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Trace level determination of D-serine in lacosamide drug substance by pre-column derivatization RP-HPLC method and conformation of N-Fmoc-D-serine derivative by LC-MS

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ABSTRACT

A reversed-phase high-performance liquid chromatographic (RP-HPLC) method was developed with UV detection and validated for the quantitative determination of D-Serine in a Lacosamide drug substance. Pre-column derivatization procedure was applied by using 9-Fluorenylmethyl-Chloroformate (FMOC-Cl) as derivatization agent. N-Fmoc-D-Serine derivative product having an absorbing maximum (Lambda max) at 265 nm. The HPLC method was developed by using an YMC-Pack Pro C18, 150 X 4.6mm, 3µm particle size analytical column. Column temperature was controlled at 25°C. This is gradient elution method with having flow rate of 1 mL/min. Developed HPLC method conditions are capable to resolve the N-Fmoc-D-Serine from unreacted FMOC-Cl and drug substance related impurities. The retention time of N-Fmoc-D-Serine is approximately 6.2 min. The method is linear, accurate in the specified range (0.7–7 ppm), and robust based on analyte (N-Fmoc-D-Serine) stability in standard and sample. Detection limit is 0.28 ppm and Quantification limit is 0.7 ppm.

KEYWORDS

D-serine;
Lacosamide;
RP-HPLC;
Pre-column derivatization.

INTRODUCTION

Lacosamide is a functionalized D-serine derivative in the R- configuration[1]. Lacosamide is chemically [R]-2-acetamido-N-benzyl-3-methoxypropionamide. Lacosamide was approved as an antiepileptic drug for adjunctive therapy of partial onset seizures in the United States and the European Union. Lacosamide acts by the mechanism of the enhancement of slow inactivation of voltage gated sodium channel. This inactivation prevents the channel from opening, and helps end the action potential. It is functionalized amino acid. Molecular weight of Lacosamide is 250.294 g/mol and molecular formula is $C_{13}H_{18}N_2O_3$. It is sparingly soluble in water and slightly soluble in acetonitrile and ethanol[2-4].

D-Serine is starting material for Lacosamide synthesis; hence, to show the absence of D-serine up to trace level in Lacosamide drug substance

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present work was taken up. In the classification of amino acids serine is non-essential amino acid and D-serine may act as a neurotransmitter in the brain[5]. Threshold for toxicological concern (TTC) acceptable criteria can now be determined for pharmaceutical substances based on their maximum daily dose and the TTC values for both long-term and short-term administrations[6-9]. By considering the Lacosamide daily dosage 400 mg TTC limit is 3.75 ppm.

Maximum daily dose of Lacosamide = 400 mg

Maximum daily intake of Lacosamide = 1.5 µg

i.e. 1.5 µg/0.4 g (400 mg) or 3.75 ppm rounded to 3.5 ppm.

To the best of our knowledge, there is no suitable HPLC method for the quantitative estimation of D-Serine up to 3.5 ppm level in the Lacosamide drug substance. But, some of the HPLC and UPLC methods are available for the estimation of related substances in Lacosamide drug substance and drug product. Some more HPLC, HPTLC and UV spectrophotometry methods are available for estimation of Lacosamide in bulk and its Tablet Dosage Forms. Lacosamide is R-Isomer, hence, to estimate the S-Isomer, chiral HPLC methods also available[10-15].

The purpose of the present research work is to develop a suitable derivative HPLC method for the determination and quantification of D-serine in Lacosamide drug substance with an establi-shed limit of 3.5 ppm. The developed HPLC method was validated with respect to specificity, LOD, LOQ, linearity, precision and accuracy. These studies are performed in accordance with established International Conference on Harmonization (ICH) guidelines[16-19].

EXPERIMENTAL

Materials and reagents

Lacosamide samples and impurities (Figure 1) were supplied by MSN Laboratories Private Limited, R&D Centre, Hyderabad, India. D-Serine, 9-Fluorenylmethyl-Chloroformate (FMOC-Cl) and Di-Sodiumtetraborate (Borax) purchased from Sigma-Aldrich, India. The HPLC grade acetonitrile and orthophosphoric acid purchased from Rankem, India. High-purity water (Milli-Q-Water) was prepared by using a Milli-Q Plus water purification system (Millipore; Milford, MA).

Equipments

Agilent 1200 series LC system equipped with quaternary pump (G1311A), Vacuum degasser (G1322A), column compartment (G1316A), auto sampler with temperature control module (G1329A) and Diode Array Detector (DAD) (G1315D) was used for method development and method validation(Agilent Technologies; Waldbronn, Germany). Data was collected and processed by using Ez chrom Elite (3.3.2 SP2) software. The LC-MS studies was performed using an Agilent 1200 series liquid chromatography coupled with Agilent 6150 single quadrupole mass spectrometer consisting of a binary pump (G4220A) with a degasser, auto sampler (G4226A), column compartment (G1316C), Diode Array Detector (DAD) (G4212A) and mass detector (G2710BA) (Agilent Technologies; Waldbronn, Germany). Data was collected and processed by using chemstation software.

Chromatographic conditions

The chromatographic column used was YMC Pack Pro C18 (150 x 4.6 mm, S-3µm, 12 nm) (YMC karasuma-Gojo Bldg, kyoto, Japan). The separation was achieved on a gradient elution. The mobile phase-A contains 1mL of ortho phosphoric acid into 1000 mL of milli-Q-water and the mobile phase-B contains a mixture of water and acetonitrile in the ratio 10: 90 (v/v). The flow rate of mobile phase was 1.0 mL/min. The HPLC gradient program (Time (min) (T) /% mobile phase-B (%B)) was set as 0.01/45, 10.0/60, 11.0/100, 22.0/100, 22.1/45 and 32.0/45. The column temperature was maintained at 25ºC. The detection was monitored at a wavelength 265 nm. The injection volume was 5.0 µL.

LC-MS conditions

LC–MS system was used for the identification of N-Fmoc-D-Serine. Xbridge C18, 50 x 4.6 mm, 3.5µm column (Waters, Ireland) was used as stationary phase. 0.1% Formic acid in (Rankem Mumbai, India) in Milli-Q-water was used as mobile phase-A. Water and acetonitrile in the ratio of

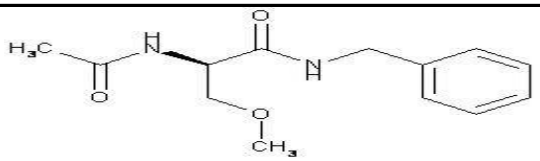
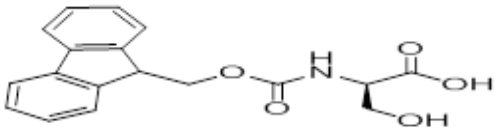
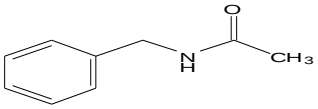
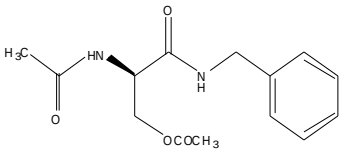
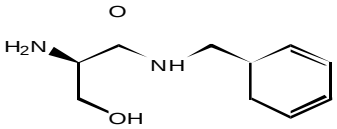
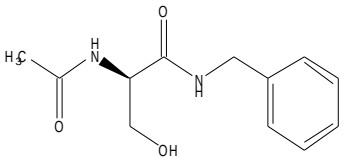
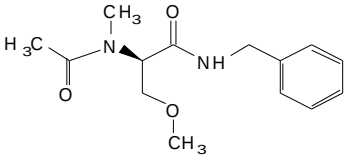
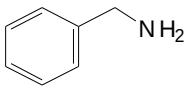
Name of the compound	Retention time in minutes	Structure
Lacosamide	2.5	
N-Fmoc-D-Serine	6.2	
Acetamide impurity	2.74	
O-Acetyl impurity	1.9	
Hydroxyamino impurity	1.22	
Hydroxy impurity	1.9	
N-Methyl impurity	2.93	
Benzylamine impurity	5.45	

Figure 1 : Chemical structures of lacosamide and related compounds

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10:90 v/v; was used as mobile phase-B. The gradient program (T/%B) was set as 0.01/45, 10.0/60, 11.0/100, 22.0/100, 22.5/45, and 28.0/45. The flow rate was 0.35 mL/min. The analysis was performed on Atmospheric pressure chemical ionization (APCI) negative mode. Capillary voltage was 4000V, Nebulizer Gas pressure was 50 psi, drying gas flow was 12 L/min, drying gas temperature was 300° C, vaporizer temperature was 250° C, fragmentor was 90 V and gain EMV was 1.0.

Solutions preparation

Preparation of borate buffer solution

Weighed accurately 4g of Di-Sodiumtetraborate (Borax) in 1000 mL of Milli-Q water.

Preparation of 9-Fluorenylmethyl-Chloroformate (FMOC-Cl) solution

Weighed accurately 4g of 9-Fluorenylmethyl-Chloroformate in 1000 mL of acetonitrile.

Diluent preparation

Borate buffer solution and FMOC-Cl solution in the ratio of 1:1 v/v.

Preparation of D-Serine stock solution

Weighed about 10 mg of D-Serine sample and transferred into 20 mL volumetric flask. Added about 10 mL of Borate buffer solution. Made up to the mark with FMOC-Cl solution and mixed well.

Preparation of 3.5 ppm standard solution

28µL of D-Serine stock solution taken into 100 mL volumetric flask, added about 50 mL of diluent, mixed well. Made up to the mark with diluent and mixed well (With respect to 40 mg/ml of test solution).

Preparation of Sample solution

Accurately weighed about 400 mg of test sample into 10 mL volumetric flask. Added about 10 mL of Borate buffer solution mix well. Made up to the mark with FMOC-Cl solution and mixed well.

Derivatization procedure

D-serine was derivatized (N-Fmoc-D-serine) at room temperature using a pre-column procedure. 10 mg of D-Serine transferred into 20 mL volumetric flask. Added about 10 mL of Borate buffer solution, then made up to the mark with FMOC-Cl solution,

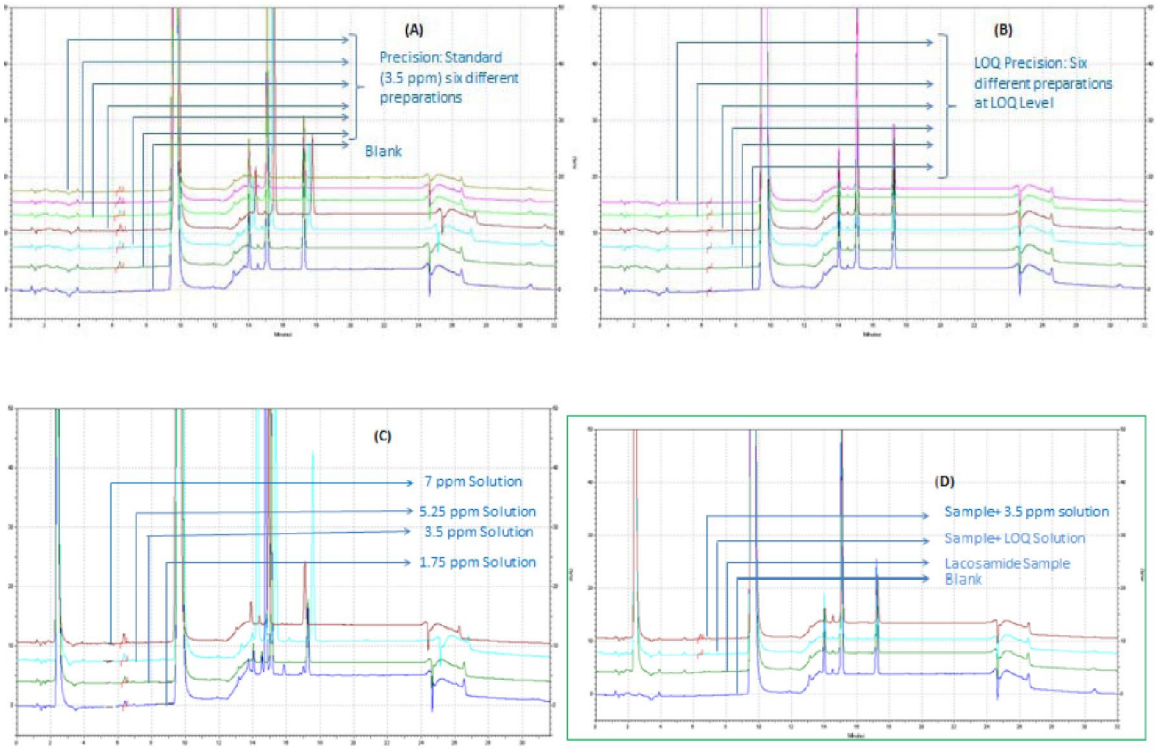

Figure 2 : Typical chromatograms of method validation: (A) Precision study at 3.5 ppm level; (B) Precision at LOQ level; (C) Linearity; (D) Spiking analysis at 3.5 ppm and LOQ level to Lacosamide sample

TABLE 1 : Precision, limit of detection, limit quantification, precision and recovery at limit quantification level, accuracy and linearity results

S.No	Name of the parameter	N-Fmoc-D-Serine derivative				
1	Precision: % RSD for 3.5 ppm standard	0.9				
2	LOD (in ppm)	0.28				
3	LOQ (in ppm)	0.7				
4	Precision at LOQ level (%RSD)	2.3				
5	% of recovery at LOQ level (in %)	90.1%				
6	Accuracy	50%	100%	150%	200%	Avg
		95.0	98.6	97.9	98.2	97.4
7	Linearity	0.9994				

and then mixed this solution by using cyclomixer. Within 5min, the reaction was completed. Then, the sample was analyzed by HPLC. The total time required for the derivatization procedure was less than 5 min.

Method Validation

The procedure is intended to be used as a limit test to monitor D-serine in drug substance. Specificity, linearity, accuracy, precision, Limit of detection (LOD), Limit of quantification (LOQ) and solution stability were established (Figure-2) (TABLE-1).

Specificity

Specificity was demonstrated by injecting diluent, drug substance sample (40 mg/mL), related impurities and D-serine 3.5 ppm standard solution. No interference was observed at the retention time of N-Fmoc-D-Serine, for the drug substance investigated. The retention times of FMOC-Cl and the N-Fmoc-D-Serine derivative product were 9.8 and 6.2 min, respectively. The peaks in the derivatized sample were identified by comparing the retention times of sample versus standard, based on m/z values, uv–vis spectra of those peaks in FMOC-Cl and the N-Fmoc-D-Serine compounds.

Precision

The precision of the method is investigated by preparing six times of 3.5 ppm of N-Fmoc-D-Serine derivative solution. The %RSD of the area of N-Fmoc-D-Serine derivative was calculated. The %RSD for the area of N-Fmoc-D-Serine derivative is 0.9 observed.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ for N-Fmoc-D-Serine derivative product were determined at a signal to noise ratio of 3:1 and 10:1 respectively, by injecting a series of dilute solutions with known concentrations. Precision study was also carried out at the LOQ level by injecting six (n=6) individual preparations and calculating the % RSD of the area for N-Fmoc-D-Serine derivative product. Accuracy study also carried out at LOQ level and calculated the % recovery for N-Fmoc-D-Serine derivative. LOD, LOQ, % RSD at LOQ level and % of recovery at LOQ level results are reported in TABLE-1. Limit of detection is 0.28 ppm with the signal to noise ratio 2.23 and Limit of Quantification is 0.7 ppm with signal to noise ratio 9.75. The % of RSD and % of recovery at the LOQ concentration is 2.3% and 90.1% respectively.

Accuracy

The accuracy of the procedure was demonstrated by the recovery studies, which were carried out by spiking aliquots of drug substance stock solution with D-serine at four levels corresponding to 50% (1.75 ppm), 100% (3.5 ppm), 150% (5.25 ppm) and 200% (7 ppm). The percentage recovery of the N-Fmoc-D-Serine derivative ranges from 95 to 98.6.

Linearity of response

Linearity test solutions for the method are prepared from a stock solution at five concentration levels from LOQ to 200% of the analyte concentration (0.7 ppm, 1.75 ppm, 3.5 ppm, 5.25 ppm and 7 ppm). The linear calibration plot for the method is obtained

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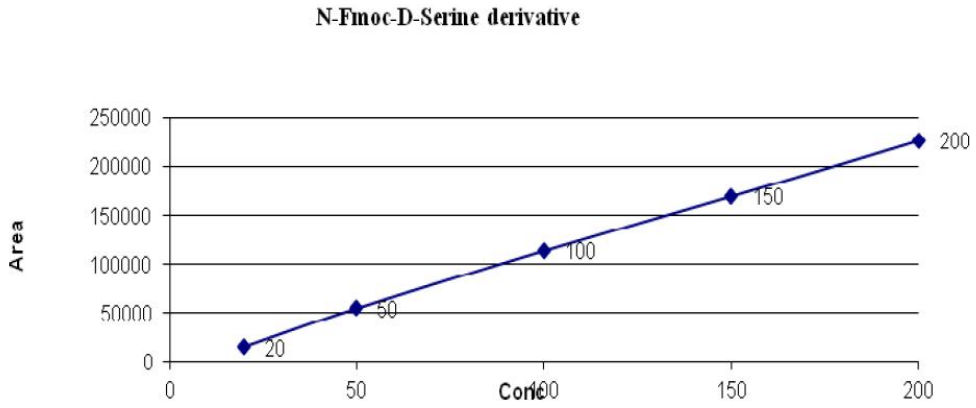

Figure 3 : Linearity chart from LOQ to 200% of specification level

over the tested calibration range (LOQ- 200%) and the obtained correlation coefficient is 0.9994. The results revealed an excellent correlation between the peak area and analyte concentration (Figure-3).

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. To determine the robustness of the method the experimental conditions were deliberately changed. The % RSD for N-Fmoc-D-Serine derivative was evaluated. The mobile phase flow rate was 1.0 mL/min; to study the effect of flow rate it was changed to 0.9 and 1.1 mL/min. The effect of column temperature was studied at 20°C and 30 °C. % RSD for peak area was less than 2 observed.

Stability of analyte solutions

The solution stability of the standard (3.5 ppm) and the representative drug substance sample spiked at 3.5 ppm with D-serine was monitored by comparing assay values over a period of 48 h against a freshly prepared standard. Both standard and sample solutions were found stable for at least 48 h at ambient condition.

Different batch analysis

Three lots of drug substance were assessed for their D-Serine content. Results of the amount of D-serine in these lots are less than the detection level, this indicates that the purification procedure was effective to remove residual D-serine in the Lacosamide or at least keep it down to trace levels.

RESULTS AND DISCUSSION

Optimization of the reaction conditions

From the literature no trace level (3.5 ppm) method for D-serine in drug substance. D-serine is less UV active amino acid and basically amino acids are not well retained in reverse phase HPLC columns. Hence, it is a challenge to develop a trace level of D-serine estimation in Lacosamide drug substance. Hence, it is decided to develop a rapid, accurate, sensitive and validated derivatization method for D-serine content in Lacosamide. The derivatization of D- serine with FMOC-Cl requires forming buffered alkaline pH to form derivative, the preliminary optimization of sample alkalinization was studied. Sodium hydroxide and several buffers (including borate, chloride, and carbonate) were tested. The alkalinization with sodium hydroxide and carbonate buffers led to the formation of an insoluble substance during the derivatization procedure. Instead, best results were achieved by using a borate buffer at pH 10. Pre column derivatization with FMOC-Cl was fast (less than 5 min), and the amino acid derivatives were stable up to 48 h. To form N-Fmoc-D-serine, 10 mg of D-Serine sample and transferred into 20 mL volumetric flask. Added about 10 mL of Borate buffer solution. Make up to the mark with FMOC-Cl solution. The mixture was stirred for 5, 10, 20, 30 and 60 minutes. At each interval, 1 mL of the mixture was pipetted into a glass vial and diluted with 1.0 mL of diluent. This diluted reaction

mixture was then analyzed by HPLC. D-serine was quantitatively converted to the N-Fmoc-D-serine in 5 min, hence it is conclude that with in the five minutes of time D-serine easily convert in to N-Fmoc-D-serine derivative. All derivatization experiments reported in this manuscript were using the 5 min optimized reaction time. We have conducted the optimization of this derivatization process at various pH and also using different mole ratio of FMOC-Cl. After extensive optimization, we found that the FMOC-Cl protection of D-serine requires pH more than 8 and this protecting group is also found be sensitive at pH more than 11. In general 1:1 mole ratio of D-serine and FMOC-Cl is required for this derivatization reaction, but it results in inconsistent. Hence we have conducted the optimization using mole ratio range from 1.0 to 2.0 and found that the 1.6 mole ratio of FMOC-Cl is enough for complete derivatization. When we conducted this derivatization using below 1.5 mole ratio, the N-Fmoc-D-Serine derivative areas not consistence (Areas are increasing, by increasing the FMOC-Cl mole ratios) when compare to 1.6 mole ratio of FMOC-Cl, whereas by using above 1.6 mole ratio the N-Fmoc-D-Serine derivative areas are unchanged (Figure-4).

Optimization of HPLC method

Initial HPLC method development trails performed by using isocratic conditions by using 0.1% orthophosphoric acid in Milli-Q-water and Acetonitrile in the ratio of 1:1 v/v as mobile phase. YMC Pack Pro C18, 150 x 4.6 mm, S-3µm, 12 nm HPLC Column was used as analytical column. Flow rate of mobile phase was 1.0 mL/min. Column temperature was maintained at 25ºC. Detection was monitored at a wavelength 265 nm. Injection volume was 5.0 µL. But these conditions Lacosamide and N-Fmoc-D-serine not separated and FMOC-Cl blank peaks are carrying to next injections. To improve the separation and avoid the carry over peaks, then tried with gradient elution. 0.1% orthophosphoric acid in Milli-Q-water was used as mobile phase-A and Acetonitrile: Water (90:10 v/v) was used as mobile phase-B. Reaming conditions were kept constant. After tried with different gradient programs, it was observed better results with (T/%B) was set as 0.01/45, 10.0/60, 11/100, 22/100, 22.1/45 and 32/45 gradient program. With this selected gradient program and chromatographic conditions Lacosamide, N-Fmoc-D-serine, unreacted FMOC-Cl peaks are well separated; all FMOC-Cl blank related peaks are eluted with in the gradient program. With this selected conditions N-Fmoc-D-serine peak related areas and retention times are is repeatable and reproducible. Hence, selected chromatographic conditions are suitable to identify and quantify the trace level of D-serine in Lacosamide drug substance.

Identification of N-Fmoc-D-Serine derivative compound by LC-MS

Derivative was confirmed by injecting the blank

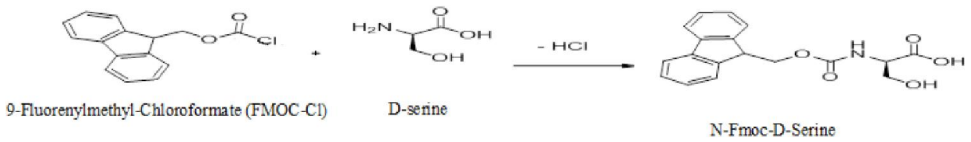

Figure 4 : Chemical reaction between FMOC-Cl and D-Serine

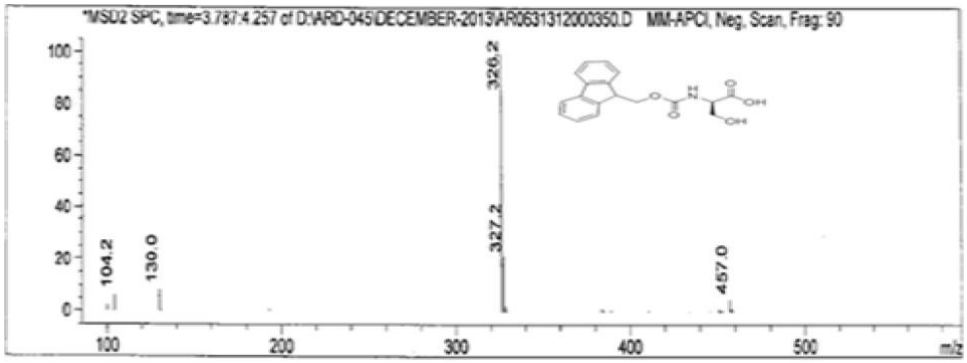

Figure 5 : LC-MS conformation of N-Fmoc-D-Serine derivative

and derivative solution individually in LC-MS, retention times of N-Fmoc-D-Serine and FMOC-Cl (blank) observed around 6.2 and 9.8 minutes respectively, m/z values identified by using mass detector, m/z for N-Fmoc-D-Serine and FMOC-Cl observed 327.2 and 258.7 respectively (Figure-5).

CONCLUSION

In this paper, a sensitive, specific, accurate and validated LC method for the determination of D-Serine in Lacosamide drug substance was established. The information presented in this study could be very useful for qualitative monitoring of D-Serine in Lacosamide drug substance and can be used to check drug quality during stability studies.

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REFERENCES

[1] http://www.rxlist.com/vimpat-drug.htm.
[2] http://en.wikipedia.org/wiki/Lacosamide.
[3] http://www.fda.gov/downloads/Drugs/DrugSafety/ucm089816.pdf.
[4] http://www.ema.europa.eu/docs/en_GB/document_library/EPAR__Product_Information / human/000863/WC500050338.pdf.
[5] H.Wolosker, E.Dumin, L.Balan, V.N.Foltyn; The FEBS J., **275**, 3514 **(2008)**.
[6] M.Venkataramanna, K.Sudhakar Babu, P.Narayanareddy; Global J of Analy Che., **2**, 198 **(2011)**.
[7] Guideline on the limits of genotoxic impurities, European Medicines Agency (EMEA), London, 28 June **(2006)**.
[8] Guidance on the limits of Genotoxic impurities, PMP/SWP/5199/02.EMEA **(2004)**.
[9] Nonclinical evaluation for anticancer pharmaceuticals S9: International conference on harmonization of technical requirements for registration of pharmaceuticals for human use, ICH **(2009)**.
[10] R.Valarmathi, S.Farisha Banu, R.Senthamarai, C.S.Dhivya Dhharshini; International journal of pharmaceutical and chemical sciences., **2**,181 **(2013)**.
[11] Sachin Shah, S.G.Vasantharaju, Arumugam Karthik, B.S.Muddukrishna; Elixir Pharmacy., **38**, 4174 **(2011)**.
[12] G.Ramanaiah, D.Ramachandran, G.Srinivas, V.Srilakshmi, Purnachanda Rao; Am. J. PharmTech Res., **2**, 556 **(2012)**.
[13] S.A.Kamdar, V.M.Vaghela, P.A.Desai; International Journal of ChemTech Research., **4**, 1193 **(2012)**.
[14] M.Srihari, Vinay Rao, K.N.Jayaveera; Der Pharma Chemica., **5**, 81 **(2013)**.
[15] M.D.Parmar, K.S.Nimavat, K.B.Vyas, D.V.N.S.Rao, R.Pande; International Journal for Pharmaceutical Research Scholars, **1**, 40 **(2012)**.
[16] International conference on harmonization, November, 2005, Validation of analytical procedures : Text and Methodology Q2(R1) USP, United States Pharmacopoeia, 'Validation of Compendial Methods', 31st Edition, United States Pharmacopeial Convention, Rockville, **(2008)**.
[17] L.R.Snyder, J.J. Kirkland, J. L.Glajch; 'Practical HPLC method development', 2nd Edition, Wiley-Interscience publication, John Wiley & Sons, Inc, 709 **(1997)**.
[18] M.Bakshi, S.Singh; J.Pharm.Biomed.Anal., **28**, 1011 **(2002)**.