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Development and validation of stability indicating RP-HPLC method for Tetrabenazine from its pharmaceutical dosage form

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Abstract-

A Sensitive, selective, rapid, precise, and economical Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method. Tetrabenazine pharmaceutical dosage form. In standard absorptivity value, determination was carried out at absorption maxima of 254 nm using methanol as a solvent where as in used. HPLC method was carried out on a column XBridgeTM C183.5µm with a mobile phase consisting of Methanol: Acetonitrile in the ratio of (50:50 v/v) at a flow rate of 1.0ml/min. Detection was carried out at 284 nm with help of photodiode array (PDA) detector. The retention time of Tetrabenazine was 3.9 min. All the determination in High Performance Liquid Chromatographic (HPLC) methods were carried out in linearity range of 2-10 µg/ml. UV- Spectrophotometric methods were checked for accuracy and precision were as High-Performance Liquid Chromatographic (HPLC) method was validated as per international conference on Harmonization (ICH) guidelines. The proposed methods can be used for the routine estimation of the drug in bulk and pharmaceutical dosage form.

Keywords: Tetrabenazine,

Introduction

Tetrabenazine is a medication primarily used to treat Huntington's disease, a genetic disorder that affects nerve cells in the brain. It works by decreasing the amount of certain chemicals in the brain, particularly dopamine and other neurotransmitters, which are responsible for communication between nerve cells. Chemically, tetrabenazine is a reversible inhibitor of the vesicular monoamine transporter 2 (VMAT2). This means it blocks the ability of nerve cells to package certain neurotransmitters (like dopamine, serotonin, norepinephrine) into vesicles for release into the synapse, where they can signal to other nerve cells. By reducing the amount of dopamine released in the brain, tetrabenazine helps to alleviate some of the symptoms associated with Huntington's disease, such as involuntary movements (chorea).It's important to note that while tetrabenazine can be effective in managing symptoms, it doesn't cure Huntington's disease or halt its progression. Additionally, it can cause side effects such as sedation, depression, and parkinsonism-like symptoms. Therefore, its use should be carefully monitored by a healthcare professional.

Tetrabenazine is a medication commonly prescribed to manage symptoms associated with Huntington's disease, a neurodegenerative disorder. It works by reducing the levels of certain neurotransmitters in the brain, primarily dopamine, thereby helping to alleviate involuntary movements (chorea) and other motor symptoms. Despite its effectiveness, tetrabenazine can lead to side effects such as sedation and depression, necessitating careful monitoring by healthcare providers.

Materials and Methods:

Materials

Analytical pure sample was procured from Sun Pharmaceuticals. Pvt. Ltd.

Table 1.: Reagents and Chemicals

Sr.No.	Chemicals	Grade
1	Acetonitrile	HPLC
2	Methanol	HPLC
3	Hydrochloric Acid	GR
4	Sodium Hydroxide	GR
5	Hydrogen Peroxide	GR
6	Double Distilled Water	GR

Table 2: Details of marketed formulation

Sr.No.	Brand name Content	Mfg. company
1	Tetrabenazine Tab 25mg	Tetrabenazine Sun Pharma

Instrument-

Shimadzu HPLC 1100 series chromatograph equipped with isocratic pump LC-10ADVP, PDA - SPD M20A detector. Calibrated glassware's were used for the whole experimental work.

Method-

Preparation of Mobile phase

The mobile phase was created by combining acetonitrile and methanol in different ratios (v/v). The prepared mobile phases were all sonicated.

Preparation of stock solutions

A. Standard stock Solution

The standard stock solution was prepared by dissolving 10.0 mg of standard Tetrabenazine in 10.0mL volumetric flask, with dilute to the mark with the mobile phase to get a concentration of 1000 g/mL. A 1mL aliquot of the aforesaid solution was pipetted out and diluted to a concentration of 100g/ML in a 10.0 mL volumetric flask with mobile phase.

Working Standard Solution

A concentration of 10 g/mL was obtained by pipetting 1 ml of the standard stock solution into a 10.0 ml volumetric flask and filling the remaining space with mobile phase

Method Development

Selection of wavelength

Working standard solution of Tetrabenazine prepared as described under 2.B, was scanned in the range of 400-200 in 1.0 cm cell against solvent blank and the spectra was recorded at 248nm. The spectrum recorded is shown in **Fig.1**

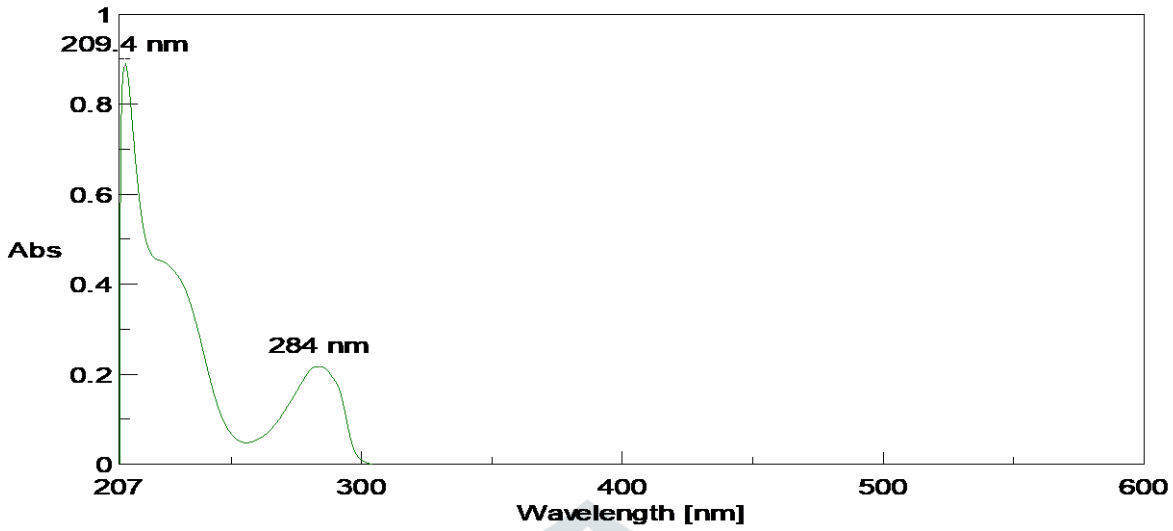


Fig 1.: UV Spectra of Tetrabenazine

Selection of Mobile phase

The mobile phase was created by combining acetonitrile and methanol in different ratios (v/v). The prepared mobile phases were all sonicated.

1. **Preliminary Optimization of Mobile Phase and Chromatographic Conditions**

   The nature of the sample will determine the best approach to use. The drug selected in its present study is non-polar in nature, therefore; reverse phase chromatography method can also be used. Here, the RP-HPLC method was selected for the initial separation owing to its simplicity, suitability, ruggedness and its wider usage. In order to achieve the optimized chromatographic condition to separate elute and quantify tetrabenazine, one or two parameters were modified at each trial and chromatograms were recorded with all specified chromatographic conditions.

HPLC Instrument and Conditions-

Shimadzu HPLC 1100 series chromatograph equipped with isocratic pump LC-10ADVP, PDA - SPD M20A detector and the column was Waters XBridge^{TM} C18 3.5µm ,tempreture was maintained at 40^{0}C. Calibrated glassware's were used for the whole experimental work.

Forced Degradation Studies

Acid Hydrolysis

An accurately weighed quantity of Tetrabenazine std. about 10.0mg was transferred in 10.0mL volumetric flasks. In flask 2,0mL of reagent (0.5N NaOH) were added and kept at room temperature for 24hrs. The stressed solutions were withdrawn after 24hrs and neutralisation was carried out. The content of the each flask was sonicated for 15min, volume was made up to the mark with mobile phase and filtered (1000ug/mL). A 1.0 mL portion of filtrate was diluted to 10.0 mL with mobile phase. A 0.6mL portion of filtrate was further diluted to 10.0mL with mobile phase(6µg/ml) was injected.

Alkali Hydrolysis

An accurately weighed quantity of Tetrabenazine std. about 10.0mg was transferred in 10.0mL volumetric flasks. In flask 2,0mL of reagent (0.5N NaOH) were added and kept at room temperature for 24hrs. The stressed solutions were withdrawn after 24hrs and neutralisation was carried out. The content of the each flask was sonicated for 15min, volume was made up to the mark with mobile phase and filtered (1000ug/mL). A 1.0 mL portion of filtrate was diluted to 10.0 mL with mobile phase. A 0.6mL portion of filtrate was further diluted to 10.0mL with mobile phase(6µg/ml) was injected.

Oxidative study

An accurately weighed quantity of Tetrabenazine about 10.0mg of Std. was transferred in10.0mL volumetric flasks. In flask 2.0mL of 3% H2O2 were added and kept at room temperature for 24hrs. The stressed solutions were withdrawn after 24hrs. The content of the each flask was sonicated for 15min, volume was made up to the mark with mobile phase and filtered (1000µg/mL). A 1.0 mL portion of filtrate was diluted to 10.0 mL with mobile phase.A 0.6mL portion of filtrate was further diluted to 10.0mL with mobile phase(6µg/ml) was injected.

Thermal degradation

An accurately weighed quantity of Tetrabenazine about 10.0mg of Std. was spread on petri dish separately and exposed to dry heat in an oven at 40 C for 3hrs. The stressed solutions were withdrawn after 3hrs. The content of the each flask was sonicated for 15min, volume was made up to the mark with mobile phase and filtered (1000µg/mL). A 1.0 mL portion of filtrate was diluted to 10.0 mL with mobile phase . A 0.6mL portion of filtrate was further diluted to 10.0mL with mobile phase(6µg/ml) was inject

Conditions	Peak area of std.	Peak area of sample	Drug Undegradation (mg)	% Undegradation
Acid	60325	58053	9.62	96.23
Alkali		57137	9.47	94.71
H2O2		56284	9.33	93.30
Thermal		45676	7.57	75.71

Method Validation

The method was validated as per Q2R1 ICH guidelines including selectivity, system suitability, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), linearity, and robustness.

1. Preliminary Optimization of Mobile Phase and Chromatographic Conditions

The nature of the sample will determine the best approach to use. The drug selected in its present study is non-polar in nature, therefore; reverse phase chromatography method can also be used. Here, the RP-HPLC method was selected for the initial separation owing to its simplicity, suitability, ruggedness and its wider usage. In order to achieve the optimized chromatographic condition to separate elute and quantify tetrabenazine, one or two parameters were modified at each trial and chromatograms were recorded with all specified chromatographic conditions. manual injection and Peak area was calculated once the chromatogram was recorded.

Study of system suitability parameter:

Following the equilibration of the column with the system suitability parameters ,mobile phase, six replicate injections of 10µL solution were injected through the manual injection and Peak area was calculated once the chromatogram was recorded.

The recorded results of system suitability parameters are displayed in Table No.3.

Sr.no.	Standard weight taken (mg)	AUC of TBZ (µV)
1	~10.0mg	113235
2		113214
3		113220
4		113228

5		113232
Mean		113225
% RSD		1.29%
Theoretical plate /Column		52054
Retention Time		3.94
Tailing factor		1.33

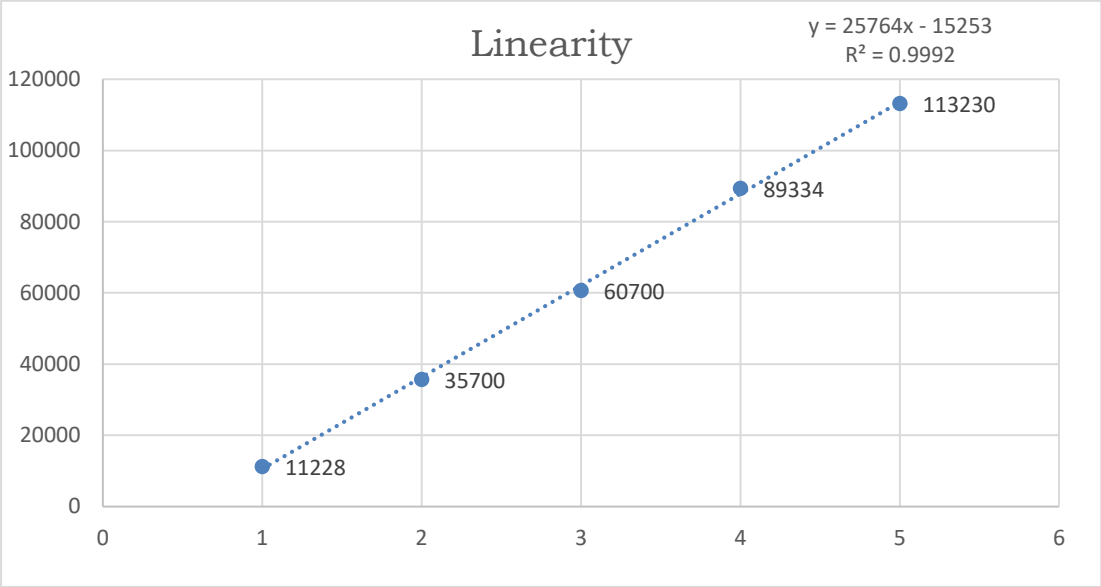
Linearity (Calibration Curve)

The process outlined earlier was followed to create standard stock solutions. Aliquots of standard stock solution were diluted in range of 2ml-10mL in 10.0mL volumetric flask and volume was made up to mark with diluent to obtained concentration ranging from 02-10µg/mL of Tetrabenazine.

The stationary phase and mobile phase were allowed to equilibrate until a stable baseline was reached.. Each of the final solution was injected separately and chromatograms recorded. The observations of area are shown in Table No.8.3.

Table no.4: Observation of linearity response of Tetrabenazine

Sr.no.	Weight of standard	Concentration (µg/ml)	AUC(µV)
1	~10.0 mg	2	11228
2		4	35700
3		6	60700
4		8	89334
5		10	113230



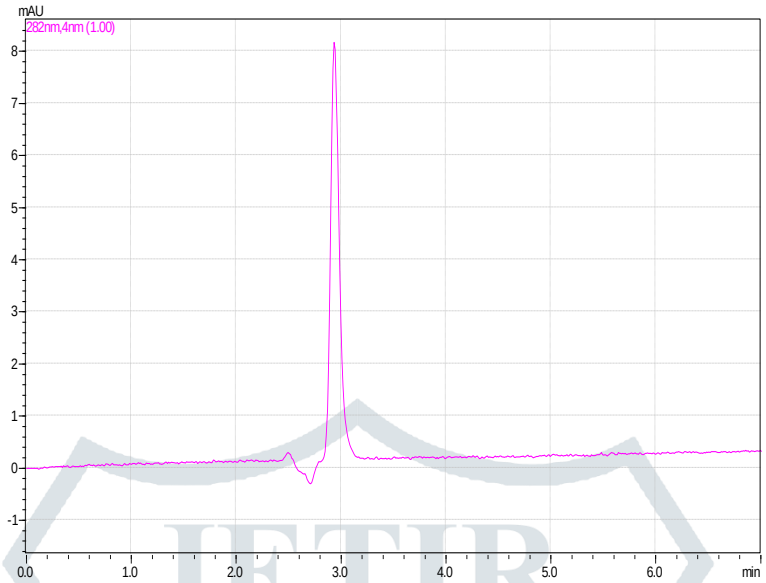
Calibration curve of Tetrabenazine

APPLICATION OF PROPOSED METHOD FOR ASSAY OF MARKETED FORMULATION

Preparation of sample.

The average weight of ten tablets was determined after they were weighed. The tablets were triturated thoroughly and mixed. an amount that was precisely weighed of tablet powder equivalent to 25 mg of Tetrabenazine was transferred to 25 mL volumetric flask and volume were made up to the mark with the mobile phase. The content was sonicated for 15 min and

filtered through Whatman filter paper. A 1.0 mL portion of filtrate was diluted to 10.0 mL with the mobile phase . Five such samples were prepared. After equilibrations of column with mobile phase 20 µL volume of the final diluted solutions were injected into the system, the representative chromatograms were recorded as shown in **Fig**



Chromatogram for Tetrabenazine in marketed formulation

Table No.5-Observations and results for marketed formulation

Sr.no.	Wt. of Std. taken(mg)	Wt. of sample taken (mg)	AUC Std. (µV)	AUC Sample (µV)	% Labeled claim
1	~10.0mg	57.1	60325	58300	100.19
2		58.0		58707	100.89
3		57.9		58032	99.73
4		58.3		58589	100.69
5		57.8		58820	101.08
				Mean	**100.51**
				±SD	**0.550**

- **Accuracy**

The accuracy of the proposed method was ascertained on the basis of recovery studies performed by the standard addition method. Results are displayed in **Table No.6**

Table no.6: Observation of Recovery Study

Sr no	Wt of tab. powder (mg)	Amt. of pure drug added (mg)	AUC		Amt. of pure drug recovered		%Recovery	
			Reported Method	Proposed method	Reported method	Proposed method	Reported method	Proposed method
1	57.1	20	1821384	146523	18.0	20.04	100.78	100.23
2	57.3	25	2460224	174623	24.0	24.69	99.78	98.79
3	57.2	30	2976921	203632	30.0	29.50	102.51	98.34
						Mean	101.02	99.12
						±SD	1.38	0.24
						%RSD	1.37	0.25

Intermediate precision

It expresses within laboratory variation. This attribute evaluates the reliability of the method in an environment different from that used during the method development phase.The sample was prepared as per procedure described under marketed formulation and analysed at intervals upto 3h for intraday study and on 1st day ,2nd day & 3rd day for interday study. The content of tetrabenazine was calculated by formula (2) and results obtained for intraday and interday studies are show in **Table no.7&8** respectively

Table no.7: Observation and result of intraday study

Sr. no.	Time (hr)	Wt. of sample taken (mg)	AUC (µV)	% Label claim
1	0	57.1	58561	100.64
2	3		58760	100.98
3	6		58693	100.87
Mean				100.83
±SD				**0.141**
%RSD				**0.14**

Table no.8: Observation and results of interday study

Sr. no.	Days	Wt. of sample taken (mg)	AUC (µV)	% Label claim
1	0	57.1	58694	100.87
2	1		58785	101.02
3	2		58746	100.96
4	3		58876	100.18
Mean				**100.72**
±SD				**0.389**
%RSD				**0.39**

Robustness

The deliberate change was made in the optimized chromatographic parameter and robustness of the method was studied by evaluating system suitability parameter data after varying the flow rate, detection wavelength, column temperature and mobile phase composition.

Table No.8: Observation Robustness Study

Sr. no.	Deliberated condition	Retention time (min)	Theoretical plate(per column)	Tailing factor
1	Standard condition (50:50)	3.944	52054	1.332
2	Flow rate 0.8ml/min	4.653	67345	1.326
3	Flow rate 1.2ml/min	2.371	41417	1.344
4	Wavelength 278	3.205	52379	1.345
5	Wavelength 289	3.267	53135	1.379
6	Mobile phase 55:45	3.963	45653	1.336
7	Mobile phase 45:55	3.740	51177	1.328
Mean				**1.341**
±SD				**0.018**
%RSD				**1.35**

Limit of Determination and Limit of Quantification

The standard deviation of Y-intercept and slop of calibration curve were used to calculate the LOD and LOQ for all the drugs using following formulas. Observation are shown in **Table no.9**

LOD= 3.3 R/S

LOQ= 10 R/S

Table no.9 : Result of LOD and LOQ

Sr.no.	LOD (µg)	LOQ (µg)
1	0.303	1.011

Conclusion-

A novel simple and fast validated RP-HPLC method is sensitive, accurate, precise, and robust. Moreover, the successfully developed by stress study and further validated methods according to ICH guidelines. Method was found to be more selective and rapid with respect to shorter runtime. The stability study was performed by exposing the drug at various thermal and humidity stress conditions. It shows degradation (not showing any additional peak) upon certain extend. Hence, developed validated stability indicating method can be employed for the routine quality control analysis of Tetrabenazine in the different laboratory stress conditions.

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