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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF ESCITALOPRAM BY RP-HPLC METHOD

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ABSTRACT

A simple, precise, accurate, rapid and sensitive Reverse-Phase High Performance Liquid Chromatography method for the estimation of Escitalopram in tablet dosage form was developed and validated. Detection was carried out at 240 nm. The mobile phase methanol: water (90:10 v/v) pH 3.0 is adjusted with formic acid at a flow rate of 1.0 ml/min. The retention time of Escitalopram was found to be 2.80 min. The standard curve was linear (R^2>0.9950) over the concentration range of 2-20 µg/ml. The analytical method developed was validated as per ICH guidelines. The selectivity, robust and reliable as accuracy, precision, recovery and other validation parameters were within the limits as specified by the guidelines. The peaks were symmetrical in nature with acceptable tailing factor. The method can be very useful for the therapeutic drug monitoring (TDM), in bioequivalence studies, for pharmacokinetics study and also in toxicology and biomedical investigations.

KEYWORDS

Escitalopram, Reverse phase HPLC, Accuracy, Precision, Robustness, LOD, LOQ and Specificity.

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INTRODUCTION

Escitalopram is used as Antidepressant[1]. Chemically it is (s)-1-[3-(dimethylamino) propyl]-1-(4-flurophynyl) 1, 3 dihydoisobenzofuran-5-carbonitrile[1]. Its molecular formula and molecular weight are $C_{20}H_{21}FN_2O$ and 324.392 g/mol respectively. Escitalopram is freely soluble in methanol, isotonic saline solution, sparingly soluble in water and ethanol. Literature survey reveals that many analytical methods such as UV spectrophotometric[2,3] and HPLC methods[3-7] are reported for determination of Escitalopram

individually from pharmaceutical dosage form and HPLC[8] methods are reported for determination of ESC with other drugs in combined dosage form.

The aim of this work was to develop simple, accurate, reproducible and sensitive method for determination of Escitalopram using RP- HPLC method.

MATERIAL AND METHODS

Chemicals and reagents

Pure sample of Escitalopram, Methanol (HPLC grade), Ortho-phosphoric acid (AR grade) and Acetonitrile (HPLC grade) were obtained from Qualigen Laboratories Pvt. Ltd., Mumbai.

Optimization of Chromatographic conditions

Optimization of the mobile phase was performed based on resolution, asymmetric factor and peak area obtained for Escitalopram. The mobile phase Acetonitrile: water (50:50, 60:40, 80:20 v/v) was tried. The combination of mobile phase methanol: water (50:50, 70:30, 80:20 and 90:10 (v/v) was also tried. Methanol: water (90:10 v/v) adjusted at pH 3.0 with formic acid at a flow rate of 1.0 ml/min was found to be satisfactory and gave symmetric and well resolved peaks for Escitalopram. The chromatogram was recorded at 240.0 nm as spectrum of Escitalopram showed maximum response at this wavelength.

Chromatogram showed symmetrical peaks with good shapes; tailing factor for Escitalopram was within range and the resolution of standard drug was satisfactory. Retention time for Escitalopram was found to be 2.80 min.

Preparation of mobile phase

HPLC grade methanol and HPLC grade water (90:10v/v) adjusted at pH 3.0 with formic acid was filtered through 0.45 µm membrane filter and sonicated on ultrasonic bath for 15 min

Preparation of standard stock solution

Escitalopram standard stock solution was prepared by transferring 5 mg of Escitalopram working standard into a 50 ml volumetric flask, approximately 20 ml of HPLC grade distilled water was added and sonicated for 20 min. The volume was made up to 50 ml with distilled water to get the concentration of 100µg/ml and filtered through a 0.45µm pore size membrane filter.

Selection of analytical wavelength

Here λ_{max} of Escitalopram was found to be 240.0 nm. Hence 240.0 nm was selected as wavelength of analysis.

Calibration curve

The appropriate aliquots of standard stock solutions were transferred to a series of 10 ml volumetric flasks and the volume was made up to mark with methanol to obtain working standard solution of concentration of 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 µg/ml for Escitalopram. Three sets of each concentration of drug were prepared separately. The standard calibration curves of Peak Area Vs concentration were plotted using the mean of these three independent observations. The concentration ranges over, which the drug obeyed Beer-Lambert's law was found to be between 2-20 µg/ml.

Analysis of Tablet formulation

The marketed tablet formulation containing 5 mg of Escitalopram was used. Accurately the average weight of twenty tablets were determined, these tablets were transferred to a clean and dry mortar and triturated into a fine powder. The quantity of powder equivalent to 1 mg of Escitalopram was weighed accurately and transferred to 10 ml volumetric flask and 2 ml of distilled water was added. The solution was sonicated for 20 min and made up to the mark with the distilled water and filtered through a 0.45µm Nylon 66 membrane filter. The solution was further diluted to get 10µg/ml and injected into HPLC.

METHOD VALIDATION

The method validation was carried out as per ICH guideline[9,10]

Specificity

It was ascertained by analyzing standard drug and sample solutions. The retention time of Escitalopram (ESC) the sample solution was confirmed by comparing with that of the respective standards.

Linearity

The linearity for ESC was selected at 0.5-12µg/ml.

Limit of detection (LOD)

LOD was estimated by the analysis of sample with known concentrations of analyte and by establishing the minimum level at which the analyte can be detected.

Limit of Quantitation (LOQ)

LOQ was estimated by the analysis of sample with known concentration of analyte and by establishing the minimum analyte can be reliably quantities.

System suitability

These parameters are used to ensure adequate performance of chromatographic system. Retention Time (Rt), Tailing factor (T), Theoretical plates (N), were measured for six replicate injections of the drug sample at concentration of 10µg/ml.

Precision

Repeatability

In this study, six replicates injections of standard solution were injected for repeatability[9,10].

Intermediate precision

The precision of the method was demonstrated by Intra-dayand inter-day precision variation studies. In the intra-day studies, six repeated injection of standard solution was made and the response factor of drug peak and % R.S.D were calculated. In the inter day variation studies six repeated injection of standard stock solution was made for after 24 hours to 48 hours and response factor of drug peak and % R.S.D was calculated[7,8].

Accuracy

Recovery studies were carried out by applying the method to drug content present in tablet dosage form to which known amount of Escitalopram was added at 80%, 100%, 120% levels. The accuracy study was measured three times at each level.

Robustness

The factors chosen for this study were the flow rate (±0.1ml/min), mobile phase composition methanol and water (90:10%v/v) and temperature 25^0C.

RESULTS AND DISCUSSION

Escitalopram was well resolved using mobile phase composition of Methanol: Water (90:10 v/v) adjusted at pH 3.0 with formic acid at flow rate of 1 ml/min, UV detection wavelength 240.0 nm and volume of injection 20µl. This method can be used for analysis of Escitalopram in tablet dosage form. The retention time for Escitalopram was found to be 2.80 min.

METHOD VALIDATION[9,10]

The method validation was carried out as per ICH guideline[9,10]

Specificity

The retention time of Escitalopram (ESC) the sample solution was confirmed by comparing with that of the respective standards.

Linearity

The linearity for ESC was selected at 0.5-12 µg/ml. The correlation coefficients were selected at 0.9990. The results are shown in Table No.1.

System suitability

The results are shown in Table No.2.

Limit of detection (LOD) and Limit of Quantitation (LOQ)

The results of LOD and LOQ are shown in Table No.3.

Precision

Repeatability

The repeatability data was expressed in terms of % R.S.D. and was found to be less than 2%. The results are shown in Table No.6.

Intermediate precision

From the data obtained the developed method was found to be precise. The results are shown in Table No.8.

Accuracy

The results of recovery study along with its statistical validation are given in Table No.9, 10.

Robustness

This study was used to determine the influence of small but deliberate variations in the chromatographic conditions.

Instruments used

S.No	Instrument	Make/Model
1	HPLC	Agilent 1120 Compact LC
2	Double Beam UV-Visible Spectroscopy	Shimadzu-1700 UV/VIS
3	Digital Balance	LC/GC
4	Ultrasonic bath	Life care

Table No.1: Linearity of Escitalopram

S.No	Concentration (µg/ml)	Peak Area* (mAU)
1	2	199526
2	4	312919
3	6	429303
4	8	546034
5	10	634126
6	12	750306
7	14	813061
8	16	901262
9	18	1032671
10	20	1173246

*Average of Three determinations

Table No.2: Standard System suitability parameters

S.No	Parameters	RP-HPLC
1	Calibration Range(µg/ml)	2-20 µg/ml
2	Detection Wavelength	240 nm
3	Retention time (min)	2.80
4	Regression equation	Y=11733x
5	Slope	11733
6	Intercept	10829
7	Theoretical plates (N)	3654
8	Tailing factor (T)	1.14
9	Coefficient correlation(r^2)	0.9956

Table No.3: LOD and LOQ value

S.No	Parameter	Conc.(µg/ml)
1	*LOD. (µg /ml)	0.031
2	*LOQ. (µg /ml)	0.23

*Average of six determinations

Table No.4: Analysis data of Tablet formulation

S.No	Label Claim (mg/tab)	Peak Area	Amount found (mg/tab)	% of Label claim
1	5	620285	5.06	98.99
2	5	615895	5.43	99.82
3	5	646985	5.01	98.55
4	5	605235	5.25	99.12
5	5	636187	5.43	99.76
6	5	604689	5.34	98.53

Table No.5: Repeatability data (Statistical evaluation)

S.No	Conc. (ng/ml)	Peak Area	Conc. Found (ng/ml)	% Purity	S. D	% R.S. D
1	10	630125	9.93	99.30	0.9538	0.9183
2	10	634528	9.94	99.31		
3	10	634159	10.02	100.24		
4	10	623012	10.09	99.55		
5	10	624615	10.13	99.68		
6	10	634126	9.92	99.27		

*Average of six determinations

Table No.6: Result of Intraday precision

S.No	Conc. (ng/ml)	Peak Area	Conc. Found (ng/ml)	% Purity	S. D	R.S. D
1	10	632116	9.94	99.41	0.00538	0.0048
2	10	612535	9.93	99.28		
3	10	624157	9.93	99.45		
4	10	616226	9.94	99.39		
5	10	628145	9.93	99.75		
6	10	619172	9.94	99.67		

Table No.7: Results of Inter day precision

Day	Peak Area	Conc.*(ng/ml)	% Purity*	S.D*	% R. S. D
D-1	612541	10	99.31	0.020736	0.0208
D-2	623461	10	99.40	0.030125	0.0302

*n=3

Table No.8: Result of Recovery Study

S.No	Level of Recovery	Amount present (mg)	Standard amount (mg)	Amt of drug recovered (mg)	%Recovery
1	80%	5	4	3.94	99.90
		5	4	3.93	99.72
		5	4	3.90	99.65
2	100%	5	5	4.96	99.82
		5	5	4.99	99.93
		5	5	4.98	99.75
3	120%	5	6	5.95	99.64
		5	6	5.96	99.81
		5	6	5.95	99.70

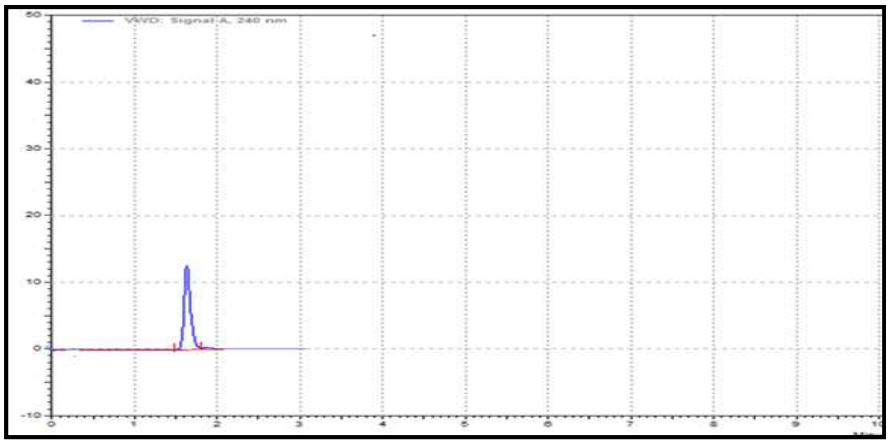

Figure No.1: Chromatogram of standard Escitalopram

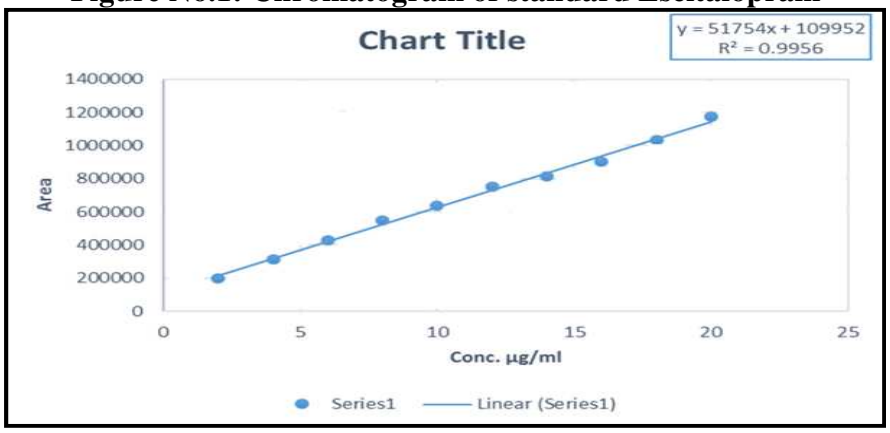

Figure No.2: Calibration curve of Standard Escitalopram

CONCLUSION

The developed HPLC method was fully validated showing satisfactory results for ICH guidelines of method validation parameters. A simple, rapid, reproducible, accurate and precise RP- HPLC method was developed for the quantitative estimation of Escitalopram in tablet dosage form. The proposed method can be conveniently used by quality control department to determine the assay of pharmaceutical preparations.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Willard H H, Merrit L L, Deam J A, Settle F A. Instrumental method of Analysis, *CBS Publishers and Distributors,* 7th Edition, 1975, 256.
2. Kasture A V, Wadodkar S G, Mahadik K R, More H M. Pharmaceutical Analysis, Instrumental method, *Nirali Prakashan,* 8th Edition, 2007, 156-168.
3. Bevkett A H, Stenlke J B. Practical Pharmaceutical Chemistry, *CBS Publishers and Distributors, New Delhi, Part-2,* 4th Edition, 1997, 85-157.
4. Ahuja S. Dong. Handbook of Practical Analysis by HPLC, *Separation Science and Technology,* 6, 1st Edition, 2000, 1-216.
5. Kirthi A, Shanmugam R. A review on bioanalytical method development validation by RP-HPLC, *J. Global Trends in*

Pharmaceutical Sciences, 5(4), 2014, 2265-2271.

6. Raja R K, Sankar G G, Rao A L. Development and validation of RP-HPLC method for estimation of Aceclofenac in tablet dosage form by ultraviolet spectrophotometry, *Indian Drugs,* 42(10), 2005, 693-695.
7. Sherikar O D, Mehta P J. Development and validation of RP-HPLC, UV Spectrophotometric and Spectrophotometric method for Estimation of Tapentadol Hydrochloride in Bulk and in Laboratory Sample of Tablet Dosage Form, *J Chemical and Pharma Research,* 4(9), 2012, 4134-4140.
8. Murali M, Satyanarayana P V. Simple validated isocratic RP –HPLC method for estimation of Tolperisone in bulk and pharmaceutical dosage form, *Der Pharma Chemica,* 3(5), 2011, 13-19.
9. Bae J W, Park Y S, Sohn U D. HPLC determination of Tolperisone in human plasma, *Arch Pharm Res,* 29(4), 2006, 339-342.
10. ICH, Q2A, Text on Validation of Analytical Procedures, *International Conference on Harmonization, Geneva,* 1996, 1-5.
11. ICH, Q2B, Validation of Analytical Procedure: Methodology, *International Conference on Harmonization, Geneva,* 1996, 1-8.
12. Code Q2A-Text on Validation of Analytical Procedure, *Step-3 Consensus Guideline, ICH Harmonised Tripartite Guideline,* 1994.
13. Muzib Y I, Reddy J R. Chowdary K P and Swathi E. Development and Validation of RP-HPLC Method for estimation of Tapentadol Hydrochloride in bulk and tablet dosage forms", *Int. J. Chemical and Analytical Science,* 4(2), 2013, 67-72.

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