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Novel Validated RP-HPLC Method For Determination Of Edoxaban Tosylate Monohydrate In Bulk And Its Pharmaceutical Dosage Form

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Abstract:

A simple, reliable, sensitive, precise, rapid, and reproducible RP-HPLC method was developed and validated for the determination of Edoxaban Tosylate Monohydrate (EDTM) in the pharmaceutical dosage form. Separation was achieved under the optimized chromatographic condition on Agilent technologies -1260 infinity system, Eclipse XDB C_{18} Column, (250 mm × 4.6 mm i.d., particle size 5 µm, maintained at ambient temperature). The mobile phase consisted of methanol: acetonitrile in the ratio 85:15 V/V. A gradient elution at a flow rate of 1 mL/ minutes using 1260 DAD detector to monitor the elute at 291.2 nm. The retention time of Edoxaban and Tosylate was found to be 3.013 minutes and 1.927 minutes respectively and the calibration curve was a linear function of the drug in the concentration range of 2-10 µg/mL (r^2 = 0.9997). The limit of detection and the limit of quantitation was found to be 0.2250 µg/mL and 0.6818 µg/mL respectively. The recovery (accuracy) studies were performed and the percentage recovery was found to be 98.8-99.89 %. Analytical validation parameters such as selectivity, specificity, linearity, precision, and accuracy were studied and the % RSD value for all key parameters was less than 2 %. Thus, the developed reversed-phase HPLC method was found to be feasible for the determination of Edoxaban Tosylate Monohydrate in bulk and pharmaceutical formulations.

Keywords: RP - HPLC, Edoxaban Tosylate Monohydrate, Validation, ICH guidelines.

1. INTRODUCTION

The chemical name for Edoxaban Tosylate Monohydrate (EDTM) is N'-(5-chloropyridin-2-yl)-N- [(1S,2R,4S)-4-(dimethyl carbamoyl)-2- [(5-methyl-6,7- dihydro-4H-[1,3] thiazolo [5,4-c] pyridine -2- carbonyl) amino] cyclohexyl] oxamide;4-methyl benzene sulfonic acid. EDTM is generally used in the treatment of systemic embolism. Rogonic et al. [1]. EDTM is a member of the novel oral anticoagulants (NOACs) class of drugs and is a rapidly acting, oral, selective factor Xa inhibitor. It does not require antithrombin III for antithrombotic activity. Edoxaban inhibits free FXa, and prothrombinase activity and inhibits thrombin-induced platelet aggregation. Inhibition of FXa in the coagulation cascade reduces thrombin generation and reduces thrombus formation. It is soluble in methanol; slightly soluble in water, ethanol, and acetonitrile [2], and the molecular structure of EDTM is presented in Figure 1.

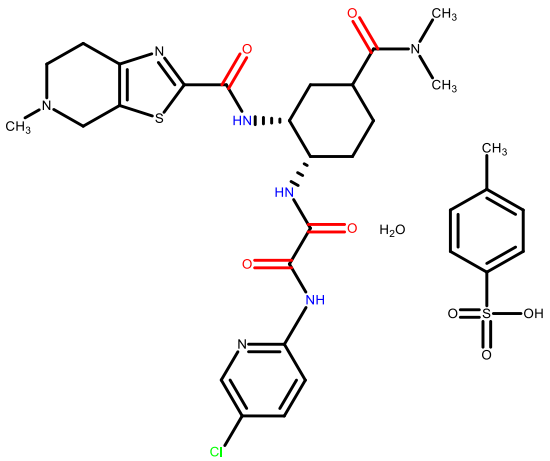

Figure 1: Chemical structure of EDTM

Literature survey revealed that not many analytical methods have been reported for estimation of EDTM individually or in combination with other drugs. The reported methods are UV Spectrophotometric [2-3], RP-HPLC [4], Bioanalytical RP-HPLC [5], HPLC by QbD approach [6], LC-MS/MS [7-10] methods. The present study was aimed to develop a simple, sensitive, rapid, and precise RP-HPLC method for the estimation of EDTM. The analytical method was validated according to ICH validation parameters [11].

2. MATERIAL AND METHODS

2.1 Chemicals and reagents:

A pure sample of Edoxaban Tosylate Monohydrate obtained from Hetero drugs private ltd, Hyderabad, India. The marketed formulation of Lixiana-30 mg tablets (EDTM 30 mg/tablet) was procured from a local pharmacy store. HPLC grade of acetonitrile was procured from Thermo Fisher Scientific India Pvt. Ltd., Mumbai, India. HPLC grade water and methanol were purchased from Merck Specialties Pvt.Ltd, Mumbai, India.

2.2 Instrumentation

The analysis was performed by using a chromatographic system, Agilent HPLC comprised of a 1260 Quaternary pump, a 20 µL injection loop, and a 1260 photodiode array detector and running on E-Z Chrome software with a reverse-phase Eclipse XDB C_{18} column having 250 x 4.6 mm internal diameter, 5 µm particle size. UV-Visible Spectrophotometer (Elico SL-210) Shimadzu electronic balance (AX-200) was used for weighing purposes. Ultra Sonicator (PCI Ltd., Mumbai) was used for the preparation and degassing of samples.

2.3 Chromatographic conditions

EDTM was analyzed with Eclipse XDB C_{18} column (250 x 4.6 mm, 5 µm particle size) for the chromatographic separation, and the column was maintained at ambient temperature. The mobile phase was composed of a mixture

of methanol and acetonitrile in the ratio of 85:15 V/V and it was delivered at a flow rate of 1.0 ml/min and detection was monitored at 291.2 nm with a PDA detector. The mobile phase was used as diluent. The injection volume was 20 µl. The run time was 10 min. The retention time of Edoxaban, Tosylate was found to be 3.013 min and 1.927 min respectively.

2.4 Preparation of standard stock and working standard solutions

The standard drug solution of EDTM was prepared by dissolving 50 mg of standard drug in 20 mL methanol in a 50 ml volumetric flask. It was sonicated for 10 minutes for the complete solubility of the drug. After dissolving the drug, the final volume was brought up to 50 ml by adding methanol to obtain an eventual concentration of 1000 µg/mL. The prepared stock solution was further diluted with methanol to get a working standard solution of 100 µg/ml of EDTM. From the working standard solution of 100 µg/mL, serial dilutions 2 µg/mL, 4 µg/ml, 6 µg/mL, 8 µg/mL & 10 µg/ml were prepared by using same solvent.

2.5 Selection of detection wavelength

The UV spectrum of diluted solutions of various concentrations of EDTM in methanol was recorded by using a UV spectrophotometer. The wavelength of maximum absorbance was scanned over a range of 200-400 nm and the maximum absorbance was found at 291.2 nm. Results of overlain UV absorption spectra are represented in Figure 2.

2.6 Preparation of Sample Solution (Tablet formulation)

Accurately 20 tablets were weighed individually and the average weight was calculated and powdered. The tablet powder equivalent to 10 mg of EDTM and it was transferred into a 100 ml volumetric flask, to that 20 ml of methanol was added and sonicated for 5 minutes at a controlled temperature to dissolve the powder, further, the volume was made up with the same solvent, and filtered through 0.45 µ membrane filter. & further dilutions were done with methanol to get eventual concentration (10 µg/ml) within the linearity range and Finally, the drug content in each tablet and also bulk drug was found by utilizing the standard graph.

3. RESULTS

3.1 Method development and optimization

The optimized HPLC conditions several mobile phases of different compositions were tested to develop an optimization of chromatographic conditions such as tailing factor, good peak shape, and theoretical plates. For the selection of the mobile phase primarily methanol: acetonitrile, methanol: water, acetonitrile: water has been tested for different compositions, flow rates, and ratios. Finally, the mobile phase consisting of a mixture of methanol and acetonitrile in the ratio of (85:15 v/v) at a flow rate of 1.0 ml/min was found to be satisfactory and proper system suitability parameter results were obtained [12]

3.2 Method Validation

The method was validated for specificity, linearity, accuracy, precision, the limit of detection, the limit of quantification, and robustness by following procedures [13-16].

Table 1: Optimized chromatographic conditions

Parameter	Condition
Instrument	Agilent High Pressure Liquid Chromatography 1260 series with GI311C Quat. pump. Diode array detector G1315D
Column	Eclipse XDB C_{18} 250 mm×4.6 mm;5.0 µm
Mobile phase and ratio	Methanol: Acetonitrile (85:15 v/v)
Detection wavelength	291.2 nm
Column Temperature	Ambient (25° C)
Injection volume	20 µl
Flow rate	1.0 min/ml
Run Time	10 minutes

3.2.1 System suitability

System suitability is an integral part of the chromatographic system. It is verification of resolution, capacity factor, tailing factor, theoretical plate count, relative retentions, etc. are calculated and compared with a standard specification of the system.

3.2.2 Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present. The specificity of an analytical method is to determine the effect of excipients and other additives that are generally present in the formulation. The test results obtained were contrasted with the results of the standard drug.

3.2.3 Linearity

Linearity is the ability (within the specified range) to obtain test results that are directly proportional to the concentration of analyte in the sample. Linearity is evaluated by visual inspection of the plot of the signal as a function of analyte concentration. If there is a linear relationship test results are calculated by regression line by the method of least squares.

The linearity of the method was determined at six concentration levels ranging from 2-10 µg/ml for EDTM. Evaluation of the drug was performed with a PDA detector at 291.2 nm, peak area was recorded for all the peaks. The correlation coefficient value of EDTM was 0.9997. The results shown that an excellent correlation exists between peak area and concentration of drug within the concentration range indicated.

3.2.4 Range

The range of analytical procedure is the interval between the upper and lower concentration of analytes in the sample.

3.2.5 Accuracy

Accuracy of an analytical method is the ‘measure of how close the experimental value to the true value’ accuracy of the method was determined by the standard addition method. A known amount of standard drug is added to the

fixed amount of pre-analyzed injection solution. Percent recovery is calculated by comparing the area before and after the addition of the standard drug. The standard addition method is performed at 80 %, 100 %, and 120 % levels. The solutions are analyzed in triplicate at each level as per the proposed method.

The accuracy of the method was determined by calculating the recovery of EDTM by the method of standard addition. A known amount of EDTM was added to a pre-quantified sample solution and the amount of EDTM was estimated by measuring the peak area ratios and by fitting these values to the straight-line equation of the calibration curve. The recovery studies were carried out three times over the specified concentration range of 80 %, 100 %, and 120 % levels. The amount of EDTM was estimated by measuring the peak area ratios by fitting these values to the straight-line equation of the calibration curve. From the above determination, percentage recovery and standard deviation of percentage recovery were calculated.

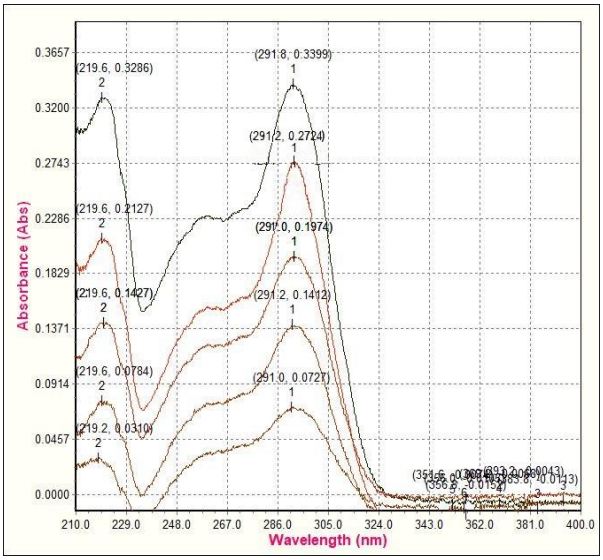

Figure 2: Overlain UV Spectra for EDTM (2-10 µg/ml)

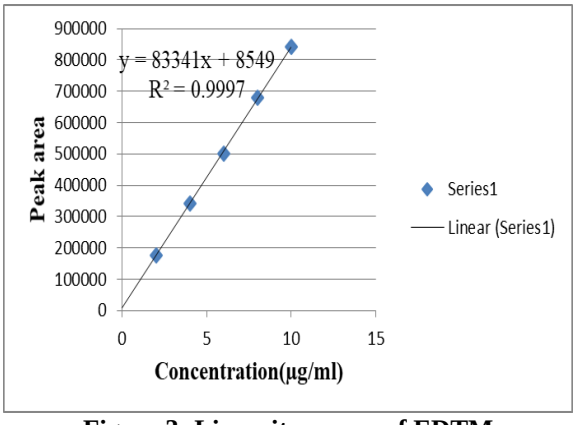

Figure 3: Linearity curve of EDTM

3.2.6 Precision

The closeness of agreement (degree of scattering) between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision, and reproducibility. The intra-day precision study of EDTM was carried out by estimating the correspondence responses six times on the same day with 100 % concentration and the inter-day precision study of EDTM was carried out by estimating the correspondence responses six times the next day with 100 % concentration.

3.2.7 Limit of detection and Limit of Quantification

Limit of detection (LOD) is defined as the lowest concentration of analyte that gives a detectable response. Limit of Quantification (LOQ) is defined as the lowest concentration of analyte that can be quantified with a specified level of accuracy and precision. For this study, six replicates of the analyte at the lowest concentration are measured and quantified.

The limit of detection (LOD) and limit of quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solution using the developed HPLC method.

3.2.8 Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of analysis concerning deliberate variations in method parameters. The robustness of the proposed method is estimated by changing the flow rate of the mobile phase and composition of the mobile phase.

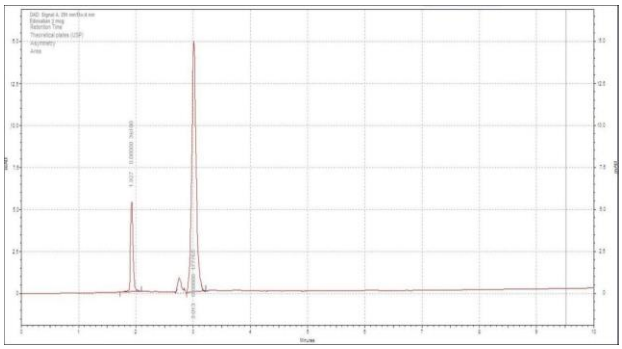

Figure 4: Standard chromatogram of EDTM (2 µg/ml).

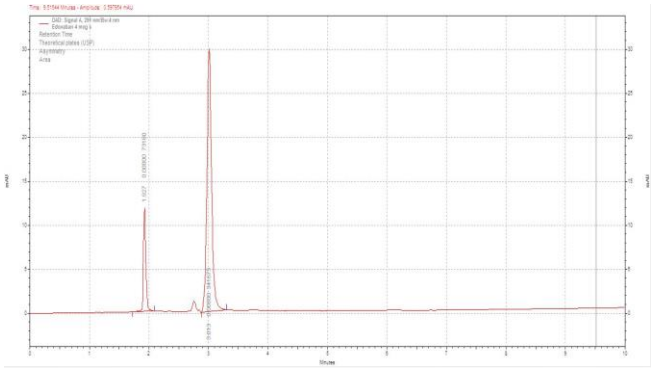

Figure 5: Standard chromatogram of EDTM (4 µg/ml).

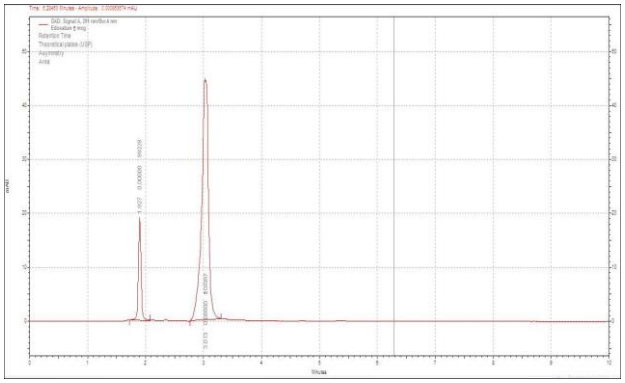

Figure 6: Standard chromatogram of EDTM (6 µg/ml)

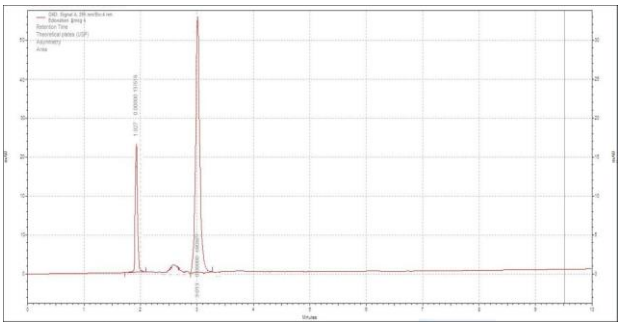

Figure 7: Standard chromatogram of EDTM (8 µg/ml)

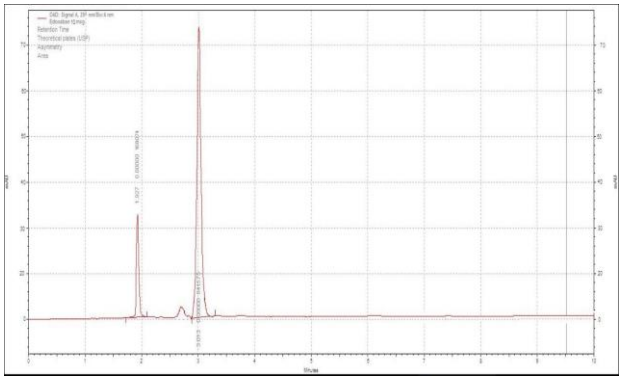

Figure 8: Stanard Chromatogram of EDTM (10 µg/ml)

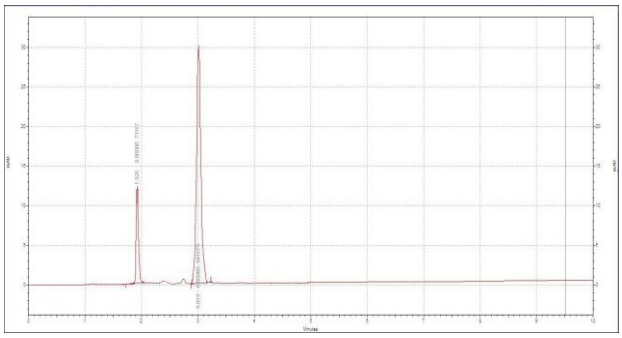

Figure 9: Sample chromatogram of EDTM (10 µg/ml)

Table 2: Linearity results of EDTM

S. No	Concentration(µg/ml)	Retention time (min)		Peak area (mV.s)	
		Edoxaban	Tosylate	Edoxaban	Tosylate
1	0	-	-	-	-
2	2	3.013	1.927	177765	36180
3	4	3.013	1.927	341675	73180
4	6	3.013	1.927	501087	99228
5	8	3.013	1.927	680879	137518
6	10	3.013	1.927	841575	168074

Table 3: Accuracy result of EDTM

S No	Level of spiking of standard	Amount added to Sample (previously analyzed) conc. (µg /ml)	Amount of standard Drug solution added (µg /ml)	Mean % recovery*	% RSD
1	80 %	2	4	99.7	0.00385
2	100 %	4	4	99.8	0.001805
3	120%	6	4	99.6	0.00119

*Average of triplicate injections

Table 4: Intraday and Inter day Precision Results of EDTM

S. No.	Intraday Precision		Inter day Precision	
	RT	Peak Area	RT	Peak Area (Mv.S)
1	3.013	501087	3.013	501067
2	3.013	501089	3.013	501079
3	3.013	501086	3.013	501085
4	3.013	501085	3.013	501058
5	3.013	501088	3.013	501063
6.	3.013	501087	3.013	501077
Average		501087		501071
Std. Dev		1.4142		10.4251
% RSD		0.000282		0.002081

Table 5: Robustness results for EDTM

S.No.	Parameter	Optimized	Used	Retention time (t R), min	Plate Count $	Peak asymmetry#	Remark
1	Flow rate (± 0.2 ml/min)	1.0 ml/min	0.8 ml/min	3.215	9,987	1.290	*Robust
			1.0 ml/min	3.013	8,600	1.250	*Robust
			1.2 ml/min	2.987	7,600	1.210	*Robust
2	Detection wavelength (±5 nm)	291.2 nm	286.2 nm	3.017	8,605	1.25	Robust
			291.2 nm	3.013	8,600	1.25	Robust
			296.2 nm	3.012	8,615	1.25	Robust
3	Mobile phase composition (± 5%) (MeOH: ACN)	85:15 v/v	80:20 v/v	2.867	8,676	1.260	*Robust
			85:15 v/v	3.013	8,600	1.250	*Robust
			90:10 v/v	3.027	8,798	1.204	*Robust

Acceptance criteria (Limits): #Peak Asymmetry < 1.5, $ Plate count > 2000, * Significant change in Retention time

Table 6: System Suitability and Validation Parameters

S. No.	Parameter	Results
1	Linearity range (µg/ml)	2-10
2	Correlation coefficient (r^2)	0.9997
3	Retention times (min)	3.013
4	Theoretical plates (N)	8,903
5	Tailing factor	1.250
6	Mean % recovery (%)	98.8 - 99.89
6	Repeatability (% RSD)	0.000282
7	Reproducibility (% RSD)	0.002081
8	LOD (µg/ml)	0.2250
9	LOQ (µg/ml)	0.6818
10	Robustness (% RSD) (0.8,1.0 &1.2 mL/min)	less than 2%
11	Assay (%) **(**Average of 6 determinations)	99.55

4. DISCUSSION

The HPLC procedure was optimized to develop an accurate assay method for the determination of EDTM in bulk and pharmaceutical dosage form by using Eclipse XDB C18 (250 x 4.6 mm internal diameter; 5 µm particle size) column with a mobile phase of methanol and acetonitrile in the ratio of 85:15 v/v. The flow rate of the mobile phase at 1.0 ml/min and the component was monitored and detected with a PDA detector at 291.2 nm. The eluted drug peaks in good shape and well resolved. The results of optimized chromatographic conditions were shown in Table 1. The retention time, the number of theoretical plates, and the tailing factor of EDTM were found to be 3.013 min and 1.927 min, 8,903 and 1.250 respectively, which indicates the efficient performance of the column.

The method was linear in the range of 2-10 µg/ml for EDTM with a correlation coefficient of 0. 9997. The regression equation of EDTM concentration over its peak area ratio was found to be Y = 83341 X + 8549, where X is the concentration of EDTM and Y is the respective peak area. The linearity results were shown in Table 2 and Figure 3.

The mean % recoveries were found to be 99.62 - 99.8 % which indicates the method is accurate. The accuracy results were shown in Table 3. The % RSD for intra-day precision and inter-day precision for EDTM were found to be 0.000282 and 0.002081, the values were less than 2 % which indicates the method is precise. The results of precision studies were shown in Table 4. The limit of detection (LOD) and limit of quantification (LOQ) for EDTM were found to be 0.2250 µg/mL and 0.6818 µg/mL respectively, which indicate the sensitivity of the method.

The robustness was performed for the flow rate variations like ±0.2 mL, wavelength variations like ± 5 nm, and mobile phase composition changes like ± 5 %. The results are summarized on evaluation of the above results, it can be concluded that the variation in flow rate, wavelength, and mobile phase composition, results are not affected significantly. Hence it indicates that the method is robust. Overall % RSD was found to be less than 2% for all the variations which indicate that the proposed method is robust. The results of the study were shown in Table 5.

The summary of system suitability parameters and validation parameters were shown in Table 6. The validated method was applied for the determination of EDTM in commercial tablet formulation that was obtained by injected six replicates of the sample solutions. The amount of drug and Mean % recovery ± SD of the assay was found to be 29.829 mg/tablet and 99.55 ± 0.40 %. 20 µl of each calibration standard solution (2, 4, 6, 8, 10 µg/ml) were injected into the HPLC system to get the chromatograms which is shown in Figure 4 to Figure 8. The average peak area and retention time were recorded.

A typical chromatogram of sample drug of EDTM was shown in Figure 9. No interfering peaks were found in the chromatogram of the formulation within the run time indicating that excipients used in the formulation did not interfere with the estimation of the drug by the proposed method.

5. CONCLUSION

The proposed Study describes a new HPLC method for the estimation of EDTM bulk and in its tablet formulation. The method was validated and found to be simple, sensitive, accurate, precise, and robust. Percentage of recovery shows that the method is free from the interference of the excipients used in the formulation. Therefore, the proposed method can be used for routine analysis of estimation of EDTM in regular quality control testing laboratories.

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