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Development and validation of stability indicating RP-HPLC method for the estimation of tetrabenazine in bulk and pharmaceutical dosage form

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ABSTRACT

A simple, efficient, and reproducible stability indicating RP-HPLC method for the determination of Tetrabenazine in pharmaceutical dosage form has been developed and validated. The separation was carried out on Thermo BDS column C_{18} (150 mm X 4.6 mm, 5 µm) column using methanol: potassium dihydrogen orthophosphate buffer (adjusted to pH 6.8 with 0.1 % OPA) in the ratio of 40:60 (v/v) as eluent. The flow rate was 1.0 ml/min and effluent was detected at 284 nm. The retention time of Tetrabenazine was 5.075 min. The linear dynamic range was 6.25-37.5 ppm for Tetrabenazine. A percentage recovery for Tetrabenazine was 99.96-100.79 %. All the analytical validation parameters were determined and found in the limit as per ICH guidelines, which indicates the validity of the method. The developed stability indicating method was also found to be precise and robust for the determination of Tetrabenazine in tablet dosage forms.

Key words: Tetrbenazine, Methanol, Potassium dihydrogen orthophosphate, Ortho phosphoric acid.

INTRODUCTION

Tetrabenazine was chemically known as (SS, RR)-3-isobutyl-9,10-dimethoxy-1,3,4,6,7,11b-hexahydro-pyrido-[2,1-a]-isoquinolin-2-one (Fig. 1). Tetrabenazine was used as an antipsychotic agent. Tetrabenazine is a reversible human vesicular monoamine transporter type-2 inhibitor. It acts within the basal ganglia and promotes depletion of monoamine neurotransmitters such as serotonin, nor-epinephrine and dopamine from stores. It also decreases uptake into synaptic vesicles.

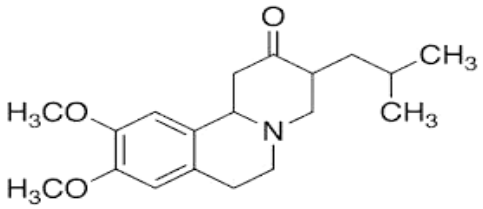

Figure.1.Chemical structure of Tetrabeazine

The stability indicating method is defined as validated quantitative analytical method that can detect the change with time in the chemical, physical or microbiological properties of the drug substance and the drug product, that are specific so that the content of active ingredient degradation can be accurately measured without interference (Bakshi and Singh, 2002). Stability testing provides information about degradation mechanisms, potential degradation products, possible degradation pathways of the drug as well as interaction between the drug and the excipients in drug product (ICH, 2003).

Literature survey revealed report of few analytical methods for estimation of Tetrabenazine. Very few analytical methods have been reported in Tetrabenazine like, UV (Osorio et al., 2013; Suneetha et al., 2014), HPLC (Robert, 1981; Reza Mehvar, 2006) and LC-MS (Derangula, 2013) methods. But the stability indicating RP-HPLC method for the estimation of Tetrabenazine is scanty. Hence, the aim of the present study was to develop a simple, precise, reliable, sensitive and selective stability indicating RP-HPLC method with UV detection for the analysis of Tetrabenazine in bulk and in pharmaceutical dosage formulation.

MATERIALS AND METHODS

Chemicals and reagents: The pharmaceutical grade pure sample of Tetrabenazine was received as gift samples from Spectrum Pharmaceutical solutions, Hyderabad. Tetrabenazine formulation was purchased from local market. Milli-Q water, HPLC grade methanol and analytical grade potassium dihydrogen phosphate, orthophosphoric acid was obtained from Qualigens Fine Chemicals Ltd., Mumbai.

Apparatus and chromatographic condition: The chromatographic separation was performed on a Waters Alliance HPLC, integrated with auto sampler and UV detector. The analytical Thermo BDS C_{18} (150 mm x 4.6

mm, 5 µ) column was used. Mobile phase was delivered at a flow rate of 1.0 ml/min and the detector wavelength was set at 284 nm. The injection volume was 10 µl and temperature was maintained at 30 ºC.

Preparation of pH 6.8 phosphate buffer: Accurately weighed 1.36 gm of potassium dihyrogen ortho phosphate was taken in a 1000 ml volumetric flask. To this about 900 ml of milli-Q water was added and sonicated. Finally the volume was made up with water and then added 1 ml of triethylamine. Then pH was adjusted to 6.8 with dilute orthophosphoric acid solution.

Preparation of mobile phase: Methanol and potassium dihydrogen orthophosphate buffers were filtered separately through 0.45 µ membrane filters. The filtered solvents were mixed in the ratio of 40: 60 (% v/v) and degassed for subjecting to sonication for 10 min and resultant solution used as mobile phase.

Preparation of diluent: Mobile phase was used as diluent. (Methanol: Phosphate buffer (pH 6.8) 40: 60 % v/v)

Preparation of Tetrabenazine standard & sample solutions

Standard solution preparation: Weighed and transferred accurately about 25 mg of Tetrabenazine working standard into 100 ml volumetric flask, added 50 ml of diluent and sonicated to dissolve and diluted to volume with diluent.

Preparation of working standard solution: From the above stock solution, 10 ml was transferred in to 100 ml volumetric flask and diluted to volume with diluent.

Sample solution preparation: Finely grinded pre weighed 20 tablets. Transferred grinded sample quantitatively equivalent to 25 mg of Tetrabenazine in to 100 ml volumetric flask, added 50 ml of diluent and sonicated to dissolve for 10 min. Latter the volume was made using diluent. Further the solution was filtered through 0.45 µ filter paper. Finally, 10 ml of filtrate was diluted to 100 ml with diluent.

Procedure: 20 µl of filtered portion of the sample and standard preparation was injected in to the chromatograph. The responses for the major peaks were recorded. The content of Tetrabenazine in each tablet was calculated & reported in Table 2.

Validation parameters: All of the analytical validation parameters for this proposed method were determined according to ICH guidelines (USFDA, 1995; USFDA, 1996; ICH, 1996; USFDA, 2000; ICH, 2001). Obtained validation parameters are presented in Table 3.

System suitability test: System suitability was ascertained by injecting 20 µl of the standard solution into the chromatographic system. Chromatogram was recorded and peak area was measured. The % RSD of the peak area and retention time for the Tetrabenazine was within the limits which indicates the suitability of the system. The suitability parameters like resolution (NLT 2.0), tailing factor (NMT 2.0), theoretical plate count (NLT 2000) and % RSD for peak area of five replicate injections of standard (% RSD NMT 2) are within limits. The results for system suitability are presented in Table 1.

Specificity: The specificity of the method was performed by separate injections of Tetrabenazine standard, sample and blank. No peaks were found in blank. The Tetrabenazine peak were appeared at retention time of around 5 in both standard and sample. Hence the proposed method was specific for Tetrabenazine. The effect of wide range of excipients and other additives usually present in the formulations in the determination under optimum conditions was investigated.

Linearity: The linearity for HPLC method was determined at six concentration levels ranging from 6.25-37.5 ppm for Tetrabenazine**.** The calibration curve was constructed by plotting response factor against respective concentration of Tetrabenazine. The plots of peak area Vs respective concentration of Tetrabenazine was found to be linear in the range of 6.25-37.5 ppm with coefficient of correlation (r^2) 0.9999 for Tetrabenazine. The linearity of this method was evaluated by linear regression analysis. The slope and intercept calculated for Tetrabenazine was given in Fig. 3.

Accuracy: Accuracy studies were performed for Tetrabenazine at three different concentration levels (80 %, 100 % and 120 %) and the mixtures were analyzed in triplicate by the proposed method. Three different samples of known concentration for Tetrabenazine were prepared and these are analyzed against standard solution. The result of

recovery analysis of Tetrabenazine was found to be in the accepted range. The obtained results are presented in Table 2.

LOD and LOQ: The limit of detection (LOD) and limit of quantification (LOQ) for Tetrabenazine was found to be 0.634 µg/ml and 1.921 µg/ml, respectively. The LOD and LOQ showed that the method is sensitive for Tetrabenazine.

Precision:

System precision: System precision was established by six replicate injections of the standard drug solution. The corresponding peak area was measured and % RSD was calculated. The obtained results are presented in Table 2.

Method precision: The method precision study was performed for six sample preparations of marketed formulations of Tetrabenazine. The obtained results are presented in Table 2.

Robustness: Robustness of the method was determined by small deliberate changes in flow rate and temperature. The content of the drug was not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method was robust.

Forced degradation studies: In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 24 h at room temperature. The result showed that for both solutions, the retention time and peak area of Tetrabenazine remained almost similar (% R.S.D. less than 2.0) and no significant degradation within the indicated period, thus indicated that both solutions were stable for at least 24 h, which was sufficient to complete the whole analytical process. Further forced degradation studies were conducted to indicate the stability of the method developed. The results of the degradation studies are presented in Table 4.

Sample preparation: Finely grinded pre weighed 20 tablets. Transferred grinded sample quantitatively equivalent to 25 mg of Tetrabenazine in to 100 ml volumetric flask, added 50 ml of diluent and sonicated to dissolve for 10 min. Latter the volume was made using diluent. Further the solution was filtered through 0.45 µ filter paper.

Acid degradation studies: To 1 ml of stock solution of Tetrabenazine, 1 ml of 2 N hydrochloric acid was added and refluxed for 30 min at 60 °C. The resultant solution was diluted to obtain 25 µg/ml solution and 20 µl of solutions was injected into the system and the chromatograms were recorded to assess the stability of sample.

Base degradation studies: To 1 ml of stock solution of Tetrabenazine, 1 ml of 2 N sodium hydroxide was added and refluxed for 30 min at 60 °C. The resultant solution was diluted to obtain 25 µg/ml solution and 20 µl of solution was injected into the system and the chromatograms were recorded to assess the stability of sample.

Thermal degradation studies: The standard drug was placed in oven at 105 °C for 6 h to study dry heat degradation. For HPLC study, the above sample was used to produce 25 µg/ml solution and 20 µl of solution was injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photo stability studies: The photochemical stability of the drug was also studied by exposing the solution to UV light by keeping the beaker in UV Chamber for 7 days or 200 Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 25 µg/ml solution and 20 µl of solution was injected into the system and the chromatograms were recorded to assess the stability of sample.

RESULTS AND DISCUSSION

The preliminary studies indicated that the desired system suitability parameters were obtained with the mobile phase containing methanol: phosphate buffer (pH 6.8) (40: 60). The mobile phase eluted the drug at lower retention time (5.075 min). The corresponding chromatogram was showed in the Fig. 2.

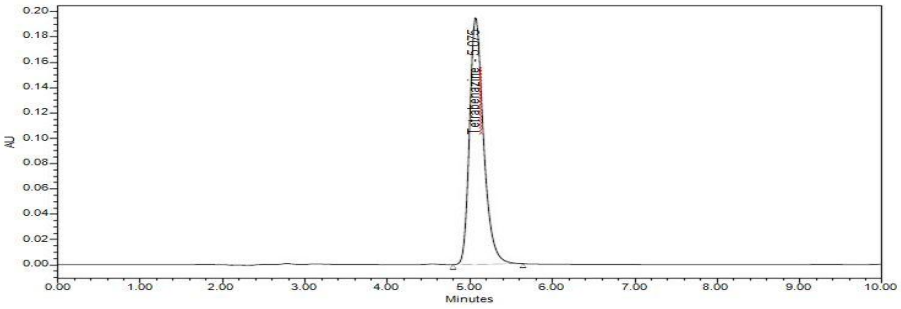

Figure.2.Typical chromatogram of Tetrabenazine

The system suitability parameters such as number of theoretical plates (N = 4449), tailing factor (1.18) and RSD of peak areas (0.437 %) were found to be within the limits (Table No: 1).

Table.1.System suitability parameters

Name	Retention time (min)	Area (mV.s)	Efficiency (th.pl)	Asymmetry	% RSD
Tetrabenazine	5.07	2296826	4449	1.18	0.437

Table.2.Assay of Terabenazine

Drug	Lable strength	% Assay
Tetrabenazine	25 mg	99.69

The calibration curve of Tetrabenazine showed good linearity in the range of 6.25-37.5 µg/ml. A linear correlation was found between chromatographic peak areas and concentrations of Tetrabenazine (r^2= 0.9999) and corresponding regression equation was y = 45831x + 8803.7 (Fig. 3).

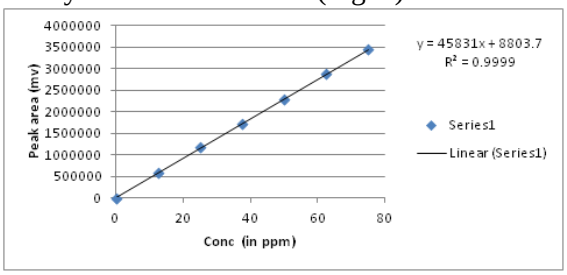

Figure.3.Calibration curve for Tetrabenazine

The method was also subjected to validation by precision, accuracy, LOD & LOQ and robustness as per ICH guidelines. All the observed values were within the acceptable range and the results are presented in Table 3. Therefore, the method attempted to evaluate the stability of the drug under various stress conditions with different rates of decomposition.

Table.3.Validation results for Tetrabenazine

Parameter	Result			
System suitability	The tailing factor of Tetrabenazine was found to be 1.15			
Linearity	The linearity range of Tetrabenazine was found to be 6.25-37.5 µg/ml. The correlation coefficient was found to be 0.999.			
Accuracy	The % recoveries of Tetrabenazine was found to be 99.75 % - 100.79 %			
System precision	The % RSD was found to be 0.45.			
Method precision	The % RSD was found to be 0.70.			
LOD and LOQ	The LOD and LOQ of Tetrabenazine were found to be 0.633 and 1.92 µg/ml, respectively			
Robustness	**Change in flow rate**			
	Flow rate (ml/min)	Area	Retention time (min)	Asymmetry
	0.8	1955181	4.232	1.12
	1.2	2818955	6.062	1.12
	Change in Temperature			
	Temp (ºC)	Area	Retention time (min)	Asymmetry
	25	2306546	4.937	1.15
	35	2308958	5.032	1.16
Ruggedness	% RSD of Day 1 was found to be 0.45 % RSD of Day 2 was found to be 0.07			

The developed method was able to detect as low as 4.31 of decomposition which was noticed with thermal heat exposure. The chromatograms observed from samples, subjected to various stress conditions, are shown in Fig. 4a to 4d. The amount of drug decomposed at various stress conditions are shown in table 4.

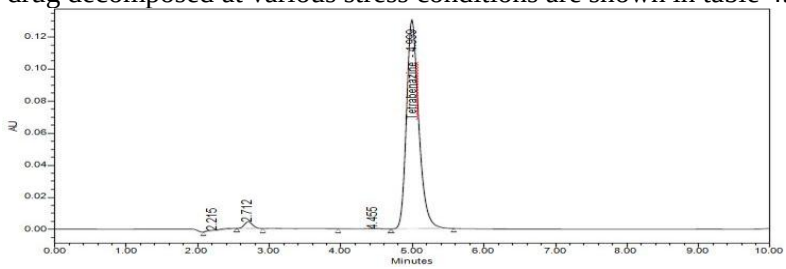

Figure.4a.Chromatogram of acid degradation studies

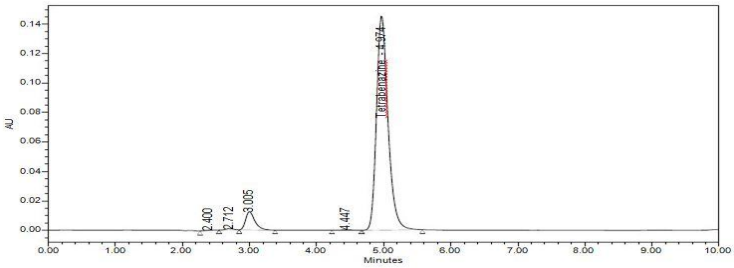

Figure.4b.Chromatogram of base degradation studies

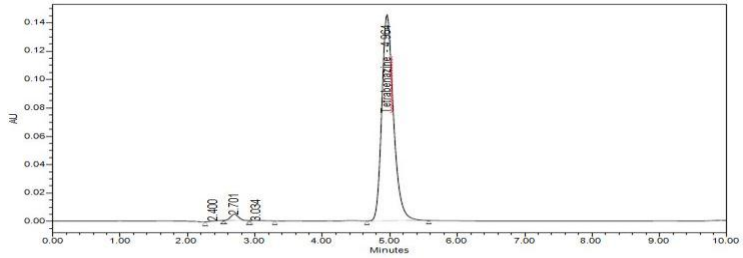

Figure.4c.Chromatogram of thermal degradation studies

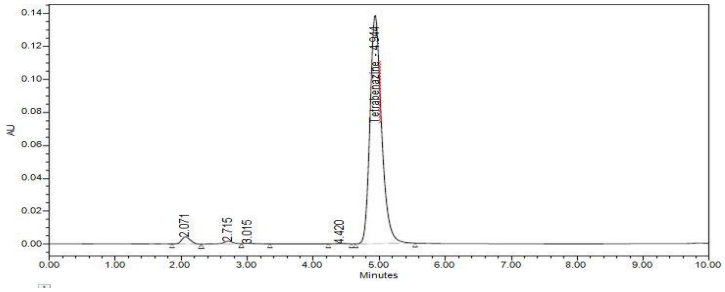

Figure.4d.Chromatogram of UV degradation studies

Table.4.Results of degradation study (% Degradation)

Compound Name	Acid	Alkali	Heat	UV
Tetrabenazine	9.19	5.31	4.31	7.83

CONCLUSION

A simple, sensitive, rapid and economical stability indicating RP-HPLC method was developed and validated for the assay of Tetrabenazine in tablet formulation. This method yielded high recoveries with good linearity and precision. It can be concluded that the proposed method is a good approach for obtaining reliable results and found to be suitable for the routine analysis of Tetrabenazine tablet formulation. In addition, we also reported the % of degradation products in various stress conditions like acid, alkali, thermal and UV.

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