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Original Article

Stability Indicating RP-HPLC Method for Determination of Drotaverine HCL and Mefenamic Acid in Pure and Pharmaceutical Formulation

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ABSTRACT

Objective: A Stability indicating Reverse-Phase liquid chromatographic method for the simultaneous estimation of Drotaverine HCl and Mefenamic acid was developed.

Method: The chromatographic assay involves the use of Hi Q C-18 W (5µm column with a simple mobile phase containing 80:20% v/v of methanol & HPLC Grade water (0.1% triethylamine at pH 3) with flow rate of 1.0mL/min with UV detection at wavelength of 250 nm.

Result: The method showed good linearity in the concentration range of 2-10 µg/mL for Drotaverine Hcl and 6-30 µg/mL for Mefenamic Acid.

Conclusion: The developed method was successfully validated as per the ICH guidelines. The stability indicating capacity was also tested and successfully applied to marketed formulation.

Keywords: Drotaverine hydrochloride, Mefenamic acid, Stability indicating, RP-HPLC, Force degradation study.

INTRODUCTION

Stability indicating assay method (SIAM)defined as an analytical method that accurately quantitates the active ingredients without interference from the degradation products, process impurities ,excipients or potential impurities .various guidelines have been issued by international conference on harmonization (ICH) to address the stability studies of drug substances and drug products. Two terms have been proposed in literature to differentiate the method that measure quantitatively the component of interest in the sample matrix without separation, and the ones where separation of the drug as well as other degradation product is done. Specific SIAM can be defined as a method that is able to measure unequivocally the drugs in the presence of

all the degradation products, excipients and additives, expected to be present in the formulation. Selective SIAM can be defined as a method that is able to measure unequivocally the drugs and all the degradation products in the presence of excipients and additives, expected to be present in the formulation.[4] Drotaverine Hcl [Figure 1] is chemically known as 1-(3,4-diethoxybenzylidene)-6, 7-diethoxy-1, 2, 3, 4-tetrahydroisoquinoline. Drotaverine hydrochloride is a highly potent spasmolytic agent. It acts as an antispasmodic agent by inhibiting the phosphodiesterase IV enzyme, specific for smooth muscle spasms and pain, and used to reduce excessive labor pain. Mefenamic acid [Figure 2] [N-(2, 3-xylyl) anthranilic acid] is an Aminobenzoate, a subclass of analgesic with Non steroidal anti-Inflammatory properties[1]. It acts by binding the prostaglandin synthetase receptors COX-1 and COX-2, inhibiting the action of prostaglandin synthetase[2,3]. It is used for the treatment of rheumatoid arthritis, osteoarthritis, dysmenorrhea, and mild to moderate pain, inflammation, and fever 4.[5]

The review of literature has suggested that there are few methods reported for estimation of selected drugs singly and in combination however, no stability indicating assay method has been reported for estimation of these drugs in combined dosage form. So the present work was undertaken with following objective to developed economical, simple, accurate, precise and reproducible stability indicating assay method for estimation of these drugs in combined dosage form with the use of different modern instruments.

EXPERIMENTALS

Reagents and chemicals

Drota HCl supplied as a gift sample by Wockhardt Pharmaceutical Ltd, Aurangabad MA Avestia Pharma Ltd. Kandivali East. All the chemicals used of HPLC Grade (Merk Ltd., Mumbai) and double distilled water was used for mobile phase preparation.

Instrument

HPLC system of yangline (S.K) Younglin (S.K) Gradient System UV Detector. UV 730 D & SP930 D Plus Intelligent HPLC Detector. With column of Hi Q C18 W (4.6mm x 250mm), 5 is used. A gradient elution is performed using mixture of Methanol & HPLC Grade water (0.1%triehylamine pH3) in the ratio of 80:20%v/v as a mobile phase at flow rated of 1 ml/min at detection wavelength of 250 nm.

Preparation of Mobile phase

A mobile phase consisted of Methanol: water (0.1%Triethylamine pH3) (80:20 v/v) was selected to achieve symmetrical peak and sensitivity.

Preparation of Stock Standard Solution (Solution A)

Standard stock solution was prepared by dissolving 10.0mg of Drota HCl and 30.0 mg of MA in 10.0 mL was water that give concentration 400 and 1200 µg/mL for Drota HCl and MA respectively.

Preparation of Working Standard Solution (Solution B)

From the standard stock solution, the mixed standard solutions were prepared using Methanol to contain 10µg/mL of Drota HCl and 30µg/mL of MA.

Selection of detection wavelength

UV detector was selected, as it is reliable and easy to set at constant wavelength. A fix concentration of analyte were analysed at different wavelengths. As per the response of analyte, 250 nm was selected.

Linearity Study

From the standard stock solution of Drota HCl and MA 0.25 mL were taken in 10 mL volumetric flask diluted up to the with Methanol such that final concentration of Drota HCl and MA in the range 2-10 µg/mL of Drota HCl and 6-30 µg/mL of MA respectively. Volume of 20µl of each sample was injected with the help of Hamilton Syringe. All measurements were repeated five times for each concentration and calibration curve was constructed by plotting the peak area versus the drug concentration.

Forced degradation studies

Forced degradation carried out by applying various stress conditions to study the effect over wide range of pH, heat, and oxidation and photo degradation using the following approach. Stress studies were conducted in aqueous solutions.

Acid Degradation

Accurately weight tablet equivalent to 10.0 mg of Drota HCl & 30.0mg of MA were dissolved in 5.0 mL of aqueous 0.1N hydrochloric acid in a separate volumetric flask and refluxed in round bottom flask on boiling water bath for 1 hr. And with heat after 3 hr.

Alkali Degradation

Accurately weight tablet equivalent to 10.0 mg of Drota HCl & 30.0mg of MA were dissolved in 5.0 mL of aqueous 0.1N sodium hydroxide in a separate volumetric flask and refluxed in round bottom flask on boiling water bath for 1hr And with heat after 3 hr.

Neutral Degradation

Accurately weight tablet equivalent to 10.0 mg of Drota HCl & 30.0mg of MA were dissolved in 10.0 mL of water in a separate volumetric flask and kept at room temperature for 1hr and with heat after 3 hr.

Oxidative Degradation

Accurately weight tablet equivalent to 10.0 mg of Drota HCl & 30.0mg of MA were dissolved in 10.0 mL of 3% H2O2 in a separate volumetric flask and refluxed in round bottom flask on boiling water bath for 1hr and without heat after 3 hr.

Thermal Degradation

Accurately weight tablet equivalent to 10.0 mg of Drota HCl & 30.0mg of MA were uniformly spread as thin layer in a separate covered Petri-dish which were then kept in oven at 60°C for 24 hrs.

Photo Degradation

Accurately weight tablet equivalent to 10.0 mg of Drota HCl & 30mg of MA were uniformly spread as thin layer in a separate covered Petri-dish which were then kept in sunlight for 3 days.

RESULTS AND DISCUSSION

HPLC Method Development and Optimization

The finally optimized chromatographic conditions are. (See table 1 and figure 3.)

Linearity

See table 2 and figure 4-5.

System suitability test

See table 4.

Force degradation studies

See table 5 and figure 6-15.

The parent drug peak was well resolved from all the degradants generated under various stress conditions. However it could not be ascertain that peaks of degradants with similar retention times under different stress condition were same chemical

entity or different. In this regard further studied may be pursued in order to isolate and characterize degradants of different stress conditions. The Drota HCl was susceptible to acid, photolytic, thermal and oxidative degradation and MA was susceptible to alkali, oxidative, thermal and photolytic degradation in the marketed formulation.

CONCLUSION

The developed HPLC technique is precise, specific, accurate and stability-indicating. Statistical analysis proves that the method is suitable for the analysis Drotaverine HCl and mefenamic acid of as bulk drug and in Pharmaceutical formulation without any interference from the excipients. This study is a typical example of a stability-indicating assay, established following the recommendations of ICH guidelines. The method can be used to determine the purity of drug available from various sources by detecting any related impurities. The method has been found to be better than previously reported methods, because of use of a less economical and readily available mobile phase, lack of extraction procedures, no internal standard, and use of the same mobile phase for washing of the column. All these factors make this method suitable for quantification of in bulk drugs and in pharmaceutical dosage forms. It can therefore be concluded that use of the method can save much time and money and it can be used in small laboratories with very high accuracy and a wide linear range.

ACKNOWLEDGEMENT

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Table 1. The finally optimized chromatographic conditions are

Mobile Phase	Mixture of Methanol & HPLC Grade water (0.1%Triethylamine) in the ratio of 80:20%v/v
Column	Hi Q C 18 W, 4.6*250mm, 5
Flow Rate	1.0ml/min
Injection Volume	20µl
Column Oven Temp	Ambient

Table 2. Linearity studies of Drota HCl

Concentration of Drota HCl [µg/mL]	Peak Area	± SD	%RSD
2	85.14	0.76	0.89
4	171.76	1.33	0.77
6	259.25	3.36	1.30
8	322.43	0.66	0.20
10	415.65	1.71	0.41

Table 3. Linearity studies of MA

Concentration of MA [µg/mL]	Peak Area	± SD	%RSD
6	195.85	0.76	0.39
12	373.94	0.49	0.13
18	527.52	0.92	1.31
24	727.75	5.74	0.79
30	872.15	7.81	0.90

Table 4. System suitability studies for Drota HCl and MA

System Suitability Parameter	Standard	Proposed Method of Drota HCl	Proposed Method of MA
Retention time (tR) (min)	5-10min	5.5	9.5
Resolution	Should be>2	0.000	12.914
Theoretical plate (N)	More than2000	5854.4	12648

Table 5. Summary of force degradation studies

Condition	% Assay Drota HCl	%Degradation Drota HCl. 1hr	3hr	%Assay MA	% Degradation MA 1hr	3hr
Initial sample	99.74	-		98.95		
0.1 N HCl	97.24	7.40	10.11	98.50	5.70	4.51
0.1 N NaoH	97.74	7.53	6.54	98.50	5.57	5.61
3%H2O2	98.44	6.65	4.18	97.90	5.69	5.90
Thermal (60) for 24hrs	98.96	-	8.05	97.37	-	5.65
Neutral	99.72	9.94	10.37	-	5.02	4.69
Sun (for 3 days)	96.45	-	8.05	98.50	-	5.65

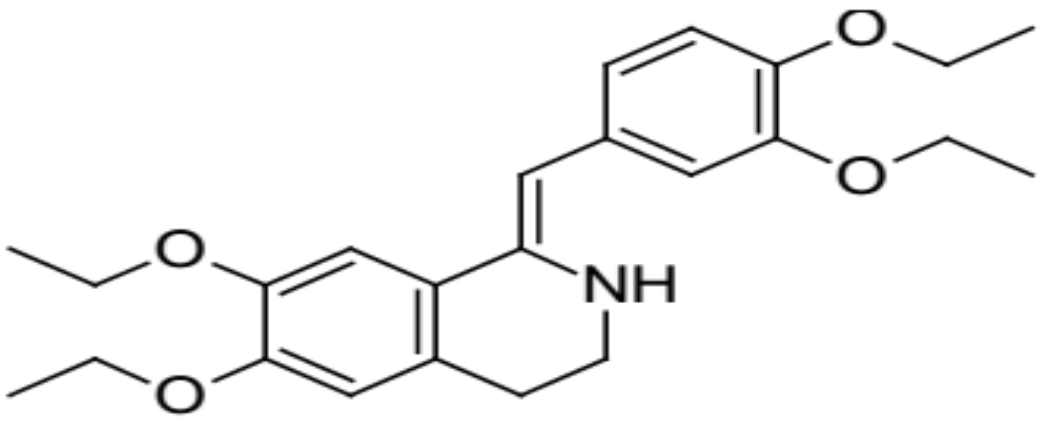

Figure 1. Chemical structure of Drotaverine Hcl {(1Z)-1-(3, 4-Diethoxybenzylidene)-6, 7-diethoxy-1, 2, 3, 4- tetrahydroisoquinoline.}

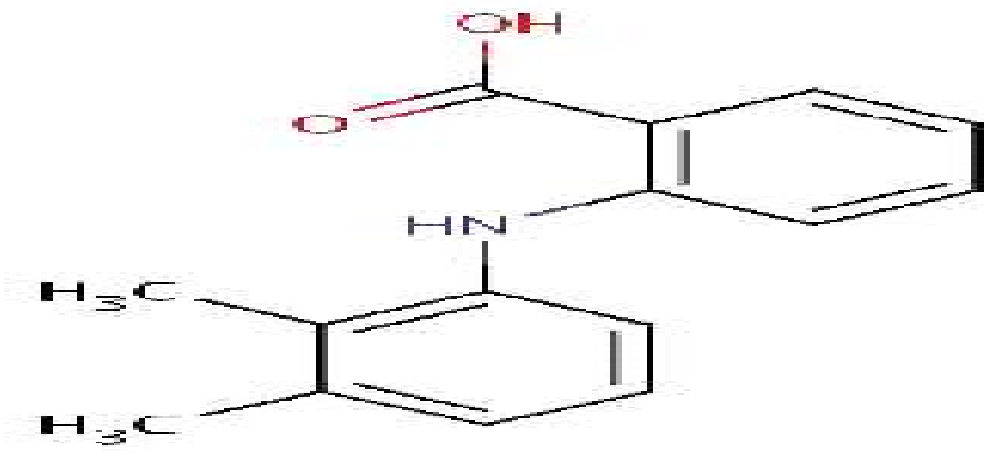

Figure 2. Chemical structure of Mefenamic Acid [N-(2, 3-xylyl) anthranilic acid]

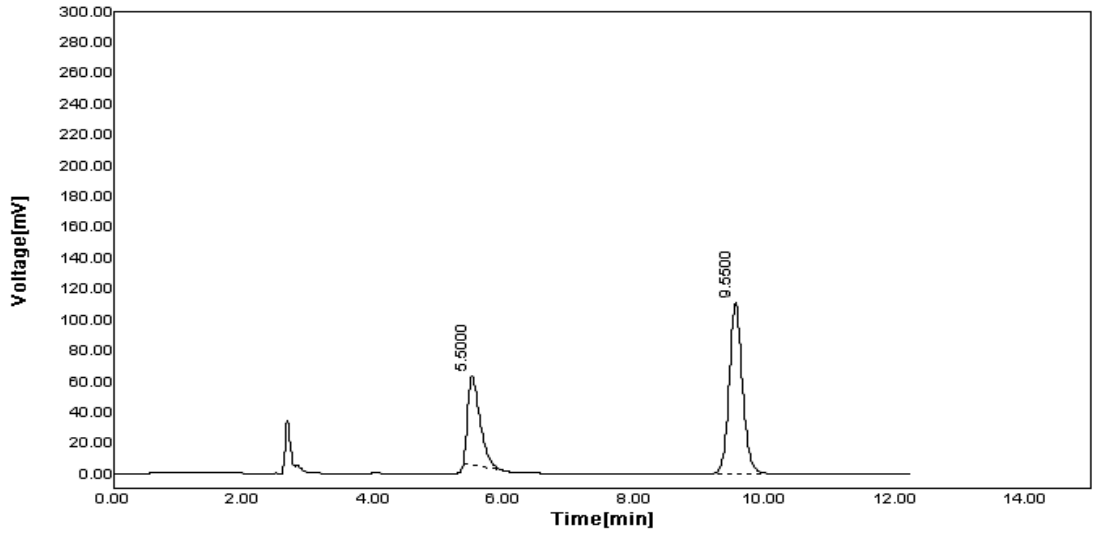

Figure 3. Optimized chromatogram of Drota HCl and MA

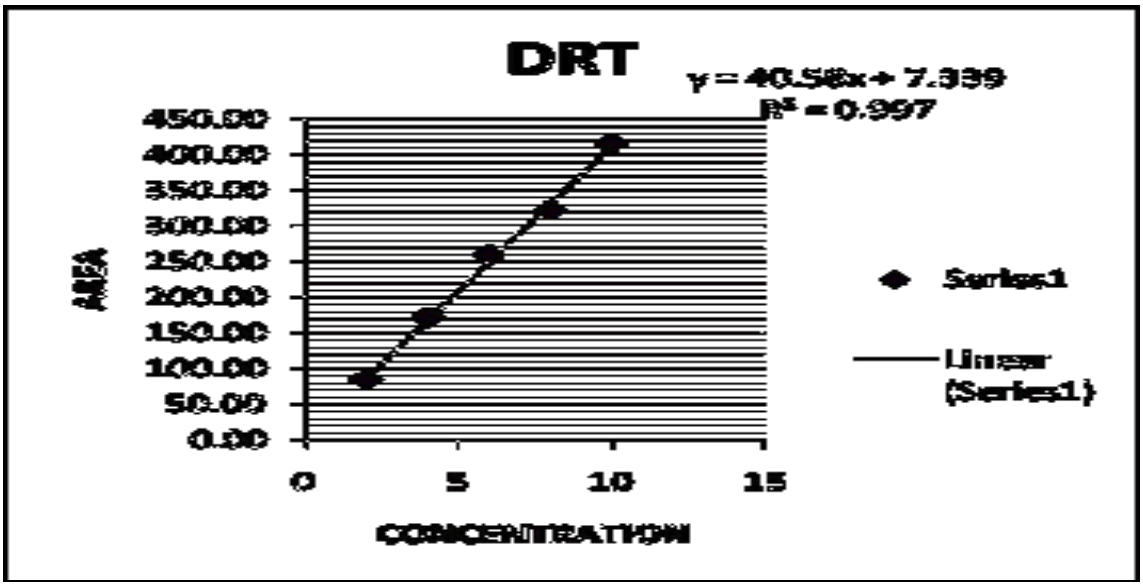

Figure 4. Linearity studies of Drota HCl

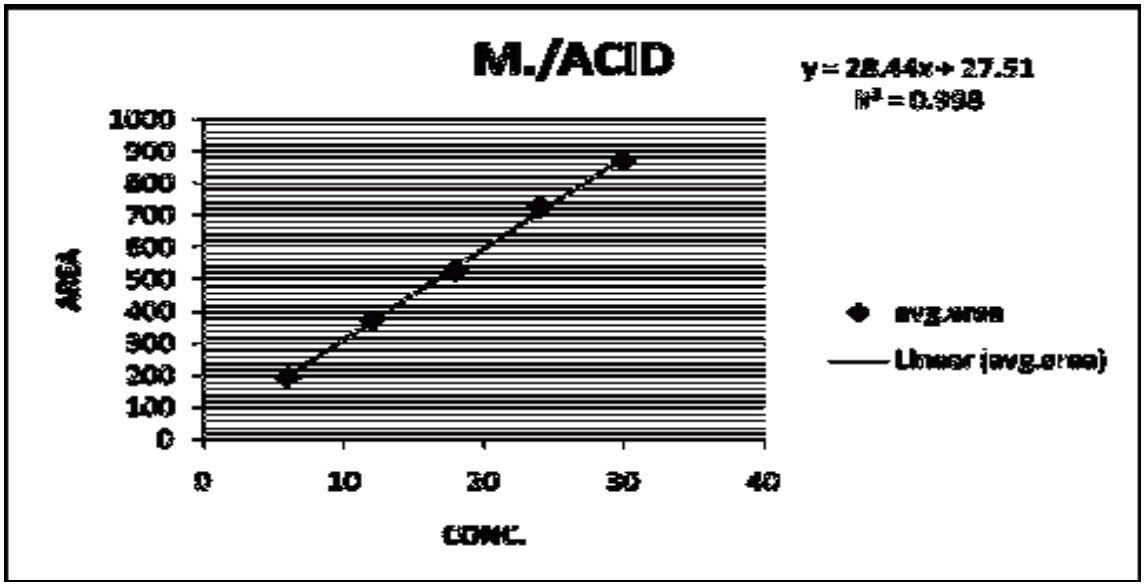

Figure 5. Linearity studies of MA

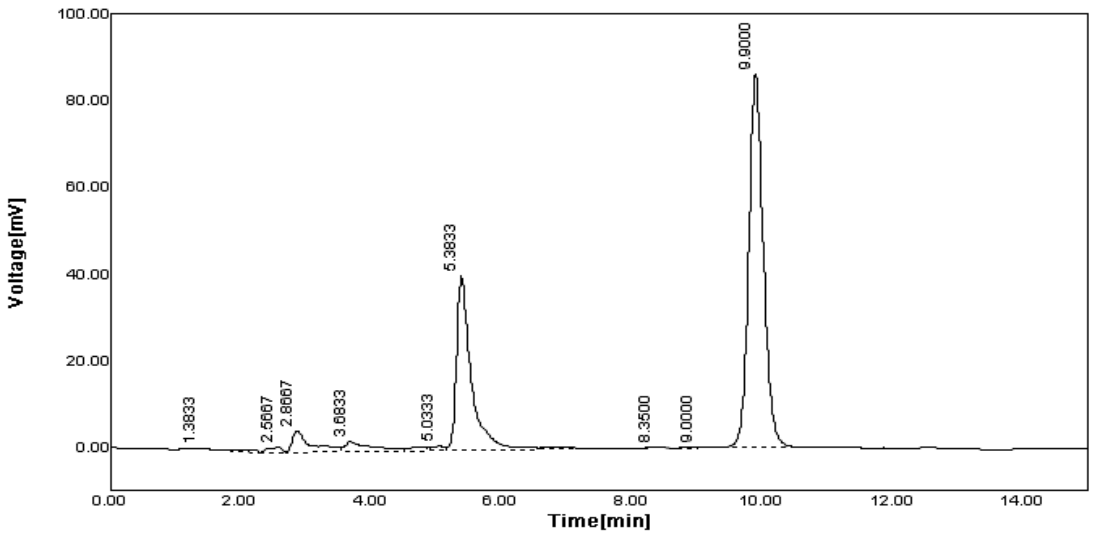

Figure 6. Chromatogram of acid degradation for 1 hr.

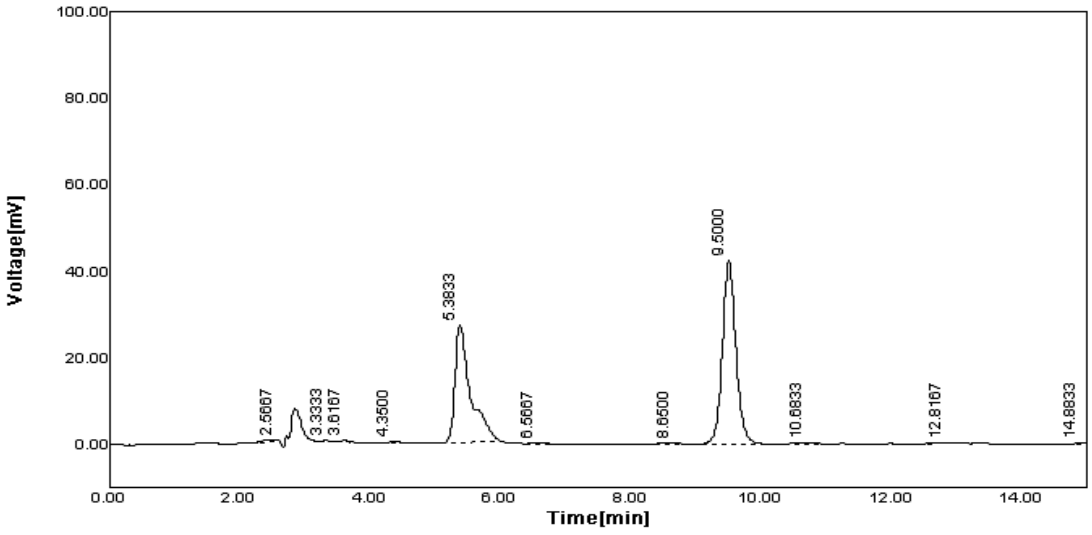

Figure 7. After 3 hrs chromatogram of acid degradation (with heat)

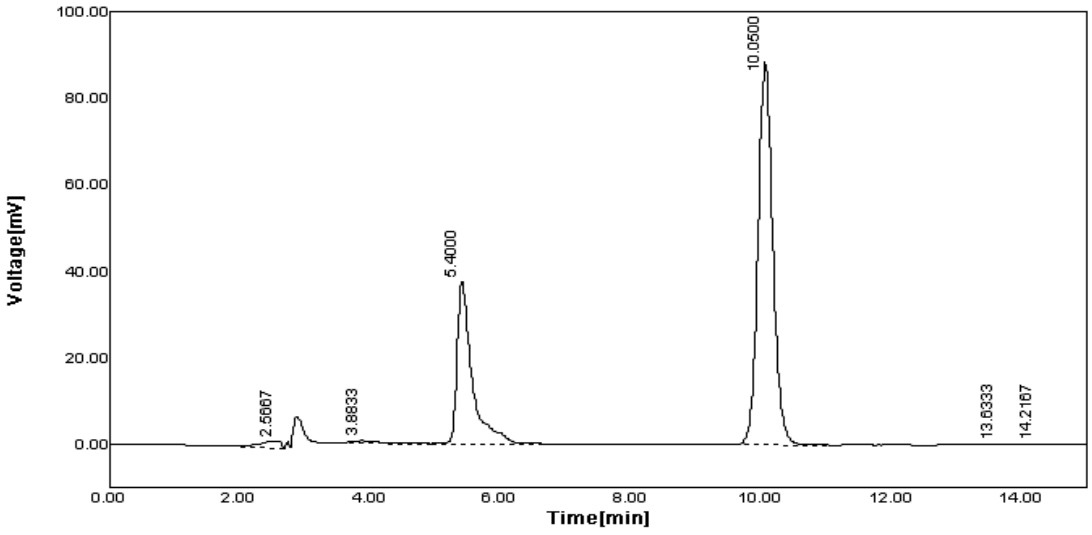

Figure 8. Chromatogram of Alkali degradation of 1 hr.

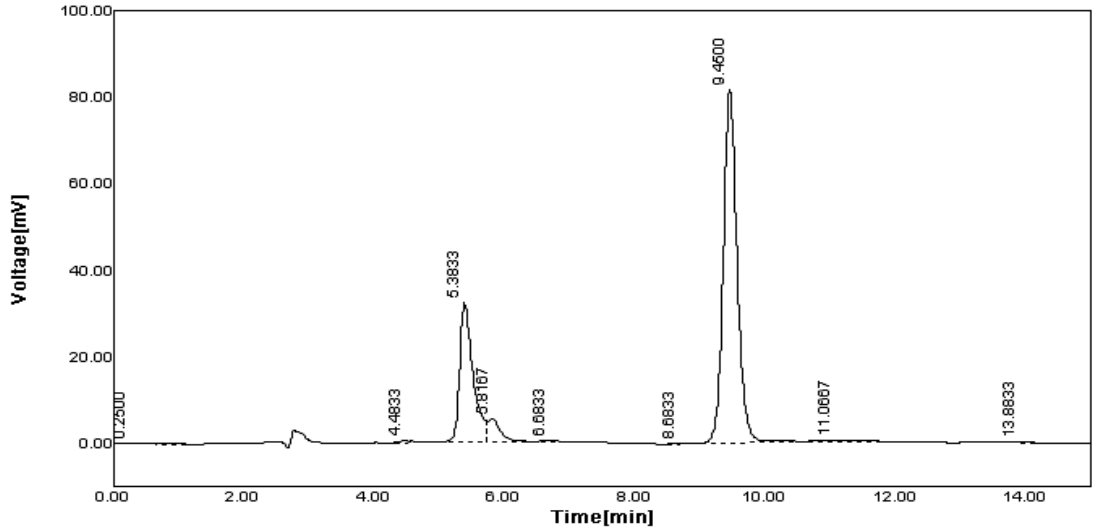

Figure 9. Chromatogram of Alkali degradation after 3 hr (with heat)

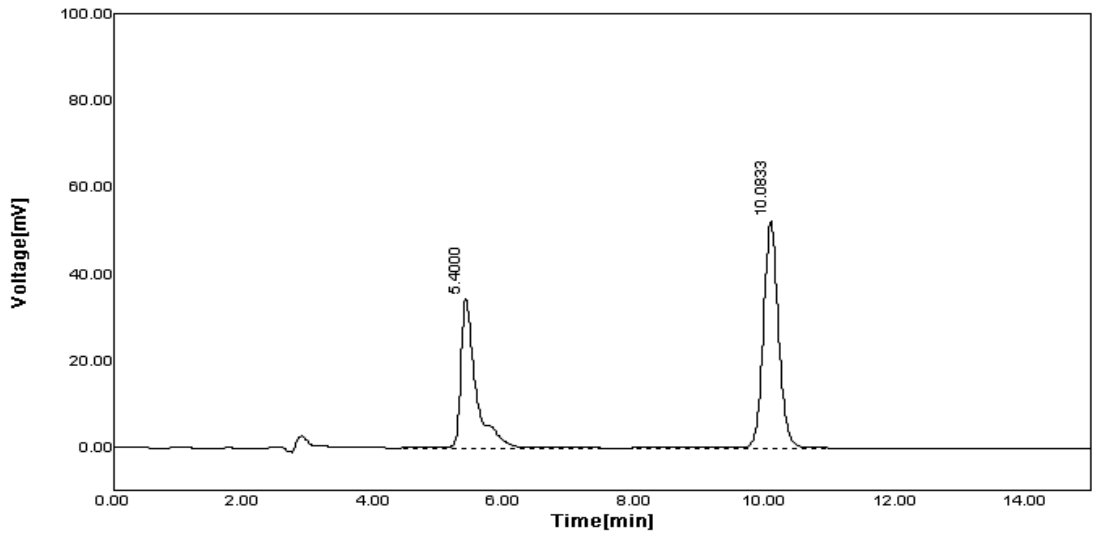

Figure 10. Chromatogram of Neutral degradation for 1 hr.

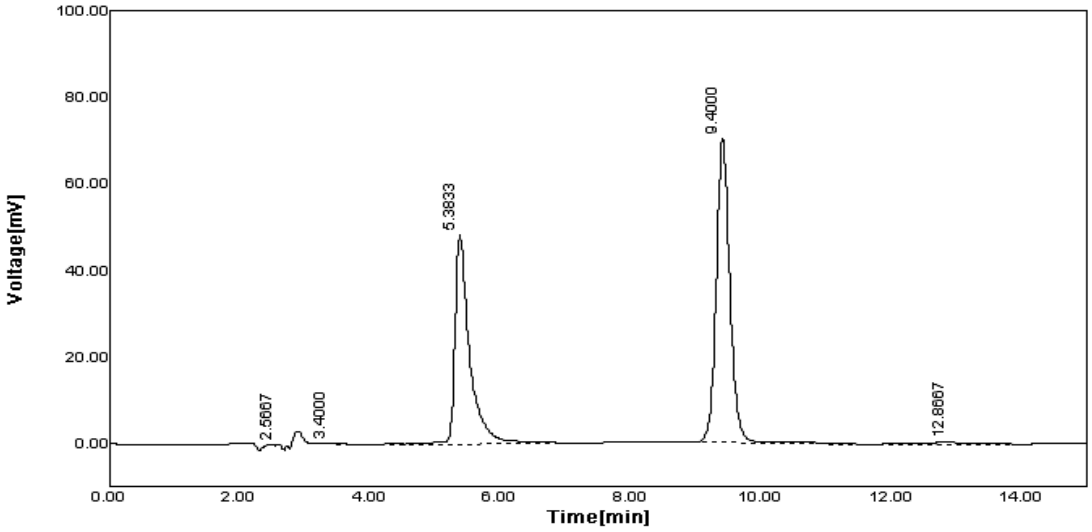

Figure 11. Chromatogram of Neutral degradation after 3 hr (with heat)

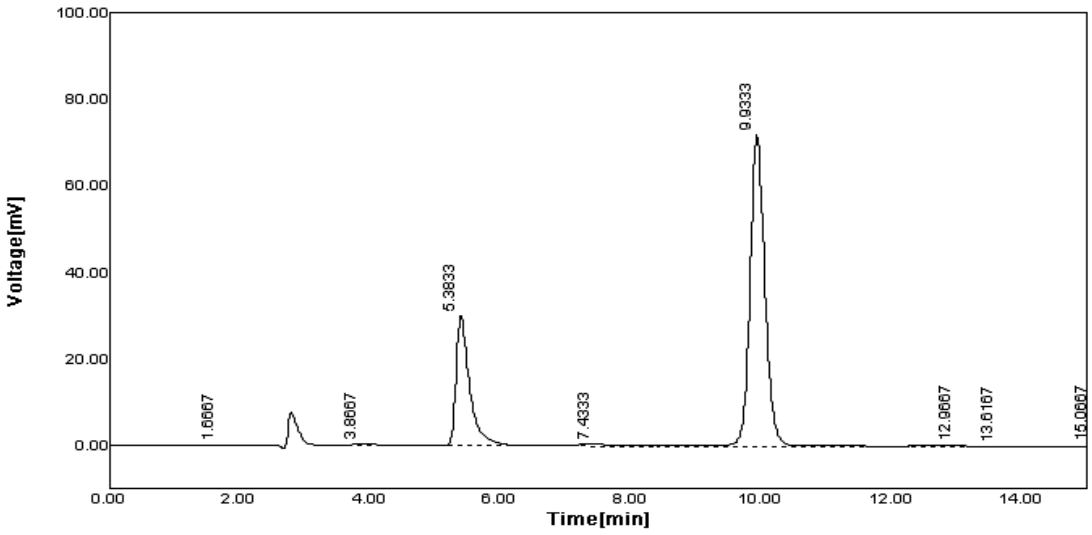

Figure 12. Chromatogram of oxidative degradation for 1 hr.

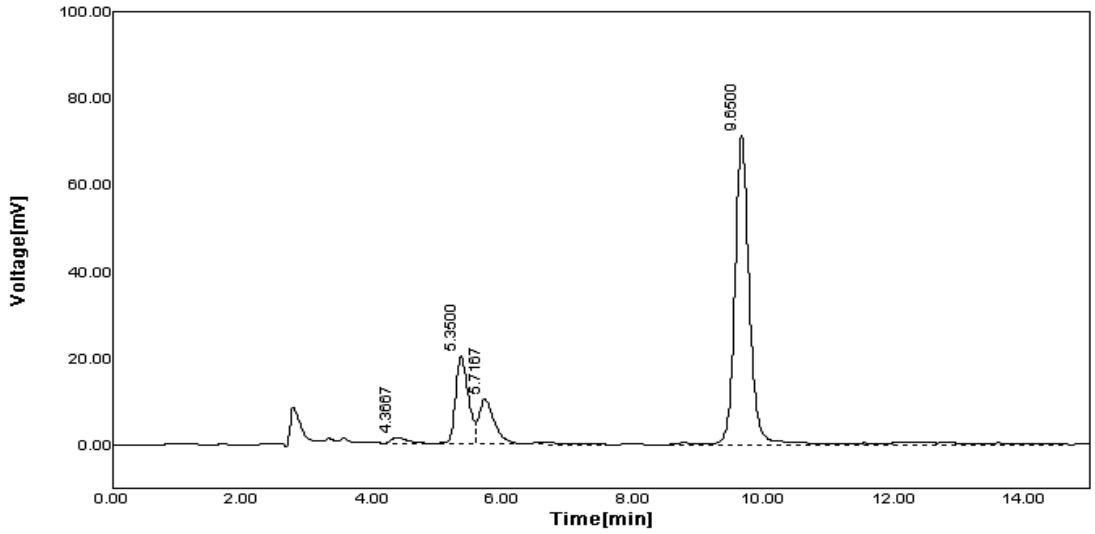

Figure 13. Chromatogram of oxidative degradation After 3 hr (with heat)

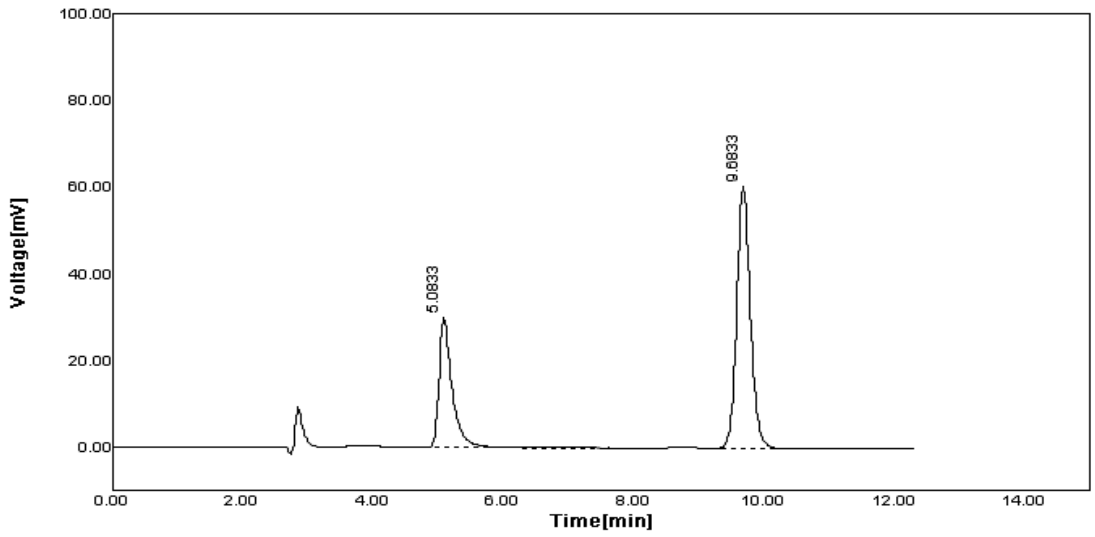

Figure 14. Chromatogram of sunlight degradation

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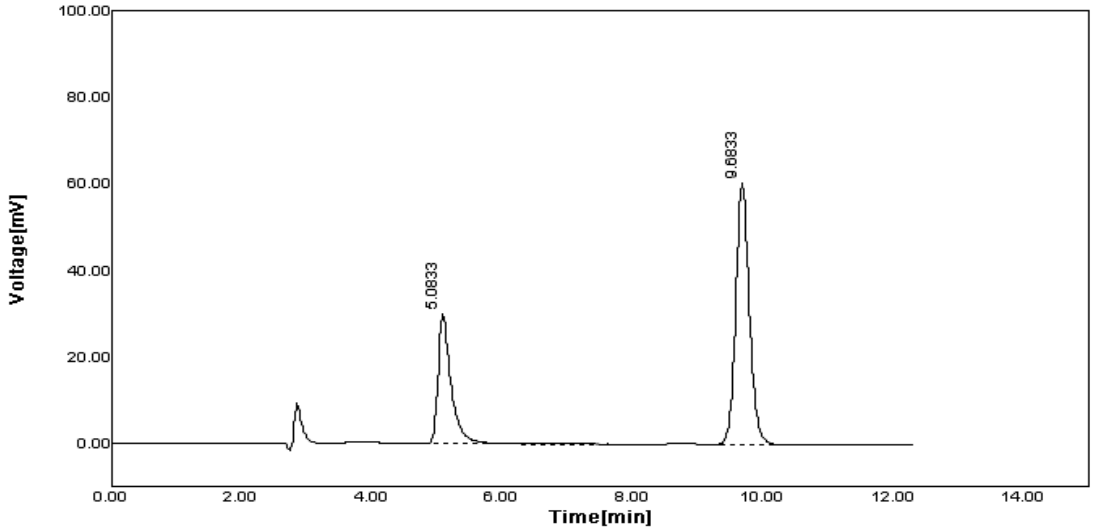

Figure 15. Chromatogram of thermal degradation