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Forced degradation study on valbenazine and application of validated stability-indicating HPLC method

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Abstract:

Valbenazine, is a medication used to treat tardive dyskinesia. It acts as a vesicular monoamine transporter 2 (VMAT2) inhibitor. The drug was subjected to forced degradation studies as per the conditions prescribed in ICH Q1 (R2) guideline. Valbenazine degraded in hydrolytic (acid and alkaline) and oxidative stress conditions. The drug was found to be stable in thermal and photolytic conditions. The novel degradation products were obtained and were well separated using an HPLC C18 stationary phase Inertsil ODS (150 × 4.6 mm, 5 µm) and mobile phase composed of Phosphate buffer (pH 6.8): Acetonitrile (70:30) pumped at 1.0 mL min−1 flow rate. The column temperature was set at 40°C and the detection at 240 nm using DAD detector. All the degradation products were isolated and characterised. A well resolved chromatographic method proposed in this study suggests that the proposed analytical method finds its application as a stability indicating assay method for the drug and can be used in routine analysis. The developed HPLC method will also suffice the suitability for impurity profiling and assay of Valbenazine in bulk drugs and pharmaceutical formulations.

Keywords: RP-HPLC, Valbenazine, ICH, Impurity, Forced Degradation Study.

INTRODUCTION:

The parent drug stability test guideline (Q1A) issued by International Conference on Harmonisation (ICH) requires that analytical test procedures for stability samples should be fully validated and the assays should be stability-indicating. Further, it is suggested that stress studies should be carried out to establish the inherent stability characteristics of the molecule such as the degradation pathways, leading to identification of degradation products and hence supporting the suitability of the proposed analytical procedures [1-3].

The aims of the present study accordingly were to establish inherent stability of Valbenazine through stress studies under a variety of ICH recommended test conditions. Further, it is suggested that stress studies should be carried out to establish the inherent stability characteristics of the molecule such as the degradation pathways, leading to identification of degradation products and hence supporting the suitability of the proposed analytical procedures.

Valbenazine, is a medication used to treat tardive dyskinesia. It acts as a vesicular monoamine transporter 2 (VMAT2) inhibitor. Valbenazine is a modified metabolite of tetrabenazine, and it is currently being approved for the treatment of various movement disorders, particularly tardive dyskinesia and chorea associated with Huntington's disease. Tardive dyskinesia has long been regarded as a consequence of anti-dopamine receptor therapy, and until 2008 with the advent of tetrabenazine, most treatments were ineffective. However, challenges in using tetrabenazine as a treatment of tardive dyskinesia included frequent dosing and safety and tolerability concerns. [40-45]

IUPAC nomenclature of Valbenazine is [(2R,3R,11bR)-9,10-dimethoxy-3-(2-methylpropyl)-2,3,4,6,7,11b-hexahydro-1H-benzo[a]quinolizine-2-yl](2S)-2-amino-3-methylbutanoate;4-methylbenzenesulfonic acid.

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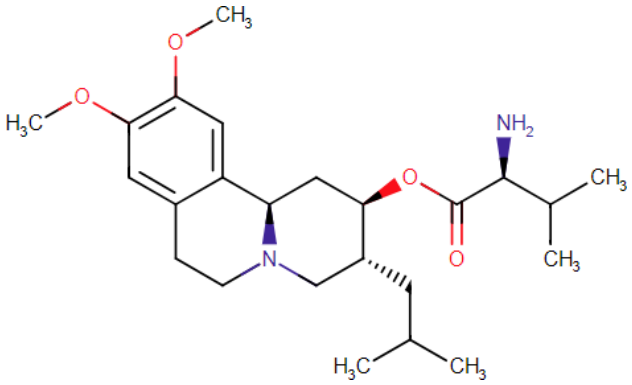

Fig-1: Chemical Structure of Valbenazine

MATERIALS AND METHODS:

Preparation of mobile phase: Accurately Weigh 1.36 g of Potassium dihydrogen ortho phosphate was transferred into 1000 ml volumetric flask. About 500 mL of double distilled water was added into the flask, dissolved the salt and finally water was added up to the mark. Then pH was adjusted to 6.80 by adding dilute sodium hydroxide solution. The mixture was sonicated for 10 minutes and then filtered through a 0.22 µm millipore filter. HPLC grade actonitrile was also filtered and degassed before use into the HPLC system.

Table-1: List of Instruments:

Sr. No.	Instruments	Make
1	HPLC	Waters e2695 separation module with PDA detector 2998
2	pH meter	Labindia
3	Analytical Weighing balance	Mettler Toledo
4	Digital ultra sonicator	Labman
5	UV – Vis Spectrophotometer	Scimadzu

Table-2: List of Chemicals:

Sr. No.	Name of Chemicals	Manufacturer/Supplier
1	Valbenazine	Jubilant generics
2	Methanol (AR grade)	Merck
3	Hydrochloric Acid (AR grade)	Rankem
4	Water (HPLC Grade)	Milli-Q
5	Acetonitrile (AR grade)	Merck
6	Sodium Hydroxide (AR grade)	Rankem
7	Potassium Dihydrogen Phosphate (AR grade)	Rankem

RP-HPLC METHOD DEVELOPMENT: [5,6, 46-49]

Standard preparation:

10 mg Valbenazine was weighed and transferred into 100 mL volumetric flask containing about 70 mL of Diluent (Water: Methanol 30:70) . The solution was sonicated for 15 min to dissolve the drug completely and the volume made up with mobile phase to get the concentration of Valbenazine of 100 mg/mL solution. Further dilution was carried to get concentration of 20, 40, 60, 80, and 100 µg/mL of Valbenazine.

Chromatographic conditions:

For quantitative analysis of Valbenazine by RP-HPLC method, the mobile phase was comprised of Potassium dihydrogen ortho phosphate (pH 6.80) and acetonitrile in the ratio of 70:30 (v/v) at a flow rate of 1.0 mL/min. The injection volume was 10 µL for standard solutions. The run time was set for 15 min. Before analysis, every standard was filtered through 0.45 µm filter tips. The mobile phase was also filtered, sonicated and degassed before use. The column eluate was monitored with a PDA detector at 240 nm. All analyses were done at ambient temperature under isocratic condition.

Procedure:
Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

METHOD VALIDATION: [4,5, 26-34]

The methods were validated for different parameters like linearity, accuracy, precision, robustness, LOD, LOQ, etc.

Linearity:
The linearity of the developed method was performed with a concentration range of 20, 40, 60, 80 and 100 µg/mL by injecting repeated thrice times. The average peak areas were plotted against respective concentration. The linearity of the proposed method was evaluated by using calibration curves to calculate coefficient of correlation and intercept values.

Accuracy:
The accuracy of the method was evaluated by determination of recovery of Valbenazine at three levels of concentrations at three times. The standard solutions were corresponding to 80%, 100% and 120% of nominal analytical concentrations.

Precision:
The precision of the method was demonstrated by intra-day and repeatability variation studies. In intra-day precision was established by analyzing three replicates over six concentrations (10µg/mL) of Valbenazine. Inter-day precision was carried out by six concentrations with three replicates for consecutive 3 days. The precision was expressed as %RSD amongst responses using the formula [%RSD = (standard deviation/mean) x 100 %].

Robustness:
Robustness of the proposed method was determined by small deliberate changes in flow rate (0.8, 1, 1.2 mL/min), change in organic composition of mobile phase ratio (±2%).

LOD and LOQ:
The limit of detection (LOD) and limit of quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions for six times. LOD was calculated as 3.3*σ/S and LOQ was calculated as 10*σ/S.

System suitability:
A standard solution of Valbenazine was prepared as per procedure and was injected six times into the HPLC system. The system suitability parameters were evaluated from standard chromatograms obtained by calculating the %RSD of retention times, tailing factor, theoretical plates and peak area from six replicate injections.

FORCED DEGRADATION STUDY OF VALBENAZINE: [5,7, 21-29]
Forced degradation of the drug was carried out as per the ICH guideline. The forced degradation study of Valbenazine was performed in acidic, alkaline and oxidant media, under UV radiation and thermal conditions.

Acidic degradation:
A 50 mL volumetric flask containing 50 mg of Valbenazine and 5 ml of 0.1M HCl was used to conduct the experiment. The mixture was neutralized with 5 ml of 0.1M NaOH after being held at room temperature for 24 hrs and the chromatogram was recorded.

Alkaline degradation:
In a 50 mL volumetric flask, 5 ml of 1M NaOH was added to 50 mg of Valbenazine. After being kept for 30 min, the mixture was neutralized with 5 mL of 1M HCl, and the chromatogram was taken.

Oxidative degradation:
By adding 5 mL of 30% H_2O_2 to 50 mg of Valbenazine in a 50 mL volumetric flask, oxidative degradation was carried out. The chromatogram was then recorded while the mixture was maintained at ambient temperature for 30 min.

Photolytic degradation:
Valbenazine was photolytically degraded by being exposed to 1.2 million Lux hours and chromatogram was recorded.

Thermal degradation:
Placed sufficient amount of Valbenazine in petri dish and covered with aluminium foil and made holes on aluminium foil with pointed object. Kept it in hot air oven at 80º C for 48 hrs. After 48 hours sample was taken out and kept in desiccator to reach at room temperature subjected Valbenazine prepared as per sample preparation method.

RESULT AND DISCUSSION:

Optimization of Mobile Phase:
The goal of method development was to produce sharp peaks for the active pharmaceutical ingredient with a resolution of more than two and a less asymmetric factor. Various mobile phases (water, methanol, acetonitrile, buffers of various pH ranges) were attempted in order to generate a distinct and well-defined drug peak. So the mobile phase ratio of Phosphate buffer (pH 6.60): Acetonitrile (70:30) which showed good chromatography and this ratio was finalized for the entire study.

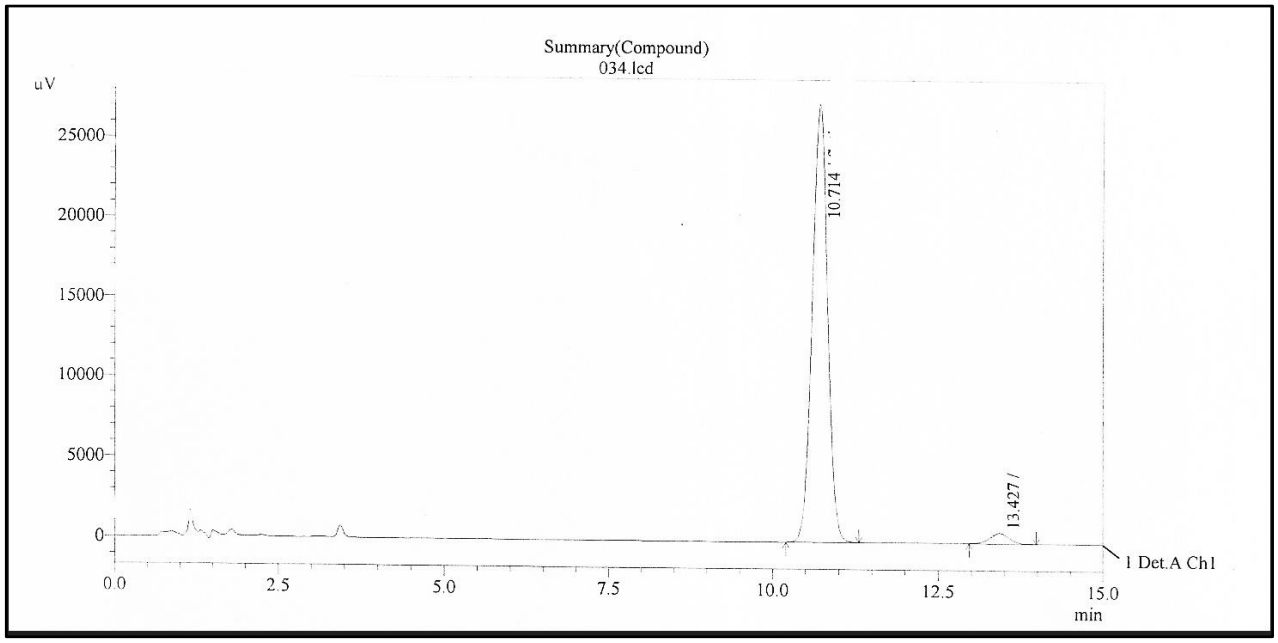

Fig-2: Optimized chromatogram of Valbenazine comprising of Phosphate buffer (pH 6.80): Acetonitrile (70:30) as a mobile phase.

System suitability:
For the system suitability % RSD for the area of 6 replicates of standard solution was found to be NMT 2.0, theoretical plates were found to be NLT 2000 and Asymmetry was found to be NMT 2.0. Hence the system suitability passes all the criteria.

Table-3: System suitability of Valbenazine

Replicates	Area	USP Tailing	USP Plates	RT	Mean	SD	%RSD
Replicate 1	61580924	1.08	15064	10.723	61574913	50079.45	0.08
Replicate 2	61631780	1.08	15075	10.71			
Replicate 3	61510307	1.07	15059	10.699			
Replicate 4	61567610	1.08	15078	10.719			
Replicate 5	61629438	1.08	15066	10.72			
Replicate 6	61529421	1.07	15055	10.678			

Linearity:
The calibration curves were obtained by plotting the peak area versus concentration over the range of 20 – 100 µg/mL with a correlation coefficient of 0.998.

Table-4: Linearity of Valbenazine

Level	Conc (µg/mL)	Area	Mean	STD DEV	% RSD
80	80	49499521	49478951	20659.579	0.042
		49458203			
		49479130			
90	90	55563330	55565902	25982.128	0.047
		55593074			
		55541301			
100	100	61594666	61597748	34888.248	0.054
		61634075			
		61564503			
110	110	67586326	67548517	38119.701	0.056
		67549130			
		67510094			
120	120	73617601	73614880	37451.707	0.051
		73650897			
		73576142			

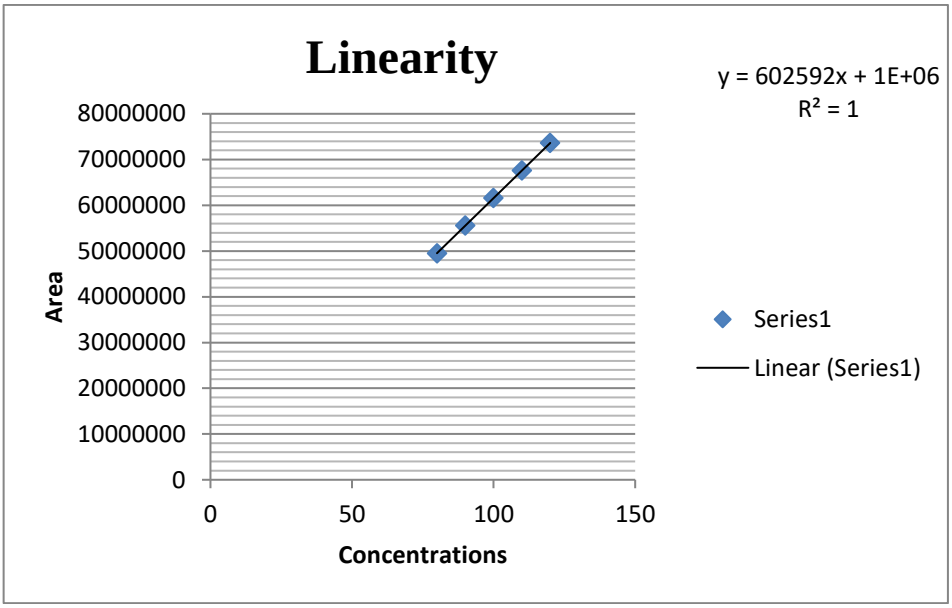

Fig-3: Calibration curve of Valbenazine

Accuracy:

Accuracy at each levels 80 %, 100 % and 120 % was prepared in triplicate and recovered concentration verses the added concentration was determined and % recovery was found within the acceptable range of 98.0 % to 102.0 %.

Table-5: Accuracy study of Valbenazine

% Accuracy	Area	Recovered Conc.	Added conc.	% Recovery	Mean Recovery
80	49461281	80.314	81	99.15	99.15
	49510734	80.395	80.5	99.87	
	49405095	80.224	81.5	98.43	
100	61609704	100.042	100.5	99.54	99.49
	61947130	100.59	100.5	100.09	
	61486034	99.841	101	98.85	
120	73593905	119.502	121	98.76	99.91
	73571910	119.466	121	98.73	
	73651765	119.596	120.5	99.25	

Precision:
Precision for six tests (About 100 µg/mL of Valbenazine) was performed and % assay for individual as well as for mean assay value was found within the acceptable criteria 98.0 to 102.0% and % RSD for 6 samples was also NMT 2.0. Precision passes the criteria, no variation found by preparing six different samples. Results are good and reproducible.

Table-6: Precision of Valbenazine

Intra Day Precision		
Sample	**Area**	**% Assay**
Sample 1	61681761	99.17
sample2	61904728	100.52
sample 3	61604785	98.56
sample 4	61527907	98.92
sample 5	61547813	99.44
sample 6	61990378	100.66
	Mean	99.55
	STD. Dev.	0.861
	% RSD	0.865
Repeatability		
Sample	**Area**	**% Assay**
Sample 1	61760794	98.8
sample2	61248281	98.96
sample 3	61970431	100.63
sample 4	61938704	98.6
sample 5	61560779	99.46
sample 6	61583710	99.01
	Mean	99.24
	STD. Dev.	0.736876
	% RSD	0.743

Robustness:

Table-7: Robustness study of Valbenazine

Change in Flow Rate								
Condition	**Parameters**	**Area**	**USP Plates**	**Change**	**RT**	**Mean**	**SD**	**%RSD**
Flow Rate	High	61594666	15064	1.2 ml	10.723	61594354	336.76	0.00
	High	61593997	15060		10.719			
	High	61594399	15070		10.721			
	Low	60664159	15044	0.8 ml	10.992	60664082	81.25	0.00
	Low	60663997	14997		10.979			
	Low	60664089	15065		10.997			
Change in Composition								
Condition	**Parameters**	**Area**	**USP Plates**	**Change**	**RT**	**Mean**	**SD**	**%RSD**
Composition	High	61585997	15051	Buffer:ACN	10.755	61585991	4.93	0.00
	High	61585988	15061		10.749			
	High	61585989	15064		10.751			
	Low	60564589	15055	Buffer:ACN	10.619	60564557	51.16	0.00
	Low	60564498	15044		10.62			
	Low	60564584	15050		10.622			

Chromatography was not compromised by changes in the wavelength, changes in the flow rate of mobile phase and changes in the column oven temperature and system suitability was found within acceptable range at each levels. So we can conclude that the method is robust.

Forced Degradation Study:
Acid hydrolysis:

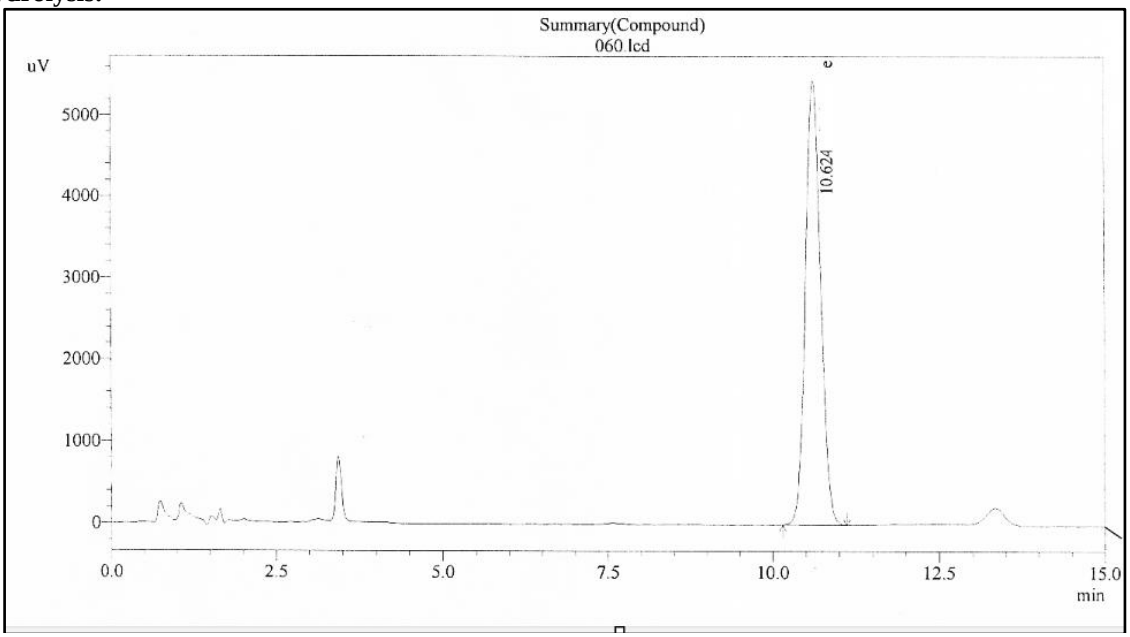

Fig-4: Chromatogram of Acid degradation for 0.1 N HCl for 24 hrs.

Chromatogram of acid hydrolysis (0.1 N HCl) performed at room temperature for 24 hrs showed 14.79 % of degradation with two degradation peaks at R.T. 2.455 for the first degradation peak and 1.974 for the second degradation peak.

Base hydrolysis:

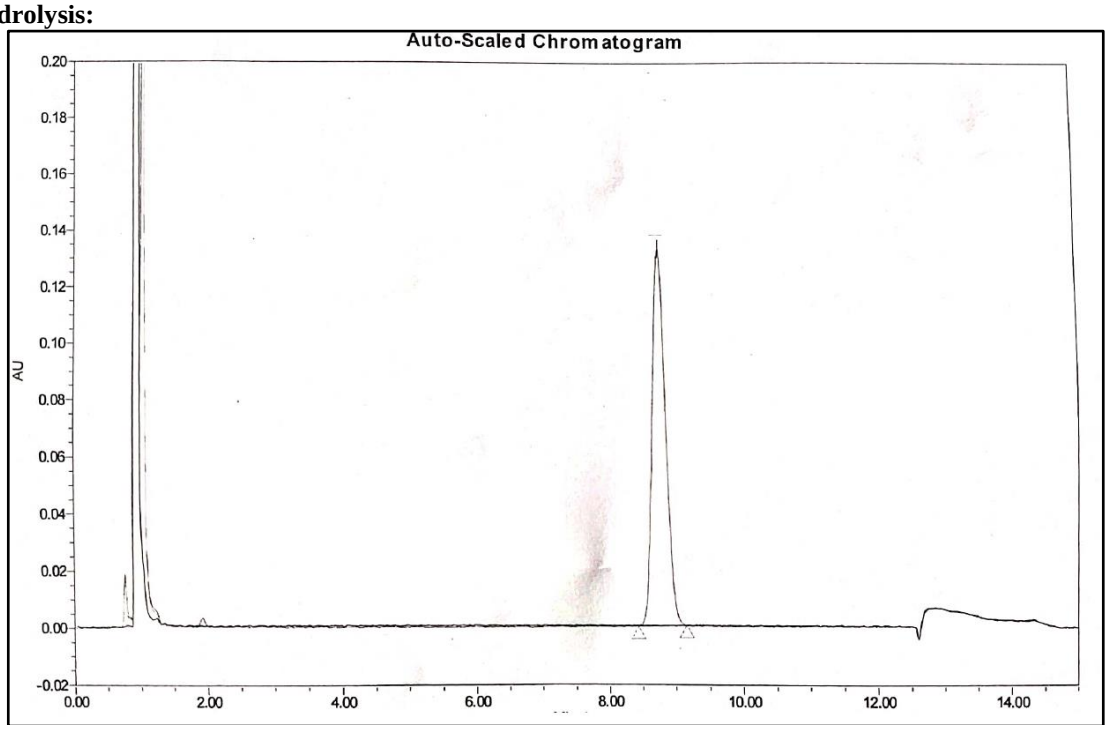

Fig-5: Chromatogram of base degradation of 1 N NaOH for 30 minutes.

Chromatogram of a base hydrolysis by using 1 N NaOH at room temperature for 30 minutes showed 9.16 % of degradation with degradation peak at R.T. 3.299 for the first degradation peak.

Oxidative degradation:

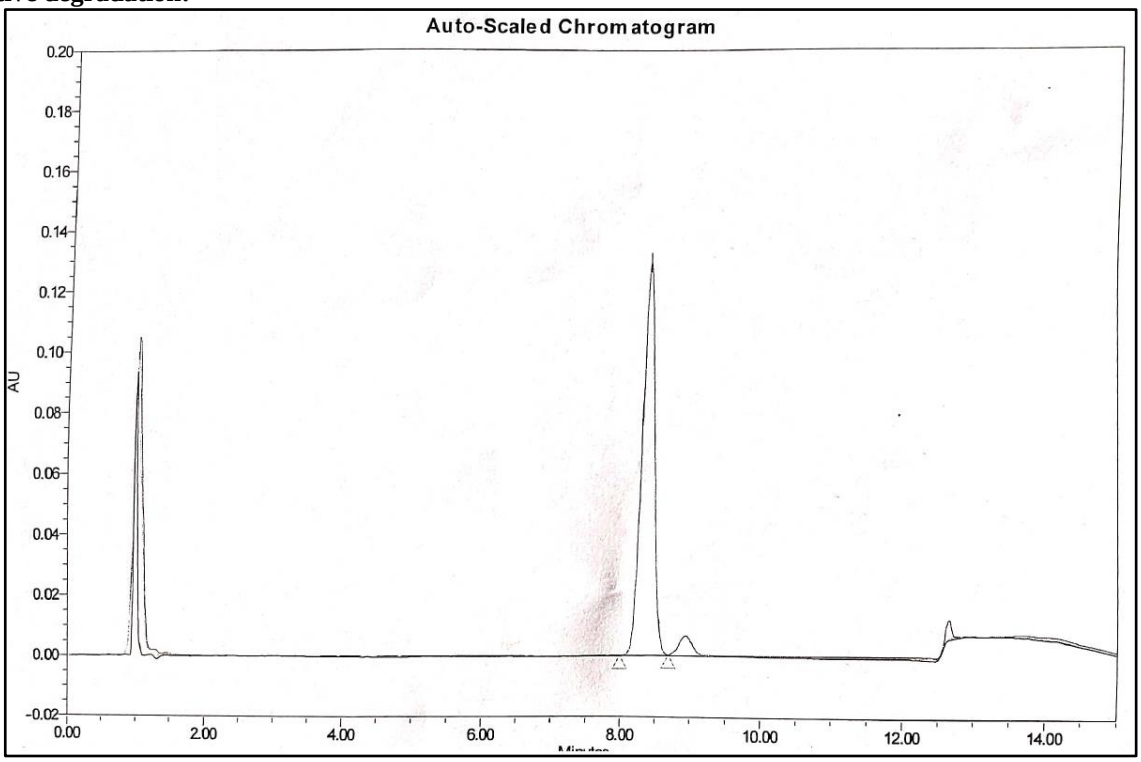

Fig-6: Chromatogram of peroxide degradation of 30% Hydrogen peroxide for 30 minutes.

Valbenazine showed 13.25 % degradation for peroxide stress study by using 30% H_2O_2 for 30 minutes at room temperature. For peroxide stress study degradation product was obtained with R.T. of 2.302.
The drug was found to be stable for thermal and photolytic degradation conditions.

SUMMARY OF STABILITY DATA:

Table-8: Summary of Forced Degradation Study of Valbenazine

Sample Name	Treatment	Exposure condition	% Assay	% Degradation
API	Valbenazine	NA	100.00	NA
	Thermal	60°C for 72 Hours	100.00	NA
	Photolytic	Sunlight for 7 Days	100.00	NA
	Acid	0.1N HCl for 24 hrs at R.T.	85.21	14.79
	Base	1N NaOH for 30 minutes at R.T.	90.84	9.16
	Peroxide	301% H_2O_2 for 30 minutes at R.T.	86.75	13.25

Conclusion:

Forced degradation of Valbenazine in various conditions like alkaline, acidic, oxidation, UV radiation and thermal degradation was observed in this investigation. The content of degradation of the drug was quantitatively analyzed by RP-HPLC. For this purpose a new RP-HPLC method was developed and validated that was also mentioned in this paper. It was found that degradation in acidic condition was greater than in alkaline and peroxide condition. In oxidation degradation of Valbenazine was significant. The drug was not degraded under exposing UV radiation and in the thermal condition.

Funding

None

Conflict of Interest

None

REFERENCES:

1. ICH Q1A (R2) Stability Testing of New Drug Substances and Products.
2. ICH Q2 (R1) Validation of Analytical Procedures
3. ICH Q3A (R2) Impurities in New Drug Substances
4. Directive 2004/27/EC of the European parliament and of the Council of 31 March 2004 amending, Directive 2001/83/EC on the Community code relating to medicinal products for human use(2004).
5. Aher P, Surana K, Ahire E, Patil D, Sonawane D, Mahajan S. Development and Validation of RP-HPLC Method for Quantitative Determination of 4-Amino Benzene Sulphonamide in Sulphonamide Hydrochloride. Trends in Sciences. 2023 Mar 15;20(6):5209-.
6. Ramakrishna Y, Chokshi A and Vijaykumar P, Development and validation of spectrophotometric methods for simultaneous estimation of Levosulpiride and Pantoprazole sodium, Int J pharma Front Research, 3(1), 54(2013).
7. Nandakishore A and Jagadeesh B, Development and validation of stability indicating RP-HPLC Method for simultaneous estimation of Levosulpiride and Rabeprazole Sodium, Int J phrma Bio Sci, 3(4), 718(2012).
8. Pani L, Gessa G L, The substituted benzamide and their clinical potential on dysthymia and on the negative symptoms of schizophrenia, Molecular Psychiatry, 7 (3), 247(2002).
9. Nuss P, Hummer M, Tessier C, The use of Amisulpiride in the treatment ofacute psychosis, Therapeutics and Clinical Risk Management, 3(1),3–11(2007).
10. Vieta E, Ros S, Goikolea, J M, Benabarre A, Popova E, Comes M, Capapey J, Sánchez-Moreno J, An open-label study of Amisulpiride in the treatment of mania, Journal of Clinical Psychiatry, 66 (5), 575(2005).
11. Thomas P, Vieta E, Amisulpride plus valproate vs haloperidol plus valproate in the treatment of acute mania of bipolar I patients, a multicenter, open-label, randomized, comparative trial, Neuropsychiatric disease and treatment, 4(3), 675(2008).
12. José Miguel Vela, Helmut Buschmann, Jörg Holenz, Antonio Párraga, Antoni Torrens, Antidepressants, Antipsychotics, Anxiolytics, From Chemistry and Pharmacology to Clinical Application(2007).
13. Köhler C, Hall H, Magnusson O, Lewander T, Gustafsson K, Biochemical pharmacology of the atypical neuroleptic Remoxipride, Acta Psychiatrica Scandinavia Supplement, 358, 27(1990).
14. Conley R, Dixon L, An Nguyen R, Taminga C, Raymond R, Remoxipride therapy in poorly responsive schizophrenics, Schizophrenia Research, 4 (3), 316(1993).
15. Vieta E, Mullen J, Brecher M, Paulsson B and Jones M, Curr Med Res Opin 21,923(2005).
16. Calabrese J.R, Keck P E, Macfadden W, Minkwitz M, Ketter T A.and Weisler R H, AmJ, Psychiatry,162, 1341(2005).
17. Bharathi Ch, Prabhakar K J, Prasad Ch S, Srinivasa Rao M, Trinadhachary G N,Handa V K, Dandala R and Naidu A ,Pharmazise, 63**,**14(2008).
18. Stolarczyk E U, Kaczmarek K, Kamil Eksanow, Kubiszewski M, Glice M, and Kutner A, Pharmaceutical Development and Technology , 14**,** 27**(**2009).
19. Belal F, Elbrashy A, Eid M and Nasar J J, Journal of Liquid Chromatography& Related Technologies, 31, 1283(2008).
20. Vasanta M, Lakshmanarao V and Suryanarayana M V, Identification, Isolation, Synthesis and Characterization of Principal Oxidation Impurities of Quetiapine. RASAYAN J.Chem.Vol, 3(4), 677 (2010).
21. Zbieta E l, Stolarczyk U, and Andrrzej Kutner, Use of Hyphenated LC-Ms/Ms Technique For Characterization of Impurity Profile of Quetiapine During Drug Development, Acta Poloniae Pharmaceutica- Drug Research, 67 (6), 599(2010).
22. Abou-Setta A M, Mousavi S S, Spooner C, Schouten J R, Pasichnyk D, Armijo OlivoS, Beaith A, Seida J C, Dursun S, Newton A S, Hartling, (https://www.ncbi.nlm.nih.gov/pubmed/23035275)(2012).
23. Leucht S, Cipriani A, Spineli L, Comparative efficacy and tolerability of 15 antipsychotic drugs in schizophrenia, a multiple treatments Meta analysis, (https://www.ncbi.nlm.nih.gov/pubmed/23810019)(2013).
24. Osser D N, Roudsari M J, Manschreck T, The psychopharmacology algorithm project atthe Harvard South Shore Program: an update on schizophrenia, Harv Rev Psychiatry 21 (1), 18(2013).
25. Poornachander T, Ramesh Kumar, Chandrasekhar B, Ramesh Ch, Pratap R, Anjaneyulu Y, and Satyanarayana B, Synthesis and characterization of impurities of an anti- psychotic drug substance Olanzapine, ARKAT USA, Inc, 195(2008).
26. Komossa K, Rummel-Kluge C, Schwarz S, Schmid F, Hunger H, Kissling W, Leucht S,Risperidone versus other atypical antipsychotics for schizophrenia, The Cochrane database of systematic reviews (1) (2011).
27. Risperidone (http://www.drugs.com/monograph/risperidone.html), The American Society of Health System Pharmacists (2011).
28. Lokhande R S, Jadhav P V, Singare P U, Development and validation of Reverse phaseHigh performance Liquid Chromatographic method for simultaneously Determination for 9- Hydroxy Risperidone, p-Fluoro Risperidone,

Unsaturated Risperidone and 6- Methyl Risperidone in Risperidone Bulk Drug, International Journal of Chemtech Applications, 2 (2), 66(2013).
29. Sattanathan P, Mosses Babu J, Vyas K, Reddy R B, Rajan S T, Sudhakar P, Structural Studiies of Impurities of Risperidone by Hyphenated Technique,
30. Bharathi Ch, Krishnamachary D, SaravanaKumar M, RamaShankar, Handa VK, Ramesh, Naidu A, Identification, Isolation and Characterization of Potential Degradation Products in Risperidone, Journal of Pharmaceutical and Biomedical Analysis, 165(2008).
31. Kane J, Honigfeld G, Singer J, Meltzer H, Clozapine for the treatment resistant schizophrenic: a double-blind comparison versus chlorpromazine/benztropine, Archivesof General Psychiatry 45 (9), 789(1988).
32. National Institute of Mental Health, What medications are used to treat schizophrenia (http://www.nimh.nih.gov/health/publications/mentalhealthmedi-cations /whatmedicationsareusedtotreatschizophrenia.shtml) (2012).
33. Lee M, Dickson R A, Campbell M, Oliphant J, Gretton H, Dalby J T, Clozapine and substance abuse in patients with schizophrenia, Canadian Journal of Psychiatry, 43, 855(1998).
34. Nagaraju G, Madhava Reddy B, Jitan A V, Synthesis and evaluation of Clozapine and its Related Compounds, International Journal of Pharmaceutical Science and NanoTechnology, 2 (4)(2010).
35. Rogowski M, Chodynicki S, Use of the preparation Cafaminol in the treatment of acute rhinitis, Wiadomości Lekarskie (Warsaw, Poland), 38 (20), 1437(1985).
36. Swiss Pharmaceutical Society Index Nominum, International Drug Directory, Medpharm Scientific Publishers(2000).
37. Berecek Kh B M, Brody M J, Evidence for a neurotransmitter role for epinephrine derived from the adrenal medulla, Am J Physiol, 242 (4), 593(1982).
38. Epinephrine and nor epinephrine, Encylopedia Britannica, Deluxe Edition, Chicago(2009).
39. Editorial, Stress, hypertension, and the heart: the adrenaline trilogy, Lancet 2, 1440(1982).
40. Pearce, J M S Links between nerves and glands, the story of adrenaline, Advances in Clinical Neuroscience & Rehabilitation, 9, 22 (2009).
41. Celander O, The range of control exercised by the sympathico-adrenal system, Acta Physiol Scand 32**,** 16(1954).
42. Lorino A M, Lofaso F, Dahan E, Coste A. Harf A, Lorino H, Combined effects of a mechanical nasal dilator and a topical decongestant on nasal airflow resistance, Chest 115 (6), 1514 (1999).
43. Morton Ian, Morton I K, Judith M Hall and Concise, Dictionary of Pharmacological Agents, Properties and Synonyms. Springer Science &Business Media(1999).
44. Whittington RM Mephentermine Sulphate as a Hypertensive Agent in General Practice, 6 (2), 336 (1963).
45. Zumpft C W, Double-blind comparison of Metizoline hydrochloride (Ellsyl) and phenylephrine in allergic and vasomotor rhinitis, Current therapeutic research, clinical and experimental 17 (1), 40(1975).
46. Delicado E G, Fideu M D, Miras-Portugal M T, Pourrias B, Aunis D, Effect of Tuamine, heptaminol and two analogues on uptake and release of catecholamines in cultured chromaffin cells, Biochemical Pharmacology 40(4), 821(1990).
47. Raoux M, Colomban C, Delmas P, Crest M, The amine-containing cutaneous irritant heptylamine inhibits the volume-regulated anion channel and mobilizes intracellular calcium in normal human epidermal keratinocytes, Molecular Pharmacology, 71(6),1685(2007).
48. Amoxapine. Martindale, the Complete Drug Reference (London, UK: Pharmaceutical Press)(2013).
49. Ban T A, Fujimori M, Petrie W M, Ragheb M, Wilson W H, Systematic studies with Amoxapine, a new antidepressant, International Journal of Pharmaco- psychiatry 17 (1),18(1982).
50. Product Information, Asendin(R), Amoxapine tablets, Physicians Desk Reference (electronic version), MICROMEDEX, Inc, Englewood, CO, USA(1999).