

Purity assessment and determination of sertaconazole in bulk and pharmaceutical formulations based on spectrophotometric and chromatographic approaches

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ABSTRACT

Stability evaluation of the drug substance is an integral part of the systematic approach to stability studies. Hence, three simple, sensitive and precise methods depending on two different techniques as UV spectrophotometry and chromatography were adopted for the task of stability indicating determination of sertaconazole (SER) in presence of its acidic degradation products. The first method is Zero-crossing first derivative (¹D) spectrophotometric one, which allows the determination of SER over a concentration range of 4-64 µg/mL at 290 nm with mean percentage recoveries 99.77±0.781. While first-derivative of the ratio spectra (¹DR) is the method of choice for determination of pure SER at a maximum 300 nm and at a trough 304 nm, with mean percentage recoveries 99.98±0.720 and 100.46±0.640, respectively. The third developed HPLC method used a RP-ZORBAX C18 column (5 µm particle size, 250×4.6 mm; id) with isocratic elution. The mobile phase was methanol: 0.2% formic acid aqueous solution (75:25, v:v); pH = 3.5 at the flow rate of 1.0 mL/min, with UV detection at 260 nm. The method could determine SER in the range of 0.8-40 µg/mL with a mean percentage recovery of 99.65±0.630. The developed methods were succeeded in the determination of SER in bulk powder, pharmaceutical dosage forms and in presence of its acidic degradation products. The results obtained were validated in compliance with ICH guidelines and compared statistically with each other and to those of the official method in the British Pharmacopoeia regarding both accuracy and precision.

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1. Introduction

Sertaconazole (SER); 1-[2-[(7-chloro-1-benzothiophen-3-yl)methoxy]-2-(2,4-dichlorophenyl)ethyl]-1H-imidazole (Figure 1) is a broad spectrum antifungal agent [1]. Sertaconazole is a member of imidazole antifungal group used topically as the nitrate with excellent activity against yeasts, dermatophytes and opportunistic fungi [2]. It is believed that it acts primarily by inhibiting the synthesis of ergosterol through inhibiting the 14 α -demethylase enzyme. Ergosterol is an important component of the cell membrane of fungi, the lack of this component leads cell injury mainly by leakage of cellular constituents in the cytoplasm from the cell.

In addition to this antifungal efficacy, it has a good safety profile, sustained cutaneous retention, and low systemic absorption, all of which make it ideal for topical applications [3]. In fact, both British and European pharmacopoeias described non-aqueous titrations for its determination [4,5] and till now, few methods have been reported for the analysis of SER as spectrophotometry [6], spectrofluorimetry [7], liquid chromatography [8-10], TLC [7], and capillary zone electrophoresis [11].

The aim of this presented work is to develop different analytical techniques as stability indicating methods for determination of antifungal drug; sertaconazole either in pure form or in presence of its acidic degradation products without prior physical separation steps. The proposed methods can be successfully applied in assay of the cited drug in pharmaceutical formulation as a routine work analysis in quality control labs.

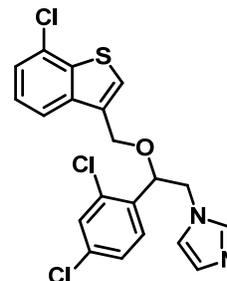


Figure 1. Chemical structure of intact sertaconazole.

2. Experimental

2.1. Instrumentation

The absorption spectra of the samples were recorded by a double beam UV-VIS spectrophotometer (UV-1800, Japan) connected to IBM compatible computer with the bundled software (UV probe software version 2.32, Shimadzu) and the spectral bandwidth was 0.1 nm. The absorption spectra were carried out using 1 cm quartz cells. HPLC was performed using a 1200 Series chromatograph equipped with a quaternary pump (No. G-1311A), UV variable wavelength detector, 20 mL injector loop, and manual injector Model No. 7725 I (Agilent Technologies, Waldbronn, Germany). The pH meter was a digital pH/MV/TEMP/ATC meter, Model 5005 (Jenco Instruments, San Diego, CA). The GC-Mass spectrometer was a Shimadzu QP-2010 (Kyoto, Japan), which was operated on electron impact (EI) mode at 70 eV.

2.2. Materials and reagents

Pure samples of sertaconazole nitrate was kindly supplied by October Pharma Pharmaceutical Co., Egypt. The purity of the samples was found to be 100.52 ± 0.427 , according to the official non aqueous method in British Pharmacopoeia [4]. Dermofix® cream, Batch No. 030409 labeled to contain 20 mg of sertaconazole nitrate per each one gram of cream and Dermofix® powder, Batch No. 0080909, each one gram is claimed to contain 20 mg of sertaconazole nitrate. Creams and powders are manufactured by October Pharma-Egypt under License of Ferrer International-Barcelona, Spain. Degraded sample was prepared by the application of accelerated acid stress condition to the intact sertaconazole nitrate. After complete degradation, the major degradation product was subjected to GC/MS spectroscopy for structure elucidation. Methanol and formic acid were HPLC grade; E. Merck (Darmstadt, Germany). Concentrated hydrochloric acid and sodium hydroxide were purchased from El-Nasr Pharmaceutical Chemicals Co., Abu-Zabaal, Cairo, Egypt. All chemical used were of the analytical grade and other organic solvents were of spectroscopic grade.

2.3. Standard solution for the intact drug (1 mg/mL)

The intact sertaconazole pure sample (0.05 g) was accurately weighed and transferred into a 50 mL measuring flask and volume was completed with methanol. Sertaconazole working solution (0.08 mg/mL) was prepared by transferring 8 mL of the standard stock solution into 100 mL volumetric flask and then the volume was made up with methanol.

2.4. Standard solution for the acidic degradation products (1 mg/mL)

The pure sertaconazole powder (0.05 g) was accurately weighed and transferred to a 100 mL round bottomed flask. Then, 20 mL of 1 N HCl were added and a reflux for 2 hrs was done. Complete degradation was followed and confirmed by the reported HPLC method [11]. Subsequently, the solution was neutralized to $\text{pH} = 7 \pm 0.2$ by 1.0 N NaOH, evaporated nearly to dryness under vacuum, cooled to room temperature (25 °C), then quantitatively transferred with methanol to a 50 mL measuring flask and completed to volume with the same solvent. A working stock solution of the acid degradation products (0.08 mg/mL) was prepared by transferring an accurate aliquot portion of the acid degradation products stock solution (1 mg/mL) into a 100 mL volumetric flask and diluted with methanol.

2.5. Procedures

2.5.1. Spectral characteristics

The absorption spectra of the intact and degraded SER samples were recorded over the range 200-400 nm, using methanol as a blank (Figure 2).

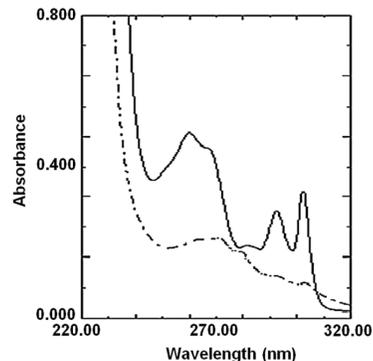


Figure 2. Zero-order spectra of pure sertaconazole (—) and its acid degradate (---), each of 40 µg/mL.

2.5.2. Construction of calibration curves for ¹D spectrophotometric method

Accurately measured volumes of intact SER working solution (0.08 mg/mL) were transferred into a series of 10 mL volumetric flasks and diluted to the mark with methanol to obtain concentrations from 4 to 64 µg/mL. The ¹D spectra of each solution was recorded using $\Delta\lambda = 4$ and scaling factor = 100. Calibration curves were obtained by plotting the peak amplitudes of ¹D at 290 nm at which zero-crossing of the degradation products was observed versus the corresponding drug concentrations, and regression equation was computed.

2.5.3. Construction of calibration curves of ¹DD spectrophotometric method

Different aliquots of intact SER working solution (0.08 mg/mL) were accurately transferred into a series of 10 mL volumetric flasks and diluted to the mark with methanol to obtain concentrations from 4 to 64 µg/mL. The absorption spectra of these solutions were divided by the absorption spectrum of 4 µg/mL of the acidic degradates (as a divisor). The obtained ratio spectra were then differentiated with respect to wavelength. The ¹DD curves were recorded at $\Delta\lambda = 8$ and scaling factor = 10. The peak amplitudes of a maximum at $\lambda = 300$ nm and of a trough at $\lambda = 304$ nm were recorded for the determination of SER in presence of its acidic degradates. The calibration curves representing the relationship between the measured amplitudes and the corresponding concentrations of the drug were constructed and the regression equations were computed.

2.5.4. Construction of the calibration curve of the HPLC method

The chromatographic separation was carried out at ambient temperature on a ZORBAX-ODS C18 column (250 × 4.6 mm, i.d.), particle size (5 µm), (Agilent Technologies, Waldbronn-Germany), with isocratic elution at 1.0 mL/min using the mobile phase; methanol:formic acid (0.2%) aqueous solution (75:25, v:v); $\text{pH} = 3.5$. The mobile phase was filtered through a 0.45 µm Millipore Corp. (Billerica, MA) membrane filter and degassed for about 15 min in an ultrasonic bath prior to use.

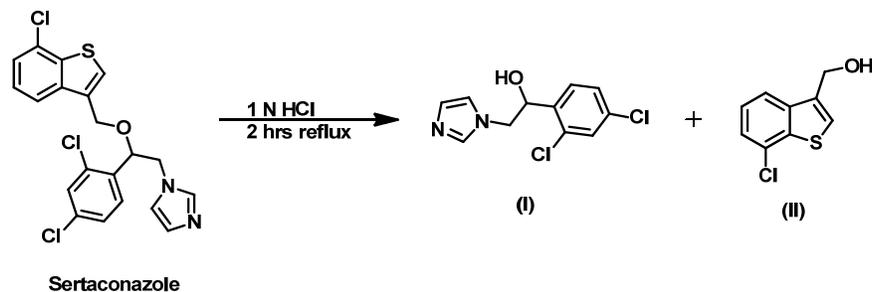


Figure 3. Suggested degradation pathway of sertaconazole.

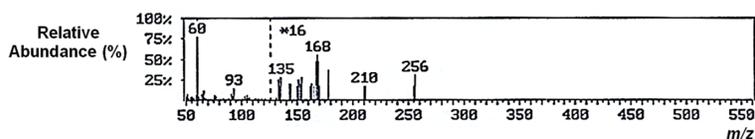


Figure 4. Mass spectrum of the acidic degradation product I.

To reach equilibrium, the analysis was usually started after passage of 50-60 mL mobile phase.

A series of working solutions containing 0.8 to 40 $\mu\text{g/mL}$ SER and 10 $\mu\text{g/mL}$ clotrimazole (CZ) as an internal standard (IS) were prepared by serial dilution of the standard working solution with the mobile phase. Then the solutions were sonicated for 5 minutes and filtered through a disposable syringe filter (0.45 μm) before column injection. 20 μL Aliquots of each solution were injected in triplicate and eluted with the mobile phase. The eluted analytes were detected at 260 nm with a sensitivity of 0.001 absorbance unit full scale. The average peak areas ratios were plotted versus the corresponding concentrations to obtain the calibration curves. Alternatively, the corresponding regression equation was derived.

2.5.5. Assay of laboratory prepared mixtures

Different aliquot portions of the intact SER working solution (0.08 mg/mL) were accurately transferred into a series of 10 mL measuring flasks, and mixed with variable portions of the acid degraded standard or working solutions to prepare laboratory mixtures containing 10-90% of the degradation products. The volume was completed with the appropriate solvent for each procedure. The mixtures were analyzed by the proposed methods and the concentrations of the intact drug were calculated from the corresponding regression equations.

2.5.6. Application to pharmaceutical products

Accurate amount of Dermofix[®] powder corresponding to 50 mg SER was transferred into 50 mL volumetric flask. About 30 mL of methanol was added and followed by sonication for 15 min. The solutions were completed to the volume with methanol, mixed well and filtered.

An accurately weighed amount of Dermofix[®] cream corresponding to 50 mg SER was transferred into a 100 mL beaker. About 20 mL of methanol was added followed by sonication in an ultrasonic bath for 15 min. The solutions were stirred for 5 min then cooled at refrigerator. This process was repeated twice. Then, the supernatant phase was quantitatively transferred to a 50 mL volumetric flask through filter paper and completed with methanol. Suitable dilutions were done using methanol for the spectrophotometric methods and the suggested mobile phase for the HPLC one.

The proposed methods were applied for the analysis of the pharmaceutical preparation solutions using the procedures mentioned before. The concentrations of SER were calculated from the corresponding regression equations.

3. Results and discussion

3.1. Identification of the degradation product

Sertaconazole is completely hydrolyzed with 1 M HCl after reflux of 2 hrs, through the splitting of the ether linkage [12,13]. The expected major degradation products I and II are obtained according to the suggested mechanism for acid degradation process of SER, and the major degradation products suggested in acidic conditions are *N*-(2-(2,4-dichlorophenyl)-2-hydroxyethyl)imidazole (I) and 7-chloro-3-hydroxy methyl benzothiazole (II), as presented in Figure 3.

The assignment of the degradation pathway and the suggested structures of the degradation products were confirmed by mass spectral data. The mass spectrum of degradation product I was characterized by the appearance of the molecular ion peak at 256 m/z which confirms the molecular weight of the suggested degradation product I, Figure 4.

3.2. Method development and optimization

The quantitative determination of SER in the presence of its acidic degradation products by conventional zero order spectrophotometry is completely hindered due to the strong spectral overlap throughout the wavelength range (Figure 2).

The focus of the present work is to develop accurate, specific, reproducible and sensitive stability indicating methods for the determination of SER in pure form, in pharmaceutical formulations and in the presence of acidic degradates.

3.2.1. Derivative spectrophotometry

Derivative spectrophotometry is considered to be an ideal stability indicating method that quantifies the standard drug alone and also resolves its degradation products as reported for antipsychotic drug, olanzapine [14]. A rapid, simple and low cost spectrophotometric method based on measuring the peak amplitude of ¹D at 290 nm (Figure 5) was developed for determination of SER in presence of its acidic degradates (Corresponding to zero crossing of the degradates).

Table 1. Regression and assay validation parameters for determination of pure samples of SER by the proposed methods.

Parameter	¹ D method	¹ DD method		HPLC method
	290 nm	300 nm	304 nm	
Linearity range, µg/mL	4-64	4-64	4-64	0.8-40
Slope ± SD	0.0435±0.0035	0.0384±0.0025	0.0465±0.0055	0.0251±0.0091
Intercept ± SD	0.0292±0.055	0.0449±0.0012	0.0827±0.0085	0.0003±0.0115
Correlation coefficient (r)	0.9999	0.9999	0.9999	0.9999
Mean recovery % ± SD	99.77±0.781	99.98±0.720	100.46±0.640	99.65±0.630
LOD a (µg/mL)	1.16	1.29	1.06	0.132
LOQ a (µg mL)	3.45	3.91	3.23	0.4
Precision (RSD %)				
Repeatability ^b	0.375-0.758-0.795	0.475-0.518-0.675	1.024-0.757-0.535	1.135-0.564-1.226
Intermediate precision ^b	0.998-0.458-0.708	1.315-1.550-0.597	1.852-1.540-0.776	0.995-0.448-1.205
Robustness data ^c				
Peak amplitude in ¹ D method				
At 290.2 nm	99.25±0.825			
At 289.8 nm	99.75±1.225			
Peak amplitude in ¹ DD method				
At 300±0.2 nm		99.85±0.998		
At 304±0.2 nm			100.05±1.245	
Methanol:0.2% formic acid (75±1:25±1, v:v)			100.55±0.556	
pH = 3.5±0.05			99.98±0.458	
Flow rate 1±0.2 mL/min			99.58±0.878	

^a Limits of detection and quantitation are determined via calculations: LOD = (SD of the response/slope) × 3.3. LOQ = (SD of the response/slope) × 10.

^b The intra-day and inter-day relative standard deviations of 8, 16 and 32 µg/mL SER; each of triplicate analysis

^c Mean recovery %±RSD (n = 5).

Table 2. Determination of SER in the laboratory prepared (L.P.) mixtures with its acidic degradation products and in dosage forms and the application of standard addition technique by the proposed methods.

Samples	¹ D-method ^a	¹ DD-method ^a		HPLC-method ^a
	290 nm	300 nm	304 nm	
Laboratory prepared mixtures, 10 to 90% degradation, (n = 5) ^b	99.96±0.585	100.06±0.900	100.31±0.759	100.84±0.246
Dermofix® cream, Batch No. 030409, (n = 4) ^b	100.28±0.839	99.88±1.093	99.91±1.040	99.92±0.721
Standard addition (n = 4) ^b	99.83±1.006	100.25±1.317	100.94±1.096	100.30±0.857
Dermofix® powder, Batch No. 0080909, (n = 4) ^b	99.21±0.419	100.56±0.735	100.37±1.121	100.05±0.325
Standard addition (n = 4) ^b	100.18±0.636	100.03±1.125	100.17±0.865	99.67±0.479

^a Recovery±RSD.

^b Sets each of 3 replicates.

In order to optimize ¹D method, different smoothing and scaling factors were tested, where a smoothing factor $\Delta\lambda = 4$ and scaling factor = 100 showed a suitable signal to noise ratio and the spectra showed good resolutions. The regression equations were computed (Table 1).

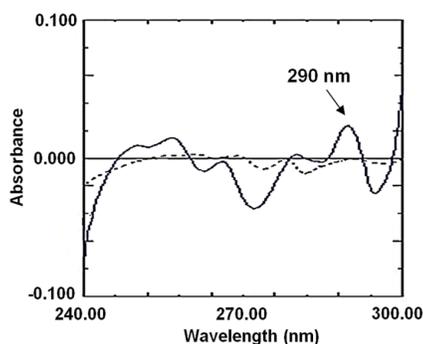


Figure 5. First derivative spectra of pure sertaconazole (—) and its acid degradate (---), each of 40 µg/mL.

The proposed method is valid for the determination of SER with good selectivity without interference of up to 90% of its acidic degradates over concentration range from 4-64 µg/mL (Table 2).

3.2.2. ¹DD method

In order to improve the selectivity of the analysis of SER in presence of its acidic degradates the ¹DD method was established. The main advantage of the method is that the whole spectrum of interfering substance is cancelled.

Accordingly, the critical measurements at the selected wavelengths are not applied anymore.

In order to optimize ¹DD method for determination of SER in presence of its degradation products, it is necessary to test the influence of the variables: divisor concentration, smoothing and scaling factors. Several divisor concentrations of the acidic degradates were tried, the best results was obtained when using 4 µg/mL of degradates as a divisor. Different smoothing and scaling factors were also tested where a smoothing factor $\Delta\lambda = 8$ nm, and scaling factor = 10 were suitable to enlarge the signals of SER to facilitate its measurement and to diminish error in reading the signal. ¹DD values showed good linearity and reproducibility at 300 and 304 nm without interference from its acidic degradates (Figure 6). Linearity of the peak amplitudes of the ¹DD curves at both wavelengths was obtained in range 4-64 µg/mL and the regression equations were computed (Table 1).

3.2.3. HPLC method

The developed HPLC method has been applied for the separation and determination of SER in the presence of its acidic degradation products. To optimize the HPLC assay parameters, the mobile phase composition was studied. A satisfactory separation was obtained with a mobile phase consisting of methanol:formic acid (0.2%) aqueous solution (75:25, v:v); pH = 3.5 at an ambient temperature with a flow rate of 1.0 mL/min, followed by UV detection at 260 nm. Complete baseline separation of SER and its degradation products was noticed in Figure 7.

The average retention times were found to be 3.8±0.1 min for intact SER and 1.4±0.2 for its degradates for 10 replicates. All peak parameters of resolution efficiency were calculated to ensure that the system is working correctly during the analysis.

Table 3. Parameters of system suitability of the developed chromatographic method for the determination of SER in presence of its degradation products.

Parameters	HPLC Method	Reference values [15,16]
Resolution (R_s)	3.69	> 1.5
Selectivity (α)	6.94	>1
Tailing factor (T)	1.07	≈ 1
Capacity factor (K')	3.75	1-10 acceptable
Column efficiency (N)	924.16	Increase with efficiency of the separation
HETP*	0.27	The smaller the value the higher the column efficiency

* Height equivalent to theoretical plate.

Overall system suitability testing was done to determine if the operating system was performing properly [15,16], and the results obtained are listed in Table 3.

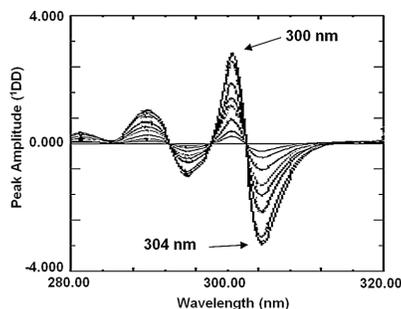


Figure 6. 1D -Ratio spectra of different concentrations (4-64 $\mu\text{g/mL}$) of sertaconazole using a spectrum of 4 $\mu\text{g/mL}$ of its degradate as a divisor.

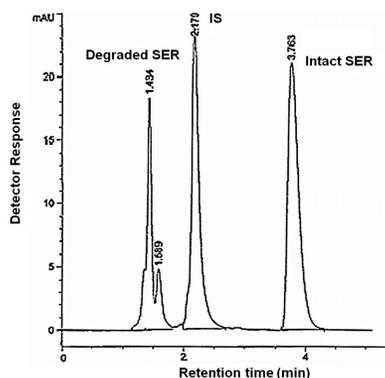


Figure 7. Typical HPLC chromatogram of laboratory prepared mixture of intact SER and its acid degradate (each of 20 $\mu\text{g/mL}$) using clotrimazole (10 $\mu\text{g/mL}$) as an internal standard.

3.3. Method validation

3.3.1. Linearity and range

Calibration curves for the proposed methods were constructed and evaluated by their correlation coefficients. Linearity of spectrophotometric methods was obtained in the concentration range of 4-64 $\mu\text{g/mL}$ of SER with mean percentage recoveries of 99.77 ± 0.781 ($n = 8$) for 1D method. While for 1DD method the mean percentage recoveries were found to be 99.98 ± 0.720 at 300 nm and 100.46 ± 0.640 at 304 nm ($n = 8$). In the HPLC method, the linearity of the detector response of SER was determined by plotting peak area ratios versus concentrations, and linear correlation was obtained in range of 0.8-40 $\mu\text{g/mL}$ with mean percentage recoveries of 99.65 ± 0.630 ($n = 7$).

The regression equations parameters of the all proposed methods were computed and given in Table 1 which shows a good linear relationship for the suggested methods as revealed by the correlation coefficients. Descriptive statistics of the regression showed low values of the standard deviation of

intercept and slope which revealed high accuracy with minimum deviations and low scattering of the calibration points [17].

3.3.2. Accuracy

The accuracy of the suggested methods was assessed by applying standard addition technique by spiking different amounts of pure sertaconazole samples to a certain concentration of the dosage forms. The mean recoveries of the added drug were calculated and illustrated in Table 2. No interference due to excipients was observed as shown from the obtained results.

3.3.3. Precision

Repeatability was evaluated by assaying freshly prepared solutions in triplicate on the same day having concentrations of 8, 16 and 32 $\mu\text{g/mL}$ of SER by the suggested spectrophotometric and HPLC methods. Intermediate precision was assessed by analyzing freshly prepared solutions of the above mentioned concentrations in triplicate in three successive days. The % Recovery and %RSD were then calculated (Table 1).

3.3.4. Specificity

In order to test the validity and applicability of the proposed methods as stability-indicating ones, recovery studies were performed by analyzing synthetic mixtures of different ratios of the intact drug and its degradation product. Results in Table 2 confirm the validity and specificity of the proposed methods.

3.3.5. Detection and quantitation limits (sensitivity)

According to International Conference on Harmonisation [ICH] recommendations [18], the approach based on the standard deviation values [S.D.] of the response and the slope was used for determining the detection and quantitation limits (LOD, LOQ). The theoretical values were assessed and given in Table 1.

3.3.6. Application to pharmaceutical formulations

The suggested methods were successfully applied for the determination of SER in creams and powered forms. The results shown in Table 2 were satisfactory and with good agreement with the labeled amounts.

3.3.7. Robustness

The robustness of the developed methods was examined by detecting the effect of small but deliberate variations of some of the most important procedure parameters such as wavelength of the peak amplitudes in 1D and 1DD method and percentage organic strength; pH and flow rate of the mobile phase in the HPLC method. None of these variables significantly affected the assay of SER and the proposed methods could be considered robust (Table 2).

Table 4. Statistical analysis of the proposed methods and the official method of Sertaconazole in the pure powder form.

Parameter	¹ D method	¹ DD method		HPLC method	Official method [4]
		300 nm	304 nm		
Mean	99.77	99.98	100.46	99.65	100.52
SD	0.781	0.720	0.640	0.630	0.427
n	8	8	8	7	5
Variance	0.610	0.518	0.410	0.397	0.182
Student's t-test	1.506 (2.201) *	1.118 (2.201) *	0.130 (2.201) *	1.835 (2.228) *	
F-test	3.352 (6.09) *	2.846 (6.09) *	2.253 (6.09) *	2.181 (6.16) *	

* Figures in parenthesis are corresponding theoretical *t*- and *F*-values at *p* = 0.05.

3.3.8. Statistical comparison to the official method

Statistical analysis of the results obtained by the suggested procedures and the official non aqueous method [4] was carried out. Table 4 shows that the calculated *t*- and *F*-values were less than the theoretical ones [19], indicating no significant differences between the proposed procedures and the reported one.

4. Conclusion

Simple newly developed ¹D, ¹DD, and isocratic HPLC methods are presented as stability indicating methods for the determination of sertaconazole in the presence of its acidic degradation products. From the previous discussion and results obtained in this work, we can conclude with 95% of confidence that the three suggested methods are simple, sensitive, selective and can be applied for quality control and routine analysis of sertaconazole in pure form, in the presence of its acidic degradation products and in the available dosage forms without any interference from the excipients. In addition, the suggested HPLC method has an advantage over the reference one [8] of being more sensitive and showing short time of analysis as the overall run time doesn't exceed 5 minutes. It offers complete separation of the drug and its degradation products which gives the opportunity for drug quantitation and purity assessment in quality control labs.

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