Research Journal of Recent Sciences _____ ISSN 2277 - 2502
Vol. **1(ISC-2011),** 281-288 **(2012)** *Res.J.Recent.Sci.*

Stability-Indicating LC Method for the Determination of Epinastine in Bulk Drug and in Pharmaceutical Dosage Form

Ahirrao V.K. and Pawar R.P.
Department of Chemistry, Deogiri Science College, Aurangabad 431004, MS, INDIA

Available online at: www.isca.in
(Received 8th Decembert 2011, revised 10th January 2012, accepted 6th February 2012)

Abstract

A novel stability-indicating LC assay method was developed and validated for quantitative determination of epinastine in bulk drugs and in pharmaceutical dosage form in the presence of degradation products generated from forced degradation studies. An isocratic, reversed phase LC method was developed to separate the drug from the degradation products, using an YMC ODS A- C18 (250 mm x 4.6 mm, 5 µm) column, and 0.05% v/v trifluroacetic acid and acetonitrile (65:35 v/v) as a mobile phase. The detection was carried out at the wavelength of 220 nm. The epinastine was subjected to stress conditions of hydrolysis (acid, base), oxidation, photolysis and thermal degradation. Degradation was observed for epinastine in base, thermal and in 30% H_2O_2 conditions. The drug was found to be stable in the other stress conditions attempted. The degradation products were well resolved from main peak. The percentage recovery of epinastine was ranged from (99.57% to 100.25%). The developed method was validated with respect to the linearity, accuracy (recovery), precision, specificity and robustness. The forced degradation studies prove the stability indicating power of the method.

Keywords: Column liquid chromatography, forced degradation, epinastine, stability indicating, method validation.

Introduction

Epinastine is chemically (*RS*)-3-amino-9,13b-dihydro-1H-dibenz(c,f)imidazo(1,5-a) azepine[1]. Epinastine is a selective H_1-receptor antagonist and also has an antiallergic effect by inhibiting the release of allergy-inducing substances such as histamine. Usually epinastine is used to treat bronchial asthma, allergic rhinitis, urticaria, eczema, dermatitis, pruritus cutaneous, prurigo and psoriasis vulgaris with pruritus [2-6].

A literature survey reveals that several methods were reported for the estimation of epinastine in plasma, serum and in tablet by high-performance liquid chromatography and by capillary electrophoresis[7-8]. Liquid Chromatographic and Ultraviolet Derivative spectrophotometric methods for determination of epinastine hydrochloride in coated tablets were reported[9-10]. Potentiometric determination of epinastine was also reported[11]. Literature survey reveals that, there is no stability-indicating HPLC assay method for determination of epinastine in bulk drug and pharmaceutical dosage form in presence of its degradants formed in stress conditions of hydrolysis (acid and alkali), oxidative, photolysis and thermal for degradation of epinastine. In the present research article, we report the development and validation of a stability indicating HPLC method for the determination of epinastine as bulk drug and pharmaceutical dosage form. It separates the drugs from the degradation products form under ICH suggested stress conditions (hydrolysis, oxidations, photolysis and thermal stress)[12-13]. We developed a rapid, robust and economic method which separates the degradation products from main peak. The main advantages of developed method are that method is useful for routine analysis in quality control labs due to short run time. The developed method is stability indicating and can be used for assessing the stability of epinastine in bulk drugs and pharmaceutical dosage form The developed method was validated with respective linearity, accuracy, precision, LOD, LOQ and robustness.

Material and Methods

Epinastine bulk drug (purity 99.8) was obtained from Transchem Ltd (Mumbai, India) and Alesion tablet (20 mg) were obtained from market. Trifluroacetic acid and Hydrochloric acid were obtained from Merck fine chemicals, India Limited. Acetonitrile, hydrogen peroxide, sodium hydroxide were obtained from Rankem laboratories, India. All chemicals and reagent were used have analytical or HPLC grade. UV cabinet was used of Kumar made, (India). Milli-Q-Water was used throughout the experiment.

Chromatographic Conditions: HPLC system used was an Agilent Technology (1100 series, Germany), system equipped with auto sampler, quaternary pump, degasser and a UV Detector. The out-put signal was monitored and processed using Agilent Chemstation software. The chromatographic column YMC ODS A (250 x 4.6 mm, 5 um, YMC Co. Ltd) was used. The instrumental setting of

flow was 1 ml/min. The injection volume was 10 µL. The detection wavelength was 220 nm.

Mobile Phase: The Mobile Phase consists of buffer and acetonitrile in the ratio of (65:35 v/v). The buffer used in the mobile phase contained 0.05%v/v Trifluroacetic acid in double -distilled water. The mobile phase was premixed and filtered through a 0.45 µm nylon filter and degassed.

Preparation of Standard stock solutions: All solutions were prepared on a weight basis and solution concentrations were also measured on weight basis to avoid the use of an internal standard. Solution of epinastine was prepared by dissolving the drug in the diluents and diluting them to the desired concentration. Diluent was composed of water and acetonitrile in the ratio of (70:30 v/v). A 75 mg of epinastine was accurately weighed, transferred in a 50 ml volumetric flask, dissolved and diluted to 50 ml with the diluent, from this stock solution 5 ml of solution transferred in to 100 ml volumetric flask and diluted to volume with diluent. This final solution contained 75 µg/ml of epinastine.

Sample solution (Tablets): Twenty tablets of Epinastine (Alesion 20) were finely ground using agate mortar and pestle. The ground material, which was equivalent to 75 mg of the active pharmaceutical ingredient, was extracted into diluent in a 50 ml volumetric flask by vortex mixing followed by ultra sonication and make up the volume by diluent. The solution was filtered through 0.45-micron filter and an appropriate concentration of sample (75 µg/ml concentration) was prepared in diluent at the time of analysis.

Specificity/ Selectivity: Specificity is the ability of the method to assess unequivocally the analyte in the presence of components, which may be expected to be present. Typically, these might include degradation products, matrix etc[14]. The specificity of the developed LC method for Epinastine was carried out in the presence of its degradation products. Stress studies were performed for epinastine bulk drug to provide an indication of the stability indicating property and specificity of the proposed method. Intentional degradation was attempted to stress condition exposing it with acid (1N hydrochloric acid), alkali (0.1 N NaOH), hydrogen peroxide (30%), heat (80^0C) and UV light (254 nm and 366 nm wavelength) to evaluate the ability of the proposed method to separate epinastine from its degradation products. For light study, study period was 48 h where as for acid, oxidation and base 3 h, for heat 24 h. Peak purity of test was carried out for epinastine by using PDA detector in stress samples. Assay studies were carried out for stress samples against epinaststine reference standard and the mass balance (% assay + % sum of all impurities + % sum of all degradants) was calculated. \The excipient mixture present in Alesion 20 tablets was injected in the optimized conditions to show the specificity of the method in formulation of epinastine.

Procedure for forced degradation study of Epinastine: Acidic Degradation: About 75 mg of epinastine was accurately weighed and dissolved in 10 ml diluent, then 10 ml of 1.0 N. HCl added and kept at 60 ^{o}C about 3 h in water bath, the solution was allowed to attend ambient temperature then solution was neutralized by 1.0 N NaOH to pH 7 and volume made up to 50 ml with diluent, from this solution 5 ml of solution transferred in to 100 ml volumetric flask and diluted to volume with diluent.

Alkali Degradation: About 75 mg of epinastine was accurately weighed and dissolved in 10 ml diluent, then 10 ml of 0.1 N. NaOH added and kept at 60^{o}C 3 h in water bath, the solution was allowed to attended ambient temperature. Then solution was neutralized by 0.1N HCl to pH 7 and volume made up to 50 ml with diluent, from this solution 5 ml of solution transferred in to 100 ml volumetric flask and diluted to volume with diluent .

Oxidative Degradation: About 75 mg of epinastine was accurately weighed and dissolved in 10 ml diluent , then 10 ml of 30% H_2O_2 solution added and kept 60^{o}C about 3 h in water bath, the solution was allowed to attended ambient temperature. Then volume made up to 50 ml with diluent, from this stock solution 5 ml of solution transferred in to 100 ml volumetric flask and diluted to volume with diluent.

Thermal Degradation: About 75 mg of drug substance kept at 80^{o}C for 48 h then the solution was prepared to achieve final concentration 150 µg/ ml.

UV Degradation: About 75 mg of drug substance were exposed to UV short (254 nm) and UV long (366 nm) light for 48 h. then the solution was prepared to achieve final concentration 150 µg/ ml..

Results and Discussion

Optimization of chromatographic conditions: The primary target in developing this stability-indicating HPLC method is to achieve the resolution between epinastine and its degradation products. To develop stability indicating method different stationary phases like C18, CN, different mobile phases containing buffers like phosphate, ammonium acetate and trifluoroacetic acid with different pH (3-5) and organic modifier (acetonitrile) were used. The data is shown in table 1 and 2.

Our objective of chromatographic method development was to achieve peak tailing factor less than 2 and retention time in between 3 to 10 minutes.

To achieve the separation of degradation products used stationary phases of C-18 and combination of mobile phase 0.05%v/v trifluroacetic acid with acetonitrile. The separation of degradation product and epinastine was achieved on YMC

ODS A column and 0.05% v/v trifluroacetic acid: acetonitrile (65:35 v/v) as a mobile phase and the column temperature 30^{o}C. The tailing factor obtained was less than 2 and retention time was also about 5 min for main peak and less than 20 min for degradation products, which would reduce the total run time and ultimately increase productivity and reduce the cost of analysis as per sample. Forced degradation study showed the method is highly specific and the entire degradation products were well resolved from the main peak. The developed method was found to be specific and method was validated as per ICH guidelines [14].

Result of forced degradation experiments: From the development studies, it was determined that aqueous solution of trifluoroacetic acid 0.05% v/v and acetonitrile in the ration of 65:35 (v/v), the flow rate of mobile phase was 1.0 ml/min. and column temperature at 30^{o}C. The analyte peak shape with less tailing, resolution from degradants and the chromatographic analysis time was less than 20 min. In optimized conditions analyte and its degradants were well separated. Typical retention time of epinastine about 5.4 min.

Though conditions used for forced degradation were attenuated to achieve degradation in the range of 10-30%, this could not be achieved in case of acidic and photolytic degradation even after exposure for prolonged duration[15]. During the initial forced degradation experiments, it was observed that alkali hydrolysis was a fast reaction for epinastine almost complete degradation occurred when 1.0N NaOH solution is used. The drug showed extensive degradation in alkali hydrolysis, oxidative condition and thermal. Table 3 indicates the extent of degradation, peak purity and assay of epinastine under various stress conditions. Chromatographic peak purity data was obtained from the spectral analysis report and a peak purity value greater than 990 indicates a homogeneous peak. The peak purity values for analyte peak in the range of 999-1000 indicating homogeneous peaks and thus establishing the specificity of assay method. Figure: 2 to 8 shows the chromatograms of diluent, epinastine tablet solution, alkali hydrolysis blank, alkali hydrolysis degraded drug product, oxidative blank, oxidative degraded drug product and thermally degraded drug product respectively.

Method Validation: Precision: Assay of method precision (intra-day precision) was evaluated by carrying out six independent assays of tablets. The intermediate precision (inter-day precision) of the method was also evaluated using two different analysts, different HPLC systems and different days in the same laboratory. The percentage of R.S.D and mean of six assay values obtained by two analysts were 0.15, 99.69 and 0.19, 99.52 respectively. Data is shown in table 4.

Accuracy (Recovery test): Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts of the drugs in the placebo.

The recovery was performed at three levels, 80%, 100%, and 120% of the label claim of the tablet (20 mg of epinastine). The recovery samples were prepared as aforementioned procedure, and then 5 ml of epinastine solutions were transferred into a 50 ml volumetric flask and volume made up with diluent. Three samples were prepared for each recovery level. The solutions were then analyzed, and the percentage recoveries were calculated from the calibration curve. The recovery values for epinastine ranged from 90.57% to 100.25% and the RSD % for nine determinations is 0.9%. Data is shown in table 3.

Linearity The linearity of the response of the drug was verified at seven concentration level, ranging from LOQ-200% of the targeted level (75 µg/ml), of the assay concentration. Linearity solutions were injected in triplicate. The calibration graphs were obtained by plotting peak area verses the concentration, data was treated by least-squares linear regression analysis. The equation of the calibration curve for analyte obtained y = 5267.5x – 4505.5, the calibration graphs were found to be linear in the aforementioned concentrations. The coefficient of determination is 0.999.

Limit of detection and Limit of quantification (LOD and LOQ): For determining the limit of detection (LOD) and limit of quantification (LOQ), a specific calibration curve was constructed using samples containing the analyte in the range of LOD and LOQ. The LOD and LOQ for the epinastine in HPLC method was 0.05 and 0.18 µg/ml respectively.

LOD and LOQ were calculated by using following equations.

LOD = Cd x Syx /b

LOQ = Cq x Syx /b

Where, Cd/Cq is coefficient for LOD/LOQ; Syx is residual variance due to regression; b is slope.

Precision at limit of quantification was checked by analyzing six test solutions prepared at LOQ level and calculating the percentage relative standard deviation of area which was less than 1.8%.

Robustness: To determine the robustness of the developed method experimental condition were purposely altered and the resolution between epinastine and base degradation product were evaluated. The flow rate of the mobile phase was 1.0 ml/min. To study the effect of flow rate on the resolution, it was changed by 0.1 units from 0.9 to 1.1 ml/min.

The effect of percent organic strength on resolution was studied by varying acetonitrile from -5 to +5%. The effect of column temperature on resolution was studied at 25 ^{o}C and 35 ^{o}C instead of 30^{o}C, while the other mobile phase components were held constant stated in chromatographic condition. The resolution between epinastine and base degradation product in robustness study was not less than 3.0 at in all conditions. Also assay was performed at each condition in triplicate. The data is shown in table 6.

Stability of analytical solution: The stability of the standard solutions and the sample solutions was tested at intervals of 24, 48 and 72 h. The stability of solutions was determined by comparing results of the assay of the freshly prepared solutions. The RSD for the assay results determined up to 72 h for epinastine was 0.47 %. The assay values were within 0.7 % after 72 h. The results indicate that the solutions were stable for 72 h at ambient temperature.

Conclusion

The developed method is stability indicating and can be used for assessing the stability of epinastine in bulk drugs and pharmaceutical dosage form. The developed method can be conveniently used for the assay determination of epinastine in bulk drugs and pharmaceutical dosage form. The developed HPLC method was specific, selective robust, rugged and precise method. The developed HPLC method can be conveniently used for assessing stability, assay, related substances and dissolution of tablets of the pharmaceutical dosage form containing epinastine in quality control.

Acknowledgment

The authors are grateful to the Transchem Ltd (Mumbai, India) for making available the samples of epinastatine hydrochloride and Department of chemistry, Deogiri College of Scinece Aurangabad, India for providing laboratory facility for this research work.

References

1. Merck Index, Merck and Co, Inc, USA, 13th edn., **(2001)**

2. Oshima K., Kazuhito, Asano K., Kanai K., Suzuki M. and Suzaki H., Influence of epinastine hydrochloride, an H1-receptor antagonist, on the function of mite allergen-pulsed murine bone marrow-derived dendritic cells in Vitro and in vivo, *Mediators of inflammation*, **(2009)**

3. Toyoda M., Nakamura M., Nakagawa H., Distribution to the skin of epinastine hydrochloride in atopic dermatitis patients, European J. of dermatology, **17(1)** 33-36, **(2007)**

4. Kanai K., Asano K., Watanabe S., Kyo Y. and Suzaki H., Epinastine hydrochloride antagonism against interleukin-4-mediated T cell cytokine imbalance in vitro, *Int. Arch Allergy Immuno.,* **140,** 43-52 (**2006)**

5. Takahashi N., Aizawa H., Inoue H., Matsumoto K., Nakano H., Hirose T., Nishima S., Hara N., Effects of epinastine hydrochloride on cholinergic neuro-effector transmission in canine tracheal smooth muscle, European Journal of Pharmacology, **358 (1)** 55-61 **(1998)**

6. Fraunfelder F.W., Epinastine hydrochloride for atopic disease, *Drugs Today*, 40 (8) 677-683 (2004)

7. *Ohtani H., Kotaki H., Sawada Y., Iga T***.,** Quantitative determination of epinastine in plasma by high-performance liquid chromatography, *J. of Chrom. B: Biomedical Sciences and Applications*, **683 (2)** 281-284 (**1996)**

8. Vera-Candioti L., Olivieri A.C., Goicoechea H.C., Simultaneous multiresponse optimization applied to epinastine determination in human serum by using capillary electrophoresis, Anal Chim Acta, **595 (1-2)** 310-318**, (2007)**

9. Ghisleni D.D**.,** Steppe M**.,** Elfrides E.S. Schapoval. Development and validation of Liquid chromatographic and ultraviolet derivative spectrophotometric methods for determination of epinastine hydrochloride in coated tablets, Journal of AOAC International , **90(5)** 1266-1271 **(2007)**

10. Saleh O.A., El-Azzouny A.A., Badawy A.M. and Aboul-Enein H.Y., A validated HPLC method for separation and determination of epinastine hydrochloride enantiomers, Journal of Liquid Chromatography and Related technologies, **33(3)** 413-422 **(2010)**

11. Basaveswara Rao M.V., Reddy B.C.K., Srinivas Rao T., and Kalyani P., Determination of epinastine hydrobromide assay by potentiometric method, Rasayan Journal of Chemistry, **2(2)** 361-363, **(2009)**

12. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, Stability testing of New Drug Substances and Products, QIA (R2), **(2003)**

13. Drug Information Branch (HFD-210), Validation of analytical procedure: Methodology, Step 4 In ICH Harmonized Tripartite Guidelines Q2B. Center for Drug Evaluation and Research, Rockville, MD, **(1996)**

14. ICH-Guidelines: Text on validation of analytical procedures. Q2 (R1)

15. Bakshi M., and Singh S., Development of validated stability-indicating assay methods critical review, *J. Pharm. Biomed Anal*., **28,** 1011-1040 **(2002).**

Figure-1
Chemical structure of epinastine

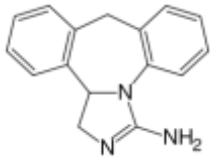

Figure-2
Chromatograms of diluents

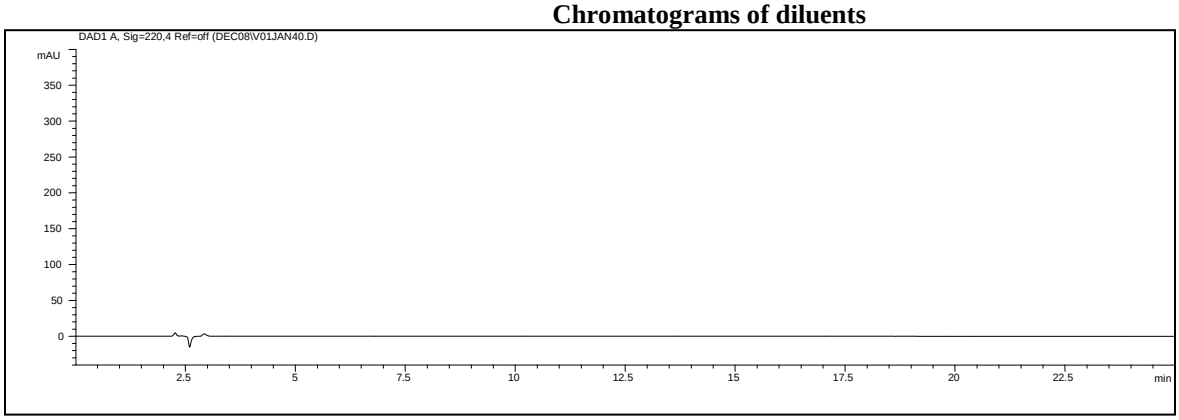

Figure-3
Chromatogram of epinastine tablet Soln.

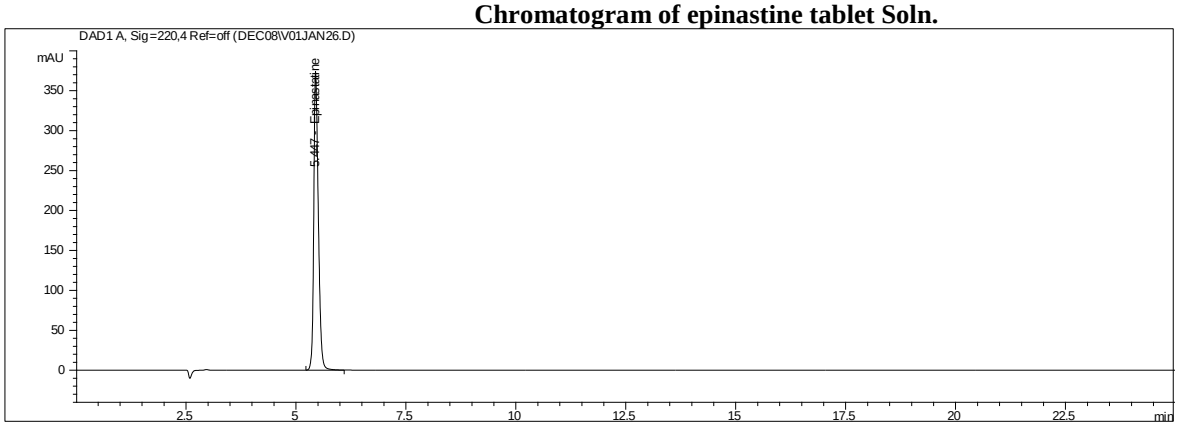

Figure- 4
Chromatogram of base hydrolysis blank

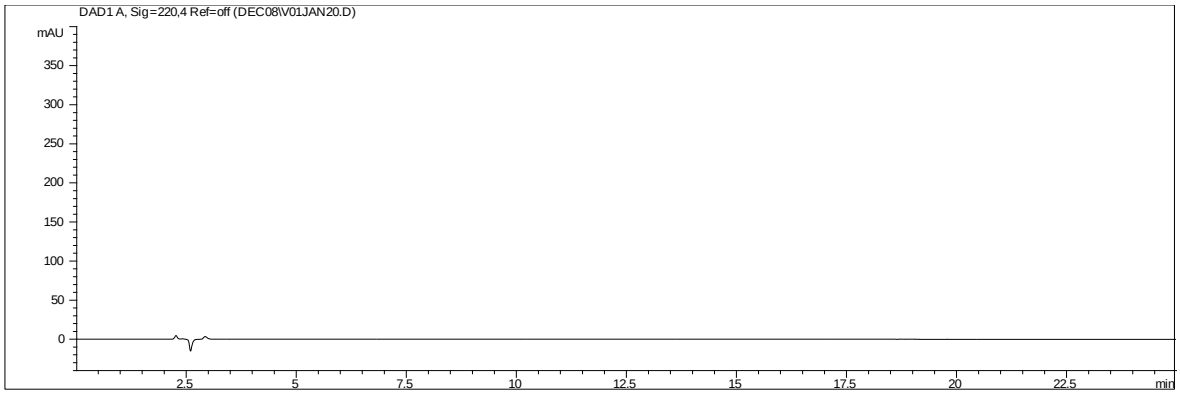

Figure- 5
Chromatogram of base hydrolysis of epinastine

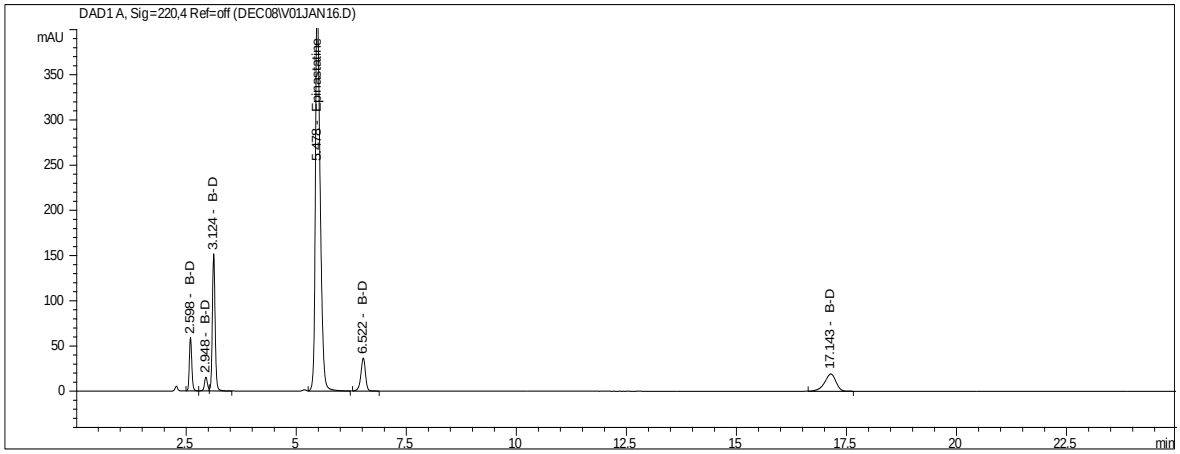

Figure-6
Chromatogram of oxidative degradation blank

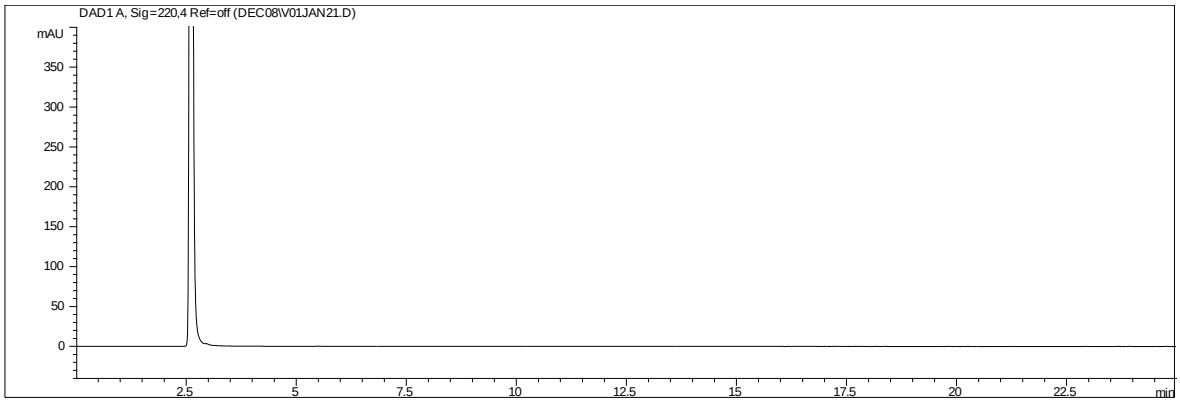

Figure- 7
Chromatogram of oxidative degradation of epinastine

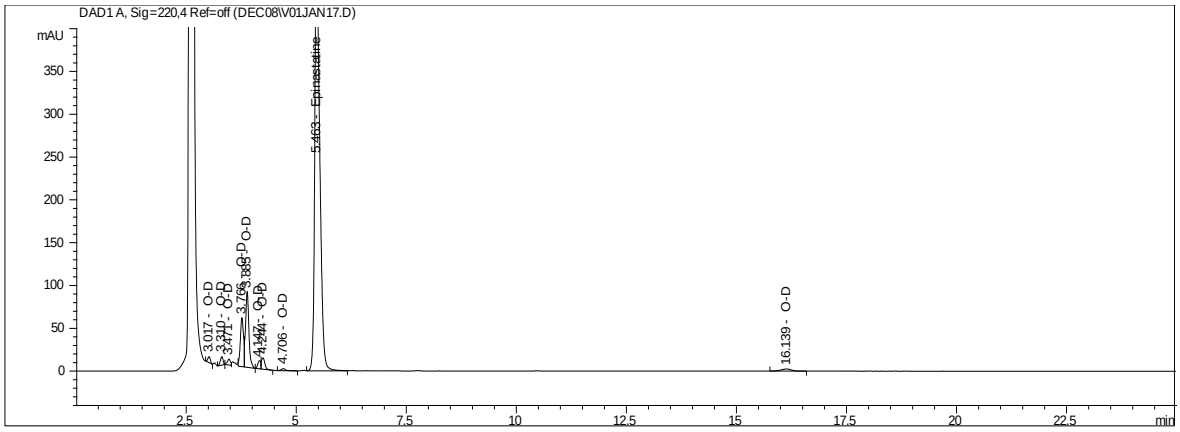

Figure- 8
Chromatogram of thermal degradation of epinastine

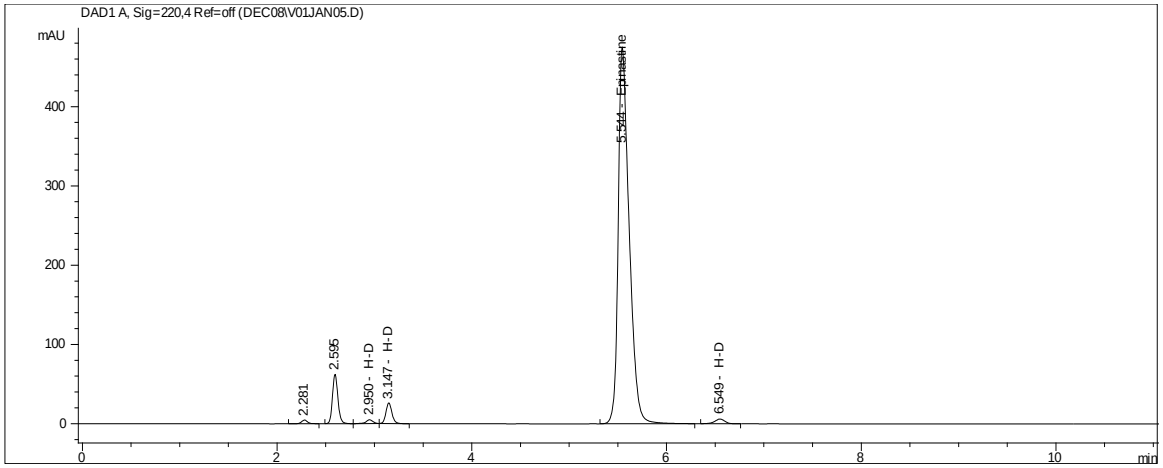

Table-1
The chromatographic behavior of Epinastine in different mobile phases

Mobile Phase	Retention time (Min.)
Water : Acetonitrile (60:40)	3.2
0.05M Ammonium Acetate pH 4.5with Acetic acid : Acetonitrile (60:40)	4.5
0.05M Ammonium Formate pH 3.5with Formic acid : Acetonitrile (70:30)	9.2
Water : Methanol (40:60)	3.9

Table-2
The chromatographic behavior of Epinastine on different columns

Mobile Phase	**Retention Time (Min)**
YMC ODS A C18;	5.4
5cm × 4.6 mm *i.d.*,5 µ particle	
Zorbax SB C18;	4.7
25cm × 4.6 mm *i.d.*,5 µ particle	
Kromosil C18;	3.6
25cm × 4.6 mm *i.d.*, 5µ particle	
Waters Symmetry C18;	3.6
25cm × 4.6 mm *i.d.*, 5 µ particle	

Table-3
Results of forced degradation study

Stress condition	**Epinastine (%) degradation**	**Peak purity[a]**	**Assay (%)**
Acidic	No degradation	999.936	99.18
Alkali	27.88	999. 706	73.26
Oxidative	18.12	999.532	81.23
Thermal	17.20	999.379	82.52
UV-short	No degradation	999.609	99.89
UV-long	No degradation	999.977	100.32

[a] peak purity values in the range of 990-1000 indicate a homogeneous peak

Table-4
Precision data

Average assay	**Assay % (n=6)**	**% RSD Standard peak area**	**% RSD (n=6)**
Chemist 1	99.69	0.12	0.15
Chemist 2	99.52	0.22	0.19

Table-5
Results of recovery experiment

Amount of standard Spiked (mg)	**Found (mg)**	**Recovery% (n=3)**
16	15.88	99.57
20	20.05	100.25
24	23.92	99.87

Table-6
Results of robustness study

Sr. no.	**Parameter**	**Variation**	**Assay % n=3**	**Resolution[a]**
1	Flow rate	a) At 0.9 ml/min	99.38	3.8
	(± 10% of the set flow)	b) At 1.1 ml/min	99.66	4.2
2	Mobile phase composition	a) At 38.5 ml	99.62	3.3
	(± 5% of organic modifier)	b) At 31.5 ml	100.04	4.5
3	Temperature (± 5° C of set temperature)	a) At 25°C	99.89	4.0
		b) At 35°C	99.53	4.4

[a] Resolution between epinastine and base degradant peak at RRT 1.2