IDENTIFICATION, ISOLATION AND CHARACTERIZATION OF UNKNOWN IMPURITY IN ASENAPINE MALEATE API BY HPLC AND MASS SPECTROMETER

Santosh Kumar[1*], Swati Goyal[2]

Article History: Received: 12.12.2022 **Revised:** 29.01.2023 **Accepted:** 15.03.2023

Abstract

The term impurity reflects unwanted chemicals that are present in APIs or that develop during formulation or upon aging of the API in the formulated drug product. The presence of such unwanted material, even in small amounts, could affect the efficacy and safety of pharmaceutical products. Impurities are substances that are present in small quantities in another substance and make it dirty or of an unacceptable quality. Moreover they might have adverse effects also. Identification, isolation and characterization of unknown impurity in Asenapine maleate Drug Substancesdone by HPLC, mass spectrometer and NMR. After thermal forced degradation of Asenapine maleate Drug Substances, a major unknown impurity observed in at RRT 2.0 with respect to Asenapine maleate in HPLC method. A chromatographic method with C8 column, ammonium acetate in water, acetonitrile and methanol as the mobile phase with gradient elution has been developed for LC-MS. Mass spectrometric studies of impurity conducted in electro spray ionization (ESI) mode.The ESI mass spectrum of this impurity at RRT 2.0 showed a protonated molecular ion [M+H]+ at m/z 282. The impurity isolated from preparative HPLC to get pure form and further molecular weight determined from the mass spectrometer and NMR studyperformed. From the NMR data and mass spectra, the structure of impurity elucidated and proven that the impurity formed from Asenapine maleate only. Asenapine maleate unknown thermal degradation impurity was confirmed as 5-chloro-2-methyl-2H-dibenzo[2,3:6,7]oxepino[4,5-c]pyrrole.This study and its findings will aid in ensuring the quality and safety of Asenapine maleate Drug Substances and Drug Products.

Keywords: Impurities, Chromatography, Structural Elucidation, LC-MS/MS, NMR.

[1*2]Department of Chemistry, Dr. A. P. J. Abdul Kalam University, Indore, Madhya Pradesh

Corresponding Author Email: santosh.chauhan81@gmail.com

DOI: 10.31838/ecb/2023.12.s3.002

1. Introduction

Asenapine is a second generation (atypical) antipsychotic agent that is taken sublingually and used in the treatment of schizophrenia and manic or mixed episodes associated with bipolar 1 disorder. Asenapineis associated with a low rate of transient and mild serum aminotransferase elevations during therapy, but has not been linked to instances of clinically apparent acute liver injury. Asenapine (a sen' a peen) is a second generation antipsychotic agent which appears to act as a dopamine type 2 (D2) and serotonin (5-HT)-2A receptor antagonist. It is a somewhat unique antipsychotic agent that has a tetracyclic structure similar to that of mirtazapine, and it is administered as a sublingual tablet, being poorly absorbed by the oral route. Several randomized controlled trials have shown that sublingual Asenapine improves symptoms of schizophrenia with effects comparable to risperidone and olanzapine. It also has beneficial activity in acute manic and mixed episodes associated with bipolar 1 disorder. Asenapine was approved for use in the United States in 2009 and is available in sublingual tablets of 2.5mg, 5mg and 10 mg under the brand name Saphris. The typical maintenance dose in adults is 2.5mg to 10 mg twice daily. Common side effects of include dizziness, somnolence, fatigue, nausea, anxiety, restlessness (akathisia) and weight gain. Rare, but potentially severe adverse reactions (mentioned in most antipsychotic and antidepressant product labels) include tardive dyskinesia, major neurologic events, neuroleptic malignant syndrome, orthostatic hypotension, seizures, neutropenia, hypersensitivity reactions, prolongation of the QTc interval and suicidal ideation or behavior[1].

Forced degradation is a process that involves degradation of drug products and drug substances at conditions more severe than accelerated conditions and thus generates degradation products that can be studied to determine the stability of the molecule. Forced degradation is an essential study that provides the knowledge and judgement necessary to develop a stability-indicating analytical method[2]. This study also helps to establish the specifications and shelf life of a drug substance or drug product[3]. Few analytical methods for identifying impurities and degradation products of drug substances and drug products have been developed [4,5] and compared[6]. As per the ICH guidelines Q3A (R2) requirement all impurities (from processing and degradation) to be identified above the specification limit[7].AsAsenapine maleate is a novel drug recently approved by the Food and Drug Administration for treatment of acute schizophrenia and for manic or mixed episodes of bipolar I disorder with or without psychotic features in adults and children aged 10–17 years.It is the antagonistic activity at the D2 receptor that is likely responsible for the antimanic properties of asenapine. Clinical trials have demonstrated that asenapine mono- and add-on therapy is effective in the short- and long-term treatment of mania associated with bipolar I disorder in adult and paediatric patients[8]. Hence, for identification of these impurities a sensitive, specific, robust and accurate method required. Few methods available for the analysis of analysis of Asenapine through RP-HPLC method[9,10].To consider these things, concentration is given to identifying unknown Asenapine degradation impurity using chromatographic systems HPLC with diode array detection (LC-DAD), mass spectrometer (MS), and NMR. In the current study,we have performed forced degradation and mass analysis with a robust, sensitive and specific method.

2. Experimental

2.1 Materials and Chemicals

HPLC-grade acetonitrile purchased from Thermo Fisher Scientific (Fair Lawn, NJ). A Milli-Q water purification system (Millipore, Billerica, MA) used for water.Trimethylamine, Dipotassium hydrogen phosphate anhydrous, Orthophosphoric acid. Asenapine API purchased from Covalent laboratories private limited, India.

2.2 Instrumentation

The LC-MS/MS system API 4000 of (Applied Biosystem Inc., California), controlled by Analyst® software used for analysis of Asenapine and Impurities. HPLC consist of Agilent 1200 series quartnary pump, a Agilent 1200 series column compartment, Agilent 1200 series autosampler, a DDA detector (Thermo Fisher Scientific Inc., Waltham, MA). NMR of Bruker 400used with Top Spin software.

2.3 Preparation of Reference Standard and Sample Solutions

Preparation of reference solution: Transfer about accurately weighed 30 mg of Asenapine reference standard in to 50 mL volumetric flask, dissolved in 5 ml methanol, sonicated, dilute the solution with Water: Acetonitrile (2:1 v/v) up to the mark. The solution injected freshly.

Preparation of Sample solutions: Transfer about accurately weighed 50 mg of Asenapine API in to 50 mL volumetric flask, dissolved in 5 ml methanol, sonicated, dilute the solution with Water: Acetonitrile (2:1 v/v) up to the mark. The solution injected freshly.

2.4 Forced Degradation Procedure

Transfer about accurately weighed 1gm of AsenapineAPI in to 10 mL volumetric flask, dissolved in 5 ml methanolsonicated to dissolve properly,add 15ml Water: Acetonitrile (50:50v/v) kept at constant heating at 110°C for 24Hrs.

2.5 Instrumental Conditions of HPLC and LC-MS

For HPLC: The analysis carried out on Inertsil C8 column (Inertsil C8-3, 250mm ×4.6 mm, 5 µm particle size). Mobile phase buffer is 0.5% trimethylamine in 10mM dipotassium hydrogen phosphate anhydrous pH 6.80 with orthophosphoric acid. Mobile phase A contained mobile phase buffers: acetonitrile: methanol in the ratio of 50:45:5 v/v/v respectively. Mobile phase B contained mobile phase buffer : acetonitrile in the ratio of 30:70 v/v respectively, the flow rate kept at 1.0 mL/min. Column oven temperature was 40 °C, and the run time was 95min. Mobile phase gradient program was as follows, time (min) /A (v/v) :B (v/v); T0.00 / 100:0, T40.0 / 100:0, T80.0 / 0:100, T85.0/ 100:0 and T95.0/100:0.

For LC-MS: The analysis carried out on Inertsil C8 column (Inertsil C8-3, 250mm ×4.6 mm, 5 µm particle size). Mobile phase buffer is 10 mM Ammonium Acetate. Mobile phase A contained mobile phase buffers: acetonitrile: methanol in the ratio of 50:45:5 v/v/v respectively. Mobile phase B contained mobile phase buffer : acetonitrile in the ratio of 30:70 v/v respectively, the flow rate kept at 1.0 mL/min. Column oven temperature was 40 °C, and the run time was 95min. Mobile phase gradient program was as follows, time (min) /A (v/v) :B (v/v); T0.00 / 100:0, T40.0 / 100:0, T80.0 / 0:100, T85.0/ 100:0 and T95.0/100:0.

2.6 Mass Spectrometry

The MS study performed by using electrospray ionization (EPI) positive ionization mode, decluster potential (DP) 23 V, entrance potential (EP) 10 V, the curtain gas: 20.0 L/h, ion source gas 1: 45.0 L/h, ion source gas 2: 45.0 L/h, ion spray voltage (IS): 5500 V, temperature (TEM): 450.0 °C. MS1 scanningof Asenapineand impuritycarried out using Mass range acquired from *m/z* 100 to *m/z* 1000 in 0.1 amu steps with dwell time of 2.0 s. Analyst software was used for data acquisition and processing. Molecular weights of all components were determined by use of protonated molecular ions ($[M+H]^+$).

3. Result and Discussion

3.1 Impurity analysisby HPLC

The presence of this unknown thermal degradation impurity was ascertained using HPLC analysis by correlating RRT study. It was observed that there is an unknown thermal degradation impurity at RRT 2.0 in the Asenapine drug substance when drug substance sample kept at 110°C for 24 Hrs. This impurity does not increase in any type of stress condition except constant heating at 110°C for 24Hrs in drug substance. Efforts are made to isolate and identify this unknown thermal degradation impurity as given below.

Thermal degradation of Asenapine drug substance performed constant heating at 110°C in water bath for 24 hoursanalyzed using the HPLC-DAD. One significant impurity peaks detected at RRT 2.0 (RT 50.7 minute). Chromatogram of Asenapine API heated at 110°C in water bath for 24 hours shown in Figure 1.

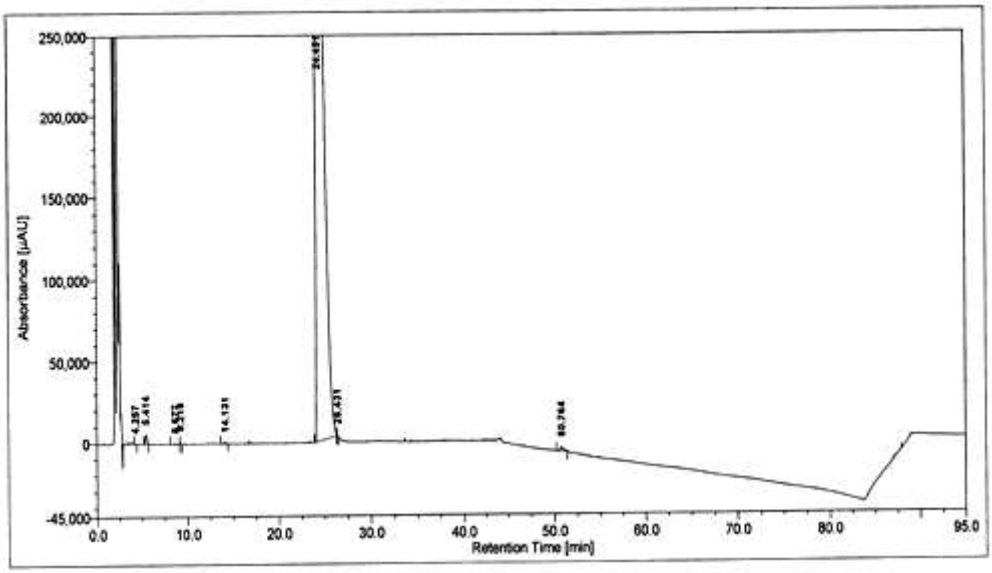


Figure 1: Typical HPLC chromatograms of Asenapine heated at 110°C in water bath for 24 hours.

3.2 Impurity analysis by LC-MS:

Fraction of Asenapine and impurity peaks collected from the HPLC run and analyzed in positive ion mode by the mass spectrometer in ESI mode. The molecular weight of Asenapine found 286.2$[M+H]^+$while molecular weight of the impurity found 282.1 $[M+H]^+$. LC-MS

chromatograms of Asenapine and impurity shown in Figure 2.

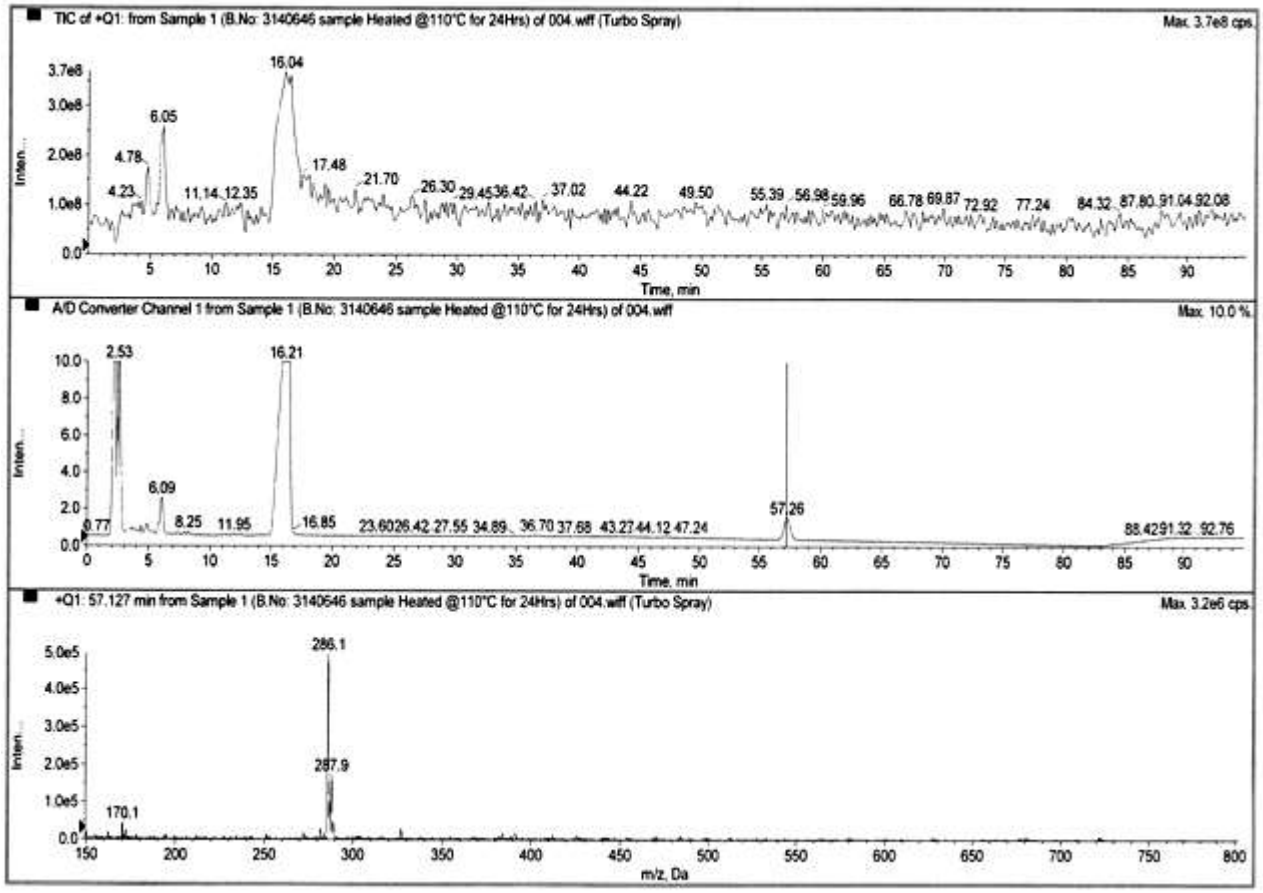


Figure 2: Typical LC-MS chromatograms of Asenapine heated at 110°C in water bath for 24 hours.

3.3 Impurity generation and purification:

This impurity was isolated from heat treated Asenapine drug substanceusing preparative LC column on Preparative HPLC. Approx20gm of Asenapinedrug substance degraded samples was taken for isolation of impurity at RRT 2.0. The preparative HPLC system used was a Shimadzu equipped with LC-8A solvent delivery module.

In the chromatographic conditions, C18 column (Inertsil ODS 3, 500mm ×30 mm i.d., 10µ particle size) was used with the flow rate of 30 mL/min. The Isocratic condition (15:85) was using mobile phase A: 20mM Ammonium acetate, Mobile Phase B: mixture of 75:25 (v/v) of methanol and acetonitrile respectively and the detection was performed at 220 nm. The isolated fractions were pooled together for individual impurity having purity 93.7%. The pooled fraction of impurity was extracted in methylene dichloride by gentle shaking, collected the methylene dichloride layer, again same extraction procedure repeated and collected the methylene dichloride layer. The collected methylene dichloride evaporated on rotary evaporator. After complete drying of the methylene dichloride collected the dried powder of impurity.

3.4 Characterization of Unknown Thermal Degradation Impurity by LC-MS and NMR

3.4.1 LC-MS

The isolated unknown thermal degradation impurity was characterized by LC-MS.The molecular weight of the impurity found 282.1 $[M+H]^+$by LC-MS.

3.4.2 NMR study

The NMR experiments were performed on Bruker spectrometers operating at 400 MHz in CDCl3.The 1H chemical shift values were reported on the δ scale in ppm, relative to Tri Methyl Silan(δ = 0.00). The Asenapine drug substance 1H NMR spectrum was performed in CDCl3 as reference taken from DMF (Version: AN/AP/00/11-12). The spectrum of isolated unknown thermal degradation impurity at RRT 2.0 is given below in figure No.-3.The chemical shift values and corresponding assignments of unknown impurity are given in table-1.

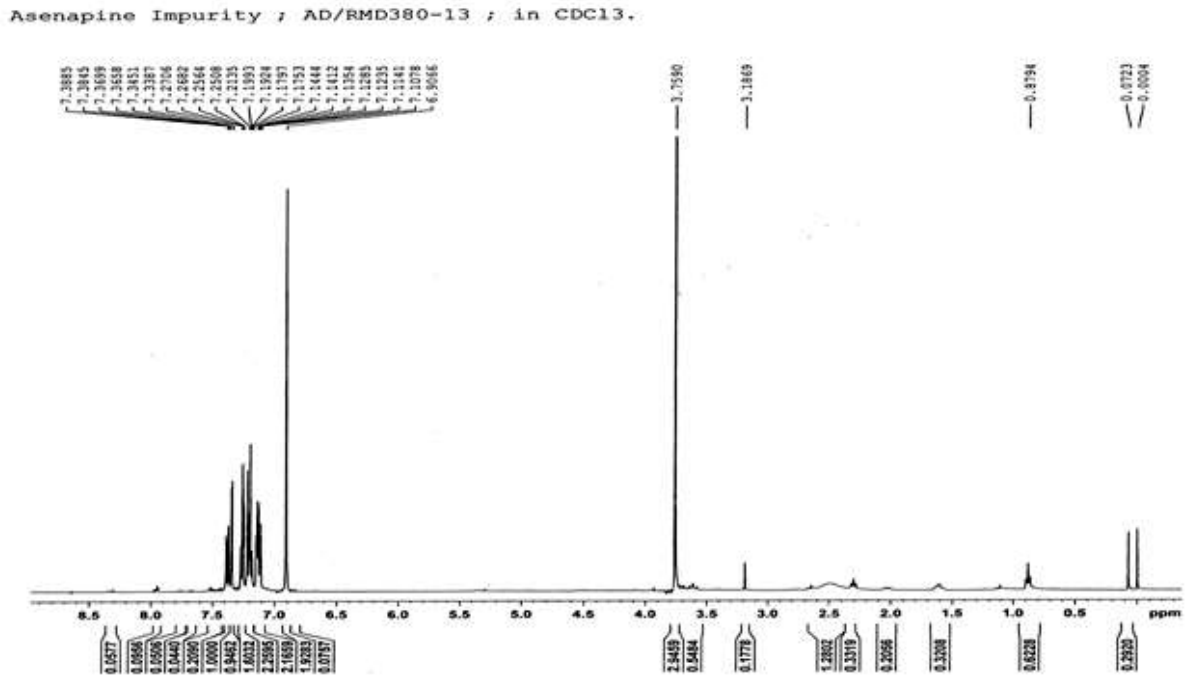


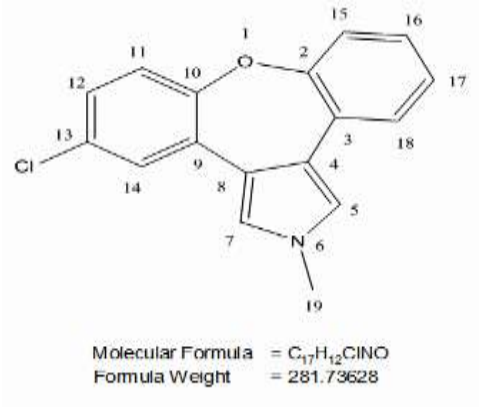
Figure 3: Typical NMR spectrum of isolated unknown thermal degradation impurity at RRT 2.0.

Table 1: Chemical shift values and corresponding assignments of unknown impurity

^{1}H NMR of Asenapine maleate impurity spectral data				
Sr. No.	Carbon Number	No. of proton	Multiplicity	Chemical Shift (in ppm)
01	19	3	s	3.76
02	7,5	2	s	6.90
03	15,17	2	m	7.10-7.14
04	11,16	2	m	27.17-7.21
05	12	1	dd	7.25-7.27
07	18	1	d	7.33-7.34
08	14	1	dd	7.36-7.38

4. Discussion

This impurity was isolated from Asenapine maleate drug substances and is identified as 5-chloro-2-methyl-2H-dibenzo[2,3:6,7]oxepino[4,5-c]pyrrole.
The ESI mass spectrum of this impurity at RRT 2.0 showed a protonated molecular ion $[M+H]^+$at m/z 282, indicating that it has molecular mass less than that of Asenapine (4 Da). Further structure confirmation of this impurity at RRT 2.0 was done by ^{1}H NMR spectrum. In the ^{1}H NMR spectrum, the significant differences were observed in pyrrolidine ring .In the aliphatic region of impurity ^{1}H NMR spectrum, 4 protons at 3.7 to 3.9ppm were disappeared due to change in pyrrolidine ring in comparison with Asenapine maleate ^{1}H NMR spectrum. While impurity spectrum was appeared 2 protons signal extra in aromatic reason at 6.91 ppm which was absent in Asenapine maleate. This indicates that change occur in pyrrolidine ring at 7,8,5&4 positions and formed pyrrole ring from pyrrolidine ring.
The proposed unknown thermal degradation impurity at RRT 2.0 is confirmed as 5-chloro-2-methyl-2H-dibenzo[2,3:6,7]oxepino[4,5-c]pyrrole.



5. Conclusion

The impurity identified with the help of highly sophisticated instrument HPLC, LC-MS/MS and NMR. After thermalforced degradation,one major unknown impurity observed in at RRT 2.0 with respect to Asenapinein HPLC method. Mass spectrometric studies of impurity conducted in ESI mode.The molecular weight of impurity found at 282.1$[M+H]^+$. The impurity isolated from preparative HPLC to get pure form and further molecular weight determined from the mass spectrometer and NMR analysis performed. The structure of impurity elucidated with LC-MS and NMR and it confirmed that the impurity formed from the drug substance. From this study, the unknown impurity was identified and characterized.The Molecular weight of the impurity 282.1 and the name of impurity is 5-chloro-2-methyl-2H-dibenzo[2,3:6,7]oxepino[4,5-c]pyrrole.This study and its outcome will aid in ensuring the quality and safety of Asenapineuse and will guide scientists for safety and efficacy studies of drug substance and drug product.

6. References

National library of medicine Clinical and Research Information on Drug-Induced Liver Injury Bethesda (MD): LiverTox: Clinical and Research Information on Drug-Induced Liver Injury,July 27, 2017.

M Blessy, Ruchi D. Patel, Prajesh N. Prajapati, and Y.K. Agrawal Development of forced degradation and stability indicating studies of drugs - A review. J Pharm Anal. 2014 Jun; 4(3): 159–165. Published online 2013 Sep 17.

Vinubhai N. Patolia. An Introduction to Forced Degradation Studies For Drug Substance & Drug Product. Pharmaceutical online, January 9, 2020.

Steven C. Stoner PharmD, BCPP, Heather A. Pace PharmD , Asenapine: A Clinical Review of a Second-Generation Antipsychotic, Clinical TherapeuticsVolume 34, Issue 5, May 2012, Pages 1023-1040.

SuhairS. Al-Nimry and Mai S. Khanfar, Validation of an RP-HPLC Method for the Determination of Asenapine Maleate in Dissolution Media and Application to Study in Vitro Release from Co-Crystals, ScientiaPharmaceutica, *Sci. Pharm.* 2021, *89*(1), 14.

Gambhire, V.M.; Ranpise, N.S. Enhanced oral delivery of asenapine maleate from solid lipid nanoparticles: Pharmacokinetic and brain distribution evaluations. Asian J. Pharm. Sci. 2018, 12, 152–161.

ICH, Q1A(R2). Stability testing of new drug substances and products. In: Proceedings of the international conference on harmonization. IFPMA, Geneva; 2012.

Eduard Vieta , José Manuel Montes A Review of Asenapine in the Treatment of Bipolar Disorder. Clinical Drug Investigation. 2018; 38(2): 87–99, published Published online 2017 Oct 24.

Aneesh T P and AiyaluRajashekaran, Stress degradation studies and development and validation of RP-HPLC method for the estimation of Asenapine maleate. International Journal of Pharmacy and Pharmaceutical Sciences,Vol 4, Issue 4, 2012.

RenukaManaguli, Lalit Kumar, AnkitaChonkar and RupeshKalidas Shirodkar Development and Validation of a Stability-Indicating RP-HPLC Method by a Statistical Optimization Process for the Quantification of Asenapine Maleate in Lipidic Nanoformulations, Journal of Chromatographic Science, April, 2016.