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LC – MS/MS Characterization of Forced Degradation Products of Tetrabenazine

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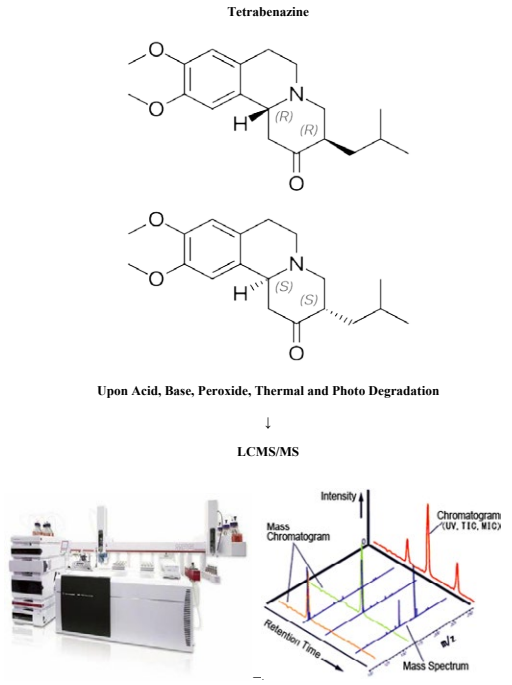
Abstract

A rapid, precise and reliable LC-MS/MS method has been developed for the identification and characterization of stressed degradation products of Tetrabenazine. Tetrabenazine is a drug, for the symptomatic treatment of hyperkinetic movement disorder. Tetrabenazine works mainly as a VMAT inhibitor. It promotes the early metabolic degradation of monoamines, in particular the neurotransmitter dopamine. Tetrabenazine was subjected to hydrolysis acidic, alkaline, neutral peroxidation, light and thermal stress conditions as per ICH-specified conditions. The drug showed degradation under peroxidation, thermal, acid and base hydrolysis stress conditions. However, it was stable to neutral stress conditions and light degradation. A total of 4 degradation products were observed and the chromatographic separation of the drug and its degradation products were achieved on Inertsil ODS-3V 150 mm × 4.6 mm, i.d., 5 µm column using 0.01 M ammonium acetate and ACN in the ratio of 640:360 as mobile phase-A and 900:100 ratio of ACN:Water as mobile phase –B. The degradation products were characterized by LC–MS/MS and its fragmentation pathways were proposed. Probable possible structures were drawn based on parent and daughter molecular ions.



Keywords: Tetrabenazine; Chromatographic separation; Characterization; Degradation; LC-MS/MS

Introduction

Tetrabenazine TBZ [1-4] a catecholamine-depleting agent initially developed for the treatment of schizophrenia, when tested for other indications, has proven to be more useful for the treatment of a variety of hyperkinetic movement disorders. Its chemical name is SS, RR-3- isobutyl-9,10-dimethoxy-1,3,4,6,7,11b-hexahydro-pyrido [2,1-a isoquinoline-2-one (Figure 1). The hyperkinetic movement disorders include neurological diseases characterized by abnormal involuntary movements such as chorea associated with Huntington's disease, tics in

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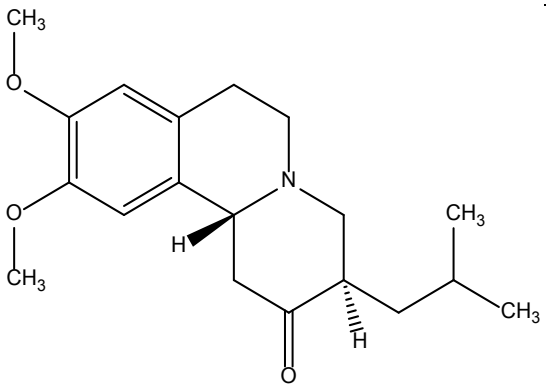


Figure 1: Chemical Structure of Tetrabenazine.

Tourette's syndrome, dyskinesias and dystonias in tardive dyskinesia, also primary dystonias and myoclonus.

It is partially polaric in nature so freely soluble in ACN: Water 60:40, v/v but partially soluble in polar solvents like, water, methanol, etc. Tetrabenazine acts mainly as a reversible high affinity inhibitor of monoamine uptake into granular vesicles of presynaptic neurons and secondary depletion at low doses, as well as a weak D_2 postsynaptic receptor blocker in high doses. TBZ depletes all three monoamines, but particularly dopamine. One in vivo study of rats showed that TBZ decreased dopamine levels by 40%, serotonin by 44%, and norepinephrine by 41% in the brain [5].

Thorough literature search reveals that many HPLC and LC–MS [6,7] methods for the determination of Tetrabenazine were reported as per ICH guidelines [8,9]. Examples of such analytical methods include spectrofluorometry [10,11], high performance liquid chromatography HPLC [12,13], UV-Visible Spectroscopy, UHPLC, UPLC-ESI-TOFMS, RP-HPLC, Gas chromatography-mass spectrometry GC-MS, Liquid chromatography-mass spectrometry LC-MS and Liquid chromatography tandem mass spectrometry LC-MS/MS [14]. Methods were available for simultaneous determination of Tetrabenazine and other pharmaceutical dosage forms and dissolution studies were also reported. Recently, liquid chromatography–mass spectrometry LC–MS/MS, evolved as versatile tool for the characterization of drug impurities, degradation products. Recent advances in Liquid chromatographic mass spectrometric detectors like ion trap, time of flight, flow injection analysis tandem mass, quadrupole time of flight resulted in successful characterization of drug substances and drug products. Recently design of experiment tools for method development on LCMS/MS for trace level identification of impurities also evolved as an efficient tool. However, so far, no study has been reported on the systematic characterization and mechanistic pathway of degradation products of Tetrabenazine under stress conditions prescribed by ICH Q1A R2. The main aim of the present study was to investigate the complete degradation behaviour of the drug and to characterize the degradation products. It was accomplished by exposing the drug to ICH-recommended stress conditions of light, thermal, hydrolysis, oxidation, acidic and basic conditions, analysing the resultant solutions to optimized LC-MS, MS/MS, MS^n and accurate mass measurements to establish the fragmentation pattern of the drug and its degradation products.

Experimental Methods

Chemicals and reagents

Tetrabenazine 99% purity samples provided by Dr. Reddy's laboratories Pvt. Ltd., Hyderabad, India. HPLC grade acetonitrile was purchased from Rankem Mumbai, India. Analytical reagent grade sodium hydroxide, hydrochloric acid, hydrogen peroxide and ammonium acetate were purchased from S.D. Fine Chemicals Mumbai, India. The millipore water used was purified by Millipore synergy Millipore, France.

Instrumentation

The HPLC was carried out on liquid chromatograph equipped with variable wavelength detector and integrator. Inertsil ODS-3V 150 mm × 4.6 mm, i.d., 5 µm column was used for separation of all the compounds. The chromatographic data was recorded using HP-Vectra Hewlett Packed, Waldron, Germany computer system with Empower acquiring software. LC–MS/MS was performed by Applied Biosystems 4000 Q trap mass spectrometer with Electrospray ionisation source in positive mode equipped with an autosampler, and diode array detector all from Applied Biosystems, United States. The data was acquired and processed using Analyst software 1.4.5. MS^n experiments were performed using a 4000 Q trap mass spectrometer equipped with an electrospray ionization source. The data acquisition and processing were under the control of analyst software.

Forced degradation procedure

Acid degradation: Taken 202.0 mg of sample into 100 mL volumetric flask, dissolved in 50 mL of Methanol and diluted up to the mark with 0.5 N aqueous HCl solution and closed the lid. Preparation of 0.5 N aqueous HCl solution: 8.5 mL of 35% HCl/200 mL of water.

Heated the above solution at 50-60°C in water bath with stirring up to 14 hr. Transferred 3.75 mL of the above solution to 10 mL volumetric flask, neutralized with 1.8 mL of 0.5 N aqueous NaOH solution and made up to the mark with diluent, and injected into the chromatographic system, and calculated the impurity content.

Base degradation: Taken 202.0 mg of sample into 100 mL volumetric flask, dissolved in 50 mL of Methanol and diluted up to the mark with 0.5 N aqueous NaOH solution and closed the lid. Preparation of 0.5 N aqueous NaOH solution: 4.0 g of NaOH/200 mL of water.

Kept the above solution at room temperature with stirring up to 62 hr. Transferred 3.75 mL of the above solution to 10 mL volumetric flask, neutralized with 1.8 mL of 0.5 N aqueous HCl solution and made up to the mark with diluent, and injected into the chromatographic system, and calculated the impurity content.

Peroxide degradation: Taken 200.6 mg of sample into 100 mL volumetric flask, dissolved in 50 mL of Methanol and diluted up to the mark with 0.3% aqueous H_2O_2 solution. Preparation of 0. 3% aqueous H_2O_2 solution: 1.0 mL of 30% H_2O_2/100 mL of water. Kept the above solution at room temperature under dark condition up to 48 hr. Diluted 3.75 mL of the above solution to 1 L with water, injected into the chromatographic system, and calculated the impurity content.

Water degradation: Taken 200.5 mg of sample into 100 mL volumetric flask, dissolved in 50 mL of Methanol and diluted up to the mark with MQ water and closed the lid. Heated the above solution at 50-60°C in water bath with stirring up to 48 hr. Diluted 3.75 mL of the above solution to 10 mL with diluent, injected into the

chromatographic system, and calculated the impurity content. All the solutions were filtered using 0.22 µm membrane filters before HPLC and LC-MS analysis. Sample was further subjected to thermal and heat degradation accordingly.

Sample preparation

The degradation products of acid and base hydrolysis were neutralized with sodium hydroxide and hydrochloric acid respectively. The samples were further diluted with diluent. All the samples were kept in refrigerator at 5°C.

Mass spectrometric conditions

The mass spectra were recorded in Electrospray ionization ESI in positive mode of detection. Nitrogen was the nebulizer and curtain gas. The ion source conditions were set as follows: Ion source temperature, 450°C; GS1: 30 psi; GS2: 35 psi; dry gas, Declustering potential: 70 ev and dwell time, 200 ms.

Results and Discussion

Optimization of chromatographic conditions

To achieve acceptable separation between the drug and its degradation products, ammonium acetate buffer was used. Aqueous ammonium acetate buffer 0.01 M as Mobile phase A and Acetonitrile:water 40:60%, v/v in gradient elution mode and Inertsil ODS-3V 150 mm × 4.6 mm, i.d., 5 µm column was used for successful separation of Tetrabenazine and its degradation products. The flow rate was 1.00 mL/min and detection wavelength was 230 nm. The runtime was 60.0 min. The gradient programme is optimised as Time/%A:0/100, 5/100, 10/92, 15/92, 20/75, 50/75, 51/100, 60/100. These optimized chromatographic conditions were used for separation of Tetrabenazine and its degradation products. The method was validated with respect to the parameters outlined in ICH guidelines Q1A R2. For LC-MS studies, same method was used as for HPLC, without replacement of buffer. The ESI source conditions were also optimized to obtain a good signal and high sensitivity. The conditions like drying gas flow, nebulizing gas flow, drying gas temperature, capillary voltage, spray voltage and skimmer voltage were optimized to maximize the ionization in the source and sensitivity even at a very low concentration to identify and characterize the degradation products.

Degradation behavior

The optimized LC-MS method is applicable for identifying the degradation products. The LC-ESI-MS total ion chromatograms TIC obtained under various stress conditions. A total of 4 degradation products were identified and characterized by tandem mass spectrometric analysis LC-MS/MS. In Figure 2a-2e, shows the typical HPLC chromatograms of the degradation products formed under a variety of stress conditions. In Figure 3 shows the typical HPLC chromatogram of the degradation products formed under neutral conditions.

Hydrolysis: The degradation products of base and acid hydrolysis were analyzed by LC–MS and the degradation products and their fragmentation ions shown in Table 1.

Oxidation: The drug was oxidized using 0.3% H_2O_2 upto 48 hrs. Under these conditions, seven degradation products were formed. The degradation products of peroxide hydrolysis were summarized in Table 2. The degradation products of thermal and photo degradation were mentioned in Table 3.

MS^n study of tetrabenazine

Initially protonation of the drug took place and the molecular ion peak at m/z 318 was observed. The protonated molecular ion with m/z 318 underwent fragmentation to give m/z values of 191 and 257 respectively.

Characterization of degradation products

Base and acid hydrolysis: Tetrabenazine degradation was poor in base hydrolysis. The basic degradation LC-ESI-MS/MS spectra were shown in Figure 4. The basic degradation products structure was shown in Figure 5. The proposed fragmentation pathways for the degradation products of Tetrabenazine in basic condition are depicted in Figure 6. The acid degradation LC-ESI-MS/MS spectra were shown in Figure 7a-7c. All the acid degradation products structures were shown in Figure 8. The proposed fragmentation pathways for the degradation products of Tetrabenazine in acidic condition are depicted in Figure 9. The acid degradants are formed at m/z: 192, m/z: 316. The structure of degradant formed at m/z: 316 could be attributed to dehydrogenation of main peak formed

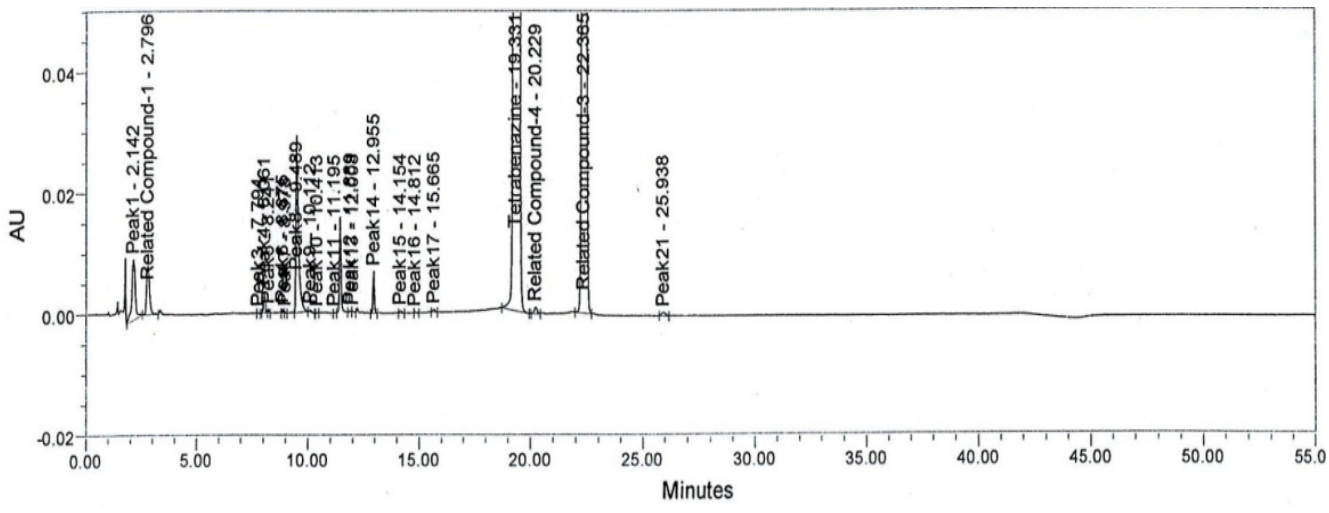


Figure 2a: HPLC chromatogram of Tetrabenazine under acid hydrolysis.

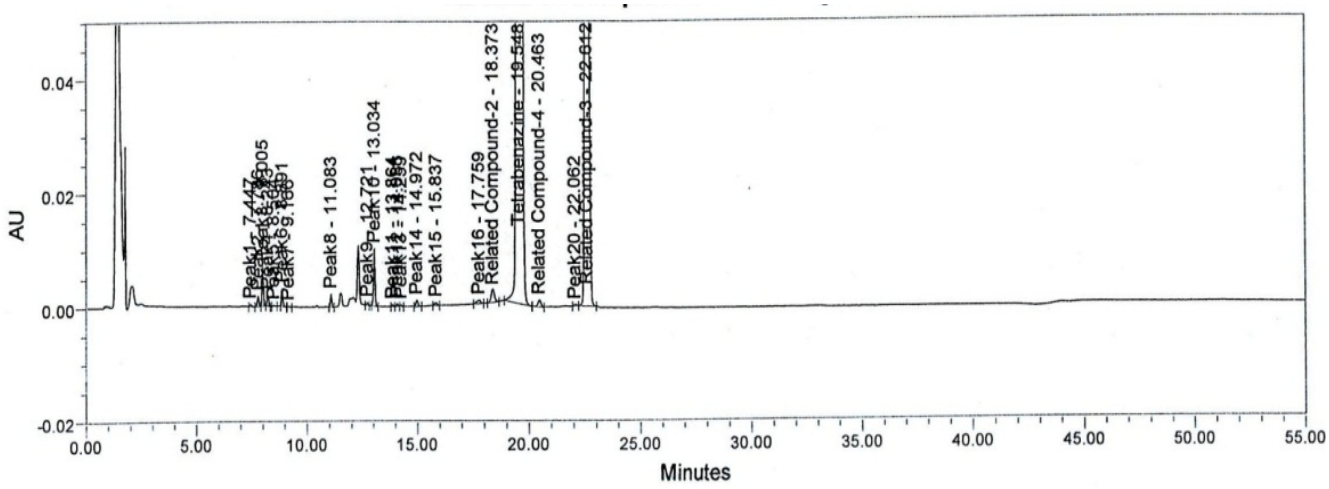


Figure 2b: HPLC chromatogram of Tetrabenazine under Base hydrolysis.

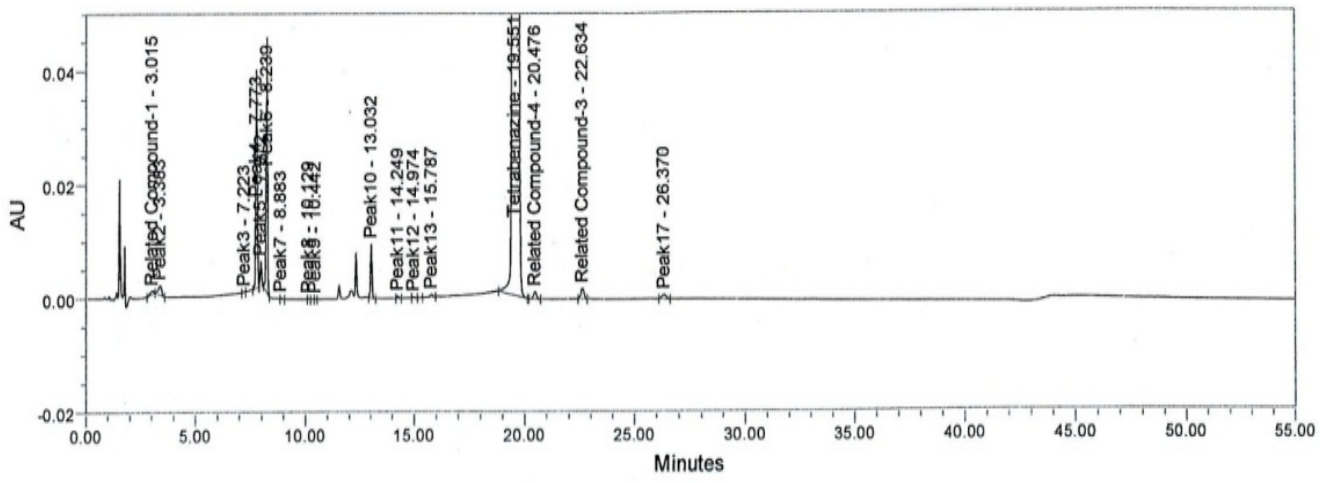


Figure 2c: HPLC chromatogram of Tetrabenazine under peroxide hydrolysis.

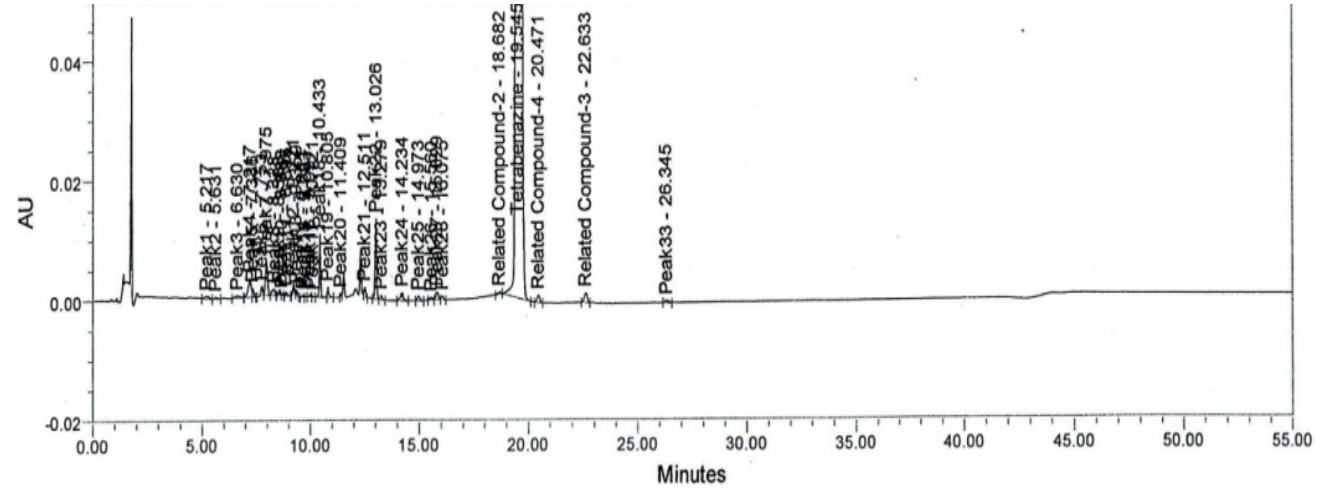


Figure 2d: HPLC chromatogram of Tetrabenazine under Light Degradation.

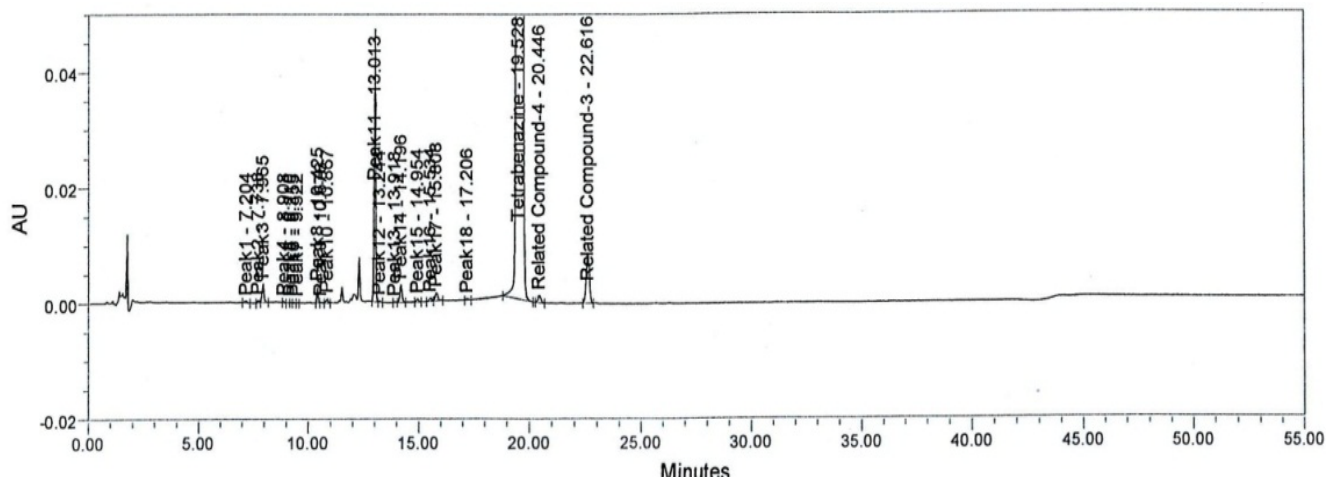


Figure 2e: HPLC chromatogram of Tetrabenazine under Heat Degradation.

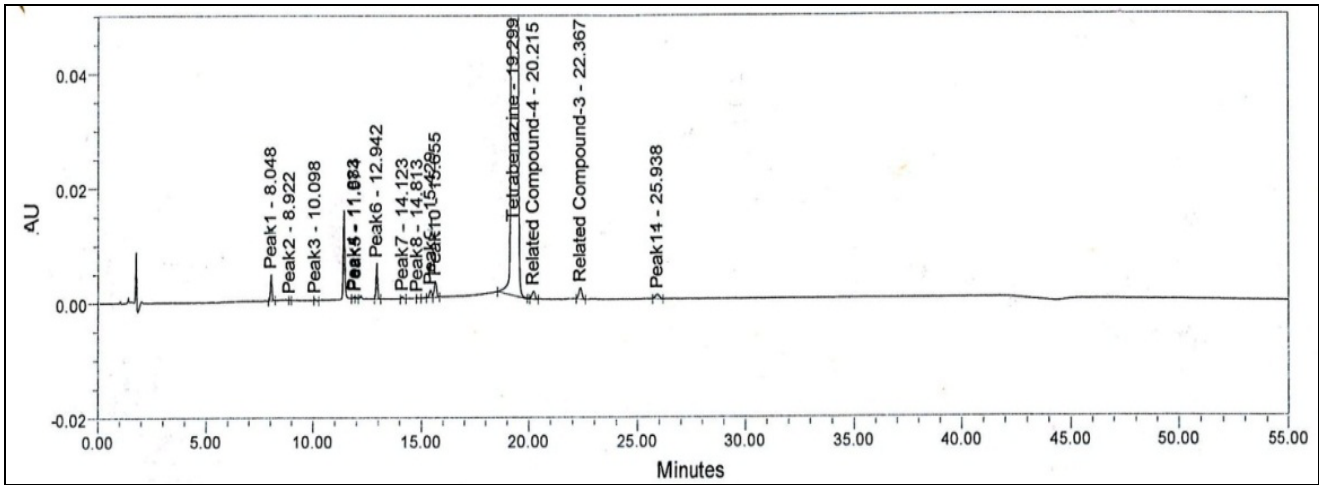


Figure 3: HPLC chromatogram of Tetrabenazine under Neutral condition.

Base Deg	LCMS RT (min)	m/z
1	20.5	318
2	23.2	318
Acid Deg	LCMS RT (min)	m/z
1	20	318
2	4	192
3	13.5	316

Table 1: Retention times and m/z values under LCMS/MS under acid and base hydrolysis.

Peroxide Deg	LCMS RT (min)	m/z
1	8.78	334
2	20.5	318

Table 2: Retention times and m/z values under LCMS/MS under peroxide hydrolysis.

Heat Deg	LCMS RT (min)	m/z
1	13.5	316
2	20.2	318
Light Deg	LCMS RT (min)	m/z
1	20.2	318

Table 3: Retention times and m/z values under LCMS/MS under heat and light degradation.

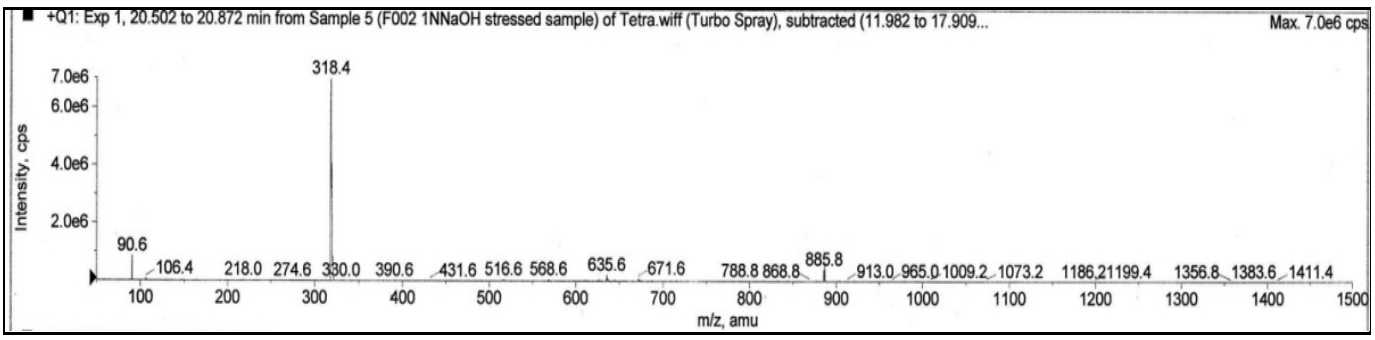
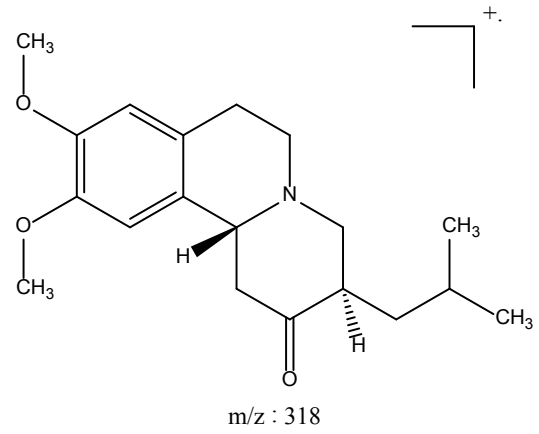


Figure 4: Mass spectrum of Tetrabenazine under base degradation at m/z: 318.



m/z : 318

Figure 5: Proposed structure of Tetrabenazine under base degradation.

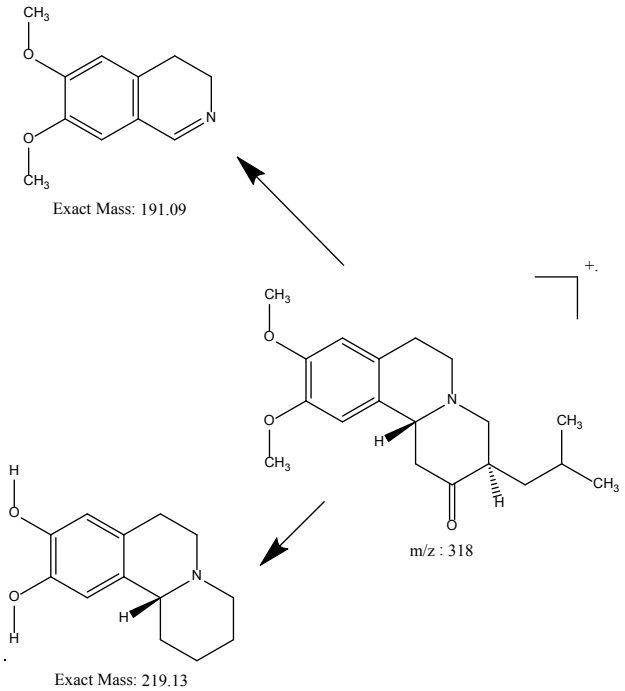


Figure 6: Proposed fragmentation structures of Tetrabenazine under base degradation at m/z: 318.

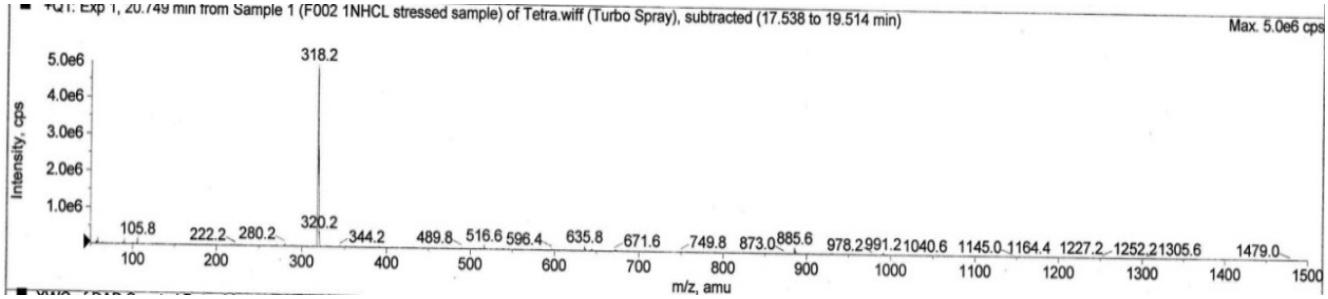


Figure 7a: Mass spectrum of Tetrabenazine under acid degradation at m/z: 318 at RT 20.5 min.

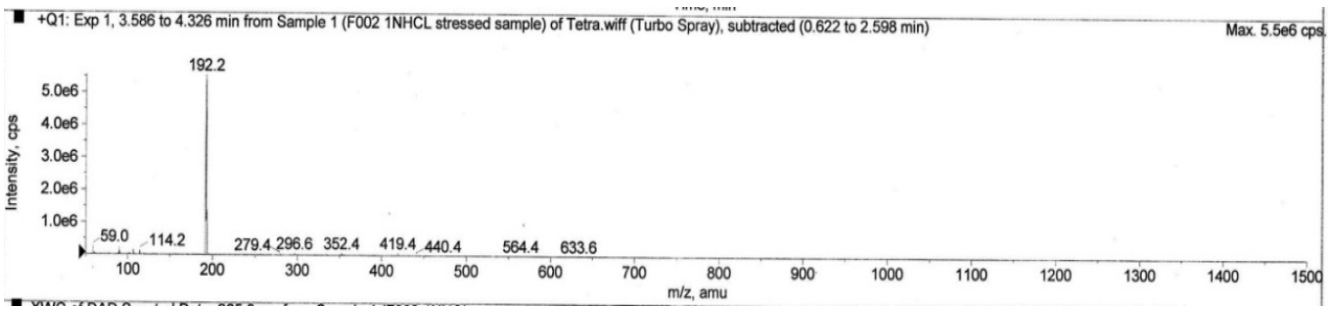


Figure 7b: Mass spectrum of Tetrabenazine under acid degradation at m/z: 192.

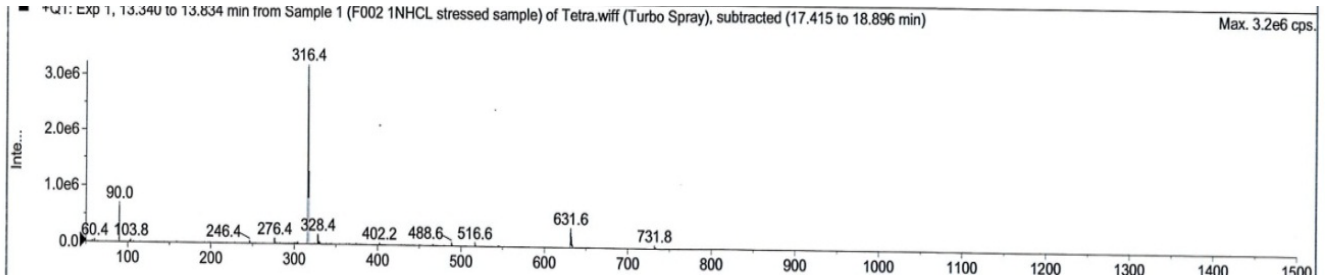


Figure 7c: Mass spectrum of Tetrabenazine under acid degradation at m/z: 316 at RT 13.5 min.

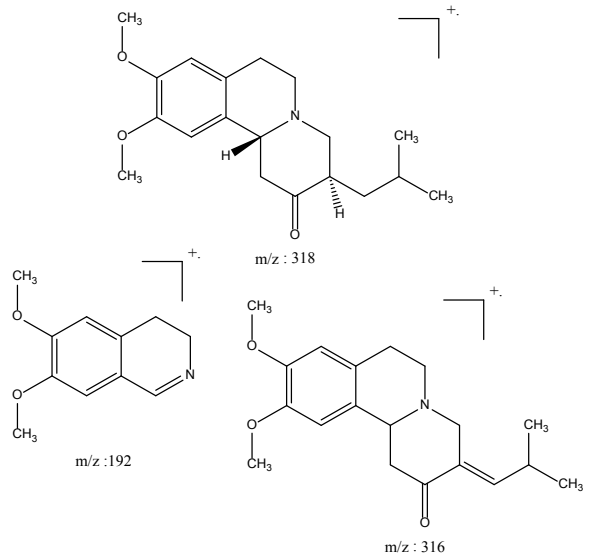


Figure 8: Proposed structures of Tetrabenazine under acid degradation.

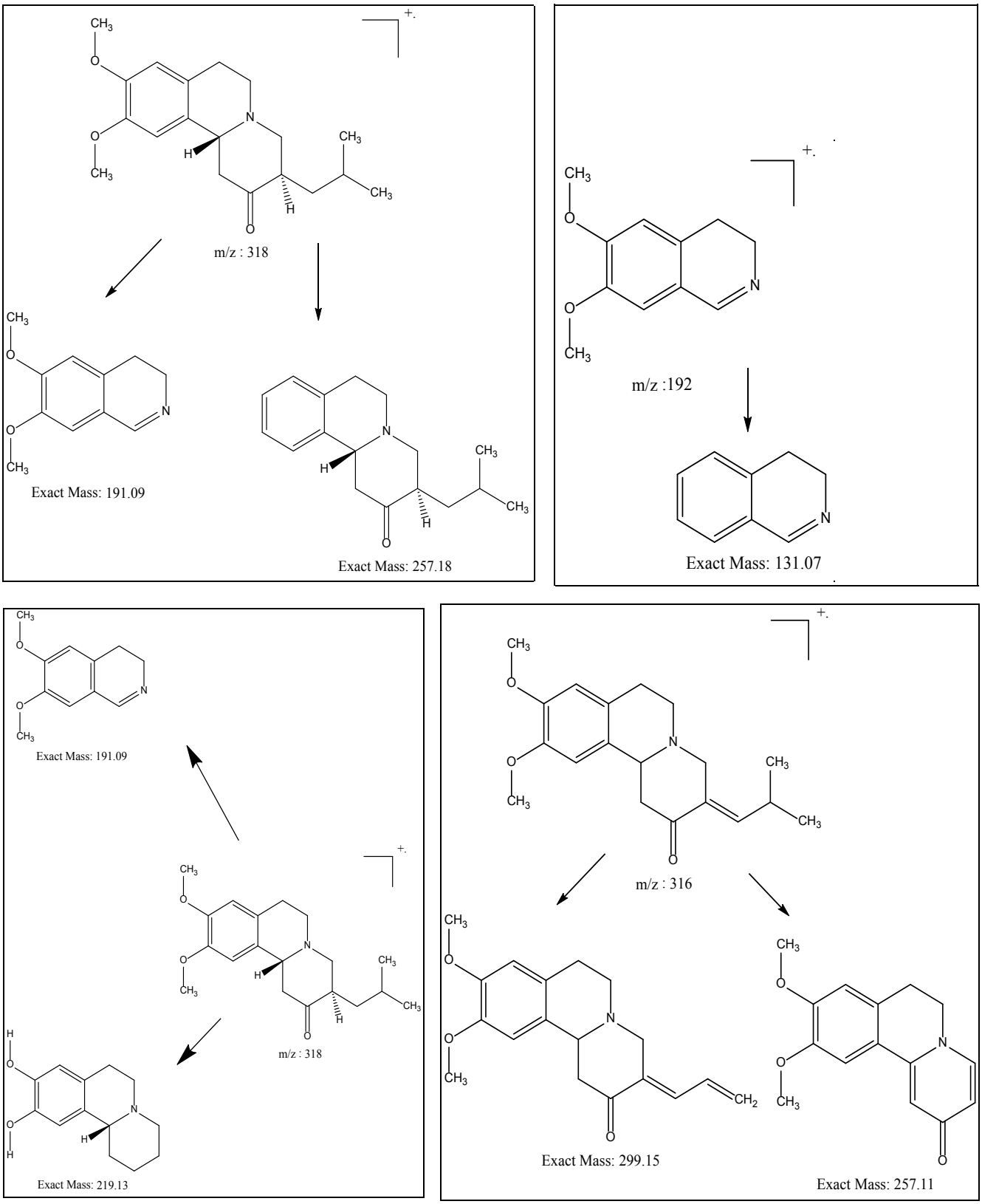


Figure 9: Proposed fragmentation structures of Tetrabenazine under acid degradation at m/z: 318, m/z: 316 and m/z: 192.

at m/z: 318. The structure of degradant formed at m/z: 192 could be attributed to the starting material in synthesis of Tetrabenazine. The fragments formed upon cleavage of degradant at m/z: 316 are 257 and 299 which could be due to loss of methyl-2-butene and demethylation respectively. The fragment formed upon cleavage of degradant at m/z: 192 is 131 which could be due to demethoxylation.

Oxidation: The oxidation of Tetrabenazine yielded two degradation products. All the degradants C_1, C_2 were formed with 0.3% H_2O_2 at room temperature for 48 hrs. All the oxidation degradation products of LC-ESI-MS/MS spectra were shown in Figure 10a and 10b. All the peroxide degradation structures were shown in Figure 11. The proposed fragmentation pathways for the degradation products of Tetrabenazine under peroxidation given in Figure 12. The structure of degradant formed at m/z: 334 could be attributed to N-oxidation of Tetrabenazine. The fragments formed upon cleavage of degradant at m/z: 334 are 191 and 259 which could be due to loss of 3,5 dimethyl-2-hexanone and isobutyl moiety respectively.

Photo and thermal degradation: No degradation observed for drug substance during photolytic study. Only one degradant impurity is formed at m/z: 316 during heat degradation study. The LC-ESI-MS/MS spectra under light degradation are given in Figure 13. The light degradation structure was shown in Figure 14. The proposed fragmentation pathway for the degradation products of Tetrabenazine under light degradation given in Figure 15. The LC-ESI-MS/MS spectra under heat degradation given in Figure 16a and 16b. All the heat degradation structures were shown in Figure 17. The proposed fragmentation pathways for the degradation products of Tetrabenazine under heat degradation given in Figure 18. The structure of degradant

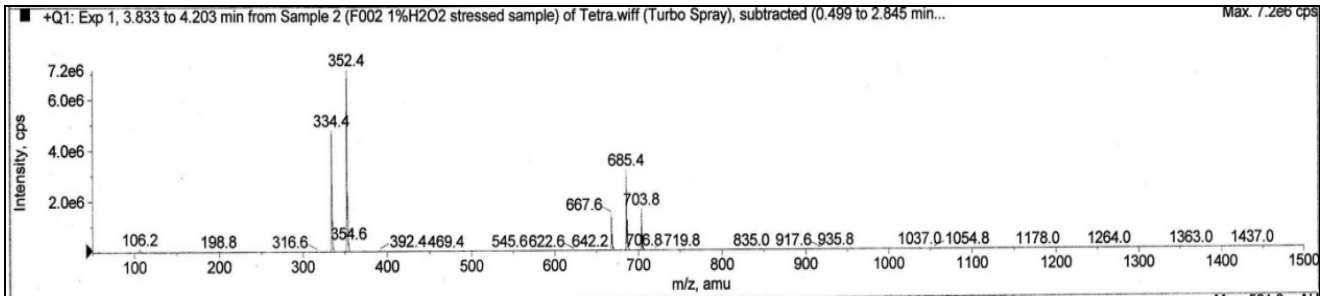


Figure 10a: Mass spectrum of Tetrabenazine under peroxide degradation at m/z: 334.

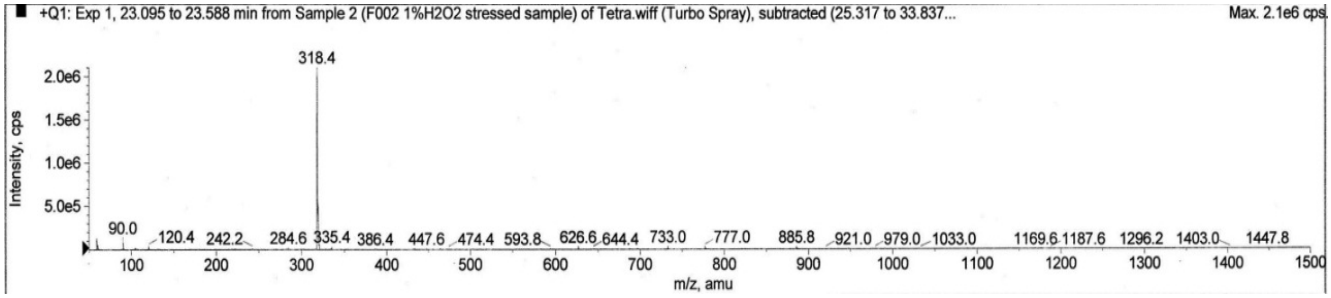


Figure 10b: Mass spectrum of Tetrabenazine under peroxide degradation at m/z: 318.

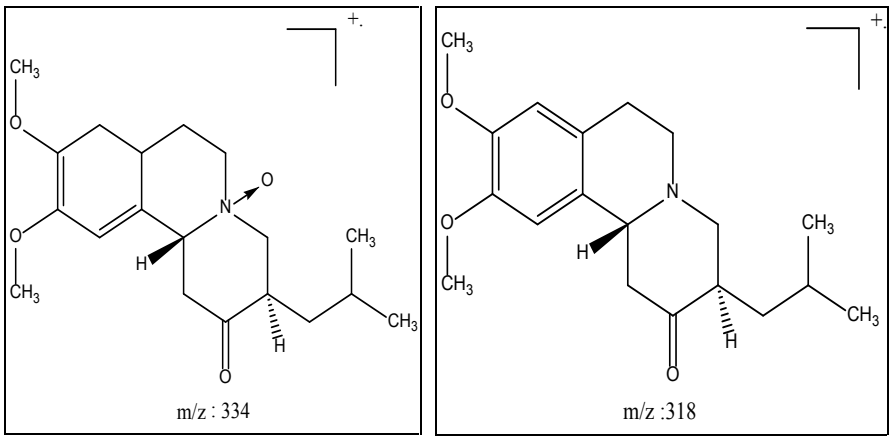


Figure 11: Proposed structures of Tetrabenazine under peroxide degradation.

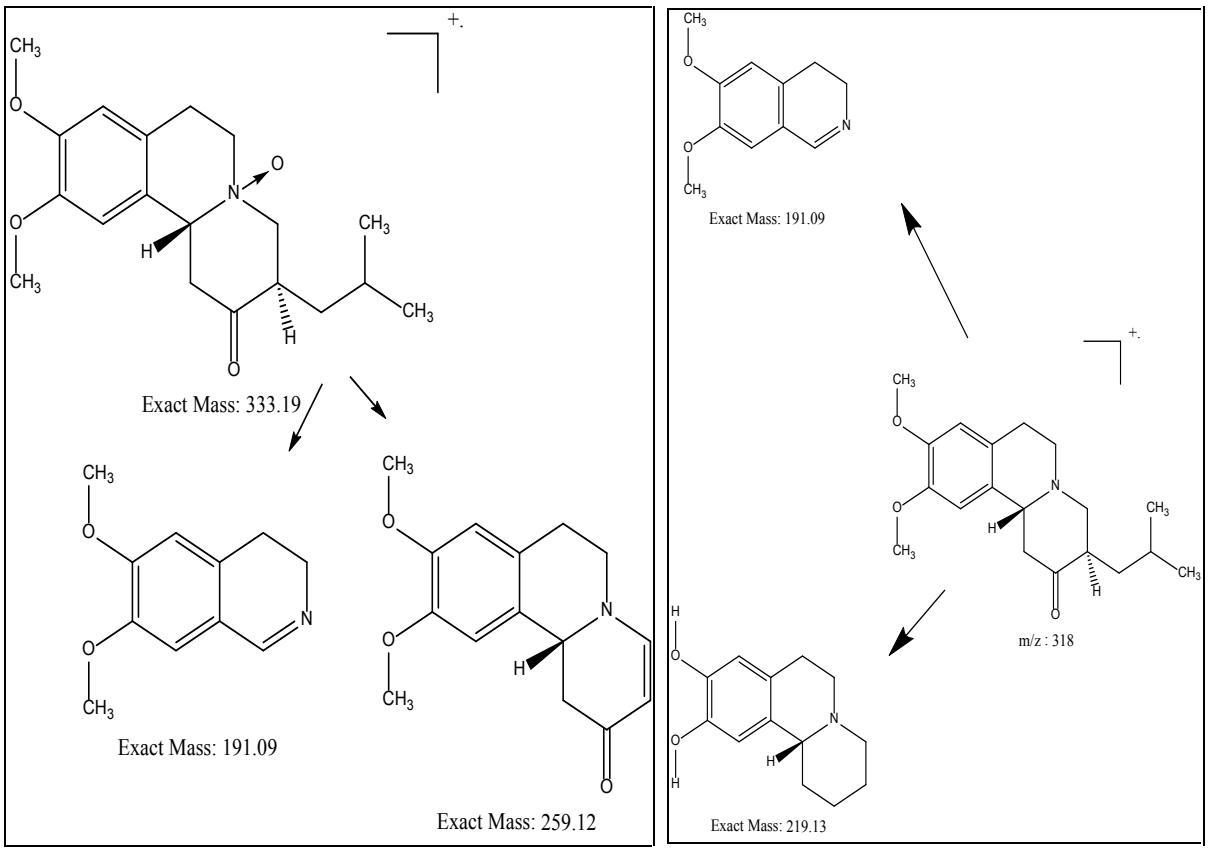


Figure 12: Proposed fragmentation structures of Tetrabenazine under peroxide degradation at m/z: 334.

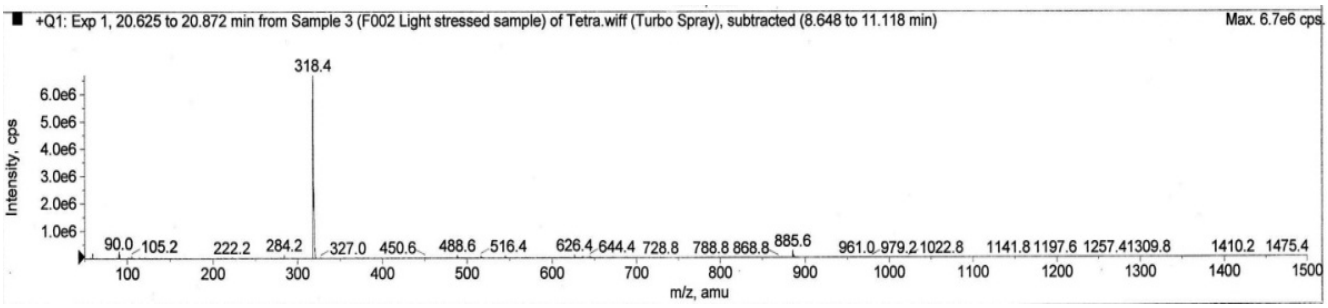


Figure 13: Mass spectrum of Tetrabenazine under light degradation at m/z: 318.

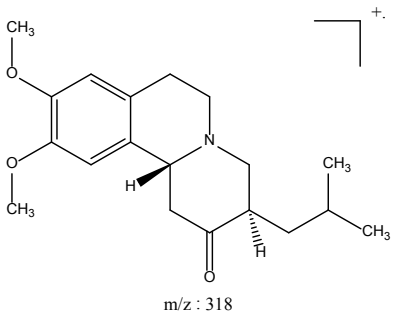


Figure 14: Proposed structure of Tetrabenazine under light degradation.

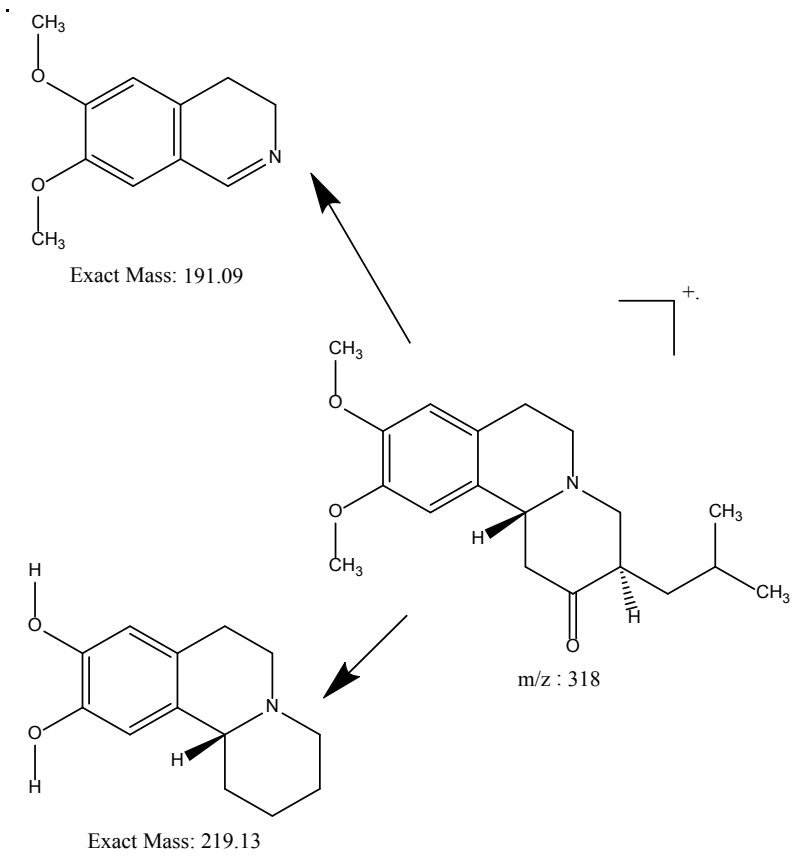


Figure 15: Proposed fragmentation structures of Tetrabenazine under light degradation.

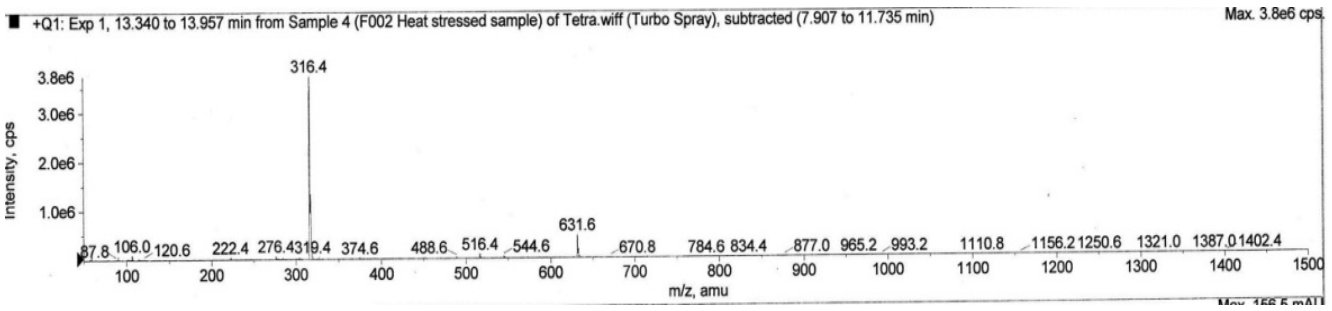


Figure 16a: Mass spectrum of Tetrabenazine under heat degradation at m/z: 316.

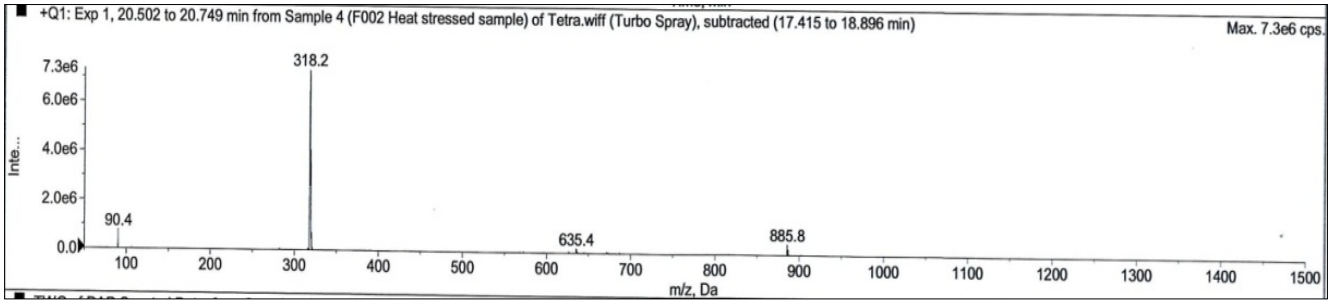


Figure 16b: Mass spectrum of Tetrabenazine under heat degradation at m/z: 318.

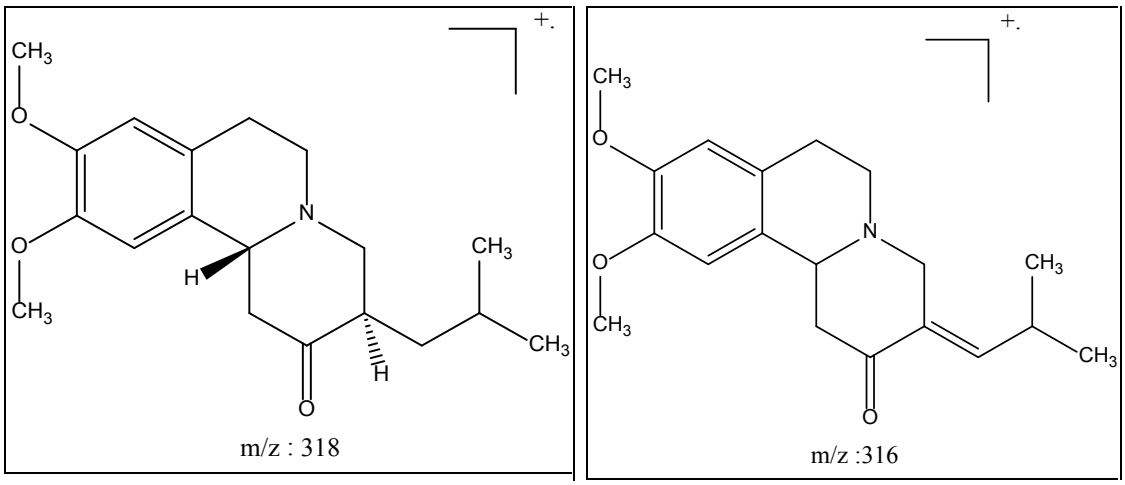


Figure 17: Proposed structures of Tetrabenazine under heat degradation.

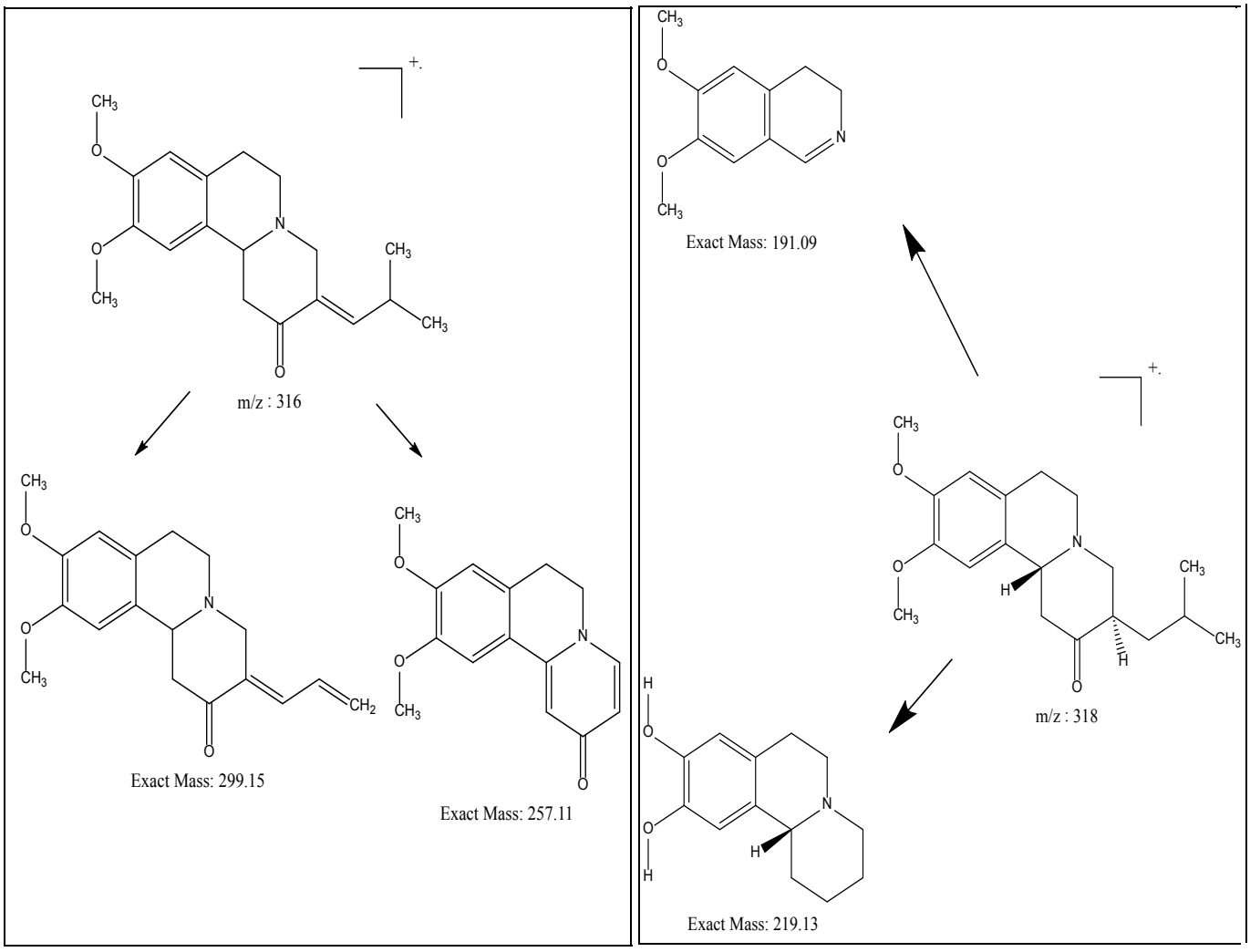


Figure 18: Proposed fragmentation structures of Tetrabenazine under heat degradation at m/z: 316 and m/z: 318.

formed at m/z: 316 could be attributed to dehydrogenation of main peak formed at m/z: 318. The fragments formed upon cleavage of degradant at m/z: 316 are 257 and 299 which could be due to loss of methyl-2-butene and demethylation respectively.

Conclusion

A robust LC-MS/MS method for stability indicating assay of Tetrabenazine was developed. The degradation behaviour of Tetrabenazine under hydrolysis acid, base and neutral, oxidation conditions was carried out according to ICH guidelines. The liquid chromatography method described in the present study can resolve all the degradation products from the Tetrabenazine as well as from each other under various stress conditions. The drug showed extensive degradation in oxidative stress, while it was stable to neutral stress conditions and mild degradation under acidic and basic stress conditions. A total of 4 degradation products were characterized and the fragmentation pathways were proposed based on LC-MS/MS data results.

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