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Original Article

Bio-Analytical Method Development and Validation of Duloxetine in Rat Plasma and its Application to a Pharmacokinetic Study by Chiral Chromatographic Technique

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ABSTRACT

Background: Duloxetine, a Serotonin-Norepinephrine Reuptake Inhibitor (SNRI), is commonly prescribed for major depressive disorder. Being a chiral compound, it exists as (R)- and (S)-enantiomers, which may exhibit distinct pharmacokinetic and pharmacodynamic characteristics. An enantioselective analysis is crucial for understanding the behaviour of individual enantiomers; however, limited research has focused on the chiral separation of duloxetine in plasma using a validated Ultra-Fast Liquid Chromatography (UFLC) method. Objective: This research seeks to create and confirm the validity of an enantioselective UFLC method for quantifying duloxetine enantiomers in rat plasma and apply it to a pharmacokinetic study in Wistar albino rats. **Materials and Methods:** A Chiral UFLC method was established using an appropriate chiral stationary phase and validated following regulatory standards. The method was then utilized for analysing plasma samples collected from Wistar albino rats after duloxetine administration. Pharmacokinetic parameters for each enantiomer were assessed. **Results and Discussion:** The proposed method successfully achieved enantioselective separation with high resolution and sensitivity. Pharmacokinetic evaluation demonstrated variations in the absorption, distribution, and elimination profiles of (R)- and (S)-duloxetine enantiomers. **Conclusion:** The validated UFLC method offers a robust approach for enantioselective analysis of duloxetine in plasma. The pharmacokinetic findings emphasize the importance of enantiomer-specific assessments in preclinical and clinical research to optimize duloxetine therapy.

Keywords: Duloxetine, Depressive disorder, UFLC method, Pharmacokinetics, Rat plasma.

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INTRODUCTION

Duloxetine, classified as a Serotonin-Norepinephrine Reuptake Inhibitor (SNRI), is generally used for treating major depressive disorder, generalized anxiety disorder, diabetic peripheral neuropathy, and other related conditions fibromyalgia (Kumar *et al.*, 2017). Its therapeutic action stems from its ability to inhibit the reuptake of serotonin and norepinephrine while also influencing dopamine levels, making it beneficial for both mood disorders and neuropathic pain management (Chhalotiya *et al.*, 2010; Kotadia *et al.*, 2012; Gajula *et al.*, 2013). Structurally, duloxetine is referred to as (3S)-N-methyl-3-naphthalen-1-yloxy-3-thiophen-2-ylpropan-1-amine it has a molecular weight of 297.4 g/mol and melts at 162°C. The compound is soluble in water, ethanol, and methanol, facilitating its analysis through various analytical methods as shown in Table 1 (Soni *et al.*, 2005; Patel *et al.*, 2008).

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Multiple analytical approaches have been developed for quantifying and assessing the stability of duloxetine in pharmaceutical formulations and biological samples. Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) is widely employed to analyse duloxetine in both bulk and tablet forms, demonstrating its reliability as a method for indicating stability (Sinha *et al.*, 2009; Bhimanadhuni *et al.*, 2012). Furthermore, Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) has been validated for quantifying duloxetine concentrations in human plasma, offering exceptional sensitivity and specificity crucial for pharmacokinetic evaluations (Gajula *et al.*, 2013; Satonin *et al.*, 2007; Johnson *et al.*, 1996).

Despite these advancements, the enantioselective separation and pharmacokinetic characterization of duloxetine enantiomers remain an area of ongoing research (Srinivasulu *et al.*, 2009). Since duloxetine exists as a racemic mixture, its enantiomers may have distinct pharmacological and metabolic profiles, necessitating precise analytical techniques for their differentiation. Chiral stationary phases in Ultra-Fast Liquid Chromatography (UFLC) have proven effective for separating and quantifying duloxetine enantiomers, enabling comprehensive pharmacokinetic studies (Kumar *et al*., 2011; Rao *et al*., 2010).

This study focuses on developing and validating a bioanalytical method using UFLC with a chiral stationary phase for the enantioselective separation of duloxetine. The method will be validated following the regulations set by the United States Food and Drug Administration (USFDA) to guarantee reliability in terms of accuracy, precision, and robustness. Furthermore, pharmacokinetic studies will be conducted on Wistar albino rats to evaluate the Absorption, Distribution, Metabolism, and Excretion (ADME) of duloxetine enantiomers. The results from this study will enhance the understanding of enantio-specific pharmacokinetics, contributing to the optimization of duloxetine therapy and improving clinical outcomes.

MATERIALS AND METHODS

Chemicals and reagents

Acetonitrile and acetate buffer, procured from Merck Life Sciences Ltd., were used in the study. S-Duloxetine hydrochloride was obtained from Aurobindo Pharma Ltd., while R-Duloxetine hydrochloride was sourced from Veerphro Laboratories Ltd., Methanol and Millipore water were supplied by Merck Specialties Pvt. Ltd., All chemicals and solvents used were of analytical or HPLC grade quality. The pure (R) enantiomer of Duloxetine HCl is available in the formulation Duvanta-20 from Intas Pharma Ltd., while the pure (S) enantiomer is marketed as Symbol 20 by Torrent Pharma Ltd.

Instrumentation

The UFLC system included a Shimadzu LC-20AD solvent delivery unit (pump) equipped with a UV detector and a 20 µL loop volume (Kyoto, Japan), with integration performed using the LC Solution program. A Shimadzu electronic weighing balance was used for precise drug weighing, A GT SONIC ultrasonic cleaner was used for sample preparation.

Chromatographic Conditions

In this chromatographic analysis, Trial 1 utilized a Lux-i-Amylose-3 column (250×4.6 mm ×5 µm) with a flow rate of 0.8 mL/min, a run time of 10 min, and a wavelength of 260 nm. The injection volume was 10 µL, and the PDA detector was used with binary gradient elution. The mobile phase consisted of Methanol, Millipore water, and Acetic Acid in an 80:20:0.02 v/v ratio. Trial 2 employed the same column with a 0.8 mL/min flow rate, a 10-min run time, and a wavelength set to 240 nm. The injection volume remained at 10 µL, and the PDA detector was used, with the mobile phase composed of Acetonitrile and Acetate buffer in a 60:40 v/v ratio. Based on the findings from Trial 2, the optimized chromatographic conditions for both analytical and bio-analytical purposes used the same column, with a 0.8 mL/min flow rate, 10-min run time, and a 240 nm wavelength. The mobile phase was adjusted to Acetonitrile and an acetate buffer (pH 3.8) in a 70:30 v/v ratio, with a 10 µL injection volume and PDA detector using binary gradient elution.

Preparation of standards and Quality Control (QC) samples

Acetate buffer pH 3.8 (0.01 M or 10 mM)

A precise quantity of 112.837 mg of sodium acetate was measured and dissolved in 517.9 mg of acetic acid. The volume was then brought up to 1000 mL using Millipore water. The pH was adjusted as needed with HCl or NaOH. Finally, the buffer solution was filtered using vacuum filtration through a membrane filter.

Stock Solutions of Duloxetine (S) and Duloxetine (R)

A 1000 µg/mL stock solution of Duloxetine (API) (S) and (R) was individually prepared by accurately weighing 10 mg of each into a 10 mL volumetric flask. To dissolve the API, 5 mL of HPLC-grade methanol was added, followed by sonication for 2 min. The solution was then diluted to the mark with HPLC-grade methanol. A 100 µg/mL working solution was prepared from each stock solution, filtered using a 0.25 µm membrane filter, and stored at 20°C.

Racemic mixture

A racemic mixture with a concentration of 100 µg/mL was prepared by combining 1 mL each of the (S) and (R) isomers and diluting the mixture to 100 mL using HPLC-grade methanol. The solution was then filtered through a 0.25 µm membrane filter. Next, 100 µL of this solution was combined with an equal volume of rat plasma and thoroughly vortexed. Afterward, 2 mL of methanol was added, and the supernatant was collected using a syringe. The sample was filtered through a 0.25 µm syringe filter before being injected into the HPLC system for analysis.

Sample preparation

Blood sampling and Plasma Collection

The rats were anesthetized using anesthetic ether in desiccators. Once fully anesthetized, they were positioned on the operation table, and blood was collected from the retro-orbital sinus using a 0.5 mm diameter capillary tube. Each blood sample Each 0.5 mL blood sample was placed into tubes containing EDTA as an anticoagulant. To separate the plasma, the samples were centrifuged at 3000 rpm for 10 min, and the supernatant was gently transferred into clean, dry tubes. The plasma samples were then stored at -20°C for future analysis. The drug was subsequently extracted from the sample's plasma using the protein precipitation method.

Linearity Concentrations for Bio-analytical Study

Linearity solutions were prepared by diluting the from the 100 µg/mL stock solution of both R and S enantiomers, 1 mL was transferred into a 10 mL volumetric flask and diluted with HPLC-grade methanol up to the calibration mark, yielding a final concentration of 10 µg/mL 10 µg/mL. Further dilutions were prepared to obtain 2, 4, 6, and 8 µg/mL concentrations. Each 100 µL of these solutions was spiked with 100 µL of rat plasma, followed by vertexing. Subsequently, 2 mL of methanol was added, and the supernatant was collected using syringes. The solutions were then filtered through 0.25 µm syringe membrane filters. Linearity was assessed by injecting the filtered solutions in triplicate into the UFLC system.

Sample solutions from formulation

A 1000 µg/mL solution of Duloxetine was prepared from two different brands (Symbal 20, Duvanta 20) by 10 mg of each compound were carefully weighed and placed into a 10 mL volumetric flask. To dissolve the drug, 5 mL of HPLC-grade methanol was added, and the mixture was sonicated for 2 min. The solution was then brought up to the mark with methanol. A 100 µg/mL concentration prepared from this stock solution was prepared, which was further diluted to obtain a 10 µg/mL solution. The final solution was filtered through a 0.25 µm membrane filter and stored at 20°C.

Racemic Mixture Sample Preparation

As sample solution of 10 µg/ml Duloxetine formulations was prepared. Then the formulation samples of 100 µL were spiked with the 100 µL of rat plasma and 100 µL of racemic Duloxetine of different concentrations. Here the formulation concentration is constant whereas racemic concentrations were changed.

Method and Validation

Calibration Curve

Standard solutions of Duloxetine, with concentrations ranging from 2 to 10 µg/mL, were spiked into separated rat plasma. The calibration curve was then constructed and obtained for the final concentration ranges of 2-10 µg/mL. The calibration curves were acquired by plotting graphs of the ratio of the peak area of Duloxetine versus the concentration of Duloxetine for both enantiomers.

Specificity and Selectivity

The specificity of the developed method was demonstrated by analyzing blank plasma obtained from six different sources. Additionally, blank plasma samples from these sources were spiked with Duloxetine for both enantiomers. The resulting samples were then analyzed using the UFLC system.

Sensitivity

The LLOQ concentration was injected into the UFLC system in five replicates, and the results were recorded.

Recovery

The percentage recovery of Duloxetine was evaluated at three QC levels: LQC, MQC, and HQC. Recovery was determined by comparing the peak area response of six replicates of extracted QC samples at each level (LQC, MQC, HQC) with the peak area response of six replicates of directly injected QC samples, which represented 100% extraction efficiency at low, middle, and high concentrations.

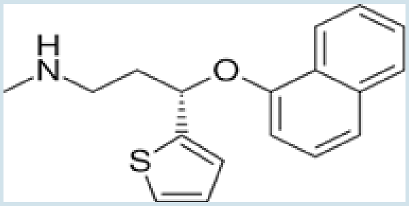
Accuracy and Precision

The precision and accuracy were evaluated by performing within-run Assays were conducted for both within-run and between-run variations at four different QC levels: LLOQ, LQC, MQC, and HQC. Six replicates of each QC sample were analyzed on the same day (within the run) and over three consecutive days (between runs). Accuracy was calculated as the percentage difference between the measured and nominal values, while precision was calculated in terms of % CV at all four QC levels.

Stability

Stability studies were conducted Stability at LQC and HQC was assessed using six replicates. Freeze-thaw stability was evaluated after three complete freeze-thaw cycles, where the samples were stored at -20°C for 24 hr and then thawed to room temperature. Benchtop stability was tested by leaving the samples at room temperature for 12 hr. The long-term stability of the analyte was

Table 1: Drug Profile.

Drug Name	Duloxetine
Chemical Structure	
Molecular Formula	C H NOS 18 19
Molecular Weight	297.4gm/mol
Category	Anti-depressant
Chemical Name	(3S)-N-methyl-3-naphthalen-1-yloxy-3-thiophen-2-ylpropan-1amine
Melting point	162°C
Solubility	Soluble in water, ethanol, methanol

also examined at LQC and HQC with six replicates examined over a 10-day period by storing the samples at -20°C.

Application to pharmacokinetic study of duloxetine in rat plasma

The validated method was successfully used to quantify duloxetine concentrations in rat plasma in the present pharmacokinetic study. The study was conducted in compliance with current Good Clinical Practice (GCP) guidelines and was approved by an authorized animal ethics committee. Male and female Wister albino rats, each weighing approximately 200-250 g, were used for the study. A single oral dose of 20 mg of duloxetine, in both racemic and enantiomeric forms, was administered to the animals. Blood samples of 0.5 mL were collected Samples were collected at specified time points: 0, 0.5, 1, 2, 4, 6, 8-, 10-, 12-, and 24 hr post-dose. The samples were placed into vacutainers containing K2EDTA as an anticoagulant. Plasma was separated by centrifugation at 3000 rpm at 4°C, and the resulting plasma was stored at -30°C until further analysis. For analysis, 200 µL of plasma was mixed with 200 µL of methanol, vortexed, and centrifuged at 9000 rpm for 15 min at 4°C. The supernatant was then filtered through a 0.2 µm syringe filter, and 10 µL of the filtered supernatant was transferred into HPLC vials for analysis. The pharmacokinetic parameters were then calculated, such as C_{max}, T_{max}, half-life (t½), AUC, and clearance, were computed from the plasma concentration data using appropriate software.

The validated method was effectively utilized in the study to measure duloxetine concentrations in rat plasma for pharmacokinetic evaluation. It was conducted following the current GCP guidelines and approved by an authorized animal ethics committee. Both male and female Wistar albino rats, weighing between 200 and 250 g, were used. A single oral dose of 20 mg duloxetine, administered in both racemic and enantiomeric forms, was given. Blood samples (0.5 mL) were collected at specified time points: 0, 0.5, 1, 2, 4, 6, 8, 10, 12, and 24 hr post-dose. The samples were placed into vacutainers with K_2EDTA as an anticoagulant, and plasma was separated by centrifugation at

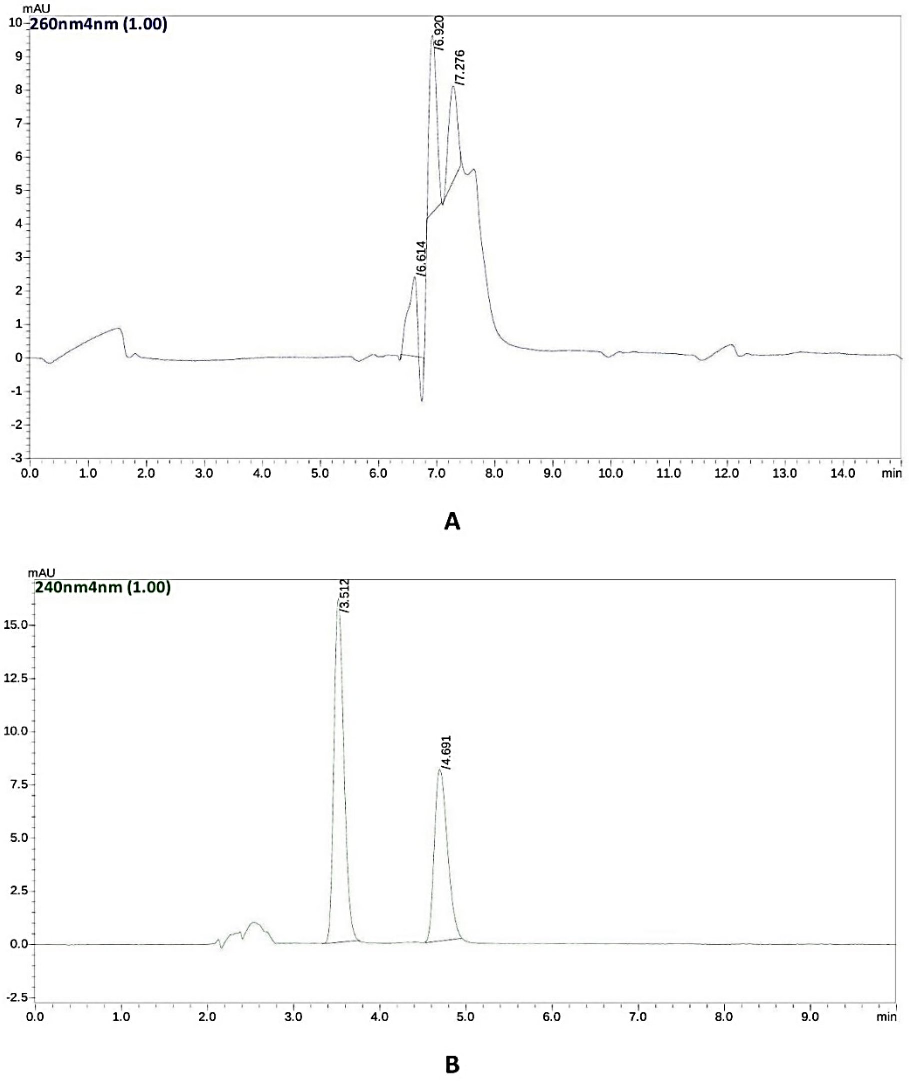


Figure 1: (A) Trail-1 Chromatogram of Racemic Mixture; (B) Trail-2: Chromatogram of DLX Racemic Mixture.

3000 rpm at 4ºC. The plasma was stored at -30ºC until further analysis. For the analysis, 200 µL of plasma was combined with an equal volume of methanol, vortexed, and centrifuged at 9000 rpm for 15 min at 4ºC. The supernatant was then filtered through a 0.2 µm syringe filter, and 10 µL of the filtrate was transferred into HPLC vials for analysis. Pharmacokinetic parameters such as C_{max}, T_{max}, half-life (t½), AUC, and clearance were derived from the plasma concentration data using appropriate software (Reddy *et al*., 2012; Chadha *et al*., 2017).

RESULTS AND DISCUSSION

Method development by RP-UFLC

During method development, various parameters were systematically assessed. Initially, the analyte's solubility and UV wavelength were determined. Chromatographic conditions were optimized to achieve effective separation of compounds. Several mobile phase compositions were evaluated, including Methanol: Millipore Water: Acetic Acid (80:20:0.02) (V/V), which resulted in merged peaks and was deemed unsuitable as shown in Figure 1(A). However, a composition of Acetonitrile: Acetate Buffer (60:40) (V/V) provided well-separated peaks with acceptable tailing factors as shown in Figure 1(B).

Different pH levels (4.0 and 5.0) were tested, but they failed to adequately separate the enantiomers. Optimal separation was achieved at pH 3.8. Various columns were examined for drug elution and related substances. Parameters such as peak area, peak height, theoretical plates, resolution, tailing factor, and run-time were assessed to optimize the buffer pH, mobile phase ratio, and flow rate. The final method used a mobile phase of Acetonitrile: Acetate Buffer (60:40) (V/V) with a flow rate of 0.8 mL/min. The optimal detector response for both the drug and its impurities was observed at 240 nm, and these conditions were also applied for the bioanalytical method validation.

Peak Purity of Duloxetine Hydrochloride

The purity of both (S)-duloxetine hydrochloride and (R)-duloxetine hydrochloride (Figure 2) was evaluated using HPLC to ensure the absence of co-eluting impurities. In both cases, the purity curves closely followed the zero line, indicating spectral consistency and peak integrity.

For (S)-duloxetine hydrochloride, the peak purity index was determined to be 0.910806, with a single-point threshold of 0.948864. Meanwhile, (R)-duloxetine hydrochloride exhibited a peak purity index of 1.0000, surpassing the single-point threshold of 0.998964. The analysis confirmed that no impurities were detected within either peak, demonstrating the method's specificity and accuracy.

These findings confirm that the chromatographic technique used is reliable for assessing the purity of duloxetine hydrochloride, making it suitable for pharmaceutical QC and regulatory compliance.

Method validation

The developed method was validated across a linear concentration range of 1.00-400.0 ng/mL. Validation parameters, including calibration curve, selectivity, specificity, sensitivity, recovery, accuracy, precision, and stability (freeze-thaw, autosampler, bench-top, and long-term stability), were assessed following USFDA guidelines.

Calibration Curve for (S) and (R) Enantiomers

Table 2 presents calibration values for (S) and (R) enantiomers, respectively, and Figure 3 depicts the corresponding calibration curves.

Specificity and Selectivity

Duloxetine enantiomer peaks showed no interference from endogenous substances. Figure 4 shows only one blank plasma interference. The retention times for (R)-Duloxetine and (S)-Duloxetine were 3.867 and 5.362, respectively, indicating that there was no notable interference between the Duloxetine enantiomers (S and R) and a racemic mixture, as shown in Figure 5.

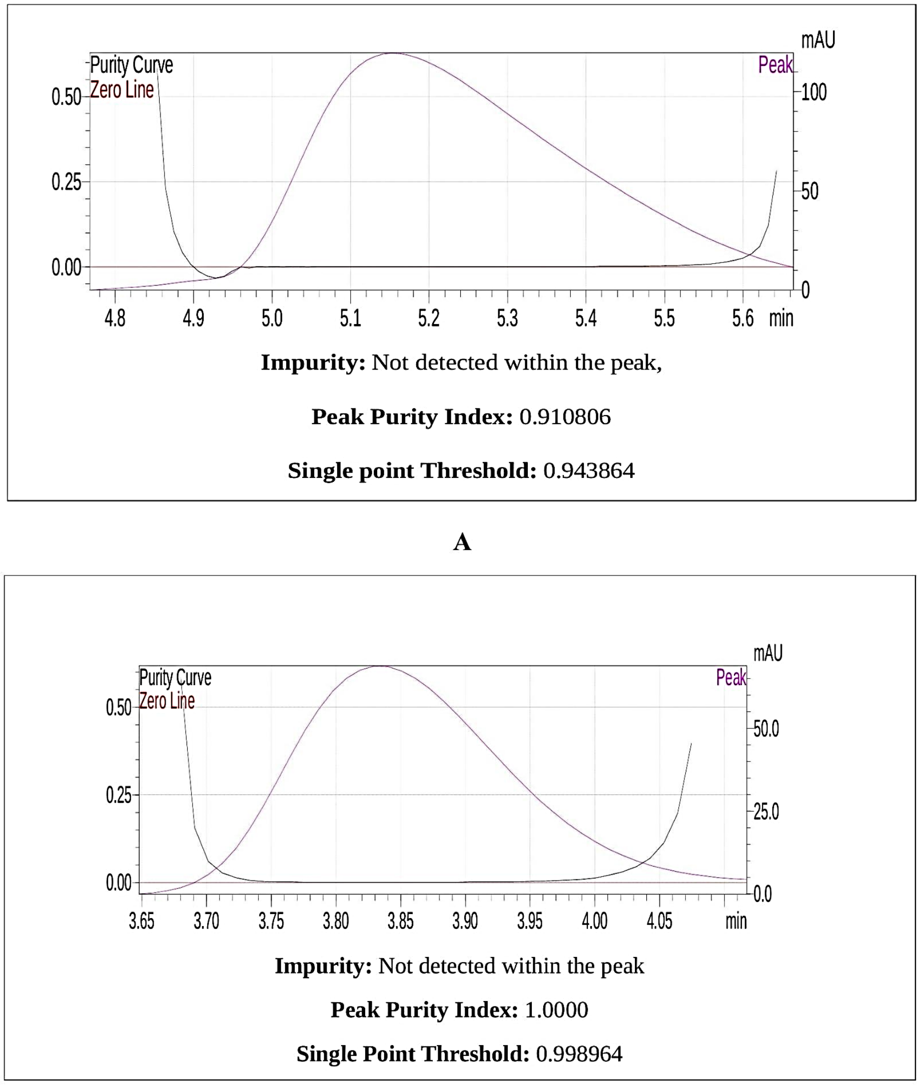
Sensitivity

The LLOQ of 10 µg/mL was found to offer good accuracy (±20% of nominal concentration) and precision (±20% CV) for Duloxetine

Table 2: Calibration Values for (S) and (R) enantiomer.

Standards	Concentration (µg/mL)	Peak Area (S)	Peak Area (R)
LLOQ	2	206037	122619
LQC	4	443544	250918
MQC	6	699608	396137.3
HQC	8	932577	501037.1
UQC	10	1183219	715012

LLOQ – Lower Limit of Quantification Sample, LQC Low-Quality Control Sample (or Low Concentration Quality Control Sample), MQC – Medium-Quality Control (or Medium Concentration Quality Control Sample), HQC – High-Quality Control (or High Concentration Quality Control Sample), UQC – Upper-Quality Control (or Ultra-High Concentration Quality Control Sample).



Impurity: Not detected within the peak,

Peak Purity Index: 0.910806

Single point Threshold: 0.943864

A

Impurity: Not detected within the peak

Peak Purity Index: 1.0000

Single Point Threshold: 0.998964

B

Figure 2: (A) Peak Purity of (S) enantiomer; (B) Peak Purity of (R) enantiomer.

enantiomers following the optimized method. The results proved the sensitivity of the developed method and could be extended to pharmacokinetic studies.

Recovery

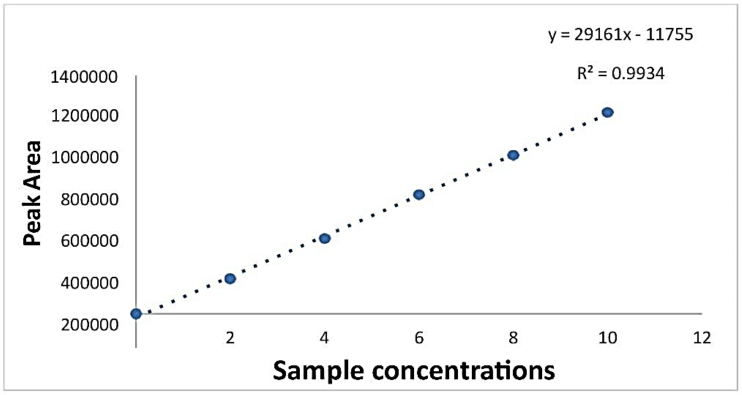
The extraction recovery of (R)-Duloxetine ranged between 80.131% and 88.528% across LQC, MQC, and HQC levels, while (S)-Duloxetine exhibited a recovery range of 79.932% to 84.759% at the same QC levels. These results indicate that the developed extraction method provided consistent and reliable recovery for Duloxetine enantiomers, as detailed in Table 3.

Accuracy and Precision

The within-run and between-run precision (%CV) for (R)-Duloxetine varied between 6.866% to 1.611%, while for (S)-Duloxetine, it ranged from 2.922% to 0.403%. The accuracy (% Relative Error or %RE) for (R)-Duloxetine varied between 64.521% and 57.311%, whereas for (S)-Duloxetine, it ranged from 35.310% to 18.701%. The precision met the acceptance criteria, with within-run and between-run precision (%CV) within ±15% at LQC, MQC, and HQC, and within ±20% at LLOQ. Similarly, the accuracy was within ±15% of the nominal concentration for LQC, MQC, and HQC, and ±20% for LLOQ. The detailed results for both within-run and between-run precision and accuracy at LLOQ, LQC, MQC, and HQC are shown in Table 4.

Stability

LQC and HQC samples were analyzed to assess Stability under bench-top conditions, freeze-thaw cycles, and long-term storage. Accuracy remained within ±15% of the nominal concentration throughout storage, ensuring analyte stability following USFDA guidelines (Table 5).



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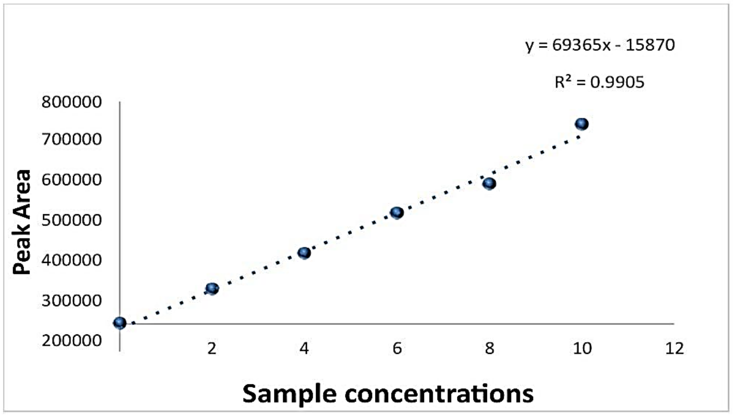

B

Figure 3: (A) Calibration curve of (S) enantiomer; (B) Calibration Curve for (R) enantiomer.

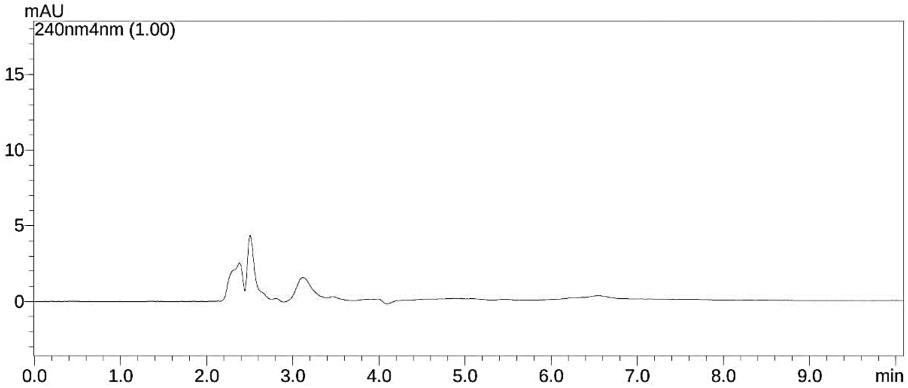

Figure 4: Chromatogram of blank rat plasma.

Table 3: Recovery Values of Duloxetine.

Quality control samples	Con (μg/mL)	% Recovery (R)- Duloxetine	% Recovery (S)- Duloxetine
LQC	4	80.131	79.932
MQC	6	83.628	81.415
HQC	8	88.528	84.759

LQC Low-Quality Control Sample (or Low Concentration Quality Control Sample), MQC – Medium-Quality Control (or Medium Concentration Quality Control Sample), HQC – High-Quality Control (or High Concentration Quality Control Sample).

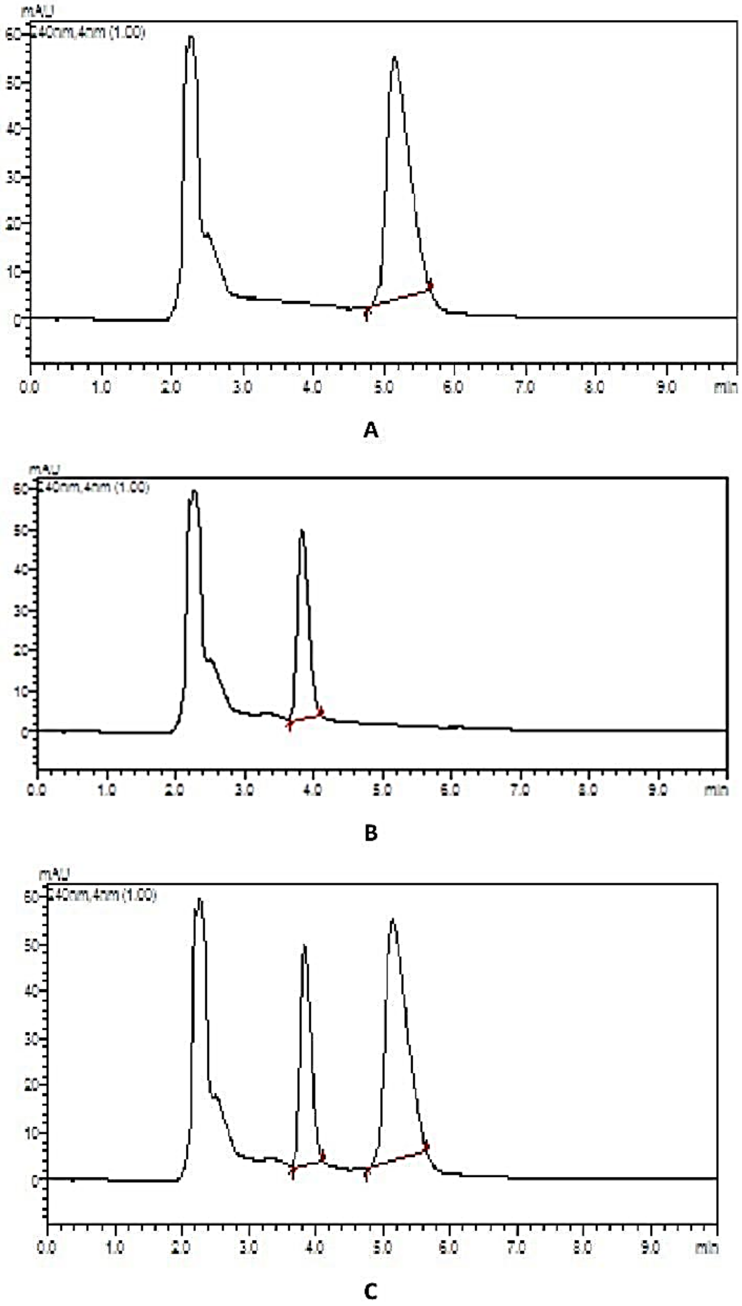

Figure 5: (A) Chromatogram of (S) Enantiomer (10 mcg); (B) Chromatogram of (R) Enantiomer (10 mcg); (C) Chromatogram of Racemic Mixture (10 mcg).

Pharmacokinetic analysis

The concentration-time profiles of Duloxetine Racemic, Duloxetine R-Enantiomer, and Duloxetine S-Enantiomer demonstrate distinct pharmacokinetic behaviors, as illustrated in Table 6.

The racemic mixture exhibited a rapid increase in concentration, reaching a peak (~30,000 units) between 5-10 hr, followed by a gradual decline, indicating systemic absorption and elimination. The R-enantiomer showed a slightly lower peak concentration (~25,000 units) with a prolonged elimination phase, suggesting a slower clearance rate. In contrast, the S-enantiomer attained a peak concentration similar to the racemic mixture (~30,000 units) but was eliminated more rapidly, indicating potential differences in metabolism or excretion pathways as shown in Figure 6. These variations in pharmacokinetic parameters highlight the differential systemic exposure of each enantiomer, which may have implications for their therapeutic efficacy and safety. The faster clearance of the S-enantiomer could influence dosing regimens, while the prolonged elimination of the R-enantiomer may lead to sustained drug exposure. These findings emphasize the need for further investigation into enantiomer-specific metabolism and their impact on clinical outcomes.

The validated analytical method successfully quantified duloxetine concentrations in rat plasma, revealing significant pharmacokinetic differences between the racemic mixture and its enantiomers. The S-enantiomer exhibited a longer half-life and larger distribution volume than the R-enantiomer, suggesting prolonged systemic presence and greater tissue penetration. In contrast, the R-enantiomer demonstrated higher systemic exposure, with greater maximum concentration and overall drug exposure, indicating its potential role in overall pharmacodynamic effects. These results are consistent with previous studies, including Reddy *et al*., (2012), who developed and validated an LC/MS/MS method for quantifying duloxetine in human plasma,

Table 4: **Accuracy Precision Values of Duloxetine.**

Quality control samples	Con (µg/mL)	(R)- Duloxetine	(S)- Duloxetine
%RE (within run)			
LLOQ	2	64.521	35.290
LQC	4	58.750	25.331
MQC	6	57.026	20.305
HQC	8	57.311	18.673
% RE (between runs)			
LLOQ	2	64.526	35.310
LQC	4	58.786	24.725
MQC	6	57.209	20.350
HQC	8	57.311	18.701
%CV (within run)			
LLOQ	2	6.866	2.922
LQC	4	2.623	1.845
MQC	6	2.854	1.014
HQC	8	1.611	0.403
%CV (between runs)			
LLOQ	2	6.860	3.012
LQC	4	2.661	2.314
MQC	6	2.486	1.057
HQC	8	1.613	0.512

LLOQ – Lower Limit of Quantification Sample, LQC Low-Quality Control Sample (or Low Concentration Quality Control Sample), MQC – Medium-Quality Control (or Medium Concentration Quality Control Sample), HQC – High-Quality Control (or High Concentration Quality Control Sample.

Table 5: **Stability Studies of Duloxetine.**

Quality control samples	Con (µg/mL)	Accuracy (%)		%CV	
		(R)-Duloxetine	(S)- Duloxetine	(R)- Duloxetine	(S)- Duloxetine
Bench-top stability					
LQC	4	96.584	2.520	94.801	2.207
HQC	8	98.172	1.863	96.320	1.527
Freeze thaw stability					
LQC	4	92.685	1.265	90.174	1.184
HQC	8	93.671	1.834	91.047	1.270
Long term stability					
LQC	4	97.850	1.316	96.401	1.273
HQC	8	98.346	0.182	97.627	0.870

LQC Low-Quality Control Sample (or Low Concentration Quality Control Sample), HQC – High-Quality Control (or High Concentration Quality Control Sample).

Table 6: Pharmacokinetic Parameters of Duloxetine and Its Enantiomer.

Parameter	Duloxetine Racemic	Duloxetine R-Enantiomer	Duloxetine S-Enantiomer	Probability (*p*-value)
T½ (h)	5.33	3.90	5.77	< 0.05
Cmax (µg/mL)	23.21	22.5	21.9	< 0.05
T_{max} (h)	6	6	6	< 0.05
$AUC_{0\text{-}t}$ (µg*h/mL)	206.01	213.057	199.18	< 0.05
$AUC_{0\text{-}\infty}$ (µg*h/mL)	1552.99	1637.85	1481.42	< 0.05
Vd (L/kg)	0.373	0.264	0.418	< 0.05
CL (L/h/kg)	0.048	0.046	0.050	< 0.05

Statistically significant $p<0.05$.
T½ – Half-life, Cmax – Maximum plasma concentration, Tmax – Time to reach maximum plasma concentration, AUC0-t – Area under the concentration-time curve from time 0 to the last measurable concentration, AUC0-∞ – Area under the concentration-time curve from time 0 extrapolated to infinity, Vd – Volume of distribution, CL – Clearance.

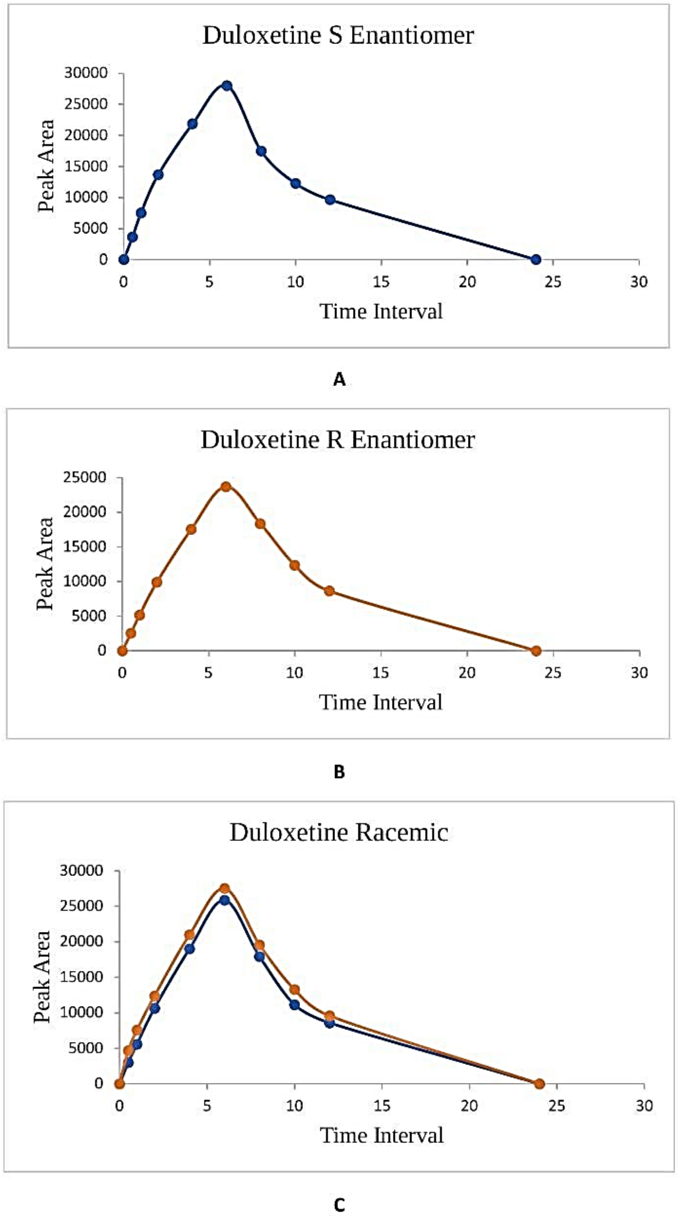

Figure 6: Peak area vs. time for duloxetine enantiomers and racemic.

and Chadha *et al*., (2017) who conducted bioanalytical method validation for duloxetine in blood serum.

The differences in clearance rates suggest that the metabolism and elimination pathways of the enantiomers differ, which could be attributed to stereoselective enzymatic processes. These findings have significant implications for enantiomeric formulations of duloxetine, as the pharmacokinetic variability between enantiomers may influence efficacy and safety. The results suggest that individual enantiomers might exhibit distinct therapeutic and adverse effect profiles, warranting further clinical investigation.

Overall, this study offers valuable insights into the pharmacokinetics of duloxetine enantiomers, emphasizing the importance of considering stereoselectivity in drug development and therapeutic applications.

CONCLUSION

A bioanalytical method for Duloxetine hydrochloride was developed using a chiral amylose column in UFLC, refined, and validated per USFDA guidelines. The method demonstrated reliable sensitivity and precision, making it suitable for pharmacokinetic studies. Significant differences were observed between the enantiomers, with the S-enantiomer showing a longer half-life and greater tissue penetration, while the R-enantiomer exhibited higher systemic exposure. These findings emphasize the role of enantiomer-specific metabolism in Duloxetine's efficacy and safety, highlighting the need for further investigation into enantiomeric formulations for optimized therapeutic outcomes.

ABBREVIATIONS

SNRI: Serotonin-Norepinephrine Reuptake Inhibitor; **UFLC:** Ultra-Fast Liquid Chromatography; **RP-HPLC:** Reverse-Phase High-Performance Liquid Chromatography; **LC-MS/MS:** Liquid Chromatography-Tandem Mass Spectrometry; **ADME:** Absorption, Distribution, Metabolism, and Excretion.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICAL CONSIDERATIONS

The IAEC of KCC BIO-LABS, Tumakuru, Karnataka gave its approval to the study. (IEC Reference Number: KCC/1AEC/53/2024).

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