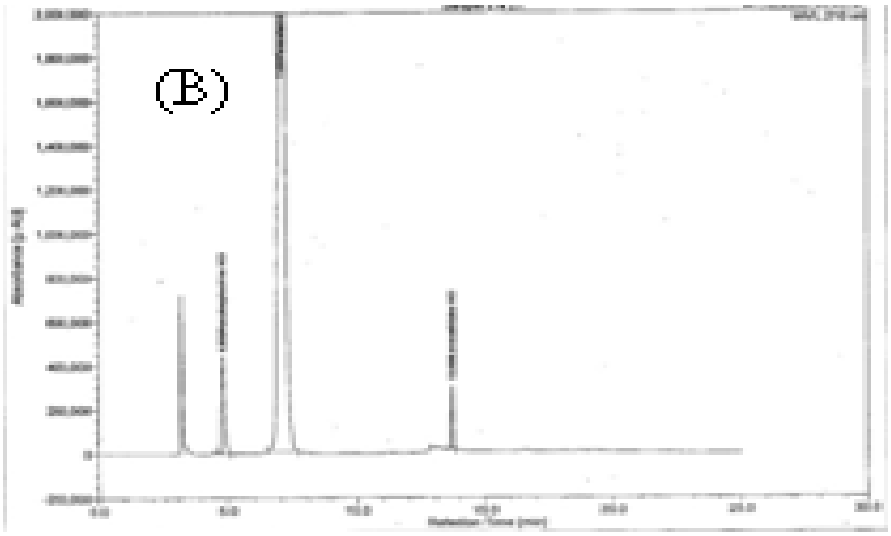
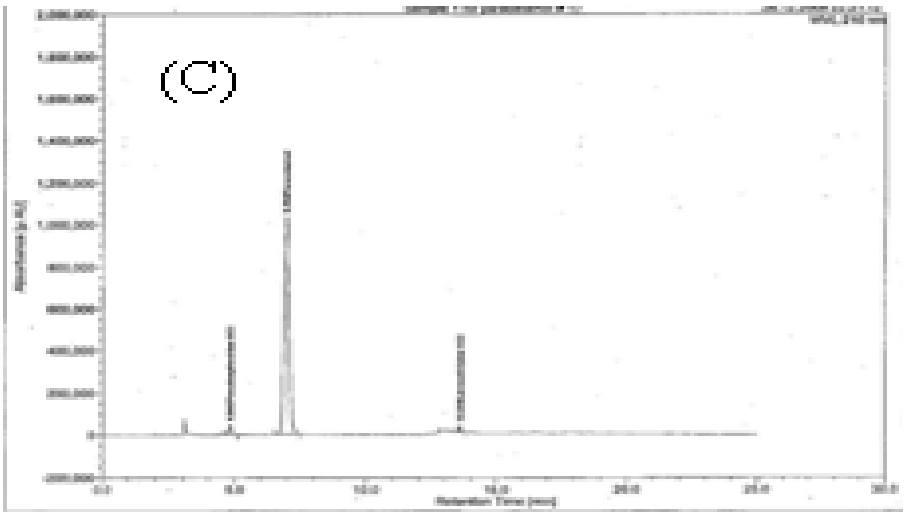
Figure 1: HPLC Chromatograms of Std.(A), Sample (B) &(C) of pseudoephedrine hydrochloride, levocetirizine hydrochloride and paracetamol







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Linearity and Range

Pseudoephedrine Hydrochloride, Levocetirizine Hydrochloride and Paracetamol show good linearity coefficient in concentration range of 60.878-179.2408 µg/ml, 106.0808-285.5408 µg/ml, and 10.4464-29.7503µg/ml for Pseudoephedrine Hydrochloride, Levocetirizine Hydrochloride and Paracetamol resp. Linearity was evaluated by preparing seven levels of working standard solutions (50%, 60%, 80%, 100%, 120%, 140%, and 150%) at that working standard solution level. The linearity of calibration graphs and adherence of the system to Beer's law was validated by high value of correlation coefficient and the S.D. for intercept value was less than 2%.

Table 2: Actual concentration range of Pseudoephedrine HCl, Paracetamol and Levocetirizine HCl.

Actual range of Pseudoephedrine HCl	Actual range of Paracetamol	Actual range of Levocetirizine HCl
60.878	106.0808	10.4464
72.854	126.0208	12.194
97.6044	163.9068	16.4441
120.5584	199.7988	20.0983
144.1112	235.6908	24.1895
167.664	269.3894	27.8239
179.2408	285.5408	29.7503

Figure 2: Linearity graph of Pseudoephedrine HCl

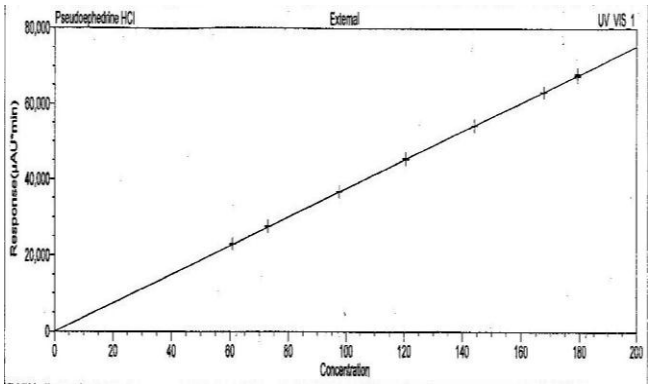

Table 3: Linearity parameters for Pseudophedrine HCl

Parameter	Result	Limit
LINEARITY COEFFICIENT 'r'	1.000	LIMIT : NLT 0.999
R Square	1.00000	-
Y' INTERCEPT	13764.495	-
% 'Y' INTERCEPT	0.5	LIMIT : NMT ± 2.0
SLOPE	22512.895	-

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Figure 4: Linearity graph of Paracetamol.

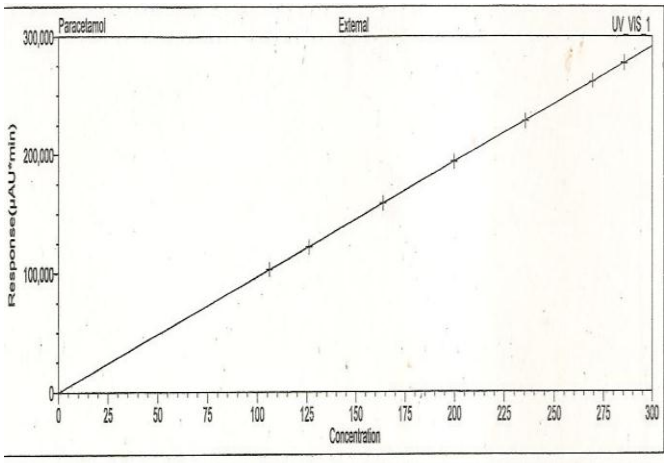

Table 4: Linearity parameters for Paracetamol

Parameter	Result	Limit
LINEARITY COEFFICIENT 'r'	1.000	LIMIT :NLT 0.999
R Square	0.99999	-
Y' INTERCEPT	-12315.686	-
% 'Y' INTERCEPT	-0.1	LIMIT :NMT ± 2.0
SLOPE	58340.531	-

Figure 5: Linearity graph of Levocetirizine HCl

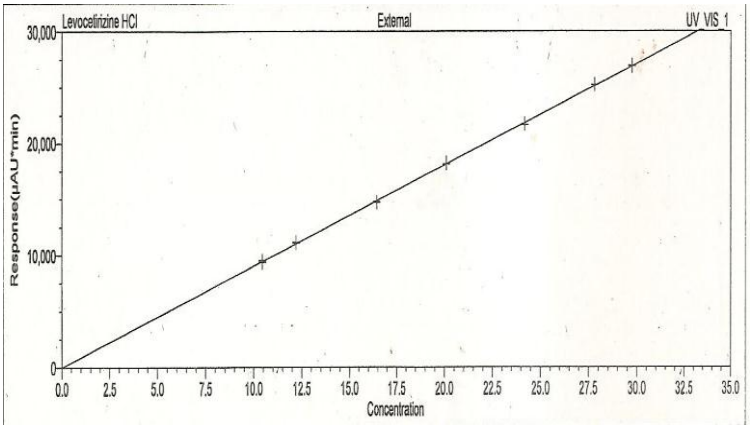

Table 5: Linearity parameters for Levocetirizine HCl

Parameter	Result	Limit
LINEARITY COEFFICIENT 'r'	1.000	LIMIT :NLT 0.999
R Square	0.99977	-
Y' INTERCEPT	-0.3	-
% 'Y' INTERCEPT	-3761.340	LIMIT :NMT ± 2.0
SLOPE	54311.351	-

Precision

Precision is the measure of how close the data values are to each other for a number of measurements under the same analytical conditions. ICH has defined precision to contain three components: repeatability, intermediate precision and reproducibility. It is normally expressed as % relative standard deviation. Precision may be considered at two levels:

Repeatability

Repeatability expresses the precision under the same operating conditions over a short interval of time. It is also termed as intra-assay precision.

Intermediate precision

Intermediate precision expresses within laboratories variations, different days, different analysts, different equipments, etc.

Precision (Intermediate precision) (Compared % variation with repeatability)

System Precision

In system precision to check system suitability parameters.std prepared in duplicates (std-1 and std-2). (Std- Pseudoephedrine HCl, Paracetamol and Levocetirizine HCl mixture) Std1- checked the system suitability parameters. Std 2- (Six Replicates) - checked % R.S.D (retention time and peak area response)

Method Precision

In method precision checked % RSD of six assay values of sample. Six samples were prepared and injected. % assay value of each sample to be calculated and % RSD of average assay value to be calculated. % variation with repeatability also be calculated.

Table 6: Data for Intermediate precision

Sample Name	Area for Pseudoephedrine HCl	% Assay	Area for Paracetamol	% Assay	Area for Levocetirizine HCl	% Assay
Spl. 1	2364739	97.9	14062958	102.3	1170081	99.7
Spl. 2	2561365	102.4	13971421	98.1	1180270	97.1
Spl. 3	2532109	102.6	13934530	99.2	1178624	98.3
Spl. 4	2497875	102.0	13953726	100.1	1190063	100.0
Spl. 5	2373400	99.4	13671319	100.6	1189975	102.6
Spl. 6	2528303	102.5	13639125	97.1	1181199	98.5
S.D.	0.598	1.994	9.188	1.838	0.094	1.877
%R.S.D.	1.97	1.97	1.85	1.85	1.89	1.89

Robustness

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Change the critical parameters like, flow rate of Mobile Phase, pH of mobile phase as shown in the table and carry out the analysis with each change in parameter. If the system suitability requirements are not achieved then discontinue the analysis for the particular change and make the relevant remarks in the experimental results. Insignificant differences in peak areas and less variability in retention time were observed in std and sample. Change in parameters as follows:

Table 7: Robustness Parameters.

Parameter	Parameter as per method	Change parameter to
Change in flow rate of Mobile Phase (± 0.1 ml/minute)	1.0 ml/minute	0.9 ml/minute
	1.0 ml/minute	1.1 ml/minute
Change in pH (± 2)	3.0	2.8
	3.0	3.2

Table 8: Robustness (At low flow rate) Samples Observations.
(Robustness (At low flow rate-1.1 ml/min)

Sample Name	Area for Pseudoephedrine HCl	% Assay	Area for Paracetamol	% Assay	Area for Levocetirizine HCl	% Assay
Spl 1	2994329	105.5	12850945	99.7	1154497	100.5
Spl 2	3011346	101.0	12865926	99.8	1153805	100.5
Spl 3	3030322	101.7	12799958	99.3	1158385	100.9
S.D.	0.184	0.612	1.299	0.260	0.011	0.224
%R.S.D.	0.61	0.61	0.26	0.26	0.22	0.22

Table 9: Robustness (At high flow rate) Samples Observations.
(Robustness (At high flow rate-1.1 ml/min)

Sample Name	Area for Pseudoephedrine HCl	% Assay	Area for Paracetamol	% Assay	Area for Levocetirizine HCl	% Assay
Spl.1	2069254	98.1	10992501	102.3	1015666	100.8
Spl. 2	2076912	98.8	10659211	99.6	1021296	101.7
Spl. 3	2083468	99.1	10580383	98.9	1019923	101.6
S.D.	0.162	0.540	9.132	1.826	0.025	0.498
%R.S.D.	0.55	0.55	1.82	1.82	0.49	0.49

Table 10: Robustness (At low pH) Sample Observations.
(Robustness (At low pH- 2.8)

Sample Name	Area for Pseudoephedrine HCl	% Assay	Area for Paracetamol	% Assay	Area for Levocetirizine HCl	% Assay
Spl. 1	2618074	98.1	11779829	101.4	1087330	99.0
Spl.2	2622558	98.8	11689764	100.9	1088194	99.3
Spl. 3	2627065	99.1	11718785	101.0	1086568	99.0
S.D.	0.072	0.239	1.473	0.295	0.008	0.155
%R.S.D.	0.24	0.24	0.29	0.29	0.16	0.16

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Table 11: Robustness (At high pH) Samples Observations Robustness (At High pH- 3.2

Sample Name	Area for Pseudoephedrine HCl	% Assay	Area for Paracetamol	% Assay	Area for Levocetirizine HCl	% Assay
Spl. 1	2756569	101.6	11638422	100.3	1066566	100.4
Spl.2	2764433	101.0	11595580	100.0	1067491	100.6
Spl. 3	2769360	101.3	11602626	100.2	1065832	100.5
S.D.	0.096	0.320	0.695	0.139	0.004	0.090
%R.S.D.	0.32	0.32	0.14	0.14	0.09	0.09

Solution Stability

Solution Stability checked for stability of standard and sample solutions. Depend on stability of standard and sample solution decided that standard or sample should be freshly prepared or not. Solution stability checked at each intervals-2, 4, 6, 8, 12, 16,20 and 24 hours. For standard solution stability, system suitability parameters was checked at each interval and for sample solution stability % assay value calculated at each time interval.std solution prepared. % RSD of assay value was calculated and % Relative difference of initial assay value along with the assay values of predetermined time interval at each time interval calculated in solution. compare the pattern of chromatogram in standard and sample solution at each time interval.

Table 12: Solution stability observations for Pseudoephedrine HCl

Sample Name	Area(Pseudoephedrine HCl)	% Assay	% Relative difference
Sample (Initial)	2291803	100.1	0.0
Sample (2 hrs)	2307554	100.8	0.7
Sample (4 hrs)	2311623	101.0	0.9
Sample (8 hrs)	2252270	98.4	1.7
Sample (12 hrs)	2247078	98.2	2.0
Sample (16 hrs)	2329275	101.8	1.6
Sample (20 hrs)	2304967	100.7	0.6
Sample (24 hrs)	2302249	100.6	0.5

Table 13: Solution stability observations for Paracetamol

Sample Name	Area(Paracetamol)	% Assay	% Relative difference
Sample (Initial)	12861804	100.4	0.0
Sample (2 hrs)	12845160	100.3	0.1
Sample (4 hrs)	12835772	100.2	0.2
Sample (8 hrs)	12852269	100.3	0.1
Sample (12 hrs)	12859720	100.4	0.0
Sample (16 hrs)	12855937	100.3	0.0
Sample (20 hrs)	12857092	100.4	0.0
Sample (24 hrs)	12858508	100.4	0.0

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Table 14: Solution stability observations for Levocetirizine HCl

Sample Name	Area(Levocetirize HCl)	% Assay	% Relative difference
Sample (Initial)	1038475	100.0	0.0
Sample (2 hrs)	1040525	100.2	0.2
Sample (4 hrs)	1032276	99.4	0.6
Sample (8 hrs)	1029715	99.2	0.8
Sample (12 hrs)	1024059	98.6	1.4
Sample (16 hrs)	1057676	101.9	1.8
Sample (20 hrs)	1052173	100.3	1.3
Sample (24 hrs)	1056058	101.7	1.7

Accuracy

For both drugs recovery studies were carried out by applying the method to drug sample to which known amount of Pseudoephedrine HCl, Paracetamol and Levocetirizine HCl) corresponding to 50, 100 and 150% of label claim had been added (Standard addition method). At each level three determinations were carried out and results obtained were compared with expected results.

Table 15: Accuracy observations for Pseudoephedrine HCl

Sample Name	Area (Pseudoephedrine HCl)	% Recovery	% R.S.D.
Acc-level 50%	1280156	102	
Acc-level 50%	1282279	101	
Acc-level 50%	1286582	102	0.92
Acc-level 100%	2574486	100	
Acc-level 100%	2578241	101	
Acc-level 100%	2430080	99	1.01
Acc-level 150%	3854637	102	
Acc-level 150%	3845107	102	
Acc-level 150%	3846400	101	0.54

Table 16: Accuracy observations for Paracetamol

Sample Name	Area (Paracetamol)	% Recovery	% R.S.D.
Acc-level 50%	7690542	102	
Acc-level 50%	7704628	99	
Acc-level 50%	7664186	99	1.43
Acc-level 100%	14340445	99	
Acc-level 100%	14325807	99	
Acc-level 100%	14324218	99	0.21
Acc-level 150%	20467059	98	
Acc-level 150%	20522183	99	
Acc-level 150%	20454467	98	0.33

Table 17: Accuracy observations for Levocetirizine HCl

Sample Name	Area (Levocetirizine HCl)	% Recovery	% R.S.D.
Acc-level 50%	600724	101	
Acc-level 50%	601569	100	
Acc-level 50%	602942	100	0.67
Acc-level 100%	1288112	102	
Acc-level 100%	1285702	100	
Acc-level 100%	1270604	101	0.70
Acc-level 150%	1703629	98	
Acc-level 150%	1707053	100	
Acc-level 150%	1706517	99	0.94

CONCLUSION

The proposed HPLC method provides accurate and reproducible results for simultaneous determination of Paracetamol, Pseudoephedrine Hydrochloride and Levocetirizine Hydrochloride in Actiflu Tablet, without any interference from the other active ingredients and excipients. Proposed LC method simultaneous estimate Paracetamol, Pseudoephedrine Hydrochloride and Levocetirizine Hydrochloride in single run with common chromatographic procedure.

The analytical method used for determination of assay of Paracetamol, Pseudoephedrine Hydrochloride and Levocetirizine Hydrochloride in Actiflu Tablet complies with the acceptance criteria of the analytical parameters such as Specificity and System suitability, linearity and Range, Precision (Repeatability & Intermediate Precision), accuracy, solution stability and robustness. Hence method stands validated. The method can be used for routine quality control and stability study analysis.

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