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Research Article

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RP-HPLC and UV Spectrophotometric methods for estimation of Sertaconazole nitrate in microemulsion

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ABSTRACT

High-performance liquid chromatography and UV spectrophotometric method were developed and validated for the quantitative determination of the bulk sertaconazole nitrate and its micro emulsion formulation. For HPLC, LC GC Qualisil BDS C18 column (4.6×250 mm, 5µm particle size) with the mobile phase consisted of acetonitrile-water (65:35%v/v) and flow rate of 1.8 ml/min were used for the analysis. The sertaconazole nitrate peak is monitored at a wavelength of 260 nm; the retention time was 20.16 min. The method is considered reliable for the determination of sertaconazole nitrate. Nearly 99.6% of sertaconazole nitrate from microemulsion formulation were recovered by applying this method with RSD 0.18% (n=9). UV spectrophotometric method was also found suitable for the estimation of sertaconazole nitrate with wavelength of 260 nm, linearity range 5-25 µg and correlation coefficient 0.9993, 99.25% of sertaconazole nitrate were recovered by this process with RSD 0.8%. The determination of sertaconazole nitrate was not affected by any of the vehicle used in the formulation of sertaconazole nitrate micro emulsion.

Key words: Microemulsion, method development, RP-HPLC, UV and sertaconazole nitrate.

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]. It has been found to have a good activity against those fungi which are resistant to other imidazoles (econazole, miconazole and clotrimazole). Sertaconazole is a highly selective inhibitor of fungal cytochrome P-450 sterol C-14 α-demethylation via the inhibition of the enzyme cytochrome P450 14α-demethylase. This enzyme converts lanosterol to ergosterol, and is required in fungal cell wall synthesis. It is soluble in ethanol and acetonitrile with (log P 5.7).

The review of literature revealed that various analytical methods involving spectrophotometry, HPLC have been reported for sertaconazole nitrate in single form and formulations. There is no method has been established for microemulsion drug delivery system. In this research article we have reported a convenient, reproducible and selective HPLC and UV methods for the estimation of sertaconazole nitrate in bulk drug as well as in topical formulation (Microemulsion). [3-11]

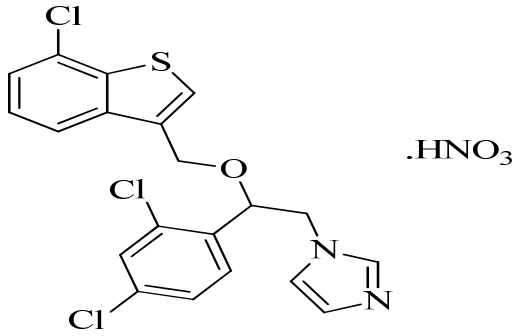


Fig 1. Chemical structure of sertaconazole nitrate

EXPERIMENTAL SECTION

Material and reagents

Sertaconazole nitrate was purchased from Hangzhou Holypharm Biotech Co. Ltd. (Zhejiang, China, Purity 99%). HPLC grade Acetonitrile and methanol were obtained from Fischer (Vadodara, Gujarat, India). Monobasic sodium phosphate was of analytical grade and was obtained from CDH all other chemical were of analytical grade.

HPLC method

The chromatographic system comprised of an Agilent 1220 series HPLC system (Agilent Technologies, USA) equipped with quaternary pump VL 400 bar, a vacuum-degasser unit and a 1024-element diode array (EDA) detector. The data was acquired and processed using Agilent Open Lab CDS (EZChrome edition, version A.04.02) software. Samples were pre-filtered using membrane filter (0.2 micron). LC GC Qualisil BDS C18 column (4.6×250 mm, 5µm particle size) protected by a Agilent Zorbax Cartridge Guard Column (4.6×12.5 mm L, 5 µm particle size packing).

Preparation of the standard solutions for standard curve

Weigh accurately 100 mg of sertaconazole nitrate and transfer to a 100 ml volumetric flask dissolved and diluted up to the mark with mobile phase to obtain stock solution containing 1.0 mg/ml of sertaconazole nitrate. Aliquots from stock solution were diluted with mobile phase to get the calibration standard solutions over the range of 20, 40, 60, 80 and 100 µg/ml of sertaconazole nitrate for HPLC method. Calibration standards were analyzed by HPLC method (n=5). The calibration curve was constructed by plotting peak area of sertaconazole nitrate against respective concentrations.

Preparation of the standard solutions for sample (Sertaconazole nitrate)

Sertaconazole nitrate solution was prepared by weighing 6,7,8,9 and 10 mg and transferred in 25ml volumetric flask and volume was made up to 25 ml with acetonitrile. Than 1 ml each solution was transferred to a 5 ml volumetric flask and volume was made with acetonitrile, final concentration of 48, 56, 64, 72 and 80 µg/ml was made. Aliquot of each solution was introduced in column.

Optimization of mobile phase

Optimization of mobile phase was performed on trial and error basis. Different mobile phase trials were carried out using methanol: 0.01M sodium phosphate buffer, ACN: 0.01M sodium phosphate buffer and methanol: ACN in different ratio and pH. ACN: 0.01M sodium phosphate buffer (40:60) pH 4 was found to full fill all criteria of system suitability. The mobile phase which consists of ACN: 0.01M sodium phosphate buffer (40:60 v/v) were found suitable and selected for further estimation. Sharp and well differentiated peak of sertaconazole nitrate was observed.

Preparation of microemulsion solution

A 2 g of microemulsion was weighed into a 25 ml beaker. A 15 ml volume of acetonitrile methanol (82:18 v/v) was added and dispersed uniformly. The resulting solution was transferred to a 50 ml flask. The flask was sonicated for 30 min by introducing it directly into an ultrasonic water bath, then, an aliquot was centrifuged at 5000 rpm for 30 min, 1 ml of the supernatant was transferred to a 10 ml flask and the required amount of acetonitrile was then added. An aliquot of this solution was transferred to a vial and 25 µl were injected into the chromatographic column.

RP- HPLC validation and system suitability parameters

The developed method was validated for different parameters like linearity, accuracy, precision, specificity, repeatability, limit of detection (LOD), limit of quantification (LOQ) and robustness. Suitability of the

chromatographic system was tested before each stage of validation. Five replicate injections of standard preparation were injected and retention time, tailing factor, number of theoretical plates, and relative standard deviation of peak area were determined.

Linearity and repeatability

Under proposed experimental conditions, the relationship between the area and the concentration of sertaconazole nitrate was studied. The calibration curve was plotted between concentrations versus area by the prepared concentration of 0-100 µg/ml of stock solution, and r^2 value was found to be 0.9998 (Table 1). Six replicate of prepared 25 µl solution of sertaconazole nitrate taken from different stock solution and measured area. The relative standard deviation (%RSD) was found to be less than 2 %, which indicates that the proposed method is repeatable.

Precision and specificity

Intraday and interday precision were carried out through replicating analysis (n=5) for concentrations (20 to 100 µg/ml). For interday precision, the analysis was carried out for three consecutive days at the same concentration level as used in intraday precision. The intraday precision was carried out by using three concentrations at different time interval in a day. The %RSD was calculated. The prepared standard, sample solutions and the blank solution were injected and check any other excipients interference occurs or not.

The results of injection repeatability (RSD <1%), intraday precision (RSD <2%) and method reproducibility (RSD <2%) proved the method to be précised.

Accuracy, LOQ, LOD and robustness

To find the accuracy of the method, the recovery experiment was carried out using the standard addition method. LOD and LOQ were determined using the equations, LOD = 3.3×SD/Sl and LOQ = 10×SD/Sl, where, SD is the standard deviation of the response and Sl is the slope of the calibration curve. The robustness of a method is its ability to remain unaffected by small changes in parameters like changes in flow rate, change mobile phase composition, change in wavelength, and change in pH. The limit of detection (LOD) and limit of quantification (LOQ) was found to be 0.04 and 0.14 µg/ml of sertaconazole nitrate, respectively.

UV Spectrophotometric method

A double beam Thermo scientific UV/Vis spectrophotometer, UV-2700 having two matched quartz cells with 1 cm light path equipped with UV Probe 2.35 software was employed in Spectrophotometric analysis of sertaconazole nitrate samples. Accurately weighed 100 mg of sertaconazole nitrate was dissolved in 100 ml of methanol to give a solution of 1 mg/ml (1000 µg/ml) concentration and this was served as a standard stock solution. Calibration standards were analyzed by UV Spectrophotometric method [12-14]

(n=5). The calibration curve was constructed by plotting absorbance of sertaconazole nitrate against respective concentrations.

RESULTS AND DISCUSSION

HPLC method

Analysis of bulk drug

The proposed methods were validated according to International Conference on Harmonization ICH Q2 (R1) guidelines [15]. The optimized HPLC elution was carried out isocratically where mobile phase acetonitrile-0.01 M sodium phosphate buffer (40:60 v/v), flow rate 1.8 ml/min, total run time of 30 min, and temperature 25°C were maintained to estimate the sertaconazole nitrate. The system suitability and validation parameters for determination of sertaconazole nitrate by HPLC method are depicted in Table 1. Fig. 2 shows standard curve with regression equation (y=mx+c), y=174.64x+68.381 and correlation coefficient 0.9998. Fig. 3 shows the corresponding chromatogram of standard sertaconazole nitrate in optimized HPLC conditions showing retention time for sertaconazole nitrate at 20.16 min. A recovery study of microemulsion was calculated by comparing the chromatographic peak areas with the calibration curve (Table 2). The retention times of the drug from standard solution and from sample solutions were identical. The comparison of chromatograms confirmed that the excipients did not interfere in the separation of sertaconazole nitrate. Peak purity check of sertaconazole nitrate peaks obtained from standard

The retention times of the drug from standard solution and from standard solution were identical. The comparison of chromatogram confirmed that the excipient did not interfere in the separation of sertaconazole nitrate.

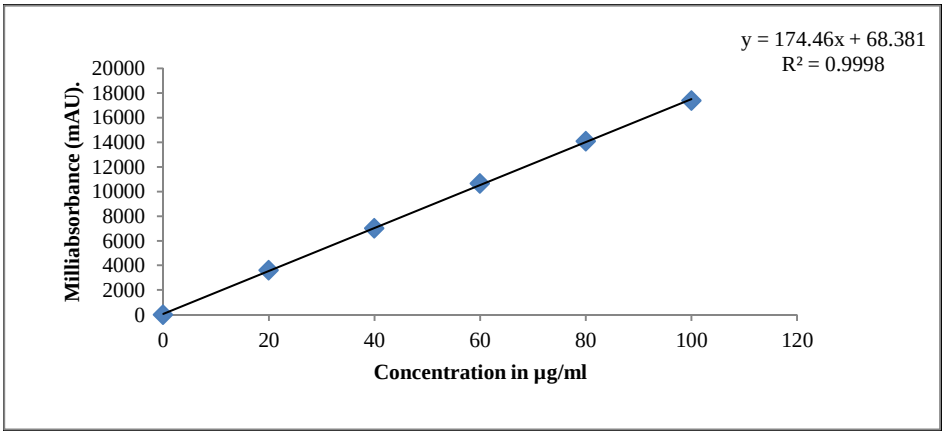
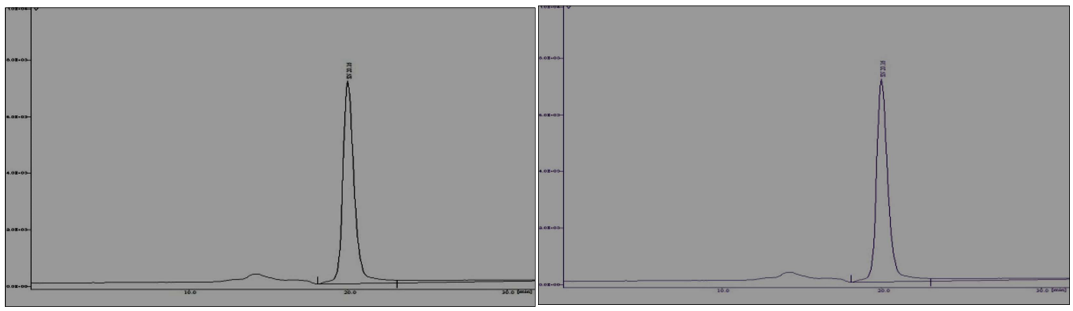


Fig 2. Standard curve of sertaconazole nitrate

Table 1: validation parameters for determination of sertaconazole nitrate by proposed HPLC method

Parameter	HPLC method
Retention time ± SD (min)	20.16 ± 0.01
Capacity factor	2
Theoretical plate	8237
Injection repeatability (% RSD, n=6)	2953
Linearity range (µg/ml)	20-100 µg/ml
Regression equation (y=mx+c)	y=174.64x+68.381
Correlation coefficient	0.9998
Intra-day precision (% RSD, n=3)	0.12 – 1.08
Inter-day precision (% RSD, n=3)	0.62 – 1.58
Reproducibility (% RSD, with different analyst)	2.25
Duration of run	30 min.
Injection volume	25 µl
Flow rate	1.8 ml/min
Detection	EDA (260nm)
Temperature	25°C

SD=Standard deviation, RSD=relative standard deviation, y=concentration of sertaconazole nitrate in µg/ml, x=peak area of sertaconazole nitrate



(a) (b)

Fig 3. HPLC chromatogram of bulk sertaconazole nitrate (a) and microemulsion (b) in optimized HPLC conditions showing retention time for sertaconazole nitrate identical at 20.16 min and no other interfering peak was found

UV spectrophotometric method

The UV absorption spectrum of sertaconazole nitrate in methanol depicted λmax at 260 nm (fig. 4). The wavelength showing the maximum absorbance 260 nm was selected for measurement of absorbance. The results of regression analysis and validation for determination of sertaconazole nitrate by UV method are shown in Table 3. The calibration curve was constructed by plotting absorbance against respective concentrations (5-25µg/ml) (Fig. 5). The variability in the precision study was found within limits proving the method to be precise. The % recovery in accuracy study was found to be 96.2 - 99.46%. The limit of detection (LOD) and limit of quantization (LOQ) was found to be 0.08and 0.24µg/ml, respectively. The working solutions of sertaconazole nitrate in methanol were found to be stable for at least five days. A recovery study of microemulsion was calculated by comparing the absorbance with the calibration curve (Table 3). The absorbance of the drug from standard solution and from sample solutions was identical. The comparison of standard and sample UV spectra confirmed that the excipient did not interfere in the separation of sertaconazole nitrate.

Table 2: Recovery studies for sertaconazole nitrate in microemulsion

Method	Amount taken (µg/ml)	Amount added (µg/ml)	% recovery*	S.D	RSD (%)
HPLC	56	55.24	98.64	0.25	0.45
		55.42	98.96	0.83	1.49
		55.76	99.57	0.67	1.2
	64	63.8	99.68	0.23	0.36
		63.2	98.75	0.16	0.25
		63.5	99.21	0.51	0.8
	72	79.2	98.91	0.28	0.35
		78.6	98.62	0.82	1.15
		79.4	99.33	0.1	1.39
UV	10	9.82	98.2	0.02	0.2
		9.75	97.5	0.04	0.41
		9.86	98.6	0.02	0.2
	15	14.92	99.46	0.03	0.2
		14.86	99.06	0.01	0.06
		14.74	98.26	0.09	0.61
	20	19.62	98.1	0.11	0.56
		19.24	96.2	0.1	0.51
		19.85	99.25	0.16	0.8

Each value is mean ± deviation of three determinations

The UV Spectrum represent standard solution of Sertaconazole nitrate (10µg/ml)

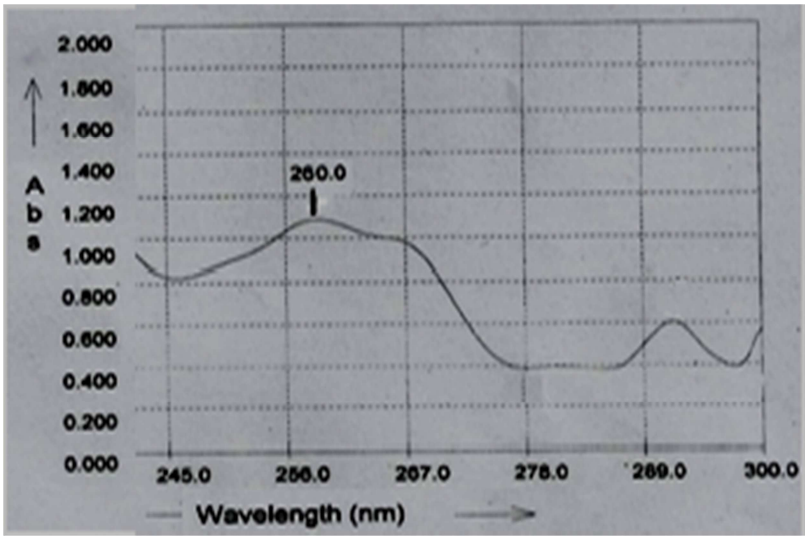


Fig 4. UV absorption spectrum of sertaconazole nitrate

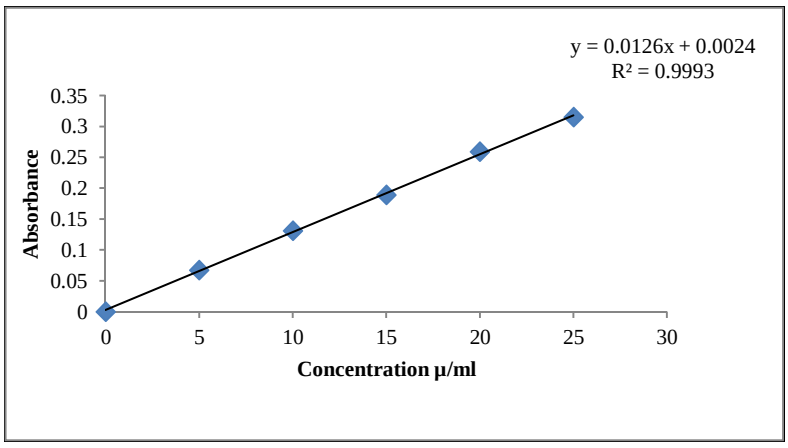


Fig 5 Standard curve of sertaconazole nitrate, where x axis shows concentration in µg/ml and y axis shows absorbance

Table 3: Validation parameters for determination of sertaconazole nitrate by uv method

Parameter	UV Spectrophotometric method
Linearity range (µg/ml)	25-May
Regression equation (y=mx+c)	y = 0.0126x+0.0024
Correlation coefficient	0.9993
Intra-day precision (% RSD, n=3)	0.59-0.81
Inter-day precision (% RSD, n=3)	1.12-1.42
Reproducibility (% RSD, with different analyst)	1.64
Instrument precision (n=7)	0.1

y=Concentration of sertaconazole nitrate in µg/ml, x=absorbance of sertaconazole nitrate at 260 nm, RSD=relative standard deviation, UV=ultra violet

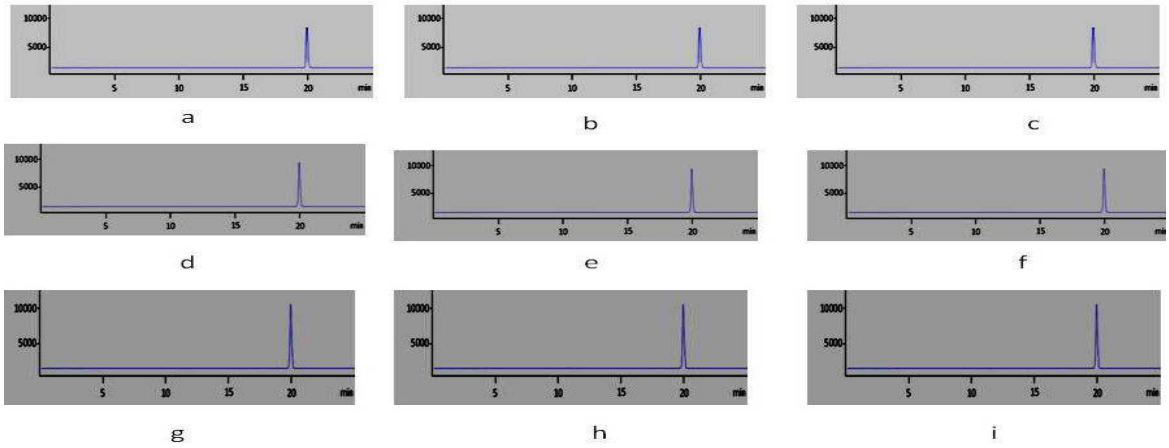


Fig 6. HPLC recovery studies for sertaconazole nitrate in microemulsion at concentration a-c, 56 µg/ml; d-f, 64 µg/ml and g-i, 72 µg/ml

CONCLUSION

The HPLC method and the UV Spectrophotometric method for the determination of sertaconazole nitrate in pharmaceutical formulations were found to be simple, rapid, precise, accurate and sensitive. Moreover, the UV method offers a cost effective and time saving alternative to HPLC method of analysis for sertaconazole nitrate from formulations. The HPLC method enables faster quantification of sertaconazole nitrate within run time of twenty minutes without interference of excipients. In summary, the proposed methods can be used for routine quality control of pharmaceutical formulation containing sertaconazole nitrate.

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