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RP-HPLC Method Development and Validation for the Estimation of Sertaconazole Nitrate in Bulk and Tablet Dosage form

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Abstract : A reversed-phase high performance liquid chromatography (RP-HPLC) method was developed and validated for the estimation of sertaconazole nitrate (STZN) in bulk and tablet dosage forms. The separation was achieved on stainless steel Purospher® STAR Hibar® C_{18} analytical column (250 mm × 4.6 mm i.d., 5.0 µm) using 0.01 M monobasic sodium phosphate and acetonitrile in a ratio of20:80 % v/v as mobile phase and at a flow rate of 1.2 mL/min. Detection was carried out using a UV detector at 260 nm. The method was validated for accuracy, precision, linearity, LOD, LOQ and robustness. Validation studies demonstrated that this HPLC method is simple, specific, rapid, reliable and reproducible. The standard curve was linear over the concentration range of 100-600 µg/mL with R^2 close to one (0.997). The limit of detection (LOD) and limit of Quantitation (LOQ) obtained for STZN were 0.00192064 µg/mL and 0.00208267 µg/mL, respectively. The developed and validated method was successfully applied for the quantitative analysis of Onabet V1 tablets. This method can be used as more convenient and efficient option for the analysis of STZN to establish the quality of the drug substance during routine analysis with consistent and reproducible results.
Key Words : RP-HPLC method, Validation, Sertaconazole nitrate, Assay.

Introduction

Sertaconazole nitrate (nitrate salt of 7-chloro-3-[1- (2, 4-dichlorophenyl)-2-(1H-imidazol-1-yl) ethoxy-methyl] benzothiophene) ($C_{20}H_{16}O_4N_3Cl_3S$), molecular weight 500.8 Da, CAS 99592–32–2) is a topical azole derivative associated with a benzothiophene matrix. This represents an important difference compared with other azoles used in the treatment of mycoses [1].It is a potent imidazole derivative antifungal drug that maintains the antifungal activity against Dermatophytoses, Tinea *versicolor*, cutaneous *candidiasis*, Seborrhoeic *dermatitis* and vaginal *candidiasis* by inhibiting the ergosterol synthesis.It has a good safety profile, sustained cutaneous retention, and low systemic absorption, all of which make it ideal for topical applications [2].

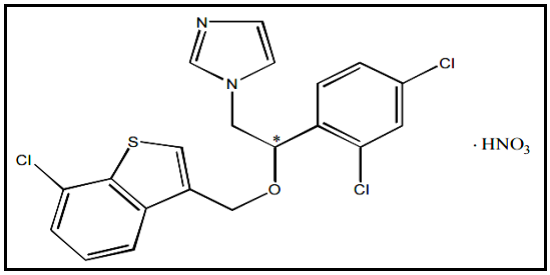

Figure 1: Structure of Sertaconazole nitrate

Literature survey reveals that UV [3],Electrophoresis [4] method has been reported for quantitation of STZN. However, these methods are bit expensive and need more sophisticated equipment and/or are time consuming.The present work describes the development of a simple, specific, rapid, reliable, precise, accurate and reproducible RP-HPLC method for the estimation of STZN in bulk and tablet dosage form. To access the reproducibility and wide applicability of the developed method, it was validated as per ICH guidelines which are mandatory also and successfully employed for the assay of STZN in their Tablet dosage form.

Experimental

Apparatus and Instruments

Chromatography was performed by using Shimadzu, LC 2010 CHTHPLC system equipped with reciprocating plunger pump, injector and UV-detector. Shimadzu (ATY 224) single pan electronic balance used for weighing purpose. Double distillation unit (EASY-STILL MARK-2000 DDQ-XL). In this study all calibrated volumetric glassware's (Borosil) were used.

Chemicals and Reagents

Sertaconazole nitrate pure drug was obtained as a gift sample from Cipla Pvt. Ltd., Mumbai. Tablets of 500 mg strength were purchased from the local pharmacy under commercial available brand name Onabet V1(Glenmark pharmaceutical), Mumbai. HPLC grade Acetonitrile were purchased from HiMedia Laboratories Pvt. Ltd., Mumbai.Monobasic sodium phosphate (0.01 M)was purchased from Loba Chemie Pvt. Ltd., Mumbai. Double distilled water (as solvent) were used in this study.

Chromatographic conditions

The separation was achieved on stainless steel Purospher® STAR Hibar® C_{18} analytical column (250 mm × 4.6 mm i.d., 5.0 µm).Mobile phase used was a mixture of 0.01 M monobasic sodium phosphate and acetonitrile in a ratio of 20:80 % v/v. The filtered mobile phase was pumped at a flow rate of 1.2 ml/min.; Column temperature was maintained at 35 ± 2°C. The eluent was detected by UV detector at 260 nm and data were acquired, store and analyzed with software class ODS-3V.

Preparation of 0.01 M monobasic sodium phosphate according to IP 2010[5]

Dissolve 1.38 g of monobasic sodium phosphatein sufficient water to produce 1000 ml.

Preparation of Standard Solution

The stock solution (1000 µg/ml) of STZN was prepared separately by dissolving accurately about 100 mg of drug in 100 ml acetonitrile HPLC grade in 100 ml volumetric flask. The solution was filtered through 0.4 µ nylon membrane filter and degassed before use.

Results and Discussion

Selection of Suitable Detection Wavelength

The working standard solution of 10 µg/mL was scanned between 400 nm to 200 nm in UV spectrophotometer against saline phosphate buffer pH 7.4as blank after baseline correction. Wavelength range selected around wavelength maxima 260 nm (Figure 2).

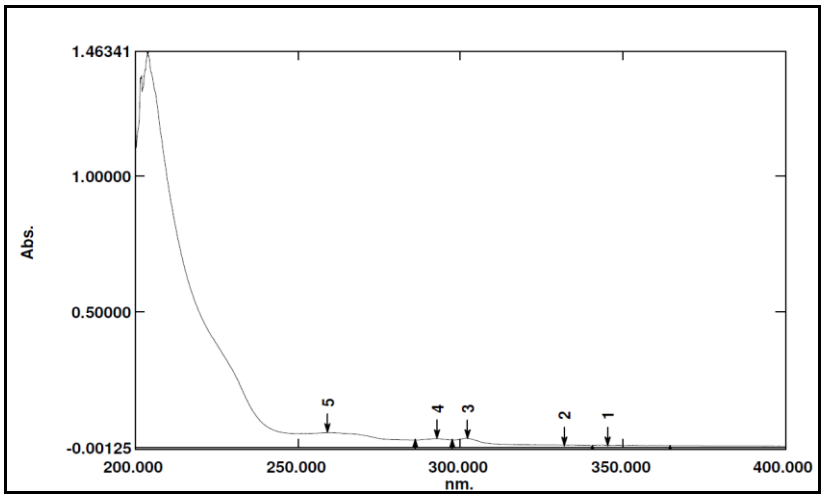

Figure 2: UV spectrum of STZN(400-200 nm)

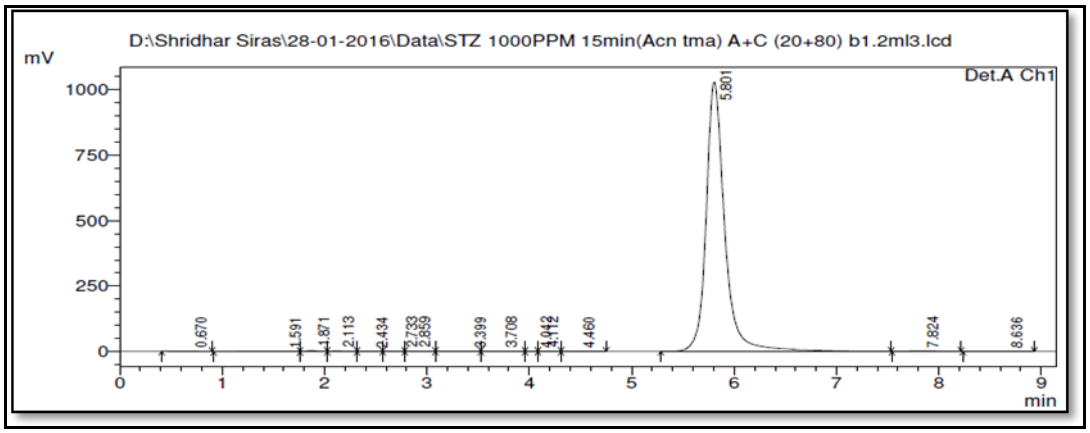

Figure 3:Typical chromatogram of STZN (1000 µg/ml) using RP- HPLC

Preparation of Calibration Curve

A standard curve was prepared by withdrawing appropriate aliquots from stock solutions into a series of 10 ml volumetric flasks. The volume was made up to the mark with mobile phase to obtain concentration range 100-600 µg/ml of STZN. Detection of STZN was performed with the UV detector set at 260 nm. Peak area was recorded and calibration curves were plotted with the peak area against the respective concentration of STZN.

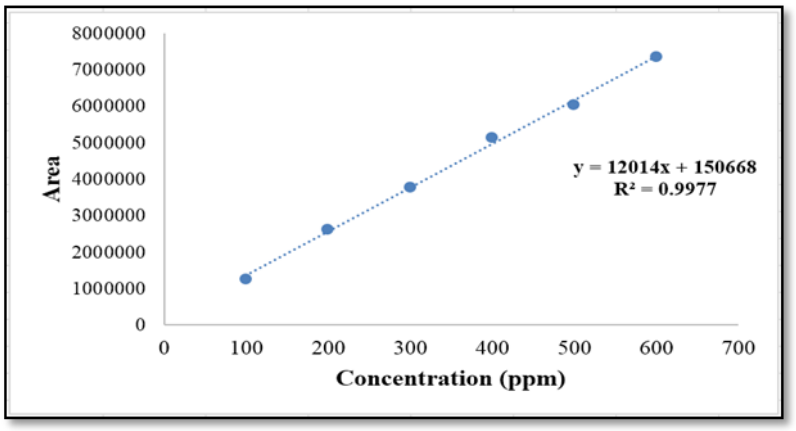

Figure 4: Standard calibration curve of STZN using RP- HPLC

Assay of Tablet Formulation

STZN containing twenty tablets of was weighed and average weight was calculated. Then tablets were crushed and powdered in a glass mortar. The tablet powder equivalent to 100 mg of STZN was accurately weighed, transferred to a 100 ml of volumetric flask containing HPLC grade acetonitrile. The solution was filtered through 0.4 µ nylon membrane filter. This solution was further diluted to obtain 400 µg/ml solutions with same solvent.The solution was injected into HPLC system. The results of the assay of tablet formulation and its statistical validation data are given in Table 1.

Table 1: Assay value of Sertaconazole nitrate tablet formulation.

Tablet formulation	Label amount	Amount taken (Equivalent Weight)	Amount found	Assay %
Onabet V1	500 mg	100 mg	99.62 mg	99.62

*average of three readings

Method Validation [6-12]

The developed chromatographic method was validated using ICH guidelines. Validationparameters performed include linearity, limit of detection and quantitation, precision, accuracy, robustness and repeatability as perICH guidelines.

1.System Suitability

Before performing the main analysis, the system suitability was evaluated. For this purpose, various parameters were calculated as per their standard procedure e.g. retention time (for STZN), theoretical plates number of the column (for column efficiency), tailing factor, relative standard deviation of peak area and retention time. The table 2 shows the result for these parameters. The column efficiency was much better for analysis i.e. ≥2000.The tailing factor was also within range. Moreover, the calculated relative standard deviation for the retention time and peak area (mean of 6 replicates) also within acceptance criteria. Depending on all these information, it reflects that the proposed method will be suitable for routine analysis.

Table 2:System suitability parameters

Sr.No	Parameters	Sertaconazole nitrate
1.	Retention time (min)	5.8
2.	Plate number	5667.01
3.	Tailing factor	1.253
4.	RSD of peak area (n=6)	0.1872
5.	RSD of retention time (n=6)	0.1254

Table 3: Calibration Curve (Linearity data) of Sertaconazole nitrate

Concentration (µg/ml)	Peak area
100	1257427
200	2612425
300	3762287
400	5128708
500	6028135
600	7344562

2. Linearity

The linearity of this method was determined at ranging from 100-600 µg/ml for STZN at 260 nm using acetonitrile as solvent.The method for estimation of STZNwas found to be linear in the range of concentrations 100-600 µg/ml withregression equation were found to be y = 12014x + 150668 and the correlation coefficient is 0.997. Linearity data shown in Table 3.

3. Precision

Precision studies were carried out to ascertain thereproducibility of the proposed method. This study dividedinto repeatability, intraday and interday.

a. Repeatability

The repeatability of method was analyzed by replicate analysis (n=6) by injecting the sample solution into the HPLC system and results werefound within acceptable limits (RSD < 2). Relative standard deviation (%RSD) was calculated (Table 4).

Table 4: Repeatability Values of Sertaconazole nitrate

Concentration (µg/mL)	Area	% RSD
400	5128708	0.14677621
400	5128707	
400	5128707	
400	5128708	
400	5128709	
400	5128708	
Avg.	5128707	
SD	0.75277265	

Table 5: Intraday Values of Sertaconazole nitrate

Concentration (µg/mL)	Area		
	1	**2**	**3**
400	5128708	5128708	5128708
400	5128707	5128712	5128707
400	5128709	5128707	5128709
400	5128707	5128709	5128710
400	5128710	5128710	5128708
400	5128712	5128708	5128711
Mean	5128753	5128754	5128753
SD	0.780651	0.82442	0.780649
%RSD	0.149307	0.286408	0.145234
Average %RSD	0.193649		

b. Intraday Precision

Intraday precision study was carried out by preparing six drug solution samples of same concentration (400 µg/ml) from stock solution and analysing it at three different times in a day. Standard deviation and relative standard deviation (%RSD) was calculated (Table 5).

c. Interday Precision

Interday precision study was carried out by preparing six drug solution samples of same concentration (400 µg/ml) from stock solution and analysing it at three different times in a day. The same procedure was followed for three different days to determine interday precision study. Standard deviation and relative standard deviation (%RSD) was calculated (Table 6).

Table 6: Interday Values of Sertaconazole nitrate

Concentration (µg/mL)	%RSD			Average% RSD
	Day 1	Day 2	Day 3	
400	0.14677221	0.13567521	0.14686211	0.14310317

Table 7: Accuracy Values of Sertaconazole nitrate

Test (µg/ml)	Accuracy level	Amount of standard drug added (µg/mL)	% Recovery	Standard deviation	%RSD
400 µg/ml	80%	320	99.58	0.67735	0.170214
	100%	400	99.74	0.75735	0.146281
	120%	480	99.79	0.78432	0.298617

3. Accuracy

The accuracy for the analytical method for STZN was determined at 80%, 100% and 120% levels of standard solutionand results were expressed in terms of % recoveries. Standard deviation and % RSD were calculated. The results were tabulated in (Table 7).

4. Robustness

The robustness of an analytical method is a measure of its capacity to remain unchanged by little but intentional variation in method parameters and provides an indication of its reliability during normal usage. Robustness of method was investigated by changing flow rate (± 2%), changing column temperature (± 5^0C), ratio of components of mobile phase and wavelength (± 2nm). Results are tabulated in Table 8.

Table 8: Data of robustness study

Parameter	% RSD of Area	Theoretical Plate Number	Tailing Factor
Flow rate + 2%	0.180256	5787	1.424
Fiow rate – 2%	0.082347	4976	1.087
Column temperature at 37^0C	0.170453	5646	0.928
Column temperature at 33^0C	0.128751	5401	0.853
Wavelength + 2nm (262nm)	0.210762	5798	1.245
Wavelength – 2nm (258 nm)	0.182461	5234	1.142

Table 9: LOD and LOQ values

Sr.No	LOD (µg/ml)	LOQ (µg/ml)
1.	0.00192064	0.00208267

5. Limit of detection (LOD) and Limit of Quantization (LOQ)

LOD and LOQ values were calculated from the dataobtained from the linearity studies. The slope of the linearity plot was determined. They were calculated as 3.3 σ/S and 10 σ/S respectively. Where σ is the standard deviation of the response (y-intercept) and S, is the mean of the slope of calibration plot. The results are tabulated in Table 9.

Table 10:Validation Parameters

Sr. no.	Parameter	Result
1	Wavelength	260 nm
2	Beer's range	100-600 µg/ml
3	Standard Regression Equation	y = 12014x + 150668
4	Correlation Coefficient (r^2)	0.997
5	Robustness(%RSD)	0.193649
6	LOD (µg/ml)	0.00192064
7	LOQ (µg/ml)	0.00208267
8	Intraday precision (%RSD)	0.01830267
9	Interday precision (%RSD)	0.14310317

A simple, specific, rapid, reliable and reproduciblemethod for the estimation of STZNin bulk and tablet dosage form has been developed and validated. The linearity range in the concentration range of 100-600µg/ ml (r^2= 0.997).It indicated that the concentrations of STZNhad good linearity. The LOD and LOQ were found to be 0.00192064 µg/ ml and 0.00208267µg/ ml respectively.The developed and validated method was successfully applied for the quantitative analysis of Onabet V1 tablets. The amount of STZNwas calculated as 99.62%. Further the precision of the method was confirmed by the repeatable analysis of solution. From the robustness study it is clear that the system suitability criteria meet with the acceptance limit. Hence the method is robust. The % RSD were found to be 0.01830267 and 0.14310317 for intraday and interday precision respectively. It indicated that the method has good precision. The percentage recovery was found to be in the range of 99-100 %. The procedure was repeated for 3 times by taking 400µg/ml as 100%.The recovery was calculated for 80%, 100% and 120%. The low % RSD value indicated that there is no interference due to excipients used in formulation. Hence, the accuracy of the method was confirmed.

Conclusion

It can be concluded from the results that the proposed method was simple, rapid, reliable, accurate, precise andmost economical for the determination of STZN in bulk and tablet dosage form. This method can be used as more convenient and efficient option for the analysis of STZNto establish the quality of the drug substance during routine analysis with consistent and reproducible results.

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