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A stability-indicating LC method for the simultaneous Determination of Levocetirizine dihydrochloride and Pseudoephedrine sulfate in Tablet dosage forms

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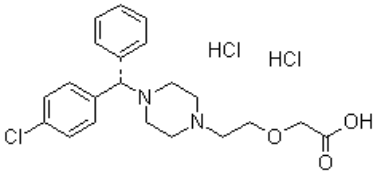
Abstract : A Stability indicating reverse phase high performance liquid chromatographic method has been developed for the simultaneous determination of Levocetirizine dihydrochloride and Pseudoephedrine sulfate in pharmaceutical formulation. The mobile phase A consisted of Potassium dihydrogen phosphate Buffer 0.05M and 1-Ocatne sulphonic acid sodium salt 0.25%, pH adjusted to 3.0 with orthophospheric acid. Mobile Phase B: Acetonitrile, Gradient elution at flow rate of 1 mL/min and Column temperature at 40°C. Detector wavelength of 242 nm using a photodiode array detector. The described method shows excellent linearity over a range of 200–10 µg ml^{-1} for Levocetirizine dihydrochloride and 7200-360 µg ml^{-1} for Pseudoephedrine sulfate. The correlation coefficient for Levocetirizine dihydrochloride and Pseudoephedrine sulfate are 0.9999. The proposed method was found to be suitable, accurate for quantitative determination and the stability study of Levocetirizine dihydrochloride and Pseudoephedrine sulfate in pharmaceutical preparations.

Keywords: Levocetirizine dihydrochloride; Pseudoephedrine sulfate; RP-HPLC; Forced degradation; Method Validation; Stability-indicating LC method .

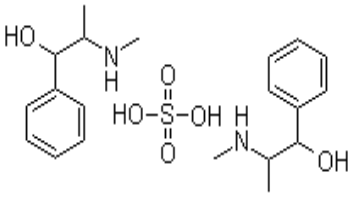
Introduction

Levocetirizine dihydrochloride is [2-[4-[(R)-(4-Chlorophenyl)phenylmethyl]-1-piperazinyl]ethoxy]-acetic acid dihydrochloride is the pharmacologically active enantiomer of cetirizine, is a potent histamine H-1 receptor antagonist.[1] Pseudoephedrine sulfate chemically [(S-(R*,R*))-alpha-(1-(Methylamino) ethyl) benzenemethanol sulfate]. Pseudoephedrine sulfate is official in USP [2]. Levocetirizine dihydrochloride is official in IP [3]. Levocetirizine Dihydrochloride is having antiallergic properties used Once daily for the treatment of allergic rhinitis. [4]

A Fixed dose combination of 180 mg of Pseudoephedrine sulfate and 5 mg of Levocetirizine dihydrochloride is available commercially as tablets,



Levocetirizine dihydrochloride



Pseudoephedrine sulfate

Fig. 1

are widely used for the symptomatic treatment of allergic rhinitis. Stability testing forms an important part of the process of drug product testing is to provide evidence on how quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, light and enables recommendation of storage conditions, retest periods and shelf life to be established. The two main aspects of drug products that play an important role in shelf life determinations are assay of active drug and degradants generated during the stability study. Stability-indicating methods have been reported for assays of various drugs in drug products containing only one active drug substance. Only few stability indicating methods are reported for the assay of combination drug products containing two or more active drug substances. The objective of this work was to develop an analytical LC procedure, which would serve as stability indicating assay method for combination drug products of Pseudoephedrine sulfate and Levocetirizine dihydrochloride.

The methods [9, 10, 11, 12] were reported for the analysis of Pseudoephedrine sulfate in combination with other drugs in Human plasma, and in tablets by LC. The methods [5, 6, 7, 8] were reported for the estimation Levocetirizine dihydrochloride in combination with other drugs in human plasma and in tablets by LC.

Simultaneous determination of Levocetirizine dihydrochloride and Pseudoephedrine sulfate of the tablets would save analysis time , also economy. In the present study attempts were made to develop a rapid, Economical, precise and accurate method for the simultaneous estimation of the ingredients of this combination in the presence of their degradants. The chemical structures of Levocetirizine dihydrochloride and Pseudoephedrine sulfate used in this work, are shown in Fig. 1**.** Where as Overlain UV spectra of Levocetirizine dihydrochloride and Pseudoephedrine sulfate are shown in Fig. 2.

Experimental

Chemicals and Reagents :

Levocetirizine dihydrochloride and Pseudoephedrine sulfate working standards were obtained from Sir Sayyed College Roshan Gate, Aurangabad, M.S., India. Orthophospheric acid, Potassium dihydrogen Phosphate (AR Grade) and Acetonitrile (HPLC Grade) were obtained from Merck Fine Chemicals(Mumbai, India). 1-Ocatne sulphonic acid sodium salt were from S.D. Fine Chem., Sodium hydroxide (NaOH), hydrochloric acid (HCl) and hydrogen peroxide (H_2O_2) were from Merck Fine Chemicals(Mumbai, India). The 0.45-um nylon filter was obtained from Advanced Micro Devices Pvt Ltd. The combination product of Levocetirizine dihydrochloride and Pseudoephedrine sulfate label claim (Levocetirizine dihydrochloride 5 mg and Pseudoephedrine sulfate 180 mg) Branded Tablets were purchased from the market. Double distilled water was used throughout the experiment. Other chemicals used were of analytical or LC grade.

Chromatographic Conditions:

The chromatographic system used was an Agilent-1100 series comprised of degasser, quaternary pump, auto injector , column compartment, photodiode array detector and the system was controlled through Empower Software. Cosmosil C8, 250 x 4.6 mm, 5 µm column was used. The mobile phase A consisted of Potassium dihydrogen phosphate Buffer 0.05M and 1-Ocatne sulphonic acid sodium salt 0.25%, pH adjusted to 3.0 with orthophospheric acid. Mobile Phase B: Acetonitrile , Gradient elution at flow rate of 1 mL/min and Column temperature at 40°C. Detector wavelength Kept 242 nm which is the isobestic point using a photodiode array detector.

Assay Standard Solution Preparation :

Levocetirizine dihydrochloride Standard Stock Solutions:

A 50 mg Standard of Levocetirizine dihydrochloride was accurately weighed, transferred into a 100 mL volumetric flask, and dissolved with Doubled distilled water.

Mixed Standard Solution:

A 90 mg Standard of Pseudoephedrine sulfate was accurately weighed, transferred into a 25 mL volumetric flask. To the same flask transferred 5 ml Standard Stock solution of Levocetirizine dihydrochloride and dissolved with Double distilled water.

Sample Preparation:

Ten tablets were weighed and finely powdered. A quantity of powder equivalent to one tablet containing 180 mg of Pseudoephedrine sulfate and 5 mg of Levocetirizine dihydrochloride was Transferred into a 50 mL volumetric flask. To this flask 10 mL of methanol were added and the solution was sonicated for 20min with intermittent shaking. The solution was cooled to ambient temperature. Then added 20 mL of double distilled water, and the solution was sonicated for 20min with intermittent shaking. The solution was cooled to ambient temperature.Then the volume was made up with double distilled water and centrifuged at 4,000 rpm for 10 min. The Centrifuged solution filtered through a 0.45-um nylon filter.

System Suitability Studies:
The resolution, number of theoretical plates, and peak asymmetry were calculated for the working standard solutions and are as shown in Table 2. The values obtained demonstrated the suitability of the system for the analysis of these drugs in combination. The typical chromatogram of the standard solution is as shown in Fig. 3.

Procedure for Forced Degradation Study of Drug Product:
Forced degradation studies were performed to demonstrate the selectivity and stability indicating capability of the proposed method. The powdered samples of Tablets were exposed to acidic, alkaline, oxidizing and thermal degradation conditions. The stress conditions engaged for degradation studies as per ICH recommendation.

Acidic Degradation:
A quantity of powder equivalent to one tablet containing 180 mg of Pseudoephedrine sulfate and 5 mg of Levocetirizine dihydrochloride was transferred into a 50 mL volumetric flask. To this flask 10 mL of methanol were added, and the solution was sonicated for 20min with intermittent shaking. Then 5 mL of 0.1 N HCl added and the mixture kept at 60◦C for 45 min in a water bath. The solution was allowed to attend ambient temperature, then solution was neutralized with 0.1 N NaOH and the volume made up to 50 mL with double distilled water .

Base Degradation:
A quantity of powder equivalent to one tablet containing 180 mg of Pseudoephedrine sulfate and 5 mg of Levocetirizine dihydrochloride was transferred into a 50 mL volumetric flask. To this flask 10 mL of methanol were added, and the solution was sonicated for 20min with intermittent shaking. Then 5 mL of 1 N NaOH added and the mixture kept at 60 ◦C for 45 min in a water bath. The solution was allowed to attend ambient temperature, then solution was neutralized with 1 N HCL and the volume made up to 50 mL with double distilled water.

Oxidative Degradation:
A quantity of powder equivalent to one tablet containing 180 mg of Pseudoephedrine sulfate and 5 mg of Levocetirizine dihydrochloride was transferred into a 50 mL volumetric flask. To this flask 10 mL of methanol were added, and the solution was sonicated for 20min with intermittent shaking. Then 5 mL of 5% H202 added and the mixture kept at Ambient Temperature for 45 min and the volume made up to 50 mL with double distilled water.

Results and discussion

Optimization of the chromatographic conditions:-
The main criteria for development of a successful HPLC method for determination of Levocetirizine dihydrochloride and Pseudoephedrine sulfate in tablet were the method should be able to determine assay of both drugs in single run , should be accurate, reproducible, robust, stability indicating, free of interference from degradation products, and straight forward enough for routine use in the quality control laboratory.

In order to optimize the LC separation of Pseudoephedrine sulfate and Levocetirizine dihydrochloride initially the retention behavior of both the components was studied in the pH range of 2.5–6.8, using mobile phases of buffer (pH 2.5–6.8) and acetonitrile, methanol as organic modifier. It was found that Pseudoephedrine sulfate eluted in void volume and more retention time Levocetirizine dihydrochloride. Hence it was decided to work by adding ion pairing reagent(1-Ocatne sulphonic acid sodium salt) in the mobile phase.

To ensure that Pseudoephedrine sulfate gives better retention, resolution between Levocetirizine dihydrochloride and Pseudoephedrine sulfate not less than 5 , the method was as fast as possible, a gradient run was optimized using buffer pH 3.0 and acetonitrile. Finally The mobile phase A consisted of Potassium dihydrogen phosphate Buffer 0.05M and 1-Ocatne sulphonic acid sodium salt 0.25%, pH adjusted to 3.0 with orthophospheric acid. Mobile Phase B: Acetonitrile , Gradient elution at flow rate of 1 mL/min and Column temperature at 40°C. Detector wavelength of 242 nm using a photodiode array detector was selected as an appropriate chromatographic conditions, which gave good resolution, acceptable peak parameters for both Levocetirizine dihydrochloride and Pseudoephedrine sulfate.

Method Validation:
As per the ICH guidelines [13], the method validation parameters were checked for linearity, precision, accuracy, limit of detection, limit of quantitation, and robustness.

Specificity:
Photodiode array detection was used as an evidence of the specificity of the method to evaluate Were complete separation of Levocetirizine dihydrochloride and Pseudoephedrine sulfate was noticed in presence of tablet excipients. In addition there was no any interference at the retention time of Levocetirizine dihydrochloride and Pseudoephedrine sulfate in the chromatogram of placebo solution. In peak purity

analysis with photo diode detector, purity angle was less than purity threshold for both the analytes. This shows that the peak of analytes was pure and excipients in the formulation did not interfere the analytes.

Linearity:
Linearity of the method was tested from 10 to 200% of the targeted level of the assay concentration (Levocetirizine dihydrochloride 100 ug mL^{-1}, Pseudoephedrine sulfate 3600 ug mL^{-1}) for both analytes. Mixed standard solutions contained 10-200 ug mL^{-1} of Levocetirizine dihydrochloride and 360-7200 ug mL^{-1} of Pseudoephedrine sulfate. Linearity solutions were injected. The calibration graphs were obtained by plotting peak area ratio against the concentration of the drugs. The equations of the calibration curves for Levocetirizine dihydrochloride and Pseudoephedrine sulfate obtained were y = 14184x +15373 and y = 332.2x + 3534 respectively. In the simultaneous determination, the calibration graphs were found to be linear in the aforementioned concentrations with correlation coefficient for Levocetirizine dihydrochloride and Pseudoephedrine sulfate are 0.9999.

Precision:
The repeatability of the analytical method was evaluated by assaying six samples solutions of Levocetirizine dihydrochloride 100 ug mL^{-1} and Pseudoephedrine sulfate 3600 ug mL^{-1}, during the same day, under the same experimental conditions. Intermediate precision was evaluated by assaying solutions on different days. Peak areas were determined and compared. Precision was expressed as percentage relative standard deviation (R.S.D%<2). From the data obtained, the developed RP-HPLC method was found to be precise and results are reported in Table:4.

Accuracy (Recovery Test) :
Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by spiking solution of known amounts of the drugs in the placebo. The recovery was performed at three levels, 50, 100 and 150% of the label claim of the tablet (180 mg of Pseudoephedrine sulfate and 5 mg of Levocetirizine dihydrochloride). Placebo equivalent to one tablet was transferred into a 50 mL volumetric flask, the amounts of Pseudoephedrine sulfate and Levocetirizine dihydrochloride at 50, 100 and 150% of the label claim of the tablet were added. Six samples were prepared for each recovery level. The solutions were then analyzed, and the percentage recoveries were calculated. The recovery values for Levocetirizine dihydrochloride and Pseudoephedrine sulfate are as shown in Table: 4.

LOD and LOQ:
The LOD and LOQ for Levocetirizine dihydrochloride and pseudoephedrine sulfate were determined at a signal to-noise ratio of 3:1 and 10:1, respectively by injecting a series of dilute solutions with known concentrations . The LOD and LOQ are as shown in Table:4.

Robustness:
The robustness of a method is the ability to remain unaffected by small changes in parameters. To determine robustness of the method, experimental conditions were purposely altered and chromatographic resolution between Levocetirizine dihydrochloride and Pseudoephedrine sulfate were evaluated. The flow rate of the mobile phase was 1.0 $mLmin^{-1}$. To study the effect of flow rate on the resolution of Levocetirizine dihydrochloride and Pseudoephedrine sulfate, it was changed to 0.2 units from 1.0 to1.2 mL min^{-1} and 0.8 mL min^{-1}. The effect of column temperature on the resolution was studied at 45 and 35 ^{0}C instead of 40^{0}C . The effect of pH Variation of Mobile phase A (Buffer) on the resolution was studied at pH 3.2 and pH 2.8 instead of pH 3.0. Robustness results are as shown in table 5.

Conclusions
The developed simple LC method for assay determination of Levocetirizine dihydrochloride and Pseudoephedrine sulfate is linear, precise, accurate and specific. The method was validated to the requirements of ICH and the results were satisfactory. The developed stability-indicating analytical method can be used for the routine analysis of production samples, where sample load is higher and high throughput is essential for faster delivery of results. Overall method provides a high throughput solution for determination of Levocetirizine dihydrochloride and Pseudoephedrine sulfate.

Table 1: Gradient Program for Levocetirizine dihydrochloride and Pseudoephedrine sulfate

Time (minutes)	%A (Buffer)	%B (Acetonitrile)
Initial	80	20
3	80	20
8	50	50
15	50	50
17	80	20
20	80	20

Table 2: System suitability studies

S.No	Parameter	Levocetirizine dihydrochloride	Pseudoephedrine sulfate
1.	Theoretical plates	99220	53335
2.	Resolution	13.93	----
3.	Asymmetry factor	1.1	0.9
4.	%RSD (Peak area)	0.10	0.10

Table 3 : Summary of forced degradation results

Stress condition/media/duration	Levocetirizine dihydrochloride Degradation(%)	Pseudoephedrine sulfate Degradation(%)
Acidic/0.1 N HCl/60^{0}C/45 min	6%	0.9%
Alkaline/1 NaOH /60^{0}C/45 min	1.2%	0.9%
Oxidative/5%H_2O_2/ambient/45 min	3.3%	0.1%
Thermal/105^{0}C/24hrs	3.0%	6.2%

Table 4. Results from validation studies

S.No.	Paramater	Levocetirizine dihydrochloride	Pseudoephedrine sulfate
1.	Linearity range (µg mL^{-1})	10-200	360-7200
2.	Correlation coefficient	0.9999	0.9999
3.	LOD (ug/mL)	0.012	0.462
4.	LOQ (ug/mL)	0.036	1.4
5.	Accuracy (%) (n=6)		
	50%	100.4	100.5
	100%	99.9	100.8
	150%	99.1	101.3
6.	Precision RSD (%) (n=6) (R.S.D %< 2)		
	Repeatability day 1	0.8	1.1
	Intermediate precision day 2	0.6	1.0

Table 5. System suitability parameters and robustness

System suitability Parameter	Sr,No.	Robustness	Levocetirizine dihydrochloride	Pseudoephedrine sulfate
Resolution.	1.	Flow rate		
		0.8 mL	13.9	----
		1.0 mL	15.6	----
		1.2 mL	17.6	----
	2.	pH of Buffer		
		2.8	16.2	----
		3.0	15.6	----
		3.2	15.2	----
	3.	Temperature variation		
		35^0C	15.2	----
		40^0C	15.6	----
		45^0C	16.1	----
Asymmetry factor	1.	Flow rate		
		0.8 mL	1.1	0.9
		1.0 mL	1.1	0.9
		1.2 mL	1.1	0.9
	2.	pH of Buffer		
		2.8	1.1	0.9
		3.0	1.1	0.9
		3.2	1.1	0.9
	3.	Temperature variation		
		35^0C	1.1	0.9
		40^0C	1.1	0.9
		45^0C	1.1	0.9
Theoretical plates	1.	Flow rate		
		0.8 mL	123940	62042
		1.0 mL	137882	65295
		1.2 mL	143924	65275
	2.	pH of Buffer		
		2.8	142610	66080
		3.0	137882	65295
		3.2	143924	66229
	3.	Temperature variation		
		35^0C	137282	63594
		40^0C	137882	65295
		45^0C	144411	66076

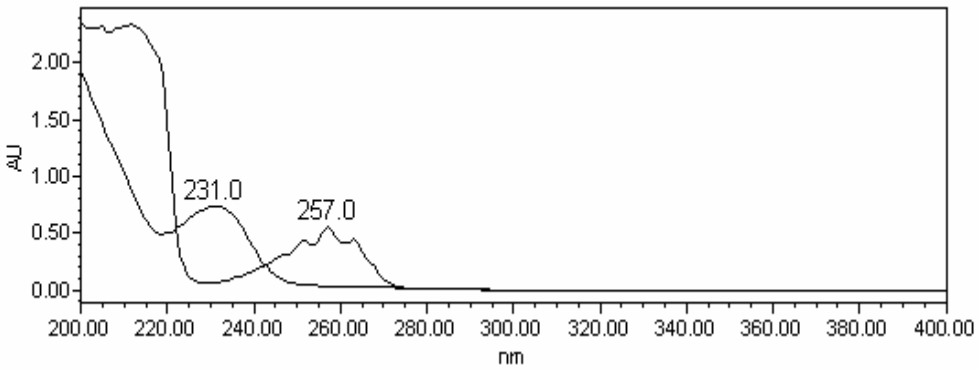


Fig. 2. Overlain UV spectra of: 1) Levocetirizine dihydrochloride, 2) Pseudoephedrine Sulfate

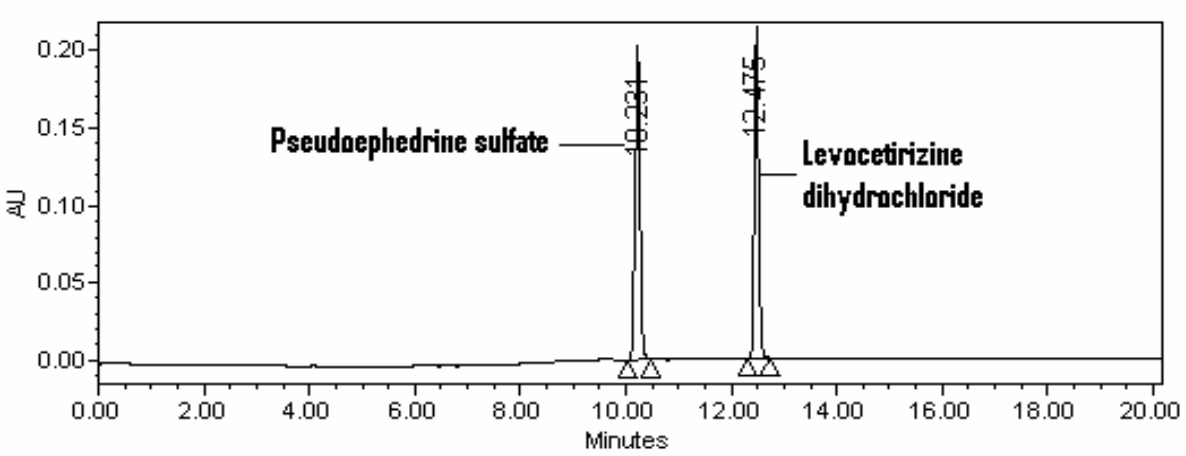


Fig. 3. A typical chromatogram of the tablet: Pseudoephedrine sulfate and Levocetirizine dihydrochloride.

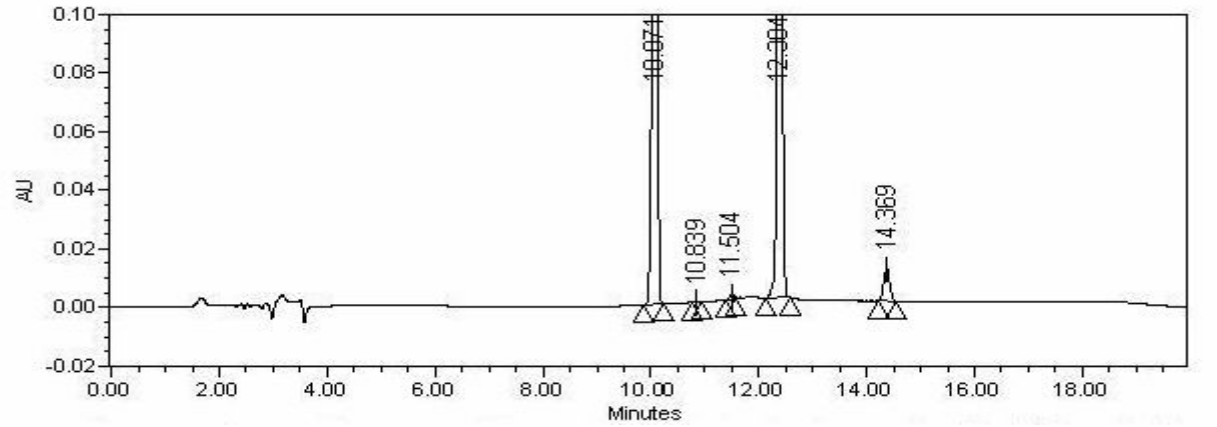


Fig. 4. Chromatogram of the acid hydrolysis degraded tablet solution.

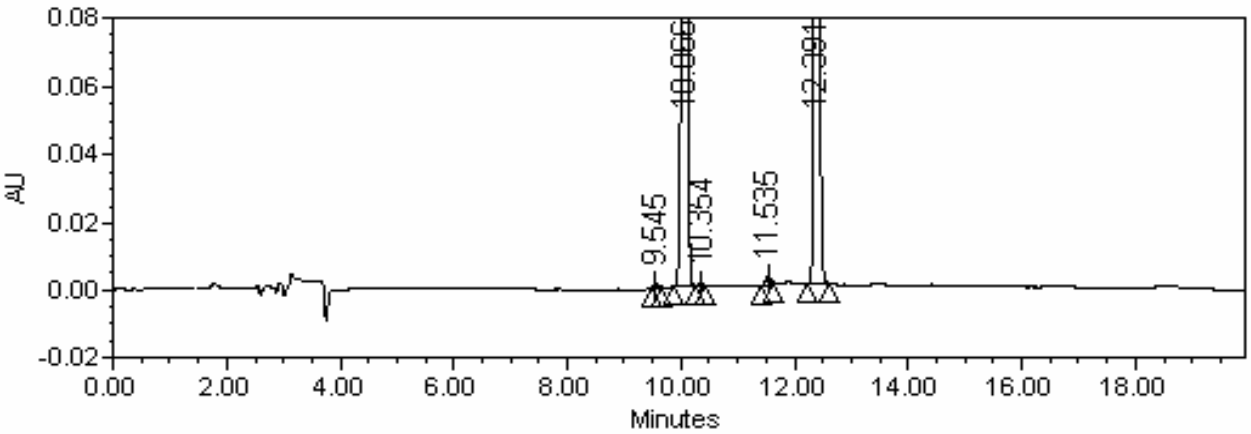


Fig. 5. Chromatogram of the Base hydrolysis degraded tablet solution

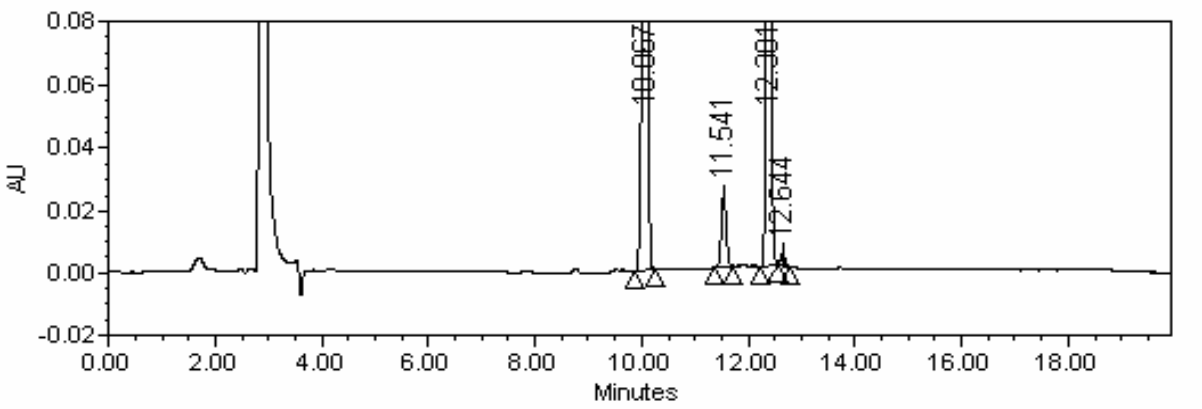


Fig.6. Chromatogram of the Oxidative degraded tablet solution.

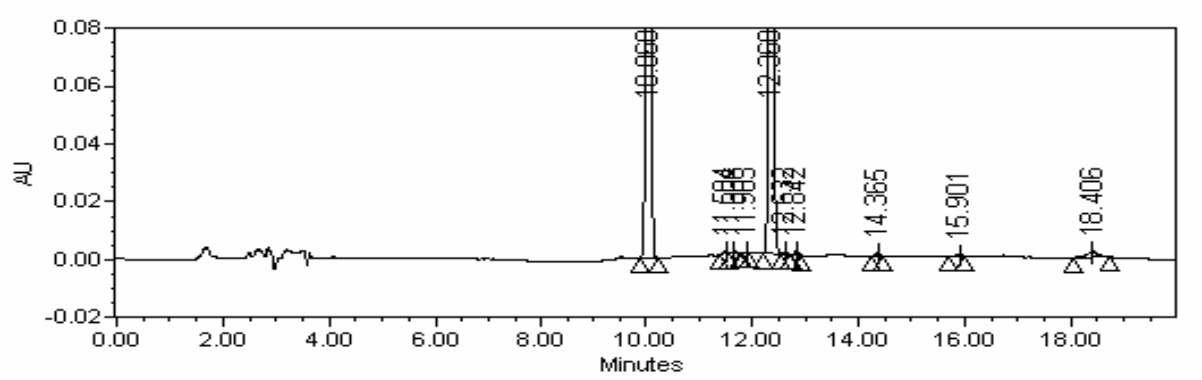


Fig. 7. chromatogram of the Thermal degraded tablet solution

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