IJPSR (2022), Volume 13, Issue 12 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 07 April 2022; received in revised form, 26 May 2022; accepted, 16 June 2022; published 01 December 2022

DEVELOPMENT AND VALIDATION OF UV SPECTROMETRIC METHOD OF ECONAZOLE NITRATE CO-CRYSTAL LOADED IN GEL FORMULATION

Priyanka S. Gala [*1], Mohammad Wais [1] and Ojaswi Ghadge [2]

Department of Pharmaceutics [1], Department of Pharmaceutical Analysis [2], HK College of Pharmacy, Oshiwara, Jogeshwari [W] Mumbai - 400102, Maharashtra, India.

Keywords:

UV Spectrometry, Econazole Nitrate, validation, Accuracy, Precision, Anti-fungal

Correspondence to Author:
Priyanka Shailesh Gala

Research student,
Department of Pharmaceutics, HK College of Pharmacy, Oshiwara, Jogeshwari [W] Mumbai - 400102, Maharashtra, India.

E-mail: galapiyu1998@gmail.com

ABSTRACT: A simple, fast, inexpensive, and reliable UV spectrometric method was developed and validated to quantify Econazole Nitrate incorporated in topical gel in the form of co-crystal. This article presents the development and validation of different methods for quantification of Econazole nitrate co-crystal loaded in the topical gel as per ICH [Q2(R1)] guidelines. In method-A, the standard and sample solutions were prepared in Methanol; in Method-B the solution was prepared in a mixture of Methanol: phosphate buffer pH7.4. Based on analysis of UV spectrum, different wavelengths were selected for analysis of drug by two methods, 220 nm was selected for method A whereas 265 nm was selected for method B. The linearity of drug was obtained in the concentration range 2-10 µg/mL (R2= 0.9988) in method-A and concentration range 20-100 µg/mL (R2= 0.9984) in Method-B. The accuracy of both method was checked by calculating % recovery at 80percent, 100percent and 120percent. The % recovery by both methods was obtained to be in the range of 80-90percent. The less values of %RSD indicate the accuracy of both methods. The method's precision was designed as an intraday, inter-day repeatability. The %RSD value is less than 2 indicates that both the methods are precise. The robustness of both methods was examined with the help of ± wavelength and two ±pH values of the buffer used. The described analytical methodology is simple, precise, accurate and robust and can be applied for the quantification of ECZN in pharmaceutical dosage forms.

INTRODUCTION: Econazole Nitrate (ECZN) C18H15Cl3N2O is a BCS class II active pharmaceutical ingredient widely used to treat topical fungal infections such as athlete's foot, pityriasis (tineaversicolor), cutaneous candidiasis. The co-crystals of Econazole Nitrate were formulated to increase the aqueous solubility of BCS class II drug [1]. Many researchers have reported analytical methods for the quantification of ECZN in medicinal dosage forms using the Titrimetric method, HPLC method, and Infrared spectroscopic method. Moreover, these methods make use of difficult and time-consuming procedures, instruments, or costlier solvent systems.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.13(12).5057-67

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.13(12).5057-67

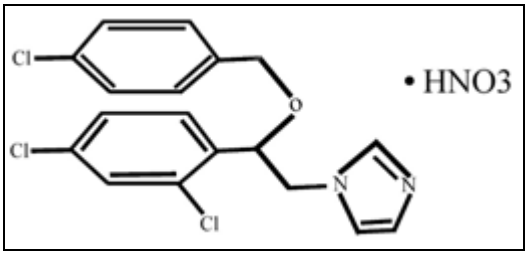

FIG. 1: STRUCTURE OF ECONAZOLE NITRATE [2]

To the best of our knowledge, the literature survey reveals very few simple UV spectrophotometric

Method for estimating Econazole Nitrate in pharmaceutical dosage forms. To detect the absorption of monochromatic radiation by solutions of the chemical compound UV spectrophotometric method has to be developed.

MATERIALS AND METHODS:

Chemicals and Reagents: A pure drug sample of Econazole Nitrate (Batch no. ECN/001/2122, Expiry date: JUL 2026) procured from Mahrshee Laboratories Pvt. Ltd., Panoli, Ankleshwar, Gujarat. as a generous gift sample, Methanol procured from Vishal chemicals, salts of phosphate buffer pH 7.4procured from Vishal chem and formulated in a laboratory.

The Solvent used for Method (A): Methanol.

The Solvent used for Method (B): phosphate buffer pH 7.4 (PBS pH 7.4): Methanol.

Instrumentation: UV-Visible double beam spectrophotometer with matched quartz cells (1cm) was used for all the spectral measurements.

Model: (Shimadzu UV-1800)- Software UV Probe 2.43.

Preparation of Stock Solution and Further Dilutions [3]:

1.1 Preparation of Stock Solution for Method (A): A standard stock solution of ECZN (1000 µg/mL) was formulated by solubilizing 10mg ECZN using Methanol in a volumetric flask. Pipette out 0.1mL from the stock solution and dilute it with Methanol to make up the volume up to 10 mL with Methanol in another volumetric flask to obtain the resulting 10 µg/mL solution. Finally, the dilutions were done from the 10 µg/mL solution to obtain 1 µg/mL to 10 µg/mL. Methanol was taken as blank, and absorbance of different dilutions was taken at 220 nm.

Preparation of Stock Solution for Method (B): A standard stock solution of ECZN (1000 µg/mL) was prepared by solubilizing 10mg ECZN using a mixture of PBS pH 7.4: Methanol in a volumetric flask. Pipette 1mL of the prepared stock solution and dilute it with diluent(B) to make the volume up to 10 mL with a mixture of Methanol: phosphate buffer pH 7.4 in another volumetric flask to obtain the resulting solution of 100 µg/mL. Finally, the dilutions were done from the 100 µg/mL solution to obtain 10 µg/mL to 90 µg/mL. The mixture of Methanol with PBS pH 7.4 was taken as blank, and the absorbance of different dilutions was taken at 265 nm.

Wavelength Selection of Maximum Absorption for Analysis [4]: The change in λmax of the drug ECZN was observed with a change in the solvent system.

Therefore, analyzing drugs at different wavelengths in different solvents was necessary. Wavelength selection for method A and method B proceeded as follows:

For Method A: A stock solution of ECZN (1000 µg/mL) was prepared using Methanol, and pipette out 1 mL of stock solution was then diluted to 10 mL with the Methanol to obtain 10 µg/mL ECZN solution.

Prepared solutions were scanned between wavelength range of 200 nm to 400 nm, using the Methanol as the diluents for the blank. The wavelength of maximum absorbance of ECZN was found to be 220nm.

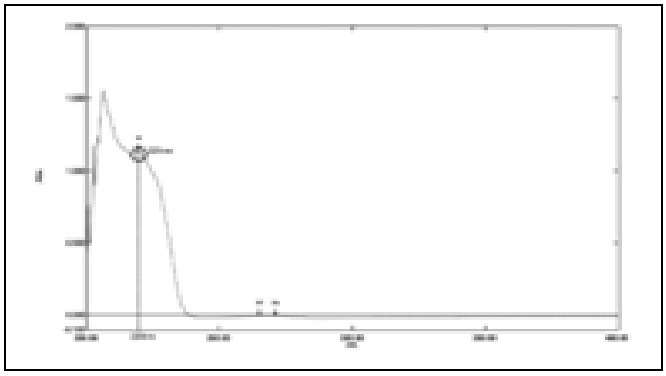

FIG. 2: UV SPECTRUM OF ECZN (METHOD A)

For Method B: Quantification of ECZN in Phosphate buffer, the stock solution of ECZN (1000 µg/mL) was prepared using PBS pH 7.4: Methanol and 1 mL of stock solution were then diluted to 10 mL with diluent(B) to obtain 100 µg/mL ECZN solution.

Prepared solutions were scanned between 200 nm to 400 nm, using a mixture of PBS pH 7.4: Methanol as a blank. The maximum absorbance of ECZ was found at λ_{max}= 265nm.

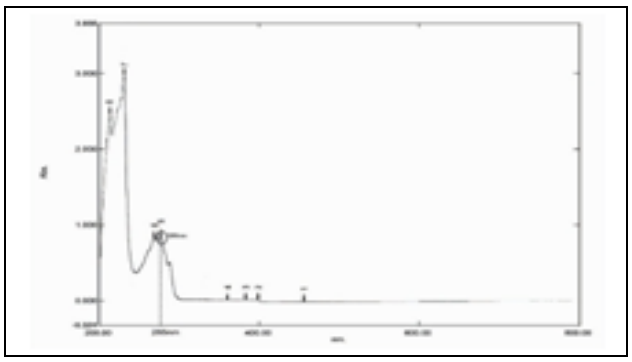

FIG. 3: UV SPECTRUM OF ECZN (METHOD B)

Analytical Method Validation: The UV method for quantifying ECZN was validated based on ICH Q2(R1) guideline. Different variables like linearity, accuracy, precision, robustness, the limit of detection (LOD), and limit of quantitation (LOQ) were evaluated [5-8].

Specificity: An examination of specificity should be done during the validation to identify tests, impurities, and the assay. The method used to illustrate specificity will depend upon the intended objective of the analytical procedure.

To illustrate that an analytical procedure is acceptable for a specific analyte is not always possible. In this case, a mixture of two or additional analytical methods is recommended to establish an acceptable result.

Method A: Formulated Co-crystals solubilized in Methanol and prepared a stock solution of 100µg/mL. To make 10µg/mL solution pipette out 1ml from the stock solution and diluted using Methanol up to 10ml in a volumetric flask. Peak was observed at 220nm.

Method B: Formulated Co-crystals solubilized in the mixture of PBS pH 7.4: Methanol and prepared a stock solution of 1000µg/mL. To make 100µg/mL solution, pipette out 1ml from the stock solution and dilute using a mixture of Methanol: PBS pH 7.4 up to 10ml in a volumetric flask. Peak was observed at 265nm.

Accuracy: Accuracy confirmed for methods A and B by percentage recovery of best-known concentrations of ECZN standard to the pre-analyzed sample solutions. The method was replicated three times for each concentration. The accuracy was accepted if %recovery exceeded 80% [7].

MethodA: Accuracy of the ECZN was determined by 80 percent, 100 percent, and 120 percent using standard and test samples. From the prepared stock solution, pipette out 1 mL and dilute up to 10 mL with Methanol to obtain 10 µg/mL solution. Prepared spiked flask using a standard of 80 percent, 100 percent, and 120 percent. Absorbance was recorded in triplicate of each concentration [3].

Method B: Accuracy of the ECZN was determined by 80 percent, 100 percent, and 120 percent using standard and test samples. From the stock solution, pipette 1 mL and dilute up to 10 mL with diluent to obtain 100 µg/mL. Prepared spiked flask using a standard of 80 percent, 100 percent, and 120 percent. Absorbance was recorded in triplicate of each concentration. Standard deviation was determined using the following formula,

$$\text{Standard Deviation } (\sigma) = \sqrt{\sum n\ i=1(xi-\bar{x})2/n}$$

Where, x = Data set values $\overline{x}$ = Mean of the data

Precision: Precision-measured closeness among the information obtained in entire experiments under the same conditions. For analytical methods, it is the closeness among the results obtained under the desired conditions. The precision aims to reveal the random error that could occur in a method.

Precision includes,

a) **Intraday Precision:** Intra-day precision was determined by analyzing (n = 9) each of the three concentrations in triplicate on the same day.

b) **Inter-day Precision:** Inter-day precision was evaluated by analyzing the same three concentrations in triplicate on three different days.

c) **Repeatability:** Repeatability was evaluated by analyzing three concentrations on the same day [9-11].

Method A:

a) **Intraday Precision:** Intraday precision is expressed within a day but in some time intervals. Intraday precision was achieved by preparing an ECZN solution of 4 µg/mL, 6

µg/mL, 8 µg/mL concentration and analyzing it three different times a day.

b) **Inter-day precision:** inter-day precision is expressed on different days using three concentrations in triplicate. Inter-day precision was achieved by preparing an ECZN dilution of 4 µg/mL, 6 µg/mL, 8 µg/mL concentration for three distinct days to determine Inter-day precision. All the concentration was performed in triplicate [11].

c) **Repeatability:** Repeatability is similar to precision which is performed under similar working conditions over a small interval of time. Repeatability was determined by preparing nine replicates of 4 µg/mL, 6 µg/mL & 8 µg/mL concentration of the sample, and the absorbance was measured.

Method B:

a) **Intraday Precision:** Intraday precision is expressed within a day but in some time intervals. Intraday precision was carried out by preparing a drug solution of 40, 60, 80 µg/mL concentration and analyzing it three different times a day.

b) **Inter-day Precision:** Inter-day precision is expressed on different days using three concentrations in triplicate. Inter-day precision was carried out by preparing a drug solution of 40 µg/mL, 60 µg/mL, 80 µg/mL concentration was followed for three different days to determine Inter-day precision. All the concentrations were performed in triplicate.

c) **Repeatability:** Repeatability is similar to precision which is performed under the same operating conditions over a short interval of time. Repeatability was determined by preparing nine replicates of 40 µg/mL, 60 µg/mL & 80 µg/mL concentration of the sample, and the absorbance was measured.

Limit of Detection (LOD) & Limit of Quantification (LOQ): Limit of detection (LOD) and limit of quantification (LOQ) are two important parameters in method validation. LOD is the lowest amount of API within the sample that may be detected, and LOQ is the lowest quantity of API within the sample that may be quantitatively determined by suitable precision and accuracy [12].

As per the ICH Guidelines:

LOD: The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated as an exact value.

LOQ: The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices and is used particularly for determining impurities and/or degradation products.

Method A and Method B: The limit of detection and the limit of quantitation were calculated using the standard deviation (σ) of response and slope (S) of the curve method. The standard deviation (σ) and slope (S) were obtained from the calibration curve and linearity data. The results of data calculation from the analysis method can be seen. Formula:

$$\text{Limit of detection} = 3.3\ \sigma / S$$

$$\text{Limit of quantification} = 10\ \sigma / S$$

Where, σ is the response S's standard deviation, the slope of curve [12].

Linearity and Range: The linearity of an analytical method is its potential to produce test results that are directly proportional to the concentration of analyte within the sample. To perform linearity, make three sets of each concentration and take absorbance in triplicate of each concentration. Therefore the obtained data were used for the linearity calibration plot mean versus absorbance.

Method A: The calibration graphs were obtained for ECZN in the concentration range 2 - 10 µg/mL at their maximum absorbance wavelength. The linearity values were shown in **Table 1**. The procedure was performed in triplicate, so the mean absorbance of each concentration is noted.

Method B: The calibration graphs were obtained for ECZN in the concentration range 20 - 100

µg/mL at their maximum absorbance wavelength. The linearity values were shown in **Table 10**. The procedure was performed in triplicate, so the mean absorbance of each concentration is noted.

Robustness: The robustness of an analytical method is a measurement of its capacity to remain not affected by a small variation in method parameters and indicates its reliability during the normal stage. Variations of pH and wavelength do the evaluation of robustness.

Method A: The determination of robustness done by variations of pH and wavelength [13]. For ECZN the absorbance values of 10 µg/mL at 219 nm, 220 nm and 221 nm were obtained.

Method B: The determination of robustness done by variations of pH and wavelength [14]. For ECZN the absorbance values of 100 µg/mL at 264 nm, 266 nm were obtained and pH7.3 phosphate buffer, pH 7.6 PBS.

Determination of ECZN in Bulk:

Method A: Accurately weighed 10 mg of ECZN was dissolved into a 100 ml volumetric flask containing 100 ml methanol to make a 100 µg/ml solution. Pipette out 0.4 ml of this solution and was transferred to a 10 ml volumetric flask and make up the volume with Methanol. The prepared dilution was scanned on a UV spectrophotometer in the range of 200 to 400 nm.

Method B: Accurately weighed 10 mg of ECZN was dissolved into a 100 ml volumetric flask containing a mixture of Methanol: PBS pH 7.4 (3: 7) to make 100 µg/ml solution.

Pipette out 4 ml of this solution was transferred to a 10 ml volumetric flask, and the volume was adjusted up to 10ml using a mixture of PBS pH 7.4: Methanol. The resulting solution was scanned on a spectrophotometer in the UV range of 200–400 nm.

Application of the Proposed Method for Pharmaceutical Formulation:

Method A: For analysis of formulated topical gel, 1g of topical gel (loaded with co-crystal of ECZN) was dissolved in a 100 ml volumetric flask, and the volume was made up to 100ml mark in a volumetric flask with Methanol to give 100 µg/ml concentration.

From the stock solution pipette out 0.4 ml and diluted with Methanol in a 10ml volumetric flask to give 4 µg/ml concentration. It was scanned on a spectrophotometer in the UV range of 200–400 nm. The spectrum was recorded at 220 nm.

Method B: For analysis of formulated topical gel, 1g of topical gel (loaded with co-crystal of ECZN) was taken in a 100 ml volumetric flask and the volume was made up to 100ml mark in volumetric flask with the mixture of Methanol: PBS pH 7.4 (3: 7) to give 100 µg/ml concentration.

From this 4 ml was then diluted to a 10 ml volumetric flask and the volume was made up with the same diluent in 10ml volumetric flask to give 40 µg/ml concentration. It was scanned on a spectrophotometer in the UV range of 200–400 nm. The spectrum was recorded at 265 nm.

RESULTS AND DISCUSSION: Results and the discussion of results have been presented separately in this section for Method A and Method B [15].

Results and the Discussion for Method A:

Specificity: Fig. 4 shows the UV spectrum of Co-crystal of ECZN formulated in the lab using excipients like Oxalic acid and Dimethyl sulfoxide.

In the proposed method, No interferences were observed due to the presence of excipients like Oxalic acid, Dimethyl sulfoxide; peak was observed at 220nm, which denoted the method was specific.

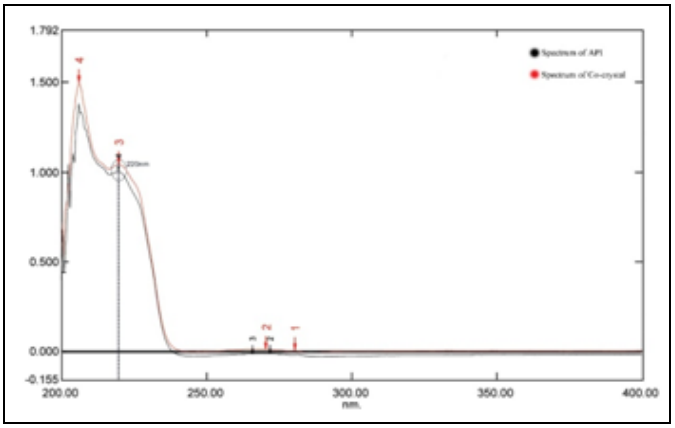

FIG. 4: SPECTRUM OF CO-CRYSTAL (METHOD A)

As observed in **Fig. 4**, there is no change in the UV spectrum and wavelength of the drug. Thus it can be concluded that the method was specific.

Linearity: The absorbance of ECZN was measured at 220nm, and calibration curves were plotted a concentration range of 2 - 10 µg/mL versus absorbance.

TABLE 1: LINEARITY OF ECZN (METHOD A)

S. no.	Concentration µg/mL	Mean of absorbance	Standard deviation	% RSD
1.	2	0.1243	0.0023	1.86%
2.	4	0.3603	0.0051	1.42%
3.	6	0.5723	0.0030	0.53%
4.	8	0.8266	0.0076	0.92%
5	10	1.0233	0.0061	0.60%

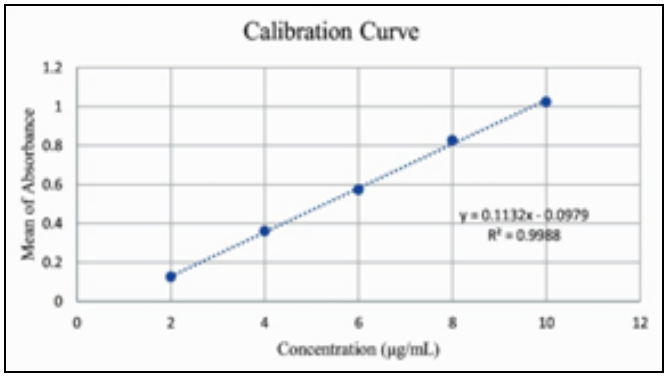

FIG. 5: PLOT OF LINEARITY (METHOD A)

The calibration curve for ECZN was linear over the concentration range of 20- 10 µg/mL, as shown in **Table 1**. The calculation equation of the regression line was found to be 0.1132x-0.0979 and the correlation coefficient was found to be 0.9988.

Accuracy: The method's accuracy was determined by calculating the mean percent recovery. It was determined as 80%, 100%, and 120% levels, and data are presented in **Table 2.**

TABLE 2: DETERMINATION ACCURACY OF ECZN (METHOD A)

S. no.	Level of recovery	Mean of Absorbance of spiked flask	Standard deviation	% Relative standard deviation	% recovery
1.	80%	0.64	0.002	0.31%	80%
2.	100%	0.747	0.0026	0.35%	86.5%
3.	120%	0.8416	0.0041	0.49%	89.37%

In this Method A, accuracy is noticeable from the data as results are near to 100 percent, and the value of SD and % Relative standard deviation were found to be < 2 %.

This result confirms that the current method is accurate.

Precision: In the proposed method, precision was studied as the repeatability of each concentration (%RSD<2) and inter-day and intraday precision (%RSD<2).

A. Intraday Precision: The data of intraday precision studies of ECZN is presented in **Table 3.**

TABLE 3: INTRADAY PRECISION STUDIES OF ECZN (METHOD A)

Concentration µg/mL	Mean	Standard deviation	% Relative standard deviation
4	0.35	0.003732	1.05%
4	0.3573		
4	0.355		
6	0.5593	0.0026	0.48%
6	0.554		
6	0.557		
8	0.88	0.001058	0.12%
8	0.878		
8	0.8796		

The %RSD of Method A for intra-day analysis of ECZN was found in the range of 0.12- 1.05%.

The observed result confirms that the intraday precision method is accurate.

B. Interday: The data of inter-day precision studies of ECZN is presented in **Table 4.**

TABLE 4: INTER-DAY PRECISION STUDIES OF ECZN (METHOD A)

Concentration µg/mL	Mean	Standard deviation	% Relative standard deviation
4	0.3506		
4	0.359	0.0042	1.20%
4	0.356		
6	0.5534		
6	0.5553	0.0035	0.64%
6	0.5603		
8	0.8783		
8	0.878	0.0041	0.47%
8	0.871		

In Method A the % RSD for Inter-day analysis of ECZN was found to be 0.47- 1.2%. The observed result confirms that the inter-day precision method is accurate.

C. Repeatability: The data of repeatability studies of ECZN is presented in **Table 5.**

TABLE 5: REPEATABILITY STUDIES OF ECZ NITRATE (METHOD A)

Concentration µg/mL	Mean	Standard deviation	% Relative standard deviation
4	0.3473	0.0031	0.91%
4	0.353		
4	0.3526		
6	0.5483	0.0063	1.15%
6	0.5553		
6	0.561		
8	0.8736	0.0029	0.33%
8	0.8783		
8	0.873		

In Method A the % RSD for repeatability of ECZN was found to be 0.33- 1.15%. The observed result confirms that the method is reproducible and accurate.

Limit of Detection and Limit of Quantification: The minimum level at which the investigated compounds can be reliably detected (limit of detection, LOD) and quantified (limit of quantitation, LOQ) was determined experimentally. The calculated data of LOD & LOQ is presented in **Table 6.**

TABLE 6: EVALUATION DATA OF LOD AND LOQ FOR ECZN (METHOD A)

S. no.	Drug	Limit of detection (µg/mL)	Limit of quantitation (µg/mL)
1.	ECZN	0.968167	2.933841

Limit of detection and Limit of quantification of the proposed UV spectrometric method were found to be 0.96 and 2.93 µg/ml, respectively as shown in **Table 6**.

Lower LOQ value indicated that the proposed method would be suitable for analyzing the samples containing even small quantities of ECZN.

Robustness: The result showed that during all variance conditions, the assay value of the test preparation solution was not affected, and it followed that of the actual.

TABLE 7: EVALUATION DATA OF ROBUSTNESS STUDY FOR ECZN (10 µG/ML) USING ± 220 NM (METHOD A)

S. no.	Wavelength 219 nm	Wavelength 221 nm
1.	0.998	1.051
2.	0.994	1.038
3.	0.987	1.064
4.	0.980	1.053
5.	0.996	1.050
6.	0.982	1.056
Mean	0.9895	1.052
SD	0.0075	0.0085
%RSD	0.77%	0.81%

The proposed Method A was found to be Robust as there is no interference from changes in wavelength.

Determination of ECZN: The concentrations of the drug were calculated from linear regression equations.

The % amount was determined between 95.75-97%. The calculated data are presented in **Table 8.**

TABLE 8: ANALYSIS OF ECZN (METHOD A)

Amount of drug weighed (mg)	Amount found (mg)	Amount found (%)
10mg	9.57	95.75
	9.7	97.00
	9.67	96.75
Mean± SD	9.64± 0.0680	96.5± 0.6614
%RSD	0.71%	0.69%

Application of the Proposed Method for Pharmaceutical Formulation (Topical Gel) for Method A: The spectrum was recorded at 220 nm. The concentration of the drug was calculated from the linear regression equation. The % amount found was between 98.25- 99.5%. The calculated data are presented in **Table 9.**

TABLE 9: ANALYSIS OF FORMULATION (METHOD A)

Amount of gel weighed (g)	Amount of drug in gel (mg)	Amount found (mg)	Amount found (%)
1	10	9.82	98.25
		9.92	99.25
		9.95	99.5
Mean± SD		9.89± 0.0680	99± 0.66
%RSD		0.69%	0.67%

Results and Discussion of the Experimental Work Have Been Presented for Method B:

Specificity: Fig. 6 shows the UV spectrum of Co-crystal of ECZN formulated in the lab using excipients like Oxalic acid and Dimethyl sulfoxide.

In the proposed method, interferences were not observed due to excipients like Oxalic acid, Dimethyl sulfoxide.

In the proposed method peak of excipients was observed at different wavelengths, and the peak of ECZN was observed at 265 nm, which denoted the method was specific.

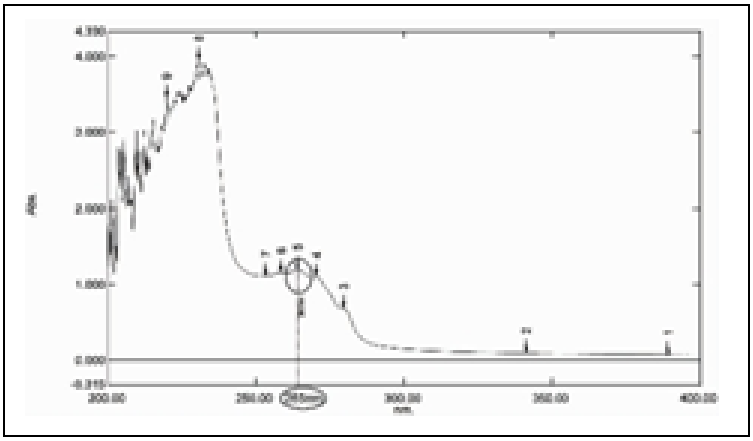

FIG. 6: SPECTRUM OF CO-CRYSTAL (METHOD B)

Linearity: The absorbance of ECZN was measured at 265nm, and calibration curves were plotted a concentration range of 20 - 100 µg/mL versus absorbance. The calibration curve for ECZN was linear over the concentration range of 20- 100 µg/mL, as shown in table 10. The calculation mentioned above equation of the regression line was found to be 0.1132x-0.0979, and the correlation coefficient was found to be 0.9984.

TABLE 10: LINEARITY OF ECONAZOLE (METHOD B)

S. no.	Concentration µg/mL	Mean absorbance	Standard deviation	%RSD
1.	20	0.245	0.0040	1065%
2.	40	0.411	0.001	0.24%
3.	60	0.59	0.002	0.34%
4.	80	0.784	0.001	0.13%
5.	100	0.9856	0.0030	0.31%

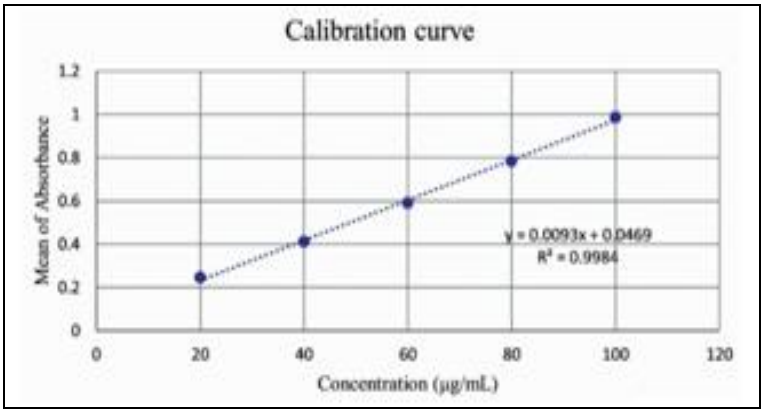

FIG. 7: PLOT OF LINEARITY (METHOD B)

Accuracy: The accuracy of the method was determined by calculating the mean percent recovery. It was determined as 80%, 100%, and 120% level and data are presented in **Table 11.**

TABLE 11: DETERMINATION ACCURACY OF ECZN (METHOD B)

S. no.	Level of recover y	Mean of Absorbance of spiked flask	Standard deviation	% Relative standard deviation	% recovery
1.	80%	0.6833	0.0041	0.61%	89%
2.	100%	0.747	0.0026	0.35%	88%
3.	120%	0.799	0.0017	0.22%	85%

In this Method B, accuracy is evident from the data as results are close to 100 % and the value of standard deviation and % RSD were found to be < 2 %. This result confirms that the current method is accurate.

Precision: In the proposed method precision was studied as repeatability (%RSD < 2) and inter-day and intraday variations (%RSD < 2); shows the high precision of the method.

A. Intraday Precision: The data of intraday precision studies of ECZN is presented in **Table 12.**

TABLE 12: INTRADAY PRECISION STUDIES OF ECZN (METHOD B)

Concentration µg/mL	Mean	Standard deviation	%Relative standard deviation
40	0.436	0.0020	0.48%
40	0.435		
40	0.439		
60	0.608	0.010	1.70%
60	0.603		
60	0.623		
80	0.756	0.0098	1.32%
80	0.74		
80	0.738		

The %RSD of Method B for intra-day analysis of ECZN was found in the range of 1.70- 0.48%. This result confirms that the intraday precision method is accurate.

B. Interday Precision: The data of inter-day precision studies of ECZN is presented in **Table 13.**

TABLE 13: INTERDAY PRECISION STUDIES OF ECZ NITRATE (METHOD B)

Concentration µg/mL	Mean	Standard deviation	%Relative standard deviation
40	0.429	0.0076	1.79%
40	0.419		
40	0.434		
60	0.607	0.0020	0.34%
60	0.610		
60	0.611		
80	0.749	0.0060	0.81%
80	0.76		
80	0.750		

In Method B the %RSD for Inter-day analysis of ECZN was found to be 0.34- 1.79%. This result confirms that the inter-day precision method is accurate.

C. Repeatability: The data on repeatability is presented in **Table 14.**

TABLE 14: REPEATABILITY STUDIES OF ECZ NITRATE (METHOD B)

Concentration µg/mL	Mean	Standard deviation	%Relative standard deviation
40	0.44	0.0070	1.62%
40	0.431		
40	0.445		
60	0.607	0.0035	0.58%
60	0.610		
60	0.603		
80	0.762	0.0040	0.53%
80	0.754		
80	0.759		

In Method A the %RSD for repeatability of ECZN was found to be 0.53- 1.62%. This result confirms that the method is repeatable and accurate.

Limit of Detection and Limit of Quantification: The proposed method LOD and LOQ were discussed in Method A. The calculated data of LOD & LOQ is presented in **Table 15.**

TABLE 15: EVALUATION DATA OF LOD AND LOQ FOR ECZN (METHOD B)

S. no.	Drug	Limit of detection (µg/mL)	Limit of quantitation (µg/mL)
1.	ECZN	11.23293	34.03917

LOD and LOQ of the proposed UV spectrometric method were found to be 11.23 and 34.03 µg/ml, respectively as shown in **Table 15**. The lower LOQ value indicated that the proposed method would be suitable for analyzing the samples containing even small quantities of ECZN.

Robustness: The result showed that during all variance conditions, the assay value of the test preparation solution was not affected, and it followed that of the actual. The proposed Method B was robust as no interference was observed from changes in wavelength and ph of phosphate buffer.

TABLE 16: EVALUATION DATA OF ROBUSTNESS STUDY FOR ECZN (100 µG/ML) USING ± PBS PH 7.4 (METHOD B) AND EVALUATION DATA OF ROBUSTNESS STUDY FOR ECZN (100 µG/ML) USING ± 265NM (METHOD B)

S. no.	Phosphate buffer pH 7.3	Phosphate buffer pH 7.5	Wavelength 264 nm	Wavelength 266 nm
1.	0.926	0.993	0.970	1.140
2.	0.922	0.979	0.970	1.143
3.	0.917	0.988	0.974	1.143
4.	0.921	0.991	0.975	1.141
5.	0.924	0.979	0.978	1.142
6.	0.929	0.996	0.977	1.143
Mean	0.9231	0.9876	0.974	1.142
SD	0.0041	0.0072	0.0034	0.0012
%RSD	0.45%	0.73%	0.35%	0.11%

Determination of ECZN by Method B: The concentrations of the drug were calculated from linear regression equations.

The % amount determined between 97.6- 98.12%. The calculated data are presented in **Table 17.**

TABLE 17: ANALYSIS OF ECZN (METHOD B)

Amount of drug weighed (mg)	Amount found (mg)	Amount found (%)
10	9.78	97.87
Mean± SD	9.76	97.6
	9.81	98.12
	9.78± 0.0251	97.86± 0.26
%RSD	0.26%	0.27%

Application of the proposed method for pharmaceutical formulation (Topical gel) for Method B: The spectrum was recorded at 265 nm. The concentration of the drug was calculated from the linear regression equation.

TABLE 18: ANALYSIS OF ECZN (METHOD B)

Amount of gel weighed (g)	Amount of drug weighed (mg)	Amount found (µg)	Amount found (%)
1	10	9.8	98.02
		9.97	99.72
		9.91	99.10
Mean± SD		9.89± 0.0862	98.94± 0.86
%RSD		0.87%	0.87%

The % amount found was between 98.02- 99.72%. The calculated data are presented in **Table 18.**

CONCLUSION: UV spectrometry's validation of Econazole Nitrate was developed using various ICH guidelines. This method resulted to be non-complicated, precise, accurate and robust can be applied for determining ECZN in pharmaceutical dosage forms. In the validation parameters of both the methods, the %RSD was less than 2%. The accuracy of current methods was confirmed by carried out accuracy parameters that showed the results within the range.

The precision of current methods was confirmed by performing intraday, inter-day precision, and repeatability. According to test results, the UV spectrometry method is one of the best methods for quantifying ECZN in pharmaceutical dosage forms.

The UV spectrometric method has been developed to quantify ECZN in topical gel formulation. The validation procedure of both methods proves that this is an accurate method for quantifying ECZN in the formulation.

ACKNOWLEDGMENTS: The authors would like to thanks
the department of pharmaceutics, HK College of Pharmacy, Oshiwara, Jogeshwari [W], Mumbai, India, for the accomplishment of the review and valuable support.

CONFLICT OF INTEREST: The authors declare no conflict of interest, financial or otherwise.

REFERENCES:

1. Heel RC, Morton P, Brogden RN, Speight TM and Avery GS: Dextranomer: A Review of its General Properties and Therapeutic Efficacy. Drugs 1979; 18(2): 89–102.
2. Sharma R and Pathak K: Polymeric nanosponges as an alternative carrier for improved retention of econazole nitrate onto the skin through topical hydrogel formulation. Pharm Dev Technol 2011; 16(4): 367–76.
3. Kumari B and Khansili A: Analytical method development and validation of UV-visible spectrophotometric method for the estimation of vildagliptin in gastric medium. Drug Res (Stuttg) 2020; 70(9): 417–23.
4. Maleque M, Hasan MR, Hossen F and Safi S: Development and validation of a simple UV spectrophotometric method for the determination of levofloxacin both in bulk and marketed dosage formulations. J Pharm Anal 2012; 2(6): 454–7.
5. Research Article UV Spectrophotometric Method Development and Validation for Estimation of Tigecycline in Spiked Human Plasma 2020; 63(15): 98–101.
6. ultaneous separation of insulin and six therapeutic analogues on a mixed mode column: HPLC-UV method development and application. J Chromatogr B Anal Technol Biomed Life Sci 2021; 1171; 2020.
7. Paul S, Barai L, Husen F, Sarker S, Pal TK and Bai P: Analytical Method Development and Validation for Estimation of Ranitidine in Solid Dosage Form by UV-Spectrophotometric Method. Orient J Chem 2020; 36(6): 1161–7.
8. Prasad AR and Thireesha B: UV-spectrophotometric method development and validation for the determination of lornoxicam in microsponges. Int J Appl Pharm 2018; 10(1): 74–8.
9. Patil SH and Janjale MV: Novel and validated titrimetric method for determination of selected angiotensin-II-receptor antagonists in pharmaceutical preparations and its comparison with UV spectrophotometric determination. J Pharm Anal [Internet] 2012; 2(6): 470–7.
10. Annapurna MM, Goutam SVS, Anusha S and Srinivas L: Development and validation of the stability-indicating LC-UV method for the determination of Cefditoren pivoxil. J Pharm Anal [Internet] 2012; 2(6): 466–9.
11. Kishore MS and Rambabu C: Development and validation of spectrophotometric method for simultaneous determination of isopropamide and trifluoperazine in tablet dosage form. Asian J Pharm 2017; 11(3): 224–9.
12. Pawar SM, Khatal LD, Gabhe SY and Dhaneshwar SR: Establishment of inherent stability of pramipexole and development of validated stability indicating LC-UV and LC-MS method. J Pharm Anal 2013; 3(2): 109–17.
13. Bakshi P, Korey A, Fowler W and Banga AK: Development and validation of an HPLC-UV method for analysis of methylphenidate hydrochloride and loxapine succinate in an activated carbon disposal system. J Pharm Anal [Internet] 2018; 8(6): 349–56.
14. Ruckmani K, Shaikh SZ, Khalil P, Muneera MS and Thusleem OA: Determination of sodium hyaluronate in pharmaceutical formulations by HPLC-UV. J Pharm Anal 2013; 3(5): 324–9.

How to cite this article:
Gala PS, Wais M and Ghadge O: Development and validation of UV spectrometric method of econazole nitrate co-crystal loaded in gel formulation. Int J Pharm Sci & Res 2022; 13(12): 5057-67. doi: 10.13040/IJPSR.0975-8232.13(12).5057-67.