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METHOD DEVELOPMENT AND VALIDATION OF VALBENAZINE IN ITS BULK AND DOSAGE FORM BY HPLC

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ABSTRACT

A simple and selective HPLC method is described for the determination of Valbenazine. Chromatographic separation was achieved on a Phenomenex C18 (250×4.6 ×5µ) using mobile phase consisting Acetonitrile : Water : Triethylamine buffer (60: 40: 0.5%) v/v with detection wavelength of 264 nm. Linearity was observed in the range 50-150 µg /ml for Valbenazine (r^2 =0.999) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. The proposed method is fully validated with parameters like accuracy, precision, linearity, limit of detection, limit of quantification, robustness and ruggedness. The proposed method is stability indicating with parameters like Acid, base, peroxide, photolytic and thermal degradation.

KEYWORDS: Valbenazine, HPLC, Method, Quantification, Formulation.

1. INTRODUCTION

Valbenazine is used to treat tardive dyskinesiain adults.[1] Tardive dyskinesia is a neurological disorder characterized by involuntary movements.[3]

Valbenazine's IPUAC name is (2R,3R,11bR)-3-Isobutyl-9,10-dimethoxy-1,3,4,6,7,11b-hexahydro-2H-pyrido[2,1-α] Isoquinolin-2-yl-L-Valine and its molecular formula is $C_{24}H_{38}N_2O_8$ and molecular weight is 418.58. Its chemical structure is shown in the Fig.1.

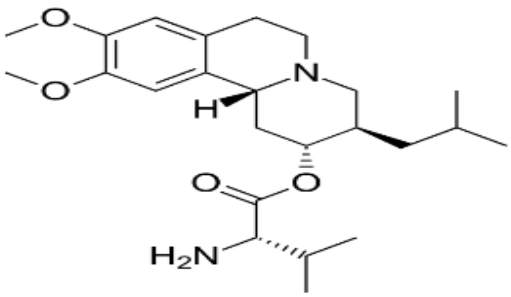

Fig. 1: Chemical structure of Valbenazine.

Valbenazine is vesicular monoamine transporter-2 inhibitor.[2] It decreases the availability of monoamine neurotransmitters by preventing their storage in synaptic vesicles. In vitro, valbenazine shows great selectivity for VMAT2 and little to no affinity for VMAT1 or other monoamine receptors.[4] This is believed to be the reason behind its therapeutic effect in tardive dyskinesia although the exact mechanism is unknown. Valbenazine is >99% bound to plasma proteins.

2. NEED FOR THE STUDY

Literature review indicates, no method is reported for quantification of bulk drug and its capsule formation till date. Only few *LC-MS*/MS methods were reported**.**

3. MATERIALS AND METHODS

Table 1: Instruments used.

UV-Visible Spectrophotometer	**Nicolet evolution 100**
UV-Visible Spectrophotometer software	Vision Pro
HPLC software	Spin chrome (LC SOLUTIONS)
HPLC	Shimadzu (LC 20 AT VP)
Ultra sonicator	Citizen, Digital Ultrasonic Cleaner
pH meter	Global digital
Electronic balance	Shimadzu
Syringe	Hamilton
HPLC Column	Inertsil ODS 3V(250x4.6mm) 5µm

Table 2: Reagents and Chemicals used.

Potassium Dihydrogenortho phosphate Dipotassium hydrogen orthophosphate	**Rankem/ AR Grade**
Acetonitrile	Merck/ HPLC Grade
Water	Merck/ HPLC Grade
Methanol	Merck/ HPLC Grade
O-Phosphoric acid	Rankem/ AR Grade

Table 3: Drugs and formulation.

Valbenazine	**Gift samples obtained from Madras pharmaceuticals, Chennai**
Ingrezza 10 mg capsules	Obtained from local pharmacy

METHODS

Preparation of Mobile phase

About 60 volumes of Acetonitrile, 40 volumes of Water and 0.5% Triethylamine buffer (60:40: 0.5%) were mixed and sonicated for 15 mins for degassing and the solution was filtered through 0.45 micron membrane filter.

Preparation of 0.5% Triethylamine: About 0.5 ml of Triethylamine was transferred into 100 ml volumetric flask and the volume was made up to mark with water.

Preparation of samples for Assay

Preparation of Standard solution

10 mg of Valbenazine was weighed and transferred in to 100 ml volumetric flask and dissolved in mobile phase and then make up to the mark with mobile phase and prepare 10 µg /ml of solution by diluting 1ml to 10ml with mobile phase.

Preparation of Sample solution

Sample name: Ingrezza 10 mg capsules

Weigh 20 capsules by removing the shell then crush with mortar and pestle then weigh a quantity of powder equivalent to 10mg of Valbenazine and transferred in to 100 ml volumetric flask and dissolved in mobile phase and then make up to the mark with mobile phase and prepare 10 µg /ml of solution by diluting 1ml to 10ml with mobile phase.

$$\% \text{ Assay} = \frac{AT}{AS} \times \frac{\text{WS}}{DS} \times \frac{\text{DT}}{WT} \times \frac{\text{P}}{100} \times \frac{\text{AW}}{LC} \times 100$$

Where,

AS: Average peak area due to standard preparation

AT: Peak area due to assay preparation

WS: Weight of Valbenazine in mg

WT: Weight of sample in assay preparation

DT: Dilution of assay preparation

DS: Dilution of standard preparation

P: Purity of Valbenazine

AV: Average weight of tablets in mg

LC: Labelled claim of Valbenazine in capsules

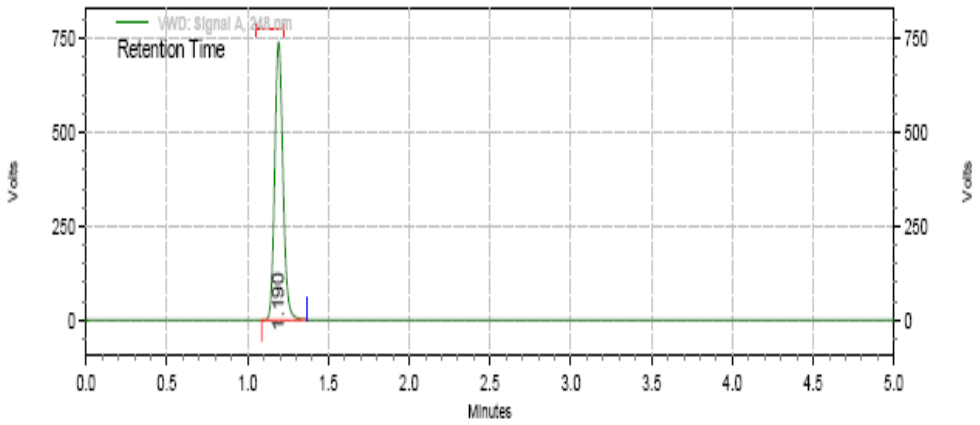

Fig. 2: Chromatogram of Assay Standard-01.

Table 4: Results for Valbenazine

Valbenazine		
	Standard Area	**Sample Area**
Injection-1	54335283	54609367
Injection-2	53884296	54791671
Injection-3	54091715	54876254
Injection-4	54660522	54122289
Injection-5	54144218	54060009
Average Area	54223207	54491918
Assay(%purity)	99.35	

Table 5: Results of assay.

Drug	**Label claim(mg)**	**Amount found(mg)**	**% Assay**
Valbenazine	10	9.85	98.5

Observation

The amount of Valbenazine present in the taken dosage form was found to be 99.35 %.

4. METHOD VALIDATION

4.1. System Suitability& System precision

To verify that the analytical system is working properly and can give accurate and precise results were evaluated by injecting six times 10µg/mL of Valbenazine and the chromatograms were recorded for the same.

Table 6: Results for system suitability of Valbenazine.

Injection	RT	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	1.189	41587410	4578	1.2
2	1.188	41585753	4582	1.1
3	1.190	41585954	4576	1.2
4	1.189	41598374	4852	1.0
5	1.187	41598854	4563	1.1
6	1.191	41588765	4577	1.3
Mean	1.189	415908512	-	-
SD	0.0014	6112	-	-
%RSD	0.11	0.01	-	-

Acceptance criteria

1. The % RSD for the retention time of Valbenazine Peaks from 6 replicate injections of each Standard solution should be not more than 2.0.
2. The % RSD for the peak area responses of Valbenazine peak from 6 replicate injections of each standard solution should be not more than 2.0%.
3. The number of theoretical plates (N) for the Valbenazine peaks is not less than 2000.
4. The Tailing factor (TP) for the Valbenazine peak is not more than 2.0.

RESULT

The plate count and tailing factor results were found to be within the limits and the % RSD Was found to be 0.1. So system is suitable and giving precise results.

4.2 Method precision

Method precision was determined by injecting sample solutions of concentration Valbenazine (10µg/mL) for six timesare prepared separately.

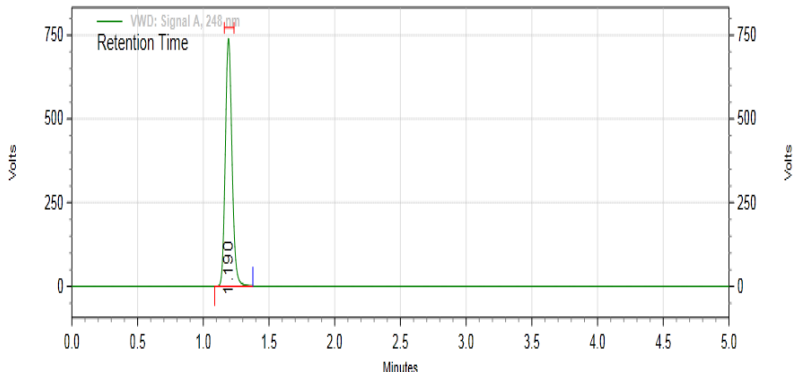

Fig. 3: Chromatogram of Method Precision-01.

Table 7: Method precision results for Valbenazine.

VALBENAZINE		
S.No.	**Rt**	**Area**
1	1.190	286.770
2	1.193	287.146
3	1.190	283.647
4	1.190	285.277
5	1.187	281.675
6	1.190	270.309
Avg	1.19	415909
Stdev	0.0019	6265
%RSD	0.16	0.015

RESULT

The % RSD of Assay for 6 Samples determinations of Valbenazine found to be within the acceptance criteria (less than 2.0%). Hence method is precise.

4.3 Linearity and range

Preparation of standard stock solution

Standard stock solutions of Valbenazine were prepared by dissolving 100 mg of Valbenazine in 100 mL of mobile phase, filter the solution using 0.45-micron syringe filter and Sonicated For 5 min, further dilutions were given in the Table.8.

Table 8: Linearity.

Preparations	**Volume from standard stock transferred in mL**	**Volume made up in mL (with mobile phase)**	**Conc. Obtained (µg/mL)**
			VALBENAZINE
Preparation 1	0.5	10	50
Preparation2	0.8	10	80
Preparation 3	1	10	100
Preparation 4	1.2	10	120
Preparation 5	1.5	10	150

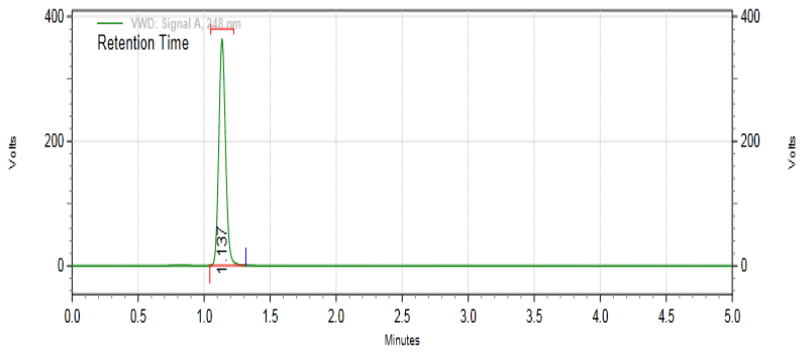

Fig. 4: Chromatogram of linearity for preparation 1.

A graph was plotted for valbenazine against the concentrations of the solutions and the peak areas (Table.9). The correlation coefficient R^2 was determined and was found to be 0.999 for Valbenazine (Fig.4).

Table 9: Linearity data of Valbenazine.

S.No	Concentration (µg/mL)	Area
1	60	21720540
2	80	34167347
3	100	44035752
4	120	52943782
5	140	67035278

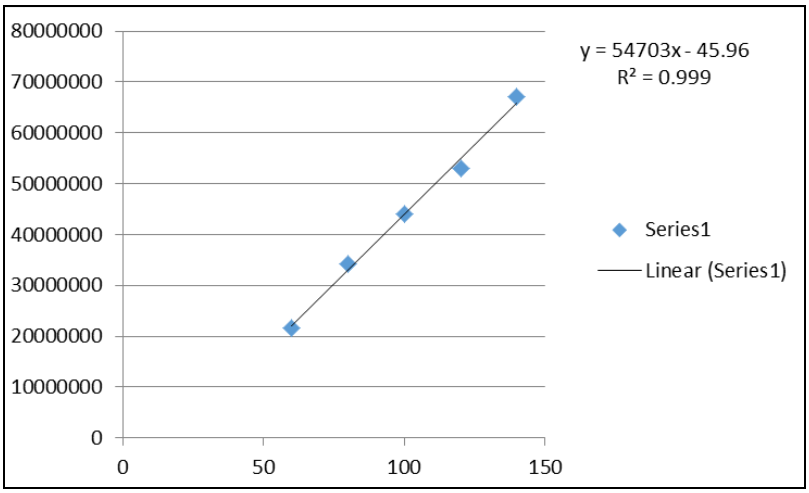

Fig.5: Graph for Linearity data of Valbenazine.

Table 10: Linearity results of Valbenazine.

S.No	Parameter	VALBENAZINE
1	Correlation coefficient	0.999
2	Slope	54703
3	Intercept	45.96

Acceptance criteria

The relationship between the concentration of Valbenazine and area of Valbenazine should be linear in the specified range and the Correlation Co efficient should not be less than 0.99.

Result

The correlation coefficient for linear curve obtained between concentrations Vs. Area for standard preparation was found to be 0.999.

4.4 Specificity

Blank solution was injected and the chromatogram was recorded for the same as given in Fig.7 Placebo solution was prepared and it was injected and the chromatogram was recorded for the same as given in Fig. 6

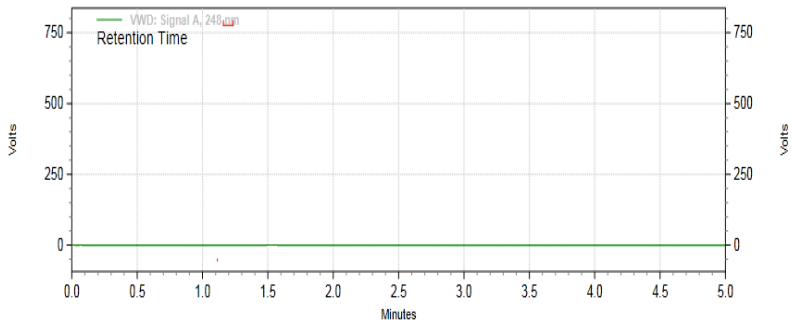

Fig. 6: Chromatogram of Placebo.

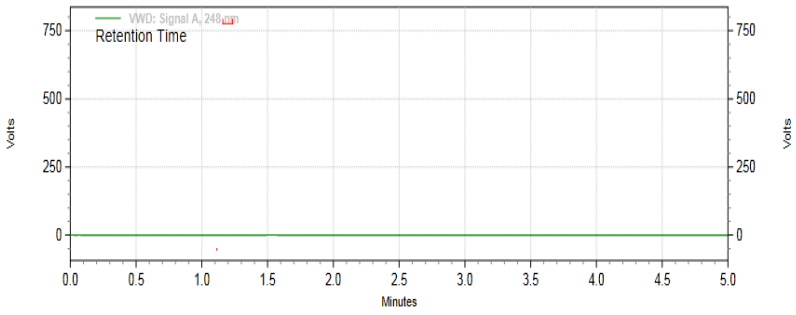

Fig. 7: Chromatogram of Blank.

Result

Chromatograms of blank and placebo solutions had shown no peaks at the retention times of Valbenazine. It was observed that diluent or excipient peaks do not interfere with the Valbenazine Peak.

4.5 Accuracy

Accuracy of the method was determined by recovery studies. To the formulation (preanalysed sample), the reference standards of the drugs **(50µg/ml, 100µg/ml and 150µg/ml)** were added at the level of 50%, 100%, 150%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shown in Table.11.

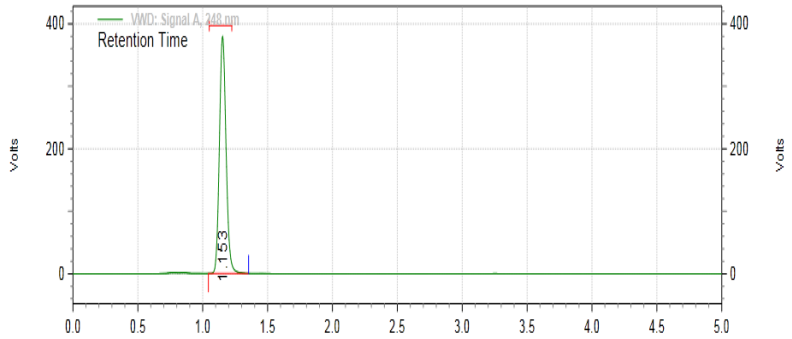

Fig. 8: Chromatogram of 50% recovery-1.

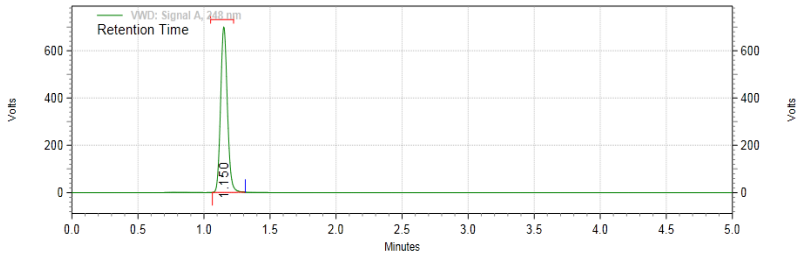

Fig. 9: Chromatogram of 100% recovery-1.

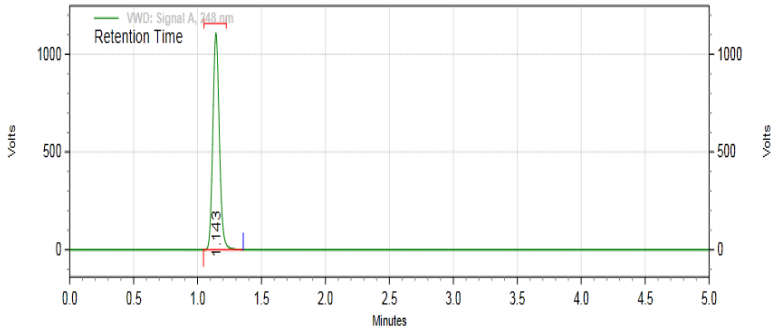

Fig. 10: Chromatogram of 150% Recovery-1.

Table 11: Results for Recovery of Valbenazine.

Recovery level	Accuracy VALBENAZINE			Average % Recovery
	Area	Average area	% Recovery	
50%	4099428	4373154	100.52	99.97
	4103816			
	4099428			
100%	4602209	4590044	99.58	
	4565673			
	4602249			
150%	4642187	4645416	99.82	
	4651875			
	4642187			

Acceptance criteria

The Average % recovery of Valbenazine should lie between 98% and 102%.

Result

The percentage mean recovery of Valbenazine was found between 99.0 to 102.0.

LIMIT OF DETECTION

$$LOD = \frac{3.3\sigma}{S}$$

$$LOD = 3.3^{*} (551277)/51766$$

$$LOD = 35.14\mu g/ml \text{ (Valbenazine)}$$

Where, σ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

Observation

The LOD for this method was found to be 35.14µg/ml (Valbenazine)

LIMIT OF QUANTIFICATION (LOQ)

$$LOQ = \frac{10\ \sigma}{S}$$

$$LOQ = 10^{*} (551277)/51766$$

$$LOQ = 106.48\mu g/ml \text{ (Valbenazine)}$$

Where, σ = the standard deviation of the respons; S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

OBSERVATION

The LOQ for this method was found to be 106.48µg/ml (Valbenazine).

4.6 Robustness

The Robustness of the method was determined. The results obtained by deliberate variation in method parameters are summarized below in Table.12.

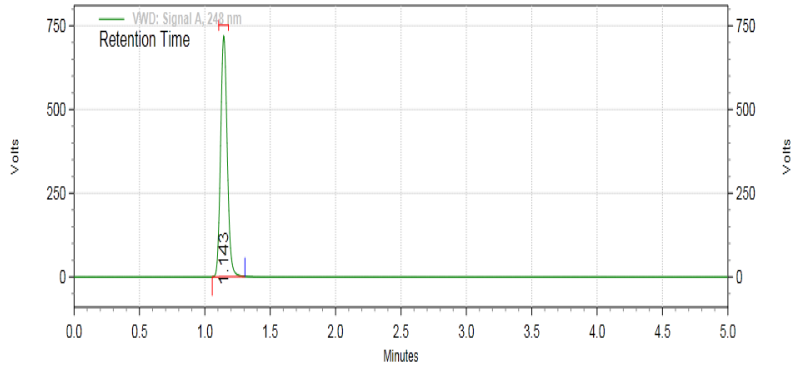

Fig. 11: Chromatogram of Temperature from 30 to 25°C.

Table 12: Results for Robustness of Valbenazine.

Chromatographic changes		Retention time(min)	Tailing Factor	Theoretical Plates
Flow rate (mL/min)	0.4	1.473	1.0	4942
	0.6	0.933	1.3	4247
Temperature (°C)	25	1.143	1.4	4467
	35	1.137	1.1	4496

Result

The tailing factor was found to be within the limits on small variation of flow rate and wavelength.

4.7 Ruggedness

The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the Assay by two different analysts.

Acceptance criteria

The % Relative standard deviation of assay values between two analysts should be not more than 2.0%.

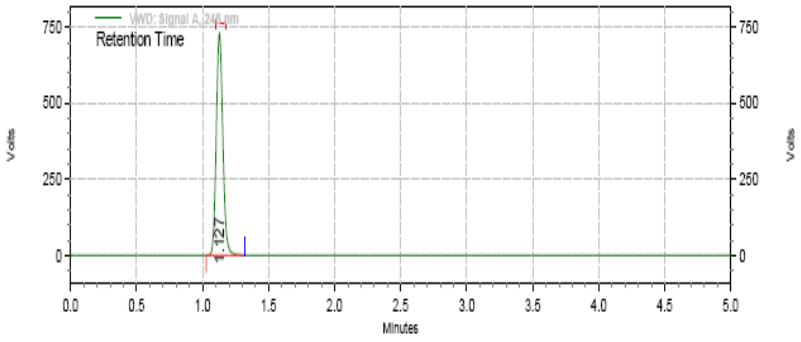

Fig. 12: Chromatogram of Analyst-1 standard.

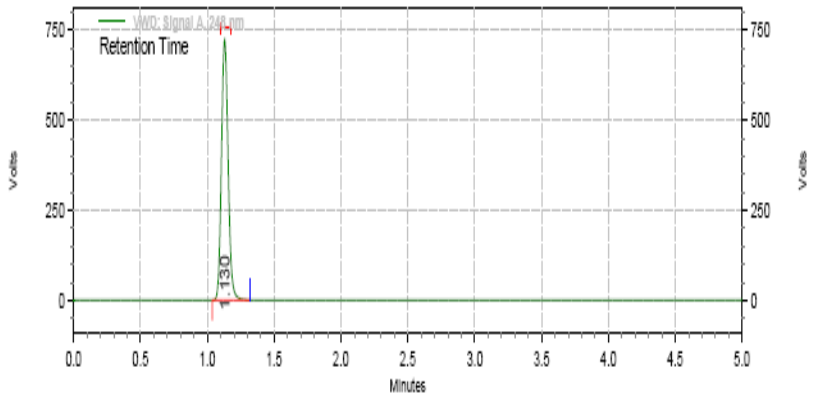

Fig. 13: Chromatogram of Analyst-1 sample.

Table 13: Ruggedness Results of Valbenazine.

VALBENAZINE	%Assay	VALBENAZINE	%Assay
Analyst 01	99.92%	**Analyst 01**	98.64%
Analyst 02	98.36%	**Analyst 02**	99.60%
%RSD	0.11	%RSD	0.28

Results

From the above results % assay and %RSD obtained acceptance criteria 2% so method is rugged

5. STABILITY STUDIES

5.1. PEROXIDE DEGRADATION

Sample solution of Valbenazine (10µg/ml) and 1 ml of 20% hydrogen peroxide (H_2O_2) was mixed. For HPLC study, 10 µl were injected into the system and the chromatogram was recorded to assess the stability of sample.

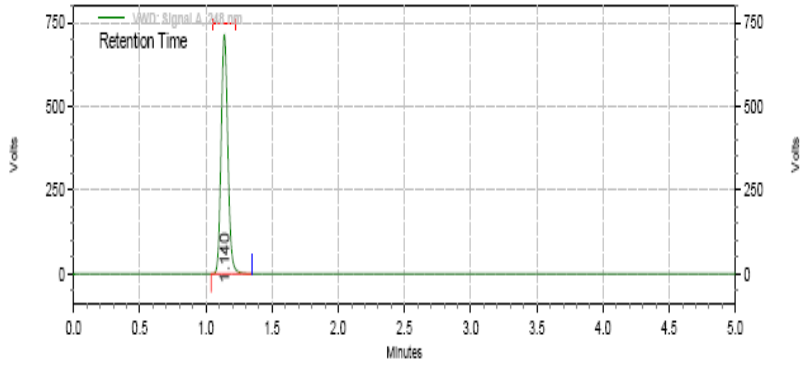

Fig. 14: Chromatogram of peroxide degradation.

5.2. PHOTOLYTIC DEGRADATION

The photochemical stability of the drug was studied by exposing the 100µg/ml solution to UV light by keeping the beaker in UV chamber for 7 days. For HPLC study, the resultant solution 10µl was injected into the system and the chromatogram were recorded to assess the stability of sample.

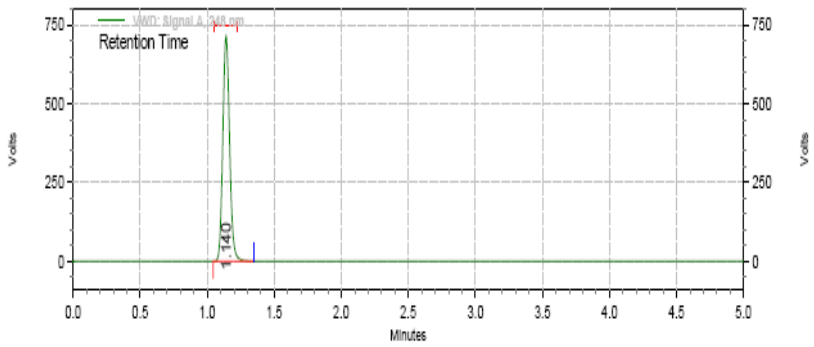

Fig. 15: Chromatogram of photolytic degradation.

5.3. ACIDIC DEGRADATION

one tablet was powdered and placed in a 50ml volumetric flask and dissolve in mobile phase up to 75% then sonicate it for 10 minutes then add 1 ml of 0.1N HCl then kept In an oven at 60^0c for 1 hour then cool and add 1 ml of 0.1N NaOH it then make up the volume up to 50ml with mobile phase, then place the sample in the vial and inject into the system to record the chromatogram.

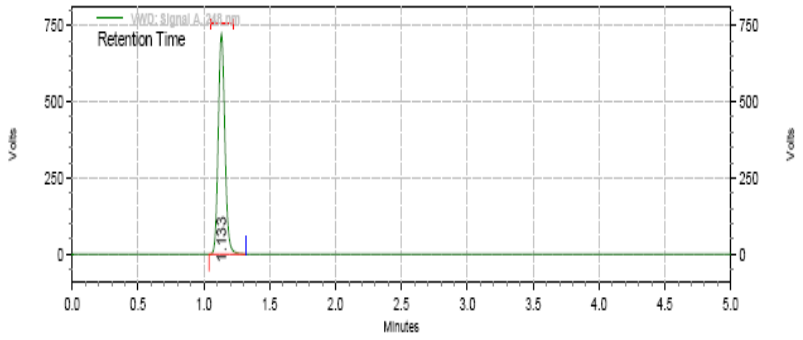

Fig. 16: Chromatogram of acidic degradation.

5.4. ALKALINE DEGRADATION

one tablet was powdered and placed in a 50ml volumetric flask and dissolve in mobile phase up to 75% then sonicate it for 10 minutes then add 1 ml of 0.1N NaOH then kept in an oven 60^0c for 1 hour then cool it and add 1 ml of 0.1N HCl then make up the volume up to 50ml

with mobile phase, then place the sample in the vial and inject into the system to record the chromatogram.

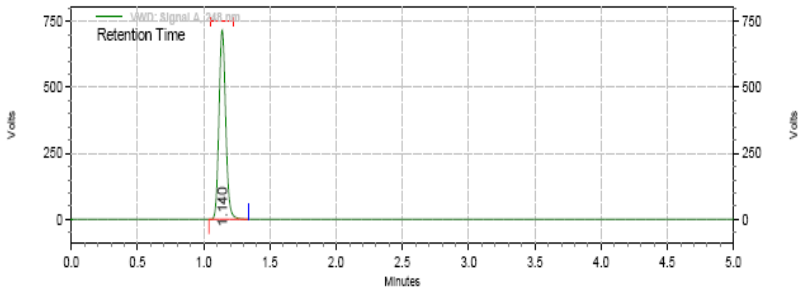

Fig. 17: Chromatogram of alkaline degradation.

5.5. THERMAL DEGRADATION

Sample solution of Valbenazine (10µg/ml) was placed in an oven at **105^{0}C** for 6 hr to study dry heat degradation. For HPLC study, the resultant solution was injected into the system and the chromatograms were recorded to assess the stability of the sample.

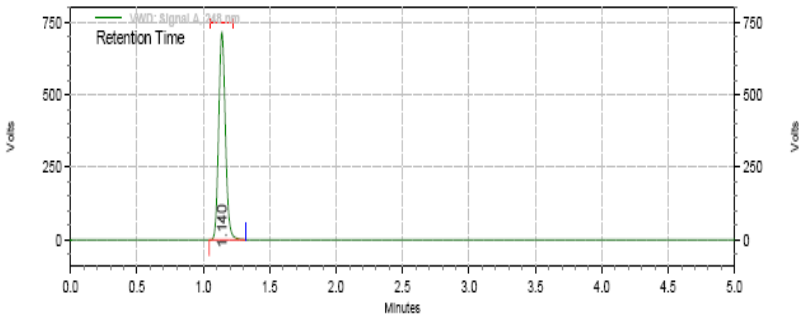

Fig. 18: Chromatogram of thermal degradation.

RESULTS

The drug was found to be degraded extensively in all conditions.

6. RESULTS AND DISCUSSIONS

6.1. Solubility Studies

These studies are carried out at 25^{0}C.

Table 14: Solubility studies.

Solvent Name	Valbenazine
Water	Sparingly Soluble
Methanol	Soluble
Acetonitrile	Soluble
Triethylamine	Soluble

6.2. Determination of Working Wavelength (λ_{max})

The wavelength of maximum absorption (λ_{max}) of the solution of the drug in mobile phase were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against mobile phase as blank. The absorption curve shows characteristic absorption maxima at 264 nm for **Valbenazine** (Fig.19), 248 nm was selected as detector wavelength for the HPLC chromatographic method.

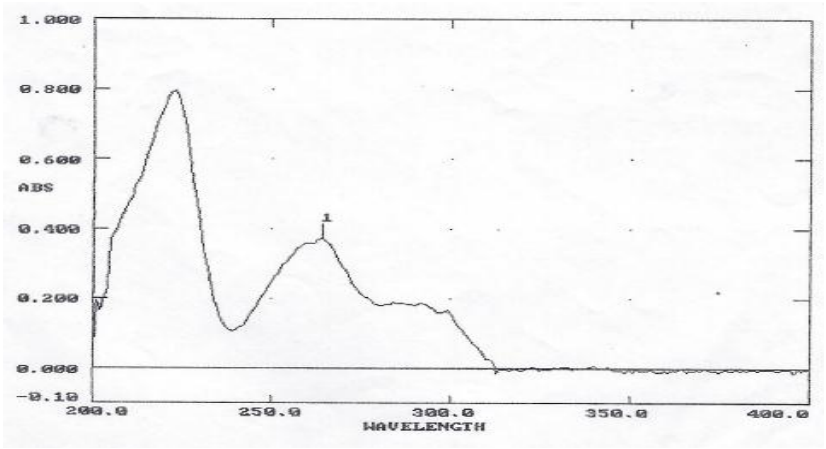

Fig. 19: UV-VIS Spectrum of Valbenazine (264 nm).

6.3 OPTIMIZED CHROMATOGRAPHIC CONDITIONS FOR ASSAY.

Table 15: Optimized condition.

Mobile phase	**Acetonitrile : Water : Triethylamine buffer (60:40: 0.5%) v/v**
Column	Phenomenex C18 (250×4.6 ×5µ)
Flow rate	1.0mL/min
Column temperature	Room Temperature (20-25°C)
Wavelength	264 nm
Injection volume	20 µL
Run time	5 min
Retention time	1.190min

7. CONCLUSION

The developed method for the estimation Valbenazine was found to be simple, precise, accurate and the high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department. The method is fully validated for all parameters and is stability indicating.

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