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Development and Validation of a new simple and Stability indicating RP-HPLC Method for the determination of Tetrabenazine and its forced degradation impurities in Bulk Drug and Pharmaceutical Dosage form

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Abstract: A novel, simple, precise and stability indicating reverse phase high performance liquid chromatography method has been developed and validated for the assay determination of Tetrabenazine in bulk drug and dosage form. LC separation was achieved on a Xterra RP18 (4.6x150) mm, 3.5 µm column in isocratic mode using buffer(0.01M K_2HPO4) and acetonitrile in the ratio 50:50(v/v) as mobile phase, pumped in to the column at flow rate 1.0 ml/min and the detection of eluent from the column was carried out using Photo Diode array detector at 210 nm. The column was maintained at 25˚c temperature and the total run time was 15min. The retention time of Tetrabenazine was 6.4min and the standard curves were linear over the concentration range of 20-150 µg/ml with r^2= 0.9999. The percentage relative standard deviation in accuracy and precision studies was found to be less than 2%. The method was successfully validated as per ICH guidelines. Validation studies demonstrated that the proposed method is specific, simple, rapid and reproducible. Forced degradations were carried out under acidic, basic, oxidation, dry heat and photolytic stress conditions. In the stressed sample chromatograms, it demonstrated the specificity of the assay method for their estimation in presence of degradation impurities. The proposed method is suitable for the routine quality control analysis of Tetrabenazine in bulk and dosage forms and is stability indicating.
Keywords: Tetrabenazine.validation.HPLC.Stability indicating.

Introduction

Tetrabenazine is a drug for the symptomatic treatment of hyperkinetic movement disorder. On august 15, 2008 the U.S. food and drug administration (FDA) approved the use of tetrabanazine to treat chorea associated with Huntington's disease (HD), the first in the US. Tetrabanazine works mainly as a VMAT-inhibitor and as such promotes the early metabolic degradation of monoamines, in particular the neurotransmitter dopamine[13]. Tetrabanazine is available as tablets at the dose of 25 mg in the market under the brand name of Revocon. Tetrabanazine is chemically (SS,RR)-3-Isobutyl-9,10-dimethoxy-1,3,4,6,7,1 1b-hexahydro-pyrido[2,1-*a*] isoquinolin-2-one with empirical formula is $C_{19}H_{27}NO_3$ and molecular weight 317.42[14].

Various methods in the literatures involve determination of Tetrabanazine in human plasma by HPLC (1, 3), LCMS/MS (2), pharmacokinetics, pharmacodynamics (4-9). However no method is available for assay of Tetrabanazine in bulk drug and pharmaceutical dosage form. In the present work we have developed a new, simple precise and stability indicating method for determination of Tetrabanazine in bulk drug and pharmaceutical dosage form.

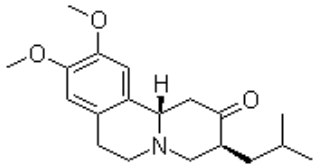


Figure 1: Structure of Tetrabanazine

Experimental

Chemicals & Reagents

Tetrabanazine is available as tablets with brand name XENAZINE was purchased from local market, containing Tetrabanazine 25mg. HPLC grade acetonitrile, AR grade dipotassium hydrogen phosphate was purchased from Merck, Mumbai. High pure water was prepared by using Millipore Milli-Q plus purification system.

Chromatographic Conditions

A Alliance e2695 separation module (Waters corporation, Milford, MA) equipped with 2998 PDA detector with empower 2 software used for analysis. Buffer consisted of 0.01M dipotassium hydrogen phosphate in water (1.74g of dipotassium hydrogen phosphate in 1000 ml of water). A Waters Xterra C18 (4.6x150) mm,3.5 µm column and isocratic mixture of solution A (Buffer) solution B (Acetonitrile) used as stationary and mobile phase respectively. The isocratic program was fixed as A: B (50:50). Acetonitrile used as diluent. The column oven maintained at 25˚c with 1.0ml flow rate. An injection volume 10µl was used. The elution compounds were monitored at 210 nm.

Preparation of Stock and standard solutions

Accurately 50mg of Tetrabanazine standard dissolved in 100ml diluent to get a concentration of 500µg/ml. Further 20ml of stock solution was taken in 100ml flask and diluted up to the mark with diluent to get concentration of 100µg/ml .

Preparation of Tablets for assay

20 tablets of Tetrabanazine were powdered and an amount of powder equivalent to 50mg of drug was weighed and transferred to the 100ml flask added 10ml diluent and placed in an ultrasonicator for 10minites made up to the volume with diluent, and filtered through a 0.45µm nylon syringe filter. 20ml of this solution was taken into 100 ml flask and diluted volume with diluent to get concentration 100µg/ml

Forced Degradation studies

Acid Degradation studies

Acid decomposition was carried out in 0.2N HCL at concentration of 500µg/ml Tetrabanazine and after refluxation for 24hrs at 80˚c, the stressed sample was cooled, neutralized and diluted as per requirement with diluents filtered and injected. The resulting chromatogram is shown in fig.3 (g). The results are tabulated in table 4.

Alkali Degradation studies

Base decomposition was carried out in 0.2N NaOH at concentration of 500µg/ml Tetrabanazine and after refluxation for 24hrs at 80˚c, the stressed sample was cooled, neutralized and diluted as per requirement

with diluents filtered and injected. The resulting chromatogram is shown in fig.3 (i). The results are tabulated in table 4.

Oxidation

Oxidation was conducted by using 4%H2O2 solution at room temperature. After 24hrs, 20ml of solution was taken in 100ml flask and diluted up to the mark with diluent to get concentration of 100µg/ml filtered and injected. The resulting chromatogram is shown in fig.3 (k). The results are tabulated in table 4.

Temperature Stress studies

1g of Tetrabanazine sample was taken into a petridish and kept in oven at 80˚c for 7 days. 50mg of sample was taken into 100 ml flask diluted volume with diluent, further 20ml to 100ml made up with diluent. The results are tabulated in table 4.

Photo stability

1g of Tetrabanazine was taken in to a petridish and kept in photo stability chamber 200 W.hr/m^2 in UV Fluorescent light and 1.2M LUX Fluorescent light. 50mg of sample was taken in 100ml flask, dissolved in diluent, further 20ml in 100ml flask diluted volume with diluent. The results are tabulated in table 4

Results and Discussion

HPLC Method Development and Optimization

The analytical method conditions were selected after testing the different parameters such as column, wavelength, aqueous and organic phase, buffer concentration, mobile phase propation, diluents, concentration of analyte, flow and other parameters. Xterra RP18(4.6x150) mm, 3.5 µm column was used because of its wide pH range(1-12), high resolution capacity and low degree of tailing. For mobile phase selection, the preliminary trials using different compositions of mobile phases containing water and acetonitrile gave poor peak shape. For improving peak shape instead of water dipotasium hydrogen phosphate and acetonitrile (50:50) and thus, better peak shape was obtained [12]. Acetonitrile used as diluents because tetrabanazine freely soluble. The detection wavelength was chosen as 210nm for Tetrabanazine because they have better absorption and sensitivity at this wavelength (fig-2). Hence selected method was best among the all trails by many aspects.

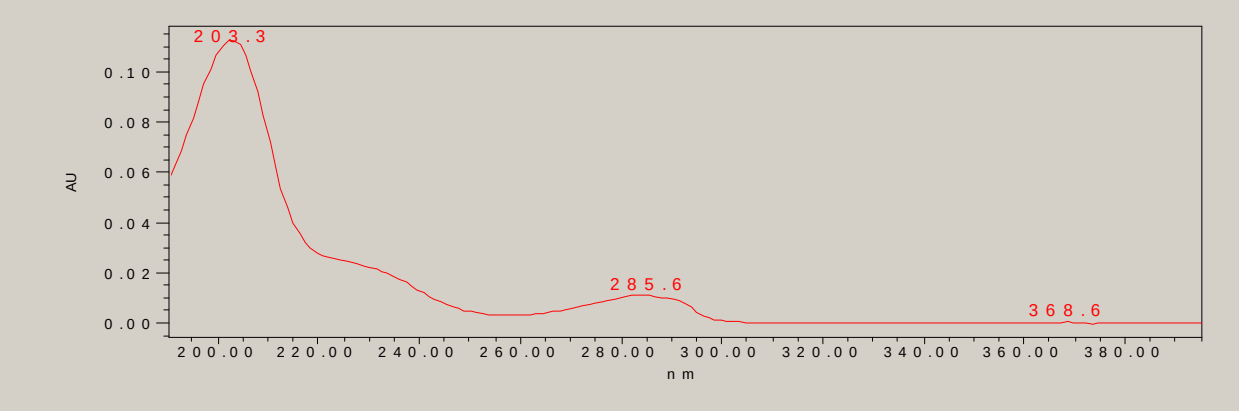


Figure 2 : wavelength spectrum of Tetrabanazine

Method Validation

The method was validated for the following parameters specificity, linearity, accuracy, limit of detection (LOD), limit of quantitation (LOQ), precision and robustness [11].

Specificity

A study to establish the interference, blank detection was conducted. Diluent was injected as per the test method. Solution of standard and sample were prepared as per test method and injected into the chromatographic system. The chromatograms of blank, standard and sample were shown in the fig a, b, c.

Precision

Precision study was established by evaluating method precision and intermediate precision study. Method precision of the analytical method was determined by analyzing six sets of sample preparation. Intermediate precision of the analytical method was determined by performing method precision on another day and another analyst under same experiment condition.The result obtained for method precision and intermediate precision are shown in table 2. The percentage of RSD was calculated. The %RSD range was obtained as 0.23 and 0.41 for method precision and intermediate precision respectively (Table 2) which is less than 2% indicating that the method is more precise.

Accuracy

The accuracy of the method was assessed by determination of recovery for three concentrations (corresponding to 50,100 and 150% of test solution concentration) covering the range of the method. For each concentration three sets were prepared and injected. The drug concentrations of Tetrabanazine were calculated, the results obtained are shown in table 2. The percentage recovery was found to be 99.58-99.99% with %RSD 0.03 - 0.14(<2.0%) indicating that the method is more accurate (table 2).

LOD and LOQ

The LOD and LOQ were determined at a signal to noise ratio of 3:1 and 10:1 respectively by injecting a series of test solutions of known concentrations within the linearity range. Precision study was also carried out at the LOQ level by injecting six pharmaceutical preparations. The LOD and LOQ were to be 0.05µg/ml and 0.18µg/ml respectively. The %RSD value was noticed to be less than 1.0% at LOQ concentration level.

Linearity

The linearity plot was prepared with six concentration levels (20, 40, 80,100,120 and 150 µg/ml of Tetrabanazine). These concentration levels were respectively corresponding to 20, 40, 80,100,120 and 150 % of test solution concentration. The results obtained are shown in table 1. The peak areas were plotted against the corresponding concentrations to obtain the calibration curve (figure 4).

Robustness

Robustness of method was checked by making slight deliberate changes in chromatographic conditions like flow rate (±0.1 ml/min) and column temperature (±5˚c). Under all the deliberately varied chromatographic conditions, the reproducibility of results was observed to be reasonably good. Hence the proposed method has good robustness for the assay of Tetrabanazine in bulk and dosage forms.

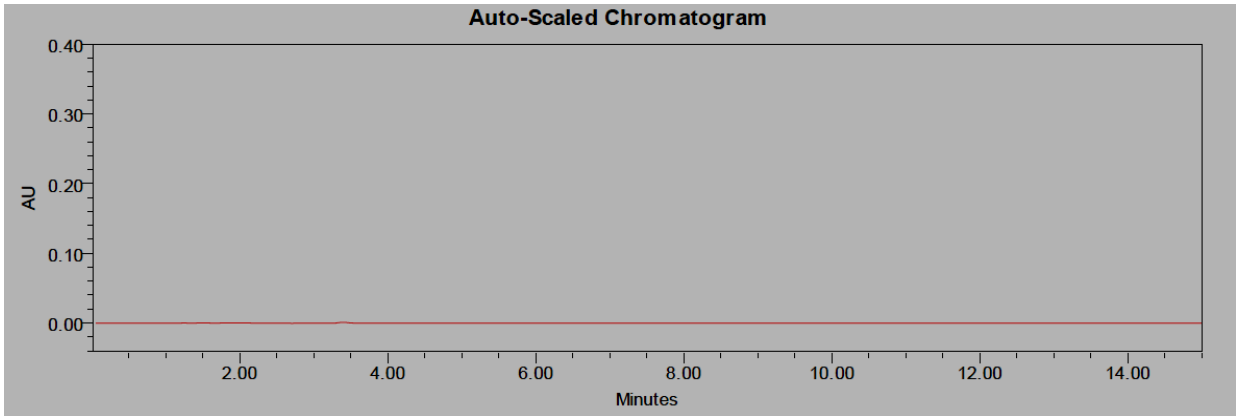
Solution stability and Mobile phase stability

Solution stability checked for stability of standard and sample solutions. Solution stability checked at each interval initial 2,4,6,8,12,16,20 and 24 hours. For standard solution stability and sample solution stability %assay value calculated at each interval. %RSD (NMT 2.0%) between initial assay value and assay value obtained at predetermined time interval calculated.

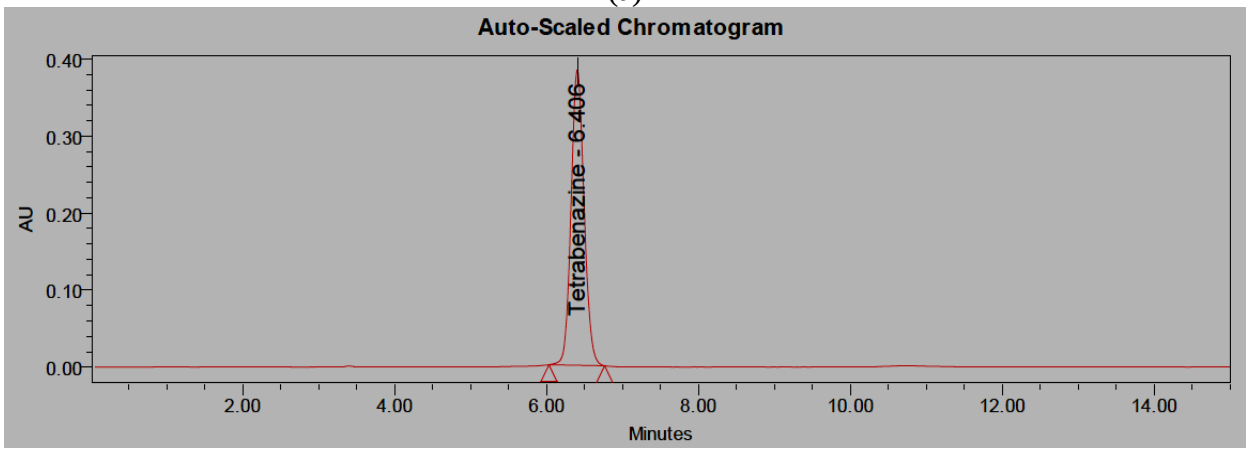
Forced Degradation Studies

Stress studies on Tetrabanazine were carried out under oxidation, thermal stress, photolysis, acid and alkali hydrolysis conditions. Significant degradation was observed in acid (fig 3g), base (fig 3i) and peroxide (fig 3k) of Tetrabanazine. There was no significant degradation of Tetrabanazine upon exposure to dry heat at 80˚c for 7days and photolysis total impurity increased to 0.15% and 0.22% which indicated that the drug was stable against these stress conditions. The developed method revealed that there was no interference[3l,3m,3n] from the impurities, degradation products and excipients to determine the assay of drug substance in pure and pharmaceutical formulation.

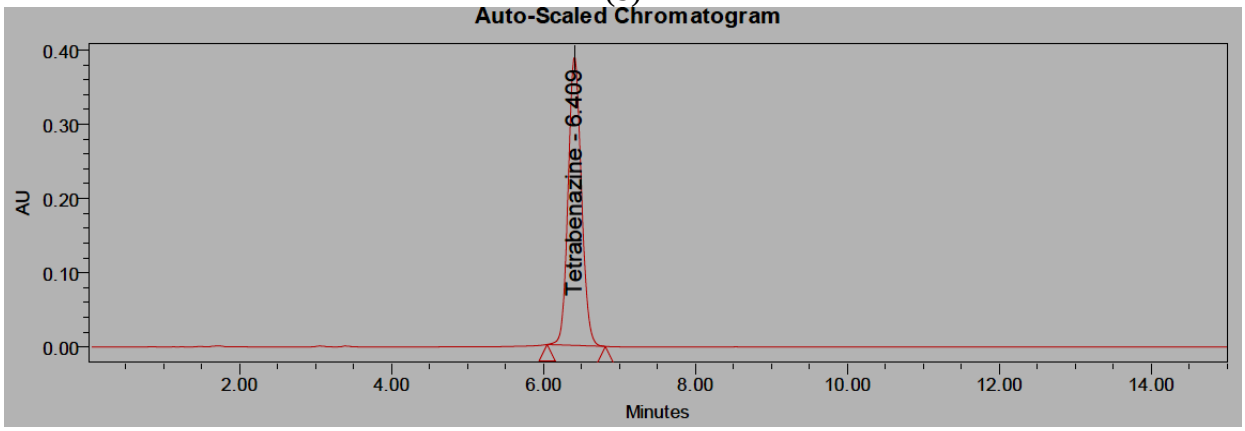
Fig-3 :


Auto-Scaled Chromatogram
AU
0.40
0.30
0.20
0.10
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
Minutes

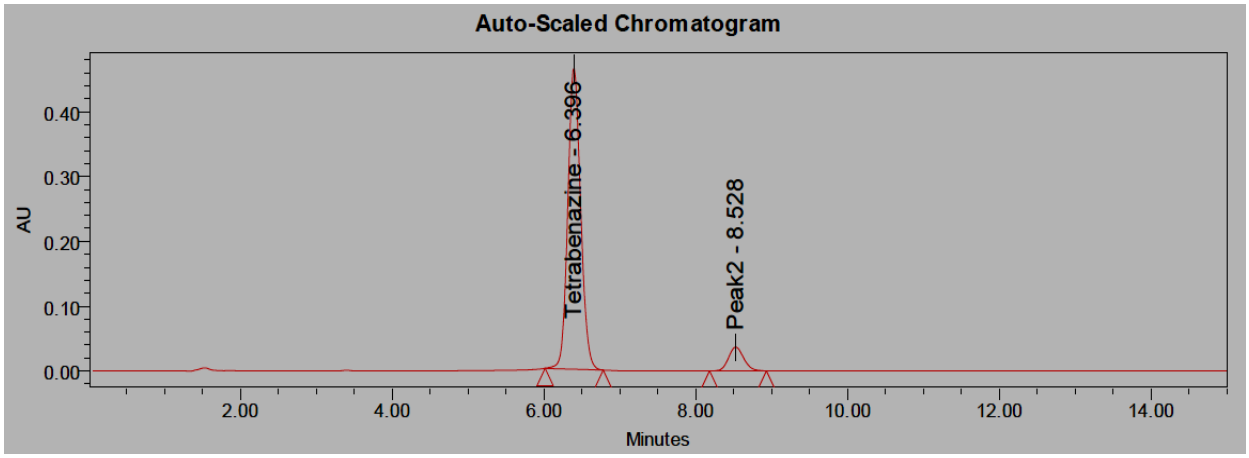
(a)


Auto-Scaled Chromatogram
Tetrabenazine - 6.406
AU
0.40
0.30
0.20
0.10
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
Minutes

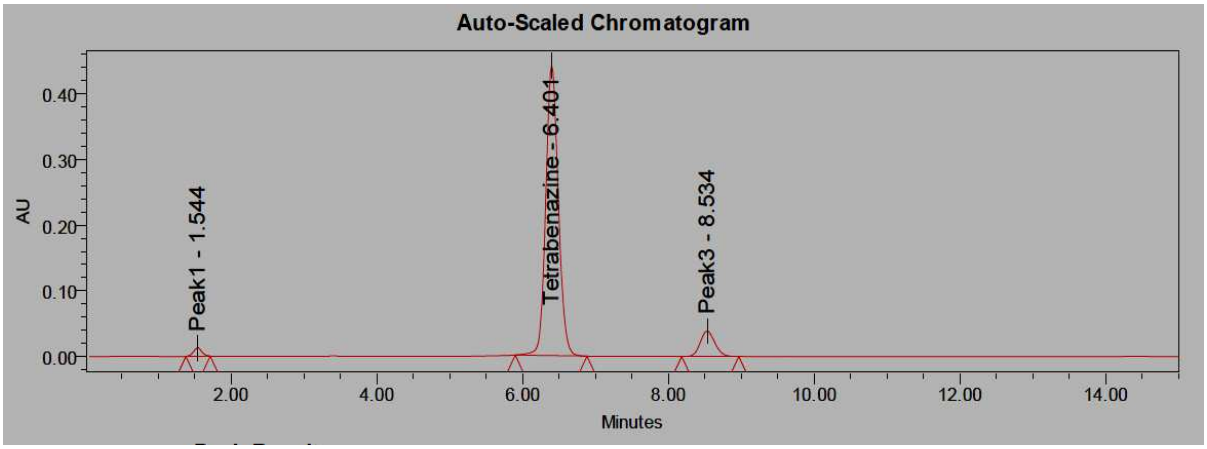
(b)


Auto-Scaled Chromatogram
Tetrabenazine - 6.409
AU
0.40
0.30
0.20
0.10
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
Minutes

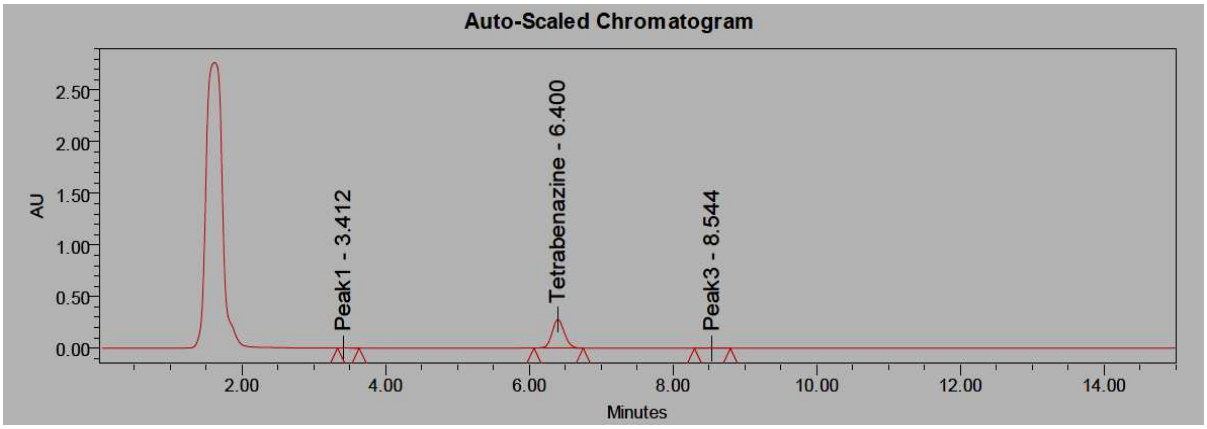
(c)


Auto-Scaled Chromatogram
Tetrabenazine - 6.396
Peak2 - 8.528
AU
0.40
0.30
0.20
0.10
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
Minutes

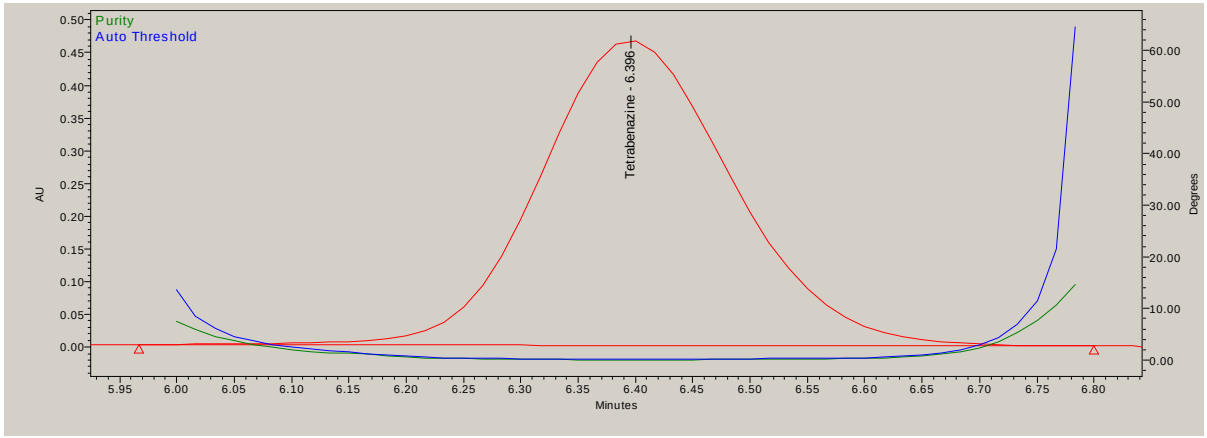
(d)


Auto-Scaled Chromatogram
AU
Minutes
Peak1 - 1.544
Tetrabenazine - 6.401
Peak3 - 8.534

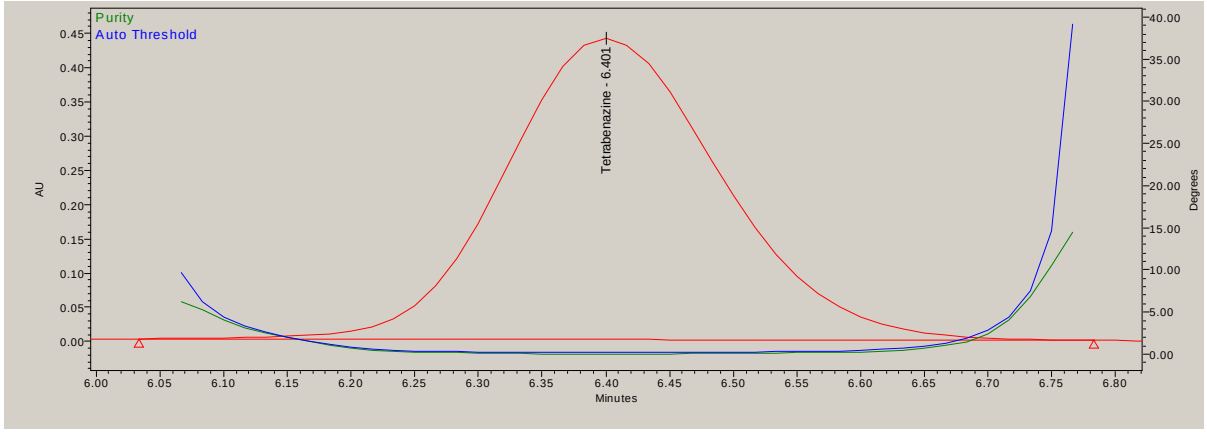
(e)


Auto-Scaled Chromatogram
AU
Minutes
Peak1 - 3.412
Tetrabenazine - 6.400
Peak3 - 8.544

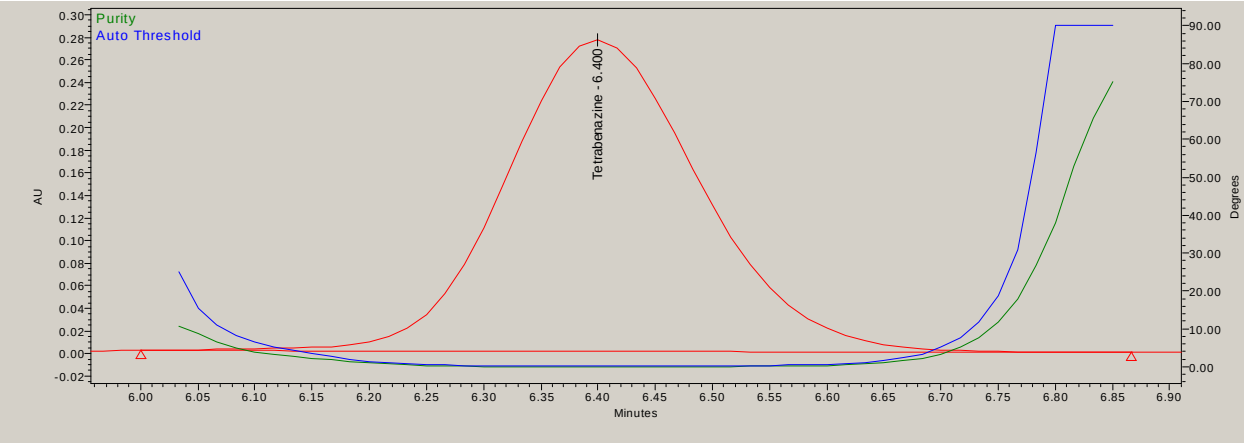
(f)


Purity
Auto Threshold
AU
Degrees
Minutes
Tetrabenazine - 6.396

(g)


Purity
Auto Threshold
AU
Degrees
Minutes
Tetrabenazine - 6.401

(h)



(i)

Fig-3 : Typical chromatograms of (a) Blank (b) Standard (c) Sample (d) Acid sample (e) Base sample (f) Peroxide sample(g)Purity plot of Acid (h) Purity plot of Base (i) Purity plot of Peroxide

Fig-4 : Linearity of Tetrabanazine

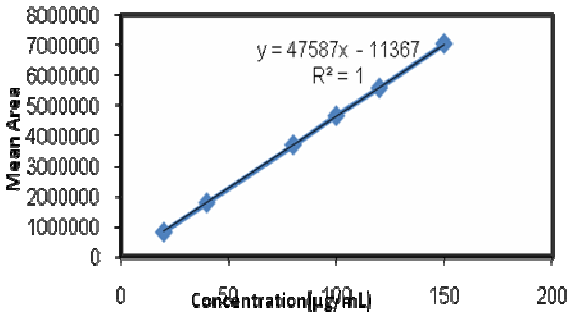


Table-1: Results for linearity of Tetrabanazine

Linearity level	%Level	Area
1	20	830128
2	40	1796200
3	80	3700601
4	100	4655446
5	120	5572996
6	150	7031764
Correlation co-efficient		0.999984
	intercept	-113679
	slope	47586.68

Table 2: Analytical Data of Proposed Method

Parameter	
LOD(µg/ml)	0.05
LOQ(µg/ml)	0.18
Linear Range(%)	20-150
Slope	47586
Correlation coefficient	0.9999
Intermediate precision(%RSD)	0.41
%Recovery(Mean±%RSD) at	
50	0.14
100	0.08
150	0.03

Table-3 forced degradation results for Tetrabanazine

Stress condition (%)	Drug recovered (%)	Drug decomposed
Standard drug	100	
Acid degradation	91.57	8.43
Alkali degradation	89.21	10.79
Oxidation degradation	90.45	9.55
Thermal degradation	99.85	0.15
Photolytic degradation	99.78	0.22

Conclusions

A new reverse phase HPLC method for assay of Tetrabanazine in bulk and pharmaceutical dosage forms is established. This method is a new, simple, precise, linear, accurate and specific. This method is free from interference of the other active ingredients and additives used in the formulation. Degradation impurities did not interfere with the retention time of Tetrabanazine, and assay method is thus stability indicating.

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