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DEVELOPMENT OF A HIGHLY SENSITIVE METHOD FOR QUANTITATIVE ESTIMATION OF POTENTIAL GENOTOXIC O-BOC IMPURITY IN LACOSAMIDE DRUG SUBSTANCES USING LC-MS

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ABSTRACT

A selective and highly sensitive liquid chromatography-mass spectrometry (LC-MS) method is developed for the determination of Sodium (tert-butoxycarbonyl)-D-serinate (O-Boc impurity) in Lacosamide drug substance. The method was partially validated as per International Council for Harmonisation (ICH) guidelines, for which limit of detection and limit of quantitation obtained were 0.302 ppm and 0.917 ppm respectively. The regression coefficient found for the linearity study was 0.9976. The % recovery of the spiked Sodium (tert-butoxycarbonyl)-D-serinate (O-Boc impurity) in drug substance obtained was in the range of 80 to 120 which ensure the accuracy of the method. The method can be adapted to determine Sodium (tert-butoxycarbonyl)-D-serinate (O-Boc impurity) in Lacosamide drug substance (API).

KEYWORD: Lacosamide, Sodium (tert-butoxycarbonyl)-D-serinate (O-Boc), development, validation, LCMS.

INTRODUCTION

Lacosamide is an anticonvulsant compound approved for the adjunctive treatment, as well as, conversion to monotherapy and monotherapy treatment of partial-onset seizures and neuropathic pain.[1] It is marketed under the trade name vimpat.[2] The chemical name for Lacosamide is (R)-2-acetamido-N-benzyl-3-methoxypropanamide (Fig.1,a). Its molecular formula is $C_{13}H_{18}N_2O_3$, which corresponds to a molecular weight of 250.294 g /mol

(Fig.1,b),[3] Sodium (tert-butoxycarbonyl)-D-serinate or O-Boc impurity is one of the process impurity that may be present in drug substance which is formed during the synthesis of Lacosamide (Fig.1). Sodium (tert-butoxycarbonyl)-D-serinate structure also shows alert with QSAR software[4], Hence Sodium (tert-butoxycarbonyl)-D-serinate (O-Boc) should be controlled in drug substances to the threshold of toxicological concern (TTC) level 1.5 µg/day in drug substances.[5] A limit for the Sodium (tert-butoxycarbonyl)-D-serinate (O-Boc) was set based on the maximum daily dose (MDD) of Lacosamide for which its limit comes out to be 3.7 ppm.

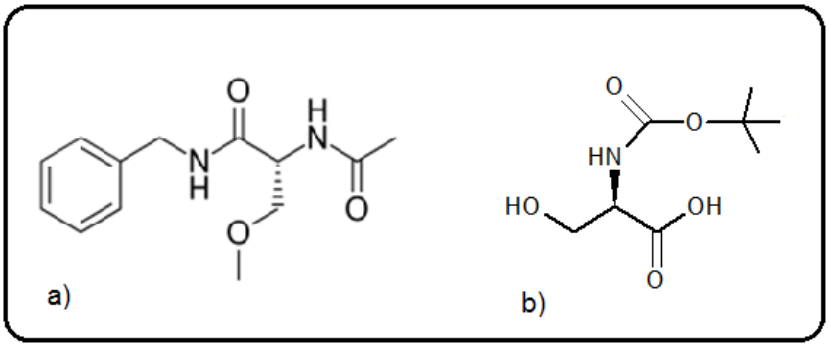

Figure-1: a) Lacosamide and b) Sodium (Tert-Butoxycarbonyl)-D-Serinate, O-Boc Impurity (Free Acid Form).

EXPERIMENTAL

Material

Lacosamide sample was obtained from chemical research and development department of Indoco while Sodium (tert-butoxycarbonyl)-D-serinate i.e. O-Boc impurity standard was qualified and characterised in-house in analytical research and development department.

Chemical and reagents

LCMS grade Methanol and Acetonitrile and Ammonium acetate (LCMS grade). Water used for the preparation of solution was from Milli-Q water purification system.

Instrumentation

The LC instrument consists of UFLC (Ultrafast liquid chromatography) equipped with a binary gradient pump, degasser, autosampler and a column oven. This LC system is hyphenated with triple quadrupole mass spectrometer along with labsolution software. The analytical balance used was of Sartorius make with model ME 235P.

Chromatographic condition

HPLC column used for the analysis was of Inertsil ODS-3 (GL Science make) having 150 mm length, 4.6 mm internal diameter and 3.0 µm particles size. The mobile phase used for elution was ammonium acetate buffer solution of 10 mM concentration and Methanol: Acetonitrile in ratio 50:50 (v/v) using Binary gradient mode of elution. The flow rate and column oven temperature were set to 1.0 mL/min and 25°C respectively. The injection volume was optimized to 20 µL.

Mass condition

The mass spectrometer was equipped with Atmospheric pressure chemical ionization (APCI) source and analysis was carried out in negative mode. Nitrogen was used as nebulizing as well as drying gas and flow rate used was 3.0 L/min and 5.0 L/min respectively. Desolvation line temperature and heat block temperature were maintained at 200°C. Quantification was done by selected ion monitoring (SIM) mode using m/z ion 204.19 for Sodium (tert-butoxycarbonyl)-D-serinate (O-Boc) is having mass 227.19 as sodium salt but in acid, for it is having mass 205.2 and same is considered for quantification.

Preparation of solutions

A mixture of water and methanol was prepared in ratio 10:90 was used as a diluent for all the preparations also taken as blank. The test sample solution was prepared of concentration 800 mg in 10 ml diluent. The standard solution of O-Boc impurity was prepared at 3.70 ppm with respect to the test solution concentration.

RESULT AND DISCUSSION

Method optimization

Several method and column were screened for the selection of technique for determination of O-Boc impurity. Neither of the methods was capable of determining the O-Boc impurity upto trace level, hence mass spectrophotometer detector was selected using liquid chromatography. The different column was optimized to non-polar octadecyl (C18) columns. O-Boc impurity gets retained on the stationary phase (C18) acetate buffer in the mobile phase. The retention time for O-Boc impurity obtained was about 10.5 minute. Due to the low concentration of O-Boc impurity, it is difficult to analyze on UV detector, hence mass detector was used for its detection and quantification. First ionization was done on APCI Souce by using mobile phase 10mM Ammonium acetate in water, buffer use to ammonium acetate due to its volatile nature and compatible with mass detector along with organic

solvent methanol: acetonitrile(50:50) and found M/Z=204.10 at negative mode. After ionization observed mass taken for SIM analysis with binary gradient elution. The divert valve was kept at on from position 4.0 to 12.3 min which will allow the O-Boc impurity to enter into the mass spectrometer. The divert valve was kept at off position from 0.01 to 4.0 min and 15.0 to 30.0 min which will not allow the higher concentration sample into the mass spectrometer and hence it will go to waste. Chromatographic run time kept was 30 minutes.

Method validation

The partial analytical method validation work was conducted according to the ICH (International Conference on Harmonization) guidelines.[6-8] The parameter with which analytical method is validated is Specificity, Limit of detection, Limit of quantitation, Linearity and Recovery.

Specificity

The specificity of the method was established by observing the Sodium(tert-butoxycarbonyl)-D-serinate (O-BOC) separated from Lacosamide peak. The method is specific for detection of O-Boc impurity, as all peaks are well separated observed in UV mode. The retention time of O-Boc impurity is about 11.013 min (Fig.2). Hence, the method has been demonstrated for specificity.

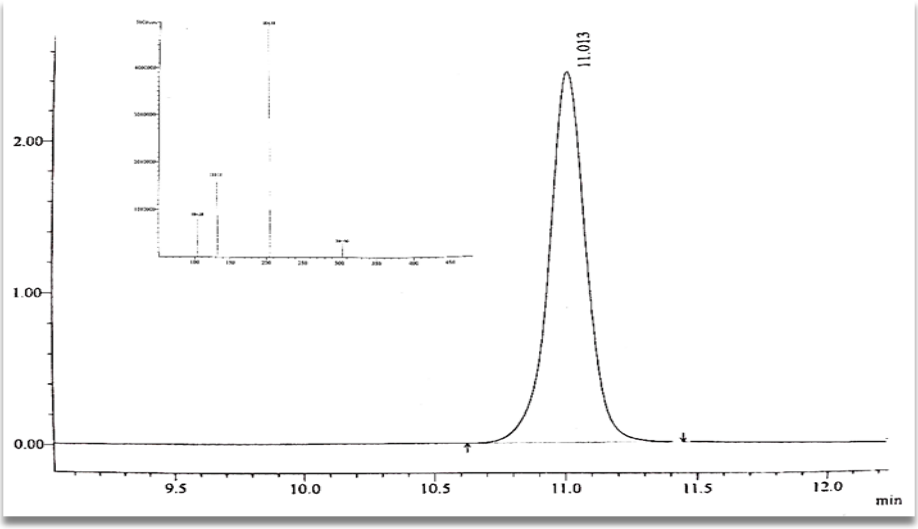

Figure 2: Linearity graph of O-Boc impurity.

Limit of detection and Limit of quantitation

Series of standard solutions of O-Boc impurity was prepared in a concentration ranging from 30% to 150% of target concentration (3.70 ppm w.r.t. sample). Limit of detection (LOD) and

Limit of quantitation (LOQ) was calculated based on a standard deviation of the regression line and slope method. Limit of detection obtained for O-Boc impurity was 0.302 ppm and Limit of quantitation 0.917 ppm.

Linearity

Series of linearity solution of O-Boc impurity was prepared from 30 to 150% of target concentration (3.70 ppm w.r.t sample). Linearity curves were drawn by plotting the peak area of O-Boc impurity against its corresponding concentration of linearity solution (Fig.3). The observed regression coefficient for linearity curve was 0.9976.

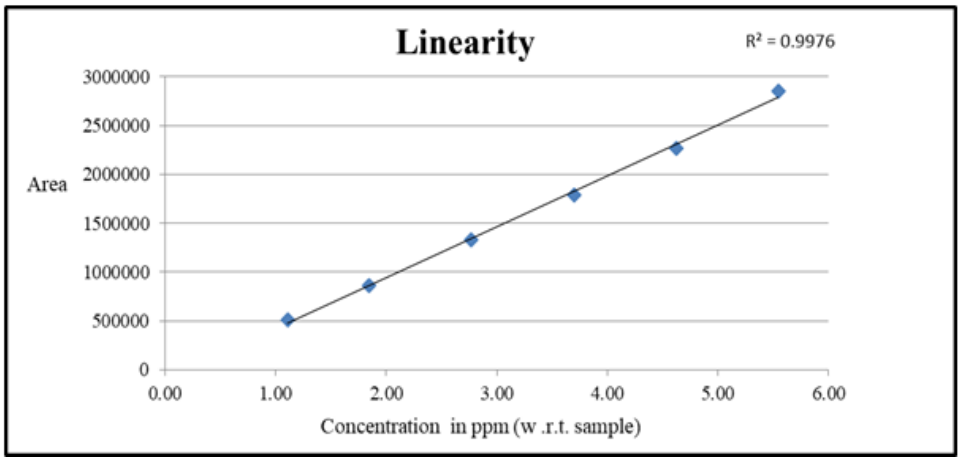

Figure 3: Linearity graph of O-Boc impurity.

Precision

System precision was established by injecting six replicate injections of standard solution where percent Relative standard deviation (%RSD) for O-Boc impurity peak area was 7.12. This proves that the method is highly precise for the detection of O-Boc impurity.

Recovery

The recovery of the method was determined for the related substances by spiking of known amounts of O-Boc impurity in drug substances at level 100% of the specified limit. The method was highly accurate for recovery of O-Boc impurity in the drug substances in the range of 80 and 120%.

CONCLUSION

An optimized LC-MS method was developed to determine the potential genotoxic Sodium (tert-butoxycarbonyl)-D-serinate (O-Boc impurity) content in Lacosamide drug substances. Since molecular mass is more specific for each compound, no interferences were observed in

Sodium (tert-butoxycarbonyl)-D-serinate (O-Boc impurity) determination due to other impurities. An advantage of this method is, its LOD value is very low. The developed method is simple and direct. This LC-MS method was partially validated which proves the method to be simple, precise, linear and accurate. Hence, this method can be for routine low-level Sodium (tert-butoxycarbonyl)-D-serinate (O-Boc impurity) analysis in pharmaceutical drug substances.

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