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Identification of Unknown Impurity by Advanced Spectroscopy Techniques in the Sumatriptan Base Which Degraded into Sumatriptan Impurity F and Sumatriptan Base

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ABSTRACT

An unknown impurity was observed in Sumatriptan base during Related Substance analysis for Sumatriptan succinate. This unknown impurity was isolated from crude sample of Sumatriptan base by using semi-preparative HPLC technique but it was not stable and gets fully degraded into two main impurities. The objective of this study is to find out unknown impurity from sumatriptan base (Active pharmaceutical ingredients) and confirmed its degradation into Sumatriptan impurity F and Sumatriptan base impurities by using advance spectroscopic Techniques.

Keywords: Sumatriptan base, Impurity degradation, HPLC, LC/MS-MS, NMR

INTRODUCTION

Sumatriptan succinate is chemically known as 3-[2-(Dimethylamino)ethyl]-N-methylindole-5-methanesulfonamide succinate (1:1). The Sumatriptan is one of the triptan [1] family drugs. The Sumatriptan used in the treatment of migraine [2,3] and help to relieve headache. Sumatriptan dose are given by four different routes oral, subcutaneous, intranasal, rectal [2]. A few methods were reported in the pharmacopeia for the analysis of Sumatripan and its related substances using chromatographic method [3-5]. Apart from those, several analytical techniques like HPLC [6,7]; HPLC-MS/MS [8] have also been reported in the literature. Sumatriptan is also useful in monitoring hemodynamic changes in the cortical and scalp surface during migraine attack and treatment by using Near-Infrared Spectroscopy System (NIRS) and laser Doppler Skin Blood Flow (SkBF) [9]. Both 50 and 100 mg Sumatriptan tablets are effective and well tolerated in the acute treatment of migraine [10]. Sumatriptan in human plasma is determined by ultra-performance liquid chromatography–tandem mass spectrometry [11]. To meet the challenges and to build high degree of purity in drug substances and drug products, it is required to carry out all the investigations for standards of drugs and impurities to get significant results. Profiling of impurities in drug substance is an important part during the manufacturing process of drug substances and drug products. Process related impurities can be arising during the manufacturing process of Sumatriptan base and their acceptance up to the certain limit are based on pharmaceutical studies or known safety data. As per regulatory guidelines, the pharmaceutical studies sample of isolated impurity can be considered for safety assessment [12]. It is therefore necessary to isolate and characterized unidentified impurities present in Active Pharmaceutical Ingredients (APIs).

One new unknown impurity at a level 2-3% was observed in HPLC analysis. In present study a new LC-MS-MS method was developed for identification of impurities present in Sumatriptan base. As per International Conference on Harmonisation (ICH) guidelines any unknown impurity present at or above 0.1% level in drug product should be well identified and characterized. Therefore this new unknown impurity was identified and characterized by different spectroscopic techniques like LC-MS-MS, HPLC, and NMR. The present work shows the identification, characterization and degradation of unknown impurity that was found to be formed during manufacturing of Sumatriptan base for Sumatriptan succinate.

MATERIALS AND METHODS

Samples and reagents

The investigated Samples of Sumatriptan API was obtained from Chemical Research Division, Ipca Laboratories Ltd. (Mumbai India.); Deionized water prepared using Mili-Q Plus purification system (Millipore, Bradford, USA). DMSO, D_2O was purchased from Merck KGaA (Darmstadt, Germany). Analytical reagent grade Trifluoroacetic acid (TFA) was purchased from Lancaster, England, Perchloric acid (70%), HPLC grade acetonitrile, Glacial acetic acid, Ammonia Solution 28-30% and Methanol were purchased from Merck India Limited (Mumbai India).

High performance liquid chromatography

Analytical method was developed using Waters HPLC system, Model Alliance 2695 separation module (Quaternary Gradient pump) equipped with a Waters 2996 Photo Array Detector and data was processed through Empower 3.0 software. The analysis was carried out on Unisphere Extend C18 column, 250 mm length × 4.6 mm i.d., 5.0 µm particle size with gradient condition for separation. Buffer was prepared by adding 2.0 ml glacial acetic acid and 0.5 ml perchloric acid (70%) in 1000 ml Mili-Q water. Adjusted pH 7.5 with ammonia solution (28-30%). Mobile phase A contain buffer and acetonitrile in the ratio 90:10 (v/v) and Mobile phase B contain buffer: acetonitrile in the ratio 10:90 (v/v) and injection volume kept 10 µl. for Sumatriptan base and 100 µl for preparative HPLC fractions. Diluent was prepared by mixing water and acetonitrile in the ratio 70:30 (v/v). The separation was achieved by gradient elution [$T_{(min)}$/A: B (%)] set as (T_0/100:00; T_{12}/85:15; T_{25}/30:70; T_{40}/20:80; T_{45}/100:00; T_{50}/100:00). Column oven temperature was kept 40ºC and sample cooler temperature 4ºC. The flow rate was kept 0.9 ml/min. UV detection was monitored at wavelength 282 nm.

Preparative high performance liquid chromatography

Waters preparative high performance liquid chromatography with Quaternary Gradient pump 2555 equipped with a Waters UV 2489 Detector (Waters, Milford, MA, USA) was used. The data was recorded using Empower 3 software. A column used for separation was Puritas Prep 100 Å ODS C18 of dimensions 30 mm × 250 mm with 5 µm particle size. Used premixed Mobile phase A consisting of Mili-Q water and acetonitrile in the ratio of 90:10 (v/v) and Mobile base B consisting 0.1% Trifluroacetic acid in Milli-Q water and acetonitrile in the ratio 80:20 and diluent 4 ml used was 0.1% Trifluroacetic acid in Mili-Q water and acetonitrile in the ratio 70:30 (v/v). Injected volume was 50 mg/4 ml and UV detection was monitored at 282 nm at a flow rate of 25 ml/min. Preparative HPLC column kept in water before injection for better retention purpose.

Mass spectrometry

The liquid chromatography-heated electrospray ionization-tandem mass Spectrometry (LC-HESI-MS/MS) analysis was carried out on Q Exactive (Thermo Scientific, Waltham, Massachusetts, United States) orbitrap mass spectrometer was used to achieve high-resolution accurate mass spectral data. The LC unit was consisted of an Ultimate 3000 quaternary gradient pump with a degasser and auto sampler. A Unisphere C18 column (250 × 4.6 mm i.d, 5 m particles) was used for chromatographic separations.

The unknown impurity present in Sumatripan base was analysed by using following chromatographic condition and mass spectrometry parameter. The mobile phase A prepared by adding 1.0 ml Trifluroacetic acid (TFA) in 1000 ml Milli-Q Water, adjusted pH 7.5 with Ammonia Solution and Mobile phase B used was Acetonitrile in a gradient mode (T_{min}/A:B; T_0/85:15; T_{12}/80:20; T_{25}/25:75; T_{40}/20:80; T_{45}/85:15; T_{50}/85:15). The flow rate was set to 0.75 mL per min with UV detector wavelength was fixed at 282 nm. The sample solution (500 ppm) was prepared in diluent Milli-Q water and Acetonitrile in the ratio 50:50 and 10 µL was injected. Column oven temperature kept 40ºC and auto sampler temperature 5ºC. In the mass parameters of LC-MS, spray voltage was kept at 4.0 kV and capillary temperature at 320ºC. Nitrogen was used as both sheath and auxiliary gas. Mass range was kept at m/z 73.50-1101. MS/MS studies were carried out by maintaining normalized collision energy at about 25% with the mass range m/z 150-1000.

The preparative HPLC fraction of Sumatriptan base was analysed by using following chromatographic condition and mass spectrometry parameter. The mobile phase A consist of 1.0 ml Trifluroacetic acid (TFA) in 1000 ml Milli-Q Water adjusted pH 7.9 with Ammonia Solution and Mobile phase B was Acetonitrile used in a gradient mode (T_{min}/A:B; T_0/95:05; $T_{14.5}$/95:05; T_{20}/78:22; T_{30}/35:65; T_{35}/95:05; T_{40}/95:05). The flow rate was set to 1.0 mL per min. The Sumatriptan liquid preparative fraction injected as such. The remaining LC-MS conditions kept same as mentioned in above analysis parameters the only change in mass range were set as m/z 100-1000. MS/MS studies were carried out by maintaining normalized collision energy at about 25% with the mass range m/z 50-620.

Nuclear magnetic resonance spectroscopy

The ^1H, ^{13}C and DEPT NMR experiments were performed on AVANCE 400 (Bruker, Fallanden, Switzerland) instrument at 300 K. The exchangeable proton were identified by D_2O exchange experiments DEPT spectral editing revealed the presence of methyl and methine groups as positive peaks while the methylene as negative peaks. Sample was run in DMSO-d6 solvent.

RESULTS AND DISCUSSION

Detection of unknown impurity and its degradation

Sumatriptan base sample was analyzed by the HPLC method as described in section 2.2. One new unknown impurity was observed at RT 20.49 min along with Sumatriptan base of RT 10.13 min (Figure 1).

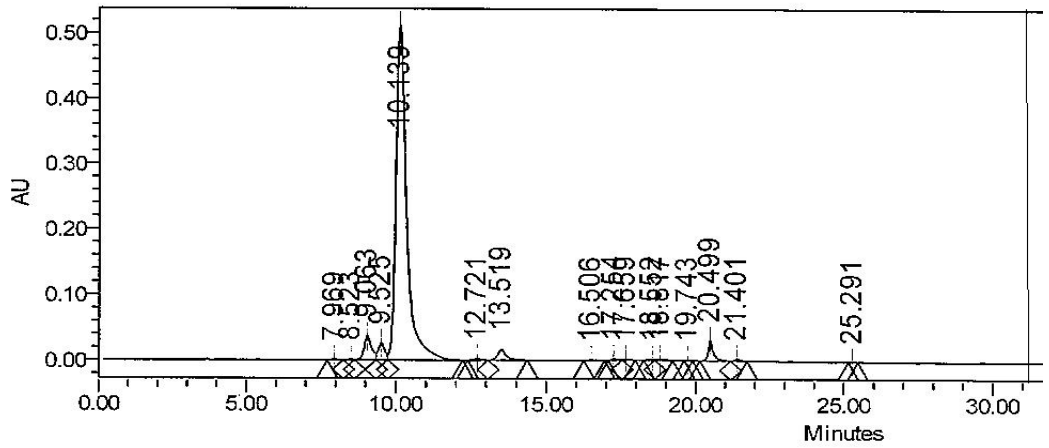


Figure 1: Typical HPLC Chromatogram of Sumatriptan base (RT 10.1) with unknown impurity (RT 20.4)

The same sample was subjected to LC-MS/MS analysis to identify the mass of unknown impurity. This impurity formed during synthesis of Sumatriptan base. The positive LC-MS spectrum (Figure 2) of the unknown impurity and Sumatriptan base exhibited molecular ion peak as $[M+H]^+$ at m/z 587.25 and at m/z 296.14 respectively.

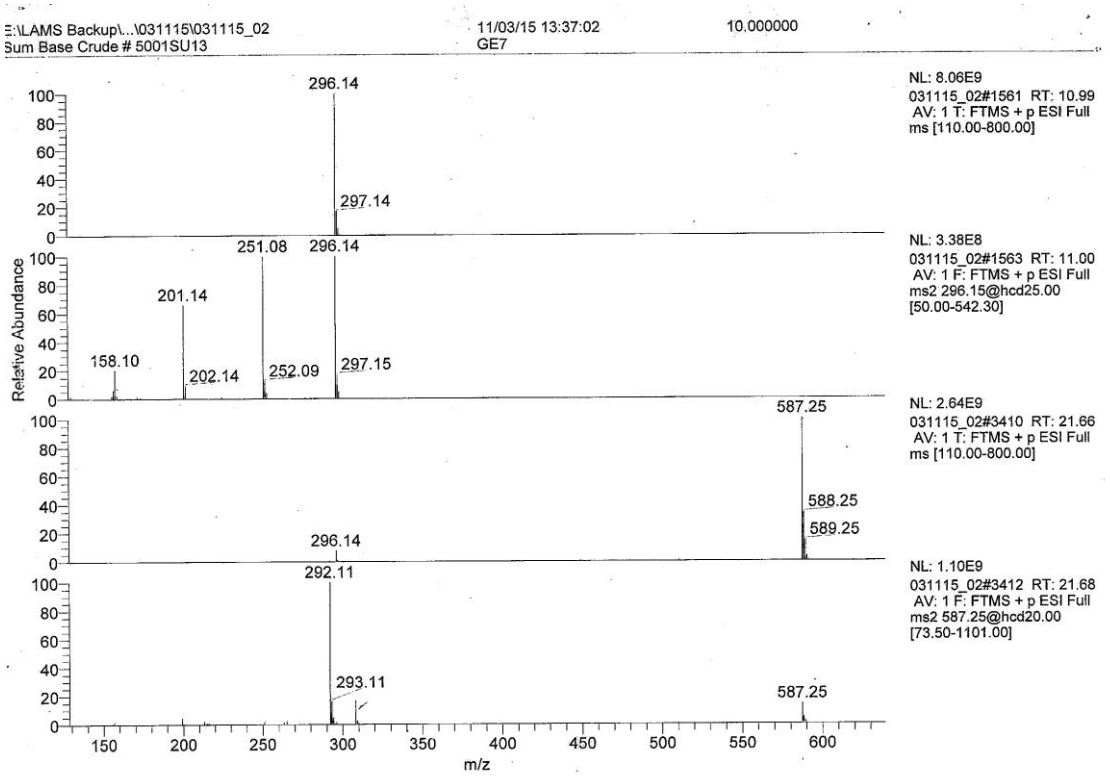


Figure 2: MS-MS of unknown impurity (MS-RT 21.6 min) and Sumatriptan base (MS-RT 10.9 min)

To confirm this new impurity with above predicted structure, it was isolated using prep HPLC. Purity of the isolated fraction (unknown impurity) was obtained 98.05% at RT 21 min (Figure 3)

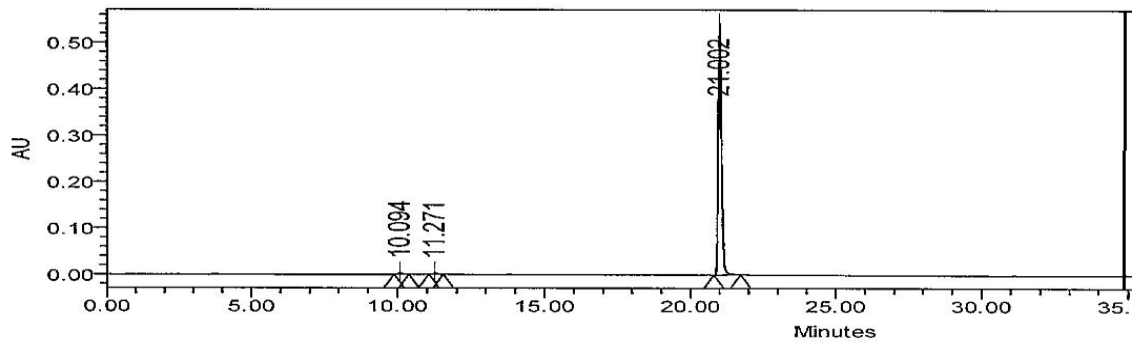
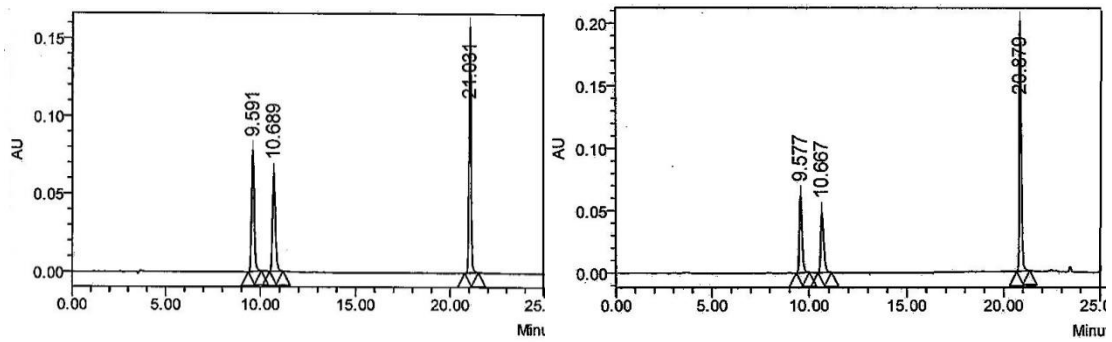
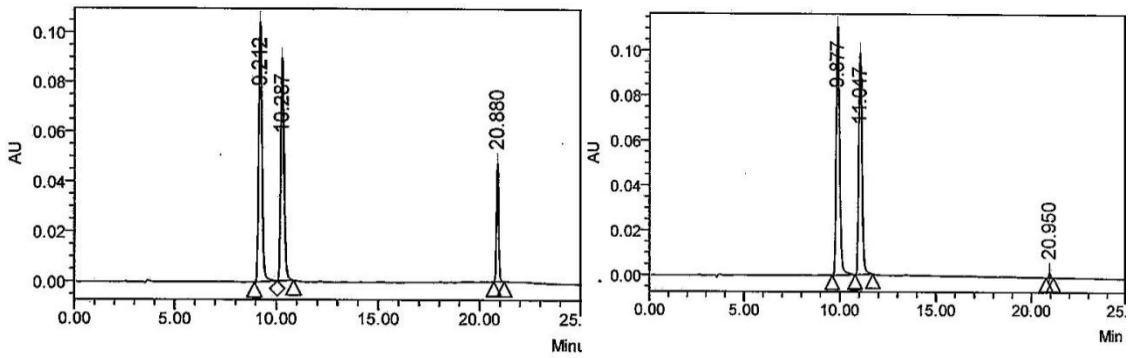


Figure 3: Typical HPLC chromatogram of isolated pure unknown impurity (RT 21.0)

Purity of this isolated impurity was monitored after intervals of 33, 56, 99 and 146 h (Figure 4) and found that this pure fraction get converted into two main peaks, one of them was at RT about 9.5 min and another peak at RT about 10.6 min (a, b, c, d). These three peaks (RT 20.49, 9.59 and 10.68) were characterized with the help of spectral techniques like HPLC (spiked study), LC-MS-MS and NMR. After 146 h this impurity RT 20.49 min fully degraded in to two impurities one at RT 9.8 min and impurity two at RT 11.0 min.



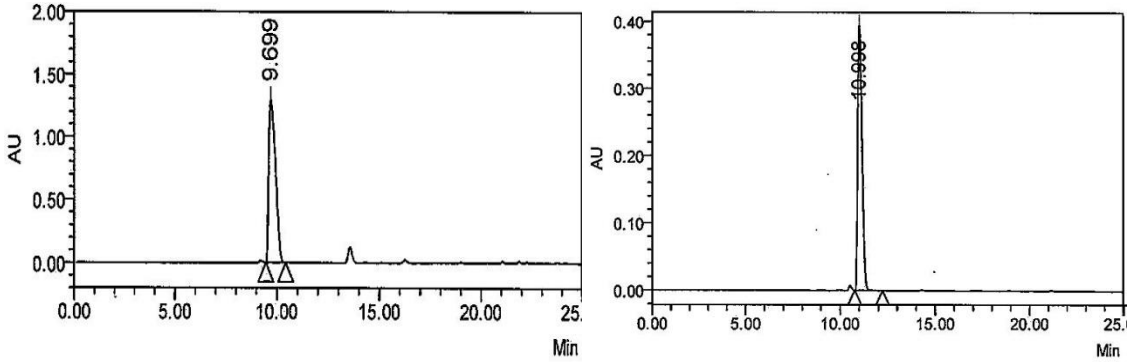
a. After 33 h b. After 56 h



c. After 98 h d. After 146 h

Figure 4: Typical HPLC chromatograms of isolated unknown impurity degraded patterns

Again preparative HPLC was done to isolate the degraded impurities of RT about 9.8 min and RT 11 min. and successfully isolated the degraded impurities with high purity (Figure 5).



a. Isolated impurity one 9.6 min b. Isolated impurity two 10.99 min

Figure 5: Typical HPLC chromatograms of isolated impurity one and two

Synthesis and Isolation of sumatriptan impurity

1-(3-(2-aminoethyl)-1*H*-indol-5-yl)-N-methylmethanesulfonamide (AIMS) 0.375 mole were mixed with Di-sodium hydrogen ortho phosphate (0.704 moles) in methanol (13 Vol.) added acetic acid (0.2 Vol.) and added sodium borohydride (0.638 moles) solution, stabilized with NaOH and 35% Formalin solution (1.125 moles) simultaneously. Stirred for 1 h. filtered the reaction mass and adjusted pH to 5.0 using Conc. HCl. Stirred for 30 min added hyflo (25% w/w) and stirred for 30 min and distilled out methanol and water under reduced pressure. Added water (16 Vol.) and stirred for 30 min, filtered through hyflo and aqueous layer washed with ethyl acetate and further treated with 10% activated carbon. Filtered through hyflo and adjusted pH to 9.6 using NaOH Solution, stirred for 30 min, filtered and suck dried. Further it was dried at 55-60ºC under vacuum to get Sumatriptan base. This Sumatriptan base sample containing 2-5% impurity at RT about 20 was isolated by Preparative liquid chromatography as mentioned in section 2.3. Similarly the degraded impurities of RT about 20.49 min into two impurities at RT 9.5 min and RT 10.6 min were isolated by above preparative HPLC method (Section 2.3).

Structures elucidation of impurity one, impurity two and unknown impurity

In ^{1}H NMR spectrum of impurity one showing total 17 protons in which 11 protons are in the aliphatic region ranging from 2.49 ppm to 4.35 ppm and 6 protons in the aromatic region from 6.8 ppm to 11.2 ppm. Signal of the protons recorded as at δ=2.52 ppm (3H). These three protons are merged with DMSO–d6. protons in his same area, δ=2.93-2.95 ppm (2H), δ=3.42-3.46 ppm (2H), δ=4.35 ppm (4H), δ=6.84-6.87 ppm (1H), δ=7.11-7.14 ppm (1H), δ=7.35-7.38 ppm (1H), δ=7.45 ppm (1H), δ=9.44 (1H), and highly deshielded proton occurs at δ=11.15 ppm (1H) corresponding to 17 protons and in ^{13}C NMR experiment it shows the δ values signals at 18.5 ppm, 29.4 ppm, 40.7 ppm, 41.9 ppm, 57.0 ppm, 106.0 ppm, 111.6 ppm, 120.7 ppm, 121.0 ppm, 124.9 ppm, 126.4 ppm, 127.8 ppm, 136.2 ppm so corresponding total 13 carbon peaks signals.

The mass spectrum of impurity one in positive ion mode exhibited molecular ion peak at m/z 280.1116 $[M+H]^{+}$ in Figure 6 indicating mass of this compound to be 279.1. LC/MS/MS spectrum for mass m/z 280.111 displayed daughter ion peaks at m/z 251.085 and 280.111.

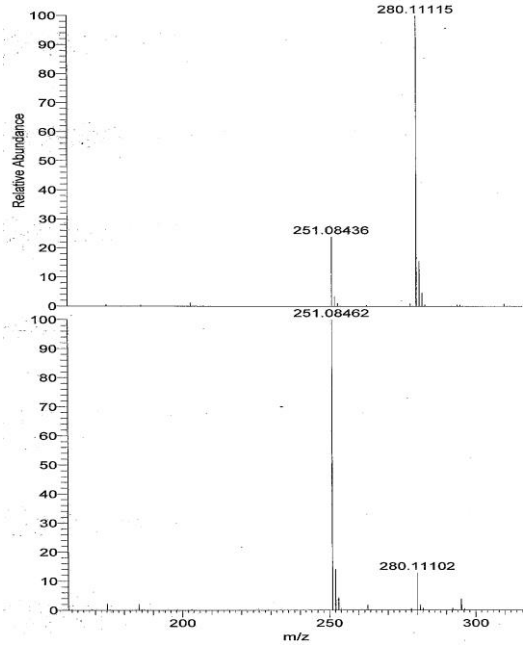


Figure 6: MS and MS–MS of imp one

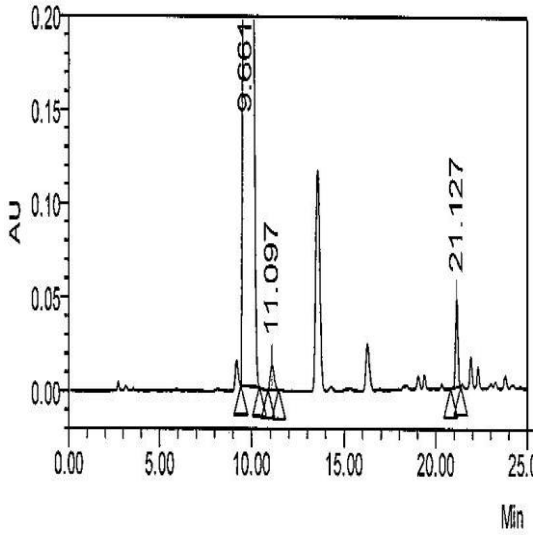


Figure 7: Degraded imp spiked with Suma imp F

Based on above spectral data the molecular formula of impurity one is confirmed as $C_{13}H_{17}N_3O_2S$ which contains 17 protons and 13 carbons which complies with structure of Sumatriptan impurity F. Impurity one was also supported by spike study as Sumatriptan impurity F (Figure 7).

In ^{1}H NMR spectrum of impurity two showing total 21 protons in which 15 are in the aliphatic region ranging from 2.10-4.4 ppm and 6 are in aromatic region ranging from 6.79-10.84 ppm. The proton NMR displayed signal at δ=2.22 ppm (6H), δ=2.51-2.55 ppm (5H), δ=2.79-2.83 ppm (2H), δ=4.36 ppm (2H), δ=6.79-6.81 ppm (1H), δ=7.07-7.10 ppm (1H), δ=7.17 (1H), δ=7.31-7.33 ppm (1H), δ=7.52 ppm (1H), and highly deshielded proton at δ=10.84 ppm (1H) corresponding to 21 protons and ^{13} C NMR of it shows δ values signals at 23.6 ppm, 29.4 ppm, 45.6 ppm, 45.6 ppm, 57.0 ppm, 60.5 ppm, 111.6 ppm, 113.2 ppm, 120.1 ppm, 121.2 ppm, 123.6 ppm, 124.2 ppm, 127.7 ppm, 136.4 ppm. Corresponding total 14 carbon peaks signals.

The mass spectrum of impurity two in positive ion mode exhibited molecular ion peak at m/z 296.14 $[M+H]^+$ (MS-RT 26.26) in Figure 8a indicating mass of this compound to be 295.14.

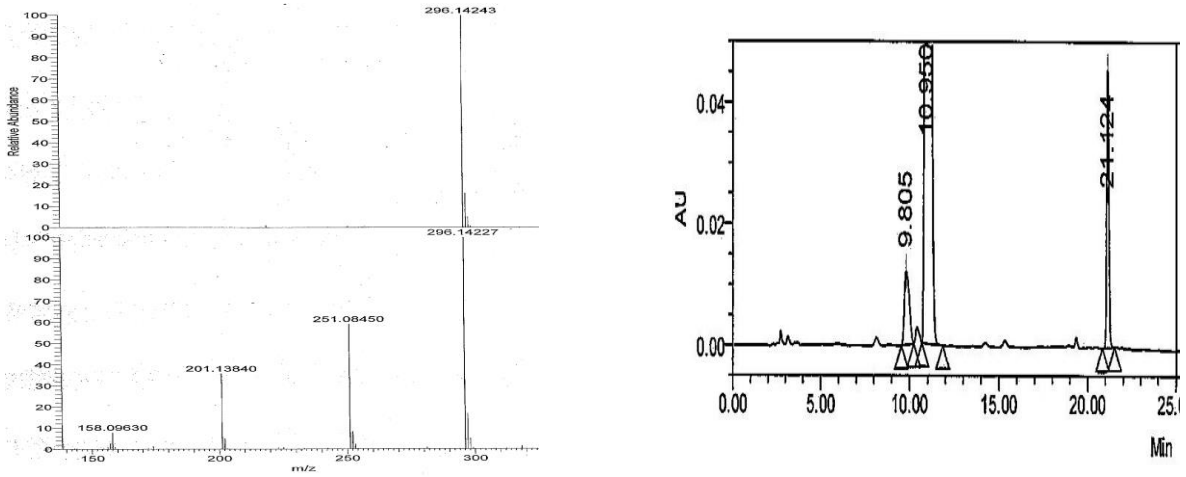


Figure 8: (a) MS and MS–MS of imp two; (b) Degraded imp spiked with Suma base

LC/MS/MS spectrum for mass m/z 296.1430 displayed daughter ion peaks at m/z 201.138, 251.08 and 296.14. This confirmed the impurity two as Sumatriptan base further supported by spike study (Figure 8b).

The probable fragmentation pattern for impurity one and two are shown in Figure 9 which matches with LC-MS data.

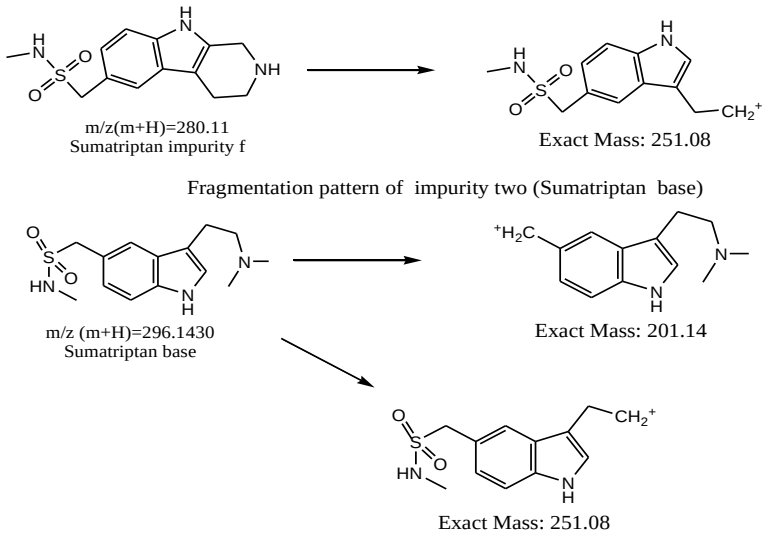


Figure 9: Mass fragmentation of impurity one and two

To avoid degradation of unknown impurity, after isolating the unknown impurity RT 21.49 min was lyophilized immediately under vacuum at temperature -105ºC on Lyophilized (Freeze Dryer) instrument for 12 h, and then performed NMR analysis. In ^{1}H NMR of unknown impurity shows total 38 protons.

These 38 protons divided in two regions; one is aliphatic region (2.5- 5.51 ppm) and second aromatic region (6.80-11.01 ppm). In this aliphatic region, total contain 28 protons and 10 protons in aromatic region. In aliphatic region out of 28 protons 6 protons is of six membered nitrogen ring, 12 protons of four methyl groups and 10 protons are of methylene (open ring) groups. In the aromatic region 6 protons is of two benzene ring, 2 protons are of –NH group (open ring) which is attached to nitrogen between CH_3 and SO_2 groups, one proton is attached to ortho position of the 5 membered nitrogen ring and one proton directly attached to nitrogen of the 5 membered nitrogen ring. The details proton values are δ=2.50-2.6 ppm (3H,a), δ=2.88 ppm (8H,b), δ=3.1-3.19 ppm (3H,c), δ=3.3-3.36 ppm (6H,d), δ=4.08ppm (2H,e), δ=4.32-4.39(4H,f), δ=5.5ppm (2H,g), δ=6.8-6.81 ppm (2H,h), δ=6.89-6.91 ppm (1H,i), δ=7.06-7.08 ppm (2H,j), δ=7.40-7.45 ppm (2H,k), δ=7.64 ppm (1H,l), δ=7.75-7.77ppm (1H,m), and δ=11.11 ppm (1H,n) corresponding total 38 proton. Also performed ^{13}C experiment which shows total 28 carbons in which four are methyl carbon, eight are methylene carbon, seven are methane carbon and nine are quaternary carbon. The four methyl group shows signals at 29.39 ppm for 2 carbons, 29.44 ppm for 2 carbon seven methane groups shows NMR signals at 111.1 ppm, 111.4 ppm, 120.6 ppm, 121.5 ppm, 124.4 ppm, 125.7 ppm, 128.3 ppm. Nine quaternary carbon showing signals at 106.4 ppm, 120.7 ppm, 122.11 ppm, 126.6 ppm, 127.8 ppm, 127.81, 136.2 ppm, 137.3 ppm and 137.4 ppm.

This isolated pure fraction of unknown impurity ~98 % at RT about 21 was subjected to LC-MS/MS analysis. The positive ion of unknown impurity showed $[M+H]^+$ ions peak at m/z 587.24701 (MS-RT 29.99 min), Figure 10 indicating the mass of this unknown impurity to be 586.24. LC/MS/MS spectrum for mass m/z 587.24701 displayed daughter ion peaks at m/z 263.08, 292.11, 308.14 and

587.246.

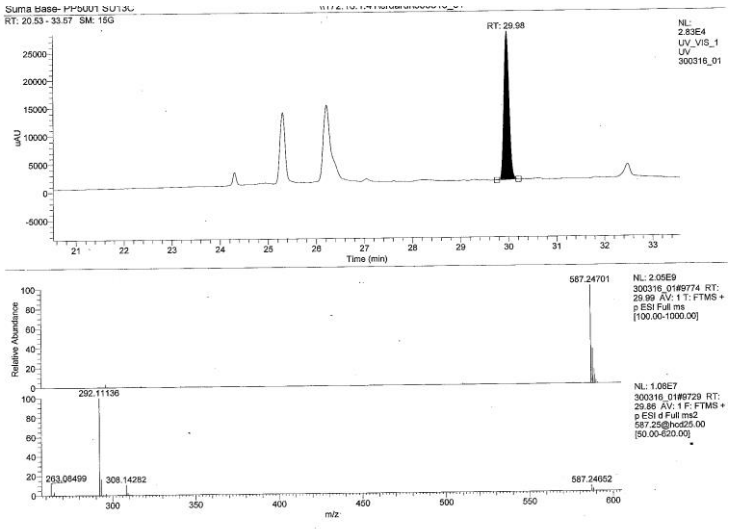


Figure 10: LC-MS/MS of degraded unknown impurity MS-RT 29.9

Above spectral data confirmed molecular formula of unknown impurity as $C_{28}H_{38}N_6O_4S_2$. Based on the spectral data the structure of unknown impurity confirmed as Figure 11.

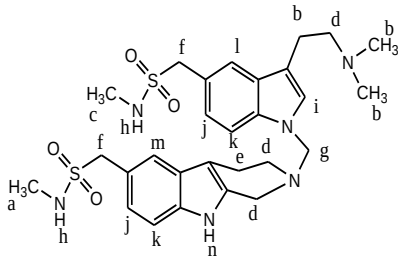


Figure 11: The structure of unknown impurity

This unknown impurity on degradation result in to two impurities one as Sumatriptan impurity F and another as Sumatriptan base (Figure 12).

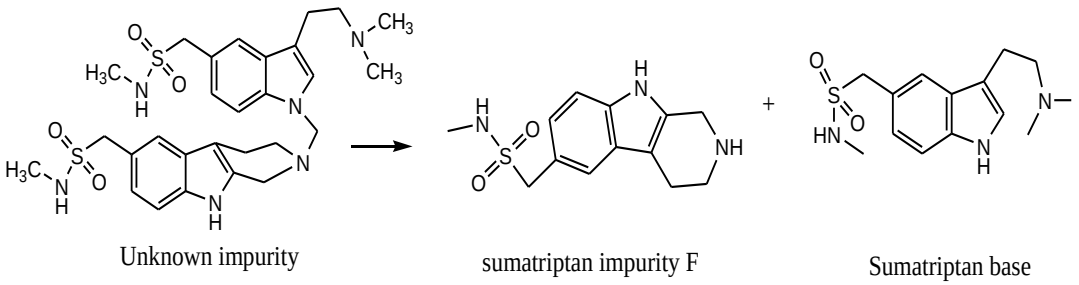


Figure 12: Chemical structure of Unknown imp and its degradations into Suma imp F and Sumatriptan base

The fragmentation pattern of above proposed structure of unknown impurity matches with the LC-MS data (Figure 13).

Hence the structure of unknown impurity confirmed as 1-(3-(2-(dimethylamino)ethyl)-1-((6-((-*N*-methylsulfamoyl)methyl)-1,3,4,9-tetrahydro-2*H*-pyrido[3,4-b]indol-2-yl)methyl)-1*H*-indol-5-yl)-*N*-methylmethanesulfonamide.

Pathway of the Sumatriptan impurity formation

The first step is the formation of 1-(3-(2-aminoethyl)-1H-indol-5-yl)-N-methylmethanesulfonamide (AIMS) by simple addition of 1-(4-hydrazinylphenyl)-N-methylmethanesulfonamide hydrochloride (HMMS.HCl) and sodium 4-chloro-1-hydroxybutane-1-sulfonate (CHBS). Methylation of AIMS using $NaBH_4$ and Formalin yielded 1-[3-[2-(dimethylamino)ethyl]-1H-indol-5-yl]-N-methylmethanesulfonamide (Sumatriptan base) with unknown impurity at about RT 20.49. The formation of Sumatriptan base and unknown impurity is shown in reaction (Scheme 1).

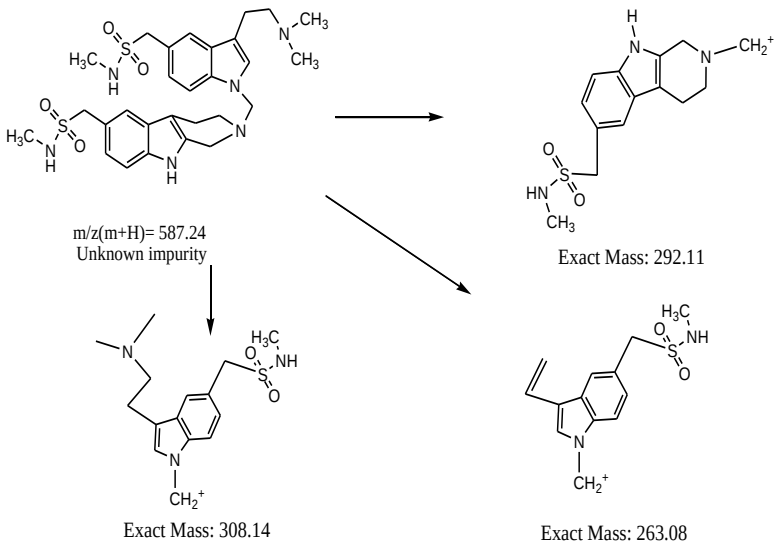
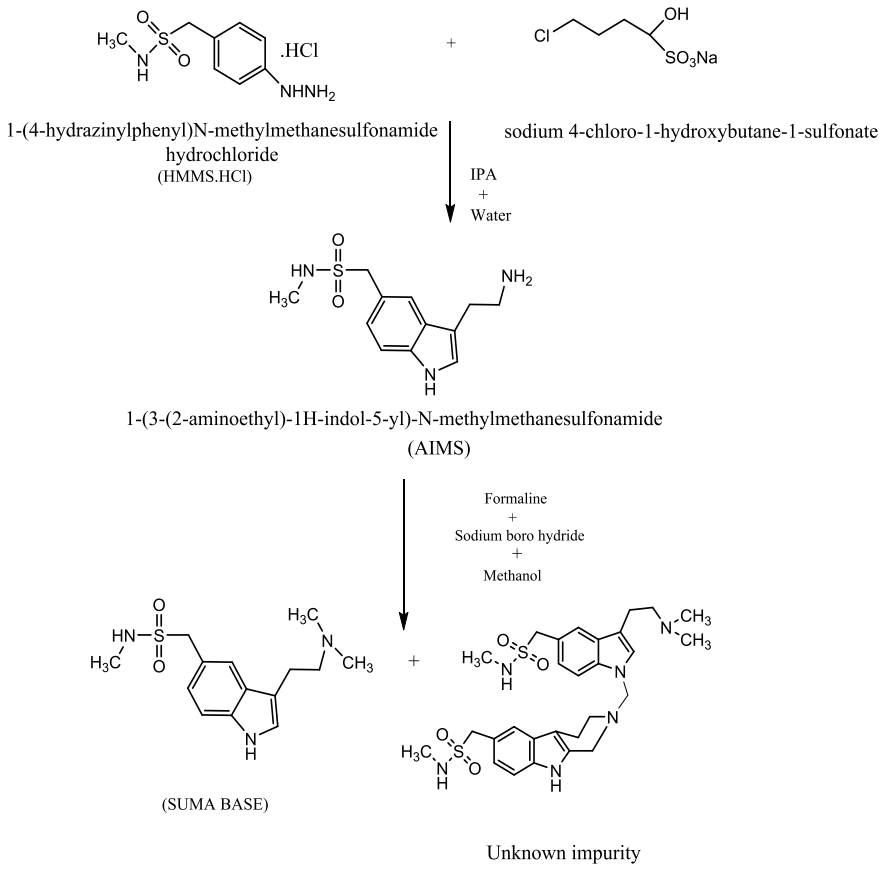


Figure 13: Fragmentation pattern of unknown impurity



Scheme 1: Formation of unknown impurity

CONCLUSION

The present investigation confirms the structure of unknown impurity as 1-(3-(2-(dimethylamino)ethyl)-1-((6-((-*N*-methylsulfamoyl)methyl)-1,3,4,9-tetrahydro-2*H*-pyrido[3,4-b]indol-2-yl)methyl)-1*H*-indol-5-yl)-*N*-methylmethanesulfonamide which degrade into two impurities as Sumatriptan impurity F and Sumatriptan base.

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REFERENCES

[1] A. Sanz, A.M.G. Carril, M.P. Matía, J.L. Novella, J. Alvarez-Buillab, ARKIVOC, **2008**, 53-58.
[2] C.J. Derry, S. Derry, R.A. Moore, **2014**, 5 CD009108.
[3] British Pharmacopoeia **2017**, Volume II, 998.
[4] USP, United states Pharmacopoeia 39 and NF-34, **2016,** 3, 5969, 5971, 5973, 5975.
[5] ICH guideline, Q3A (R2), **2006**.
[6] Z. Ge, E. Tessier, L. Neirinck, Z. Zhu. *J. Chromatog. B,* **2004,** 806, 2, 299.
[7] R.J. Majithiya, J.B. Majithiya, Manish L Umrethia, Murthy R S Rayasa. *Ars. Pharmaceutica.,* **2006**, 47, 2, 199.
[8] http://www.dionex.com/en-us/webdocs/5455-AN504_LPN1429.pdf
[9] Y. Watanabe, H. Tanaka, I. Dan, K. Sakurai, K. Kimoto, R. Takashima, K. Hirata. *Neurosci. Res.*, **2011,** 69, 60.
[10] S. Narendra, V. Pfaffenrath, L. Rice, C. Stat, D. Boswell, L. Black, M. Jones, *Clin. Therapeut.*, **2001**, 23, 2, 260.
[11] S. Jeong Ju, P. Jeonghyeon, B. Min Ho, L. Mi-sun, S. Sook Jin, L. Joomi, P. Sung Min, L. Hae Won, Y. Young-Ran, *J. Chromatog. B.*, **2013,** 919-920, 38.
[12] EP, European Pharmacopoeia, **2014,** Volume II, 3352.