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Der Pharma Chemica, 2014, 6(2):343-351
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Stability indicating HPLC method for the quantification of bepotastine besilate and its related substances*

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ABSTRACT

A reverse phase liquid chromatographic (RP-LC) method was developed for the quantification of the related impurities of Bepotastine besilate drug substance. The method was optimized using buffer (prepared by dissolving 1.0mL H_3PO_4 (85%) taken in 1000mL milli-Q-water and then pH was adjusted to 3.0 with dilute triethylamine solution) as mobilephase-A, and Acetonitrile : Methanol : water in the ratio of 70:20:10 v/v/v as mobile phase-B. The flow rate was set at 1.0 mL min^{-1}, wavelength at 225nm respectively and the column temperature was maintained at 45°C. The capability of stability indicating method developed was demonstrated by studying the degradation products generated during the forced degradation studies under the following conditions i) water hydrolysis, ii) at 75% relative humidity, iii) oxidative, iv) thermal v) photolytic, vi) acid, vii) base, and viii) photolytic degradation. The developed method can be used for the determination of synthetic and degradation impurities in the regular quality control analysis for the drug substance.

Keywords: Bepotastine besilate, Reverse phase liquid chromatography, Stability-indicating methods, method development, Method validation, Stress conditions, ICH.
**MSNRD Communication No.018*

INTRODUCTION

The biological activity of chiral substances often depends upon their stereochemistry. A large percentage of commercial and investigational pharmaceutical compounds are enantiomers, and many of them show significant enantioselective differences in their pharmacokinetics and phar macodynamics [1-3]. Analysis of the enantiomeric purity of chiral drug candidates has become very important particularly in the pharmaceutical and biological fields, because few enantiome rs of racemic drugs have relatively different pharmacokinetic properties and diverse pharmacol ogical or toxicological effects [4-7], apart from this the International Conference on Harmoniz ation (ICH) guide-lines [8-10] emphasizes that the purity and assay of drug susceptible to change during storage, must be determined by using validated stability- indicating methods, which can selectively determine the drug in presence of its process (including the other isomers) and degradation impurities. Bepotastine (Talion) is an antihistamine[11]. It was approved in Japan for use in the treatment of allergic rhinitis and urticaria/pruritus in July 2000 and January 2002, respectively, and is marketed by Tanabe Seiyaku Co., Ltd. under the brand name TALION. Histamine H_1 receptor (H_1R) antagonists, or antihistamines, are often used for treating allergic disorders such as seasonal rhinitis. Antihistamines mainly act on peripheral tissues, but can induce sedation as a central side-effect. This

undesirable side-effect is caused by blockade of nerve transmission in the histaminergic neuron system which projects from the tuberomammillary Bepo Beponucleus in the posterior hypothalamus to almost all cortical areas[12-15]. First-generation antihistamines that can easily penetrate the blood–brain barrier (BBB), such as diphenhydram ine and d-chlorpheniramine, tend to occupy a large proportion of postsynaptic H_1Rs as demonstrated by positron emission tomography (PET) [12, 17-19]. PET also reveals that second-generation antihistamines (mildly or slightly sedative antihistamines), such as cetirizine and olopatadine, can slightly penetrate the BBB and occupy some H_1Rs[12,17, 20-21]. Users who take these second-generation antihistamines at doubled or tripled doses to achieve desired effects may suffer from dose-related cognitive impairment. Third-generation antihistamines (truly nonsedative antihistamines), such as fexofenadine and ebastine, hardly penetrate the BBB and do not occupy H_1Rs even at high doses, as demonstrated by ^{11}C-doxepin PET [20]. Thus, the sedative property of antihistamines can be evaluated in terms of H_1R occupancy (H_1RO). Such variations in BBB permeability are caused by various factors, including differences in lipophilicity, molecular size and actions of drug transporters. Bepotastine besilate ({d-(S)-4-[4-[(4-chlo rophenyl) (2-pyridyl)methoxy]piperidino} butyric acid monobenzenesulphonate, betotastine besilate, CAS 125602-71-3, TAU-284 or Talion), a new second-generation antihistamine dev eloped in Japan, is now used as an oral tablet for allergic rhinitis and chronic urticaria [22-24]. Previous studies have demonstrated its excellent antiallergic effects compared with other antihistamines such as ketotifen, cetirizine, epinastine and terfenadine [25-29], whereas only a few studies have shown its central effects [29-30]. Only one available animal behavioural study by Kato and colleagues has demonstrated that Bepotastine is a highly specific drug to H_1R, having no significant binding affinity for histamine H_3, adrenergic α_1, α_2, β, dopaminergic D_2, serotonergic $5HT_2$, muscarinic or benzodiazepine receptors, and that it poorly penetrates the BBB [30]. Takahashi and colleagues first conducted a double-blind, placebo-controlled, crossover study to measure subjective sedation and psychomotor activities following administration of bepotastine, cetirizine, fexofenadine and olopatadine [29], where Bepotastine had the least sedative effect [29]. Chemically, Bepotastine besilate is designated as (+)-4-[[(S)-p-Chloro-alpha-2-pyridylbenzyl]oxy]-1- piperidine butyric acid monobenzenesulfonate. The empirical formula is C21H25ClN2O3.C6H6O3S and its molecular weight is 547.06 and the chemical structure of Bepotastine besilate is shown in (Fig.1).

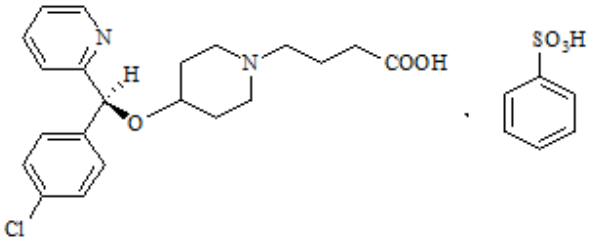

Fig-1: Bepotastine Besilate

Chemical name: (+)-4-[[(S)-p-Chloro-alpha-2-pyridylbenzyl]oxy]-1-piperidine butyric acid monobenzenesulfonate. Recently, A rapid andsensitive method method for estimation of bepotastin in human plasma by LC-MS/MS method was published[31]. UPLC–MS/MS Method for Determination of Bepotastine in Human Plasma method is available[32]. However extensive survey revealed that stability indicating HPLC method for quantitative determination of Bepotastine besilate and its related impurities in active pharmaceutical ingredient was not reported till date. Therefore it was felt necessary to develop an accurate, rapid, and specific stability indicating method for the determination of 'Related substances' of Bepotastine besilate. We have developed a new accurate and stability indicating HPLC method for the determining the 'Related substances' of Bepotastine besilate.

MATERIALS AND METHODS

Chemicals and Reagents

Bepotastine Besilate and its impurities viz. Condensed ether, Condensed ester, Isopropyl ester and n-Butyl ester impurities were obtained from MSN Laboratories Private Limited, Hyderabad, India. HPLC-grade of acetonitrile and AR grade of Ortho phosphoric acid, Triethylamine, hydrochloric acid, sodium hydroxide and hydrogen peroxide (30%) were obtained from Rankem, New Delhi, India. Milli Q Millipore (USA) purification system was used to prepare high pure water.

HPLC Instrumentation and Conditions

The method development attempts, forced degradation studies and the method validation was performed in Agilent 1200 series LC systems with a diode array and variable wave length detectors (Agilent Technologies, Waldbronn, Germany). The data were collected and processed using Ez chrom Elite software. The peak homogeneity was studied by using Agilent 1200 series DAD detector.

Chromatographic conditions

The chromatographic separation was optimized in the Symmetry shield RP-18 column with the dimension of 250mm x 4.6 mm and 5µm as particle size. A gradient elution was involved with the buffer (1.0mL H3PO4 (85%) in 1000mL of milli Q water (100%) and pH adjusted to 3.0 with triethylamine solution) as mobilephase-A, and acetonitrile : methanol : water in the ratio of 70:20:10 (*v/v/v)* as mobile phase B. The HPLC gradient program was set as (time/% mobile phase- B) 0.01/25, 25/64, 27/64, 27.5/25, 35/25. The flow rate of the mobile phase and the column temperature was set as 1.0 mL min^{-1} and 45°C. The detection wave length was optimized at 225 nm, 5µL injection volume. A mixture of Mobile phase-A and acetonitrile 1:1v/v was used as diluent, and diluent used for needle wash purpose.

Preparation of standard solutions:

Mobilephase-A and Acetonitrile in the ratio of 1:1v/v was used as diluent. A standard solution (Reference solution) 0.003mg/mL of Bepotastine besilate solution was prepared in the diluent. A stock solution with the blend of Condensed ether, Condensed ester, Isopropyl ester and n-Butyl ester impurities was also prepared in diluent for the preparation of system suitability solution (0.15% solution with respect to 3.0mg/mL Bepotastine besilate test concentration).

RESULTS AND DISCUSSION

Method development and optimization

The HPLC method was optimized so as to obtain stability– indicating method that it could resolve degradation impurities from Bepotastine besilate. Different stationary phases with different selectivity were used for the determination of Bepotastine besilate and it's impurities as the initial attempts. However good peak shape with less peak width and the resolution of all the related impurities were achieved satisfactorily in Symmetry shield RP-18 column with the dimension of 250mm x 4.6 mm and 5µm as particle size. A gradient elution was involved a buf fer (1.0mL H3PO4 (85%) in 1000mL of milli Q water (100%) and pH adjusted to 3.0 with triethylamine solution) as mobilephase-A, and acetonitrile : methanol : water in the ratio of 70:20:10 (*v/v/v)* as mobile phase B. The HPLC gradient program was set as (time/% mobile phase- B) 0.01/25, 25/64, 27/64, 27.5/25, 35/25. The flow rate of the mobile phase and the column temperature was set as 1.0 mL min^{-1} and 45°C. The detection wave length was optimized at 225 nm, 5µL injection volume. A mixture of Mobile phase-A and acetonitrile 1:1v/v was used as diluent, and diluent used for needle wash purpose.

The system suitability parameters are resolution between any two adjacent known impurities and known impurity and bepotastin peak should not be less than 1.5, detector sensitivity for reference solution the S/N ratio should not be less than 30 and tailing factor not more than 2.0 and Theoretical plates should not be less than 3000 for Bepotastine standard peak. Inhibit the integration of peak at about~ 4.0 retention time .Which is due to Benzene sulphonic acid The developed method is specific for Bepotastine besilate and its degradation products. The structures and chemical names of the impurities are given below:

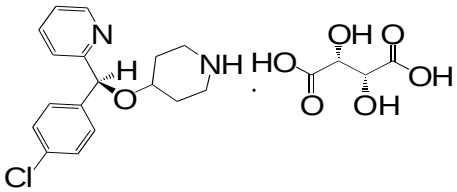

Fig-2 : Condensed ether

Chemical name : (S)-2-((4-chlorophenyl)(piperidin-4-yloxy)methyl)pyridine L-tartrate.

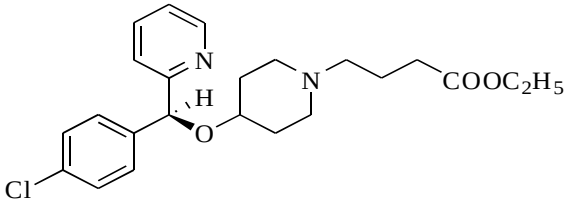

Fig-3 : Condensed ether

Chemical name : (S)-ethyl 4-(4-((4-chlorophenyl)(pyridin-2-yl)methoxy) piperidin-1-yl)butanoate.

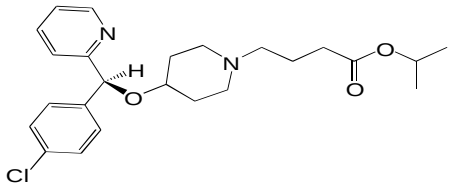

Fig-4: Isopropyl ester impurity

Chemical name : (S)-isopropyl 4-(4-((4-chlorophenyl)(pyridin-2-yl)methoxy)piperidin-1-yl)butanoate.

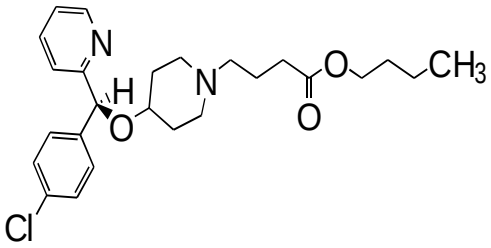

Fig-5: n-Butyl ester impurity

Chemical name : (S)-butyl 4-(4-((4-chlorophenyl)(pyridin-2yl)methoxy)piperidin-1-yl)butanoate.

Chromatograms: A typical chromatogram of Blank, Reference solution, System suitability solution are given below:

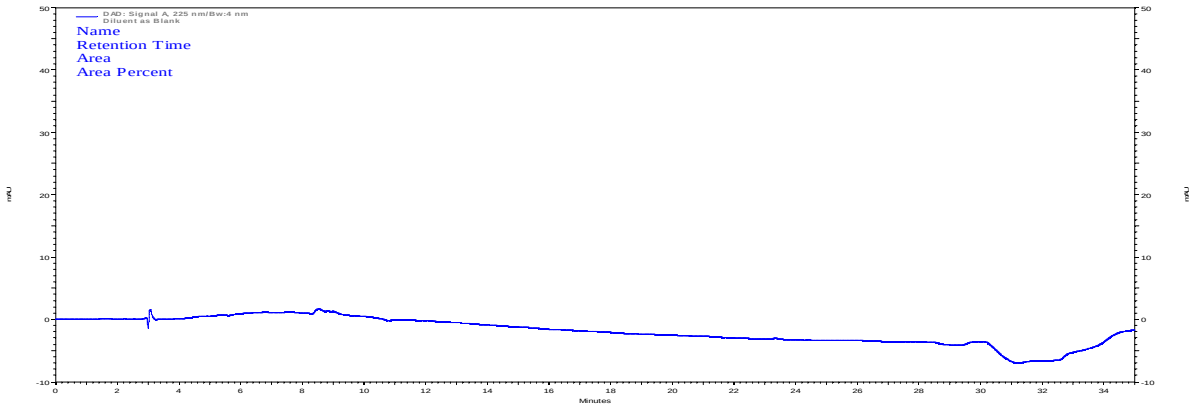

Fig 6: Diluent

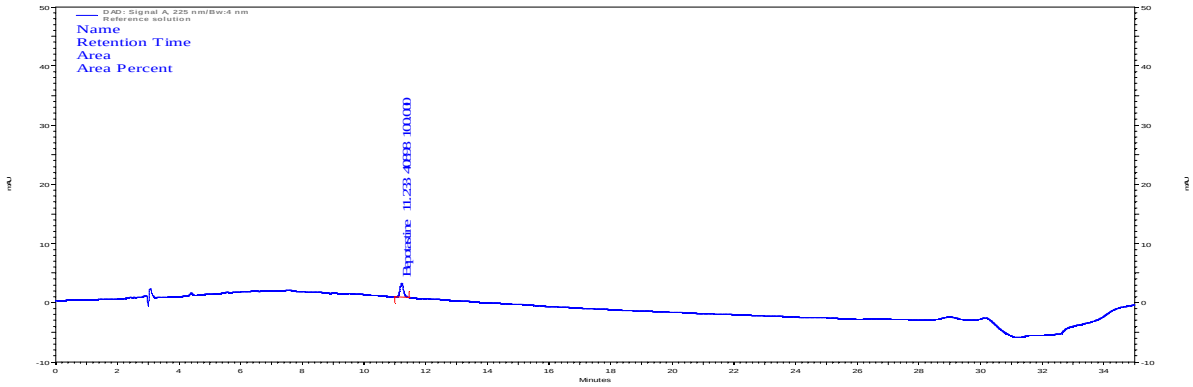

Fig 7: Reference solution

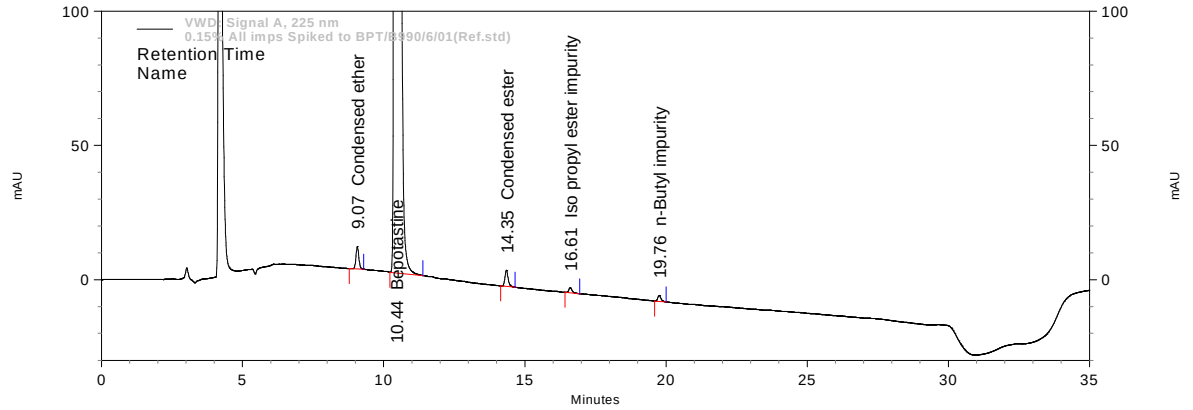

Fig 8: 0.15% all impurities spiked to Bepotastine besilate sample

Table-1: Validation data of the developed method

Parameter	Condensed ether.	Condensed ester.	Isopropyl ester.	n-Butyl ester.	Bepotastine
DL (%)	0.0020	0.0020	0.0025	0.0027	0.0022
QL (%)	0.0080	0.0081	0.0098	0.0108	0.0086
Method Precision (%RSD)#	2.12	2.45	1.94	2.23	---
Intermediate precision (%RSD)#	5.17	5.45	7.18	4.91	---
Accuracy[a] (% recovery) at:-					
QL	102.4	96.0	104.1	104.5	
50%	99.6	99.3	98.7	94.9	
75%	101.7	99.4	102.7	100.3	
100%	101.3	100.4	102.6	99.7	
125%	96.8	95.3	98.5	98.8	
150%	93.1	97.4	96.7	96.4	

[a] *Carried at QL,50%,75%, 100% and 150% level with respect to specification (0.15%)*

Method validation Results

The developed method was validated as per ICH guidelines and the results are given (Table I). Stress testing of the drug substance can help to identify the degradants, which in turn help to evaluate the stability-indicating nature of the developed method. The specificity of the developed HPLC method for Bepotastine besilate was determined in the presence of its process and degradation impurities. All the stressed samples of Bepotastine besilate and all degradation impurities were well resolved from one another and from Bepotastine besilate. The analysis was carried out by HPLC with Diod array detector. The chromatographic peak purity tool, applied to Bepotastine besilate and its impurities peaks, demonstrated that all the peaks were pure in all cases conform the absence of other impurities co-eluting in the same retention time and there by signifying the specificity and stability indicating nature of the method. The detection limit (DL) and quantification limit (QL) for Condensed ether, Condensed ester, Isopropyl

ester and n-Butyl ester were determined at a signal to noise ratio of 3:3 and 10:1 respectively, by injecting a series of dilute solutions with known concentration. Precision study was carried at QL level by injecting six times and calculating the percentage of R.S.D of area of Condensed ether, Condensed ester, Isopropyl ester and n-Butyl ester. Linearity test solutions for purity determination were at six concentration levels from QL to 150 % of the specification level (0.15%). Peak area versus concentration data was performed by least-squares linear regression analysis. Standard addition and recovery experiments were conducted to determine accuracy of impurities quantitation in bulk drug samples. The study was carried out in triplicate at QL, 50%, 75%, 100%, 125% and 150% level with respect to specification 0.15%. The percentages of recoveries for impurities were calculated.

The robustness of developed method was determined by altering experimental conditions purposely and evaluating the resolution between Bepotastine besilate, Condensed ether, Condensed ester, Isopropyl ester and n-Butyl ester. Flow rate was changed by $\pm$ 0.1 units, pH was varied by $\pm$ 0.2 units and column temperature was studied at 40°C and 50°C instead of 45°C in all above varied conditions the components of the mobile phase were held constant and no significant change (relative error less than 5%) of relative retention time was observed.

Significant changes were not observed in the contents of Condensed ether, Condensed ester, Isopropyl ester and n-Butyl ester. The stability data confirmed that sample solutions were stable up to 48hrs. The system suitability was established in terms of resolution between any two adjacent known impurities and known impurity and bepotastin peak should not be less than 1.5, detector sensitivity for reference solution the S/N ratio should not be less than 30 and tailing factor not more than 2.0 and Theoretical plates should not be less than 3000 for Bepotastin standard peak, when a 3.0mg/ml Bepotastine besilate solution spiked with 0.15% of Condensed ether, Condensed ester, Isopropyl ester and n-Butyl ester impurities were injected.

Table 2. Summary of forced degradation results

S.No	Stressed conditions		Duration	% of Total imp	% of Condensed ether	% of Condensed ester	% of Isopropyl ester	% of n-Butyl ester
1	Normal		---	0.07	0.00	0.00	0.06	0.00
2	Thermal at 60°C		10 days	0.08	0.00	0.00	0.07	0.00
4	At 75% Relative Humidity		10 days	0.08	0.00	0.00	0.07	0.07
5	Under Sunlight		50 hours	0.08	0.00	0.00	0.07	0.00
6	*Photo Degradation*	UV direct	200watt hours/square meter	0.08	0.00	0.00	0.07	0.00
		UV indirect		0.08	0.00	0.00	0.07	0.00
		LUX direct	1.2 million LUX hours	0.08	0.00	0.00	0.07	0.00
		LUX indirect		0.08	0.00	0.00	0.07	0.00
7	Acid hydrolysis (5.0N HCl at RT)		After 48hrs	0.05	0.00	0.00	0.00	0.00
8	Base Hydrolysis (3.0N NaOH at RT)		After 48 hrs	0.05	0.00	0.00	0.00	0.00
9	Oxidation (10% H_2O_2 at RT)		After 48 hrs	5.93	0.39	0.00	0.05	0.00
10	Water Hydrolysis (at 60°C±5°C)		After 48 hrs	0.13	0.00	0.00	0.06	0.00

Forced degradation studies

The stability indicating power of the developed method was studied by conducting forced degradation studies on Bepotastine besilate. Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities. The specificity of the developed HPLC method for Bepotastine besilate was determined in the presence of its impurities, and degradation products. Forced degradation studies were also performed on relative Humidity study stress at 75% Relative humidity for 10 days. The thermal stress was done at 60 °C for 10 days. The under photolytic stress studies conducted for 50 hours at under sunlight. The photolytic stressed studies were performed for UV Direct (200 watt hours/square meter), UV Indirect (200 watt hours/square meter), Lux direct (1.2 million LUX hours) and Lux in direct (1.2 million LUX hours). Water hydrolysis was performed for 48 hours at 60 °C. The acid stress was performed at 5.0 N HCl at the concentrated sample solution at ambient temperature for 48 hours and base stress was performed at 3.0N NaOH for 48 hours at ambient temperature and the oxidation stress was done using 10% hydrogen peroxide for 48 hours an ambient temperature. Stressed samples of Bepotastin besilate generated were checked for peak purity of by using Agilent diod array detector (DAD). The peak purity is within the

limit obtained in all stressed samples, demonstrates the analyte peak homogeneity. The Forced degradation studies results are given (Table-2).

Results of forced degradation studies

Significant degradation was observed in Bepotastine besilate stressed sample that were subjected to very sensitive in Peroxide hydrolysis degradation and stable in Acid hydrolysis, Base hydrolysis, Sunlight degradation, Thermal, 75% relative humidity and Water at 60°C±5°C, Photo degradation(U.V direct and indirect, Lux direct and indirect). Peak purity test results derived from Diode array detector, confirmed by that Bepotastine besilate peak is homogeneous and pure in all the analyzed stress samples. The Acid, base, Peroxide and water Degradation chromatograms are given below:

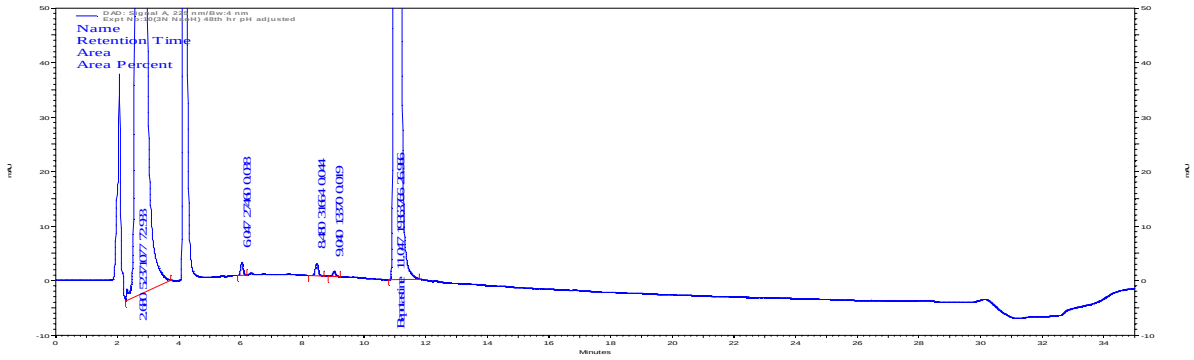

Fig 9: Typical chromatogram of 3.0N NaOH degradation solution

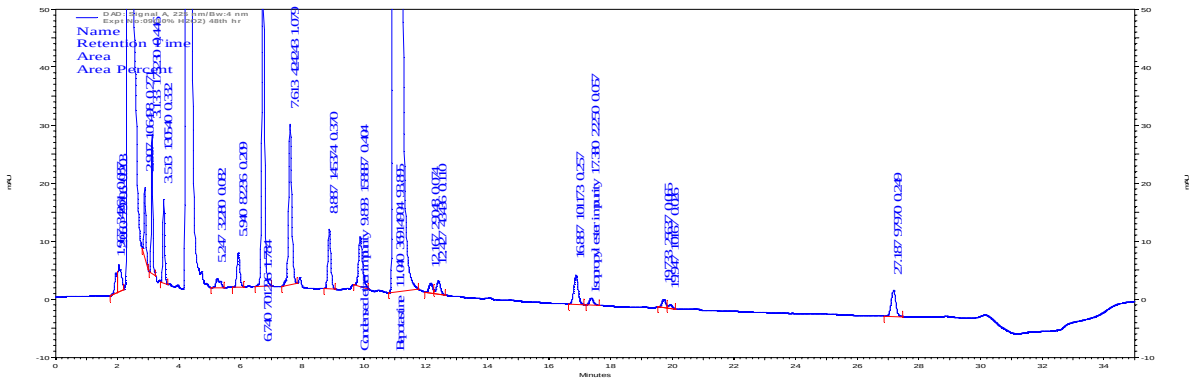

Fig 10: Typical chromatogram of 10% H2O2 degradation solution

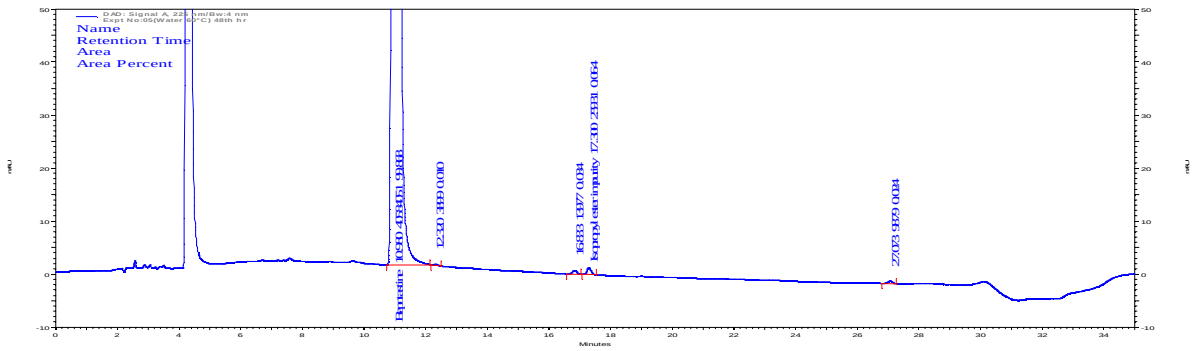

Fig 11: Typical chromatogram of degradation solution of Water at 60°

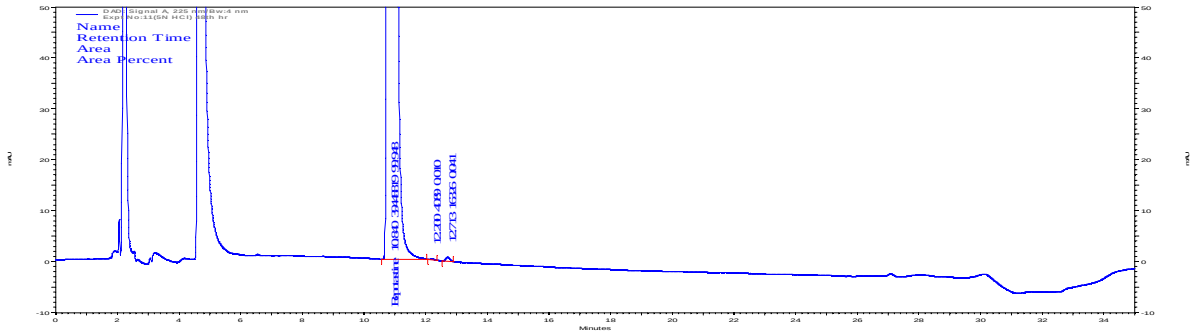

Fig 12: Typical chromatogram of 5.0N HCl degradation solution

Solution stability and mobile phase stability

Solution stability and mobile phase stability provides an indication of its reliability during normal usage during the storage of the solutions used in the method. The solution stability of Bepotastine besilate was established for 48 hours. The solution stability studied by using Bepotastine besilate sample and injected for every 12 hours. The content of impurities and Bepotastine besilate were quantified at each interval up to the study period. The mobile phase stability was also established by quantifying the freshly prepared sample solutions against freshly prepared reference standard solutions for every 12 hours up to 48hrs. During the study period the prepared mobile phase was stable up to 48hrs at room temperature.

CONCLUSION

The developed stability-indicating analytical method for related substance determination of Bepotastine besilate and its impurities is precise, accurate, linear and specific. The validation carried out for the method in accordance with the ICH requirements are satisfactory. The developed method can be used conveniently for the routine analysis of production samples and also to check the stability of bulk samples of Bepotastine besilate during its storage. The same method can also be attempted for the drug products for the getting the information of impurities and degradation products at lower level.

Acknowledgments

One of the authors Narasimha Reddy thanks Dr. Chakravarthy. I.E, for guiding him in his PhD work. The authors wish to thanks Dr. MSN Reddy (MSN laboratories Pvt.Ltd) for providing facilities to do research work.

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