

## QUANTITATIVE DETERMINATION OF DULOXETINE HCL IN HUMAN PLASMA BY GC-FID METHOD

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### ABSTRACT

**Objectives:** Objective of our present study was to develop a novel gas chromatographic method for quantitative estimation of Duloxetine HCl (DUL) in human plasma and to validate the developed method following USP guidelines.

**Methods:** Duloxetine HCL (DUL) was spiked in to human plasma and the extracted DUL was derivatized with Ethyl chloroformate (ECF) to obtain volatile product. Elution was carried out on Rtx-5 capillary with a dimension of 30 m X 0.25 mm. Eluates were monitored using a flame ionization detector (FID). The elution was carried out at an initial temperature of 60° C for 2 min and temperature was increased to 85° C at the rate of 2° C min<sup>-1</sup>, maintained for 5 min. Carrier gas pressure was programmed to 85 Kpa.

**Results:** DUL eluted at 2.7 minutes. The linear calibration range for was observed between 5-50 µg mL<sup>-1</sup>. The method was found to be accurate with 99.1 % recovery, precise as the inter day and intraday precision was shown at an RSD of 0.234 % and 0.224 % respectively. The LLOD and LLOQ were found to be 0.11 µg and 0.34 µg respectively.

**Conclusion:** A novel specific, accurate, precise gas chromatographic method was developed for quantitative estimation of DUL in human plasma and validated. The developed method is suitable and economic for routine analysis and pharmacokinetic studies of DUL.

**Keywords:** Gas chromatography, Duloxetine HCl (DUL), Human plasma, Ethyl chloroformate (ECF), Flame ionization detector (FID).

### INTRODUCTION

Neuropathic pain caused by damage in the peripheral and central nervous system, may be more difficult to treat than other types of pain. This is because standard analgesics often do not provide significant relief. Neuropathy is associated with considerable impairment of quality of life. [1]

Duloxetine HCl (DUL) [(S)-N-methyl-3-(naphthalen-1-yloxy)-3-(thiophen-2-yl) propan-1-amine] is a serotonin and norepinephrine reuptake inhibitor. It is effective for major depressive disorder and generalized anxiety disorder. It can also relieve the symptoms of painful peripheral neuropathy, particularly diabetic neuropathy, and it is used to control the symptoms of fibromyalgia. [2, 3]

Even though HPLC [4-5], Spectroscopical [6-10], fluorimetric [11] methods are reported, a thorough literature search has revealed that no gas chromatographic methods available for determination of Duloxetine HCl in bulk drugs and pharmaceutical formulations and in human plasma. Hence there is a scope for development of suitable gas chromatography methods for the determination of DUL in bulk and pharmaceutical formulations in human plasma.

Alkyl chloroformates in aqueous alcohol medium enable amines and amino acids to be converted into derivatives amenable to gas chromatographic analysis in seconds. The reaction is simple and sample overheating is not required. The reaction enables faster conversion of hydrophilic compounds to organophilic ones which is suitable for GC analysis [9]. A number of drugs are analyzed by the GC method after derivatization with Ethyl chloroformate [10-15]. As the drug is polar and non volatile, the amine group of the drug is derivatized using ethyl chloroformate as reagent.

The present work aims for the development and validation of sensitive and rapid capillary GC determination of DUL after derivatization with ethyl chloroformate (ECF) with FID determination.

### MATERIALS AND METHODS

#### Chemicals and Reagents

DUL was obtained from Kanvista Formulations, Hyderabad, (India). Ethyl chloroformate, chloroform, orthophosphoric acid, methanol, acetonitrile, pyridine (HPLC grade) from Merck (India), human plasma from JSS Hospital, Mysore, were used for the analysis.

#### Instrumentation and chromatographic conditions

GC studies were carried out on SHIMADZU model 2014, method development and validation was performed on coupled with a split/split less injector, GC and FID. Rtx-5 capillary column (cross bond of 5 % diphenyl and 95 % dimethyl polysiloxane) with dimension of 30 m × 0.25 mm was used.

The injection port and detector temperature were set to 160 °C and 250 °C respectively. All injections were made manually in split less mode. The oven temperature was adjusted to 60 °C for 2 min. Temperature was gradually increased to 80 °C at the rate of 2 °C min<sup>-1</sup> was maintained for 5 min. Carrier gas pressure was maintained at 80.0 Kpa.

#### Preparations of standard solutions

A stock solution of Duloxetine HCl of concentration 1 mg/mL was prepared in methanol and diluted to obtain serial dilutions of 5 to 50 µg/mL. The solutions were kept at low temperature (4 °C) protecting from direct sunlight.

#### Sample preparation

40 µL of Duloxetine HCl solutions of concentrations 5, 10, 20, 30, 40 and 50 µg/mL were spiked into five different polypropylene centrifuge tubes containing 500 µL human plasma. To each tube 50 µL of 10 % ortho phosphoric acid and 1ml acetonitrile were added. Tubes were vortexed for 10 min; followed by centrifugation for 10 min. Supernatant layers were collected and evaporated at 50° C. The drug residue was dissolved in 500 µL methanol and derivatized following the procedure given below.

3 mL of 1M sodium carbonate and 2 mL of ethyl chloroformate were mixed and added 2 ml of Duloxetine HCl, sonicated for 10 minutes at room temperature. 2 mL of chloroform was added and mixed for 10 minutes. Chloroform layer was collected and various dilutions were prepared according to linearity. 2 µL of the test solutions were injected into the GC column after filtration.

20 Dulane capsules (20 mg, Sun Pharma) were used to determine the recovery of Duloxetine HCl in its capsule formulation. An amount of powder equivalent to 20 mg of Duloxetine HCl was dissolved in 100 mL volumetric flask. The solution was sonicated for 20 min and filtered through 0.2 µm membrane filter paper. 40 µL of the above solution was spiked into a polypropylene centrifuge tubes containing

500  $\mu$ L human plasma. The solution was derivatized with ethyl chloroformate as above.

### Method validation

#### Specificity

The specificity of the method was determined by analyzing blank plasma, without the addition of DUL. It was done to show that the method has no interference from plasma.

#### Linearity range

DUL Spiked in human plasma and derivatized concentrations were plotted against the peak area and the best fit line was obtained by linear regression analysis of the resultant curve. The linearity equation ( $y=mx+c$ ) and the regression coefficient was calculated. The wide range of concentrations was investigated by injecting solutions containing 1.0 to 100  $\mu$ g/mL.

#### Precision and accuracy

Five analytical batches were used to assess the precision and accuracy of the method. Each batch contained a single set of calibration standards and six replicates of regular quality control (QC) samples at three concentrations (8, 10 and 12  $\mu$ g/mL). The assay precision for each QC level was determined as the relative standard deviation (RSD) of the measured concentrations and the accuracy was expressed as percentage of the mean of measured concentrations over the nominal concentration. The intra and inter-batch precisions were required to be below 15 %, and accuracy within  $\pm$  15 %. For LOQ, the precision required should be below 20 % and accuracy within  $\pm$  20 %.

#### Stability studies

The stability of Duloxetine HCl was determined by measuring concentration change in control sample over time. The plasma

control samples after derivatization were stored in polypropylene plasma tubes at -20  $^{\circ}$ C. Stability was tested by subjecting the plasma controls to freeze-thaw cycles and storage for 24 hrs at room temperature.

## RESULTS AND DISCUSSION

### Method development

#### Optimization of chromatographic conditions

The chromatographic conditions were optimized through several trails to achieve good resolution and symmetric peaks of analyte. The column pressure was found to be necessary for good sensitivity and peak shape. Elution of the analyte was at retention time of 2.7 min.

#### Optimization of extraction

Five organic solvents orthophosphoric acid, acetonitrile, methanol, ethyl chloroformate, pyridine and chloroform were used in the extraction process. 10 % ortho phosphoric acid and 1ml acetonitrile was found to be optimal as it produced clean chromatogram for a blank plasma sample.

The compounds DUL reacted with ECF to form a volatile product (Figure 1), and eluted from a capillary GC column, each having single peak. The reaction was carried out in the presence of 1M sodium carbonate solution to provide basic medium. The reaction mixture was sonicated at room temperature (30 $^{\circ}$ C) for 5-20 min at an interval of 10 min and the optimum response was observed within 15 min.

Chromatogram obtained for DUL and blank plasma with derivatising agents are shown in Figure 2 & 3. On the basis of the chromatograms obtained, characteristic retention times were determined for the drug as the basis for qualitative identification.

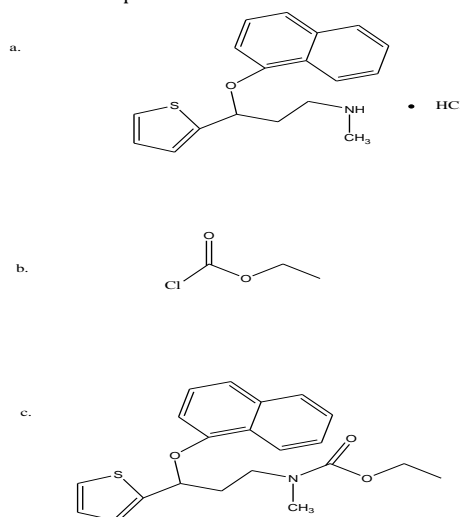


Fig. 1: Structure of the (a) Duloxetine HCl (b) ECF (c) Derivatized DUL

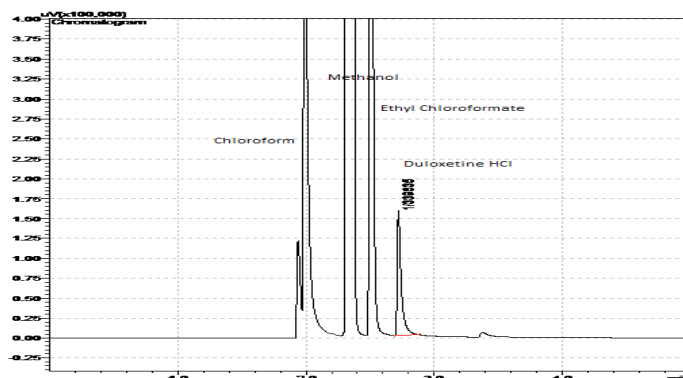


Fig. 2: Chromatogram of Duloxetine HCl (DUL) in human plasma

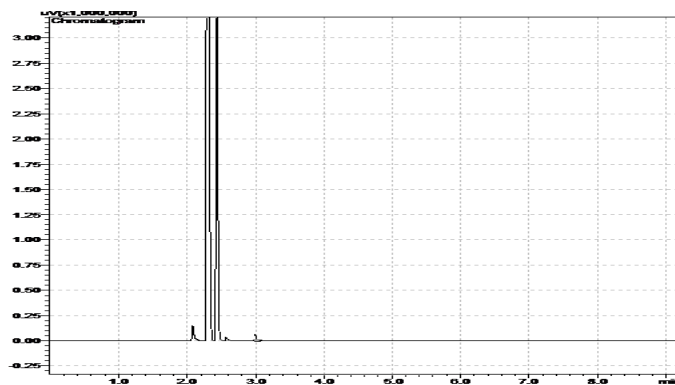


Fig. 3: Chromatogram of blank human plasma with derivatizing agents

### Method Validation

After the method conditions were established as described above, method was validated for accuracy, precision, limit of detection and limit of quantification, linearity and recovery following USP guidelines.

The linearity of peak area response versus concentration for DUL spiked in human plasma was studied between concentration ranges of 5-50 µg/mL. The calibration curve constructed was evaluated by its correlation coefficient. The calibration equation from six replicate experiments,  $y = mx + c$  ( $r^2 = 0.9871$ ), demonstrated the linearity of the method.

Accuracy was determined as percentage recovery ( $n=5$ ) of human plasma spiked DUL samples at three concentrations (80, 100 and 120 % of the amount expected) achieved by spiking placebo with a reference standard. The results are shown in Table 1.

The precision of the analytical method was determined by repeatability (intra-day) and intermediate precision (inter-day).

Three different concentrations of human plasma spiked DUL samples (8, 12, 12 µg/mL) were analyzed six times in one day for intra-day precision and once daily for three days for inter-day precision. The RSD value for intra-day precision was 0.234 % and for inter-day precision was 0.224 %. The values are summarized in Table 2.

The limit of detection (LOD) and quantification (LOQ) were determined. Quantitative analysis of the drugs was performed under the conditions established. DUL was analyzed in medicinal products in the form of immediate release capsule. The selectivity of the method was evaluated by comparing retention time values in chromatograms obtained from the analyzed product with those in the chromatograms obtained from reference standard. There were no additional peaks found in the chromatogram. Validation and system suitability parameters for the gas chromatographic determination of Duloxetine HCl are reported in Table 3 & 4. Plasma control samples were found to be stable for 24 hrs (Table 5).

Table 1: Recovery Studies results of DUL

Name of the Drug	Amount of formulation powder added (µg/ml)	Amount of pure drug taken (µg/ml)	Amount recovered (µg/ml) n=3	% recovery	Average % recovery
Duloxetine HCl	10	8	7.3	97.4	99.1
	10	10	10.2	96.6	
	10	12	11.2	103.2	

Table 2: Interday and Intraday Precision report for gas chromatograph for determination of Duloxetine HCl

Parameters N = 9	Interday Precision	Intraday Precision
Mean peak area	360164	362567
SD	1102.52	1013.8
%RSD	0.234	0.224

Table 3: Validation report for gas chromatograph for determination of Duloxetine HCl

S. No.	Validation parameters	Duloxetine HCl
1	Retention time (min)	2.7
2	Linearity range (µg/ mL)	5 - 50
3	Linearity ( $R^2$ )	0.9871
4	Recovery (%)	99.1
5	% RSD	0.36
6	LLOD (µg)	0.11
7	LLOQ (µg)	0.34

Table 4: System suitability parameters of GC

S. No.	Parameter	Results	
		Gabapentin	Duloxetin
1	Theoretical plates/meter	55123	54797
2	HETP	0.000234	0.000453
3	Tailing factor	1.1	1.09

Table 5: Stability studies on DUL

S. No.	Number of hours stored	Recovery in % N = 6
1.	1	97.4
2.	2	97.2
3.	4	96.5
4.	8	96.0
5.	12	95.3
6.	24	94.7

## CONCLUSION

In the present work, a simple, sensitive and accurate GC-FID method for the determination of DUL in human plasma has developed and validated. The method described in the present report has been effectively and efficiently used to analyze DUL pharmaceutical dosage form and DUL human plasma without any interference from the pharmaceutical excipients. Therefore, the GC-FID method can be used for the routine QC analysis of DUL in pharmaceutical preparations.

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