International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (3): 1269-1275
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Pharmaceutical Sciences | Open Access | MCI Approved
UGC Approved Journal

Isolation and Characterization of Zolmitriptan Unknown Impurity by Chromatographic and Mass Spectroscopy

G.P. Narasimha Reddy[1*], T. Srinivasulu Reddy[1], Vijay Kumar. R[2] and P. Raghuram[3]

[1]Department Of Chemistry, Sri Krishnadevaraya University, Anantapur, A.P., India.
[2]Appco Pharma LLC, Somerset, Newjersey, USA.
[3]Quality Assurance, Regulatory Affairs and Pharmacovigilance, Sciegen Pharmaceuticals Inc., NY, USA.

Received: 18 Mar 2019 / Accepted: 20 Apr 2019 / Published online: 1 Jul 2019
***Corresponding Author Email:** narasimhagp@gmail.com

Abstract

One unknown impurity in Zolmitriptan film coated tablet was detected during the stability studies by a simple High Performance liquid chromatographic method whose percentage area was ranged from 0.30 to 0.50 % in different stability batches. 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 allow us to proposed 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 and 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. 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 testing's of drug product. 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) [2]. 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 throughout its shelf life is the appearance of impurities in formulation during accelerated and long term stability studies.

Zolmitriptan-[4-({3-[2-(dimethyl amino) ethyl]-1*H*-ind ol-5-yl}-1,3-oxazolidin-2-one] is a synthetic tryptamine derivative [Fig.1.]. It is a potent selective serotonin receptor agonist used in the treatment of acute migraine attacks [1]. During the stability testing of Zolmitriptan Film coated 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 [2]. Zolmitriptan is used to treat migraines [3]. It helps to relieve headache, pain, and other migraine symptoms (including nausea, vomiting, sensitivity to light/sound) [4]. Prompt treatment helps you return to your normal routine and may decrease your need for other pain medications. HPLC retention time of the unknown impurity did not coincide with any available impurity standard of Zolmitriptan [5-8]. Several methods have been reported for the determination and analysis of Zolmitriptan in bulk drug, dosage form and biological fluids [5]. Few spectrophotometric methods for the determination of Impurities in Zolmitriptan were described earlier [6-10]. An LC-MS method have been described for the determination of Zolmitriptan in plasma [7].

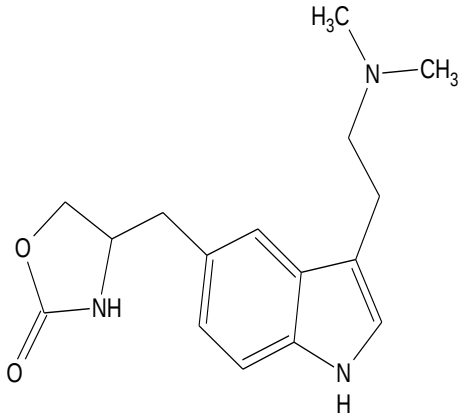

Fig. 1: Structure of Zolmitriptan

Objective:

The main objective of this study was to identify the unknown impurity in zolmitriptan product through isolation, identification and characterization. In the present work, 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 proposed 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.

EXPERIMENTAL METHOD

Samples and Chemicals

The investigated stability 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

Stability samples of Zolmitriptan film coated tablets were analyzed on a waters Alliance 2690 HPLC system equipped with Waters 2487 UV detector. UV spectra of all the peaks were recorded at 225 nm. 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 30 V and Extraction cone voltage at 4V. Nitrogen was used as both desolvation and nebulizing gas. Cone gas flow maintained at 50 L/hr and desolvation gas flow maintained at 500L/hr. MS/MS studies were carried out by maintaining Collision Energy at 15 and mass range 100-1000 amu. The purification of impurity was carried out using preparative LC (Agilent Technologies-1200 series Waldbrom, Germany) equipped with binary gradient pump, Multi wavelength 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 x 4.6mm, with 5µm particle size (Waters, USA) was used for Chromatographic separation. Flow rate of mobile phase was maintained at 1.0 mL /min. and injection volume was 10µL. Mobile phase A used for separation was 1 mL TFA in 1 l of water (pH 9.85 with ammonia solution) & Mobile phase B consist of 1 mL 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 Fig. 2.

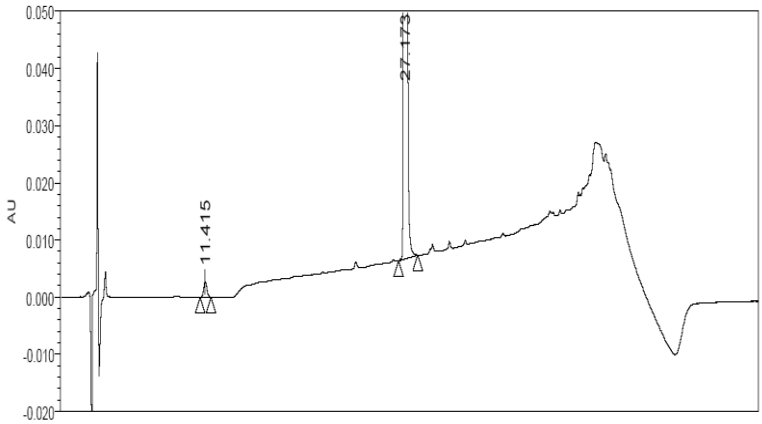

Fig.2: HPLC Chromatogram of Zolmitriptan Stability sample

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 stability samples of Zolmitriptan film coated 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 Fig. 3.).

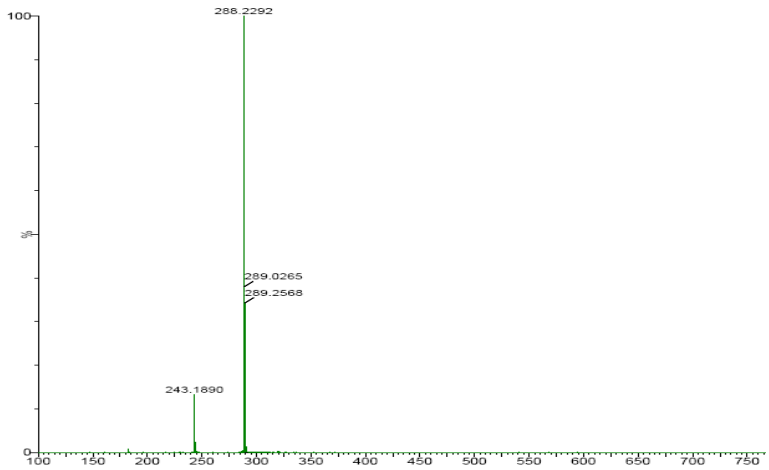

Fig.3a LC/MS/MS spectra of Zolmitriptan

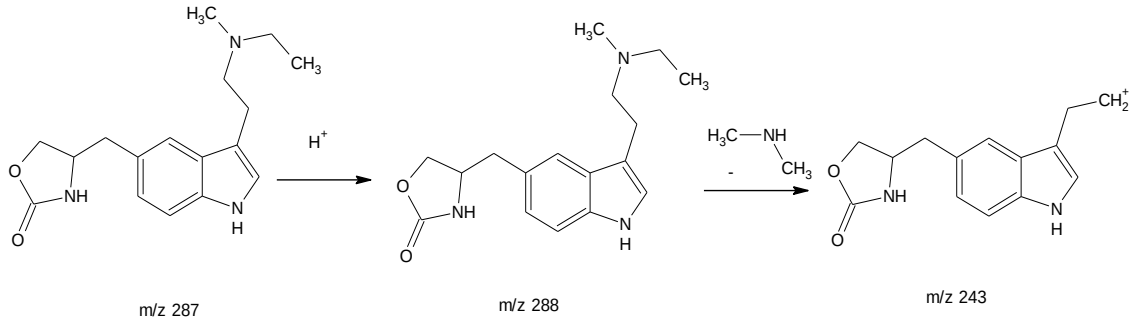

Fig.3b: Proposed fragmentation mechanism for product ion of Zolmitriptan

Mass spectra of unknown impurity showed molecular ion peak $[M+H]^+$ at m/z 304 and fragment of m/z 243 amu was also obtained with the impurity (Fig. 4.). This showed that the impurity had structural similarity to the Zolmitriptan and had mass 16 amu 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 Fig. 4.).

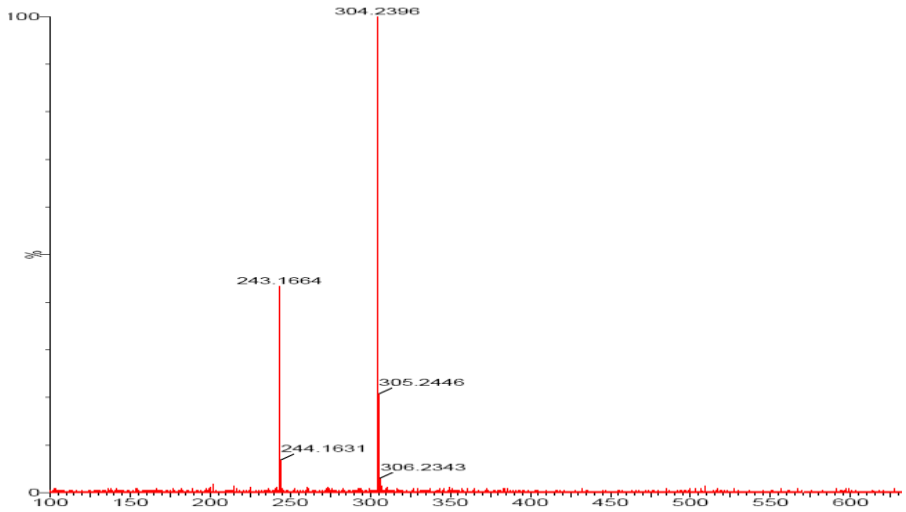

Fig.4a: LC/MS/MS spectra of impurity

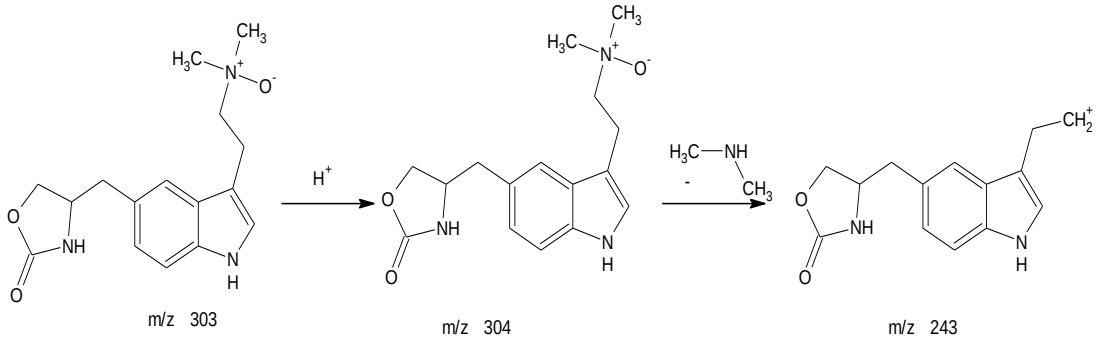

Fig.4b: Proposed fragmentation mechanism for product ion of impurity

Synthesis of Impurity:

Impurity was synthesized by oxidation of Zolmitriptan using mCPBA. 1g of Zolmitriptan API was dissolved in 100 mL of DCM in a round bottom flask. To it 1 g of mCPBA 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. 3 g of sodium bicarbonate was added to it and the solution was filtered and the solid precipitate left above was further washed with about 100 ML of DCM. Filtrate was evaporated on rotavapor under vacuum 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 stability 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 fig. 5).

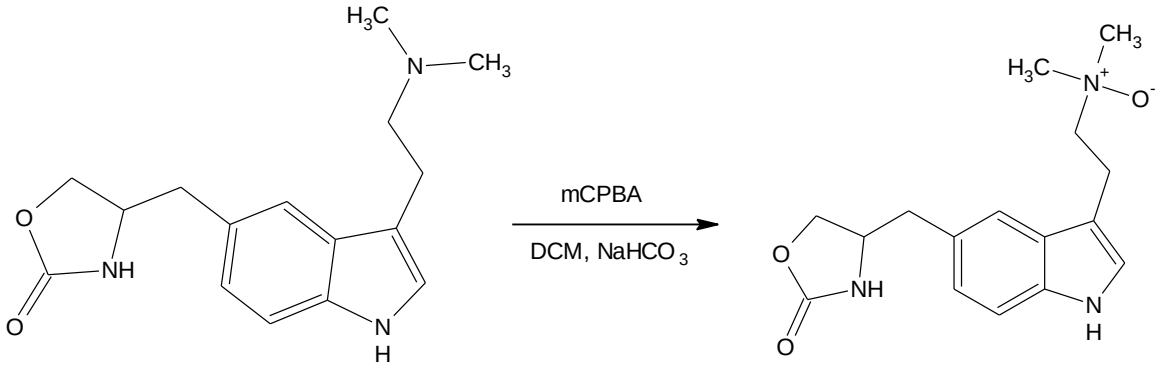

Fig.5: Scheme for synthesis of impurity

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 x 19 mm i.d, particle size 7µ was used for the separation. Mobile phase A used for isolation was Ammonim Formate (pH 4.0; 10 mM) 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 30 $mLmin^{-1}$. Detection was monitored at 225 nm. 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 fig.6. This sample was used further for spectroscopic studies.

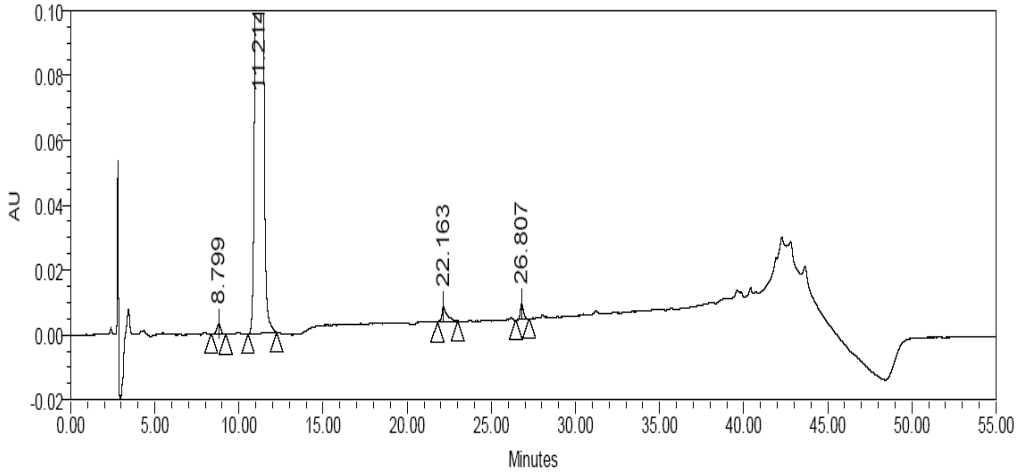

Fig.6: HPLC Chromatogram of isolated lyophilized impurity

Structure elucidation of impurity:

Table 1: NMR spectral assignments for impurity

Position	No. of Protons	Proton Chemical Shift	13C 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	-

Refer the structural formula for numbering (Fig. 6).

CONCLUSION

Zolmitriptan unknown impurity was isolated and identified as per the scientific characterization approach. Degradation path was predicted and detail discussion was represented in results and discussion. In this study 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, NMR spectral data. Unknown impurity was within the ICH guidance acceptable limit. Identified impurity and Zolmitriptan was well separated and quantified.

REFERENCES

1. E.L. Spierings, J.L. Brandes, D.B. Kudrow, J. Weintraub, P.C. Schmidt, D.J. Kellerman, S.J. Tepper. Randomized, double-blind, placebo-controlled, parallel-group, multi-center study of the safety and efficacy of ADAM zolmitriptan for the acute treatment of migraine. *Cephalalgia*. 38(2):215-24 (2018). DOI: 10.1177/0333102417737765
2. Impurities in new drug products Q3B(R2), *ICH guidelines*, 2006, Step-4 version, 1-14.
3. R. Kumria, B.E. Al-Dhubiab, J. Shah, A.B. Nair. Formulation and evaluation of chitosan-based buccal bio adhesive films of zolmitriptan. *Journal of Pharmaceutical Innovation*. 13(2): 133-43 (2018). DOI: 10.1007/s12247-018-9312-6
4. S.J. Tepper, D.W. Dodick, P.C. Schmidt, D.J. Kellerman. Efficacy of ADAM Zolmitriptan for the Acute Treatment of Difficult-to-Treat Migraine Headaches. Headache: *The Journal of Head and Face Pain*. 509-517 (2019). DOI: 10.1111/head.13482
5. J. Nguyen, H. Lewis, A. Queja, A.N. Diep, G. Hochart, M. Ameri. Pharmacokinetics and Skin Tolerability of Intracutaneous Zolmitriptan Delivery in Swine Using Adhesive Dermally Applied Microarray. *Journal of pharmaceutical sciences*. 107(8):2192-7 (2018). DOI: 10.1016/j.xphs.2018.05.001

6. P. Girotra, S.K. Singh, G. Kumar. Development of zolmitriptan loaded PLGA/ poloxamer nanoparticles for migraine using quality by design approach. *International journal of biological macromolecules*. 85:92-101 (2016). DOI: 10.1016/j.ijbiomac.2015.12.069
7. C.T. Uppuluri, J. Devineni, T. Han, A. Nayak, K.J. Nair, B.R. Whiteside, D.B. Das, B.N. Nalluri. Microneedle-assisted transdermal delivery of Zolmitriptan: effect of microneedle geometry, in vitro permeation experiments, scaling analyses and numerical simulations. *Drug development and industrial pharmacy*. 43(8):1292-303 (2017). DOI: 10.1080/03639045.2017.1313862
8. S. Maddela, B.N. Nalluri. Development of zolmitriptan mouth dissolving films: formulation variables, mechanical properties, and in vitro drug release studies. development. 12(4), 273-279 (2019).
9. B. Patel, B.N. Suhagia, A.G. Jangid, H.N. Mistri, N. Desai. Systematic evaluation of matrix effect and cross-talk-free method for simultaneous determination of zolmitriptan and N-desmethyl zolmitriptan in human plasma: a sensitive LC-MS/MS method validation and its application to a clinical pharmacokinetic study. *Biomedical Chromatography*. 30(3):447-58 (2016). DOI: 10.1002/bmc.3568
10. N.S. Dighe, S.K. Hodgar, D.S. Musmade. Development and Validation of RP-HPLC Method for the Identification of Process Related Impurities of Zolmitriptan. *J Anal Pharm Res*. 4(1):00094 (2017). DOI: 10.15406/japlr.2017.04.00094