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ORIGINAL ARTICLE

RP-HPLC BIOANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF ESCITALOPRAM IN HUMAN PLASMA

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ABSTRACT : For the simultaneous measurement of escitalopram in human plasma using reverse-phase high-performance liquid chromatography (RP-HPLC), a solid-phase extraction technique was created. As an internal standard, tinidazole was employed. Using 0.2% ortho phosphoric acid and acetonitrile in a 65:35% v/v ratio as the mobile phase, samples were separated on an Enable C18 column at a flow rate of 1.0 ml/min at room temperature. At 240 nm, detection was done. For escitalopram, calibration plots were linear ($R^2 > 0.9960$) across the 100–800 ng/ml concentration range. In accordance with ICH requirements, all analytical validation parameters were established. Since accuracy, precision, recovery, and other validation criteria fell within the guidelines' defined bounds, the bioanalytical method that was created was robust, selective, and dependable. For escitalopram, the overall recovery rate was 99.75%. The intra-day precision was 5.34%, and the inter-day precision was 6.31%. Human plasma pharmacokinetics was successfully studied using the proven approach. The technique can be highly beneficial for biomedical research, bioequivalence studies, and therapeutic drug monitoring (TDM).

Key words : Escitalopram, human plasma, RP-HPLC, solid phase extraction, serotonin reuptake inhibitor.

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INTRODUCTION

Escitalopram is a widely prescribed selective serotonin reuptake inhibitor (SSRI) used in the management of major depressive disorder and generalized anxiety disorder (Burke, 2002). It is the S-enantiomer of citalopram and exhibits higher selectivity and potency toward the serotonin transporter, resulting in improved therapeutic efficacy and tolerability (Rao, 2007). Due to its extensive clinical use and narrow therapeutic considerations in certain patient populations, accurate quantification of escitalopram in human plasma is essential for pharmacokinetic studies, bioavailability and bioequivalence assessments, therapeutic drug monitoring, and clinical toxicology investigations (Kennedy *et al,* 2009; Waugh and Goa, 2003).

Reverse phase high-performance liquid chromatography (RP-HPLC) is one of the most reliable and widely employed analytical techniques for the determination of drugs in biological matrices (Sakhreliya, *et al,* 2012). The method offers advantages such as high sensitivity, specificity, reproducibility, and suitability for routine laboratory analysis (Bairagi and Ghosh, 2020). However, analysis of escitalopram (Fig. 1) in human plasma presents analytical challenges due to the complex biological matrix, low drug concentrations and potential interference from endogenous components and co-

Table 1 : Accuracy and Intraday precision studies of escitalopram oxalate.

S. no.	Conc. of drug (ng/ml)	Mean peak Area*	Accuracy (%)	RSD (%)
1.	200	199526	99.2	4.98
2.	400	312919	100.3	3.57
3.	600	429303	99.9	5.32

*Average of six determinations.

Table 2 : Accuracy and Interday precision studies of escitalopram oxalate.

S. no.	Conc. of drug (ng/ml)	Mean peak Area*	Accuracy (%)	RSD (%)
1	200	191206	99.6	5.78
2	400	336188	99.2	6.31
3	600	419568	100.08	6.11

*Average of three determinations.

Table 3 : Ruggedness studies for escitalopram oxalate.

Drug	Concentration (ng/ml)	Mean peak area	%RSD
	Day I analyst – I		
Escitalopram oxalate	200	195392	2.76
	Day II analyst – II		
Escitalopram oxalate	200	199527	4.98

**Average of six determinations.

minutes, respectively, according to the RP-HPLC method for estimating escitalopram in human plasma. The Solid Phase Extraction method (SPE) was used to extract the medication from plasma, demonstrating a significant decrease in plasma interference. The developed approach was validated in accordance with FDA and ICH criteria. The obtained precision and accuracy values fell within acceptable bounds. The intraday approach has an accuracy range of 99.2% to 100.3% and a precision range of 3.57% to 5.34%. The accuracy and precision of the inter-day approach range from 99.5% to 100.08% and 5.78% to 6.31%, respectively. The depicted calibration curve was linear across the drug's concentration range of 100 ng/ml to 800 ng/ml. Escitalopram oxalate's regression equation was y=0.006x+0.216, and its correlation coefficient was 0.995, both of which were within acceptable bounds. The findings of the system suitability studies were favorable. Based on all of these findings, the current approach yielded symmetric peak shape, excellent resolution, and a shorter retention time.

CONCLUSION

The created RP-HPLC method was ultimately determined to be a very straightforward, dependable, accurate, sensitive and precise method for estimating escitalopram oxalate in human plasma based on the current work. Using solid phase extraction, the peaks for the drug of interest and internal standard were symmetrical in nature with an appropriate tailing factor, and they were well resolved from one another without any plasma interferences. The technique can be applied to therapeutic drug monitoring units, bioequivalence and bioavailability studies, pharmacokinetic research, and toxicological investigations of escitalopram oxalate. It is also appropriate for routine quantitative analysis in pharmaceutical dose forms.

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