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Simultaneous determination of escitalopram oxalate and eszopiclone in their combined mixture by ultraviolet spectrophotometry (Dual wavelength method)

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ABSTRACT

A simple, accurate, and precise dual wavelength spectrophotometric method was developed for simultaneous determination of Escitalopram oxalate (ESC) and Eszopiclone (ESZ) in their mixtures. The principle for dual wavelength method is "the absorbance difference between two points on the mixture spectra is directly proportional to the concentration of the component of interest". The method based on determination of ESZ at 304.0 nm using its absorptivity value and ESC at 238.0 nm after deduction of absorbance due to ESZ. The two drugs follow Beer-Lambert's law over the concentration range of 5-50 µg/mL for ESC and 3-18 µg/mL for ESZ. The % estimation of the drugs was found near to 100 % representing the accuracy of the three methods. The recovery of the ESC and ESZ were found near to 100 %. Validation of the proposed methods was carried out for its accuracy, precision, specificity and ruggedness according to ICH guidelines. The proposed methods can be successfully applied in routine work for the determination of ESC and ESZ in combined dosage form.

KEYWORDS

Escitalopram oxalate;
Eszopiclone;
Dual wavelength method;
UV spectrophotometric method;
Validation.

INTRODUCTION

ESC[1-2] is an orally administered selective serotonin reuptake inhibitor. It is the pure *S* enantiomer of racemic bicyclic phthalane derivative of citalopram. Chemically it is S-(+)-1-[3-(dimethylESCno)propyl]-1-(p-fluorophenyl)-5 phthalancarbonitrile oxalate. Various analytical methods, such as spectroscopic method with clonazepam, stability indicating HPTLC, colorimetric method, HPLC method with clonazepam[3-18] have been reported.

Eszopiclone (ESZ)[1-2] is a nonbenzodiazepine hypnotic agent used as a treatment for insomnia. Eszopiclone is the active stereoisomer of zopiclone, and belongs to the class of drugs known as cyclopyrrones. It is chemically [(7S)-6-(5-chloropyridin-2-yl)-5-oxo-7H-pyrrolo[3,4-b]pyrazin-7-yl] 4-methylpiESZazine-1-carboxylate. ESC and ESZ are available in a single

dosage form but not in combination therapy in the market. Fixed dose combination therapy of ESC and ESZ is indicated for the treatment of insomnia. Eszopiclone also enhances the effect of Escitalopram in insomnia and anxiety. Various analytical methods, such as estimation of eszopiclone in bulk and in formulation by simple UV and difference spectroscopic methods, HPLC method, Stability indicating HPLC have been reported[19-20].

No spectrophotometric methods have been reported in the literature for the simultaneous determination of Escitalopram oxalate and Eszopiclone in their mixtures. It would therefore be beneficial to provide accurate, precise, and reliable methods for simultaneous determination of Escitalopram and Eszopiclone. The present work describes a analytical procedures for the quantitation of Escitalopram in co-formulation with Eszopiclone using dual wavelength spectroscopy method.

EXPERIMENTAL

Instruments and apparatus

All absorption spectra were recorded with a UV-1700 PC UV/Vis double beam spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cells (Shimadzu, Japan), CP224S analytical balance (Sartorius) and ultra sonic cleaner (Frontline FS 4) were used throughout the practical.

Reagents

Escitalopram oxalate and Eszopiclone pure powder were donated by Sun Pharma. Ltd., Gujarat. Methanol was purchased from SDfine Chemicals and were of analytical grade.

Preparation of ESC and ESZ standard solution

ESC (10mg) and ESZ (10mg) were accurately weighed and transferred to two separate 100 ml volumetric flask, dissolved in methanol to obtained stock solution of 100 µg/ml each.

Preparation of ESC and ESZ sample solution

To determine the content of ESC and ESZ in commercial tablets (each individual tablet containing 10 mg ESC and 2 mg ESZ),A quantity of powder 10 mg of ESC and 2 mg of ESZ was weighed accurately and transferred to a 50 ml volumetric flask and the volume was made up with the solvent. It was sonicated for 30 minutes and then filtered through 0.5 µm whatman paper.

Development of the method

Standard stock solutions of 100 µg/ml were prepared for both the drugs using a methanol as solvent. From these stock solutions appropriate dilutions of ESC (10µg/ml) and ESZ (6 µg/ml) were prepared and scanned over the range of 200-400 nm and the overlain spectra was observed for development of suitable method for analysis. The overlain spectra of ESC and ESZ were shown in Figure 1.

Method validation

(a) Linearity

Linearity of the proposed method was verified by analyzing five different concentrations in the range of 5-50 µg/ml for ESC and 3-18 µg/ml for ESC and ESZ, respectively. Each concentration was made in triplicate.

(b) Accuracy

The accuracy of the method was performed by conducting the recovery studies (50, 100 and 150%) of pure drugs from mixtures, by standard addition method. The actual and measured concentrations were then compared.

(c) Precision

The intra day precision of the developed method was evaluated by analyzing samples of three different concentrations of ESC (5, 10 and 15 µg/ml) and ESZ (3 6 and 9 µg/ml) in triplicates on the same day. The inter day precision was evaluated from the same concentration on three consecutive days, precision was evaluated from the same concentration by three different analysts.

(d) Stability

Stability was observed by scanning the drug solutions in selected solvent system in time scan mode of UV spectrophotometer for 12 hours.

Limits of detection (LOD) and quantification (LOQ)

The detection limit is determined by the analysis of

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samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected. The quantitation limit is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision.

The LOD of the both the drugs were found by trial and error method (visual). The lowest concentration of range was selected as LOQ.

Analysis in mixtures

To determine the content of ESC and ESZ in mixtures (mixtures containing 10 mg ESC and 2 mg ESZ). A quantity of powder equivalent to 10 mg of ESC and 2 mg of ESZ was weighed accurately and transferred to a 50 ml volumetric flask and the volume was made up with the solvent. It was sonicated for 30 minutes and then filtered through 0.5 µm whatman paper.

RESULTS AND DISCUSSION

Method development and validation

The overlain spectrum of the drugs suggested that a dual wavelength spectrophotometric method was the most suitable method for simultaneous determination of ESC and ESZ. In Dual wavelength method the diluted solutions were scanned over the wavelength range of 200 - 400 nm. From the overlain spectra (Figure 1), wavelengths 304.0 λmax of ESZ and 238.0 nm the λmax of ESC were selected for quantitation by proposed method. For studying Beer's law, two series of different concentrations in range of 5-50 µg/mL for ESC and 3-18 µg/mL for ESZ were prepared from stock solutions. The calibration curves were constructed at 304.0 and 238.0 nm for ESZ and ESC, respectively. The absorptivities (A1%, 1 cm) of both the drugs at both the selected wavelengths were determined. The quantitative determination of ESZ is carried out by using A (1%, 1cm) value at a 304.0 nm where ESC, interfering substance does not have any absorption and quantitation of ESC is carried out by subtracting absorption due to ESZ, interfering drug in the overlapping region of spectrum, on the basis of its absorption ratio at two wavelengths.

Linearity

The calibration curves of ESC and ESZ were linear in the range of 5-50 µg/ml and 3-18 µg/m,l respectively. The regression equations of calibration curves were Y_{ESC} = 0.0431x + 0.015, r2 =0.9995 and Y_{ESZ} =0.0516x - 0.0043, r2 =0.9998 for ESC and ESZ, respectively.

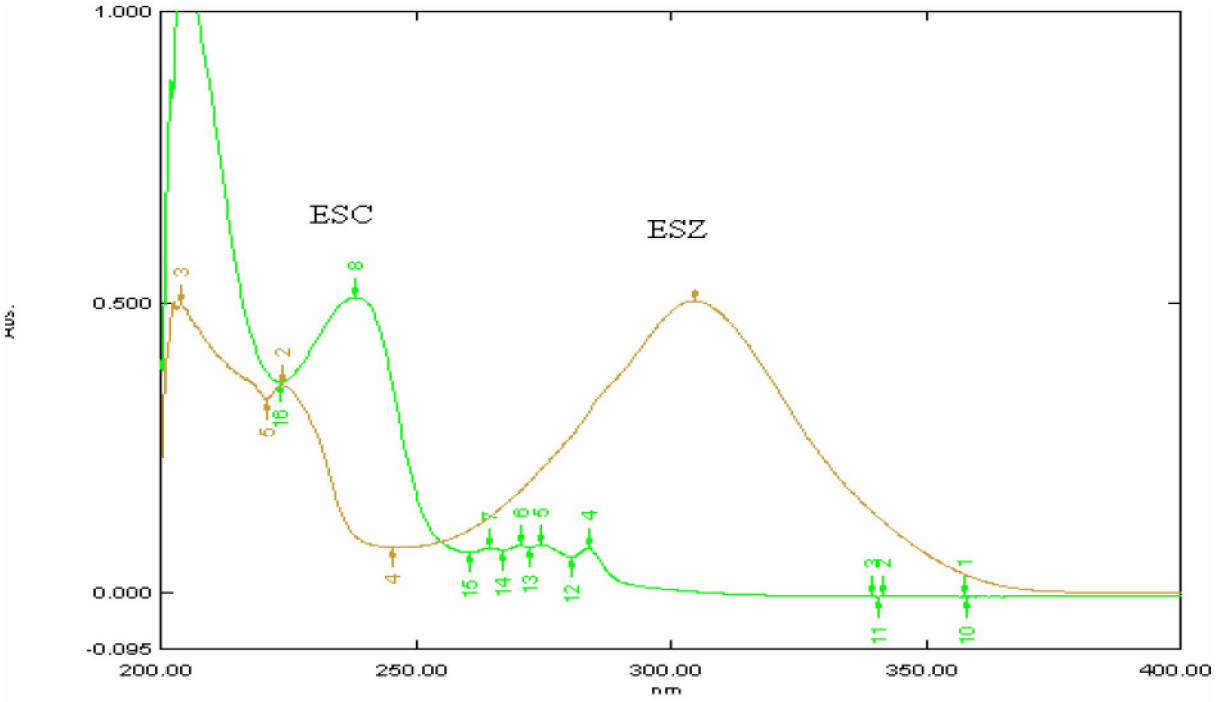

Figure 1 : Overlain absorption spectra of ESC and ESZ.

Accuracy

The percentage recovery was calculated of pure drugs from mixtures by standard addition of pure drugs at three known concentrations (80, 100 and 120%) and excellent recoveries were obtained at each level. The respective % recovery and %RSDs for ESC at three levels (80, 100 and 120 %) were found 99.66 ± 0.14, 98.37 0.37 and 101.1 ± 0.12, respectively. The respective % recovery and %RSDs for ESZ at three levels (80, 100 and 120) were found 99.66 ± 0.19, 100.62 ± 1.13 and 99.70 ± 0.45, respectively. The results of accuracy studies are shown in TABLE 1.

Precision

The intraday precision showed a relative standard deviation (R.S.D. %) of 0.10-1.16% for ESC and 1.14-2.13 % for ESZ. The inter day precision showed a R.S.D. % were 0.49-1.36% and 0.24-0.64% for ESC and ESZ, respectively. Intra day, inter day precision of method is illustrated in TABLE 2.

Analysis in formulations

The proposed UV method was applied for the determination of ESC and ESZ in their mixtures and the results are shown in TABLE 3. The high percentage recoveries (99.45-100.10) and low %CV (0.12-1.13) values confirm the suitability of the proposed method for the routine determination of these components in combined formulation. The data are shown in TABLE 3.

TABLE 1 : Application of the standard addition technique to the analysis of ESC and ESZ in their combined mixture by the proposed method.

Proposed methods	Amount of drug taken (µg/ml)		Amount of drug added (µg/ml)		Amount of drug found (µg/ml)		% Recovery (n^a=3) ± SD^b	
	ESC	ESZ	ESC	ESZ	ESC	ESZ	ESC	ESZ
Dual wavelength method	10	6	5	3	14.95	8.98	99.66 ±0.14	99.66±0.19
	10	6	10	6	1+.95	12.05	99.37 ±0.37	100.62±1.13
	10	6	15	9	25.11	14.97	101.1 ±0.12	99.7±0.45

[a]n is number of determinations, [b]SD is a Standard deviation

TABLE 2 : Summary of validation parameters for the proposed methods.

Proposed Methods	Drug	Parameters			
		LOD[a] µg/ml	LOQ[b] µg/ml	Interday (*n* = 3) (RSD[c], %)	Intraday (n^d = 3) (RSD[c], %)
Dual Wavelength method	ESC	2.5	5.0	0.49-1.36	0.10-1.16
	ESZ	1.5	3.0	0.24-0.64	1.14-2.13

[a]LOD is Limit of detection, [b] LOQ is Limit of quantification, [c] RSD is Relative standard deviation, [d] n is number of determinations.

TABLE 3 : Assay results for tablets using the proposed method.

Proposed methods	Mix.	Amount of drug added (mg)		Amount of drug found (mg)		% Amount found (n^a=3) ± SD^b	
		ESC	ESZ	ESC	ESZ	ESC	ESZ
Dual wavelength method	1	10	2	10.19	1.92	100.80±1.54	99.08±0.14
	2	10	2	10.18	1.95	100.76±0.12	99.41±0.85

[a]n is number of determinations [b]SD is a Standard deviation.

CONCLUSION

The proposed dual wavelength method gives accurate and precise results for determination of ESC and ESZ in mixtures and is easily applied for routine analysis. The most striking feature of the dual wavelength method is its simplicity and rapidity. Method validation has been demonstrated by variety of tests for linearity, accuracy, precision and stability. The developed method has several advantages, as it is simple, accurate, precise and economical. The proposed method was successfully applied to determination of these drugs in commercial tablets.

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