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Isolation and characterization of the zolmitriptan unknown impurity by chromatographic and mass spectroscopy

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ABSTRACT

The control of pharmaceutical impurities is currently a critical issue to the pharmaceutical industry. In Zolmitriptan tablet one unknown impurity was observed above identification threshold as mentioned in ICH guidelines. This unknown product was analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS) to determine its molecular weight. Comparison of fragmentation pattern of the protonated species of unknown impurity and Zolmitriptan were made us to determine the possible structure for impurity and its fragments. A detailed study was done to characterize the impurity and it was further synthesized, subsequently characterized and was co injected with the sample and was found to be co-eluting with the impurity in the sample. Impurity was subsequently isolated using preparative scale chromatography and its structure was confirmed using MS/MS derived structural information combined with ^{1}H proton, ^{13}C-NMR & IR analysis. Based on the spectral data impurity was characterized as (4-4-[[3-[2-(Dimethyloxidoamino) ethyl]-1H-indol-5-yl] methyl-2-oxazolidinone).

KEYWORDS

Zolmitriptan;
Stability studies;
Mass spectroscopy;
Impurity identification;
Impurity isolation;
Impurity characterization.

INTRODUCTION

Impurity profiling, i.e. identification, structure elucidation and quantitative determination of impurities and degradation products in bulk drug materials and pharmaceutical formulations is one of the most important fields of activities in modern pharmaceutical analysis. The reason for the increased importance of this area is that unidentified, potentially toxic impurities are health hazards and in order to increase the safety of drug therapy, impurities should be identified and determined by selective methods[1]. To assure the quality, safety and efficacy of the drug product stability testing is the primary tool. Regulatory authorities in each country use their own standards for stability testings of drug product[2]. Efforts are being made to unify these approaches, as exemplified by International Conference on Harmonization (ICH) guidelines for stability testing for drug substance and drug product ICH Q1A(R2)[3]. Physical, Chemical, biological and microbiological testing of drug substance are performed to assess stability. One of the evaluation criteria for evaluation of stability of drug product through out its shelf life is the appearance of impurities in formulation during accelerated and long

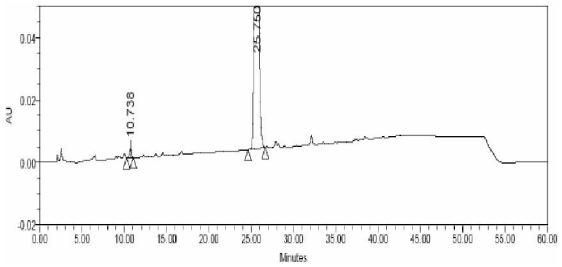


Figure 1 : HPLC Chromatogram of stability sample of lansoprazole delayed release capsule

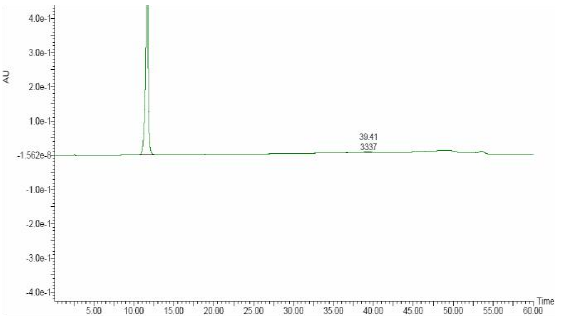
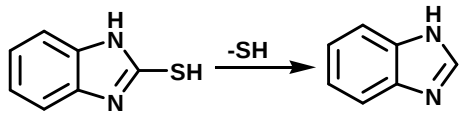


Figure 3 : HPLC chromatogram of isolated impurity

term stability studies.

Zolmitriptan- [4-({3-[2-(dimethylamino) ethyl]-1*H*-indol-5-yl}-1, 3-oxazolidin-2-one] is a synthetic tryptamine derivative (Figure 1). It is a potent selective serotonin receptor agonist used in the treatment of acute migraine attacks[4]. In Zolmitriptan tablets containing 2.5 mg of active substance one unknown impurity comes at 0.30% level, which was crossing the limit for identification threshold as mentioned in ICH Q3 A(R2) guidelines[5]. HPLC retention time of the unknown impurity did not coincide with any available impurity standard of Zolmitriptan. Several methods have been reported for the determination and analysis of Zolmitriptan in bulk drug, dosage form and biological fluids[6-8]. Few spectrophotometric methods for the determination of impurities in Zolmitriptan were described earlier[9-11]. An LC-MS method has been described for the determination of Zolmitriptan in plasma[12].

In the present paper, we describe the identification of unknown impurity of Zolmitriptan in drug product using HPLC-ESI-MS. Positive ion mode was employed to obtain the protonated $[M+H]^+$ ions of the molecular species and the fragments. This allowed us to propsed fragmentation pathway of the impurity. Novelty of the present work is to identify impurity by LCMS/MS, synthesis of impurity in reasonable yield followed by isolation on preparative Liquid Chromatography and characterization of impurity by MS/MS, IR and ^1H & ^{13}C-NMR techniques.



Formula weight =150.202 **Formula weight =118.136**

Figure 2 : Mechanism for the formation of fragment ion from impurity

EXPERIMENTAL

Samples and chemicals

The investigated samples of an Experimental Formulation of Zolmitriptan film coated tablets were obtained from formulation R&D Department, Jubilant Organosys Ltd., Noida, India. LC-grade water (resistivity less than 18.0 MΩ cm at 25°C) was prepared by purifying distilled water with a Milli-Q water purification system from Millipore (Malsheim, France). Methanol and Acetonitrile (gradient grade for chromatography) and AR grade ammonia solution was purchased from s d fine-chem limited (SDFCL-Mumbai, India). AR grade Trifluoroacetic acid was procured from Spectrochem Pvt. Ltd. (Mumbai, India). Ammonium Formate was purchased from Merck India Limited. Formic acid was purchased from RFCL Ltd. (New Delhi, India).

Instrumentation

Samples of Zolmitriptan coated tablets were analyzed on a waters Alliance 2690 HPLC system equipped with Waters 2487 UV detector. Wavelength of detection for analysis was 225nm. LC-MS/MS analysis was carried out on Water Alliance 2690 Liquid chromatograph coupled with Q-Tof Micromass system (Waters). Positive Electron Spray ionization (ESI mode) technique was used for the analysis of samples. Capillary voltage was maintained at 4000 V, Sample cone voltage at 30V and Extraction cone voltage at 4V. Nitrogen was used as both desolvation and nebulizing gas. Cone gas flow maintained at 50L/hr and desolvation gas flow maintained at 500L/hr. MS/MS studies were carried out by maintaining Collision Energy at 15 and mass range 100-1000amu. The purification of impurity was carried out using preparative LC (Agilent Tech-

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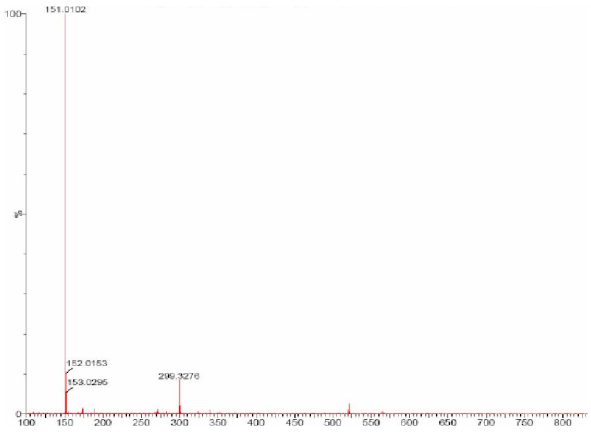

Figure 4(a) : Mass spectrum of impurity in ES+ve mode

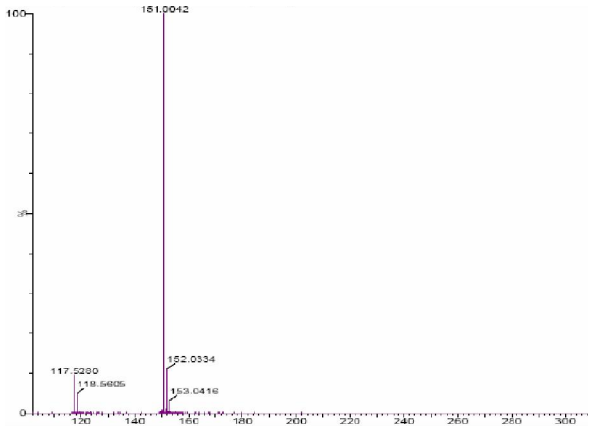

Figure 4(b) : MS/MS spectrum of impurity in ES+ve mode

nologies-1200 series Waldbrom, Germany) equipped with binary gradient pump, multiwavelength detector, sample manager and fraction collector. The ^{1}H and ^{13}C NMR spectrum was recorded on Bruker 400 MHz instrument. The IR Spectra of isolated impurity was recorded in the solid state as KBr powder disc using Thermo Electron, Nicolet Avatar 370 DTGS FT-IR spectrometer.

RESULT AND DISCUSSION

Method development for impurity identification using LC-UV and MS/MS

For the identification of unknown impurity X Terra RP-18 Column 250mm×4.6mm, with 5µm particle size (Waters, USA) was used for Chromatographic separation. Flow rate of mobile phase was maintained at 1.0mL/min. and injection volume was 10µL. Mobile phase A used for separation was 1mL TFA in 1 liter of water (pH 9.85 with ammonia solution) & Mobile phase B consist of 1mL TFA in mixture of Acetonitrile-Methanol (pH 9.85 with ammonia solution) (850:150, v/v). Binary Gradient program was used and method was able to detect all the impurities. Following gradient was applied % Mobile Phase B (time, min): 10(0), 10(10), 60(40), 10(45), 10(55). Apart from the Zolmitriptan peak observed at RT 27.1 min. unknown impurity peak elutes at 11.4 min. A typical chromatogram is shown in figure 2.

Further investigation was done to identify and characterize the unknown impurity using High performance liquid chromatography coupled with Q-Tof mass spectrometer.

Since the mobile phase employed for LC-UV analysis of samples of Zolmitriptan tablets consisted of volatile buffer, same was used for LC-MS analysis and all the conditions used for HPLC –UV analysis were employed for LC-MS/MS analysis also using same gradient program. Mass spectral data of Zolmitriptan showed a molecular ion peak $[M+H]^+$ at m/z 288 (molecular mass of Zolmitriptan is 287). The MS/MS spectrum for the protonated Zolmitriptan molecule showed fragment at m/z 243. Formation of daughter ion peak of m/z 243 attributed to the loss of N, N Dimethyl Amine from the Zolmitriptan (Mass spectra of Zolmitriptan and fragmentation behavior Showed in figure 3).

Mass spectra of unknown impurity showed molecular ion peak $[M+H]^+$ at m/z 304 and fragment of m/z 243amu was also obtained with the impurity (Figure 4). This showed that the impurity had structural similarity to the Zolmitriptan and had mass 16amu more than the Zolmitriptan moiety. As fragment of m/z 243 was also obtained with the impurity it showed that there was addition of oxygen to the N, N Dimethylamine group (Mass spectra of impurity and its proposed fragmentation behavior Showed in figure 4).

Synthesis of impurity

Impurity was synthesized by oxidation of Zolmitriptan using meta Chloro perbenzoic acid. 1g of Zolmitriptan API was dissolved in 100 mL of DCM in a round bottom flask. To it 1g of meta Chloro perbenzoic acid was added while maintaining the reaction condition below 0°C and the solution was stirred for 3 hrs. on magnetic stirrer. After 3 hrs. appox. 3g of sodiumbicarbonate was added to it and the so-

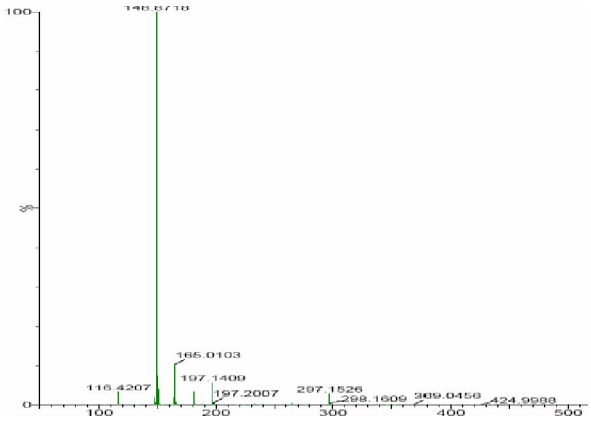

Figure 5 : Mass spectrum of impurity in ES-ve mode

lution was filtered and the solid precipitate left above was further washed with about 100mL of DCM. Filtrate was evaporated on rotavapor under vaccum at 35°C to get solid mass. Rxn mass so obtained contain appox. 40% of the required impurity. Retention time, MS and MS/MS data of the synthesized impurity matched well with the impurity coming in the samples. As the purity of the impurity was 40% in the reaction mass it was further purified on preparative LC to get pure fraction. Proposed synthetic scheme for impurity (see Figure 5).

Method development for impurity isolation using preparative LC

To isolate the impurity from Zolmitriptan reaction mass a newly developed reverse phase chromatographic method was employed using Agilent 1200 Series Auto purification System Consisting of binary gradient pump, multiwavelength detector, sample manager and fraction collector (Agilent Technologies Waldbrom, Germany). Waters symmetry C18 column 300mm×19mm i.d, particle size 7µ was used for the separation. Mobile phase A used for isolation was Ammonim Formate (pH 4.0; 10mM) and Mobile Phase B used was Methanol. Following gradient was applied % Mobile Phase B (time, min): 10(0), 25(10), 25(15), 80(25). Flow rate was maintained at 30mLmin^{-1}. Detection was monitored at 225nm. Injection volume for preparative LC was 5000µL. The retention time for Zolmitriptan and unknown impurity were observed at 9.0 min. and 11.8 min., respectively. The collected fractions were combined and concentrated on rotavapor at 35°C and dried under high vacuum using lyophilizer to obtain solid product. Chromatographic purity of the isolated impurity sample was determined by HPLC and found to be 98%, respectively as shown in figure 6. This sample was used further for spectroscopic studies.

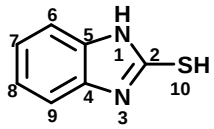

Figure 6 : Structure of impurity 1*H*-benzimidazole-2-thiol

TABLE 1 : NMR spectral assignments for impurity

Position	No. of Protons	Proton chemical shift	^{13}Chemical shift
1	1	12.5	-
2	-	-	168.5
3	-	-	-
4	-	-	132.6
5	-	-	132.6
6	1	7.1	109.9
7	1	7.0	122.7
8	1	7.0	122.7
9	1	7.1	109.9
10	1	3.3	-

Structure elucidation of impurity

The isolated impurity was subjected to structural analysis using MS, LCMS/MS, ^{1}H and ^{13}C NMR and FTIR spectroscopic methods. IR spectrum of impurity exhibited a broad peak at 3418cm^{-1}. Additionally one signal was observed in the proton NMR spectrum at 10.2ppm. This observation indicates the presence of indole NH group in the proposed structure. Presence of fragment of 243 in the MS/MS spectrum of the impurity added further support to the conclusion that indole nucleus of the Zolmitriptan is also present in the impurity. One sharp peak was observed at 1631cm^{-1} due to >C=O stretching vibration and the presence of same was further confirmed by signal at 159 ppm in ^{13}C spectrum of impurity. Aliphatic asymmetric and symmetric stretching >C-H stretching appeared in the range 2817-2924cm^{-1}. Detailed description of ^{1}H and ^{13}C is given in TABLE 1. Taken together all these data support the structure of impurity that was proposed on the basis of MS/MS data. Based on the above spectral data the molecular formula of impurity was confirmed and the corresponding structure was characterized as (4-4-[[3-[2-(Dimethyoxidoamino)ethyl]-1H-indol-5-yl]methyl-2-oxazolidinone).

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CONCLUSION

The study of impurity profile of Zolmitriptan has been carried out by LC/MS and LC/MS/MS. Preliminary structure assignments for the unknown impurity was made on the basis of mass spectral data. On the basis of proposed structure it was synthesized and the complete characterization of the compound was carried out by various spectroscopic studies after preparative chromatographic isolation.

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REFERENCES

[1] Bartos Dorottya, Gorog Sandor, Current Pharmaceutical Analysis, **4**, 215-230 **(2008)**.
[2] S.Ahuja; 'Impurities Evaluation of Pharmaceuticals', New York, Marcel Dekker, **(1998)**.
[3] ICH Topic Q1A (R_2) Stability Testing of New Drug Substance and Products, Proceedings of the International Conference on Harmonization, London, **(2003)**.
[4] R.Yates, K.Niarn, R.Dixon, J.V.Kemp; J.Clin. Pharmacol., **42**, 1244-1250 **(2002)**.
[5] ICH Topic Q1A (R_2) Stability Testing of New Drug Substance and Products, Proceedings of the International Conference on Harmonization, London, **(2003)**.
[6] Jun Chen, Jiang Xin Guo, Jiang Wen Ming, Ni Mei, Gao Xiao Ling, Zhang Oi Zhi; J.Pharmaceutical and Biomed.Anal., **35**, 639-645 **(2004)**.
[7] Z.Aydogmus, I.Inanli; J.AOAC Int., **90**, 1237-1241 **(2007)**.
[8] B.M.Rao, M.K.Srinivasu, G.Sridhar, P.R.Kumar, K.B.Chandrasekhar, A.Islam; J.Pharm.Biomed. Anal., **39**, 503-9 **(2005)**.
[9] E.M.Clement, M.Franklin; Journal of Chromatography. B., **766**, 339-343 **(2002)**.
[10] M.K.Srinivasu, B.Mallikarjuna Rao, G.Sridhar, P.Rajender Kumar, K.B.Chandrasekhar, A.Islam; J.Pharmaceutical and Biomed.Anal., **37**, 453-460 **(2005)**.
[11] Y.Z.Hu, T.W.Yao, X.J.Wang; Journal of Zhejiang Univ., Medical Sciences, **33**, 37-40 **(2004)**.
[12] Zunjian Zhang, Fengguo Xu, Yuan Tian, Wei Li, Guoguang Mao; J.Pharmaceutical and Biomed. Anal., **813**, 227-233 **(2004)**.