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"Development and Validation of a Robust UHPLC Method for Quantitative Analysis of Sertaconazole Nitrate in Pharmaceutical Formulations"

Miss. Jagruti R. Girase*, Mr. Devendra Surendra Mahale, Dr. Sunila A. Patil, Dr. S. P. Pawar
P.S.G.V.P. Mandal's College of Pharmacy Shahada (Maharashtra)

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ABSTRACT
Sertaconazole nitrate, a synthetic antifungal agent, is effective in treating superficial and systemic fungal infections, demonstrating broad-spectrum activity against dermatophytes, pathogenic yeasts, and Aspergillus species. This study details the development and validation of a UHPLC method for the quantification of Sertaconazole nitrate in pharmaceutical formulations. Utilizing an Agilent 1260 Infinity II HPLC system with a Kromasil C18 column, the optimized chromatographic conditions were determined as follows: isocratic mode with a mobile phase of acetonitrile and 0.1% OPA in water (60:40 V/V), a flow rate of 1.0 ml/min, detection at 260 nm, and a column oven temperature of 40 °C. The method validation adhered to ICH Q2(R1) guidelines, encompassing parameters such as system suitability, specificity, linearity, accuracy, precision, robustness, LOD, and LOQ. The method showed a linear response within the tested range with an LOD of 0.433 µg/ml and an LOQ of 1.313 µg/ml. Accuracy was confirmed with recovery rates between 98.70% and 101.13%, and precision tests yielded RSD values below 2%. Filtration studies indicated no significant interference from the filters used, and stability studies confirmed that the solution remained stable over 24 hours. Specificity tests showed no interference from the excipients. Robustness testing, which included variations in wavelength, flow rate, and column temperature, demonstrated the method's reliability under varying conditions. This validated UHPLC method is precise, accurate, specific, and robust, making it suitable for routine quality control of Sertaconazole nitrate in pharmaceutical formulations.

Keey Word: Sertaconazole nitrate, UHPLC method, pharmaceutical formulations, method validation, ICH Q2(R1) guidelines, Accuracy and precision

I. INTRODUCTION

Sertaconazole nitrate chemically, 1- [2 -[(7 -chloro-1-benzothiophen -3- yl) methoxy] -2 -(2, 4- dichlorophenyl) ethyl] imidazole (Fig.1), is a new synthetic antifungal drug used for the treatment of superficial and systemic fungal infections. It has been found to have wide spectrum activity against topical fungi (Trichophyton microsporum, Epidermophyton), pathogen yeasts (Candida albicans, C. tropicalis, C. spp., Malasseria furfur) and Aspergillus [1,2]

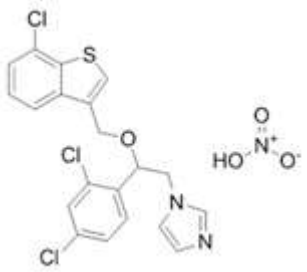

Fig 1. Chemical structure of Sertaconazole nitrate

II. MATERIAL AND METHOD

Material
Sertaconazole nitrate drug taken from Vidisha lab

Instrumentation and software
An Agilent 1260 Infinity II HPLC system with DEAX02386 pump and autosampler with UV–visible detector served as the chromatographic system (DEACX16446). For data collection and processing, the chromatograms were registered using Openlab EZ Chrome Workstation on a Windows-based computer system. Alcaftadine concentrations were determined using a Kromasil C18 column (250 mm X 4.6 mm i.d. 5µm) column.

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Methods

Selection of analytical wavelength

Methanol as a blank and Sertaconazole standard solution (20 PPM) was scanned from 400 nm to 200 nm. Absorption maxima was determined for drug. Sertaconazole showed maximum absorbance at 260 nm shown in results. {figure 2}.

Method Development by UHPLC

Preparation of standard stock solution for Chromatographic development:

In order to prepare stock solution, weighed accurately 20 mg Sertaconazole and transferred into 20 ml volumetric flask, added 15 ml of Methanol and sonicated to dissolve the standard completely and diluted up to the mark with Methanol (1000 PPM). Further diluted 0.4 mL to 20 mL with methanol. (20 PPM)

Selection of analytical wavelength for UHPLC method development: Analytical wavelength for the examination was selected from the wavelength of maximum absorption from the spectrophotometric analysis and it was 260 nm**.** {figure 2}.

Method Validation by UHPLC

a] Development and optimization of HPLC method: After all experimental trials and with reference to the acceptance criteria for various system suitability parameters, the conditions were optimized for the estimation of Sertaconazole bulk drug and its dosage form and result shown in {Figure 3 and Table 1}.

b] Preparation of System suitability test (Sertaconazole standard solution):

Weighed about 20 mg of Sertaconazoleand transferred in 20 mL volumetric flask, added 15 mL of Methanol, sonicated to dissolve it, made volume up to the mark with Methanol.Pipette out 1 ml from standard stock solution and transferred into 20 ml volumetric flask and made volume up to the mark with mobile phase. **(50 µ/mL working concentration),** chromatograms were recorded. {Figure 3}.

A]Analysis of marketed Test sample:

Marketed test sample Having Name Onabet cream 2% w/w are selected for analysis and for doing validation

Sample preparation of Marketed test sample:

Weighed the Test Sample (1000 mg) equivalent to 20 mg of Sertaconazole and transferred to clean and dried 100 mL of volumetric flask. Added 70-75 mL of Metahnol, sonicated for 10 minutes with intermittent shaking. After 10 minutes allow to cool the solution to room temperature and made volume up to the mark with Methanol. Filtered the solution through suitable 0.45 µ syringe filter discarding 3-5 mL of initial filtrate.Further diluted 5 ml of filtered stock solution to 20 ml with mobile phase. (50 mcg of Sertaconazole), injected the resultant solution and chromatograms were recorded and results are recorded. {Table 2}.

Formula for % Assay calculation:

$$\% \, Assay = \frac{Sertaconazole \; Spl \; area}{Sertaconazole \; Std \; avg \; area} \; X \; \frac{Sertaconazole \; STD \; wt \; (mg)}{20} \; X \; \frac{1}{20} \; X \; \frac{100}{sample \; weight \; (mg)}$$

$$X \; \frac{20}{5} \; X \; \frac{Avg \; wt \; of \; sample \;\; (mg)}{Label \;\; claim \;\; of \;\; Sertaconazole} \; X \; 100$$

B] Method Validation Parameters

FILTRATION STUDY:

Filtration study of an analytical procedure checks the interference of extraneous components from filter, deposition on filter bed and compatibility of filter with sample.

This study was conducted with Sertaconazole Test sample.

Filtration study carried out with unfiltered and filtered test solution. During filtration activity 0.45 µm PVDF and 0.45 µm Nylon syringe filters used by discarding 5 mL of aliquot sample. {Table 3}

STABILITY OF ANALYTICAL SOLUTION

Stability study was conducted for standard and test sample solution. Stability study was performed at normal laboratory conditions.

The solution was stored at normal illuminated laboratory conditions and analyzed after 12 hours and 24 hours. {Table 4}

SPECIFICITY:

Specificity is the ability to access unequivocally the analyte in the presence of components which may be expected to be present.

Following solution shall be prepared and injected to prove the specificity nature of the

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method. (Checked peak purity for standard and test sample solution) {Table 5}

I. Blank (Mobile phase as Blank)
II. Placebo
III. Sertaconazole Standard solution
IV. Tablet test sample solution

Placebo solution preparation:

Weighed 980.0 mg of placebo material (Which is equivalent to 20 mg of Sertaconazole) and transferred to clean and dried 100 mL of volumetric flask. Added 70-75 mL of Methanol, sonicate for 10 minutes with intermittent shaking. After 10 minutes allow to cool the solution to room temperature and made volume up to the mark with Methanol. Filtered the solution through suitable 0.45 µ Nylon syringe filter discarding 3-5 mL of initial filtrate. Further dilute 5.0 ml of filtered stock solution to 20 ml with mobile phase, injected the resultant solution and chromatograms were recorded.

LINEARITY AND RANGE

Linearity Sertaconazole stock solution:

Weighed 25 mg of Sertaconazole and dissolved in 50 mL with Methanol. (500 PPM) {Figure 4}

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

Detection limit:

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

Quantitation limit:

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.

As per ICH Q2R1 guidelines LOD and LOQ was determined by using the approach Based on the Calibration Curve in which residual standard deviation of a regression line was calculated and determined the LOD and LOQ by using following formula:

$LOD = 3.3\ \sigma / S$

$LOQ = 10\ \sigma / S$

Where,

σ = residual standard deviation of a regression line

S = Slope of regression line

ACCURACY (% RECOVERY)

Accuracy will be conducted in the range from 50 % to 150 % of working concentration. Solution of each accuracy level was prepared in triplicate. Calculated % Recovery for each sample, Mean % recovery for each level and overall recovery and also calculated % RSD for each level and % RSD for overall recovery. {Table 6}

Procedure for preparation of Accuracy sample solution:

Take clean and dried 9 volumetric flask of 100 mL. Weighed aprrox 980.0 mg of placebo and transferred in each 100 mL volumetric flask. Weighed Sertaconazole API as per accuracy level and transferred in same 100 ml volumetric flask. Add 70-75 ml of Methanol sonicate it for 10 minutes with intermittent shaking. Allowed to cool the solution at room temperature and made the volume up to the mark with Methanol. Filter the solution through suitable 0.45 µ Nylon filter discarding 5 mL of filtrate. Further dilute 5 ml of filtrate to 20 ml with mobile phase.

PRECISION

Precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous test under the prescribed conditions. Precision is of two types, Repeatability and Intermediate precision. It is performed on tablet test sample. {Table 7}

Repeatability:

Preparation of sample solution (6 Samples prepared):

Weighed the Test sample (1000 mg) equivalent to 20 mg of Sertaconazole and transferred to clean and dried 100 mL of volumetric flask. Added 70-75 mL of methanol, sonicate for 10 minutes with intermittent shaking. After 10 minutes allow to cool the solution to room temperature and made volume up to the mark with Methanol. Filtered the solution through suitable 0.45 µ Nylon syringe filter discarding 3-5 mL of initial filtrate.Further dilute 5 ml of filtered stock solution to 20 ml with mobile phase. (50 mcg of Sertaconazole), injected the resultant solution and chromatograms were recorded.

Intermediate precision

It is performed by doing analysis on another day to check reproducibility of results. Samples prepared in same manner as that of Repeatability parameter (6 Samples prepared).

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ROBUSTNESS

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage{Table 8}.

III. RESULT AND DISCUSSION

Selection of analytical wavelength

Fig. No. 2 UV spectrum of Sertaconazole

Parameter	Description
Mode	Isocratic
Column Name	Water Cortex 18, 150 mm X 4.6 mm, 2.7 µm
Detector	UV Detector
Injection Volume	20 µl
Wavelength	260 nm
Column Oven temp	40ºC
Mobile Phase	Acetonitrile : 0.1% OPA in Water (60:40 %V/V)
Flow Rate	1.0 ml/min
Diluent	Mobile phase
Run time	5 Minutes

Table 1: Optimized Chromatographic Conditions

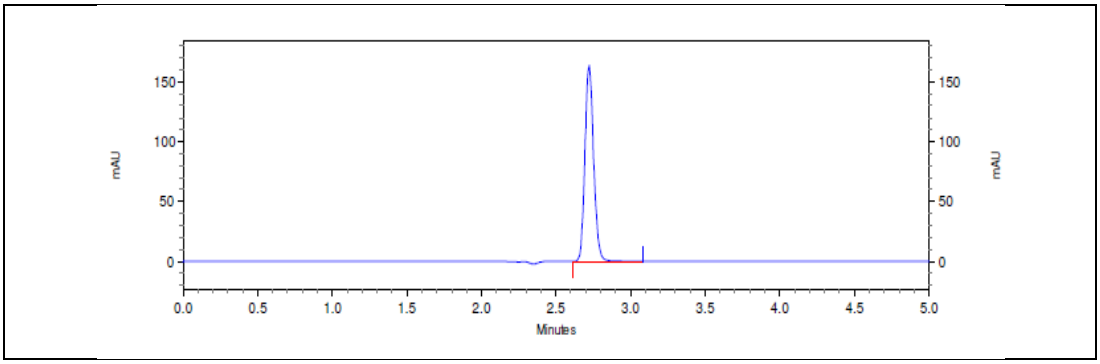

Figure 3: Typical chromatogram of Optimized Trial

System Suitability Acceptance Criteria:

Relative standard deviation of the area of analyte peaks in standard chromatograms should not be more than 2.0 %. Theoretical plates of analyte peak in standard chromatograms should not be less than 2000. Tailing Factor (Asymmetry) of analyte peaks in Standard Chromatograms should be less than 2.0 in figure 3

Analysis of Marketed Test samples (Assay)

a) Onabet cream 2% w/w:

Sample	Area	% Assay	Mean Assay
Sample 1	5390256	98.90	98.20
Sample 2	5310214	97.51	

Table No.2 Assay results of Onabet cream 2% w/w:

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Validation of UHPLC method

The optimized method for estimating Alcaftadine was validated for the following parameters using ICH Q2(R1) guidelines.[6-12]

FILTRATION STUDY:

Filtration study of an analytical procedure checks the interference of extraneous components from filter, deposition on filter bed and compatibility of filter with sample. Performed on test sample.in table 3

Sample description	Area	% Absolute difference
Unfiltered	5458963	NA
0.45 µ PVDF filter	5410280	0.89
0.45 µ Nylon filter	5424758	0.63

Table 3: Result of Filter study

SOLUTION STABILITY: Stability study was conducted for Standard as well as Test Sample. Stability study was performed at normal laboratory conditions. The solution was stored at normal illuminated laboratory conditions and analyzed at initial, after 12 hours and 24 hours.in table 4

Sample solution			Standard solution		
Time point	**Area**	**% Absolute difference**	**Time point**	**Area**	**% Absolute difference**
Initial	5458412	NA	**Initial**	5460325	NA
12 Hours	5407586	0.93	**12 Hours**	5428234	0.59
24 Hours	5396858	1.13	**24 Hours**	5412360	0.88

Table 4: Results of Solution stability:

SPECIFICITY: Specificity is the ability to access unequivocally the analyte in the presence of components which may be expected to be present.in table 5

Blank, standard solution prepared and injected to check peak purity.

Description	Observation
Blank	No interference at R.T. of Sertaconazole due to blank
Placebo	No interference at R.T. of Sertaconazole due to placebo
Standard solution	Peak purity was 0.988
Test Solution	Peak purity was 0.986

Table 5: Results of Specificity

Linearity and Range

Linearity of an analytical method is its ability to elicit test results that are proportional to the concentration of analyte in samples within a given range.in figure 4

$Y = M X + C$

$Y = 108641.1963 x + -12462.43789$

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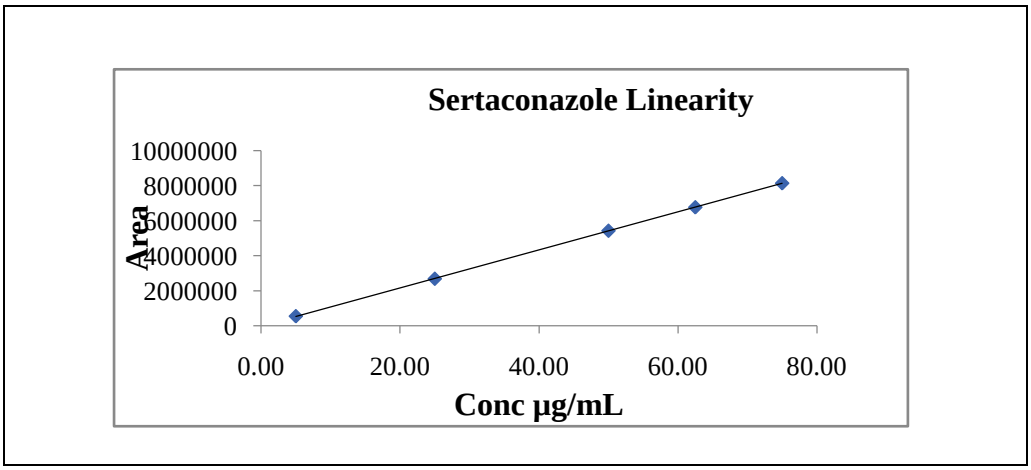

Figure 4: Calibration curve of Sertaconazole

Limit of Detection (LOD) and Limit of Quantitation (LOQ):
σ =14264.27181 (Residual standard deviation of a regression line)
s = 108641.1963 (Slope)
Detection limit (LOD):
LOD = 3.3 σ / S
LOD = 3.3 x 14264.27181 / 108641.1963
LOD = 0.433 µg/mL

Quantitation limit (LOQ):
LOQ = 10 σ / S
LOQ = 10 x 14264.27181 / 108641.1963
LOQ = 1.313 µg/mL

ACCURACY (RECOVERY):
The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The accuracy of an analytical method is determined by applying the method to analysed samples to which known amounts of analyte have been added.In table 6

Overall Recovery: 99.56 %
% RSD for Overall Recovery: 0.824

Level	Area	Recovered conc (µg/mL)	Added conc (µg/mL)	% Recovery	Mean Recovery	% RSD
50	2752560	25.29	25.50	99.18	99.46	0.8790
	2760259	25.36	25.25	100.44		
	2768460	25.43	25.75	98.76		
100	5531305	50.82	50.25	101.13	100.02	1.0810
	5440254	49.98	50.50	98.97		
	5467891	50.23	50.25	99.96		
150	8084785	74.27	75.25	98.70	99.21	0.4951
	8103250	74.44	75.00	99.25		
	8164710	75.01	75.25	99.68		

Table No.6 Result and statistical data of Accuracy of Sertaconazole

PRECISION
Precision was performed on test sample. HPLC method was precise. The result shown in table 7

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Sr. No	Parameters	Intraday Precision	Intraday Precision
1	Mean	98.67	98.668
2	STD	1.475100	1.30033
3	%RSD	1.495	1.318

Table 7: Result of Intra- day and Inter- Day Precision for Sertaconazoltest sample assay

ROBUSTNESS:

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage in table 8.

Following changes made under Robustness:

- Change in Wavelength
- Change in flow rate
- Change in column oven temperature

Change in Parameter	R.T.	Standard area	Asymmetry	Theoretical plates
Wavelength by +3 NM (263 NM)	2.71	3924045	1.11	8391
Wavelength by -3 NM (257 NM)	2.72	7339586	1.13	8518
Flow rate by +10% (1.1mL/min)	2.46	4926035	1.13	7689
Flow rate by -10% (0.9mL/min)	3.02	6043258	1.15	9563
Column oven temp by +2ºC (42 ºC)	2.73	5403695	1.12	8603
Column oven temp by -2ºC (38 ºC)	7.72	5426891	1.15	8514

Table 8: Result of Robustness study

IV. CONCLUSION:

The developed and validated UHPLC method for quantifying Sertaconazole nitrate in pharmaceutical formulations is precise, accurate, specific, and robust. Utilizing an Agilent 1260 Infinity II HPLC system with a Kromasil C18 column, the optimized conditions include an isocratic mobile phase of acetonitrile and 0.1% OPA in water (60:40 V/V), a flow rate of 1.0 ml/min, detection at 260 nm, and a column oven temperature of 40°C. The method validation, adhering to ICH Q2(R1) guidelines, demonstrated satisfactory results across various parameters, including system suitability, specificity, linearity, accuracy, precision, robustness, LOD, and LOQ. The method showed excellent linearity, with an LOD of 0.433 µg/ml and an LOQ of 1.313 µg/ml. Recovery rates ranged between 98.70% and 101.13%, and precision tests yielded RSD values below 2%. The method also proved reliable under different conditions and showed no significant interference from excipients or filtration processes. Therefore, this UHPLC method is suitable for routine quality control of Sertaconazole nitrate in pharmaceutical formulations.

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