

# Comparison of Analytical Spectrophotometric Methods for the Determination of Tetrabenazine in Tablets

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## ABSTRACT

**Background:** Tetrabenazine was approved in the year of 2008 by USFDA. It was indicated mainly as an antipsychotic drug but now-a-days it is used for the treatment of hyperkinetic disorders. **Objective:** The objective of the study was to develop a simple and cost-effective spectrophotometric methods for the determination of tetrabenazine in tablets. **Materials and Methods:** The UV-Visible spectrophotometric studies carried out by Shimadzu UV 1800 with UV probe software and FTIR studies using Bruker alpha with opus software. The UV spectrum at 284 nm (Method A) and visible spectrum at 454 nm (Method B) are recorded. The FT-IR band of carbonyl group in tetrabenazine appeared at 1700  $\text{cm}^{-1}$  with two-point baseline between 1650-1750  $\text{cm}^{-1}$  (Method C). **Results: Method A:** The UV method was proved linear over the range of 10–50  $\mu\text{g/mL}$  with correlation coefficient  $r^2=0.9988$  and mean recovery of 99.02% to 101.54%. **Method B:** The colorimetric method involves oxidative reaction of tetrabenazine and it yields green color chromogen. The linear concentrations over the range of 3–15  $\mu\text{g/mL}$  with correlation coefficient of  $r^2=0.9981$  and mean recovery of 99.93% to 100.50%. **Method C:** The FT-IR method was showed linear

over the range of 5–25  $\mu\text{g/mg}$ , with correlation coefficient  $r^2=0.9998$  and mean recovery of 100.75% to 100.91%. The results of tetrabenazine tablets showed good agreement with their label claim. The results of ANOVA declare there was no significant difference between these spectrophotometric methods. **Conclusion:** Hence, these spectroscopic methods can be used for routine quality testing analysis of tetrabenazine in pharmaceutical laboratories.

**Key words:** Tetrabenazine, Quantitative Analysis, UV, Colorimetry, FT-IR, Validation.

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## INTRODUCTION

Tetrabenazine ( $\text{C}_{19}\text{H}_{27}\text{NO}_3$ ), (9, 10-Dimethoxy-3-(2-methylpropyl)-1, 3, 4, 6, 7, 11b-hexahydro benzo[a]quinolizin-2-one) (Figure 1), is a dopamine depleting agent indicated for schizophrenia treatment initially.<sup>1</sup> Many studies have shown that tetrabenazine is effective to treat hyperkinetic movement disorders.<sup>2</sup> As per the regulatory body of United States Food and Drug Administration (USFDA) tetrabenazine is indicated for chorea associated with Huntington's disease.<sup>3</sup>

Extensive literature survey reveals that previous studies have been reported for the determination of tetrabenazine employing UV,<sup>4</sup> spectrofluorimetry,<sup>5</sup> HPLC,<sup>6,7</sup> LC-MS.<sup>8,9</sup> Now-a-days most of the analytical studies related to the techniques of LC/MS, UPLC, GC and HPLC either in raw material or formulation leads to more cost or either it may be use of high grade solvents or time consumption. Based upon these aspects of cost and the process to determine the quantity in the dosage form we are aimed to develop a new analytical method for the determination of tetrabenazine in tablet dosage forms using UV, Colorimetry and FT-IR spectrophotometric techniques. The objective of the study was to develop a simple and cost-effective methods by using the different spectrophotometric techniques. The Validation of these three spectroscopic developed methods was done as per regulatory guidelines.<sup>10,11</sup> The results of the pharmaceutical dosage form of tetrabenazine were statistically compared with analysis of variance (ANOVA) studies.

## MATERIALS AND METHODS

### Materials

Tetrabenazine, was obtained from Sun Pharma, Mumbai, India. The tablets of tetrabenazine was obtained from local market under the trade

name of Revocon-25 mg. All the chemicals and reagents used in these studies are of analytical grade and potassium bromide was FT-IR grade and it was from Merck, India.

### Instrumentation

UV and Colorimetric studies were carried out using a Shimadzu model of 1800 double beam UV-Vis spectrophotometer, using 10 mm quartz cells. The data analyzed using UV probe software. The FT-IR studies carried out in mid infrared (IR) region (4000-400  $\text{cm}^{-1}$ ) using the Bruker alpha model with opus software. The analysis performed by accumulating 16 scans per FT-IR spectrum at an optimum resolution of 4  $\text{cm}^{-1}$ .

### Preparation of standard solutions

#### Method A & B (UV Spectroscopic and Colorimetric method)

The standard stock solution of tetrabenazine 1000  $\mu\text{g mL}^{-1}$  was prepared in methanol. For 'Method A' further dilutions of aliquots of standard stock solution were carried out with water to reach the concentration range of 10-50  $\mu\text{g mL}^{-1}$  for tetrabenazine and it was measured at 284 nm. For 'Method B', the standard solutions were prepared by dilution of aliquots of the standard stock solution and the final concentration brought to 100  $\mu\text{g mL}^{-1}$ . Aliquots of drug solution ranging from 0.3-1.5 mL were taken separately into 10 mL volumetric flasks and then 1.0 mL of Hydrochloric acid (1.0 M), 1.0 mL of Ferric chloride (0.4% w/v) and 1.0 mL of Potassium ferricyanide (0.2% w/v) were added and boiled for 10 mins and it was allowed to cool at room temperature (25 °C) for 5 mins. The remaining volume was adjusted with distilled water. The absorbance of drug solution was measured at  $\lambda_{\text{max}}$  454 nm against the corresponding

reagent blank.

#### Method C (FT-IR Spectroscopic method (green method))

The standard of tetrabenazine was grinded in a mortar to homogenize the powders properly. Weighed 1 mg of tetrabenazine with 99 mg of potassium bromide were transferred to 13 mm die and pressed near 10,000 lbs for 5 mins to obtain a pellet. The pellet of drug was then placed in the FT-IR for recording the spectra. Before each scan of the analysis of drug sample, fresh background spectrum was taken with the same instrumental conditions. A set of five standards of tetrabenazine with KBr in the form of pellet were prepared in concentration range of 5.000-25.000 mg. Proper mixing of tetrabenazine with KBr should be done then only the pellet having the uniformly distributed drug. The calibration plots were carried out by focusing on carbonyl absorption band at  $1700\text{ cm}^{-1}$  to achieve the best linearity and regression results.

### Assay of Tetrabenazine in Tablets

Twenty tablets of tetrabenazine were weighed and crushed to a fine powder. An accurately weighed amount of tablet powder equivalent to 10 mg of tetrabenazine was transferred to a 100 mL volumetric flask and add 50 mL of methanol to dissolve the sample and sonicated for 10 min, followed by addition of same solvent to made up to the volume (Sample stock). After filtration, an aliquot of 5 mL of this solution was transferred into a 50 mL volumetric flask and marked up to volume with water to produce a final concentration of  $10\text{ }\mu\text{g mL}^{-1}$  (Method-A). For colorimetric method the above procedure has been followed. After filtration, an aliquot of 0.6 mL of this solution was transferred into a 10 mL volumetric flask, 1.0 mL of Hydrochloric acid (1.0 M), 1.0 mL of Ferric chloride (0.4 % w/v) and 1.0 mL of Potassium ferricyanide (0.2 % w/v) was added and heated for 10 min. and made up to volume with water to produce a final concentration of  $6\text{ }\mu\text{g mL}^{-1}$  (Method-B). 20 tablets of Tetrabenazine were triturated after taking their average weight. The tablet powder equivalent to 10 mg was transferred to the volumetric flask and dissolved with chloroform. The resulting solution was sonicated for 10 min and supernatant was filtered through whatmann filter paper no. 41. Filtrate was evaporated and from the residue obtained 10 mg was accurately weighed, made up to 1000 mg with dried KBr to produce a final concentration of  $10\text{ }\mu\text{g mg}^{-1}$  (Method-C).

### Method validation

The developed analytical method was validated as per the regulatory requirements of USP and ICH guidelines.<sup>10,11</sup>

#### Linearity

For method A, the appropriate dilutions were made from stock solution ( $100\text{ }\mu\text{g mL}^{-1}$ ) of tetrabenazine with water in order to produce a final concentration of 10-50  $\mu\text{g/mL}$ . For method B, transferred 0.3 mL, 0.6 mL, 0.9 mL, 1.2 mL and 1.5 mL of the working standard solution ( $100\text{ }\mu\text{g mL}^{-1}$ ) of Tetrabenazine in to 10 mL volumetric flask and in each volumetric flask add 1.0 mL of Hydrochloric acid (1.0 M), 1.0 mL of Ferric chloride (0.4 % w/v) and 1.0 mL of Potassium ferricyanide (0.2 % w/v) was added and boiled for 10-15 min. and make up to volume with water to produce a concentration over the range of 3-15  $\mu\text{g/mL}$ . For method C, proper dilutions were done from working standard of tetrabenazine using potassium bromide and prepared the concentrations over the range of 5-25  $\mu\text{g/mg}$ . Each concentration of the linearity in the above three spectrophotometric studies were analyzed in triplicate and the results assessed using linear regression method.

### Specificity

Specificity studies were performed for these developed methods using all the components of tetrabenazine tablets excepting the drug. The placebo solutions were scanned from 400 to 200 nm (Method A), 800 to 400 nm (Method B) and checked for any interference or overlaps or any degradations in the absorbance at all tested wavelengths. For method C, the wavenumber selected for analysis was specific for tetrabenazine and observed any excipient interference.

### Precision

The precision of the method was determined by repeatability (intra-day precision) and intermediate precision (inter-day precision) and was expressed as relative standard deviation (% RSD) of a series of measurement. The repeatability was evaluated by assaying six samples of each pharmaceutical formulation, at the concentration  $30\text{ }\mu\text{g mL}^{-1}$  (Method A),  $15\text{ }\mu\text{g mL}^{-1}$  (Method B) and  $10\text{ }\mu\text{g mg}^{-1}$  (Method C) during the same day. The intermediate precision was carried out in three different days.

### Accuracy

Accuracy was determined by the recovery test that consisted on adding known amounts of reference solution to the sample solutions (prepared according to "Sample preparation"). For method A, the concentrations of 8, 10 and  $12\text{ }\mu\text{g mL}^{-1}$  were prepared using the working standard solutions of tetrabenazine and it was added to a prequantified test mixture of Tetrabenazine  $10\text{ }\mu\text{g mg}^{-1}$ . For method B, the concentrations of 4.8, 6 and  $7.2\text{ }\mu\text{g mL}^{-1}$  were prepared using the working standard solutions of tetrabenazine and it was added to a prequantified test mixture of Tetrabenazine  $6\text{ }\mu\text{g mg}^{-1}$ . For method C, the concentrations of working standard of Tetrabenazine 8, 10 and  $12\text{ }\mu\text{g mg}^{-1}$ , were added respectively to a prequantified test mixture of Tetrabenazine  $10\text{ }\mu\text{g mg}^{-1}$ . At each level, three analyses were performed and the mean percentage recovery, % RSD value were determined for the above three methods.

### Limit of Detection and Quantification

The limit of detection (LOD) and limit of quantification (LOQ) were separately calculated based upon the response obtained on the standard deviation for the three methods.

### ANOVA

These spectrophotometric methods were compared and it was applied for the determination of tetrabenazine in tablet dosage forms statistically by ANOVA showing non-significant difference ( $P > 0.05$ ).

## RESULTS AND DISCUSSION

### Method development and optimization

#### Method A

Tetrabenazine was soluble in methanol and hence the solvent methanol was chosen for analysis and the prepared concentrations of tetrabenazine was scanned over the range of 400-200 nm. After the evaluation of the spectrum, the wavelength of 284 nm was selected for measurement, due to the adequate molar absorptivity of tetrabenazine in this region (Figure 2).

#### Method B

The colorimetric determination of tetrabenazine involves the oxidative reaction. It involves the reaction in between ferric salt and potassium ferricyanide under acidic conditions and the solution is boiled for 10-15 min. to produce a green colored chromogen with wavelength of 454 nm. The reaction involves the reduction of iron (III) by tetrabenazine to iron (II),

which subsequently reacts with ferricyanide to give a green colored chromogen in acidic medium. The optimized conditions were chosen based upon the response of the concentration of the reagents, volume of addition of reagents and the order of addition of reagents. The concentration of hydrochloric acid was used over the range of 0.5 M to 1.5 M and the volume of hydrochloric acid was tested over the range of 0.5 mL to 1.5 mL. The concentration of ferric chloride was performed over the range of 0.2 to 1.0 % (w/v) of ferric chloride and the volume of addition was tested it on 1.0 mL to 3.0 mL. The concentration of potassium ferricyanide was done over the range of 0.1 to 0.3 % (w/v), and then the volume of addition was 1.0 mL to 3.0 mL was evaluated. The color chromogen complex was studied based upon the boiling temperature for 10 to 30 min, based upon the trails we chosen the 10-15 min. is sufficient for the formation of color complex. Based upon the trail measurements, volume of addition of reagents and order of addition of reagents finally we chosen 1 mL of 1 M of Hydrochloric acid, 1 mL of 0.4 % (w/v) ferric chloride, 1 mL of 0.2% (w/v) solution of potassium ferricyanide and the solution is boiled for 10-15 min for the formation of green color chromogen and which it is stable for a period of more than 3 h (Figure 3, 4).

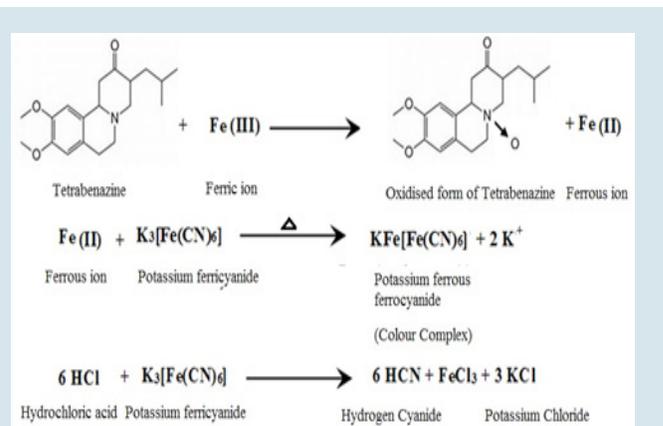


Figure 3: Scheme-I of the chromogen formation.

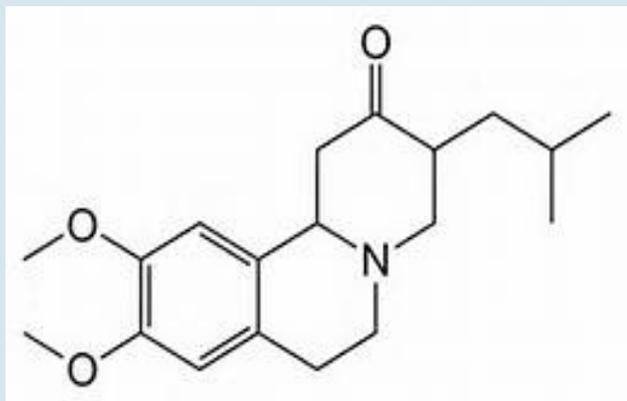


Figure 1: Chemical Structure of Tetrabenazine.

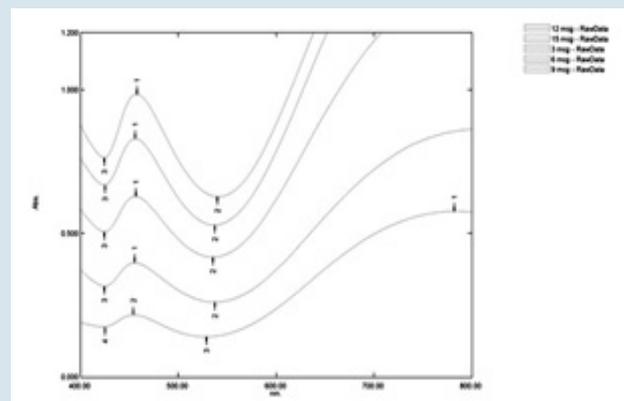


Figure 4: Overlay spectrum of Tetrabenazine (Colorimetric Method).

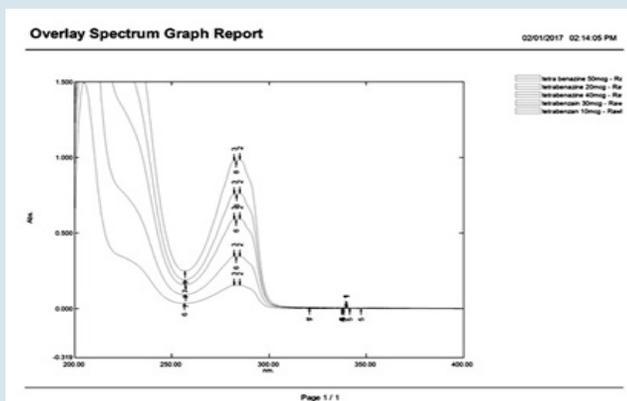


Figure 2: Overlay spectrum of Tetrabenazine (UV Method).

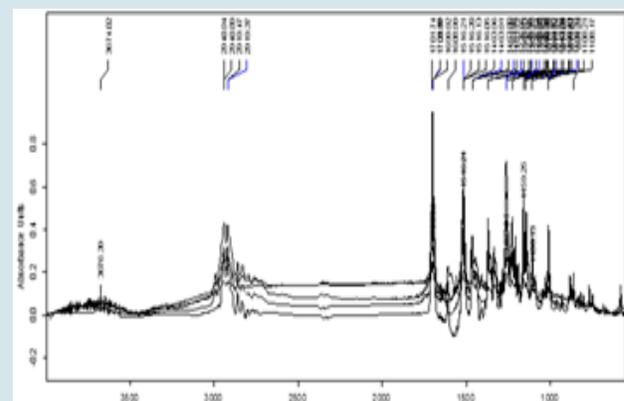
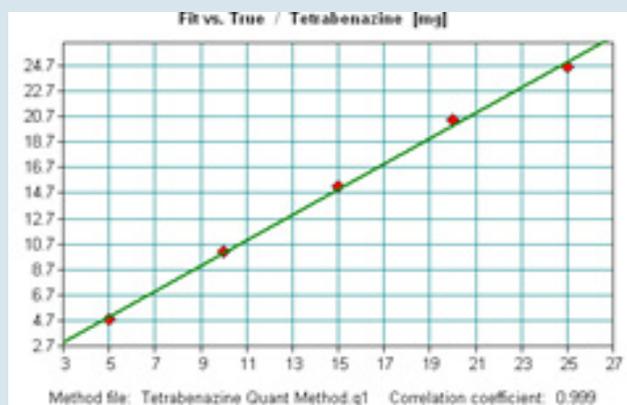


Figure 5: Overlay spectrum of Tetrabenazine (FT-IR Method).



**Figure 6:** FT-IR Calibration Plot of Tetrabenazine.

### Method C

The FT-IR spectrum of tetrabenazine obtained from absorbance mode and prepared the pellet using the solid phase technique, (KBr pellet or pressed pellet technique) shows well defined absorption bands with relatively high intensity over the range of 4000-400  $\text{cm}^{-1}$ . The absorption band of C=O (Carbonyl group) was chosen and the peak was integrated over the range of 1690  $\text{cm}^{-1}$  to 1710  $\text{cm}^{-1}$ . The result showed that the carbonyl group was clear and intense peak at their allowable range. Based upon the S/N ratio 16 scans and resolution of 4  $\text{cm}^{-1}$  was selected (Figure 5, 6).

### Linearity

A linear relationship was found between the concentration and the response of UV, colorimetric and FT-IR methods. The regression analysis data are presented in Table 1. The values of the LOD and LOQ was determined by statistical evaluation and the results were showed in Table 1.

### Specificity

The absorption spectra of method A & B demonstrates there is no potential interference of the tablet excipients at 284 nm and 454 nm. The results obtained for method C showed no potential interference at selected wavenumber. Hence, these spectrometric methods are specific.

### Precision

The precision data of these studies of tetrabenazine are tabulated in Table 2. Three methods presented R.S.D. values lower than 2.0% assuring a good precision.

### Accuracy

Accuracy of these studies were calculated based on the mean recovery studies. Three methods exhibited mean recoveries ( $n = 9$ ) which are close to 100% demonstrating an adequate accuracy. The results of accuracy of tetrabenazine are tabulated in Table 3.

### Analysis of tablets

The quantitative results of these developed methods i.e., UV, colorimetric and FT-IR methods are shown in Table 4. The results indicate that these three spectroscopic studies show the good agreement with the label claim of the tablet dosage form.

**Table 1: Optical characters of three spectrophotometric methods of Tetrabenazine in tablet dosage forms**

Parameter	UV Method	Colorimetry	FT-IR
$\lambda_{\text{max}}$ (nm)	284	454	1700
Beer's law range ( $\mu\text{g/ml}$ )	10-50	3-15	5-25
Molar absorptivity	0.0207	0.0640	0.0350
Correlation coefficient, $r^2$	0.9988	0.9981	0.9998
Slope (b)	0.021	0.066	0.035
Intercept	0.053	0.020	0.080
Sandell's sensitivity ( $\mu\text{g/ml}^{-1}/0.001\text{A}$ )	0.064	0.013	0.019
LOD	0.608 $\mu\text{g/mL}$	0.420 $\mu\text{g/mL}$	0.1288 $\mu\text{g}$
LOQ	1.842 $\mu\text{g/mL}$	1.274 $\mu\text{g/mL}$	0.3903 $\mu\text{g}$
Stability of chromogen	-	3 h	-
Reaction time	-	10 to 15 min	-

**Table 2: Precision results of spectrophotometric methods of Tetrabenazine.**

Intra-day Precision						
Mean of Absorbance*						
Method	Conc. ( $\mu\text{g/ml}$ )	Morning	Afternoon	Evening	SD	%RSD
A	30	0.6338	0.6368	0.6355	0.0042	0.65
B	12	0.7757	0.7738	0.7670	0.0088	1.23
C	15	0.5543	0.5531	0.5531	0.0010	0.21
Inter-day Precision						
	Day-1	Day-2	Day-3			
A	30	0.6338	0.6276	0.6273	0.0047	0.75
B	12	0.7757	0.7720	0.7772	0.0083	1.07
C	15	0.5543	0.5532	0.5545	0.0022	0.33

\*Average of 6 determinations

**Table 3: Accuracy results of spectrophotometric methods of Tetrabenazine.**

S. No.	Spike level (%)	Amount of standard*		% Mean recovery	SD	% RSD
		Added	Found			
METHOD-A						
1	80	8	8.0317	99.02	1.2311	1.24
2	100	10	10.0317	100.15	0.2887	0.29
3	120	12	12.1217	101.54	0.9141	0.90
METHOD-B						
1	80	4.8	4.80	99.93	0.625	0.625
2	100	6	6.03	100.50	0.41944	0.417
3	120	7.2	7.20	99.95	0.41667	0.417
METHOD-C						
1	80	8	8.0839	100.75	1.0921	1.08
2	100	10	10.1939	100.91	1.0017	0.99
3	120	12	12.1039	100.81	0.8347	0.83

\*Average of 3 determinations.

**Table 4: Assay of Tetrabenazine.**

Dosage form	Label claim	Method	Mean (% Amount)*	% RSD
Tablet (Revocon)	25 mg	UV	99.68	0.49
		Colorimetry	100.06	0.13
		FT-IR	99.76	0.28

\*Average of 6 determinations.

**Table 5: Results of the ANOVA (Single factor method).**

Groups	Count	Sum	Average	Variance
UV	6	598.09	99.68	0.24
Colorimetric	6	600.34	100.06	0.02
FT-IR	6	598.56	99.76	0.08

Source of Variation	SS	df	MS	F Calculated	F tabulated
Between Groups	0.47	2	0.23		
Within Groups	1.67	15	0.11	2.07	3.68
Total	2.17	17	-		

The calculated value of F is 2.07; Here  $F_{\text{calculated}} = 2.07 < F_{\text{tabulated}} = 3.68$  ( $P = 0.05$ ).

## Comparison between analytical Spectrophotometric methods

The results of the ANOVA of tetrabenazine in three spectroscopic studies were compared using statistical analysis. The results are shown in the Table 5. The developed and validated spectroscopic methods provided similar results for Tetrabenazine quantitation. Hence, these three spectroscopic studies can be applied directly to the quality control department of oral pharmaceutical preparations of Tetrabenazine.

## CONCLUSION

This work presents a simple and validated UV-spectrophotometric method, colorimetric method and FT-IR Spectroscopic method (green method) for the determination of Tetrabenazine in pharmaceutical dosage forms. These studies were validated as per the regulatory requirements and the results showed that these studies are simple, precise and accurate. These studies also provides the advantages over the other alternative analytical studies like HPLC in the aspects of time and cost. There is no significant difference in between these developed spectroscopic methods and it was declared by the ANOVA test. Therefore, these proposed methods are suitable and it can be conveniently used for the routine quality control of Tetrabenazine.

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## CONFLICT OF INTEREST

The author declared no conflict of interest.

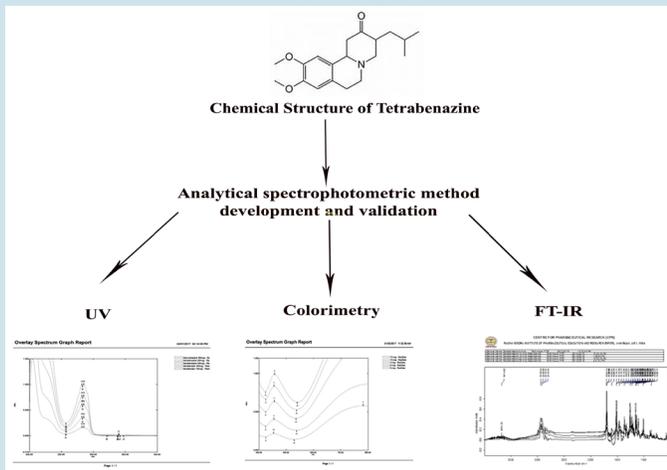
## ABBREVIATION USED

%; Percentage; %W/V: Percentage weight by volume;  $\mu\text{g}$ : Microgram; ANOVA: Analysis of variance; **cm**: Centimeter; **Df**: Degree of freedom; **FT-IR**: Fourier Transform Infrared Spectrometer; **GC**: Gas chromatography; **h**: Hour; **HPLC**: High pressure liquid chromatography; **ICH**: International Council on Harmonisation; **KBr**: Potassium bromide; **LC/MS**: Liquid chromatography-mass spectrometry; **M**: Molarity; **mg**: Milligram; **min**: Minute; **mL**: Millilitre; **MS**: Mean of Squares; **nm**: Nanometer;  $^{\circ}\text{C}$ : Degree centigrade; **RSD**: Relative standard deviation; **SD**: Standard deviation; **SS**: Sum of squares; **UPLC**: Ultra pressure liquid chromatography; **USFDA**: United states food and drug administration; **USP**: United states of Pharmacopoeia; **UV**: Ultraviolet.

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## PICTORIAL ABSTRACT



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