Development And Validation Using Protein Precipitation Extraction For The Estimation of Dexlansoprazole In Spiked Human Plasma By Liquid Chromatography Tandem Mass Spectrometry

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***Abstract*-** To develop a simple, precise and accurate LC-MS/MS method for the estimation of dexlansoprazole in spiked human plasma with cost effective protein precipitation extraction technique. The validation of the proposed method was done according to the ICH and FDA guidelines. Omeprazole was used as the internal standard (IS). The method was developed in isocratic separation mode using Zorbax SB C_{18} column (4.6 x 150 mm, 3µm) as a stationary phase and the mobile phase consists of 0.5 mM Ammonium acetate buffer, adjusting the pH to 3.5 using glacial acetic acid and acetonitrile in the ratio of 30:70% V/V with a flow of 0.5 mL/min. Detection in UV was at 284 nm and it was carried out by triple quadrupole mass spectrometry with electrospray ionization in positive mode. Proton adducts at m/z 369.95 > 251.95 and 346.00 > 198.05 to monitor Dexlansoprazole and Omeprazole. The method was found to be linear over a concentration range of 0.5-3000 ng/mL with a regression analysis of 0.9994. The percentage recovery of the present method was found to be 99.54 ± 0.28%. The method was found to be stable. The developed LC-MS/MS method will be suitable for the analysis of dexlansoprazole and applied for routine analysis in different quality control and research laboratories. It will be more precise, accurate and cost effective while comparing to the reported method.

***Index Terms*-** Dexlansoprazole, Omeprazole, Human Plasma, LC-MS/MS, Validation, FDA, Electrospray Ionization

I. INTRODUCTION

Dexlansoprazole [DLP] {(R) - (+) – 2 - ([3- methyl – 4 - (2, 2, 2 trifluoroethoxy) pyridine – 2 – yl] methylsulfinyl) - 1H - benzo [d] imidazole)} is a proton pump inhibitor (**Figure 1**) of the antisecretory, substituted benzimidazole class. It is having a molecular mass of 369.69 g/mol. It is an enantiomer of lansoprazole [1,2,9,10]. It is available commercially as delayed-release capsules (30 and 60 mg). It suppresses the final stage of acid secretion by specifically blocking the (H+/K+)- ATPase present in the gastric parietal cell. It is used to treat the healing of erosive esophagitis, in maintaining of healed erosive esophagitis and non-erosive gastroesophageal reflux disease [3-4] (GERD) associated with heartburn. In January 30, 2009, it was accepted by FDA.

Literature survey reveals that Dexlansoprazole was estimated by HPLC [5-8] and tandem mass spectroscopy has been previously reported in bulk and human plasma. It was done for determination of DLP, simultaneous estimation and for pharmacokinetic study.

There is no reported cost effective method for the estimation of DLP by LC-MS/MS method and hence, a sensitive and validated bioanlytical method for the estimation of DLP as per FDA guidelines [13-16]was undertaken.

II. Materials and Methods

2.1. Materials

DLP used as working standard was provided as a gift sample from Indian Pharmacopoeia Commission, New Delhi, India and Omeprazole (**Figure 2**) used as internal standard was purchased from Drugs testing laboratory, JSS College of Pharmacy, Ooty, India. Acetonitrile was analytical reagent grade used for LC-MS/MS purchased from Sigma Aldrich, Ammonium Acetate from Rankem Fine Chemical Limited and Water of LC-MS/MS grade from Milli-Q RO system (Millipore, Bedford, USA) were used.

2.2. Instrumentation

LC system coupled with tandem quadrupole mass spectrometry (Shimadzu 8030, Tokyo Japan) equipped with electrospray ionization (ESI) interface, LC-20AD pump, SPD-M20 PDA detector, CTO-20AC column oven, CBM-20 alite controller and SIL-20AC autosampler was used. The data were recorded using Lab solution data station software. Isocratic separation was achieved using Zorbax SB C_{18} column (4.6 x 50 mm, 3µm) as a stationary phase and the mobile phase consists of (0.5 mM) Ammonium acetate (pH 3.5): acetonitrile (30:70 V/V) with a flow of 0.5 mL/min and injection volume of 10 µl was employed.

2.3. Selection of mass range

A 1000 ng/mL of DLP and OMP was infused into the mass spectrometer directly and the conditions for operation were optimized. Obtained transitions were 369.95→251.95 (**Figure 5**) and 346.00→198.05 m/z (**Figure 6**) were used to monitor DLP and OMP (IS) **.**

2.4. Preparation of solution

About 0.77 g of ammonium acetate was weighed and dissolved in 200 ml Milli-Q RO water to get 0.5 mM. The pH was adjusted to 3.5 with glacial acetic acid.

2.5. Preparation of standard solutions

DLP and OMP solution was prepared by dissolving accurately about 10 mg in methanol and making up the volume to 10 mL with acetonitrile and this solution was refrigerated at 2-8

°C. Spiking of DLP solution was done and dilutions were made to obtain working standards **(Figure 5,6**), calibration curve and quality control samples and were stored in refrigerator.

2.6. Preparation of sample solution and extraction

Extraction of DLP from plasma was done by protein precipitation extraction technique. Plasma is stored in containers hermetically sealed at -60 °C till analysis. A 500 µl of aliquot was taken in eppendorf for analysis which was mixed with 100 µl OMP as IS with the help of a vortex mixer. From the above solution 20 µl was taken and DLP is extracted by using acetonitrile as optimized precipitating solvent (acetonitrile). The sample was vortexed for a min and then centrifuged for 20 min at 7000 rpm at room temperature. Supernatant was transferred for LC-MS/MS analysis. (**Figure 3,4**)

2.7. Method Validation

Validation of the method for specificity, linearity, accuracy, precision, range, quantitation limit, and detection limit, robustness and system suitability as per the ICH and FDA guidelines [11-16].

2.7.1. Specificity

The analyte response measurement in the presence of other drugs, excipients and their potential impurities to demonstrate the specificity**.**

2.7.2. Linearity

The average of six determinations at ten concentration levels covering the range of 0.5-3000 ng/mL for DLP, the evaluation of linearity was performed. Calculation of the coefficient correlation, slope and intercept values was done by using calibration curve for linearity evaluation. The same have been designed.

2.7.3. Accuracy

Determination of accuracy was demonstrated for standard addition method from recovery studies according to ICH guidelines. The pre-analyzed samples were spiked with standard drug DLP.

2.7.4. Precision

Evaluation of precision was carried out by inter-day and intra-day comparision study samples consisted of three levels concentration (six replicates) of LLOQ, low (LQC), medium (MQC) and high (HQC) quality controls, i.e. 0.3, 1.5, 750, 2000 ng/mL, respectively. Report used for precision was from the regressed concentration of the percent relative standard deviation (%RSD).

2.7.5. Limit of detection (LOD) and limit of quantification (LOQ)

Determination of LOD and LOQ was assessed by the signal-to-noise ratio. LOD ratio was 3:1 whereas LOQ of the drug could be quantified with minimum peak area in the ratio of 10:1.

2.7.6. Robustness

The alteration in the condition of the experiment like operators, the source of reagents, similar type column and optimized conditions like pH, mobile phase ratio and flow rate were used as assessment tool for the robustness monitoring.

2.7.7. Matrix effect

In quantitative analysis by mass spectrometry with electrospray ionization (ESI-MS), a major limitation is matrix effects in which the coextracted matrices with the analytes can cause change in the signal response, causing either enhancement or suppression of the in teased signal. Moreover it also may influence on the quality of results in terms of recovery.

III. Results and discussion

3.1. Specificity

To determine that the excipients used are not interfering with the main compound peak, test for specificity needs to be done. No peaks were eluted along with the retention time of DLP in addition to that it was very well resolute by compound. Hence, the developed method results showed that it was selective for determination of DLP in the formulation.

3.2. Linearity

The evaluation of the method to be linear was by six determinations at ten concentration levels with a range of 0.5-3000 ng/mL for DLP. A calibration curve was found to be linear with a mean regression of equation ($y = 0.0035x + 0.1196$, $r^2 = 0.9994$, S.D.=1.28) respectively, where the response factor is the Y and the analyte concentration in ng/mL was the X (**Figure 11**).

3.3. Accuracy

The accuracy of the method was carried for three quality control (LLOQ, LQC, MQC and HQC) samples by standard addition method, and the accuracy was found to be 99 ± 0.12 %**.** Application of the developed method for the estimation of DLP (**Table 1**).

3.4. Precision

Calculation of the method for precision was carried by the intra-day and inter-day precision studies at three different concentrations and they were found to be within the limits (**Table 1**).

3.5. Limit of detection and Limit of quantification

The lowest limit detected for the method for DLP was at 0.1 ng/mL based on the signal-to-noise ratio 3:1. Due to the increase in the sensitivity of the method, quantification was done at 0.5 ng/mL for DLP. This method found to have a high percentage recovery at low concentration at the acceptable limit (**Table 1**) (**Figure 9 and 10**).

3.6. Robustness

When alteration in the condition of experiment was done, no notable changes in the parameters of chromatograph were observed, proving that the developed method was found to be highly robust.

3.7. Stability studies

Exposing to various stress condition the evaluation of stability was performed. According to international stability guidelines the test performed are bench top stability, freeze thaw stability and long term storage stability for spiked plasma samples, autosampler stability for the processed sample and stock solution stability for the stock solution. 1 mg/mL DLP stock solution was refrigerated for 7 days and kept for 8 hours in room temperature. Refrigeration of DLP in plasma for 20 days at -20°C and for 18 hours in room temperature. Bench top stability was achieved by keeping the samples for 8 hours in room temperature. Three cycles of freeze and thaw stability was performed. DLP proc.essed

sample stability was done for 60 hours in autosampler in room temperature

3.8. Recovery and Matrix effect

The mean recovery of DLP from the spiked human plasma was 99.95, 100.02 and 97.01 respectively. The obtained value evidenced that, the no matrix influenced was observed in plasma sample analysed. Ion suppression is indicated in the values. Therefore, the present method is reliable. The protein precipitation method has capacity to remove the maximum plasma bounded.

IV. Conclusion

A novel simple, precise, accurate and a validated, liquid chromatography-tandem mass spectroscopy method have been developed and validated. The developed method can be successfully applied for the estimation of DLP in the human plasma.

CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

ACKNOWLEDGMENT

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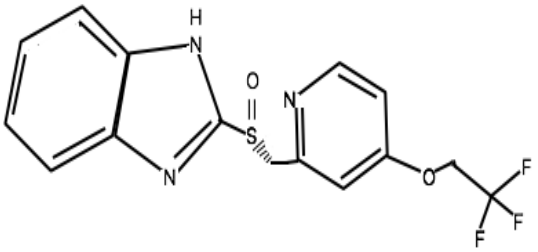


Figure 1. Dexlansoprazole

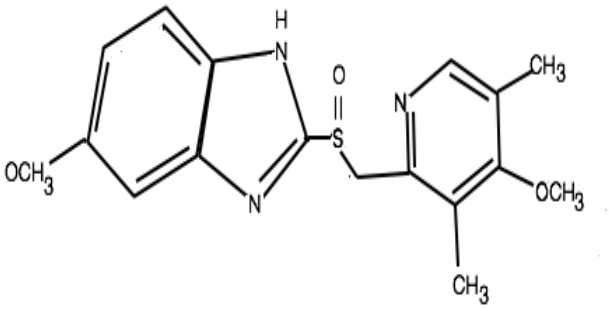


Figure 2. Omeprazole

Table 1: Accuracy and precision for the determination of dexlansoprazole.

QC samples (ng/mL)	Mean Conc. found (ng/mL)	Intraday		Inter-day	
		Accuracy (% N)	Precision (% CV)	Accuracy (%N)	Precision (% CV)
15	12.83	96.00	2.83	84.0	2.23
750	720.7	96.81	2.78	96.36	2.92
2000	1994	98.83	0.96	98.14	2.03

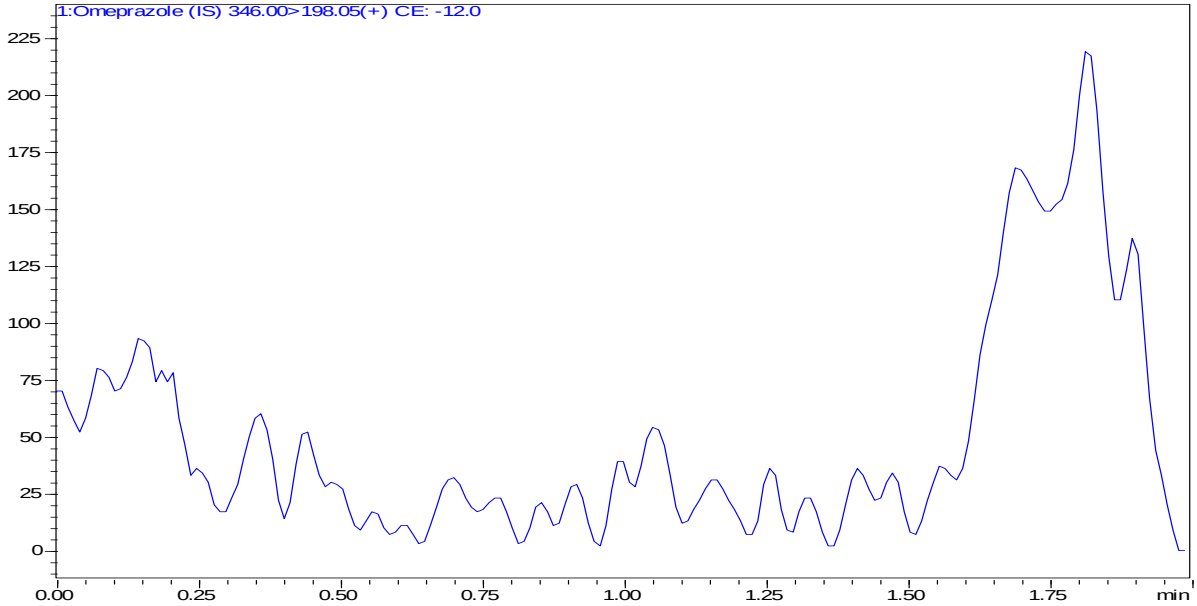


Figure 3. Typical LC-MS/MS separation Blank plasma and IS spectra

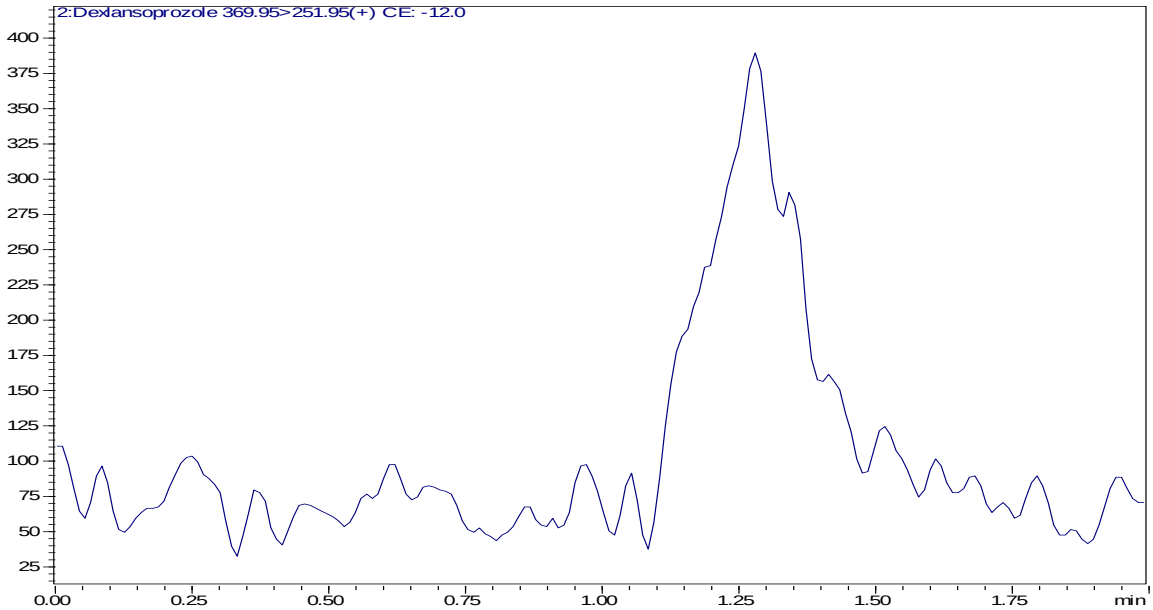


Figure 4. Typical LC-MS/MS separation Blank plasma and DLP spectra

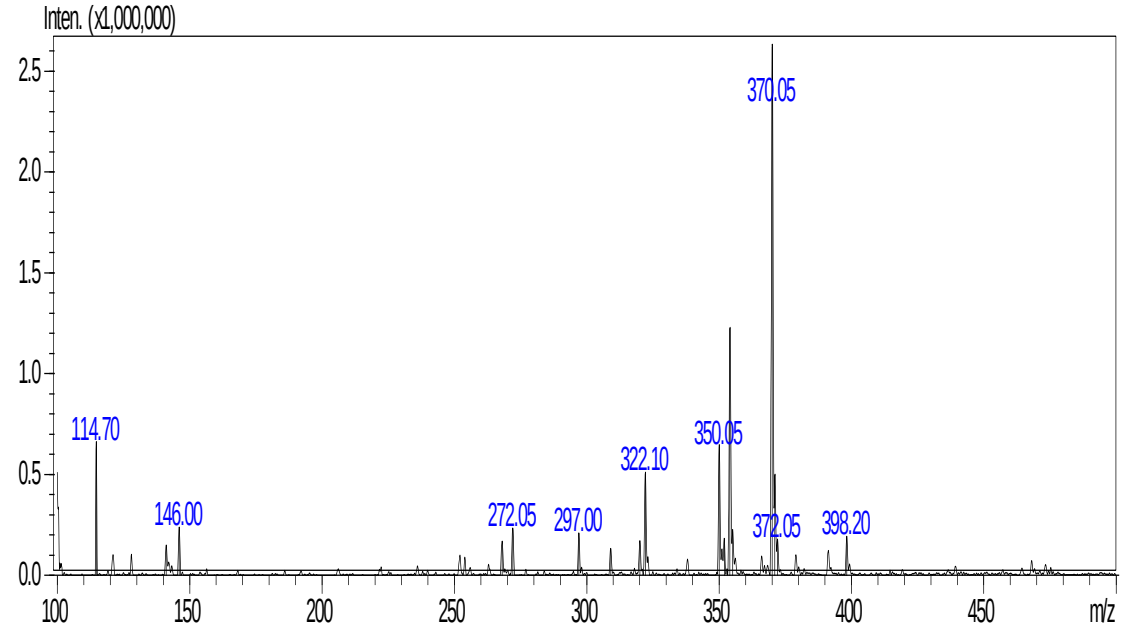


Figure 5. Typical mass spectra of DLP

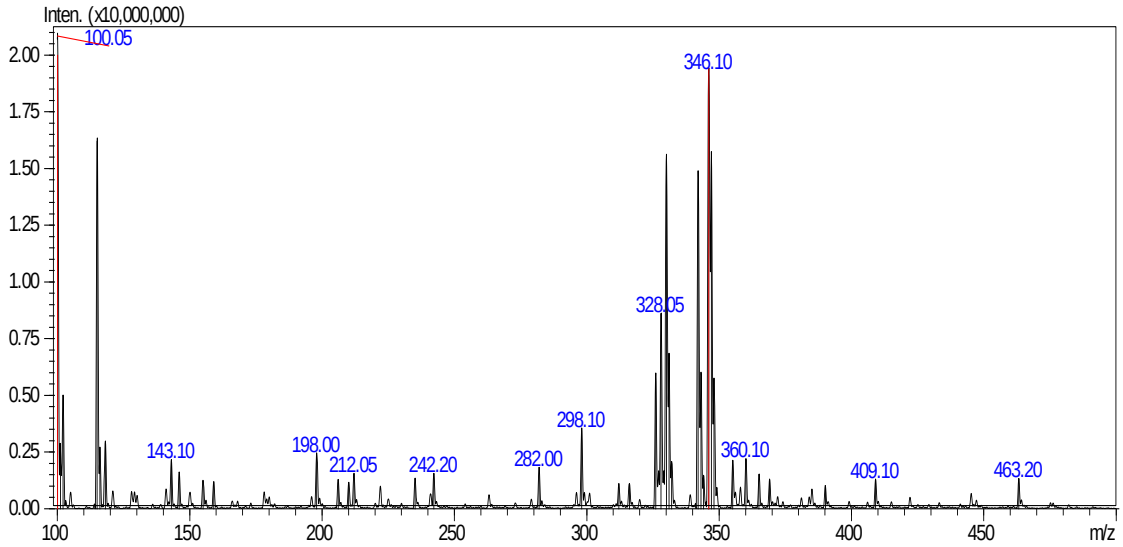
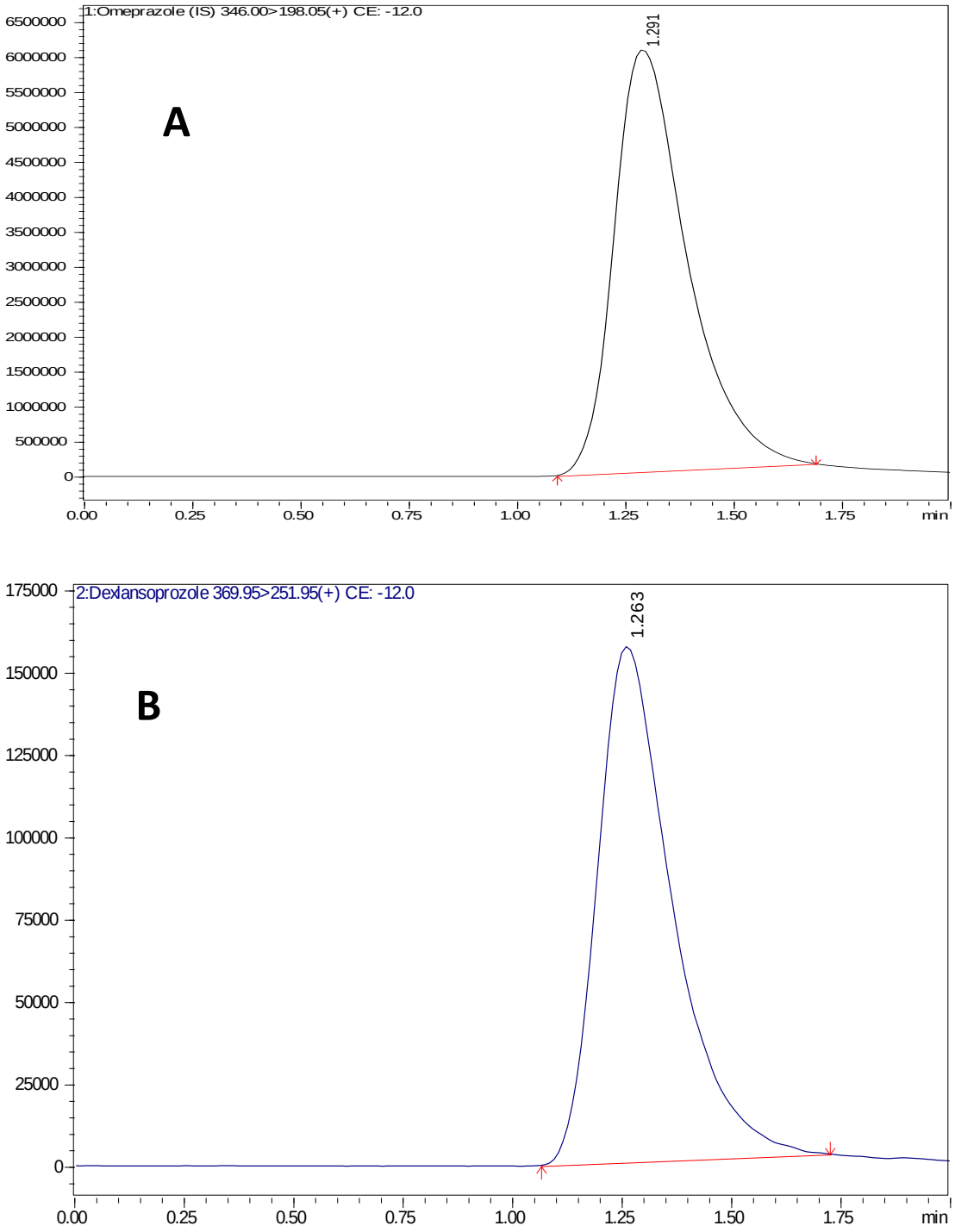


Figure 6. Typical mass spectra of OMP



(B)

Figure 7. Typical LC-MS/MS Standard chromatogram – (A) OMP (B) DLP

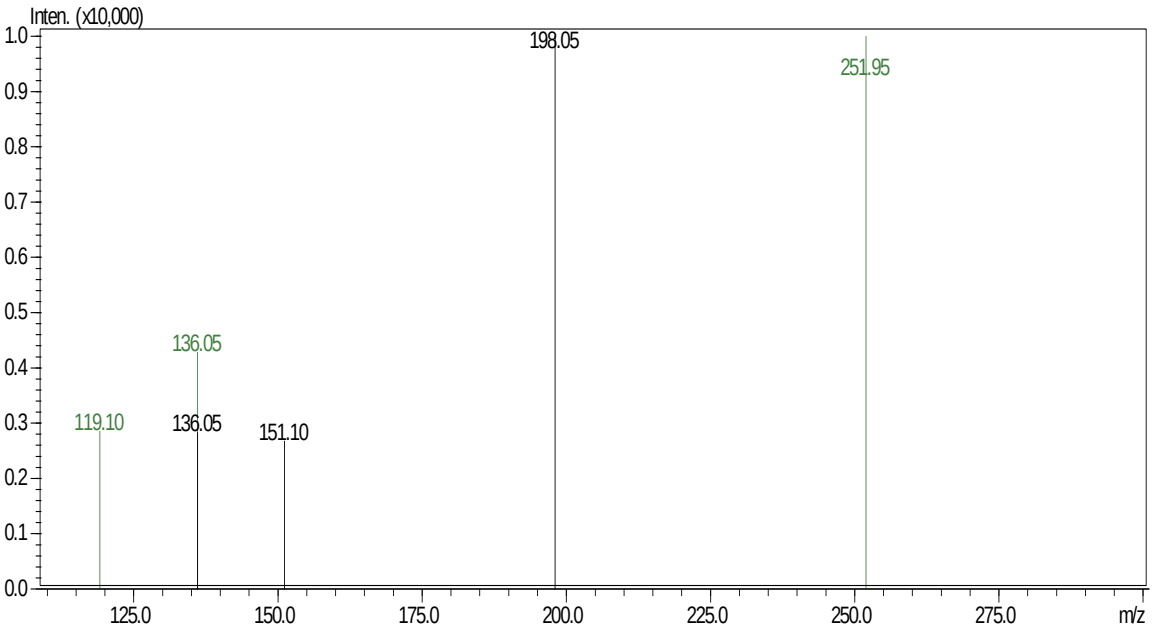


Figure 8. Typical LC-MS/MS spectra of PI scanning

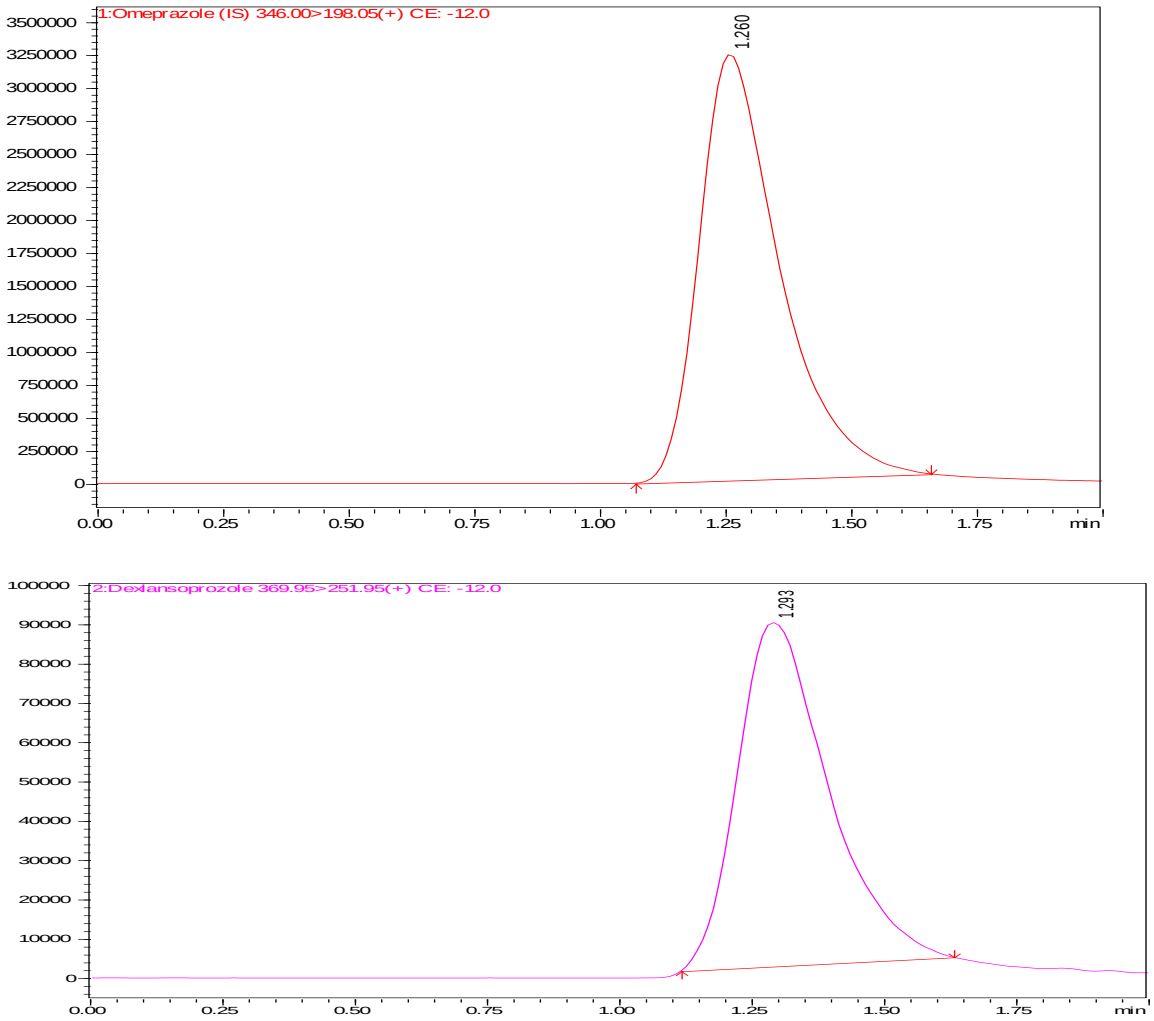


Figure 9. Typical LC-MS/MS spectra for LOQ samples

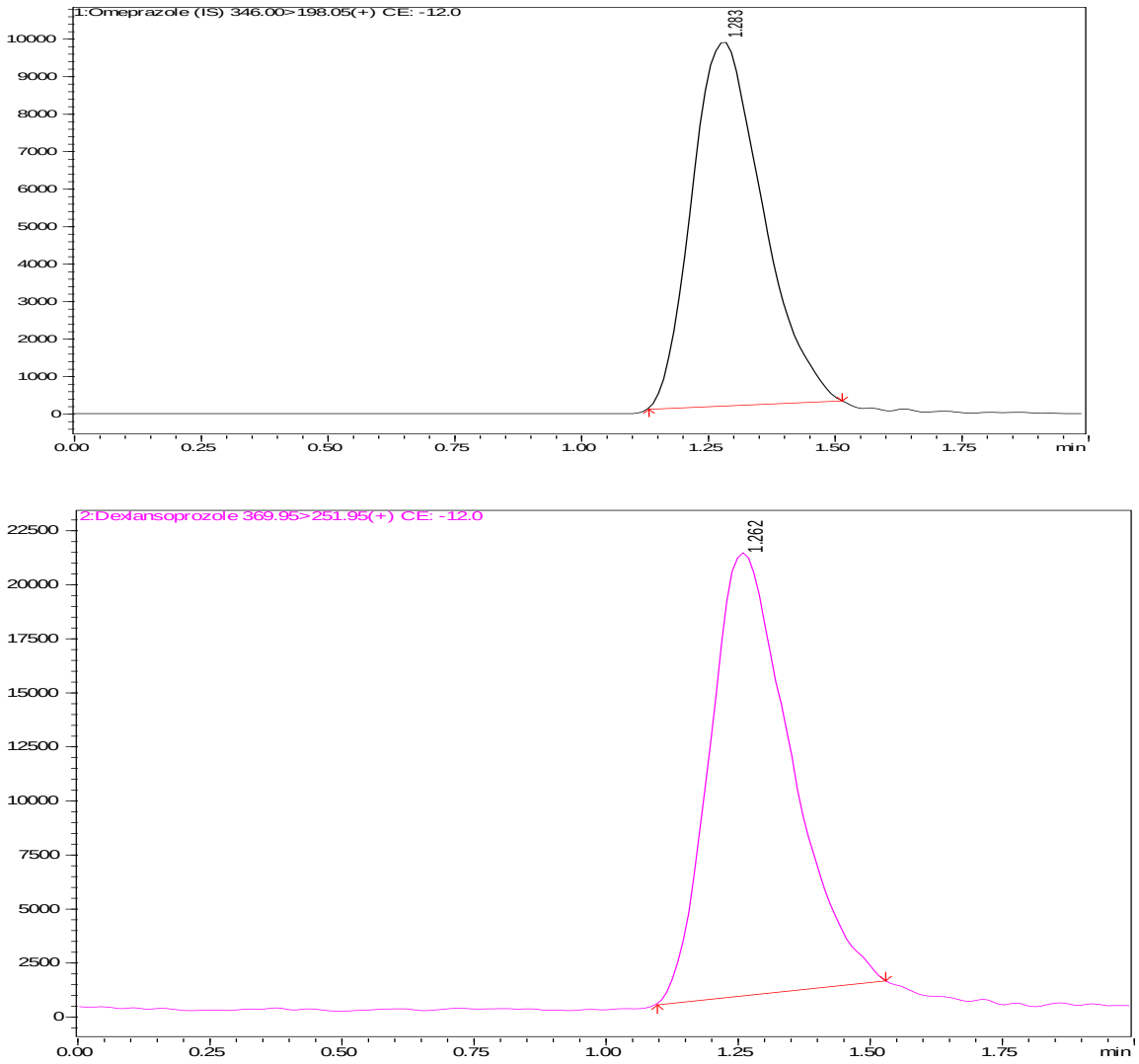


Figure 10. Typical LC-MS/MS spectra for LOD samples

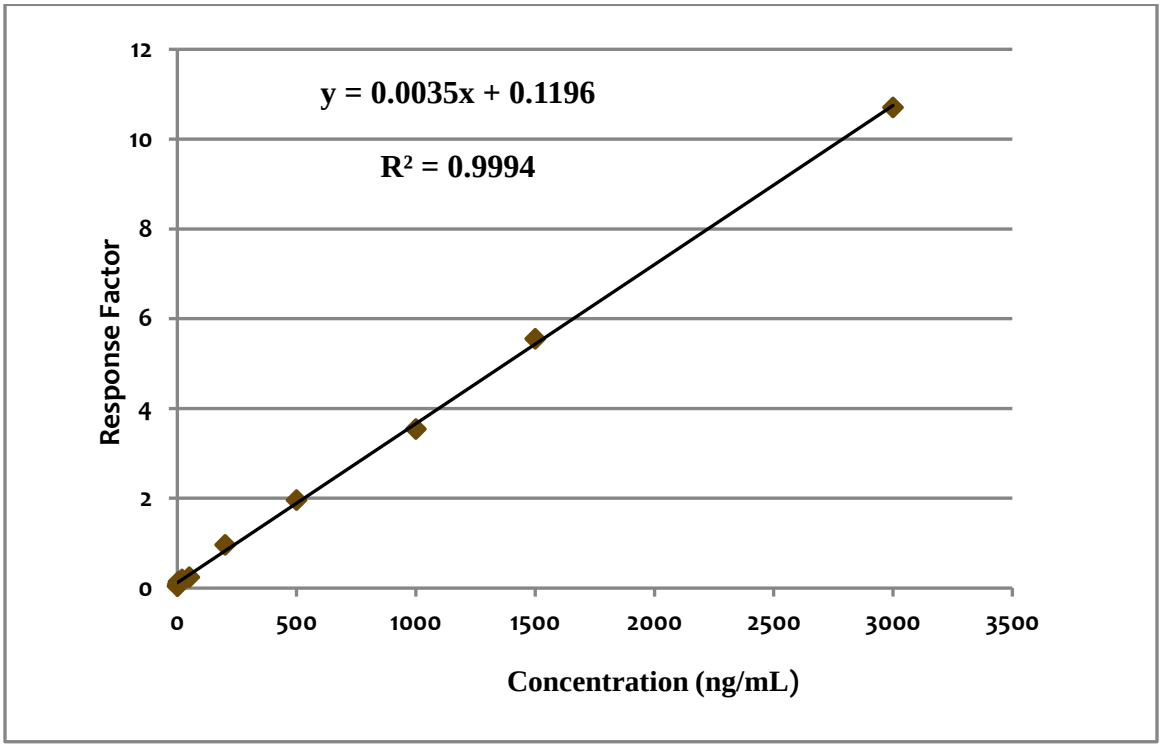


Figure 11. Linearity curve of DLP in plasma