Analytical Method Development And Validation Of Econazole Nitrate by using RP-HPLC

Ms. Pranali Pinjari, Mr. Krunal Kanase, Dr. Vijaya Barge, , Dr. Amit Kasabe,Ms.Komal Kendre, Almisba Shaikh

Research Scholar, PDEA's Shankarrao Ursal College of Pharmaceutical Sciences and Research Centre, Kharadi, Pune

Assitant Professor, PDEA's Shankarrao Ursal College of Pharmaceutical Sciences and Research Centre, Kharadi, Pune

Vice Principal & Professor, PDEA's Shankarrao Ursal College of Pharmaceutical Sciences and Research Centre, Kharadi, PAssitant Professor , PDEA's Shankarrao Ursal College of Pharmaceutical Sciences and Research Centre, Kharadi, Pune

Research Scholar, PDEA's Shankarrao Ursal College of Pharmaceutical Sciences and Research Centre, Kharadi, Pune
Research Scholar, PDEA's Shankarrao Ursal College of Pharmaceutical Sciences and Research Centre, Kharadi, Pune

Abstract

A simple and accurate method was developed for the determination and validation of the Econazole Nitrate. HPLC system used was JASCO system equipped with model PU 4180 RHPLC pump, Rheodyne sample injection port (20 µl), JASCO UV-4075 UV-VIS detector and ChromNAV CFR chromatography software (version 2.0). Separation was carried out on HiQSil C18 (250 mm × 4.6 mm, 5 µm) column using Acetonitrile:Methanol (85:15v/v) as mobile phase at flow rate of 1.2 mL/min. Samples were injected using Rheodyne injector with 20 µL loop, Detection was carried out at 225nm.

The HPLC linear regression analysis results for calibration plots demonstrated a good relationship with (R^2 = 0.9994). The method has been validated for its accuracy, Recovery, Robustness and documented. The LOD and LOQ were found to be 1.12 & 2.59 µg/m/ respectively.

Keywords- Econazole nitrate, Method Development, Validation,

Introduction

Econazole Nitrate is an imidazole derivative and broad-spectrum antimycotic agent with fungistatic properties. Econazole nitrate inhibits biosynthesis of ergosterol, thereby damaging the fungal cell wall membrane and altering its permeability which leads to a loss of essential intracellular components. Additionally, Econazole Nitrate inhibits the biosynthesis of

triglycerides and phospholipids as well as oxidative and peroxidative enzyme activity, which may aid in cell necrosis and death. It is also active against some gram-positive bacteria. The treatment of different dermatomycoses uses this antifungal agent.

Econazole nitrate is an antifungal drug containing imidazole ring which interacts with 14 demethylase a cytochrome P-40 enzyme which converts to lanosterol to ergosterol. Econazole inhibits the ergosterol synthesis which is the essential component of fungal cell membrane, as a result of increased cellular permeability, fungal cells die because cellular components leak out of the cells. Econazole Nitrate is incompletely absorbed after being administered orally due to it's low solubility. Additionally, it can be used topically to treat skin infections like tinea and cutaneous candidiasis.

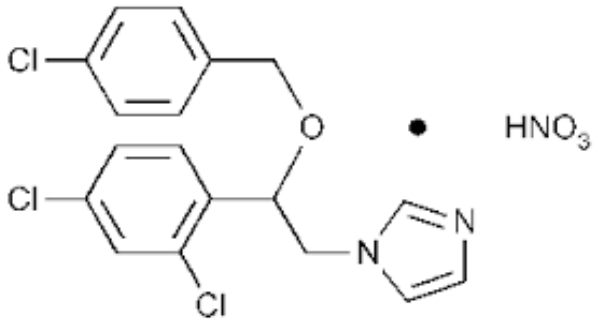

Figure. Structure of Econazole Nitrate

Analytical method development

Determination of Lambda maximum

Preparation of stock solution of Econazole nitrate

Econazole nitrate (100 mg) in a 100mL volumetric flask and 100 mL of methanol to it and it was vortexed (Eltek) for 2 minutes. This was the main stock accounting for concentrations of 1000 μg/mL. A diluted solution was used to scan in UV-Spectrophotometer in the range of 200-400nm, taking methanol as blank.

The lambda maximum for Econazole Nitrate was found to be 225nm.

Instrumentation and Chromatographic Conditions

HPLC system used was JASCO system equipped with model PU 4180 RHPLC pump, Rheodyne sample injection port (20 µl), JASCO UV-4075 UV-VIS detector and ChromNAV CFR chromatography software (version 2.0). Separation was carried out on HiQSil C18 (250 mm × 4.6 mm, 5 µm) column using Acetonitrile: Methanol (85:15v/v) as mobile phase at flow rate of 1.2 mL/min. Samples were injected using Rheodyne injector with 20 µL loop, Detection was carried out at 225nm. All weighing were done on Shimadzu balance (Model AY-120)

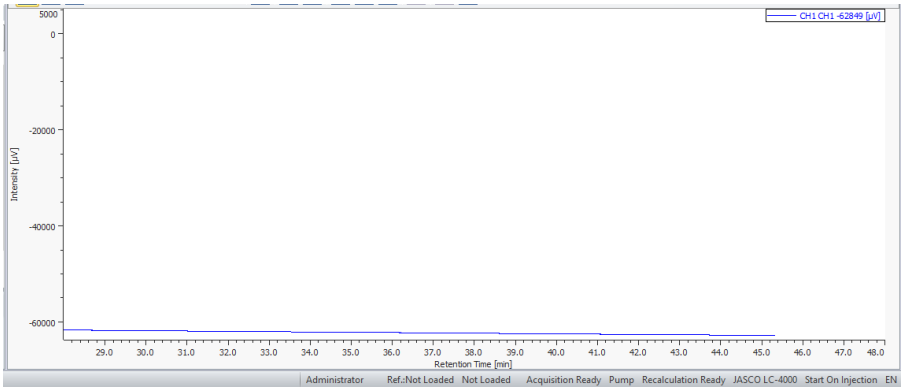

Figure 1: HPLC chromatogram of blank.

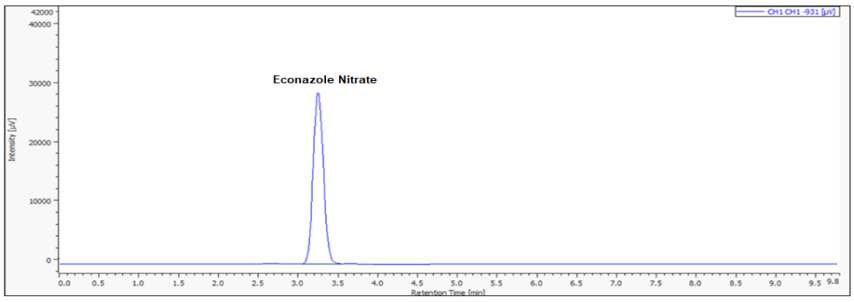

Figure 2: HPLC chromatogram of standard Econazole nitrate.

The retention time was found to be 3.32 with distinct peak.

MATERIALS AND METHODS

Material

Econazole nitrate standard is procured from Solanki Suppliers (Pune, India). Chemicals utilized for method development are of HPLC grade includes Methanol, water were purchased from Merck (India) Ltd.

Preparation of mobile phase

The preparation of mobile phase was done by mixing methanol with ACN in the ratio of 85:15 v/v. Filtered the solution through 0.45µ filter.

Diluent preparation

Mobile phase used as diluents.

Preparation of standard stock solution

100mg of Econazole nitrate standard was transferred into 100ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 1 ml of the above solution into a 10mlvolumetric flask and make up to volume with mobile phase and performed the subsequent dilutions.

Preparation of test solution

100mg equivalent of Econazole nitrate API standard was transferred into 100ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 1 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase and performed the subsequent dilutions.

Selection of analytical wavelength

It is the characteristic of a compound which helps to provide the electronic structure of the compound or analyte. The structural analysis of Econazole nitrate was carried out under UV ranging from 200-400nm using the standard solution.

Method Validation

Linearity:

The linearity of the developed method was studied over the concentration ranges between 10-30µg/ml. The aliquots of 5, 10, 15, 20, 25 and 30µg/ml were prepared by diluting standard stock solution of 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 ml with mobile phase. The obtained concentrations were injected into the chromatographic system. Calibration curve of

Econazole nitrate was constructed by plotting peak area versus used concentration of Econazole nitrate. To assure the concentration range studied is linear the regression equation and correlation coefficient were evaluated.

Accuracy

Accuracy was carried out by % recovery studies at three different concentration levels. To the pre-analysed sample solution of Econazole nitrate, a known amount of standard drug powder of Econazole nitrate was added to 80, 100, 120% level.

Precision method

By studying the changes in the inter-day and intra-day determined the precision of the method. In the intra-day studies, six repeated injections of standard solution was made and % RSD were calculated. In the inter-day variation studies, six repeated injections of standard solution were made for six consecutive days and %RSD were calculated.

Limit of Detection and Limit of Quantitation

Based on the standard deviation of response of the calibration curve the LOD and LOQ of the drug was determined separately.

Robustness

Robustness of the method was tested by small but deliberate variations of flow rate, mobile phase composition and wavelength.

RESULTS AND DISCUSSION

Selection of wavelength maxima

The solution of Econazole nitrate was scanned between ranges 200- 400nm. UV spectra of the drug show maximum absorbance at 225nm.

Method development

The proposed chromatographic method was found to be suitable for effective separation of Econazole nitrate with good resolution, peak shape given in the figure. The mobile phase composed of Acetonitrile: Methanol in ratio of 85:15 % v/v, at a flow rate of 1.2 ml/min was selected as it gave well resolved peaks of standard Econazole nitrate. The optimum wavelength 225nm selected for detection and quantitation.

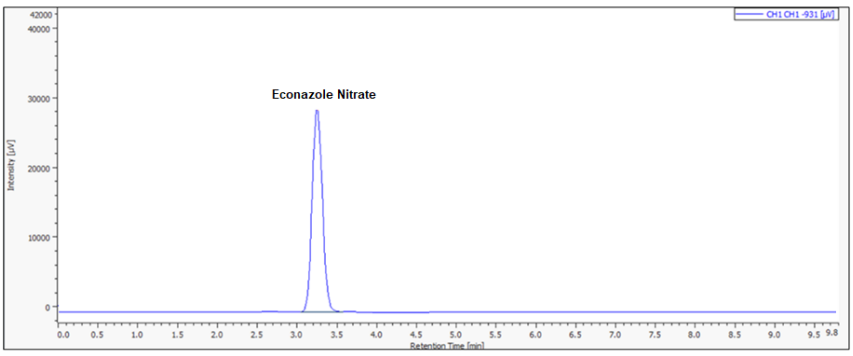

Figure 3: HPLC Chromatogram with resolved peak of Econazole nitrate

Method validation

Linearity

The calibration curves were found be linear for the concentration range of 5-30ppm. The standard working curve equation for drug was found to be y = 2768.4x + 670.53 with correlation coefficient value R^2 = 0.9994. The results of linearity are given in Table and Figure.

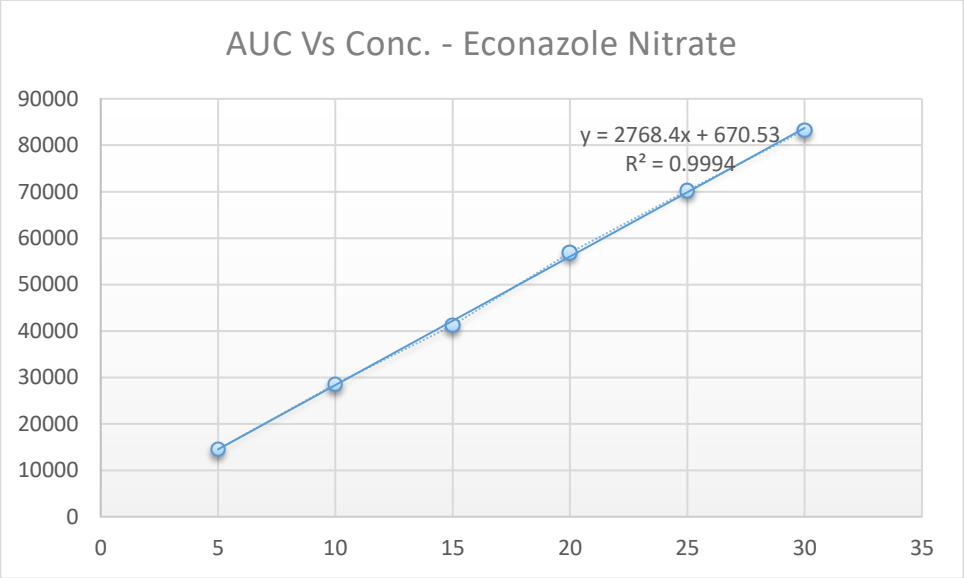

Figure 4: Linearity curve of standard Econazole nitrate

Table 1: Linearity data of Econazole nitrate

Concentration µg/mL	Area
5	14512
10	28567
15	41215
20	56897
25	70254
30	83256

Recovery studies

The mean % recovery at 80, 100, 120 % of the test concentration along with its statistical validation for drug Econazole nitrate given in Table. The % recovery at 80, 100, and 120 % was found to be 101.25, 99.8, and 101.25. It was confirmed that the developed method was accurate as the percent recovery was in the range of 100%.

Table 2: Recovery data of Econazole nitrate

Level (%)	Drug Conc (mg)	Amt recovered (mg)	% Recovery
80%	8	8.1	101.25
100%	10	9.98	99.8
120%	12	12.15	101.25

Precision

The repeatability of sample application and measurement of peak area were expressed in terms of % RSD and was found to be less than 2.0%. The % RSD of intra-day precision was found to be 0.009, 0.006 and 0.004 % RSD of interday precision was found to be 0.011, 0.009 and 0.004. The results of precision studies are shown in Table.

Table 3: Precision study (intra- day) of Econazole nitrate

Conc µg/mL	Area	AVG	%RSD
10	28745	28624.6667	0.48911778
	28658		
	28471		
15	42156	42370.3333	0.50863294
	42587		
	42368		
20	56874	56624.3333	0.94181701
	56987		
	56012		

Conc, Concentration; AVG, average; RSD, Relative standard deviation

Table 4: Precision study (inter-day) of Econazole nitrate

Conc µg/mL	**Area**	**AVG**	**%RSD**
10	28456	28608	0.50714043
	28623		
	28745		
15	41545	41711.6667	1.87815881
	41025		
	42565		
20	56897	56486	0.63400044
	56241		
	56320		

Conc, Concentration; AVG, average; RSD, Relative standard deviation

Limit of Detection (LOD) and Limit of Quantification (LOQ)

This data showed that the sensitivity of method to determine the drug Econazole nitrate. The Minimum concentration level at which the analyte can be reliable detected (LOD) &quantified (LOQ) were found to be 1.12 & 2.59 µg/m/ respectively.

Robustness

Robustness of method was measured by multiple injections of a homogenous sample containing Econazole nitrate by changing flow rate 1.0 mL/min and 1.4 mL/min, mobile phase composition ACN: Methanol ratio 84:16 and 86:14, wavelength i.e., 224nm and 226nm. The method was found to be robust in the range of deliberate changes made.

Table 5: Robustness study with change in flow rate of Econazole nitrate

Flow rate mL/min	Conc µg/mL	Area	AVG	%RSD
1.0	20	55421	55836.67	1.565403
1.0		55248		
1.0		56841		
1.4	20	55789	55411.67	0.590198
1.4		55210		
1.4		55236		

Conc, Concentration; AVG, average; RSD, Relative standard deviation

Table 6: Robustness study with change in concentration of mobile phase of Econazole nitrate

Mobile phase (Methanol: 01% OPA)	Conc µg/mL	Area	AVG	%RSD
84:16	20	56321	56542.67	0.545612
84:16		56895		
84:16		56412		
86:14	20	56321	56367	0.171372
86:14		56478		
86:14		56302		

Conc, Concentration; AVG, average; RSD, Relative standard deviation

Table 7: Robustness study with change in Wavelength of Econazole nitrate.

Wavelength nm	Conc µg/mL	Area	AVG	%RSD
224	20	56320	56334	0.251217

224		56482		
224		56200		
226	20	56874		
226		56231	56376	0.786936
226		56023		

CONCLUSION

A HPLC method developed has been validated as per ICH guidelines in terms of accuracy, precision, linearity, robustness, limit of detection and limit of quantitation, for the determination of Econazole Nitrate API. A good linear relationship was observed in concentration ranges of 5 and 30µg/ml. The correlation coefficient was 0.9994. The inter day and intraday precision results were good enough to say that the method developed is precise and reproducible. Accuracy studies revealed that mean recovery after spiking experiments was 100.7%, an indicative of accurate method. Accordingly, it can be concluded that the developed method is accurate, precise, linear, and robust.

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