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An Applicable Method for the Estimation of Tetrabenazine by Simple RP-HPLC in Tablet Dosage Form

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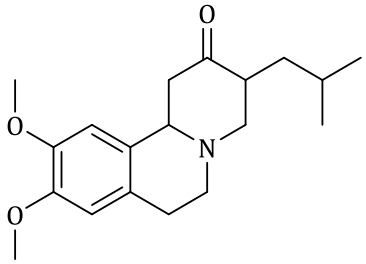
ABSTRACT

The aim of this work was to develop and validate the assay test for Tetrabenazine in the pharmaceutical dosage forms by HPLC. The assay method by HPLC was found to be linear in the concentration range of 20 to 100 µg/mL. The mobile phase was composed of acetonitrile and buffer pH: 6.8 potassium dihydrogen phosphate (60:40 v/v) the flow rate of 1 mL/min using UV detection at 215 nm. The results of the analysis were validated by recovery studies. The percentage recovery method was found to be 99.33-100.87%. The LOD and LOQ were found to 0.04 µg/mL and 0.15 µg/mL. All the parameters of validation were in the acceptable range. This developed method was successfully applied to estimate the amount of Tetrabenazine in the tablets.

GRAPHICAL ABSTRACT



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Introduction

Tetrabenazine (Figure 1) is a drug for the symptomatic treatment of the hyperkinetic movement disorder. The hyperkinetic movement disorders include neurological disease characterized by abnormal involuntary movement, such as Chorea associated with Huntington's disease. On August 2008 FDA approved the use of tetrabenazine to treat Chorea associated with Huntington's disease, the first in the USA [1, 2]. Tetrabenazine is a benzoquinoline pharmacophore that selectively depletes monoamines and improves hyperkinetic movement symptoms [3]. Tetrabenazine has been used for clinical purposes in several countries decades. Tetrabenazine is a potent blocker of vesicular monoamine transporter [4, 5]. There are few analytical methods published in the literature to detect the drug in biological fluids or to perform quality control of the medication [6]. Chemical name for the tetrabenazine is a 3-Isobutyl-9, 10-dimethoxy-1,3,4,6,7,11b-hexahydro-2*H*-pyrido[2,1a]isoquinolin-2-one [2] with molecular formula $C_{19}H_{27}NO_3$ and molecular weight 317.42. Various methods in the literature involve a determination of tetrabenazine by HPLC, LC/MS, pharmacokinetics, pharmacodynamics, UV [7]. Analysis plays important role in the formulation development of any drug product. The aim of this study was to develop and validate a simple and rapid RP-HPLC method to quantify tetrabenazine in a tablet dosage form.

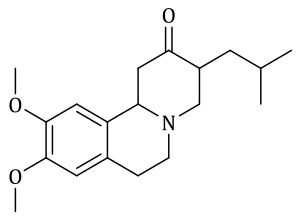

Figure 1: chemical structure of tetrabenazin

Experimental

Materials and Methods

Reagents and Chemicals

The standard tetrabenazine (purity, 99.9%) was obtained from Inke pharmaceutical lab in Spain. Acetonitrile (CHEM-LAB, Belgium) and Water used were of HPLC grade. Potassium dihydrogen phosphate, orthophosphoric acid, and triethylamine analysis grade were purchased from the Merck Company.

Instrumentation and Chromatographic condition

The reversed phase-High Performance Liquid Chromatography (RP-HPLC) method was developed on an HPLC system. The HPLC system consists of a quaternary pump (Agilent 1260 Infinity Quaternary LC System) equipped with a UV/VIS detector. The separation was on Phenomenex® C8 column (150 mm × 4.6 mm, 5 µ). Data were analyzed by using Chemstation software.

The analyte was monitored with UV/VIS detector at 215 nm. The HPLC was operated in an isocratic elution mode with the mobile phase consists of acetonitrile and buffer pH: 6.8 potassium dihydrogen phosphate (60:40 v/v). This analysis was done in the room temperature. The flow rate of elution was 1.0 mL/min. An ultrasonic irradiation was used for the sonication of the mobile phase, standard solution and sample solution. A typical chromatogram showing the separation of the drug (Figure 2).

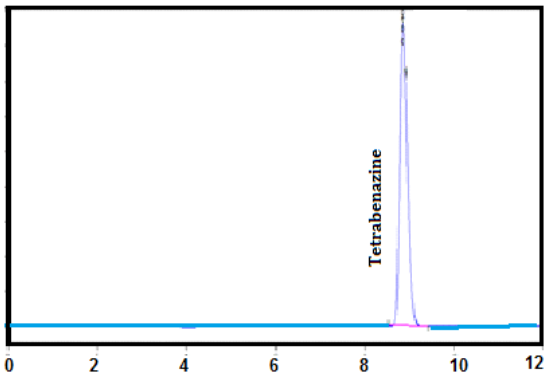

Figure 2: chromatogram of tetrabenazine

Preparation of Solutions

Preparation of solution section is divided into the preparation of buffer solution, standard solution and sample solution. The details of each section are followed.

Buffer solution

Dissolved 1.36 gr potassium dihydrogen phosphate in 1000 mL of water. Added 1 mL triethylamine. Adjusted the pH to 6.8 with orthophosphoric acid. Filtrated this solution through 0.45 µm nylon membrane and degassed it.

Preparation of Standard solution

Accurately weighed and transferred about 25.0 mg of tetrabenazine reference standard into a 100 mL volumetric flask. Dissolved and diluted to volume with mobile phase. Pipetted out 4 mL of this solution into a 20 mL volumetric flask and makeup to mark with mobile phase. Filtrated this solution through 0.45 µm PTFE filter.

Sample Preparation

Twenty tablets were weighed and powdered. Tablet powder equivalent to 25.0 mg of tetrabenazine was weighed and transferred to 100 mL volumetric flask and diluted with the mobile phase. Sonicated for 10 min and filtered so as to get solution having concentration 250 µg/mL. 4 mL this solution was further diluted with mobile phase to get the final concentration 50 µg/mL of tetrabenazine. Filtrate this solution through 0.45 µm PTFE filter.

Result and discussion

Validation of Assay Method

Validation of the method was divided into system suitability, linearity and rang, precision, accuracy, selectivity, limit of detection (LOD) and limit of quantification (LOQ) studies. The details of each section were followed.

System Suitability Study

The chromatographic parameters, including peak area, retention time, theoretical plates, tailing factor, and peak symmetry factor, were calculated. Five replicates of a standard solution of the Tetrabenazine was injected to check the system suitability. All results are presented in Table 1.

Table 1: System suitability parameters of standard chromatogram obtained to tetrabenazine

Standard	Retention time	Area	Theoretical plates	Symmetry	USP Tailing
1	8.890	2457.41	12128	0.7186	1.27
2	8.892	2459.73	11927	0.7199	1.25
3	8.892	2459.95	12128	0.7198	1.27
4	8.892	2458.35	11950	0.7205	1.26
5	8.894	2456.95	11985	0.7213	1.27
Average	8.892	2458.48	12024	0.7200	1.26
SD	0.001	1.34	97.52	0.001	0.009
RSD %	0.02	0.05	0.81	0.14	0.71

Linearity and Range

Some concentrations of calibration standard, namely: 20, 30, 50, 60, 80 and 100 µg/mL were prepared using the stock standard solution. Chromatograms of tetrabenazine in various concentrations showed in Figure 3. The linear regression line was used to determine the linearity and concentration of the samples. Calibration data are presented in Table 2. A calibration curve was prepared by plotting concentration of the tetrabenazine on X-axis and their respective area under curve (AUC) on the Y-axis. The calibration curve is shown in Figure 4.

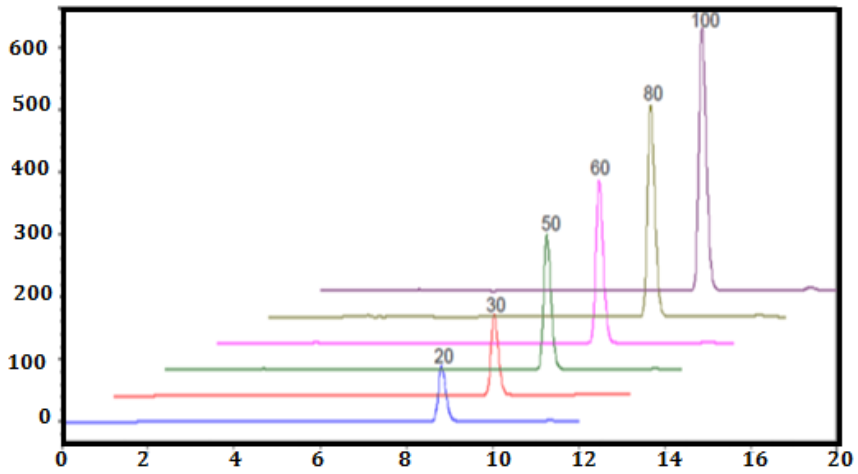

Figure 3: Chromatograms of tetrabenazine in various concentrations for linearity test

Table 2: Linearity data of tetrabenazine by HPLC

Concentration (µg/mL)	Inject 1	Inject 2	Inject 3	Mean	SD	RSD %
20	1071.58	1071.44	1070.76	1071.26	0.44	0.04
30	1570.46	1588.53	1593.96	1584.32	12.30	0.78
50	2616.18	2624.20	2628.83	2623.07	6.40	0.24
60	3164.21	3163.91	3164.07	3164.06	0.15	0.00
80	4183.15	4177.38	4122.21	4160.91	33.64	0.81
100	5199.38	5165.87	5192.61	5185.95	17.72	0.34

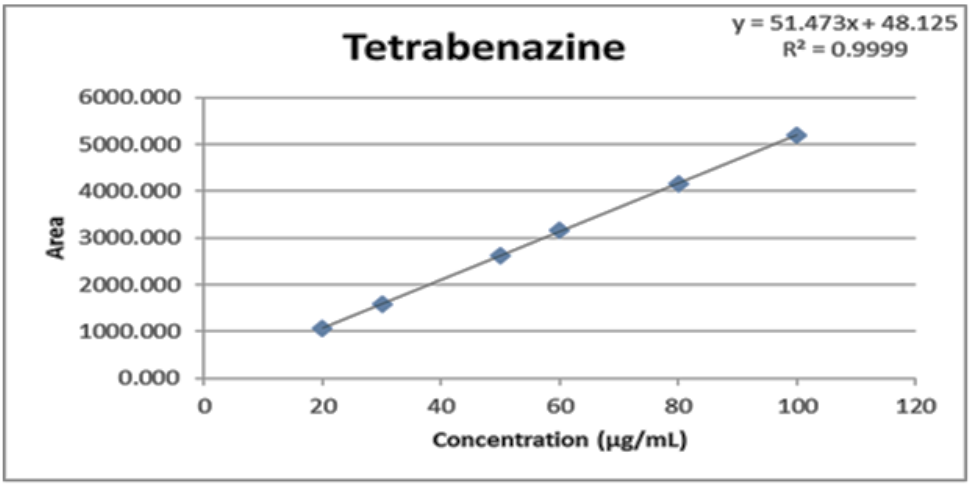

Figure 4: Calibration curve for tetrabenazine

Precision

The precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analysis of three different concentrations40, 50 and 60 µg/mL for tetrabenazine three times on the same day.

The intermediate precision of the method was checked by repeating studies on three different days (Table 3).

Accuracy (Recovery Studies)

The accuracy of the analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted true value. Accuracy studies were performed at three different levels (80%, 100%, and 120 %) by the standard addition method and the samples were analyzed in triplicate by the proposed method. The recovery studies were carried out by adding a known amount of pure drug Tetrabenazine at three different levels. The results obtained are given in Table 4.

Table 3: Intermediate precision on three different days

	Day 1			Day 2			Day 3		
Concentration (µg/mL)	40	50	60	40	50	60	40	50	60
Mean peak area	1977.51	2458.42	2981.53	1981.85	2456.82	2992.83	1985.30	2454.87	2995.49
SD	7.73	1.50	16.47	0.30	3.25	0.60	2.91	0.04	2.45
RSD %	0.39	0.06	0.55	0.02	0.13	0.02	0.15	0.001	0.08

Table 4: Determination of accuracy by percentage recovery method

Level of addition (%)	Amount of pure drug added (mg)	Amount of pure drug Recovered (mg)	recovery %
80	8	8.07	100.87
100	10	9.96	99.60
120	12	11.92	99.33
mean			99.93
SD			0.822
RSD %			0.823

Selectivity

Defining its ability to measure accurately and specifically the analyte of interest without interference from the blank and other ingredients in the matrix. In Figure 5 is shown any interference excipients with tetrabenazine peak.

LOD and LOQ

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected, but not necessarily quantified as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of the analyte in a sample which can be quantitatively determined with suitable precision and accuracy. Signal-to-noise ratios of 3:1 and 10:1 were obtained for the LOD and LOQ respectively.

The LOD and LOQ were found to 0.04 µg/mL and 0.15 µg/mL.

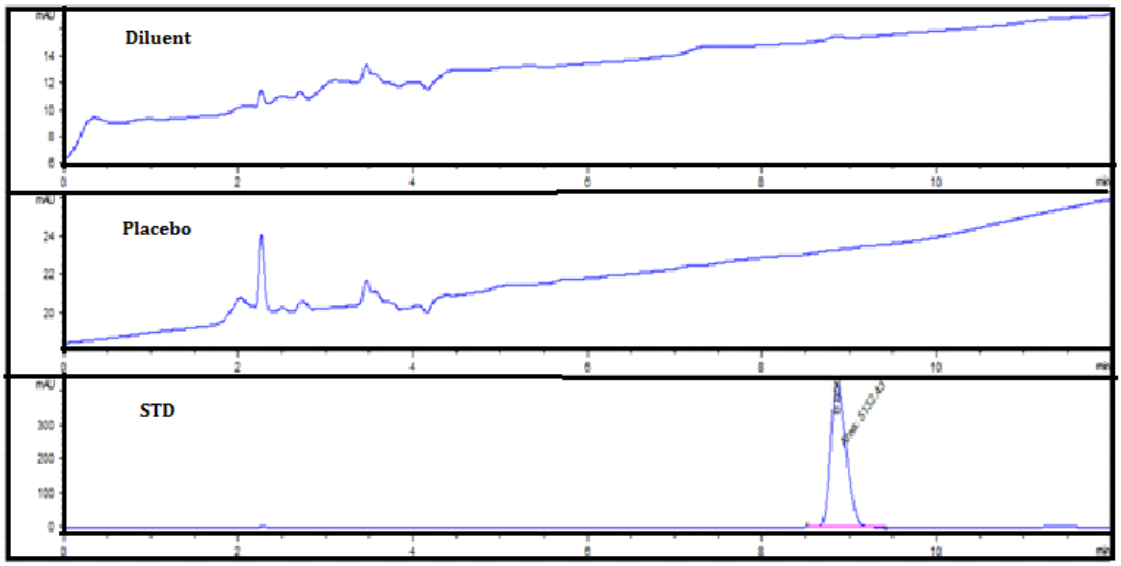

Figure 5: Diluent, placebo and standard solution chromatogram

Conclusion

The present method for the determination and validation study developed with the isocratic mode of RP-HPLC. The proposed HPLC method is rapid, sensitive, precise, and accurate for the determination of the tetrabenazine and can be reliably adopted for routine quality control analysis of tetrabenazine in its tablet dosage forms. This method is free from interference of the other active ingredients and additives used in the formulation. Degradation impurities does not interfere with the retention time of tetrabanazine, and assay method is thus stability indicating. This method can be recommended for routine and quality control analysis of tetrabenazine in the pharmaceutical dosage forms.

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Disclosure Statement

No potential conflict of interest was reported by the authors.

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Authors' contributions

All authors contributed toward data analysis, drafting, and revising the paper and agreed to responsible for all the aspects of this work.

Conflict of interest

The authors declare that they have no conflicts of interest in this article.

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