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DETERMINATION OF ESCITALOPRAM OXALATE AND ETIZOLAM IN TABLET DOSAGE FORMS BY RP-HPLC METHOD

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ABSTRACT

A simple and selective LC method is described for the determination of Escitalopram oxalate and Etizolam in tablet dosage forms. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of 30 volumes of ammonium acetate buffer, 40 volumes of acetonitrile and 30 volumes of Methanol with detection of 238 nm. Linearity was observed in the range 60-140 µg/ml for Escitalopram oxalate (r^2 =0.999) and 6-14 µg /ml for Etizolam (r^2 =0.996) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

KEY WORDS: Liquid chromatography (LC), RSD Relative standard deviation, r^2 correlation coefficient.

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INTRODUCTION

Pharmaceutical analysis simply means analysis of pharmaceuticals. Webster' dictionary defines a pharmaceutical is a medical drug. A more appropriate term for a pharmaceutical is active pharmaceutical ingredient (API) or active ingredient to distinguish it From a formulated product or drug product is prepared by formulating a drug substance with inert ingredient (excipient) to prepare a drug product that is suitable for administration to patients. Research and development (R&D) play a very comprehensive role in new drug development and follow up activities to ensure that a new drug product meets the established standards is stable and continue to approved by regulatory authorities,assuring that all batches of drug product are made to the specific standards utilization of approved ingredients and production method becomes the responsibility of pharmaceutical analysts in the quality control (QC) or quality assurance department . The methods are generally developed in an analytical R&D department and transferred to QC or other departments as needed. At times they are transferred to other divisions.

Escitalopram, the S-enantiomer of citalopram, belongs to a class of antidepressant agents known as selective serotonin-reuptake inhibitors (SSRIs). Despite distinct structural differences between compounds in this class, SSRIs possess similar pharmacological activity. As with other antidepressant agents, several weeks of therapy may be required before a clinical effect is seen. SSRIs are potent inhibitors of neuronal serotonin reuptake. They have little to no effect on norepinephrine or dopamine reuptake and do not antagonize α- or β-adrenergic, dopamine D2 or histamine H1 receptors. During acute use, SSRIs block serotonin reuptake and increase serotonin stimulation of somatodendritic 5-HT1A and terminal autoreceptors. Chronic use leads to desensitization of somatodendritic 5-HT1A and terminal autoreceptors. The overall clinical effect of increased mood and decreased anxiety is thought to be due to adaptive changes in neuronal function that leads to enhanced serotonergic neurotransmission. Side effects include dry mouth, nausea, dizziness, drowsiness, sexual dysfunction and headache. Side effects generally occur within the first two weeks of therapy and are usually less severe and frequent than those observed with tricyclic antidepressants. Escitalopram may be used to treat major depressive disorder (MDD) and generalized anxiety disorder (GAD). The antidepressant, antiobsessive-compulsive, and antibulimic actions of escitalopram are presumed to be linked to its inhibition of CNS neuronal uptake of serotonin. Escitalopram blocks the reuptake of serotonin at the serotonin reuptake pump of the neuronal membrane, enhancing the actions of serotonin on 5HT1A autoreceptors. SSRIs bind with significantly less affinity to histamine, acetylcholine, and norepinephrine receptors than tricyclic antidepressant drugs. Escitalopram is one of a class of antidepressants known as selective serotonin reuptake inhibitors (SSRIs). It is used to treat the depression associated with mood disorders. It is also used on occassion in the treatment of body dysmorphic disorder and anxiety. The antidepressant, antiobsessive-compulsive, and antibulimic actions of escitalopram are presumed to be linked to its inhibition of CNS neuronal uptake of serotonin. In vitro studies show that escitalopram is a potent and selective inhibitor of neuronal serotonin reuptake and has only very weak effects on norepinephrine and dopamine neuronal reuptake. Escitalopram has no significant affinity for adrenergic (alpha1, alpha2, beta), cholinergic, GABA, dopaminergic, histaminergic, serotonergic (5HT1A, 5HT1B, 5HT2), or benzodiazepine receptors; antagonism of such receptors has been hypothesized to be associated with various anticholinergic, sedative, and cardiovascular effects for other psychotropic drugs. The chronic administration of escitalopram was found to downregulate brain norepinephrine receptors, as has been observed with other drugs effective in the treatment of major depressive disorder. Escitalopram does not inhibit monoamine oxidase. Labeled indications include major depressive disorder (MDD) and generalized anxiety disorder (GAD). Unlabeled indications include treatment of mild dementia-associated agitation in nonpsychotic patients.

Etizolam (marketed under the brand name Etilaam, Etizola, Sedekopan, Etizest, Pasaden or Depas) is a benzodiazepine analog. The etizolam molecule differs from a benzodiazepine in that the benzene ring has been replaced by a thiophene ring, making the drug a thienodiazepine. It possesses amnesic, anxiolytic, anticonvulsant, hypnotic, sedative and skeletal muscle relaxant properties. Etizolam is not authorized for medical use in the U.S. However, it currently remains unscheduled and is legal for research purposes.

Quality investigation plays a very important role in quality specification establishment of chemical drugs. The number of drugs introduced into the market every year .very often there is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. Hence, standards and analytical procedures for these drugs may not be available in the pharmacopoeias. It becomes necessary, therefore to develop newer analytical methods for such drugs. Method development involves considerable trial and error procedures. The most difficult problem usually is where to start, what type of column is worth trying with what kind of mobile phase. Single dosage forms with combination of drugs are widely used today due

to their advantages and their simultaneous estimation of individual component is a challenging task (1-9).

Aim of the study is to develop new RP HPLC method for the simultaneous estimation of Escitalopram Oxalate and Etizolam pharmaceutical dosage form.

MATERIALS AND METHODS (10, 11)

Determination Of Working Wavelength (λmax)

In simultaneous estimation of two drugs isobestic wavelength is used. Isobestic point is the wavelength where the molar absorptivity is the same for two substances that are interconvertible. So this wavelength is used in simultaneous estimation to estimate both drugs accurately.

Preparation of standard stock solution of ESCITALOPRAM

10 mg of Escitalopram was weighed and transferred in to 100ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

Preparation of standard stock solution of ETIZOLAM

10 mg of etizolam was weighed in to 100ml volumetric flask and dissolved in Methanol and then dilute up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

Preparation of samples for Assay

Preparation of mixed standard solution

weigh accurately 10mg of escitalopram and 10 mg of etizolam in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 10 µg/ml of escitalopram and etizolam is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Tablet sample

10 tablets (each tablet contains Etizolam-05 mg Escitalopram -50 mg) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of etizolam and escitalopram (µg/ml) were prepared by dissolving weight equivalent to 10 mg of etizolam and escitalopram and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 10ml with mobile phase. Further dilutions are prepared in 5 replicates of 10µg/ml of etizolam and escitalopram was made by adding 1 ml of stock solution to 10 ml of mobile phase.

Standard solutions were prepared as per the test method and injected into the chromatographic system. The system suitability parameters like theoretical plates, resolution and asymmetric factor were evaluated.

Validation

Standard solutions were prepared as per the test method and injected into the chromatographic system. The system suitability parameters like theoretical plates, resolution and asymmetric factor were evaluated.

RESULTS AND DISCUSSION

The wavelength of maximum absorption (λ_{max}) of the drug, 10 µg/ml solution of the drugs in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The isobestic point was found to be 227 nm for the combination.

The amount of escitalopram and etizolam present in the taken dosage form was found to be 100.08 % and 98.50 % respectively (Table-1).

Table-1Assay Results

	ESCITALOPRAM		ETIZOLAM	
	Standard Area	**Sample Area**	**Standard Area**	**Sample Area**
Injection-1	2306.966	2324.553	479.552	477.204
Injection-2	2393.229	2402.442	490.035	497.287
Injection-3	2368.996	2374.124	499.05	493.789
Injection-4	2324.494	2323.438	484.363	469.318
Injection-5	2398.839	2401.217	488.215	498.57
Average Area	2358.505	2365.155	488.243	487.2336
Standard deviatuion	39.24303		13.`17308	
%RSD	1.655898		1.551382	
Assay(%purity)	**100.08**		**98.50**	

The % RSD for the retention times and peak area of escitalopram oxalate and etizolam were found to be less than 2%. The plate count and tailing factor results were found to be satisfactory and are found to be within the limit (Table-2 and 3).

Table-2 Results for system suitability of escitalopram oxalate

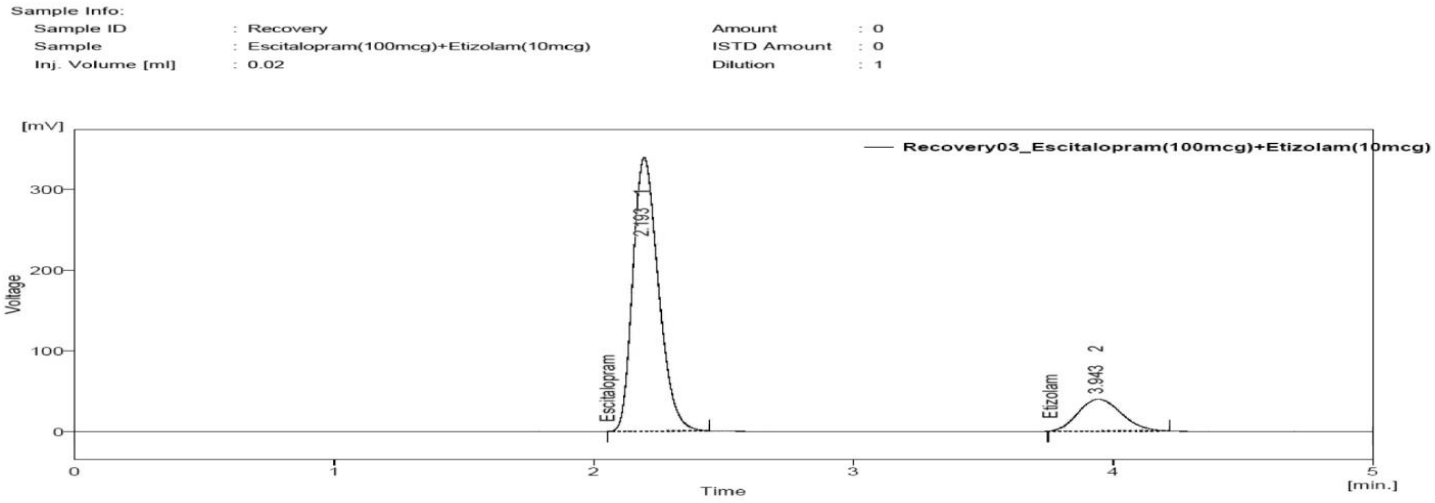
Injection	Retention time (min)	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	2.223	2306.966	4407	1.375
2	2.247	2393.229	4458	1.458
3	2.213	2368.996	4385	1.417
4	2.250	2324.494	4627	1.478
5	2.263	2389.839	4494	1.417
Mean	2.2392	2356.705	-	-
SD	0.020572	39.03348	-	-
%RSD	0.916876	1.652961	-	-

Table-3 Results for system suitability of etizolam

Injection	Retention time (min)	Peak area	Theoretical plates	Tailing factor
1	3.967	479.552	3138	1.375
2	3.983	490.035	3042	1.300
3	3.967	499.050	3016	1.293
4	3.970	484.363	3273	1.282
5	4.013	488.215	3345	1.220
Mean	3.98	488.243	-	-
SD	0.019596	7.25588	-	-
%RSD	0.491375	1.483148	-	-

The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparations of escitalopram oxalate and etizolam is 0.999 and 0.996. The relationship between the concentration of escitalopram oxalate and etizolam and area of escitalopram oxalate and etizolam is linear in the range examined since all points lie in a straight line and the correlation coefficient is well within limits.

The percentage mean recovery of escitalopram oxalate and etizolam is 99.19 % and 99.89 % respectively (Fig-1-3).



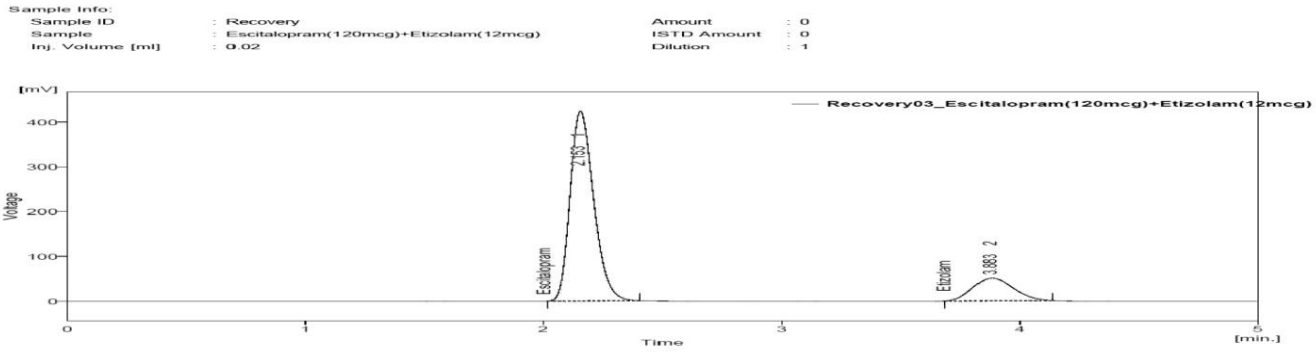
Result Table (Uncal - Recovery03_Escitalopram(100mcg)+Etizolam(10mcg))

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.193	2327.418	339.101	83.6	89.5	0.11
2	3.943	461.334	39.592	16.4	10.5	0.19
	Total	2788.752	378.693	100.0	100.0	

Column Performance Table (From 50% - Recovery03_Escitalopram(100mcg)+Etizolam(10mcg))

	Reten. Time	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	2.193	0.110	1.500	0.00	4203	42026	-
2	3.943	0.187	1.250	0.00	3472	34723	6.942

Figure-1 Chromatogram of 100% recovery



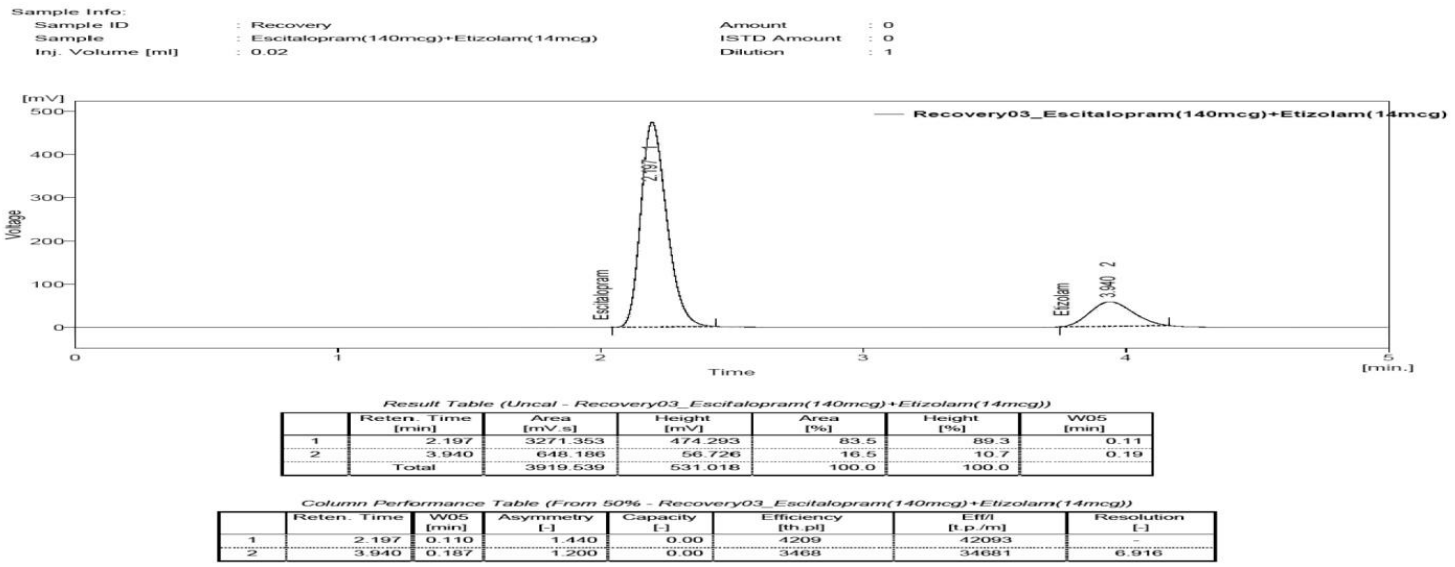
Result Table (Uncal - Recovery03_Escitalopram(120mcg)+Etizolam(12mcg))

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.153	2944.655	423.284	83.1	89.3	0.11
2	3.883	597.534	50.931	16.9	10.7	0.19
	Total	3542.190	474.215	100.0	100.0	

Column Performance Table (From 50% - Recovery03_Escitalopram(120mcg)+Etizolam(12mcg))

	Reten. Time	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	2.153	0.113	1.440	0.00	4999	49999	-
2	3.883	0.190	1.244	0.00	3314	33143	6.712

Figure-2 Chromatogram of 120% recovery



Result Table (Uncal - Recovery03_Escitalopram(140mcg)+Etizolam(14mcg))

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.197	3271.353	474.293	83.5	89.3	0.11
2	3.940	648.186	56.726	16.5	10.7	0.19
	Total	3919.539	531.018	100.0	100.0	

Column Performance Table (From 50% - Recovery03_Escitalopram(140mcg)+Etizolam(14mcg))

	Reten. Time	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	2.197	0.110	1.440	0.00	4209	42093	-
2	3.940	0.187	1.200	0.00	3468	34681	6.916

Figure-3 Chromatogram of 140% recovery

The LOD for this method was found to be 4.61µg/ml and 14.0 µg/ml for etizolam. The LOQ for this method was found to be 0.21µg/ml for escitalopram oxalate and 0.63µg/ml for etizolam.

CONCLUSION

From the above experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation Escitalopram oxalate and Etizolam was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories studies in near future.

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