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Method Development and Validation of Stability Indicating RP-HPLC Method for Simultaneous Estimation of Escitalopram Oxalate and Clonazepam in Bulk and its Pharmaceutical Formulations

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ABSTRACT

This article refers to simple isocratic reverse-phase high-performance liquid chromatographic method (RP-HPLC) developed for the simultaneous quantification of Escitalopram Oxalate (EST) and Clonazepam (CZP) in active pharmaceutical ingredient and pharmaceuticals. The separation of the two drugs was attained using a C_{18} column (250mm×4.6mm, 5µ) as a stationary phase. The mobile phase was used as a mixture of methanol; acetonitrile; and 0.05M potassium dihydrogen orthophosphate buffer (pH 4 adjusted by orthophosphoric acid) with an isocratic ratio of 40:20:40 v/v. Detection was made by using PDA detector at 210 nm. Escitalopram Oxalate (RT= 4.428 minutes) and Clonazepam (RT= 6.532 minutes) were separated in a single chromatographic run with resolution of 8.719. The calibration plot indicated good linear relationship with r^2 = 0.998 for Escitalopram Oxalate in concentration range of 32 µg/ml - 48 µg/ml and r^2 = 0.999 for Clonazepam in concentration range of 16 µg/ml - 24 µg/ml. The retrievals for Escitalopram Oxalate and Clonazepam were found to be 99.75% and 99.00%, respectively. The established analytical method was validated and found acceptable as per ICH guidelines for linearity, precision, accuracy, specificity, limit of detection, limit of quantification, robustness and stability. Escitalopram Oxalate and Clonazepam individually as well as in combination were exposed to different stress conditions like acid, base, thermal, photolytic and oxidation degradation and peaks of a degraded product were well determined from peaks of pure drug. This method is modest, quick and appropriate for routine quality control analysis.

Keywords: Reverse Phase – HPLC; Escitalopram Oxalate; Clonazepam; Validation; Degradation study.

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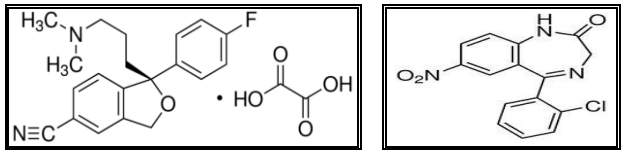
INTRODUCTION

Escitalopram oxalate (EST) (Figure 1a) is chemically known as S-(+)-1-[3-(dimethyl-amino) propyl]-1-(p-fluro-phenyl)-5-phthalancarbonitrile oxalate, which fits to the class of compounds known as antidepressant and is the S-enantiomer of racemic citalopram[1]. Escitalopram is spontaneously soluble in dimethlysulfoxide (DMSO), methanol and, sparingly soluble in ethanol and water. Escitalopram is insoluble in heptane but slightly soluble in ethyl acetate.

Escitalopram oxalate originates in category of oral selective serotonin reuptake inhibitor (SSRI) and proved highly potent with *in vitro* and *in vivo* studies. Escitalopram mainly used for the management of generalized Anxiety Disorder and major depressive disorder. Escitalopram works by specific competitive inhibition of the membrane transporter of serotonin[2]. As per studies Escitalopram discovered to be more than twice as potent as citalopram and is the highly selective drug in its class[2-3].Several analytical methods have been developed for the estimation of escitalopram oxalate in pharmaceutical formulations and/or biological fluids include liquid chromatography coupled with mass spectrometry[4].



(a) **(b)**

Figure 1: Chemical Structures of (a) EST (b) CZP

Clonazepam (CZP) (Figure 1b) is a derivative of benzodiazepine related to diazepam, with distinct antiepileptic properties[5]. It is official in BP[6] and USP[7]. Chemically clonazepam is 5-(2-chlorophenyl)-1, 3-dihydro-7-nitro-2H-1,4-benzodiazepine-2-one. Clonazepam is a light yellow crystalline powder that is practically odorless. It is freely very soluble in acetone, ethanol and methanol and insoluble in water.

Clonazepam is known as commercial drug to treat the depression associated with anxiety and highly used for the treatment of seizure and anxiety disorders. It works by Allosteric interactions between gamma-aminobutyric acid (GABA) receptors and central benzodiazepine receptors and potentiate the effects of GABA. GABA being an inhibitory neurotransmitter, helps to increase inhibition of the ascending reticular activating system (RAS)[8].

The literature survey reveals the accessibility of analytical methods for the quantitative estimation of Escitalopram Oxalate (EST) and Clonazepam (CZP) individually, or combination of these drugs with other drugs typically using chromatographic methods with different detectors such as electrochemical[9] or mass spectrometry detection[10-12] fluorescence[13], gas chromatography with electron-capture[14] or mass spectrometry[15] detection. The present study describes the development and validation of a stability indicating RP-HPLC method for the simultaneous estimation of EST and CZP in active pharmaceutical ingredient and marketed tablet formulation. The developed method was effectively applied for the routine analysis of EST and CZP in bulk and marketed tablet formulation.

MATERIAL AND METHOD

Standards of Escitalopram Oxalate and Clonazepam and marketed formulations were received as a gift sample from Consern Pharma Pvt. Ltd., Sahnewal, Punjab, India. All the chemical, solvents and reagents were HPLC and analytical grade procured from Merck chemicals, Mumbai, India. Water purified via milli-Q 0.45 µ Millipore nylon filter. The marketed formulation (ESTax Plus) used for analysis was procured from Consern Pharma Pvt. Ltd., Sahnewal, Punjab, India.

Instrumentation and chromatographic conditions:

Analysis was performed on a Shimadzu HPLC (LC-2010) equipped with Prominence LC-Gradient quaternary pump (LC-20AD), auto sampler, online degasser, sampler cooler and a SPD-20A prominence PDA detector. Operation data acquisition and analysis were performed by using LC solution software. The analytical column C_{18} column (250mm×4.6mm, 5µ) was used for separation. The isocratic elution was done with mobile phase of acetonitrile; methanol; and 0.05M potassium dihydrogen orthophosphate buffer (pH 4 adjusted by orthophosphoric acid) with an isocratic ratio of 40:20:40 v/v. Detection was performed by using PDA detector at 210 nm. The flow rate of 1.0 ml min^{-1} and created a back pressure of approx. 1039 psi. The buffer for mobile phase was prepared by dissolving 2.72 Potassium di-hydrogen orthophosphate in 400 ml of water and the pH was adjusted to 4 (±0.1) with orthophosphoric acid. Mobile phase was filtered through 0.45µ Millipore nylon filter under vacuum and ultrasonicated for 10 minutes prior use. The column was maintained at 35^0C temperature, detection was carried out using PDA detector at 210 nm and injection volume of 20 µl was used for analysis.

Standard solutions and calibration graphs for standardization:

For Stock standard solutions, 100 mg EST and 50 mg CZP working standard were separately weighed and transferred into 100 ml volumetric flasks respectively. Further, 60 ml of mobile phase was added in both volumetric flasks and sonicated for 5 minutes and the volume was made with mobile phase. Aliquots of standard sub-stock solutions (1ml) of EST and CZP were pipette out accurately in 25 ml volumetric flask and standard concentration of 40µg/ml and 20µg/ml were prepared respectively using mobile phase.

Sample preparation:

Sample was prepared by weighing accurately 20 tablets of combination of EST & CZP in pastel mortar and crushed. Weigh accurately sample powder about equivalent to 1/20 mg of CZP/EST and transfer them to 50ml of volumetric flask. The samples were sonicated for 10 minutes with 5ml of water and then add 30 ml of mobile phase and shake to dissolve and make volume with Mobile Phase. The above sample solutions were filtered through 0.45µ Millipore nylon filter paper and used for estimation.

For Escitalopram: Dilute 1ml from filtered solution to 10ml volumetric flask with mobile phase.

Procedure: Measure area of both standard and sample and calculate the result by comparison. Each mg of Escitalopram oxalate eq. to 0.782751 mg of Escitalopram.

Method validation:

System Suitability Test:

The system suitability test was conducted to calculate the accuracy of the system for the analysis, using six replicate injection of a reference solution of EST and CZP. The parameters measured were number of theoretical plates, retention time, peak area and tailing factor.

Specificity:

Specificity is the ability of the method to estimate the response of the analyte in the presence of its degradation products and potential impurities. The quantity of drugs was determined by taking chromatograms by using appropriate dilutions[16-24].

Linearity and range:

Calibration curves were prepared by plotting concentrations versus peak area of EST and CZP. It was verified from 80% to 120% of standards concentration using five calibration levels of 80%, 90%, 100%, 110 %, and 120%, (*i.e.* 32,36,40,44 and 48 µg/ml for EST and 16,18,20,22 and 24 µg/ml for CZP). For evaluation of data, method of linear regression was used[16-24].

Precision:

Three replicates of standard solution using with different concentrations were used for Precision analysis of the analytical method. It was demonstrated by intermediate precision (interday precision) and repeatability (intraday precision) of the solutions[16-24].

Accuracy:

It is measure of closeness of experimental values to the true value. The previous analyzed samples of EST/CZP (10mg/0.5mg) were spiked with extra 50%, 100% and 150% of the standard EST/CZP, and the proposed method was used for re-analysis of mixtures. The experiment was

conducted in triplicate. RSD (%) and standard error mean (%) were calculated for each concentration. The recovery of the drug was ensured at different levels in the formulations[23].

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

The limit of detection (LOD) and limit of quantification (LOQ) were used to describe the smallest concentration of an analyte that can be reliably measured by an analytical procedure as per ICH guidelines. By using the visual evaluation method, LOD was estimated by constant detection of the analyte at the minimum level. LOQ's were expressed as the minimum concentration of standard analytes that can be reproducibly calculated with acceptable precision and accuracy.

Robustness:

Robustness of the analytical method referred to its ability to remain unaffected by minute, but deliberate changes alterations in method parameters[16]. Robustness was studied by analyzing the effect of small and deliberate changes made in chromatographic conditions like, temperature of column, flow rate, pH and composition of mobile phase[25].

Assay of marketed preparation:

The prepared sample preparations of EST and CZP were tested on HPLC and percentage assay was calculated using following formulas.

For EST:

$$\frac{\text{Area of sample}}{\text{Area of standard}} \times \frac{\text{Concentration of standard}}{\text{Concentration of sample}} \times \frac{\text{Average weight of tablet}}{\text{Claim of EST}} \times 100 = \%\text{age assay of EST}$$

For CZP:

$$\frac{\text{Area of sample}}{\text{Area of standard}} \times \frac{\text{Concentration of standard}}{\text{Concentration of sample}} \times \frac{\text{Average weight of tablet}}{\text{Claim of CZP}} \times 100 = \%\text{age assay of CZP}$$

Forced degradation study:

Acid Hydrolysis:

Standards of EST (100mg) and CZP (100mg) were accurately weighed and transferred into three sets of 250ml round bottom flasks. About 20ml of 2N Hcl was added to all flasks and refluxed on heated mantle for 45 min at 80 ^{0}C[24].

Alkali Hydrolysis:

Standards of EST (100mg) and CZP (100mg) were accurately weighed and transferred into three 1N NaOH was added to all flasks and refluxed on heated mantle for 60 min at 80 ^{0}C[24].

Oxidative Degradation:

Standards of EST (100mg) and CZP (100mg) were accurately weighed and transferred into three sets of 250ml round bottom flasks. About 20ml of 6% H_2O_2 was added to all flasks and refluxed on heated mantle for 2 hr. at 80 ^{0}C[24].

Thermal Degradation:

Standard of EST (100mg) and CZP (100mg) was accurately weighed and transferred into Petri dish individually and spread into thin layer with spatula; this Petri dish was placed in the hot air oven for 1 hour at 80^{0}C. Heated drug samples of EST (100mg) and CZP (100mg) were taken into 100ml volumetric flask and dissolved in diluent. Volume was made up to the mark using diluent. 1ml of above sample was transferred into 10 ml volumetric flask and diluted up to the mark using diluent. It was filtered through 0.45µ Millipore nylon filter and filtrate was used for chromatographic analysis[24].

Photolytic Degradation:

Standard of EST (100mg) and CZP (100mg) was accurately weighed and transferred into petri dish individually and spread into thin layer with spatula; this petri-dish was put inside the UV Chamber for 1 hour. UV-exposed drugs samples of EST (100mg) and CZP (100mg) were taken into 100ml volumetric flask, and dissolved in diluent. Volume was made up to the mark using diluent. 1ml of above sample was taken into 10ml volumetric flask in a few ml of mobile phase and sonicated for 10min; this solution was cooled to the room temperature and made up the volume up to the mark using mobile phase. It was filtered through 0.45µ Millipore nylon filter and filtrate was used for chromatographic analysis[24].

RESULTS AND DISCUSSION

Method Optimization:

In the current study, separation of two analytes was carried out using Reversed-phase LC-method. Mobile phase used in study consist of mixture of organic solvents i.e. methanol: acetonitrile and aqueous buffer. Firstly, the column used was a C_{18} column (250mm×4.6mm, 5µ) as a stationary phase. The mobile phase was used as a mixture of acetonitrile; methanol; and 0.05M potassium dihydrogen orthophosphate buffer with an isocratic ratio of 40:30:30 v/v. Detection was performed by using PDA detector at 210 nm. The flow rate was maintained at 0.8 ml/minute which does not show enough resolution between analytes. After that, another column was used a C_{18} column (250mm×4.6mm, 5µ) as a stationary phase. The mobile phase was used as a mixture of acetonitrile; methanol; and 0.05M potassium dihydrogen orthophosphate buffer (pH 4 adjusted by orthophosphoric acid) with an isocratic ratio of 40:20:40 v/v. Detection was performed by using PDA detector at 210 nm. that showed adequate resolution between EST and CZP. To optimize the chromatographic parameters, the effect on changing the composition and pH (range from pH 3-6) of mobile phase was studied on the peak asymmetry, theoretical plates, retention time, capacity factor (k') and resolution. The selection of the concentration of potassium phosphate buffer

(pH 4) and the composition of mobile phase (acetonitrile: methanol: buffer :: 40:20:40 v/v) was done on the basis of attaining good baseline, adequate separation and sharp peaks in a minimum run time. Detection wavelength used at 210 nm in PDA detector. The injection volume was 20µl as well as column temperature was kept at 35°C temperature with the run time of 15 minutes.

Method Validation:

Method validation is the proof needed to ensure that an analytical method can produce results which are reliable and reproducible and which are fit for the purpose intended. The parameters that need to be demonstrated are system suitability test, precision, accuracy, specificity, linearity and range, limit of detection and limit of quantification and robustness as per ICH guidelines.

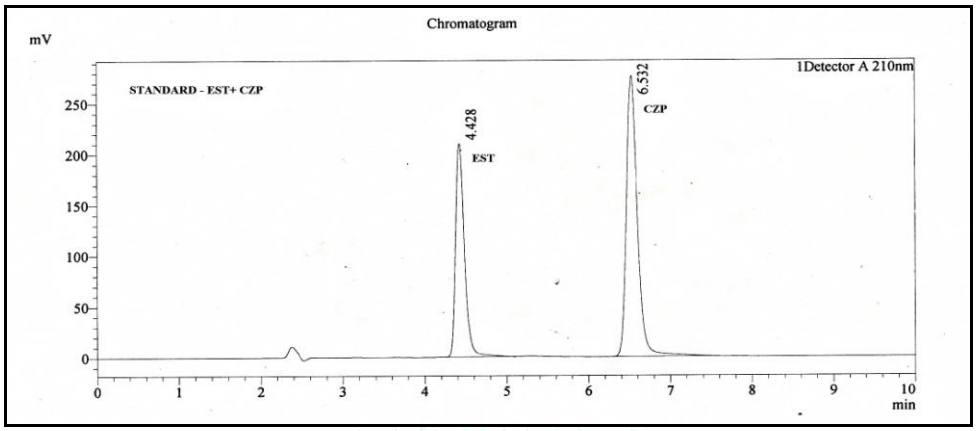


Figure 2: Standard peaks of EST and CPZ

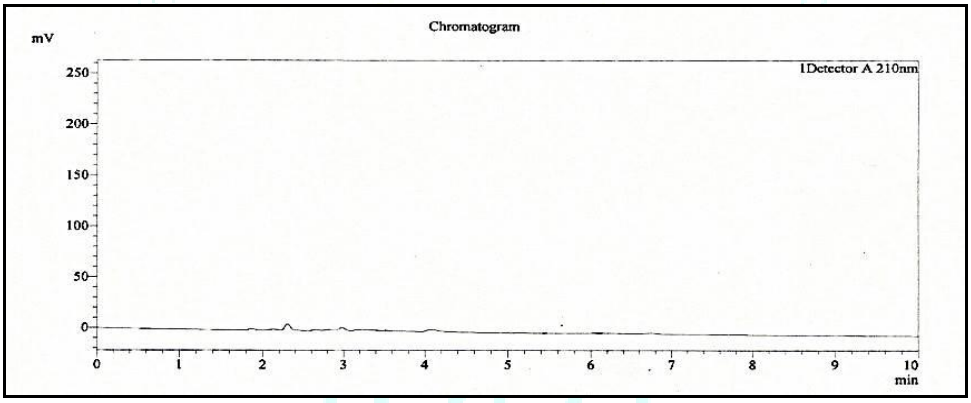


Figure 3: Specificity

System Suitability Tests:

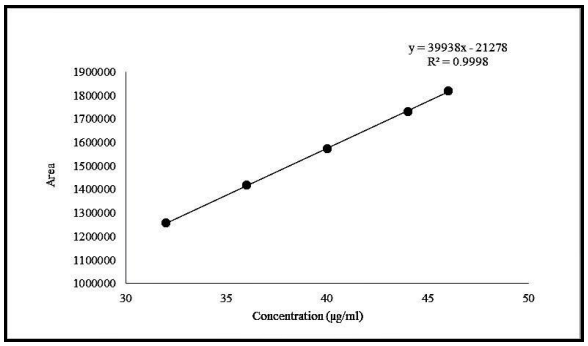
The system suitability test was performed to ensure the adequacy, validity and feasibility of the analytical method with instrument and also ensures the resolution between peaks of different analytes. The system suitability tests was applied to a representative chromatogram to confirm the various parameters such as peak area, peak asymmetry, capacity factor, theoretical plates, resolution, retention time and repeatability of the chromatographic system and ensures that the equipment, electronics and analytical operations for the samples analyzed could be constituted as an integral system that can be evaluated as a whole. The RSD of peak areas of five consecutive injections was found to less than 2%, as 1.28 % for EST and 1.05% for CZP, thus showing good repeatability, and excellent chromatographic and environmental conditions (Table 1). The resolution between the peaks of analytes indicates good separation from each other, as resolution found to be greater than 2. Theoretical plate number (N) and capacity factor (k´) demonstrated good column efficiency (Figure 2).

Specificity:

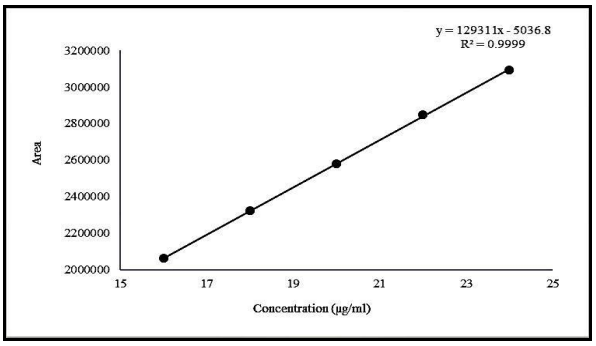
The specificity of a method demonstrates the presence of impurities or excipient. Specificity was determined by injecting placebo preparation, diluents, standard solution and sample preparation. No peak was interfering with retention time of analyte peaks (Figure 3). This clearly indicates that, excipients don't interfere with analytes and the assay is specific for EST and CZP.

Linearity and range:

The linearity of an analytical procedure is its ability (within a range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. The linearity range is 32 µg/ml to 48 µg/ml for EST with a correlation coefficient of 0.9998 and 16 µg/ml to 24 µg/ml for CZP with a correlation coefficient of 0.999 (Figure 4). The linearity of EST and CZP was found to be in range.



(a)



(b)

Figure 4: Linearity plots of (a) EST and (b) CZP

Precision:

The precision of an analytical procedure is the spreading of results from a replicates sets of measurements. The precision, was studied by calculating the RSD value. The intermediate precision composed of two parameters: intraday (n = 6) and interday (n = 6). The RSD values of intraday precision was 0.284 and 1.965 for EST and CZP, respectively and of interday precision was 0.168 and 1.442 for EST and CZP, respectively (Table 2). The RSD value for intermediate precision was found to be <2%, which confirms that the proposed method is précised.

Table 1: System Suitability Test Parameters

Parameter	Active Drugs			
	EST		CZP	
Retention time	Average	4.420	Average	6.535
	Standard Deviation	0.065	Standard Deviation	0.043
	RSD %	0.811	RDS %	0.981
Peak Area	Average	1578765.89	Average	2589768.33
	Standard Deviation	23243.43	Standard Deviation	49342.19
	RSD %	1.28	RDS %	1.05
Tailing Factor	Average	1.429	Average	1.528
	Standard Deviation	0.016	Standard Deviation	0.098
Theoretical plates	Average	5276.5	Average	4834
Resolution	Average	8.419		
	Standard Deviation	0.045		

Table 2: Precision (Intra-day and inter-day study)

Sample (Standard)	Intra-day study		Inter-day study	
	EST (area)	CZP (area)	EST (area)	CZP (area)
S1	1576589	2654253	1580143	2653423
S2	1572342	2533542	1574232	2539353
S3	1572987	2605423	1576453	2602232
S4	1583543	2578426	1581668	2586783
S5	1579934	2576546	1578342	2579245
S6	1573541	2512548	1578753	2579475
Mean	1576489.333	2576789.667	1578265.167	2590085.167
SD	4467.42112	50636.99272	2641.961348	37346.93717
RSD (%)	0.283377821	1.965119364	0.167396544	1.441919272
SEM	1823.824289	20672.54794	1078.580494	15246.8839

Table 3: Accuracy (Recovery study)

Analyte	Concentration (µg/ml)	Amount added		Amount recovered ± SD	Recovery (%)	Average recovery (%)	SEM	RSD
		(%)	(µg/ml)					
EST	40	80	32	31.92 ± 0.055	99.51	99.75	0.031	0.369
		100	40	39.89 ± 0.060	99.45		0.034	0.302
		120	48	48.07 ± 0.061	100.29		0.035	0.244
CZP	20	80	16	15.28 ± 0.283	98.81	99.00	0.163	0.477
		100	20	19.39 ± 0.330	99.24		0.190	0.417
		120	24	22.97 ± 0.271	98.97		0.156	0.273

Accuracy:

The accuracy of the analytical procedure expresses the closeness of mean values for a replicate set of a result to the true or accepted value. It can also be termed as trueness. The accuracy was assessed by the recovery experiments that were performed by the standard addition method. The recoveries obtained were 99.75 and 99.00 for EST and CZP, respectively (Table 3). Recovery between 80-120% indicates that the developed analytical method is accurate for determination of pharmaceuticals on combinations.

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

The LOD and LOQ were determined from slops of linear regression curves. LOD and LOQ were found to be 0.028 and 0.079μg/ml for EST and 0.015 and 0.092 μg/ml for CZP respectively (Table 4).

Table 4: Results of LOD and LOQ

Parameter	EST (µg/ml)	CZP (µg/ml)
LOD	0.028	0.015
LOQ	0.079	0.092

Robustness:

Robustness of analytical method refers to its ability to remain unaffected when subjected to small changes in method parameters. The method was determined by deliberately varying parameter like flow rate and pH of mobile phase used for estimation (Table 5).

Table 5: Robustness

Parameter altered	Retention time		Peak Area		Theoretical Plates	
	EST	CZP	EST	CZP	EST	CZP
Optimized chromatographic conditions	4.988	6.598	1579832	2567354	5693	4765
Increased flow rate (1.1mLmin−1)	5.132	6.943	1582354	2616232	5701	4892
Decreased flow rate (0.9mLmin−1)	4.311	6.054	1569267	2494654	5634	4423
Increased pH (4.5)	6.213	7.409	1398212	2398761	6231	5987
Decreased pH (3.5)	4.032	6.121	1425234	2416754	5453	5301

Assay of EST and CZP as tablet in comparison to standard:

The validated method was applied for the simultaneous quantification of these drugs in the marketed formulation and the percentage assay of EST was found to be 99.01% and for CZP found to be 99.57% in comparison to the standard of both drugs (Table 6).This clearly justify the assay in between 90-110% of the label claim.

Table 6: Percentage assay of EST and CZP tablets in comparison to standard

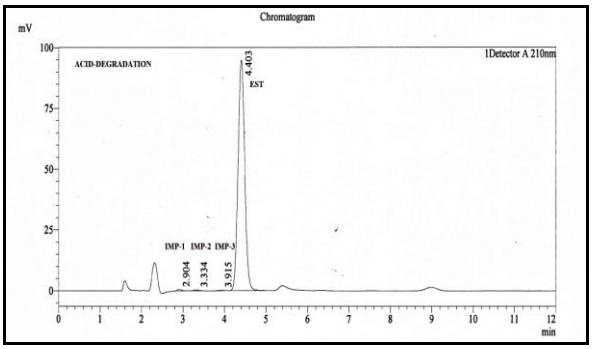
Drugs		Concentration (mg/ml)	Peak Area	%age Assay
Standard	EST	0.05124(including factor)	1583653	--
	CZP	0.02016	2560917	--
Sample	EST	0.04048	1582398	99.01
	CZP	0.0202	2559671	99.57

Table 7: Degradation of EST and CZP at different stress conditions:

Stress condition	Drugs	Peak Area	% Assay of drug after degradation	% Degradation
Acidic Degradation	EST	1058954	66.26	32.75
	CZP	387211	15.03	84.54
Basic Degradation	EST	326721	20.44	78.57
	CZP	274904	12.74	86.83
Oxidative Degradation	EST	973361	60.76	38.25
	CZP	2553810	99.34	0.23
Thermal Degradation	EST	1579226	98.81	0.20
	CZP	2475282	96.51	3.01
Photolytic Degradation	EST	1542957	96.54	2.47
	CZP	2668810	99.19	0.38

Forced degradation study (Stability study):

Forced degradation study plays a key role in development of stability indicating method. This study justified useful information about the degradation pathway of drug substances and drug products that could be form during storage. It was concluded by performing stress testing that the method was specific for EST and CZP. Both drugs were observed to be highly unstable for acidic, basic conditions while CZP is stable in oxidative stress conditions in comparison to EST, which is still unstable as per current study. So, under these conditions, we should be very careful during analysis. Hence, special storage conditions should be provided for the dosage form. The chromatograms of individual drugs as well as in combination after force degradation study under various stress conditions are shown in Figures 5-9. The percentage assay and percentage degradation of both EST and CZP observed for current degradation study is mentioned in Table 7 in comparison to assay values of EST and CZP without degradation (Table 6).



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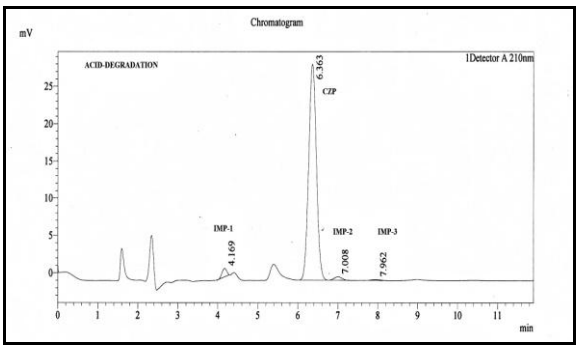

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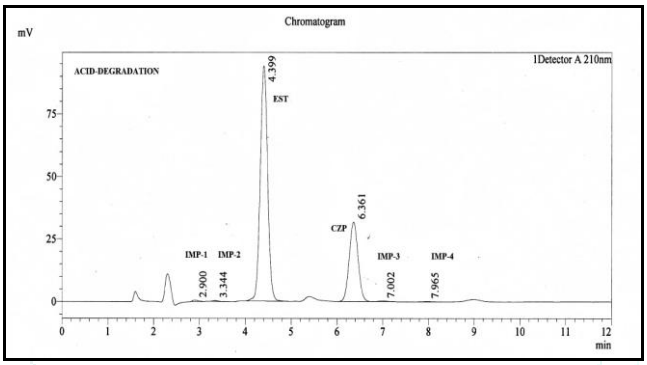
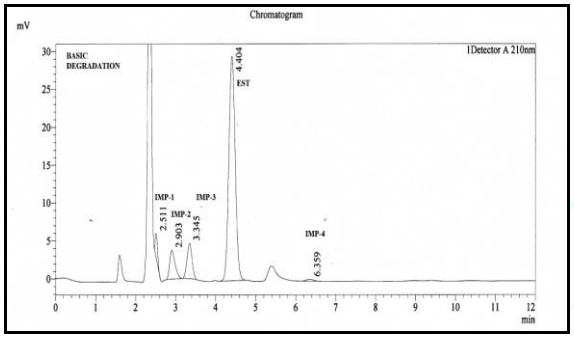
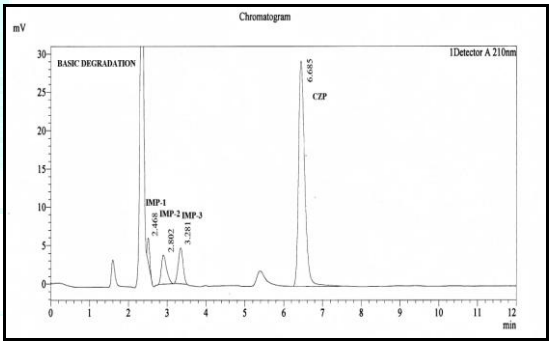


(c)

Figure 5: Acidic degradation of (a) EST (b) CZP (c) EST & CZP tablets



(a)



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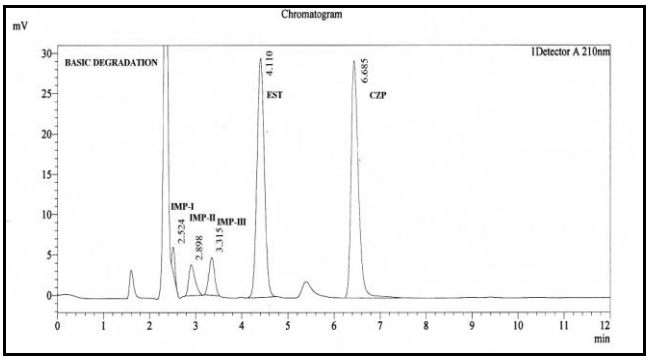
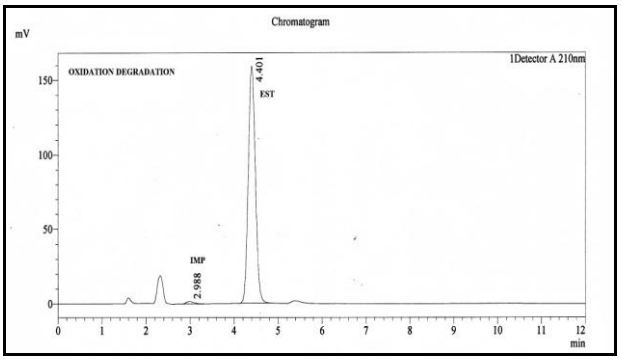
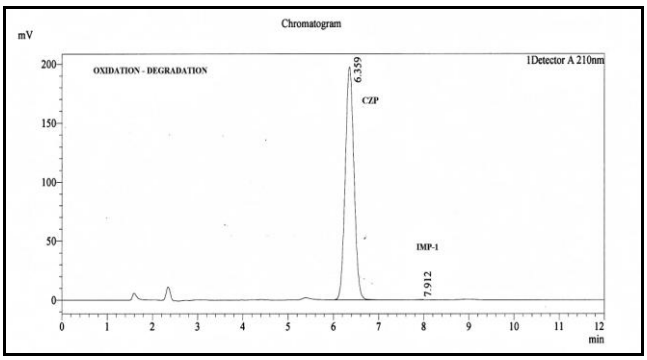


(c)

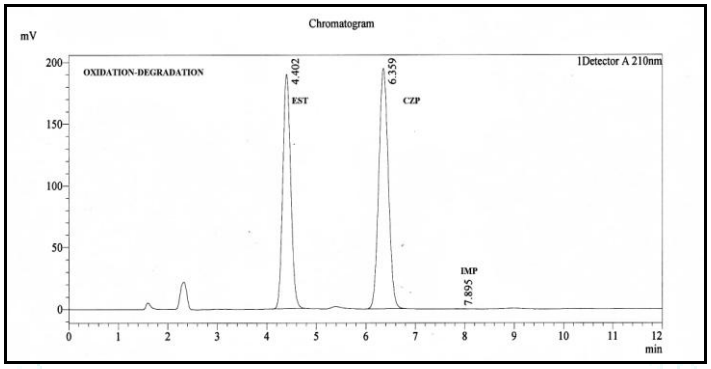
Figure 6: Basic degradation of (a) EST (b) CZP (c) EST & CZP tablets



(a)

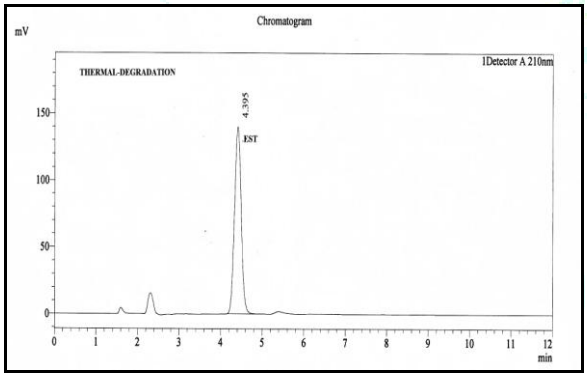


(b)

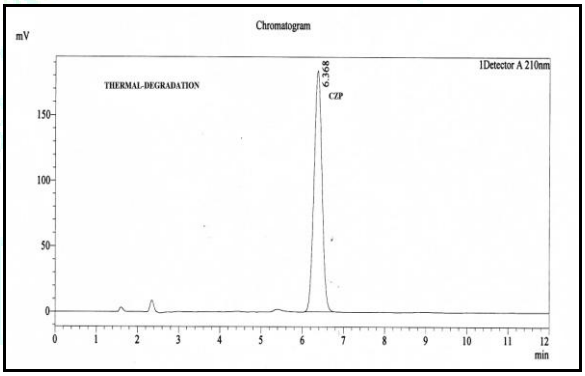


(c)

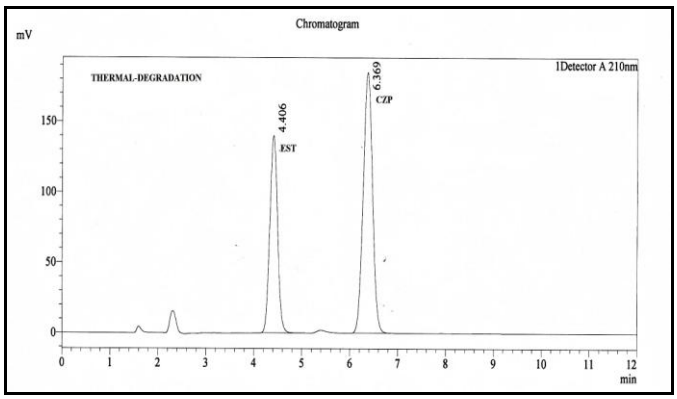
Figure 7: Oxidative degradation of (a) EST (b) CZP (c) EST & CZP tablets



(a)



(b)



(c)

Figure 8: Thermal degradation of (a) EST (b) CZP (c) EST & CZP tablets.

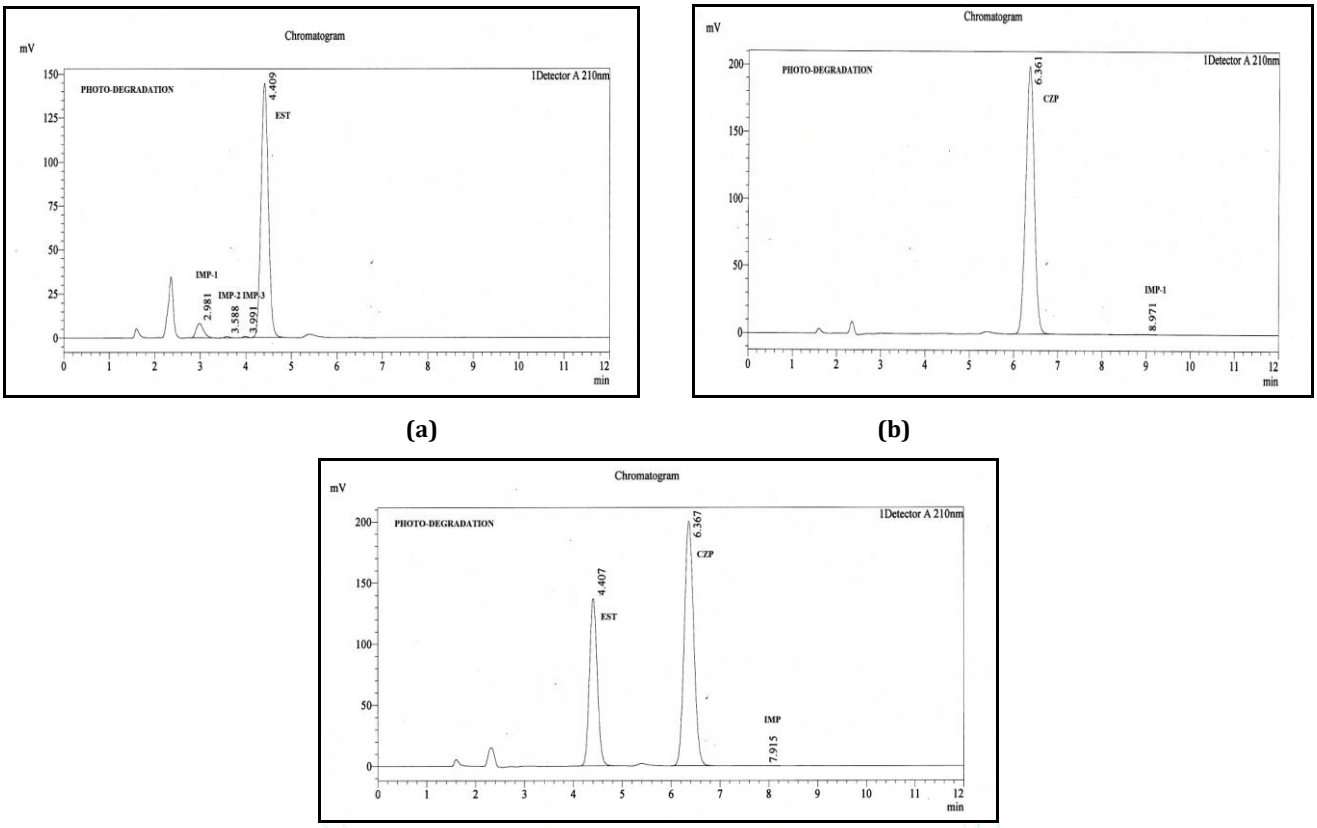


Figure 9: Photolytic degradation of (a) EST (b) CZP (c) EST & CZP tablets

CONCLUSION

A validated stability indicating Reverse phase HPLC method has been developed for estimation and quantification of EST and CZP as tablet dosage form in combination as per ICH guidelines. The mobile phase is simple to prepare and economical. The validated results showed that this method was specific, sensitive, linear, precise, accurate and robust. It can be concluded that this newly developed RP-HPLC method can be successfully applied for routine analysis for the estimation of combination of EST and CZP in tablet as well as bulk dosage form in pharmaceutical industry. Degradation studies justified the method specificity for its intended application. Therefore, the proposed method can be used for routine analysis of two drugs in their combined pharmaceutical dosage form.

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REFERENCES

1. Waugh J, Goa KL, Escitalopram-A review of its use in the management of major depressive and anxiety disorders, CNS Drugs, 2003; 17(5):343-362.
2. Kasper S, Stein DJ, Loft H, Nil R, Escitalopram in the treatment of social anxiety disorder-Randomized, placebo-controlled, flexible-dosage study, British Journal of Psychiatry, 2005; 186(3):222-226.
3. Rao N, The clinical pharmacokinetics of escitalopram, Clinical Pharmacokinetics, 2007; 46(4):281–290.
4. Singh S, Shah H, Gupta S, Jain M, Sharma K, Thakkar P, Shah R, Liquid chromatography–electrospray ionisation mass spectrometry method for the determination of escitalopram in human plasma and its application in bio-equivalence study, Journal of Chromatography B, 2004; 811:209–215.
5. Martindale KP, The Extra Pharmacopoeia - Martindale, The Pharmaceutical Press, London, 1999.
6. British Pharmacopoeia, 3 Vol, Medicines and Health Care Products Regulatory Agency London; 2018:383-384.
7. United States Pharmacopoeia, 41-NF 37, Rockville: The United States Pharmacopoeial Convention; 2018:1021-1021.
8. Monti JM, Pandi-Perumal SR, Möhler H, GABA and Sleep, Molecular, Functional and Clinical Aspects, Springer, Basel. 2010.
9. García-Fernández MA, Fernández-Abedul MT, Costa-García A, Determination of buprenorphine in biological samples by high performance liquid chromatography with electrochemical detection, Chromatographia, 2001; 53(11-12):704-708.
10. Panchal JA, Maheshwari DG, Review on chromatographic and spectrophotometric estimation of escitalopram oxalate and eszopiclone in bulk and in different dosage form, European Journal of Pharmaceutical and Medical Research, 2017; 4(1):183-191.
11. Kumar V, Singh HP, Rathore RPS, Method development & validation of escitalopram oxalate & etizolam by UV-spectrophotometry, International Journal of Institutional Pharmacy and Life Sciences, 2015; 5(2):12-42.
12. Kakde RB, Satone DD, Spectrophotometric Method for Simultaneous Estimation of Escitalopram Oxalate and Clonazepam in Tablet Dosage Form. Indian Journal of Pharmaceutical Sciences, 2009; 71(6):702-705.
13. Liu SY, Liu KS, Kuei CH, Tzeng JI, Ho ST, Wang JJ, Simultaneous determination of buprenorphine and its prodrug, buprenorphine propionate, by high-performance liquid chromatography with fluorescence detection: application to pharmacokinetic studies in rabbits, Journal of Chromatography. B, 2005; 818:233-239.
14. Everhart ET, Cheung P, Shwonek P, Zabel K, Tisdale EC Jacob, P, Mendelson J, Jones RT, Subnanogram-concentration measurement of buprenorphine in human plasma by electron-

capture capillary gas chromatography: application to pharmacokinetics of sublingual buprenorphine Clinical Chemistry, 1997; 43:2292-2302.
15. Lisi AM, Kazlauskas R, Trout GJ, Gas chromatographic-mass spectrometric quantitation of urinary buprenorphine and norbuprenorphine after derivatization by direct extractive alkylation, Journal of Chromatography B, Biomedical Sciences and Applications, 1997; 692(1):67-77.
16. Rajamanickam V, Santhosam D, Sridharan D, Thenmozhi A, Simultaneous determination of risperidone and Trihexhyphenidyl hydrochloride from bulk and tablet dosage form by RP-HPLC, Asian Journal of Research in Chemistry, 2010; 3 (3):549–551.
17. Dabir J, Mary Mathew E, Moorkoth S, Analytical method development and validation of RP-HPLC method for simultaneous estimation of N-acetyl cysteine and cefexime from its fixed dose combination, Research Journal of Pharmacy and Technology, 2016; 9(7):835-842.
18. Vinyas M, Velivela S, Yadav G, Pati NB, Gupta VRM, Analytical Method Development and Validation of alogliptin by RP-HPLC Method, Research Journal of Pharmacy and Technology, 2016; 9(7):775-778.
19. Patel RB, Patel MR, Dubey N, Dubey NN, and Patel BG, HPTLC method development and validation: Strategy to minimize methodological failures, Journal of Food and Drug Analysis, 2012; 20:561–571.
20. Kumar M, Rao JR, Yadav SS, Sathiyanarayanan L, Vikas, Development and validation of a stability-indicating HPTLC method for analysis of bumetanide in the bulk drug and tablet dosage form, Research Journal of Pharmacy and Technology, 2010; 3(1): 239-243.
21. International Conference on Harmonization (ICH), Validation of analytical procedures: text and methodology Q2(R1)., Geneva, Switzerland, 2005.
22. Reddy Thumma PK, Deepthi PN, Sudheer B, Reddy PP, Kandukuri S, Banji D, Formulation, evaluation and stability studies for the sustained release mucoadhesive microcapsules of gliclazide, Research Journal of Pharmacy and Technology, 2013; 6(11):1242-1246.
23. Agrawal H, Kaul N, Paradkar AR, Mahadik KR, The ICH guidance in practice: stress degradation studies on indinavir sulphate and development of a validated specific stability-indicating HPTLC assay method. Farmacopia, 2004; 59(9):729–738.
24. Mahalingam V, Vijayabaskar S, Kalaivani RA, Somanathan T, Analytical method development and validation for the analysis of donepezil hydrochloride and its related substances using ultra performance liquid chromatography, Research Journal of Pharmacy and Technology, 2017; 10(8):2743-2749.
25. Shetti P, Venkatachalam A, Stability indicating HPLC method for simultaneous quantification of trihexyphenidyl hydrochloride, trifluoperazine hydrochloride and chlorpromazine hydrochloride from tablet formulation. Journal of Chemistry, 2010; 7:299-313.