

Electron Paramagnetic Resonance Method for the Quantitative Assay of Ketoconazole in Pharmaceutical Preparations

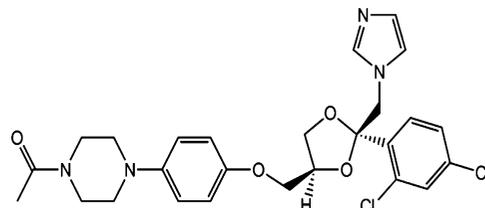
Mohamed A. Morsy,* Salah M. Sultan, and Hatim Dafalla

Chemistry Department, King Fahd University of Petroleum & Minerals, Dhahran 31261, Saudi Arabia

In this study, electron paramagnetic resonance (EPR) is used, for the first time, as an analytical tool for the quantitative assay of ketoconazole (KTZ) in drug formulations. The drug was successfully characterized by the prominent signals by two radical species produced as a result of its oxidation with 400 $\mu\text{g/mL}$ cerium(IV) in 0.10 mol dm^{-3} sulfuric acid. The EPR signal of the reaction mixture was measured in eight capillary tubes housed in a 4 mm EPR sample tube. The radical stability was investigated by obtaining multi-EPR scans of each KTZ sample solution at time intervals of 2.5 min of the reaction mixing time. The plot of the disappearance of the radical species show that the disappearance is apparently of zero order. The zero-time intercept of the EPR signal amplitude, which should be proportional to the initial radical concentration, is linear in the sample concentration in the range between 100 and 400 $\mu\text{g/mL}$, with a correlation coefficient, r , of 0.999. The detection limit was determined to be 11.7 ± 2.5 $\mu\text{g/mL}$. The method newly adopted was fully validated following the United States Pharmacopeia (USP) monograph protocol in both the generic and the proprietary forms. The method is very accurate, such that we were able to measure the concentration at confidence levels of 99.9%. The method was also found to be suitable for the assay of KTZ in its tablet and cream pharmaceutical preparations, as no interferences were encountered from excipients of the proprietary drugs. High specificity, simplicity, and rapidity are the merits of the present method compared to the previously reported methods.

Ketoconazole (KTZ), *cis*-1-acetyl-4-[4-[[2-(2,4-dichlorophenyl)-2-(1H-imidazole-1-ylmethyl)-1,3-dioxolan-4-yl] methoxy] phenyl] piperazine (Scheme 1) is a highly effective broad spectrum antifungal agent.^{1–4} Recently, new drugs in which KTZ is

Scheme 1. Ketoconazole (KTZ) Molecular Structure



coordinated to transition metals such as Ru, Rh, Cu, Au, and Pt result in a remarkable enhancement of the biological activity as antitrypanosoma cruzi.^{5–8} KTZ is also used to treat a wide variety of superficial and systemic mycoses and has the advantage over other imidazole derivatives of producing adequate sustained blood levels following oral administration.⁹

Several methods for the assay of KTZ have been reported, including potentiometric methods that are approved standard methods in the British Pharmacopeia (BP) and United States Pharmacopeia (USP).^{10,11} Both methods are based on the potentiometric titration of the KTZ against 0.1 mol dm^{-3} perchloric acid in an anhydrous acetic acid media.

Intensive investigations were performed to assay KTZ using different spectrophotometric methods. Farhadi and Maleki¹² selected 1,10-orthophenanthroline (Phen) for indirect spectrophotometric determination of KTZ based on its oxidation with Fe(III) to produce a colored $[\text{Fe}(\text{Phen})_3]^{2+}$ complex which absorbs at 512 nm. Khashaba et al.¹³ suggested simple spectrophotometric and spectrofluorimetric methods for the determination of antifungal drugs such as clotrimazole, econazole nitrate, KTZ, miconazole, and tolnaftate. Similarly, Abdel-

* To whom correspondence should be addressed. E-mail: mamorsy@kfupm.edu.sa

- (1) Urbina, J. A.; Payares, G.; Molina, J.; Sanoja, C.; Lizardi, K.; Piras, M. M.; Piras, R.; Pérez, N.; Wincker, P.; Ryley, J. F. *Science* **1996**, *273*, 969.
- (2) Urbina, J. A.; Vivas, J.; Lizardi, K.; Molina, J.; Payares, G.; Piras, M. M.; Piras, R. *Chemotherapy* **1996**, *42*, 1294.
- (3) Brener, Z.; Cancado, J. R.; Galvao, L. M. C.; Luz, Z. M. P.; Filardi, L. S.; Pareira, M. E. S.; Santos, L. M. T.; Cancado, C. B. *Mem. Inst. Oswaldo Cruz Rio J.* **1993**, *88*, 149.

- (4) Cabe, R. E. Mc.; Remington, J. S.; Araujo, E. G. *Trans. R. Soc. Trop. Med. Hyg.* **1987**, *81*, 613.
- (5) World Bank. World Development Report. *Investing in Health*; Oxford University Press: Oxford, U. K., 1993.
- (6) Sánchez-Delgado, R. A.; Navarro, M.; Lizardi, K.; Atencio, R.; Capparelli, M.; Vargas, F.; Urbina, J. A.; Bouuille, A.; Hubert, A. J.; Noels, A. F. *Inorg. Chim. Acta* **1998**, *275*, 528.
- (7) Navarro, M.; Lehmann, T.; Cisneros-Fajardo, E. J.; Fuentes, A.; Sanchez-Delgado, R. A.; Silva, P.; Urbina, J. A. *Polyhedron* **2000**, *19*, 2319.
- (8) Navarro, M.; Cisneros-Fajardo, E. J.; Lehmann, T.; Sánchez-Delgado, R. A.; Atencio, R.; Silva, P.; Lira, R.; Urbina, J. A. *Inorg. Chem.* **2001**, *40*, 6879.
- (9) Sánchez-Delgado, R. A.; Lizardi, K.; Rincón, L.; Urbina, J. A.; Hubert, A. J.; Noels, A. F. *J. Med. Chem.* **1993**, *36*, 2041.
- (10) *British Pharmacopoeia Volume I & II Monographs: Medicinal and Pharmaceutical Substances, Ketoconazole*; TSO: London, U.K., **2007**.
- (11) *United States Pharmacopoeia*; USP 29-NF 24 p 1216; USP 30-NF25 p 680; USP Press: U.S.A.
- (12) Farhadi, K.; Maliki, R. *Anal. Sci.* **2001**, *17*, SUPPLEMENT, i867.
- (13) Khashaba, P. Y.; El-Shabouri, S. R.; Emara, K. M.; Mohamed, A. M. *J. Pharm. Biomed. Analysis* **2000**, *22*, 363.

Gawad¹⁴ established that KTZ reacts with iron(III) chloride in the presence of potassium thiocyanate to form a pink complex (2:1) that is soluble in 1,2-dichloroethane with a maximum absorbance at 510 nm.

Vojic et al.¹⁵ studied the acid–base equilibria of the diprotic, slightly hydrosoluble base KTZ in homogeneous and heterogeneous water systems at 25 °C at a constant ionic strength of 0.1 mol dm⁻³ NaCl solution. The acidity constants, K_{a1} and K_{a2} , served to calculate the solubility and the distribution of the equilibrium forms of KTZ as a function of pH. On the basis of the distribution of the equilibrium forms of KTZ and the absorbance at 225 nm, a spectrophotometric method for the determination of its content in commercial tablets was developed. Jalali et al.^{16,17} described a spectrofluorimetric determination of KTZ from different complex formulations that has been applied to low concentration pharmaceutical preparations and blood serum samples, respectively. Kedor-Hackmann et al.¹⁸ developed a spectrophotometric method for quantitative determination of KTZ in commercial and simulated emulsion formulations using a zero-crossing method at 257 nm with methanol as the background solvent. Abou-Attia et al.¹⁹ developed a spectrophotometric method for the determination of three pharmaceutical piperazine derivatives, namely ketoconazole, trimetazidine hydrochloride, and piribedil, by the formation of yellow-orange complexes with iron(III) chloride. El-Ragehy et al.²⁰ quantified KTZ by measuring complexes formed with copper(II) or cobalt(II) spectrophotometrically.

Arranz et al.²¹ described a capillary zone electrophoresis (CZE) method for simultaneous determination of a mixture of three imidazolic antifungal drugs. Abdel-Moety et al.²² developed a high-performance liquid chromatographic technique for the determination of clotrimazole, KTZ, and fluconazole, in pure forms and in pharmaceutical formulations.

Recently, electron paramagnetic resonance spectroscopy was successfully utilized in our laboratories as an analytical tool for the investigation of the free radicals generated in different systems including fermented natural leaves,^{23–25} degraded plastics,²⁶ and irradiated alanine,²⁷ and for the assay of chlorpromazine in drug formulations.²⁸

The present work is based on monitoring the radical systems formed as the result of oxidation of the KTZ with cerium(IV) in sulfuric acid media.

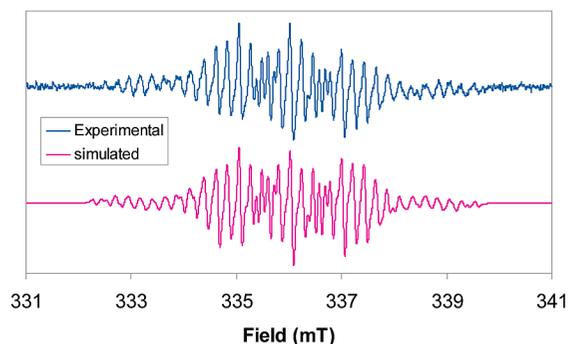


Figure 1. EPR experimental (top) and simulated (bottom) spectra for the oxidation of 400 µg/mL KTZ in 0.1 mol dm⁻³ sulfuric acid with 400 µg/mL cerium(IV). Spectrometer settings are as follows: microwave frequency, 9.49397 GHz; microwave power, 5 mW; center field, 337 mT; sweep width, 10 mT; resolution, 4096 points; receiver gain, 2.5×10^2 ; modulation frequency, 100 kHz; modulation amplitude, 0.1 G; time constant, 30.0 ms; sweep time, 120 s.

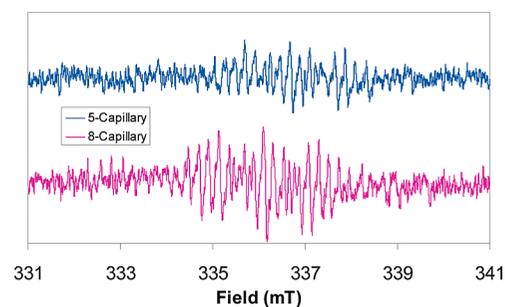


Figure 2. EPR signal using 5 (top) and 8 (bottom) capillary tubes housed in a 3 and 4 mm ESR sample tube, respectively, for the reaction mixture of 0.5 mmol dm⁻³ of KTZ and 50 µg/mL Ce(IV) after 2.5 min.

EXPERIMENTAL SECTION

KTZ Generic Samples. It is considered as the standard treatment for seborrheic dermatitis,²⁹ being the active ingredient of Nizoral tablets and cream, as well as of an antidandruff shampoo. Due to its high permeability but low aqueous solubility, this drug is classified as a class II active substance, since its dissolution properties in the gastrointestinal tract are insufficient under normal conditions.^{29,30} Therefore, acidified double-distilled deionized water was used throughout for the preparation of the KTZ solutions and all other dilutions.

KTZ Proprietary Samples. Twenty tablets from proprietary drugs were accurately weighed, crushed, and powdered. The amount of the powder containing the appropriate weight to give 1000 µg/mL KTZ was dissolved in about 40 mL of acidified water, heated for 3 min, filtered, and then diluted to a 100 mL volume in a volumetric flask after being cooled. Further, samples were made by successive dilutions of this sample.

The cream sample solutions were prepared by dissolving a precise amount of the cream in hot 0.10 mol dm⁻³ sulfuric acid. The excipients were separated by filtration, and the filter paper was washed three times with the same acid. The filtrate and wash solutions of the cream samples were transferred quantitatively into a 100 mL volumetric flask and diluted to the mark with the same acid to give a 1000 µg/mL KTZ solution. Other diluted solutions are prepared from this stock.

- (14) Abdel-Gawad, F. M. *IL Farmaco* **1997**, *52*, 119.
 (15) Vojic, M.; Popvic, G. V.; Sladic, D. M.; Pfendt, L. J. *Serb. Chem. Soc.* **2005**, *70*, 67.
 (16) Jalali, F.; Afshoon, A. J. *Fluoresc.* **2008**, *18*, 219.
 (17) Jalali, F.; Afshoon, A.; Shamsipur, M. *Chem. Anal. (Warsaw)* **2007**, *52*, 115.
 (18) Kedor-Hackmann, E. R. M.; Santoro, Ma. I. R. M.; Singh, A. K.; Peraro, A. C. *Braz. J. Pharm. Sci.* **2006**, *42*, 1.
 (19) Abou-Attia, F. M.; Issa, Y. M.; Abdel-Gawad, F. M.; Abdel-Hamid, S. M. *IL Farmaco* **2003**, *58*, 573.
 (20) El-Ragehy, N. A.; EL-Saharty, Y. S. *J. AOAC* **2001**, *84*, 563.
 (21) Arranz, A.; Echevarria, C.; Moreda, J. M.; Cid, A.; Arranz, J. F. *J. Chromatogr., A* **2000**, *871*, 399.
 (22) Abdel-Moety, E. M.; Khattab, F. I.; Kelani, K. M.; Abou Al-Alamein, A. M. *IL Farmaco* **2002**, *57*, 931.
 (23) Morsy, M. A.; Khaled, M. J. *Agric. Food Chem.* **2001**, *49*, 683.
 (24) Morsy, M. A.; Khaled, M. *Spectrochim. Acta, Part A* **2002**, *58*, 1271.
 (25) Morsy, M. A. *Spectros. Int. J.* **2002**, *16*, 371.
 (26) Morsy, M. A.; Shwehdi, M. H. *Spectrochim. Acta, Part A* **2006**, *63*, 624.
 (27) Al-Karmi, A. M.; Morsy, M. A. *Radiat. Meas.* **2008**, *43*, 1315.
 (28) Sultan, S. M.; Al-Turabi, M. O.; Hwang, J. S. *Talanta* **2000**, *51*, 327.

- (29) Shamsipur, M.; Farhadi, Kh. *Chem. Anal.* **2001**, *46*, 387.
 (30) Shamsipur, M.; Farhadi, Kh. *Analyst* **2000**, *125*, 1639.

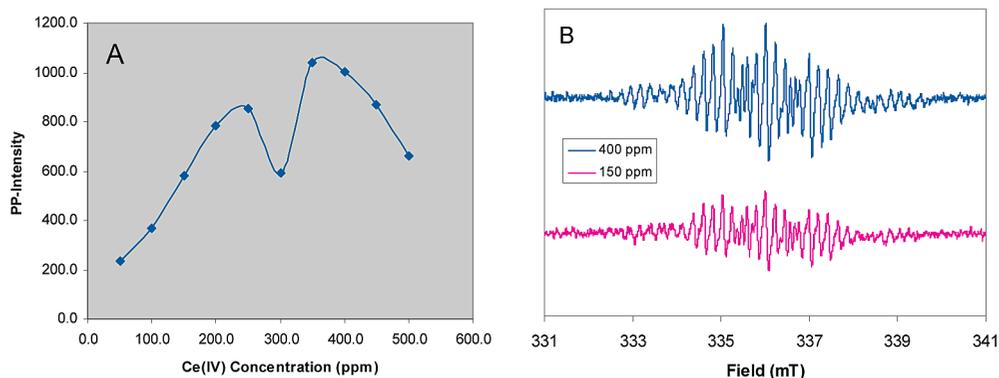


Figure 3. Effect of different Ce(IV) concentrations on the observed EPR signal amplitude in the range of 50–500 ppm (A) and a representative EPR spectra before and after the saddle point at 300 ppm (B) of the reaction mixture containing 0.5 mmol dm^{-3} of KTZ after 2.5 min.

For the shampoo sample, an accurately weighed portion of the KTZ shampoo equivalent to 10 mg of the drug was dissolved in 0.10 mol dm^{-3} sulfuric acid, filtered, and washed with hot acid. The supernatant liquid was made up to a volume of 100 mL in a volumetric flask and kept as the stock solution, from which other working solutions were prepared.

Sulfuric Acid Solution (0.1 mol dm^{-3}). A stock solution of 0.1 mol dm^{-3} H_2SO_4 (98% specific gravity 1.84 kg dm^{-3} , Merck, U.K.) was prepared the usual way. Working solutions were prepared by dilution.

Cerium(IV) Solution ($1000 \mu\text{g/mL}$). A stock solution was prepared by dissolving a sufficient amount of dried cerium(IV) ammonium sulfate [$\text{Ce}(\text{NH}_4)_4\text{SO}_4 \cdot \text{XH}_2\text{O}$] (Fluka AG, CH-9470 Buchs, Switzerland) in 0.1 mol dm^{-3} sulfuric acid and diluting to 1000 mL with the same acid solution in a calibrated flask.

Electron Paramagnetic Resonance (EPR) Spectroscopy. A JEOL ER-series EPR spectrometer operating at X-band frequency and a modulation frequency and amplitude of 100 kHz and 0.1 G, respectively, were used. The data were transferred to a PC and saved in a format so that they could be read by EXCEL, a program with which the data were analyzed. Microwave frequencies were measured with a Hewlett-Packard 5342 A microwave frequency counter.

RESULTS AND DISCUSSION

Figure 1 shows two spectra: one of them for the radicals produced as a result of the oxidation of pure KTZ with cerium(IV) in 0.1 mol dm^{-3} sulfuric acid and the second one presents its simulated spectrum. It is worth mentioning here that the simulated EPR spectra has been generated utilizing the WinSim EPR software from NIEHS.³¹ The resultant simulated spectrum indicates that the drug undergoes fragmentation of the two ends of the KTZ compound producing imidazole and acetamide radical species. The spectral pattern of the former radical is well in agreement with a previously reported spectrum of a delocalized unpaired electron on an imidazole ring in a lophine-X anion radical.³² The simulation also indicates that the imidazole and the acetamide radicals contribute to the total signal area by 83.3% and 16.7%, respectively.

Furthermore, the simulation indicated that the imidazole hyperfine splitting arises from two semiequivalent nitrogen atoms

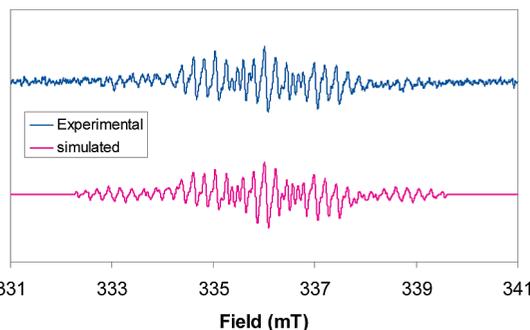


Figure 4. EPR experimental (top) and simulated (bottom) spectra for the oxidation of $400 \mu\text{g/mL}$ KTZ in 0.1 mol dm^{-3} sulfuric acid with $150 \mu\text{g/mL}$ cerium(IV).

with hyperfine constants $a_N = 1.41 \pm 0.02 \text{ mT}$ that will produce five distinct main groups with an intensity ratio of 1:4:6:4:1. These groups are due to three distinct sets of proton coupling, two on an imidazole ring and the third on an *N*-methylene group, with coupling constants $a_H = 0.28 \pm 0.03$ (2 protons), 1.58 ± 0.02 (1 proton), and 0.49 ± 0.03 (2 protons) mT, respectively. On the other hand, the acetamide nitrogen produces three groups of $a_N = 0.97 \pm 0.02 \text{ mT}$; each of the group is due to two equivalent sets of protons around the amide functional group. A set consists of four protons with coupling constants equal to $0.43 \pm 0.02 \text{ mT}$ and the other one of two protons with a coupling constant equal to $0.21 \pm 0.02 \text{ mT}$.

The lifetime of these radicals (about 15 min) is sufficient to allow one to search for the best EPR signal intensity using the univariate method.^{33,34} The variables optimized are sulfuric acid concentration, sampling and sample size using capillary tubes, and cerium(IV) concentration. Testing a series of acid concentration in the range of $0.05\text{--}0.40 \text{ mol dm}^{-3}$, holding the concentration constant at 0.5 mmol dm^{-3} KTZ, demonstrated that the optimal acid concentration is 0.1 mol dm^{-3} .

For the sampling and sample size, the study was limited to commercially available capillary tubes of 75 mm in length, 0.5–0.9 mm in internal diameter (i.d.), and $40 \mu\text{L}$ in full volume, because of the reported independency of signal intensity on the microwave power (MP) using a $100 \mu\text{L}$ flat cell.^{35,36} Different numbers of tubes were housed in regular EPR sample tubes of different

(33) Idris, A. M.; Assubaie, F. N.; Sultan, S. M. *Microchem. J.* **2006**, *83*, 7.

(34) Gardiner, W. P. *Statistical analysis methods for chemists*; The Royal Society of Chemistry: London, England, 1997.

(35) Minakata, K.; Suzuki, O. *Anal. Chem.* **2002**, *74*, 6111.

(31) Duling, D. R. *J. Magn. Reson.* **1994**, Series B, *104*, 105.

(32) Ueda, H. *J. Phys. Chem.* **1964**, *68*, 1304.

Table 1. Recovery Tests of KTZ Solution Systems

recovery level	amount spiked,		active substances		
	mg/mL	fixed time signal	amount found ^a , mg/mL	% recovery ^b	average recovery, %
1 (80%)	0.2020	1551	0.2018	99.89	100.6
	0.2010	1545	0.2013	100.2	
	0.1990	1558	0.2023	101.7	
2 (90%)	0.2250	1923	0.2289	101.7	101.4
	0.2280	1944	0.2304	101.1	
	0.2260	1931	0.2294	101.5	
3 (100%)	0.2520	2218	0.2503	99.34	100.0
	0.2500	2215	0.2501	100.1	
	0.2480	2209	0.2497	100.7	
4 (110%)	0.2750	2556	0.2749	99.98	100.1
	0.2730	2557	0.2750	100.7	
	0.2760	2553	0.2747	99.54	
5 (120%)	0.3010	2904	0.3003	99.76	99.34
	0.3030	2895	0.2996	98.89	
	0.3020	2901	0.3001	99.36	

^a mg/mL found = ((fixed time signal – standard fixed time signal)/slope of regression analysis). ^b Percent recovery = amount found/amount spiked × 100.

internal diameter. The variation of the tube's number indicated that the maximum EPR signal had been achieved using eight capillary tubes housed in a 4 mm EPR sample tube. This type of sampling enhances the spectral intensity compared to that of five capillary tubes housed in a 3 mm EPR sample tube to about 55% (Figure 2).

To examine the effect of cerium(IV) concentration on the EPR signal, a series of cerium(IV) solutions with a concentration ranging between 50 and 400 µg/mL was tested using the eight capillary tubes in a 4 mm ESR sample tube of 0.10 mol dm⁻³ sulfuric acid reaction mixture at a fixed time of 2.5 min. The variation of the signal amplitude with cerium(IV) concentrations is shown in Figure 3A. The variation of cerium(IV) concentration resulted in a peculiar behavior showing two overlapping parabolas intersecting when the cerium(IV) concentration reached 300 µg/mL, thus developing a saddlelike shape. This is most probably due to the presence of different radical species based on the concentration range of Ce(IV) Figure 3B. The simulated spectra in Figure 1 above indicated that a ratio of 83.3% and 16.7% for the imidazole and the acetamide radicals, respectively, were obtained in a reaction mixture with a concentration higher than 300 ppm cerium(IV).

At a concentration lower than 300 ppm cerium(IV), explicitly at 150 µg/mL, the simulation results indicated that the same radical species exist but with opposite distribution (see Figure 4). The ratio of the radicals at 150 µg/mL are 14.5% and 85.5% for the imidazole and the acetamide radicals, respectively.

These findings are in good agreement with the results obtained in the titrimetric method for the assay of KTZ using a tri-iodide oxidant where the obtained results from the determination of an unreacted tri-iodide ion in filtrate solutions clearly indicated that each mole of KTZ reacts with two moles of I₃⁻ ions.³⁷ Additional

Table 2. Tablet and Cream Samples' Precision Test of KTZ Solution Systems

trial #	ATW, mg	amount spiked, g		fixed time signal		assay, mg	
		tablet	cream	tablet	cream	tablet	cream
1	250.0	0.2510	0.2510	2378	1997	267.5	224.7
2		0.2510	0.2530	2392	2017	269.1	225.1
3		0.2510	0.2500	2375	1998	267.2	225.7
4		0.2530	0.2510	2376	1965	265.2	221.1
5		0.2500	0.2520	2377	1958	268.5	219.4
6		0.2540	0.2520	2373	1958	263.8	219.4
					mean	266.9	222.6
					SD	2.020	2.930
					% RSD	0.7600	1.320

evidence was reported earlier in the cyclic voltamograms of doxazosin and trazodone.^{29,30,38,39}

Analytical Appraisals. Under the optimal conditions investigated above, a series of solutions of different KTZ concentrations was scanned at different fixed times between 2.5 and 12.5 min with time intervals of 2.5 min using 200 µg/mL or 400 µg/mL Ce(IV) solutions. Regardless the type of radical species, the plot of the disappearance of the radical species shows that the disappearance is apparently of zero order. Moreover, the zero-time intercept of the EPR signal amplitude, which should be proportional to the initial radical concentration, is linear in the sample concentration. The reaction system with a larger quantity of acetamide radicals was linear in the KTZ concentration range between 100 and 200 µg/mL while the richer reaction system of imidazole radicals was linear over much wider range of KTZ, 150 to 400 µg/mL.

EPR Method Validation. The EPR method of KTZ drug formulation is validated following the USP monograph protocol.¹¹ The linearity was demonstrated for the standard solution over a range of 50–150% of a solution containing 250 µg/mL KTZ using 400 µg/mL cerium(IV) and 0.1 mol dm⁻³ sulfuric acid. The zero-time intercepts of the EPR signal disappearance produce a linear relation in the KTZ sample concentration in the range of 150–350 µg/mL, with a correlation coefficient, *r*, of 0.999 and a relative standard deviation of 1.5% of three runs with the following calibration equation:

$$I = (13.74 \pm 0.14)C - (1222 \pm 255)$$

(36) Burns, D. T.; Flockhart, B. D. *Philos. Trans. R. Soc. London, Ser. A* **1990**, *333*, 37.

(37) Farhadi, K.; Maleki, R. *J. Pharm. Biomed. Anal.* **2002**, *30*, 1023.

(38) Arranz, A.; Fernandez de Betono, S.; Moreda, J. M.; Cid, A.; Arranz, J. F. *Analyst* **1997**, *122*, 849.

(39) Kauffmann, S. M.; Vire, J. C.; Patriarche, G. J. *Electrochim. Acta* **1987**, *32*, 1159.

Table 3. EPR and USP Methods for Assaying KTZ in Tablet Samples

drug proprietary name	supplier	contents (mg)/tablet	mean recovery \pm RSD, % ^a		t ^b test value
			EPR method	USP ^c method	
Nizoral	Janssen Pharmaceutica N. V., Turnhoutseweg 30, B-2340 Beerse, Belgium	ketoconazole 225	101.4 \pm 0.3	100.3 \pm 1.2	0.3
		ketoconazole 250	100.0 \pm 0.1	99.50 \pm 1.3	0.1
		ketoconazole 275	100.1 \pm 0.1	100.1 \pm 1.1	0.1

^a Relative standard deviation for three replicates. ^b Student *t* test values. ^c United States Pharmacopoeia.

where *I* is the intercept of the linear plot of the radical decay and *C* is concentration in micrograms per milliliter. The detection limit was determined to be $11.7 \pm 2.5 \mu\text{g}/\text{mL}$ using the equation: $\text{DL} = (3.3 \times s/S \pm t_{p,v} \sigma/s)$, where DL is the detection limit, *s* is the standard deviation of responses, *S* is the slope of the calibration curve, and the uncertainty in DL is amplified by the confidence interval for σ/s . The large intercept value is certainly due to the formation of an irreversible fragmentation reaction once the reaction mixture takes place. This phenomenon has been observed by the formation of an instantaneous brown color upon mixing, which slowly decays with time.

The recovery tests were performed against the standard solution by preparing the drug without excipients at an 80, 90, 100, 110, and 120% level of the final sample concentration. The results are listed in the Table 1 and indicate that the recovery test of the EPR assay approaches 100% (average recovery = $100.3 \pm 0.8\%$).

The precision of the EPR assay procedure was also investigated by the determination of the standard deviation (SD) of the repeatability of the method for six determinations of tablet and cream samples under the same analytical conditions. The listed results in Table 2 indicated that the EPR is also highly reproducible as the precision of the results obtained for the tested tablet and cream samples were found to be quite reasonable, %SD =

0.76 and 1.32%. In shampoo samples, no radical was detected, most probably due to the quenching ability of the shampoo excipients.

The accuracy of the method was investigated by assaying the same batch of proprietary drugs of KTZ using the EPR method and the standard USP method. The results obtained are chemometrically compared by calculating the student *t* test values introduced in Table 3. All calculated *t* test values were found to be less than the tabulated values at the 99.9% confidence level, indicating accuracy of the EPR method. In addition, the results obtained also indicate that the present method suffers no interferences from the excipients already existing in these formulations.

CONCLUSIONS

The EPR spectroscopic technique has proven to be successful for the quantitative determination of KTZ in tablet and cream preparations. The method thus adopted was found to be precise, accurate, specific, and fast and consumed less reagents compared to the previously reported methods.

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