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Stability-Indicating Methods for the Determination of Ornidazole in The Presence of its Degradate According to ICH Guidelines

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Abstract

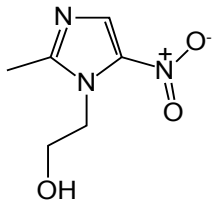
Four simple, sensitive, selective and precise methods were developed for the determination of Ornidazole (OZ) in presence of its degradation product. The first method was based on first derivative spectrophotometry D^1 and measuring the peak amplitude of D^1 spectra at 290.4 and 332 nm. The second method was depended on measuring the peak amplitude of the first derivative of the ratio spectra DD^1 at 288.5 and 328 nm. The third method was the mean centering of the ratio spectra one (MCR), which allowed the determination of OZ in presence of its degradate and the concentration of OZ was determined by measuring the amplitude at 312.8 nm. Separation and determination of OZ by HPLC in the forth method was achieved using Lichrosorb RP-18 column and acetonitrile: water, (50:50 v/v), 0.2% triethylamine, the pH was adjusted to 4 using o-phosphoric acid. The flow rate was 1 mL min^{-1}. Beer's law was obeyed in concentration range 5–30 µg/ml for the first three methods. The linearity range in the forth method was 2-20 µg/ml. The proposed methods were used to determine OZ in its pure powdered form with mean percentage recoveries of 99.86 ± 1.249% and 99.98 ± 0.868% for OZ at 290.4 and 332 nm respectively, in D^1 method. In DD^1 method, the mean percentage recoveries were 100.11 ± 1.020% and 100.15 ± 1.043% at 288.5 and 328 nm respectively. While in MCR and HPLC methods, the mean percentage recoveries were 100.09 ± 0.387% and 100.00 ± 1.302% respectively. The degradation product was obtained in alkaline stress condition, separated, and identified by LC-MS spectral analysis, from which the degradation product was confirmed. The four methods were validated according to International Conference on Harmonization. The four methods were found to be specific for OZ in presence of up to 80% of its degradation product in the first three methods. The four proposed methods were successfully applied for the determination of OZ in Tibezole® tablets. Statistical comparison between the results obtained by these methods and the reported method for the determination of the drug in its pharmaceutical formulation was done, and it was found that there was no significant difference between them.

Keywords: Orindazole; Derivative; Ratio derivative; Mean centering spectrophotometry; HPLC

Introduction

It is considered as antibacterial and antiprotozoal. OZ (Figure 1a) is converted into an active form by reduction of its nitro group, this binds to DNA and prevent nucleic acid formation; it is a bacteriostatic [1,2]. Ornidazole used for treatment of bacterial vaginosis, trichomoniasis, genitourinary infections in women and men due to *Trichomonas vaginalis*, amoebiasis (all intestinal infections due to *Entamoeba histolytica*, including amoebic dysentery, all extra intestinal forms of amoebiasis, especially amoebic liver abscess), Giardiasis (lambliasis). It is also used in infections due to anaerobic bacteria (such as septicaemia, meningitis, peritonitis, postoperative wound infections, puerperal sepsis, septic abortion, and endometritis). OZ also used in the treatment of prophylaxis during surgical interventions, particularly those involving the colon, and in gynaecological operations [2].

Many analytical methods were reported for detection and determination of ornidazole in bulk powder, pharmaceutical formulations alone or in combination with other drugs and/or in biological fluids using titremetry [3], spectrophotometry [4-6], HPLC [7-15], HPTLC [16,17], GC [18], voltammetry [19-21] and Chemiluminescent method [22]. None of the reported methods was concerned with the determination of the intact drug in presence of its degradation product. This paper presents a study of alkaline, acidic, oxidative, thermal and photo degradation of OZ, followed by the development of 4 stability-indicating procedures for the determination of the drug in its pure form, in presence of its degradate and in tablet formulations. The scientific novelty of the present work is that the methods used are simple, rapid, and selective. The focus of the present study was to develop and validate different methods for the determination of OZ in its tablets dosage form.



Molecular formula=C6H9N3O3; Molecular weight=171.15

Figure 1a: Structure of Orindazole.

Experimental

Instrumentation

- A double beam UV–visible spectrophotometer (SHIMADZU,

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Japan) model UV-1601 PC with quartz cell of 1 cm path length, connected to IBM compatible computer. The software was UV-PC personal spectroscopy software version 3.7. The spectral bandwidth was 2 nm and wavelength-scanning speed 2800 nm/min.

- UV-lamp with short wavelength 254 nm (USA).
- An Agilent 1100 series HPLC instrument (Agilent,Waldbronn, Germany), with an isocratic pump model G 1310 A pump; (Agilent, Waldbronn, Germany), connected with UV-Visible spectrophotometric detector model G 1314 A, set at 311 nm, (Agilent, Waldbronn, Germany), Injection was performed with a A Rheodyne injector model 7225/7725I, (Rohnert park, CA., USA) equipped with 20 µl injector and Hamilton syringe (100 µl). The instrument was connected to an IBM compatible PC, bundled with Merck- Hitachi model D-7000 HPLC System Manager Chromatography Data Station Software, HP 800 inkjet printer, and Lichrosorb RP-18 (250 mm×4.6 mm i.d) column particle size (5 µm); (USA).

Reference sample

Ornidazole (OZ) pure sample was kindly supplied by El Pharonia-Pharmaceuticals, New Borg El-Arab City, Alexandria, A.R.E; it was certified to contain 99.90% w/w according to the manufacturer's method.

Pharmaceutical formulation

Tibezole Tablets, batch no. 1319003, are labeled to contain 500 mg OZ and were manufactured by El Pharonia-Pharmaceuticals, New Borg El-Arab City, Alexandria, A.R.E. and obtained from the local market.

Degraded sample

Degraded OZ sample (OZ Deg) was prepared by accurately weighing 50 mg of OZ pure sample, refluxed with 50 mL 0.5 N NaOH for 15 minutes and neutralized with 1 N HCl, then transferred quantitatively to 100-mL measuring flask and the volume completed to the mark with methanol. Complete degradation was confirmed by TLC using methanol: chloroform: ether (1:3:9 by volume) as the mobile phase.

Reagents

Methanol, Ether, Chloroform (Prolapo, VWR, international, West Chester, PA), Sodium hydroxide, Hydrochloric acid (Merck, Dramstadt, Germany), Acetonitrile: HPLC grade, (Merck, Darmstadt, Germany), Water for HPLC and Orthophosphoric acid: HPLC grade.

Standard solutions

- Stock standard solution of OZ in the concentration of 0.5 mg mL^{-1} for D^1, DD^1, MCR and HPLC methods. Prepared by accurately weighing 50 mg of OZ into 100-mL measuring flask and completing the volume with methanol.
- Stock solution of OZ Deg in the concentration of 0.5 mg mL^{-1} for D^1, DD^1, MCR and HPLC methods. Prepared by accurately weighing 50 mg of OZ Deg into 100-mL measuring flask and completing the volume with methanol.
- Working standard solution of OZ and working solution of OZ Deg in the concentration of 0.05 mg mL^{-1} for D^1, DD^1, MCR and HPLC methods. Prepared by an additional dilution of the respective stock solutions with methanol.

Laboratory prepared mixtures containing different ratios of OZ and its degradation product for D^1, DD^1 and MCR methods

Aliquots (5.4–1.2 mL) of OZ equivalent to 270–60 µg were accurately transferred from its working standard solution (0.05 mg mL^{-1}) into a set of 10-mL measuring flasks to which aliquots (1.2–5.4 mL) of OZ Deg equivalent to 60–270 µg from its working solution (0.05 mg mL^{-1}) were accurately added. The volumes were then completed with methanol to prepare mixtures containing from 10–80% of OZ Deg.

Procedures

Construction of the calibration graphs for (D^1), (DD^1), (MCR) and HPLC methods for D^1 method: aliquots of OZ working standard solution (0.05 mg mL^{-1}) equivalent to 50-300 µg mL^{-1} were accurately transferred into a series of 10-mL measuring flasks and the volume was completed to the mark with methanol. The prepared solutions were scanned against methanol and the first derivative spectra (D^1) of the scanned spectra were recorded using $\Delta\lambda$=4 and scaling factor =10. Calibration curves were then constructed by plotting the values of the peak amplitude of D^1 spectra at 290.4 and 332 nm (corresponding to zero absorbance of degradate) versus the corresponding concentrations and the regression parameters were computed.

For DD^1 method: The zero-order spectra of the prepared solutions were divided by a standard spectrum of 25 µg mL^{-1} OZ Deg and the first derivative of the ratio spectra (DD^1) were then obtained with $\Delta\lambda$=4 and scaling factor=10. Calibration curves were constructed by plotting the peak amplitude at 288.5 and 328 nm versus the corresponding concentrations of OZ and the regression parameters were computed.

For MCR method: The zero-order spectra of the prepared solutions were scanned in the range of 200-400 nm. The scanned spectra of OZ were divided by the normalized absorption spectrum of OZ Deg and the obtained ratio spectra were then mean centered. The calibration curve for OZ was constructed by plotting the mean centered values at 312.8 nm versus the corresponding concentrations of OZ.

For HPLC method: Aliquots (0.4-4.0 ml) of OZ working standard solution (0.05 mg mL^{-1}) equivalent to 20-200 µg, were accurately transferred into set of 10-mL measuring flasks and the volume was then completed to the mark with the mobile phase. Using a 100-µL syringe, a 20-µL volume of each solution was injected in triplicate into the liquid chromatography at ambient temperature under the following chromatographic conditions:

- Stationary phase: Lichrosorb RP-18 column (250 mm×4.6 mm I.D), particle size is 5 µm.
- Mobile phase: acetonitrile: water, (50:50 v/v), 0.2% triethylamine, the pH was adjusted to 4 using o-phosphoric acid (The mobile phase was filtered using 0.45 µm membrane filters and degassed by ultrasonic vibrations for 30 minutes prior to use).
- Flow rate: 1 mL/min.
- Wavelength: 311 nm.
- The sensitivity: was set at 0.001 AUFS.
- Column temperature: 25°C.

A linear calibration curve was constructed for OZ relating the relative peak area (calculated following the external standard technique

using an external standard of 6 μg mL^{-1}) of OZ, to the corresponding concentrations, and the corresponding regression equation was computed.

Application of the proposed methods for the analysis of laboratory prepared mixtures of intact drug and its degradation product for (D^1), (DD^1) and (MCR) methods

The absorption spectra of the laboratory prepared mixtures were recorded. Then the procedures were completed as described under construction of the calibration graphs. The concentrations of OZ were calculated by substituting in the corresponding regression equation.

Application of the four proposed methods to the analysis of OZ in pharmaceutical preparation

Twenty Tibezole® tablets [labeled to contain 500 mg OZ] were accurately weighed and finely powdered. An accurate weight of the powdered tablets equivalent to 50 mg of OZ was transferred into a 100-mL measuring flask and extracted with 50 mL methanol in an ultrasonic bath for 30 min., diluted to volume with the same solvent and filtered. Suitable dilutions were made using methanol to prepare a tablet solution containing 10 μg mL^{-1}OZ, then the procedure was completed as described under construction of the calibration graphs. The concentrations of OZ were calculated from the corresponding regression equation.

Results and Discussion

By reviewing the reported stress stability testing of OZ as stated by the ICH guidelines, Monika et al. proved that OZ is liable to acid, alkaline, oxidative and photo degradation.

In this work, we were concerned with the alkaline degradation of OZ as it is completely degraded under very mild conditions.

Upon trying the previously reported conditions for alkaline degradation (0.1 N NaOH at zero time) [9] the drug is not completely degraded as confirmed by TLC [using methanol: chloroform: ether ((1:3:9) by volume) as the mobile phase.

So, several trials have been made to obtain complete hydrolysis of OZ and it was found that upon refluxing with 0.5 N NaOH at 80°C, complete degradation occurred after 15 minutes and the degradation process was followed by TLC.

In the present work and according to Salman and Sümer [23] the alkaline hydrolysis of OZ resulted in the removal of the nitro group and furthermore losing its pharmacological antibacterial activity [1], thus it was of a potential task to develop different stability indicating methods.

This was confirmed by treating the cooled degradate solution with standard sulfanilamide, the violet color produced indicated the presence of the nitrite ions in the solution. Also the spectrum of the degradation product showed the disappearance of the conjugation caused by the nitro group which resulted in the disappearance of its λ_{max}, (Figure 2), further more the degradation products were subjected to LC- MS analysis, (Figure 3), which clarify the disappearance of the molecular ion peak of the drug at 219 m/z and the appearance of two new molecular ion peaks corresponding to the two degradates at 157 and 174 m/z, (Figure 1b).

Trials have been made to separate the two degradation products by TLC and HPLC, using several systems with different ratios and different flow rates in case of HPLC methods. Unsuccessful results were obtained; therefore we consider the solution of the degradation as one product.

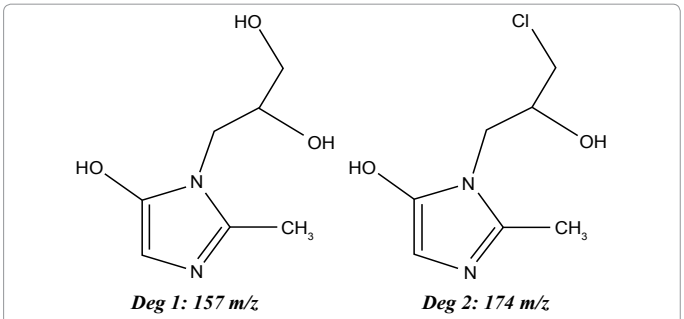

Figure 1b: Structure of Orindazole Degradation products 1 & 2.

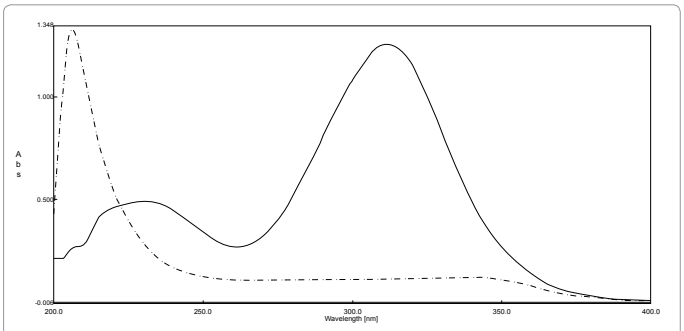

Figure 2: D^0 of 30 μg mL^{-1} of OZ (——) and OZ Deg(-·-·-·-) in methanol.

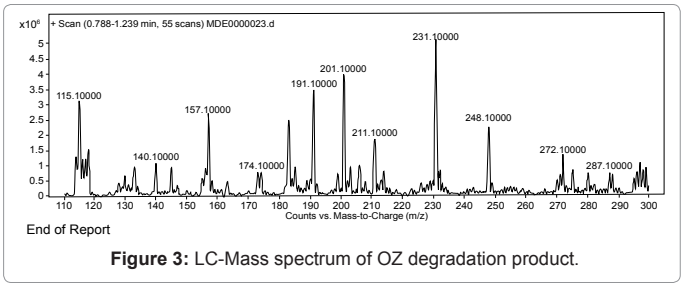

Figure 3: LC-Mass spectrum of OZ degradation product.

The degradation process was also monitored by HPLC [Column: C-18; mobile phase: water: acetonitrile (60:40 v/v); detection wavelength: 311 nm; flow rate: 1 mL min^{-1}] (Figures 4-7).

First-derivative (D^1), first derivative of the ratio spectrum (DD^1) and mean centering of the ratio spectra (MCR) spectrophotometric methods

The zero-order spectra of OZ and its degradation product show an overlap, (Figure 2, that prevents the use of direct spectrophotometric analysis of the drug in the presence of its degradation product. In an attempt to resolve this overlap, derivative, derivative ratio and mean centering of the ratio spectra methods were applied. Upon examining the first derivative spectra of OZ and degradate, (Figure 8), it was noticed that OZ could be determined at 290.4 nm and 332 nm, where degradate showing no interference.

Linearity of the peak amplitude at 290.4 nm and 332 nm with the concentrations of OZ was studied and calibration curves were constructed.

The proposed procedure was found to be valid in the range of 5-30 µg mL^{-1}, and the regression equations found to be:

$D^1_{290.4}$ = 0.0097C + 0.0011 r = 0.9998

D^1_{332} = 0.012C - 0.0047 r = 0.9999

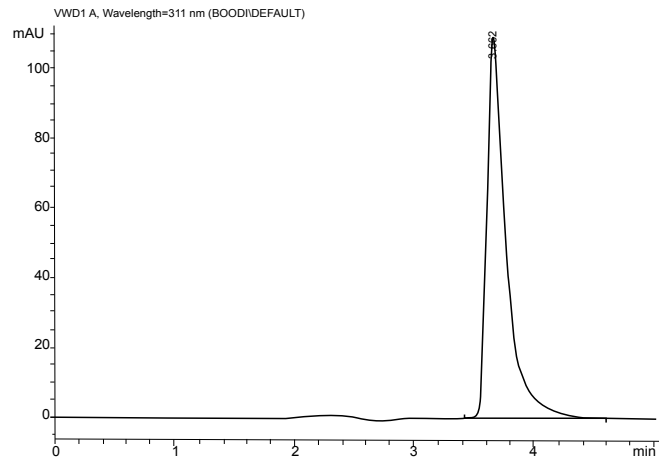

Figure 4: HPLC chromatogram of 10 µg/ ml OZ in 0.5M NaOH, at zero minute.

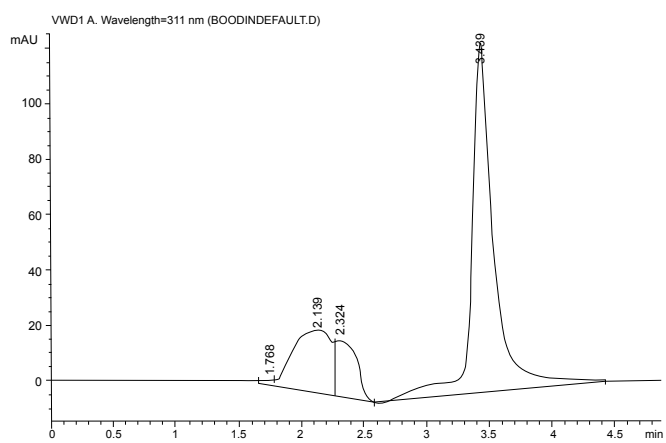

Figure 5: HPLC chromatogram of 10 µg/ ml OZ in 0.5M NaOH, after 5minutes

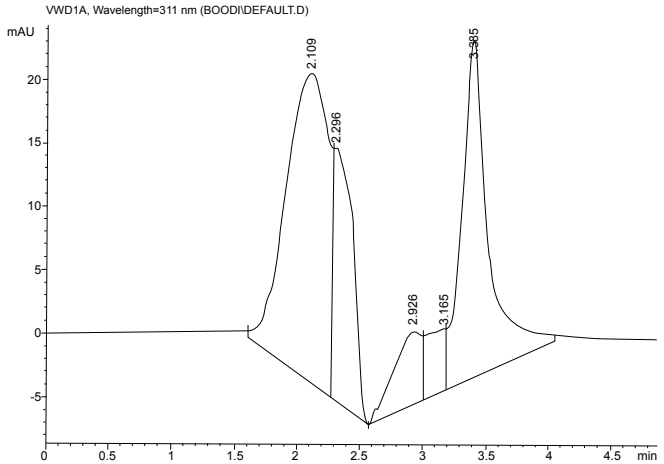

Figure 6: HPLC chromatogram of 10 µg/ ml OZ in 0.5M NaOH, after 10 minutes.

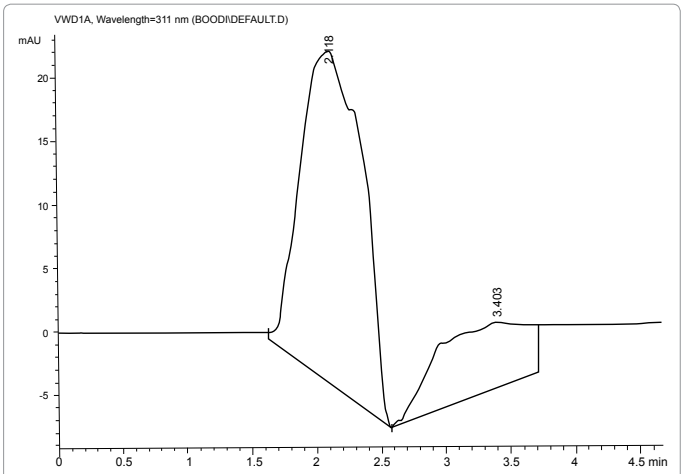

Figure 7: HPLC chromatogram of 10 µg/ ml OZ in 0.5M NaOH, after 15 minutes.

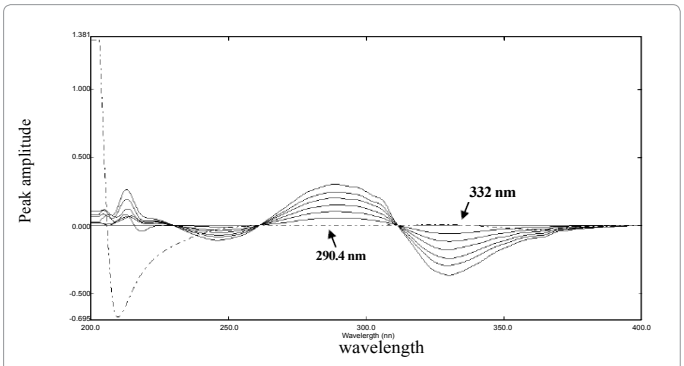

Figure 8: D^1 of (5-30 µg mL^{-1}) of OZ (——) and 30 µg mL^{-1} of OZ Deg (-·-·-·) in methanol.

Where, $D^1_{290.4}$ and D^1_{332} is the peak amplitudes at 290.4 nm and 332 nm, respectively, C are the concentration of OZ in µg mL^{-1} and r is the correlation coefficient.

The proposed method was successfully applied for the determination of OZ in its pure powdered form with mean percentage recovery of 99.86 ± 1.249% and 99.98 ± 0.868% at 290.4 nm and 332 nm, respectively (Table 1).

Also, in order to achieve good resolution and remove this interference, DD^1 method was established. The main advantage of this method is that the whole spectrum of the interfering substance is cancelled. In order to optimize DD^1 method, several divisors were tested as 5, 10, 15, 20, 25 and 30 µg mL^{-1} along with the normalized OZ Deg spectrum. The best results were obtained on using a standard spectrum of 25 µg mL^{-1} OZ Deg as a divisor. The absorption spectra of OZ in the concentration range of 5-30 µg mL^{-1} were divided by the absorption spectrum of 25 µg mL^{-1} OZ Deg to obtain the ratio spectra, (Figure 9), then the first derivative of the obtained ratio spectra were then calculated using Δλ=4 and scaling factor=10 as shown in Figure 10.

DD^1 values showed good linearity and reproducibility at 288.5 and 328 nm, the corresponding regression equations were found to be:

$DD^1_{288.5}$ = 0.0992C - 0.0015 r = 0.9998

DD^1_{328} = 0.1248C - 0.0305 r = 0.9997

Parameter	D^1 method		DD^1 method		MCN method	HPLC method
	290.4 nm	332 nm	288.5 nm	328 nm		
Range	5-30 µg mL^{-1}		5-30 µg mL^{-1}		5-30 µg mL^{-1}	2-20 µg mL^{-1}
Slope	0.0097	0.0120	0.0992	0.1248	0.0530	0.1334
Intercept	0.0011	-0.0047	-0.0015	-0.0305	-0.0039	-0.0318
SE of the slope	8.463×10^{-5}	8.443×10^{-5}	0.0010	0.0014	0.0001	0.0009
SE of the intercept	0.0016	0.0016	0.0193	0.0271	0.0021	0.0113
Correlation coefficient(r)	0.9998	0.9999	0.9998	0.9997	1	0.9998
LOD	0.5364	0.4345	0.6178	0.6894	0.1281	0.3884
LOQ	1.6255	1.3167	1.8798	2.0889	0.3881	1.1769
Accuracy (mean ± RSD %)	99.86 ± 1.249	99.98 ± 0.868	100.11 ± 1.020	100.15 ± 1.043	100.09 ± 0.387	100.00 ± 1.302
Precision	0.847	0.853	0.508	0.582	0.631	0.935
Repeatability %						
Intermediate precision %	0.977	0.733	0.615	0.743	0.584	0.749

Table 1: Validation parameters of the proposed method for the determination of OZ pure samples.

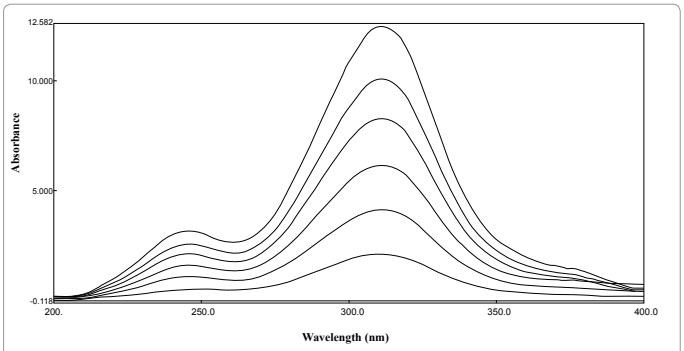

Figure 9: Zero order of ratio spectra of (5-30 µg mL^{-1}) of OZ using 25 µg mL^{-1} of OZ Deg as a divisor.

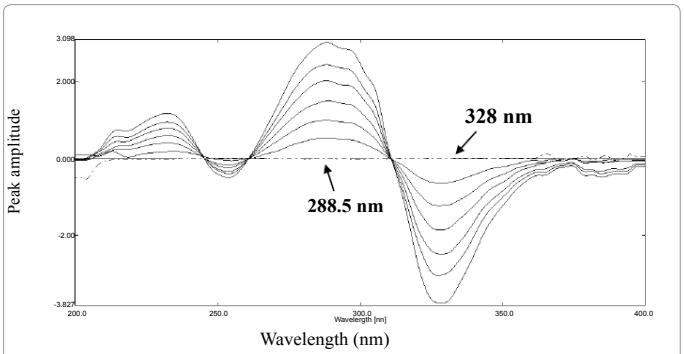

Figure 10: DD^1 of OZ (——) (5-30 µg mL^{-1}) and 15 µg mL^{-1} of OZ Deg (-·-·-·), in methanol, using 25 µg mL^{-1} of OZ Deg as a divisor.

Where, $DD^1_{288.5}$ and DD^1_{328} are the peak amplitudes at 288.5 nm and 328 nm, respectively, C is the concentration of OZ in µg mL^{-1} and r is the correlation coefficient.

The proposed method was successfully applied for the determination of OZ in its pure powdered form with mean percentage recoveries of 100.11 ± 1.020% and 100.15 ± 1.043% at 288.5 nm and 328 nm, respectively (Table 1).

Also, mean centering method was applied to resolve this overlap so, for the determination of OZ, the absorption spectra of the standard OZ solutions with different concentrations were recorded in the range of 200-400 nm, divided by the normalized OZ Deg spectrum and the ratio spectra were obtained (Figure 11).

The obtained ratio spectra were then mean centered and the concentration of OZ was determined by measuring the amplitude at 312.8 nm (corresponding to a maximum wavelength) (Figure 12).

The calibration curve relating the mean centered (MCN) values at 312.8 nm to the corresponding concentrations of OZ was constructed. The proposed method was found to be valid in the concentration range of 5-30 µg mL^{-1}, and the regression equation was found to be:

$$MCN_{312.8} = 0.053C - 0.0039 \quad r = 1$$

Where, MCN is the mean centered values at 312.8 nm, C is the concentration of OZ in µg mL^{-1} and r is the correlation coefficient.

The effect of divisor concentration on the analytical parameters such as slope, intercept and correlation coefficient of the calibration graphs was also tested. Different concentrations of divisor were tested but it was observed that changing the concentration had no significant effect on their linear calibration range and the calculated analytical parameters. Therefore, a normalized spectrum of OZ Deg was used as a divisor spectrum in the proposed method.

The proposed method was successfully applied for the determination of OZ in its pure powdered form with mean percentage recovery of 100.09 ± 0.387% (Table 1).

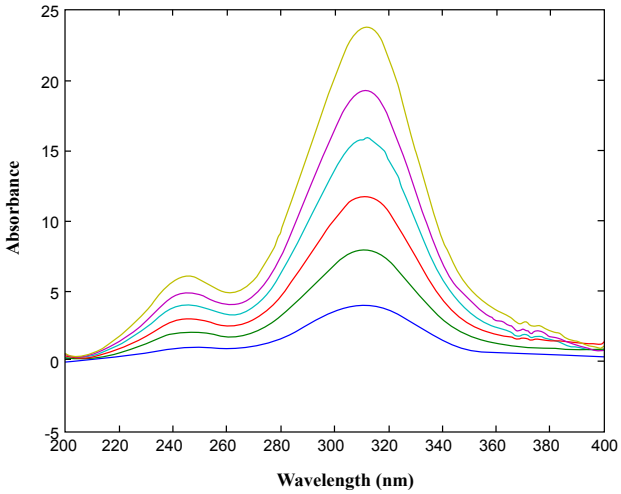

Figure 11: Ratio spectra of OZ (5-30 µg mL^{-1}), using OZ Deg normalized spectrum as a divisor.

HPLC method

In this work, a RP-HPLC method is described for the determination of OZ in presence of OZ Deg without prior separation. This method aims to develop a simple stability indicating HPLC assay for the analysis of OZ.

In order to optimize the proposed HPLC method, all the experimental conditions were investigated. Several trials were carried out to obtain good and optimum separation of OZ from its degradation products. Different composition mobile phases with different ratios were tried such as methanol: water (50:50, v/v), and acetonitrile: water (50:50, v/v) best resolution was obtained upon using acetonitrile: water (50:50, v/v, 0.2% triethylamine, pH adjusted to 4 using O-phosphoric acid) with a flow rate of 1 mL min^{-1} and a detection wavelength 311 nm.

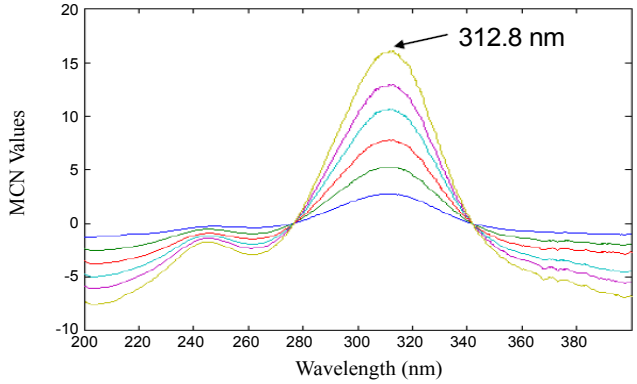

Figure 12: Mean centered ratio spectra of OZ (5-30 µg mL^{-1}).

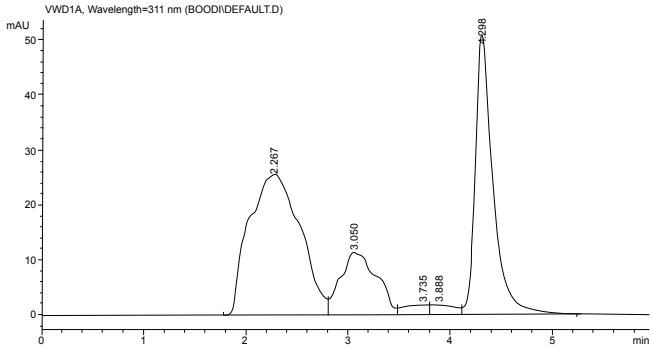

Figure 13: HPLC chromatogram of a resolved mixture of 10 µg mL^{-1} OZ (t_R=4.298) and 10 µg mL^{-1} OZ Deg (t_R=2.267 and 3.050), mobile phase is acetonitrile: water (50:50 v/v, 0.2% triethylamine, pH adjusted to 4 using o-phosphoric acid).

Parameters	OZ	Deg	limit
Retention time (t_R)	4.298	3.050	
Resolution (R)	5.16		Rs>2
Tailing factor (T)	1.17		T<2
Capacity factor (K')	9.745	6.625	1<K' <10
Selectivity factor (α)	1.471		α>1
Column efficiency (N)	9552.59	1575.114	N>2000
Height equivalent to theoretical plate (HETP)	0.026	0.159	

Table 2: System suitability parameters of the proposed HPLC method.

Upon applying the previously described HPLC optimum experimental conditions, good and efficient separation was observed between OZ and its degradation product (Figure 13).

Linear relationship was obtained for OZ between the relative peak areas and the corresponding concentrations. The regression equation was computed and found to be:

A = 0.1334C - 0.0318 r = 0.9998

Where, A is the relative peak area, C is OZ concentration in µg mL^{-1} and r is the correlation coefficient.

System suitability was checked by calculating different parameters such as capacity factor, tailing factor, column efficiency (N), selectivity and resolution factors, where the system was found to be suitable relative to the reference values as shown in table 2.

The proposed HPLC method was successfully applied for the determination of OZ in pure powdered form with mean percentage recovery of 100.00 ± 1.302%.

The specificity of the D^1, DD^1 and MCR methods was proved by the analysis of laboratory prepared mixtures containing different ratios of OZ and its degradate and satisfactory results were obtained for OZ in presence of degradate up to 80% (Table 3).

The four proposed methods have been successfully applied for the determination of OZ in Tibezole® tablets. The validity of the methods was further assessed by applying the standard addition technique, (Table 4).

Mixture No.	Degradate %	Taken OZ g mL^{-1}	D^1 method				D^1 method				MCN method	
			OZ (µg mL^{-1})				OZ (µg mL^{-1})				OZ (µg mL^{-1})	
			At 290.4 nm		At 332 nm		At 288.5 nm		At 328 nm		At 312.8 nm	
			Found	Recovery %	Found	Recovery %	Found	Recovery %	Found	Recovery %	Found	Recovery %
1	10	27	27.31	101.15	27.39	101.44	27.01	100.04	26.80	99.26	27.31	99.75
2	20	24	24.42	101.75	24.23	100.96	24.07	100.29	24.38	101.58	24.42	98.88
3	30	21	21.23	101.10	21.31	101.48	21.35	101.67	21.12	100.57	21.23	99.42
4	40	18	18.24	101.33	18.23	101.28	18.28	101.56	18.28	101.56	18.24	99.04
5	50	15	15.14	100.93	15.14	100.93	15.16	101.07	15.18	101.20	15.14	99.22
6	60	12	12.05	100.42	12.23	101.92	12.14	101.17	12.11	100.92	12.05	101.00
7	70	9	9.06	100.67	9.14	101.56	9.16	101.78	9.07	100.78	9.06	100.21
8	80	6	6.07	101.17	6.06	101.00	6.09	101.50	6.05	100.83	6.07	98.29
Mean RSD%				101.07 0.401		101.32 0.343		101.14 0.640		100.84 0.728		99.48 0.845

Table 3: Determination of OZ in presence of its degradate in laboratory prepared mixtures by the D^1, DD^1 MCN methods.

Parameter	D[1] method				DD[1] method				MCR method		HPLC method	
	At 290.4 nm		At 332 nm		At 288.5 nm		At 328 nm		At 312.8 nm			
	Found % [a]	Recovery of standard added % [b]	Found % [a]	Recovery of standard added % [b]	Found % [a]	Recovery of standard added % [b]	Found % [a]	Recovery of standard added % [b]	Found % [a]	Recovery of standard added % [c]	Found % [a]	Recovery of standard added % [c]
Tibezole® tablets 500 mg OZ/tablet B.N.1319003	99.90 ± 1.032	101.12 ± 0.629	100.86 ± 0.954	100.17 ± 0.606	100.62 ± 0.452	100.87 ± 0.300	99.88 ± 0.501	98.91 ± 0.600	98.40 ± 1.204	97.72 ± 0.648	100.97 ± 0.504	100.74 ± 0.372

[a]Average of four determinations
[b]Average of four determinations
[c]Average of three determinations

Table 4: Results obtained by applying the proposed methods for the determination of OZ in Tibezole® tablets and results obtained by applying standard addition technique.

Parameter	D[1] method		DD[1] method		MCR method	HPLC method	**Reported HPLC method
	At 290.4 nm	At 332 nm	At 288.5 nm	At 328 nm			
Mean	99.9	100.86	100.62	99.88	98.40	100.97	99.78
SD	1.031	0.962	0.455	0.500	1.185	0.509	0.852
n	4	4	4	4	4	4	4
Variance	1.063	0.925	0.207	0.250	1.404	0.259	0.726
Student's t	0.137 (2.45)*	1.284 (2.45)*	1.328 (2.45)*	0.155 (2.45)*	1.44 (2.45)*	1.028 (2.45)*	
F	1.464 (9.2766)*	1.274 (9.2776)*	3.507 (9.2766)*	2.904 (9.2776)*	1.934 (9.2776)*	2.803 (9.2776)*	

* The values in parentheses are the corresponding tabulated values at p=0.05.
** HPLC method (C-18, using acetonitrile: water 38:62 v/v consisting of triethylamine and adjusted to pH 3.6 with 5% O-phosphoric acid, flow rate 0.8 mL min[1], 254 nm).

Table 5: Statistical comparison of the results obtained by the proposed methods and the reported HPLC Method [25] for the analysis of OZ in Tibezole® tablets.

The results obtained for the analysis of OZ in its pharmaceutical dosage form were statistically compared with those obtained by applying a reported method [24]. The values of calculated t and F are less than the tabulated ones, which reveals that there is no significant difference between the two methods with respect to accuracy and precision (Table 5).

The repeatability and intermediate precision was evaluated by assaying freshly prepared solutions of the drug in triplicate on the same day and on three successive days respectively at concentrations of 10, 20, and 30 µg/ml for the D[1], DD[1] and MCN methods and at concentrations 4, 8, and 12 µg/ml for the HPLC method. RSD% (Table 1) showed the precision and the ruggedness of the methods.

Validation [25] of the proposed methods was constructed by determining the linearity, range, accuracy and precision (Table 1).

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