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Reverse Phase Liquid Chromatographic Method for the Quantification of Di-*P*-Toluoyl-*D*-Tartaric Acid in Escitalopram Oxalate Drug Substance

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Abstract

A simple and rapid high performance liquid chromatographic method has been established to quantify the optically active precipitant Di-*p*-Toluoyl-d-Tartaric acid (DPTTA) at very low level in Escitalopram oxalate drug substance. The method is subsequently validated to prove its suitability, sensitivity and repeatability. The high performance liquid chromatographic (HPLC) method is developed in such a way that to enhances the detection level and minimizes acquisition time by using suitable buffer of 0.02% (v/v) of Orthophosphoric acid pH 3.0±0.5 and Acetonitrile as eluent in isocratic mode. The retention time of DPTTA is about 4.0 min and the total acquisition time was less than 15 min. The optimized method was validated to prove its performance characteristics by demonstrating selectivity, sensitivity (limit of detection and quantification), linearity, precision and accuracy. The experimentally established limit of detection and quantification was found to be 0.040 µg mL^{-1} and 0.120 µg mL^{-1} respectively and the overall percent accuracy (recovery) of the samples evaluated at different concentration levels was found to be 99.0, indicating the sensitivity and accuracy of this optimized HPLC method by citing the guideline requirement.

Keywords:

Liquid chromatography; di-*p*-toluoyl-d-tartaric acid; escitalopram oxalate, development; validation

1. Introduction

Citalopram is a well known antidepressant that has now been in the market for several years as selective centrally acting serotonin (5-HT) re-uptake inhibitor (SSRI). The *S*-enantiomer of racemic Citalopram is known as Escitalopram, comparatively more potent than *R*-enantiomer. Effectively high affinity for the treatment of major depressive episodes and generalized anxiety disorders explaining the pharmacological and clinical effects of Escitalopram [1-6]. Having IUPAC name as (*S*)-(+)-[3-(Dimethylamino)propyl]-1-(4-Fluorophenyl)-1,3-Dihydroisobenzo-Furan-5-carbonitrile Oxalate. The molecular formula is $C_{20}H_{21}FN_2O.C_2H_2O_4$ and molecular weight is 414.43. The chemical structures are also shown in serial no.4 of Fig 1. There are several publications available for the synthesis of Escitalopram [7-8], in view of that Chiral resolving acid DPTTA plays a major role to produce S-enantiomer of Citalopram.The activity of DPTTA during synthesis of Escitalopram drug substance also discussed in Fig. 1.

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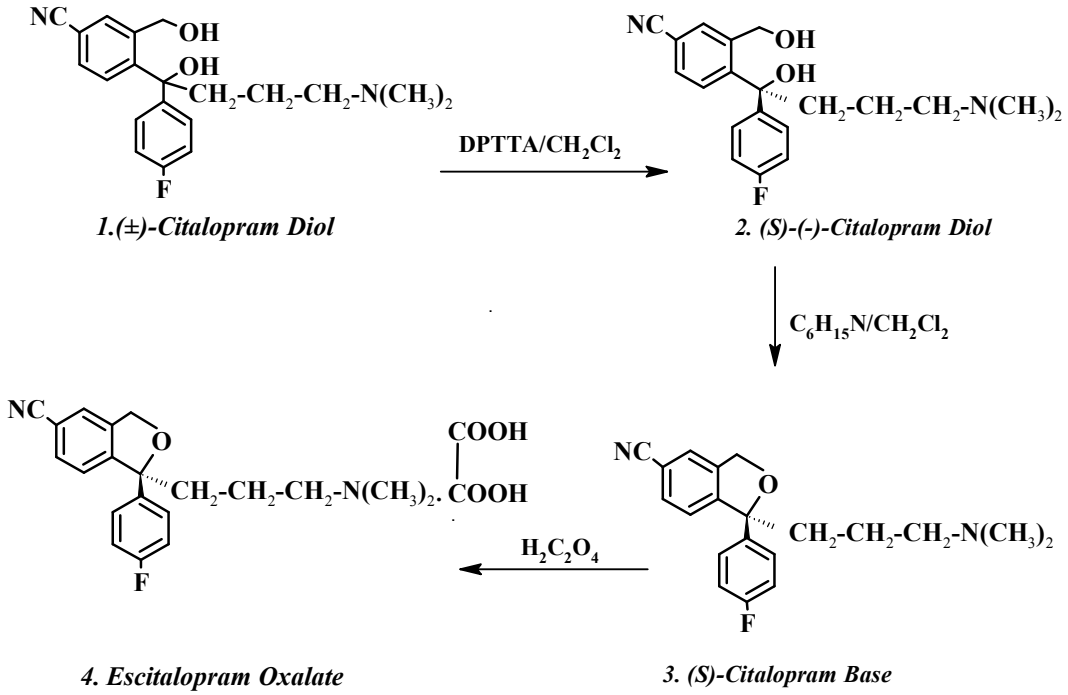

Fig. 1. Mechanistic activity of DPTTA during Escitalopram oxalate drug substance preparation.

Various publications available globally in the literature search for the determinations of Escitalopram by HPTLC [9], Capillary Electrophoresis [10], Colorimetric [11], HPLC and Spectrophotometric [12], Fluorometric and Thin Layer Chromatography Densitometry [13] and by LC/ESI-MS and NMR [14]. But all these publication and study were related to main analyte and its process/degradation impurities. The organic impurities can arise during the manufacturing process and storage of the drug substance or products, the criteria for their acceptance up to certain limits are based on pharmaceutical studies or known safety data [15]. In view of that, the drug product available in the market is directly consumed by human being based on the prescription, so therefore these should be of good quality and highest purity. So monitoring of DPTTA in Escitalopram oxalate drug substance is essential for preserving the desired quality of active moiety of the compound. The aim for this paper work is to develop a rapid and economical method for DPTTA determination in Escitalopram oxalate. By employing simple isocratic HPLC method [16], the elution pattern was established without co-elution of other impurities.

2. Experimental

2.1. Reagents and Chemicals

All chemicals and reagents were of analytical purity grade unless stated otherwise, reference standard of DPTTA and investigation samples of Escitalopram oxalate were gifted by Aurobindo Pharma Limited Research Centre, Hyderabad, India. Oxalic acid and Orthophosphoric acid were procured from (E.Merck Limited, Mumbai India), LC grade Acetonitrile purchased from Merck (Mumbai, India) and water obtained from Milli-Q purification system. Triethyl amine purchased from Spectrochem Chemicals (Mumbai, India).

2.2. Chromatographic Condition

The HPLC system consisted of Waters alliance 2695 separation module equipped with a 2996 photodiode array detector connected with Empower software for data acquisition and processing (Waters, Milford, USA). Detector wavelength was set at 240 nm and the mobile

phase consisted of 0.02% orthophosphoric acid adjusted to pH 3.0±0.5, with Triethylamine and Acetonitrile, in the ratio 50:50 (v/v). Routine degassing of the buffer was performed by passing it through a 0.45 µm membrane filter (Millipore).The mobile phase was pumped in isocratic mode, at a flow rate of 1.0 mL min^{-1}. The injection volume was 20 µL, using Symmetry C_8 column, 150 mm x 4.6mm, 5µm (Waters, Inc. USA) at 25°C. Standard and sample solutions were prepared with diluent (degassed mixture of Milli-Q water and Acetonitrile in the ratio 1:1 (v/v)). The peak homogeneity was expressed in terms of peak purity values using Empower software, 2996 Photodiode array detector at 240 nm with PDA scan range of 200 nm to 400 nm.

2.3. Standard stock and sample solution

Standard solution was prepared by dissolving accurately weighed 50 mg of DPTTA in 50 mL. Diluted 1 mL of this solution in to a 100 mL volumetric flask (10 µg mL^{-1}) and used as stock solution. Further, 1mL of this solution was diluted to 10 mL(1 µg mL^{-1}) as standard solution, filtered through 0.45µ or finer porosity membrane filter.

Sample solution was prepared by dissolving accurately weighed 10 mg of drug substance in 10 mL, filtered through 0.45 µm or finer porosity membrane filter.

2.4. Impurity stock solution

The each impurity of Escitalopram oxalate were weighed about 1.0 mg accurately and prepared with diluent for Impurity-I, Impurity-II, Impurity-III, Impurity-IV, Impurity-V, Impurity-VI and oxalic were taken to 10 mL volumetric flask. Make up to volume with the diluent solution (100 µg mL^{-1}).

2.5. Summary of method development

The buffer, organic modifier (acetonitrile and methanol) and column stationary phase were selected based on the molecule nature. Based on the various trials, preliminarily the buffer was prepared by dissolving 0.05 mol L^{-1} of diammonium hydrogen phosphate in 1000 mL water to this 1 mL of Triethylamine was added, mixed well and adjusted separately between pH 2.5 to 3.0±0.1 with 20% (v/v) orthophosphoric acid. By using waters make, Symmetry C_8 250 mm (I.D) x 4.6 mm (Dimension) 5 µm (particle size) column and acetonitrile as organic modifier. This preliminary method shows best suit for DPTTA determination without interference, so validation for the same was conducted as per guideline. Based on the view of cost and time consumption, the optimized method was switched over to a simple acidic buffer containing 0.02% (v/v) ortho-phosphoric acid and 0.1% triethylamine with pH 2.8 to 3.0 by altering the concentration of organic modifier. Acetonitrile shown to be good in peak symmetry, plate counts in variable concentration of buffer. The solvent concentration varied from 10% to 55% level in the mobile composition without altering buffer concentration. Based on the elution pattern the method was optimized in such a way that the separation and baseline were good with no co-elution of other impurities with DPTTA. In the described experiment, the effect of pH and organic modifier on retention and elution were studied. From the overall experimental condition it was concluded that 2 mL of orthophosphoric acid buffer taken into 1000 mL water and adjusted to pH of 3.0 ± 0.05 with triethylamine filtered through 0.45 µm membrane filter and actonitrile in the ratio 50:50 (v/v) were mixed and degassed in ultrasonically for about 10 minutes is used as mobile phase respectively. The chromatographic system using symmetry C_8 column 150 mm (I.D) x 4.6 mm (dimension) 5 µm (particle size) was selected. Hence, it is concluded that the best result were achieved when the temperature was ambient (working room temperature was 20°C to 30°C). Fig. 2 represents a typical chromatogram of DPTTA standard.

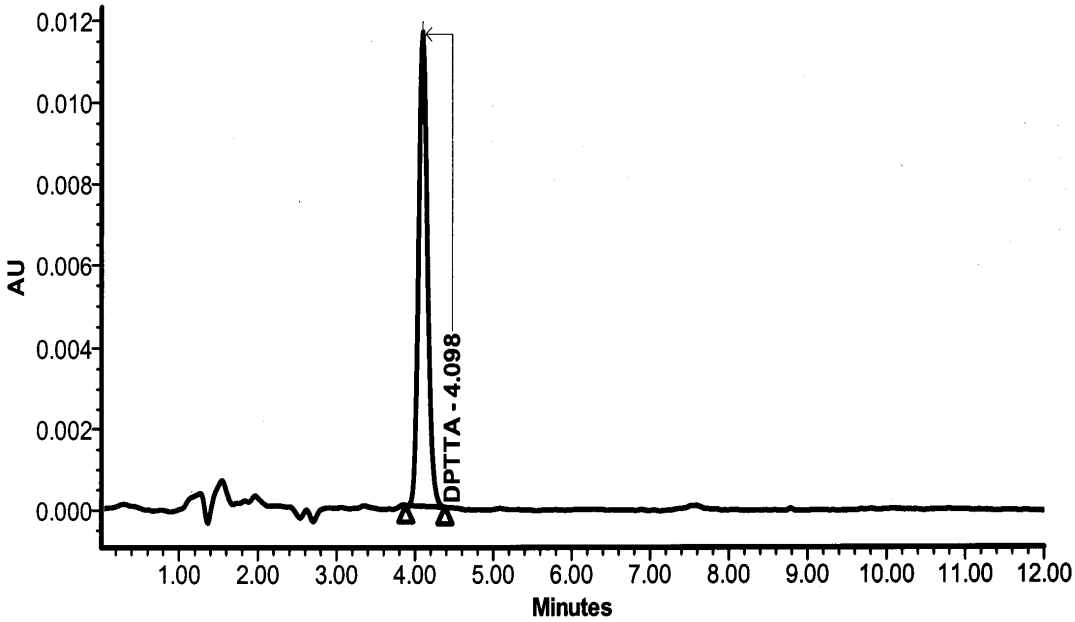

Fig. 2. Typical chromatogram of DPTTA standard

The proposed HPLC method was validated [17] for selectivity, sensitivity, linearity, accuracy, limit of detection and limit of quantification, intermediate precision, stability of sample solution, stability studies and forced degradation studies.

3. Results and Dicussion

3.1. Method Validation

3.1.1. Selectivity

The sample solutions of impurities, sample and standard were prepared at 0.1% (w/w) concentration based on Escitalopram and injected into the chromatographic system to identify the retention time. The retention time of DPTTA was found to be about 4.0 min. The sample was found to contain DPTTA at very low level, and therefore, the sample (Escitalopram) was spiked with DPTTA at 0.1% (w/w) level (Control sample) and sample spiked with other known impurities of Escitalopram including DPTTA (spiked sample). It is confirm that no co-eluting peak was observed due to other known related impurities of Escitalopram oxalate drug substance with the analyte peak under investigation, thereby indicating that the method is selective for determining the content of DPTTA. In view, Figure 3 describes the representative chromatogram obtaind from Diluent, Standard, As such sample, sample spiked with DPTTA (Control sample) and sample spiked with DPTTA along with known related impurities of Escitalopram oxalate (Spiked sample).

A system suitability rule has been established from the above experiment for the following parameters, retention time at about 4.0 minutes, peak tailing should not be more than 1.5 and plate counts should not be less than 4000. Therefore, the Table 1 summarized the system suitability and peak purity results obtained from the above experiment.

3.1.2. Linearity

By measuring area responses at different levels of DPTTA over the range of 5% to 150% of analyte concentration the linearity data were validated. Required concentrations of solutions were prepared from stock solution for different level of 0.050, 0.100, 0.250, 0.501, 0.751, 1.001, 1.502 and 2.002 µg mL^{-1}, correlation co-efficient was found to be 0.9998. The

statistical parameters slope, intercept, residual standard on deviation response and correlation co-efficient values were calculated in Table 2.

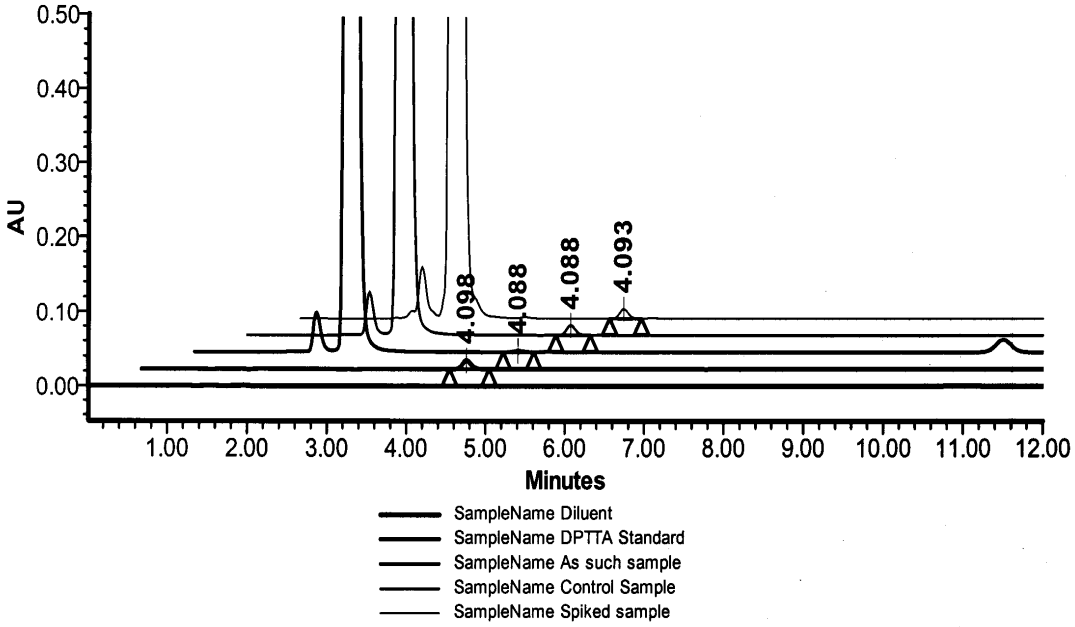

Fig. 3. Representative chromatogram obtained from Diluent, Standard, As such sample, Control sample and Spiked sample.

Table 1, Experimental data of system suitability and selectivity

Components	DPTTA	
System suitability		
Retention Time (R_T) [a]	4.04 minutes	
Peak Tailing [a]	1.1	
Plate counts [a]	5450	
Selectivity [b]		
	Peak Angle	Purity Threshold
Standard	0.542	0.821
Control sample	0.171	0.427
Spiked sample	0.197	0.464

[a] Average experimental observation,
[b] Criteria for peak purity: Purity angle should be less than purity threshold

Table 2, Experimental data obtained from Linearity analysis

Component	DPTTA
Calibration range (μg mL^{-1})	0.050 - 2.002
Calibration Points	8
Slope	93405
Intercept	988
STEY X	1124
Correlation co-efficient (CC)	0.9998
Residual sum of square (r^2)	0.9997

The area and concentration were treated by least square linear regression analysis plot [Area count in terms of Area count (AU) at Y-axis Vs Concentration (µg mL^{-1}) at X-axis] as shown in Fig. 4.

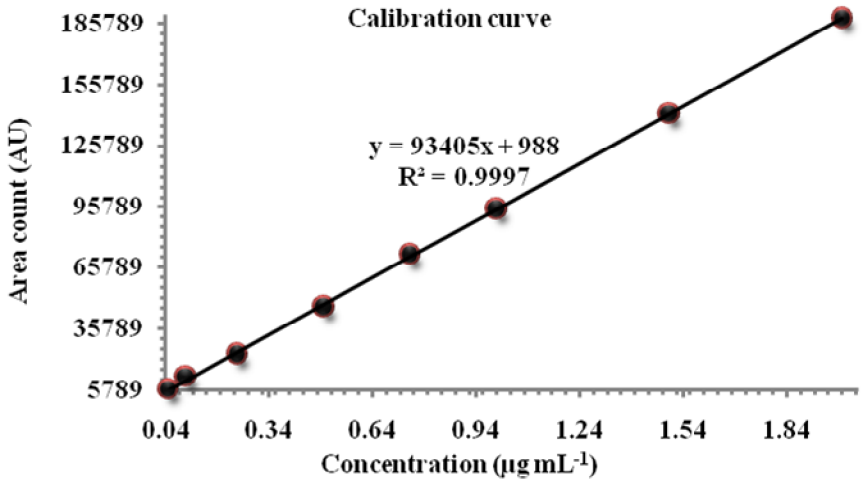

Fig. 4. Correlation curve obtained from the linearity experiment.

3.1.3. Sensitivity

To predict the limit of detection (LOD) and limit of quantification (LOQ) the solutions were prepared from known stock concentration. The predicted values obtained from a linear regression line performed at lower concentration levels using slope (S) and residual standard deviation (S.D).The limit of detection and quantification predicted was found to be 0.004% (w/w) and 0.012% (w/w) respectively, by using the calculation.

3.3*STEY.X/SLOPE*100/Sample concentration (for LOD) and,

10*STEY.X/SLOPE*100/Sample concentration (for LOQ)

Each predicted level was verified for precision by analyzing six replicate measurements. The percentage relative standard deviation for six replicate measurements at predicted LOD and LOQ concentration levels was found to be 10.1 and 2.2, respectively, verifying the predicted values.

3.1.4. Precision

The method was assessed by six replicate injections of DPTTA standard solution (1 µg mL^{-1}) into chromatographic system, and the percentage relative standard deviation of response for six replicate measurements was found to be 0.8 for the repeatability of the system. Reproducibility of the method (Method precision) was demonstrated by preparing six replicate sample preparations by spiking known concentration (0.1% (w/w)) of DPTTA in random selection of one batch of Escitalopram oxalate drug substance. The samples were analyzed as per method, and the content of DPTTA was determined. The values obtained from the above experiment were found to be 0.094% (w/w) with %RSD value of 1.8 has shown good repeatability for analytical experiment. The degree of reproducibility is known as ruggedness, obtained by the analysis of the same sample concentration (which is used in the method precision) under a variety of conditions using different series of column, with different user on different day by using new standard also found to be 0.098% (w/w) with %RSD value of 2.1 also proves that the method is rugged for the determination of DPTTA under the experimental conditions.

3.2. Stability of Sample Solution

The Escitalopram oxalate drug substance were spiked with known concentration of DPTTA with respect to sample concentration (0.1% (w/w)) was stored at 25 ± 2°C temperature conditions, were injected into chromatographic system at different time intervals. The content of DPTTA was determined at each interval, the sample solution was found to be stable over a period of 15 hours. The % difference between the peak area obtained at initial and different time interval was found to be less than 3.2. However, it is observed from the experimental condition the stability of the sample was found to be stable for at least 15 hour at room temperature (~25°C).

3.3. Accuracy

The recovery studies during the method was evaluated by preparing sample solution spiked with known amount of DPTTA at different concentration levels in the range between 20%, 50%, 100% and 150% with respect to Escitalopram concentration. Each concentration of sample solution was prepared in triplicate and analyzed as per the method. The percent recovery of DPTTA was found to be in the range of 98.3 to 99.6, mean percent recovery was 98.9, when calculated against the known added amount, indicating that the method is accurate. Table 3, describes the experimental results obtained from accuracy analysis.

Table 3. Experimental data obtained from Accuracy analysis

Component	Specification level [c]			
Amount (% w/w)	20%	50%	100%	150%
Added [d]	0.0198	0.0496	0.0991	0.1487
Found [d]	0.0197	0.0488	0.0987	0.1463
% Recovery [e]	99.5	98.3	99.6	98.4
%R.S.D [f]	1.0	1.0	1.5	1.0
Overall statistical data				
Mean	98.9			
SD	1.0			
% R.S.D	1.1			
95% Confidence level (±)	± 2			

[c] specification level 0.1%, [d] n=3, average of three determinations
[e] Average experimental determination, [f] overall %R.S.D.

3.4. Robustness

To assess the robustness of the method, experimental conditions were deliberately altered for pH (2.8-3.2), temperature (20 °C-30 °C), flow of mobile phase (0.9-1.0 mL min^{-1}) and wavelength (235-245 nm). The result obtained from the robustness indicated that, the experimental method parameters were tolerance limit with minor changes to optimize the method.

3.5. Stability Studies

To present stability studies on Escitalopram oxalate drug substance for the determination of DPTTA content, the analysis were conducted on samples from variable sources of temperature and humidity storage of accelerated (40°C/75%RH), long term (25°C/60%RH) and refrigerated (5°C±3 °C) storage condition [18]. The results obtained from the above storage conditions are found to below 0.009% and 0.011%, respectively. Hence

formation of DPTTA in Escitalopram oxalate drug substances resulting as process related impurity, in view of that the sample shows no degradation profile with respect to storage at different conditions of temperature and humidity. The experimental condition shows precise results with good repeatability on inter and intra day with other analyst and different chromatograph shows the method is rugged for the determination of DPTTA content.

3.6. Forced degradation studies

The experiment further studied on different degradation variables to comply the parameters as per guideline for the sample kept under light, thermal and humidity for dry exposure. Liquid phase degradation using acid, base and peroxide of different concentration also conducted to prove the method is stability indicating shows no degradation on DPTTA in Escitalopram oxalate drug substance. The UV light exposed up to 200 watts/sq.mtr, fluorescent light exposed up to 1.2 million lux hour, Humidity exposure at 25°C/92%RH, thermal exposure at 105 °C and 60 °C with the time duration of 72 hours and hydrolysis using aqueous media up to 10 hour at room temperature were conducted, the samples were analyzed as per the method. The result obtained from the chromatographic data shows no significant degradation for DPTTA and found to 0.012% (w/w). In addition, wet stress condition also conducted using 30% H_2O_2, 5 mol L^{-1} hydrochloric acid, 5 mol L^{-1} sodium hyroxide were added separately for sample taken into different reservoir and analyzed as per method condition, the observation shows no degradation for DPTTA and the values were found to be 0.009% to 0.011% (w/w). In the case of Base degradation with 5 mol L^{-1} sodium hydroxide at 85°C for 15 minutes shows more degradation observed for Escitalopram and its related impurities peak but no interference for DPTTA. Hence, the method is found to be selective and stability indicating with respect to forced degradation data.

4. Conclusion

The proposed simple methodology for quantitative determination of DPTTA in Escitalopram oxalate drug substance is rapid, accurate, precise and selective .The method provided satisfactory validation data for the tested parameters as per the ICH guidelines. Hence the proposed method may be conveniently used in bulk manufacturing for the quantification of DPTTA content in Escitalopram oxalate drug substance.

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