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# Validated LC- MS Bioanalytical Method for the Estimation of Duloxetine Hydrochloride in Human Plasma

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**ABSTRACT**

*Duloxetine Hydrochloride is a selective serotonin reuptake inhibitor used for depression. In the proposed method, duloxetine hydrochloride was determined from human plasma using LC-MS method. Liquid-Liquid extraction method was used for the estimation of duloxetine hydrochloride. The drug was found to be stable in plasma and various parameters of bioanalytical validations were executed using LC-MS as per ICH guidelines and the proposed method was found to be sensitive, selective, economic and reproducible for the estimation of duloxetine in human plasma.*

**Keywords**: Duloxetine hydrochloride, validation, bioanalytical method, atmospheric pressure ionization, depression

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## INTRODUCTION

Duloxetine Hydrochloride is a selective serotonin and noradrenalin reuptake inhibitor used in the treatment of depression. It is chemically (+)-(S)-N-methyl-γ-(1-naphthyloxy)-2-thiophenepropylamine hydrochloride with empirical formula $C_{18}H_{19}NOS$•HCl and having molecular weight of 333.88. The structural formula is shown in fig 1.Duloxetine hydrochloride is slightly brownish white solid and slightly soluble in water.

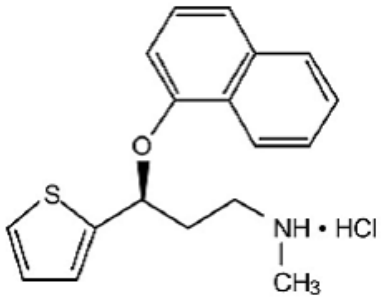


**Fig 1. Duloxetine Hydrochloride**

The half-life of duloxetine is 12 hours and reaches steady-state plasma concentrations after three days of dosing. Duloxetine is eliminated via CYP1A2 and CYP2D6[1]. The literature survey revealed that a stability-indicating

HPLC method was reported using C-8 column at 40°C consisting of phosphate buffer (pH 2.5)-methanol-tetrahydrofuran in the ratio of 50:40:10, flow rate of 1 ml/min at a wavelength of 232 nm. [2]. It was reported such that duloxetine is stable to dry heat, photo-degradation, oxidation and basic conditions attempted [3]. Various drug and excipient interactions of duloxetine were determined [4]. The reported RP-HPLC method for duloxetine hydrochloride(DLX), in which DLX was subjected to stress conditions of acid, base, oxidation, wet heat, dry heat, and photo-degradation and estimated with Phenomenex $C_{18}$column using acetonitrile, methanol, 0.032 M ammonium acetate buffer (55 + 05 + 40, v/v/v) at a flow rate of 1.0 mL/min at 40°C[5-6]. Duloxetine was analyzed using eletrospray ionization(ESI) mass spectrometer [7]. Duloxetine was determined using multiple reactions monitoring (MRM) to quantify duloxetine and internal standard (I.S.) [8]. The literature survey revealed that there is no simple method available for the estimation of duloxetine from human plasma. The present aim of the proposed study is to develop a simple liquid-liquid extraction method for estimation of duloxetine in human plasma. These methods were validated as per ICH guidelines [9].

**II) Materials and methods**

Methanol, Acetonitrile, Ammonium Acetate were purchased from SD Fine Chemicals, India. Expired human plasma was collected from local blood bank. Shimadzu HPLC, Remi Centrifuge, Labindia pH/Con conductivity meter, spinchrom C-18 column and LCMS API 3000 were selected for the method.

**Method development**

0.005M ammonium acetate is dissolved in 1 liter of triple distilled water and degassed with bath sonicator. Furthermore, this solution was filtered through 0.45 micron Millipore membrane. The mobile phase was prepared by adding 90 ml of methanol and 0.0025M ammonium acetate. The DLX determinations were performed at a flow rate of 1 ml per min with 65:35 and having standard injection volume 20 microliter.

**Duloxetine Stock Solution**

Accurately weighed quantity 10 mg of Duloxetine was transferred to 10 ml standard volumetric flask and volume was made with methanol to produce a final concentration of 1 mg/ml. The prepared stock solution was stored between 4 and 8 °C and used in three days.

**Duloxetine D5 Stock Solution (Internal Standard)**

Accurately weighed quantity 1 mg of Duloxetine D5 was transferred to 1 ml standard volumetric flask and volume was made with methanol to produce a final concentration of 1 mg/ml. The prepared stock solution was stored between 4 and 8 °C and used in three days.

**Preparation of various concentrations**

200 µL of the plasma was transferred into 10 ml glass stoppered tubes, 30 µL (2001.14ng/mL of Duloxetine D5) IS solution was added to it and vortexed.

5 ml of TBME (Methyl tert-butyl ether) was added and centrifuged at 5000 rpm for 10 minutes at 5 °C. Then supernatant was evaporated to dryness. These samples were diluted further with 500 µL of mobile phase and injected 20µL into HPLC.

Atmospheric Pressure Ionization 3000 was used for the analyses of duloxetine and duloxetine D5. The m/z was found to be 298.24 and product was 154.52 in fig.2 ;303.29 and product was 159.21 in fig.3.

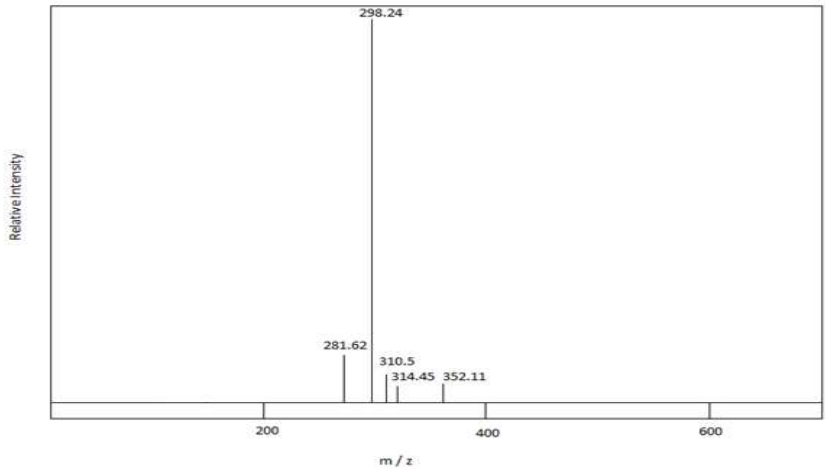


**Fig.2. Mass spectrum of Duloxetine Hydrochloride**

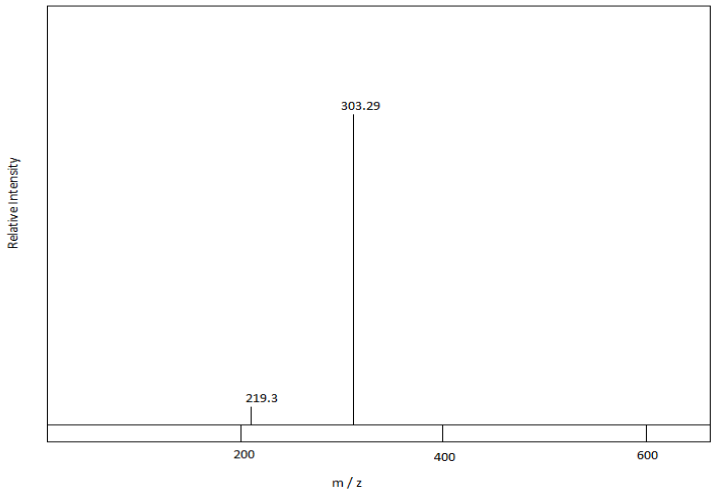


**Fig.3. Mass spectrum of Duloxetine D5**

**III) Validation parameters**

**Linearity**

The linearity of proposed analytical method is in which the response is proportional to the concentration of drug in samples within a given range. The correlation coefficient should be greater than 0.98, with ± 20% standard deviation in LLOQ.

**Precision and Accuracy**

Precision refers to the degree of repeatability or reproducibility of the analytical method. In this proposed method, five bioanalytical samples were selected for batch precision and accuracy.

Accuracy is defined as the ratio of average quality control to the nominal concentration and multiplied with 100. Precision is the standard deviation of each concentration to the average quality control in a batch and multiplied with 100. The permissible limit is ±15% and ±20% and for LLOQ the value is ±20%.

**Stability of standard stock solution**

The prepared stock solutions kept at temperature 2 - $8^0$C and analyzed at 0 and 6 hours. The response should be between 85% and 115%. This is determined by ratio of average response at six hours to average response at zero hour and multiplied with 100.

**Stability of spiking solution analyte and Internal Standard**
The MQC spiking concentrations from stock and internal standard were taken for the study and kept at temperature 2- $8^0$C and calculated by taking ratio of average response at six hours to the average response at zero hour and multiplied with 100.

**Freeze thaw stability**
The three freeze thaw cycles were performed for LQC and HQC after one day freezing. The actual concentration after freeze thaw was determined using the ratio of average concentration of either LQC or HQC to the nominal concentration of LQC or HQC and multiplied with 100.

**Recovery**
The standard IS was spiked into blank matrices samples and analysed and further average percent recovery was determined using the ratio of average response of extracted to non-extracted response and multiplied with 100.

**IV) Results and Discussion**
**Linearity**
Concentration-response Linearity Data for Duloxetine was shown in the table 1.

**Table 1. Obtained concentration with relative standard deviation**

| Mean±Std.Dev | Relative Std.Dev |
|---|---|
| 0.49±.01 | 2.04 |
| 1.01±.04 | 3.96 |
| 2.54±.02 | 0.78 |
| 4.97±0.14 | 2.81 |
| 10.42±0.13 | 1.24 |
| 29.97±0.42 | 1.40 |
| 60.23±0.35 | 0.58 |
| 116.21±1.53 | 1.31 |
| 177.91±1.72 | 0.96 |
| 194.41±1.22 | 0.62 |

Correlation coefficient (r) was greater than 0.99 in the concentration range of 0.49ng/mL to 194.41ng/mL for Duloxetine.

**Precision and accuracy**
The precision of the method was evaluated using LLOQ QC, LQC, $MQC_1$, $MQC_2$ and HQC and mentioned in the following tables.

**Intra Batch Precision and Accuracy for DLX**

**Table 2. Intra Batch Precision and Accuracy**

| | Mean±Std.Dev | Relative Std.Dev |
|---|---|---|
| LLOQ | 0.47±0.12 | 3.54 |
| LQC | 1.51±.08 | 2.95 |
| MQC1 | 40.53±0.77 | 1.95 |
| MQC2 | 103.28±1.32 | 1.29 |
| HQC | 168.42±1.48 | 1.01 |

**Standard Stock Solution Stability at Room Temperature (25 ± 2 °C):**
Spiking solution stability was determined by injecting six samples of middle concentration of standard stock solution. The samples were further analyzed and average concentration was determined.

**Table 3.Stock Solution Stability of Duloxetine (25 ± 2 °C):**

| Hours | %Stability |
|---|---|
| 0 | 100.00 |
| 9 | 99.21 |

**Stock Solution Stability at 2 - 8 °C:**
Spiking solution stability was determined by injecting six samples of middle concentration of standard stock solution. The samples were further analyzed and average concentration was determined.

**Table 4.Stock Solution Stability of Duloxetine at 2 - 8 °C**

| Days | %Stability |
|---|---|
| 0 | 100.00 |
| 4 | 99.84 |

**Freeze-thaw Stability**
The freeze-thaw concentrations were determined and compared with freshly spiked calibration concentrations which are equivalent tothe concentrations of accuracy and precision.

**Table 5.Freeze Thaw Stability Data of Duloxetine (IV Cycles)**

| | Mean±Std.Dev | Relative Std.Dev |
|---|---|---|
| LQC | 1.52±0.05 | 3.2 |
| HQC | 166.86±1.49 | 0.89 |

**Bench Top Stability**
The prepared calibration concentrations spiked with plasma were kept in ambient conditions and determined for its drug content. The drug content is evaluated after 10 hours,

**Table 6.Bench Top Stability Data of Duloxetine for 10 hours**

| | Mean±Std.Dev | Relative Std.Dev |
|---|---|---|
| LQC | 1.55±0.04 | 2.55 |
| HQC | 164.24±3.12 | 1.89 |

**Recovery**
Six samples of LQC, MQC2 and HQC and extracted samples were analyzed. The extracted concentrations of drug were compared against the non-extracted samples.

**Table 7.Recovery of Duloxetine from Human Plasma**

| Quality Control | Average± Std. Dev |
|---|---|
| Extracted LQC | 25014.5±598.14 |
| Non Extracted LQC | 28874.4±841.51 |
| Extracted LQC | 1555451.8±14875.9 |
| Non Extracted LQC | 1772478.65±24581.17 |
| Extracted LQC | 2414103.8±47588.5 |
| Non Extracted LQC | 2785403.49±41542.27 |

## CONCLUSION

The quantitative estimation of Duloxetinehydrochloride using LC-MS/MS in human plasma was established. The literature survey revealed that many combination analytical methods were found but single LC MS method was not reported with good precision and accuracy. Various extraction procedures like solid phase and protein precipitation available though, liquid-liquid extraction application on to Duloxetine was simple and do not involve complex preparation steps. Freeze-thaw stability and bench-top stability were shown that there is no change in the drug concentration. The proposed method is suitable for analysis of Duloxetine in human plasma.

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