RESEARCH ARTICLE

Enhanced Detection and Quantification of Impurities in Erythromycin Tablets Using RP-HPLC

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ABSTRACT

In this present work very simple, highly rapid, highly accurate as well as more precise gradient RP-HPLC process is developed also validated to separate foreign substances (IMP) present in Erythromycin (ERT-A) tablets pharma dosage form. Chromatographic disengagement is drifting out on WatersX-Terra RP 18 (250 mm x 4.6 mm I.D., 3.5 µm particle size) at 65°C is recycled to this separation. Mobile Phase-A consists of Buffer solution which is prepared by taking 35.0000g of di-potassium hydrogen phosphate in 1000.00mL of triple distilled water. pH is noted at 7.0 by using dilute O-phosphoric acid, purified over 0.45 µm membrane filter) acetonitrile as well as water in ratio of 5v: 35v:60v Phosphate buffer pH 7.0, water and acetonitrile in ratio of 5v:45v:50v is used as mobile Phase-B. The rate of flow and injection volume was 1.00mL/min and 100.00µL respectively. Analysis is lugged out beneath gradient circumstances includes time in min./A (v/v): B(v/v); T0/100:00, T45/100:00, T47/0:100, T63/0:100, T65/100:00, and T70/100:00. 215 nm is the wavelength. The ERT-A degrades under different circumstances. Total degradation substances are well resolved by observed peaks from ERT. This process is observed as linear. The cumulative %RSD values are identified, found that they are within the range. By utilizing this analysis revealed that this process is more accurate, highly specific, more selective, highly precise, robust, and also useful in development of process as well as quality check in flawless dosage form manufacture.

Keywords: Erythromycin, HPLC, forced degradation, LOD, LOQ, Accuracy.

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INTRODUCTION

Erythromycin (ERY) therapeutically substances belongs macrolide antibiotics class, originally invented scientist named as McGuire et al.[1] the molecular formulae is $C_{37}H_{67}NO$ and molecular weight is 733.94 g/mol also produced by biosynthesis in the interim fermentation by species of Gram-positive Saccharo polysporaerythraea, heretofore restricted as Streptomyces erythraeus.[2,3] Erythromycin is pragmatic to treat numerous infections as well as provides an gesture to a non-infectious pathology. Habitually, its use has been to different respiratory infections, prophylaxis of neonatal conjunctivitis, along with chlamydia.[4] Santhosh Kumar Ettaboina et al.,[5] used mobile phases A as Buffer consists of pH as 9.0 using dibasic Potassium Phosphate, tertiary butyl alcohol, Acetonitrile as 800v/v, 170v/v also 30 v/v, purified Water is utilized as a mobile phase B; Acetonitrile is utilized as a mobile phase C. For this analysis authors are utilized Agilent PLRP-S, 250mm x 4.6mm, 8µm, 1000A°. Wavelength is measured at 215nm also authors discussed about topical formulation only. SalikaJeelani et al.,[6] proposed this method for separation of various impurities in this proposed drug by UHP-QDa with Column as Waters XBridge C18 (100mm × 4.6mm, 3.5µm), maintained the wavelength at 215 nm. Mobile phase is 0.4 % ammonium hydroxide in water as well as methanol. Chirag J Patel et.al.,[7] proposed method to determine the low level of erythromycin. For this analysis used BEH C18, (50 mm x 2.1 mm, 1.7 µm) and 210 nm. B. Habibi et al.,[8] proposed with to separation with an Asahipak Shodex ODP-50 4E column (250mm × 4.6mm i.d.,5µm particles) column with HPLC, measurement was taken at Wavelength 210 nm and authors used this method to conviction of erythromycin, clarithromycin, as well as azithromycin residues in fish muscles. Fahimeh Kamarei et al.,[9] utilized X-Terra C18 analytical column UV detection at 205 nm for Assay of erythromycin only. Jacqueline Wardrop et al.,[10] A C18 Polymeric column is used with UV detection at 205 nm. During the study not discussed about forced degardation studies. K. Griessmannet et al.,[11] Method is used at 215 nm as UV detection and Column: polymer X column (4.6 mm 25.0 cm, 7 µm). The method is only limited to regulate Erythromycin without reckoning of foreign substances. Lakshmana Rao A et al.[12] quantified Erythromycin utilizing Hypersil BDS C18 column (150mm x 4.6 mm I.D., 5µm particle size) UV detection is performed 224nm, proposed method doesn't have any forced degradation data and method is only to assay of Erythromycin in

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Erythromycin tablet dosage forms. Zhiling Cao et al,[13] performed this chromatographic analysis by Inertsil C18 ODS column at wavelength of UV detector is 215nm. Total four related substances, includes Z isomer of erythromycin A oxime, erythromycin A, erythromycin A-6, 9-hemiketal and erythromycin A-6, 9-9, 12-spiroketal are subjected to separaton as well as measured the beneath isocratic elution. But forced degradation study is not performed. A. S. Rathore et.al.,[14] developed as well as validated method to quantification of Isotretinoin also ERY in bulk drug as well as topical gel formulation This separation is lugged out on Merck TLC aluminum sheets of silica gel 60 F254, (20 × 10 cm) with 250 µm thickness. Used this technique for TLC and not have studied about ERY and its related impurities. Verified the USP ERY Tablets and not found any related substance method for Erythromycin and Its related impurities.[15] The related substance method available in current USP monograph of ERY API.[16] European pharmacopeia ERY API monograph listed related impurities is verified for API but placebo peaks and blanks peaks was not separated.[17] To address these gaps in the existing literature, the objective of our study is to devise straightforward as well as expeditious stability-indicating process tailored to quantification of ERT and its associated foreign substances in Tablets (OSD) formulations. By using literature reviews authors are carried out this method to development of ERY and it's associated impurities in Tablets (OSD) formulations. Further developed method was validated as per ICHQ(2A) guidelines.[18]

MATERIALSAND METHODS

Standard and Impurities

ERT (93.9% purity), Anhydro ERT A (Imp d) (92.2% purity) (Fig 2), ERT A enol ether (Imp. E) (94.5% purity)(Fig 3), Pseudo ERT A enol ether (Imp. F) (91.2% purity) (Fig 4), ERT Imp. H (ERT A 3´´-N-oxide) (Purity 86.7%) (Fig 5)and ERT Imp. L (3"-N-demethyl-3"-N-formyl ERT A (89.7% purity) (Fig 6) were taken as a gift samples by Dr Reddy's Laboratory, Hyderabad, India .ERT Related Compound N (Imp. B) (95.3 % purity) (Fig 7) were obtained from USP.

Experimental

Procured analytical/HPLC-grade 3^0 butyl alcohol by Alfa Aesar labs. From spectrum Chemicals, Mumbai, India monobasic potassium phosphate, dibasic potassium phosphate, along with O-Phosphoric acid as AR grade. Both the HPLC-grade methanol as well as acetonitrile are acquired by J. T. Baker. K_2PO_4Di-potassium hydrogen phosphate along with O-phosphoric acid acquired by Merck Life Sciences. The required pure water is Milli-Q which is utilized for this total experimental analysis. This chromatographic analysis is focused by Waters Alliance 2695 Module from Waters Corporation-USA. The HPLC system was composed of various components, including a QSM, an auto sampler a column oven, and a PDA detector. LC column used the Waters X-Terra RP 18 by particle size of 3.5 µm as well as proportions of 4.6 mm x 250 mm, which was manufactured by Waters.

Optimization of Chromatographic circumstances

Mobile Phase-A consists of a composition of buffer solution as 5v/v, acetonitrile as 35v/v, as well as water as v/v. Mobile Phase-B consists of pH 7.0 phosphate buffer at as 5v/v, 45v/v water, and 50v/v acetonitrile. The rate of flow rate is identified as 1.00mL/min. Volume injected is 100 µL. Temperature is nursed at 65°C where as sample temperature is nursed at 10°C. Wavelength detected at 210 nm. Run time was 70 minutes. The results obtained are represented in the table 1.

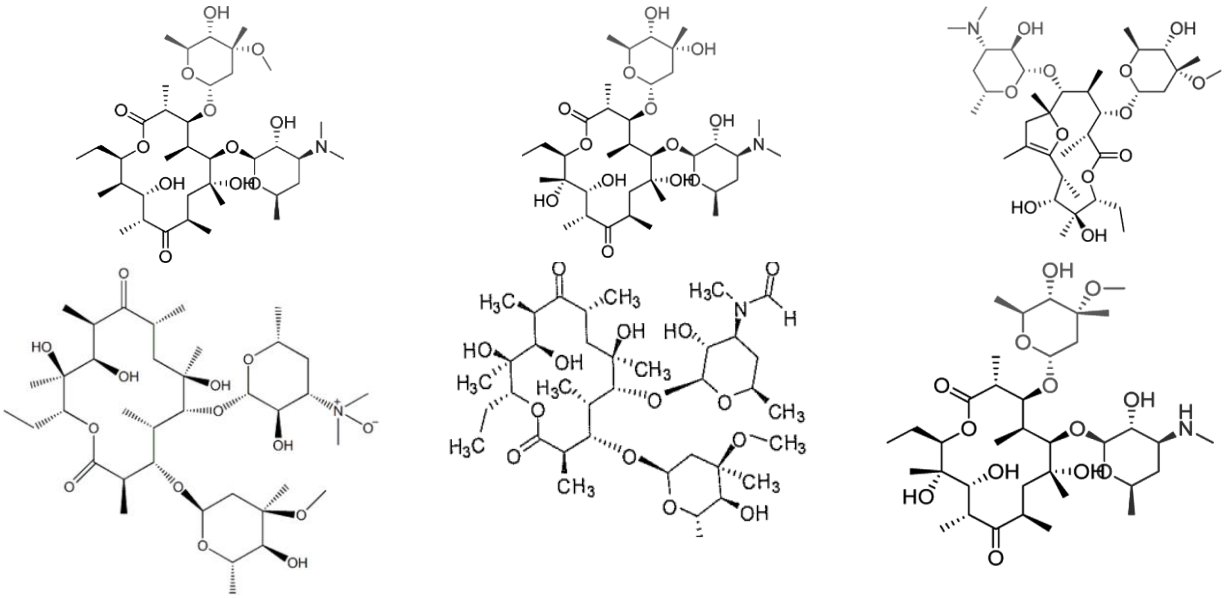

Figure 2: AnhydroERT A (foreign substance. D), ERT A enol ether (foreign substance E), Pseudo ERT A enol.ether (foreign substance F), ERT A 3´´-N-oxide (foreign substance H) ERT Imp. L, ERT Related Compound N (foreign substance B), (3"-N-demethyl-3"-N-formyl ERT A)

Preparation of solutions

For this analysis prepared various solutions includes solution of diluent, diluted standard solution, resolution solution as well as retention time solution, solution of sample, and Impurity spiked sample solution. By using the said solutions the following chromatograms are obtained and represented in the figure 3 reveals that the chromatograms of resolution and diluted standard and

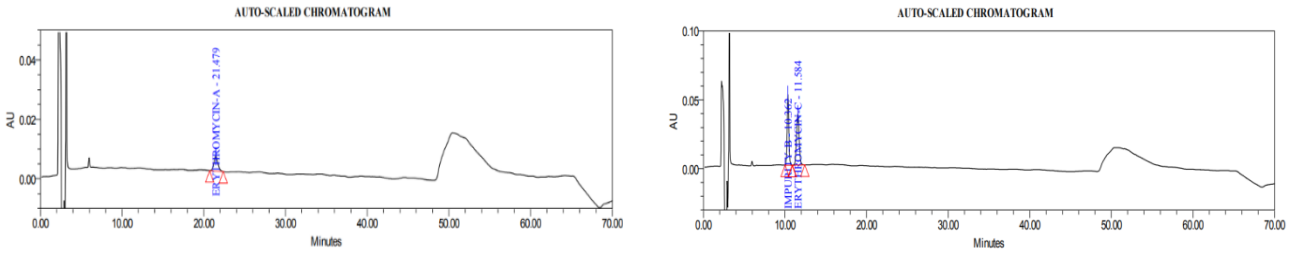

Figure 3: ERT standard and Resolution solution chromatograms

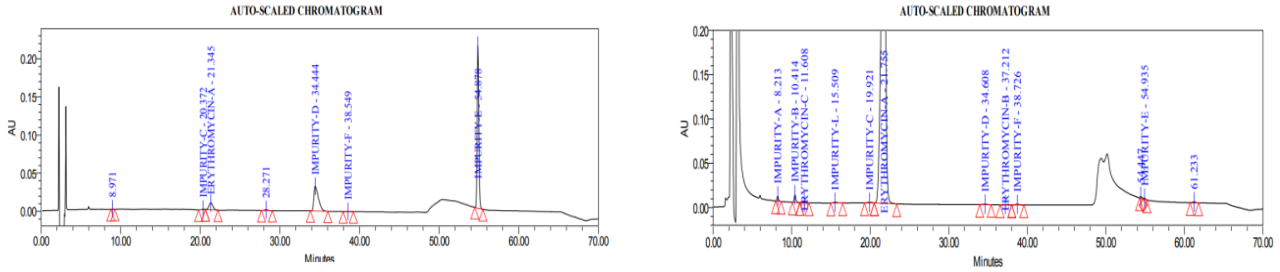

Figure 4: Chromatograms of ERT A enol ether (Imp. E) and Spiked sample with Impurities

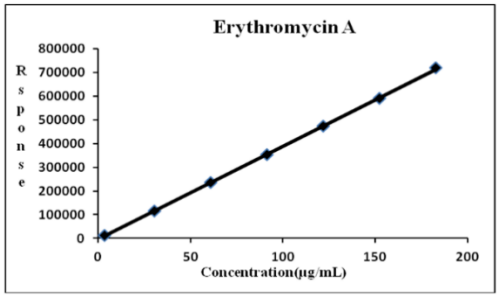

Figure 5: Linearity graph of ERT A

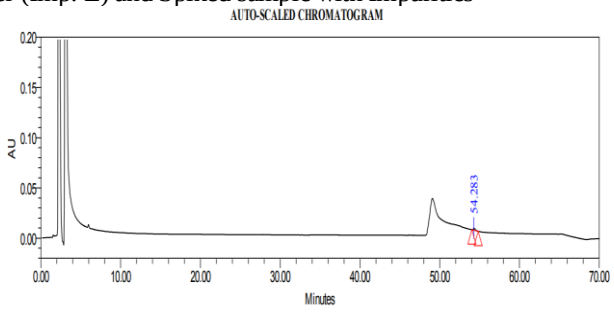

Figure 6: Typicalchromatogramof Placebo solution

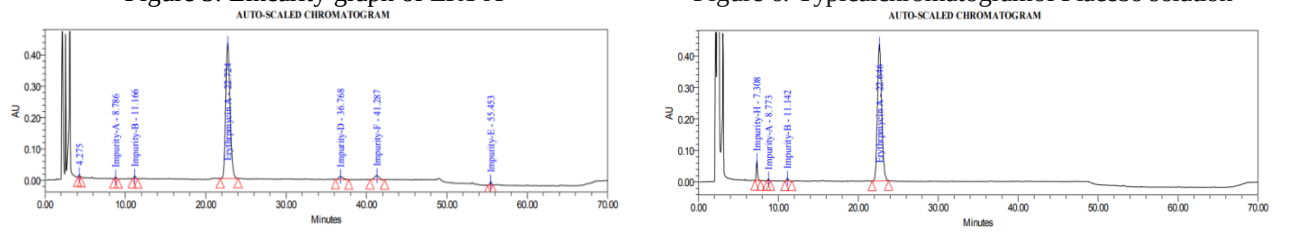

Figure 7: Typical chromatogram of Base and Peroxide degradation sample

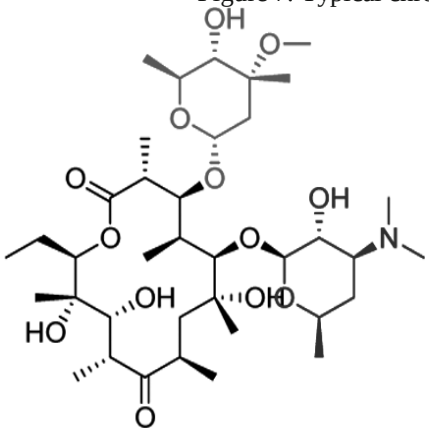

Figure1: Chemical Structures of ERT

figure 4 represents impurities present in the ERT sample.

RESULTS AND DISCUSSION

System suitability

For this parameter we began by sequentially injecting four distinct solutions into the HPLC system: a Blank solution (a single injection), the Standard solution, the Imp.-E Retention Time (RT) solution, and the Resolution solution. Each solution was carefully prepared. A comprehensive analysis of the system suitability results confirms that the method is indeed suitable and reliable for further evaluation. The detailed results can be found in table 2.

LOQ and LOD

These parameters are performed at signal-to-noise ratios 3:1 and 10:1. After introducing a course of reagents which are diluted containing admitted strengths of components of

Table 1: Gradient Program

Time (min)	Mobile phase A as % v/v	Mobile phase B as % v/v
0	100	0
45	100	0
47	0	100
62	0	100
65	100	0
70	100	0

Table 2: System suitability results

System suitability parameters	Obtained Result
The resolution between ERT Related Compound N (Impu B) and ERT C in resolution solution.	2.4
Tailing factor from diluted standard chromatogram (First replicate injection of Standard)	1.2
Relative standard deviation to replicate inoculations of diluted standard preparation.	0.4

Table 3: LOD and LOQ results

Name of the Imp.	LOD (%)	LOQ (%)	LOQ	
			Precision (% RSD)	Accuracy
ERT A 3 N-oxide(Imp.-H)	0.013	0.034	2.9	97.5
ERT Related compound N (Imp.-B)	0.008	0.029	3.6	98.3
3-N-demethyl-3-N-fromyl ERT A(Imp.-L)	0.002	0.006	0.0	98.8
Anhydro ERT A (Imp.-D)	0.055	0.120	3.2	105.3
Pseudo ERT A enol ether (Imp.-F)	0.005	0.013	0.0	95.3
ERT A enol ether(Imp.-E)	0.001	0.003	0.0	102.4
ERT A	0.029	0.090	3.2	97.8

target into matrix of placebo. To assess the both parameters named as precision as well as accuracy, a study is conducted at LOQ concentration. Total six separate spiked preparations (n = 6) were accurately prepared, as well as obtained values are erect to consistently meet criteria for the validation which is specified in Table 3.

Linearity

For this parameter this process is established through validation process by injecting solutions at six levels of strength, spanning by LOQ to 150% of level of specification for each and every component of interest, including foreign substances like H, B, L, D, F, E, and ERT A. This evaluation involved performing a least-squares linear regression analysis by plotting peak area in contrast to data strength, resulting in a linear regression coefficient exceeding 0.998. Linearity graph to ERT A is referred as Figure 5, and a summary of the results pertaining to the method's linearity is provided in Table 4.

Precision and accuracy

This precision for method is assessed by analyzing by preparing total six ERT Tablets Formulation samples and spiking ERT related foreign substances (Foreign substance H, Foreign substance B, Foreign substance L, Foreign substance D, Foreign substance F, Foreign substance E) level of specification test concentration. %RSD is calculated to all impurities. Intermediate precision study was conducted with peculiar analysts over disparate days, employing different systems as well as columns. %RSD values for all impurities were calculated, and the cumulative %RSD for both method precision along with intermediate precision was determined. Similarly, method precision and intermediate precision were evaluated for an unknown Imp. (ERT A) spiked into a placebo. Accuracy testing involved the preparation of a sample of ERT Tablets solution by spiking impurities at equivalents ranging by LOQ - 150% of the Imp. specification level in test solution. Additionally, ERT A, an unknown Imp., was spiked into the placebo at the specification level. The % recovery conscience are constitute to be within acceptable limits of 85.0% to 115.0%.The consequences of this process validation to precision, intermediate precision, as well as accuracy were summarized in Table 5.

Specificity

Impurities H, B, L, D, F, E, and ERT A are measured at their target levels. The resulting chromatogram was analyzed at the Rt of each component of interest. The results demonstrate that this prospective process is more specific to precise reckoning of ERT and its related foreign substances in tablet formulations. Chromatograms for placebo and impurities spiked into the test solution are provided in Figure 6 for the typical placebo chromatogram for the typical chromatogram of impurities spiked in the test solution

Forced Degradation Study

For this analysis forced degradation studies are carried out with Acid hydrolysis, Base hydrolysis, Oxidation, Thermal stress, Humidity, Photolytic conditions revealed that percentage of total ignominy, indicative of major ignominy foreign substances hammered in relation to proportionate detainment, Mass equity calculations, purity in peak assessments. Findings from stress circumstances were contracted in Tables 6 and 7. Notably, significant degradation was observed under base and peroxide conditions. Figure 7 represents the chromatogram of the base and peroxide degradation.

Robustness

These deliberate modifications were carried out to assess the impact on system suitability parameters as well as % recoveries, in comparison to this method's precision values. The results revealed that all these variations remained within a range of ± 10%. Consequently, the findings from

these experiments demonstrate robustness for analytical process.

Stability of Mobile Phase and Sample Solutions

Stability assessments were conducted for the samples of control, spiked, and dilute standard used in foreign substances determination, all under room temperature conditions. Evaluation stability for solution was carried out employing freshly prepared standard solution/s. The percentage resumption are compared among initial time (T) and time T + h hours. The results designated that sample solution remained stable in a time interval of 1 hour in room temperature also 9 hours when stored in the refrigerator. Diluted standard solutions were found that this is stable upto 7 days time interval at both room temperature as well

Table 4: Linearity Results

Parameter	Foreign substance H	Foreign substance B	Foreign substance L	Foreign substance D	Foreign substance F	Foreign substance E	ERT-A
Range(µg/mL)	1.176-59.5637	1.2096-119.9923	0.2373-24.6840	4.6696-58.4710	0.4837-62.5121	0.1193-180.3196	3.6077-182.7917
Correlation coefficient	0.999908	0.999916	0.999920	0.999328	0.999991	0.999959	0.999929
Intercept	690.8571	1375.1608	3816.1817	332.6382	6178.3838	14906.4015	3606.4752
Slope	3653.02050	3589.70139	45564.31996	1700.68122	41571.27261	48011.16791	3920.95129

Table 5: Accuracy and Precision results

Parameter	Foreign substance H	Foreign substance B	Foreign substance L	Foreign substance D	Foreign substance F	Foreign substance E	ERT-A
LOQ Accuracy (Mean, 95% Confidence low & High)	97.5, 96.8 & 98.2	98.3, 88.8 & 107.8	98.8, 93.4 & 104.2	105.3, 104.0 & 107.2	95.3, 102.0 & 102.8	102.4, 94.5 & 96.1	97.8, 95.5 & 100.1
50% Accuracy (Mean, 95% Confidence low & High))	97.9, 96.8 & 99.0	98.6, 97.6 &99.6	97.4, 95.9 & 98.9	96.5, 91.9 &94.7	100.7, 99.4 & 102.0	104.6, 102.5 & 106.7	100.9, 100.3 & 101.5
100% Accuracy (Mean, 95% Confidence low & High)	96.4, 93.5 & 99.3	101.2, 98.5 & 103.9	98.6, 95.6 & 101.6	102.8, 92.3 & 113.3	104.3, 102.9 & 105.7	102.7, 100.0 & 105.4	103.6, 101.8 & 105.4
150% Accuracy (Mean, 95% Confidence low & High)	99.2, 98.0 & 100.4	98.3, 97.1 & 99.5	97.8, 96.6 & 99.0	91.2, 90.0 & 92.4	100.9, 100.3 & 101.5	103.3, 102.1 & 104.5	105.5,104.9 & 106.1
Repeatability (%RSD)	1.1	1.4	1.4	6.9	0.8	1.0	1.0
Intermediate precision (%RSD)	3.0	1.5	3.0	4.2	0.4	0.9	0.3
Cumulative (%RSD)	5.1	4.0	3.8	5.8	2.8	4.3	3.1

Table 6.Forced Degradation Results individual Impurity and Single maximum Impurity

S. No.	Name of Degradation	%Known Impurities						
		Foreign substance H	Foreign substance B	Foreign substance L	Foreign substance D	Foreign substance F	Foreign substance E	Single Max Imp.
1	Test As such	---	0.8758	---	---	---	0.0189	---
2	Acid degradation	---	0.9544	---	---	---	0.4015	---
3	Base Degradation	---	0.8620	---	4.8203	0.3061	0.0721	0.5201
4	Peroxide Degradation	4.7654	0.9629	---	---	---	---	---
5	Water Degradation	---	0.8734	---	1.0762	---	---	---
6	Thermal Degradation	---	0.8245	0.0402	---	0.2946	0.1516	0.2805
7	Photolytic degradation	---	0.8914	---	2.4222	---	---	---
8	Humidity Degradation	---	0.9269	---	---	---	---	---

Table 7.Mass balance Results and peak Purity Results

Mode of degradation	%Assay of ERT	% Net degradation	%Mass balance (Between 95.0 to 105.0)	ERT (Limit PA<PT)	
				PA	PT
Control sample	101.4	-	–	0.515	2.552
Stressed with 1mL, 0.1N HCl for 5 minutes at bench top	98.3	2.8834	100.8	2.611	2.763
Stressed with 2mL, 0.1N NaOH for 5 minutes at bench top	97.3	5.6859	102.6	1.537	3.236
Stressed with 1mL, 3% Peroxide for 20 minutes at bench top	97.0	4.8336	101.4	0.753	2.256
Stressed with 2mL, water, for 5 hours at bench top	98.4	1.0549	99.1	0.330	2.240
Dry Heating at 105° C for 48 Hrs	103.1	0.6967	103.4	0.826	2.407
Photolytic in a time interval of 200 watt Hours/ Square meter and in a time interval of 1.2 million lux hours)	101.6	2.8834	101.1	0.677	1.875
Humidity (90% RH at 25°C in a time interval of 7 Days)	100.2	0.0322	99.8	0.804	2.857

as in refrigerator. Similarly, the resolution solution exhibited stability for 7 days when stored in the refrigerator. Furthermore, the mobile phase remained stable for 7 days when kept at room temperature. This process implement selective quantification for ERT as well as related degradation commodities without interfering by blank as well as placebo, thereby affirming this process as stability-indicating character.

CONCLUSION

This process for assessing related substances in ERT Tablets has undergone a comprehensive validation process, covering various critical parameters. The validation confirmed its effectiveness in terms of system suitability, specificity (interference from placebo and degradation products), linearity, method precision, ruggedness (intermediate precision), accuracy, establishment of LOD and LOQ, precision at LOQ, accuracy at LOQ, range (linearity, precision, and accuracy), stability of solutions (benchtop stability of standard and samples, as well as refrigerator stability of samples), mobile phase stability (on bench top), and robustness (variation in flow rate, column temperature, acetonitrile variation in Mobile phase-A, buffer discrepancy in Mobile phase-A, buffer discrepancy in Mobile phase-B, aqueous discrepancy in Mobile phase-B, pH discrepancy of buffer). This method was found to be linear from strength range 3.6-182.8 for ERT-A,1.18-59.563 for IMP-H, 1.21-120.0 for IMP-B,0.23-24.69for IMP-L,4.67-58.47 for IMP-D, 0.48-62.51 for IMP-F, and 0.12-180.32 for IMP-E. This proposed method has demonstrated a high degree of specificity, selectivity, and robustness, making it exceptionally well-suited to separate and quantification of foreign substances in ERT tablets solution.Conflicts of Interest

The authors are stated that they don't have either conflict of interest among authors.

Ethical approval

This present research work did not contain each study with human participants accomplished by any of authors.

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