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Development and Validation of Spectrophotometric Methods for Quantitative Estimation of Oxfendazole in Presence of Its Alkali-induced Degradation Product: A Comparative Study

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ABSTRACT

Objectives: This study aimed to develop and validate three simple, accurate, selective, reproducible and sensitive spectrophotometric methods for the determination of oxfendazole in the presence of its alkali-induced degradation product without preliminary separation. **Methods:** (A) first derivative spectrophotometry (^{1}D) method at 298.9 nm, (B) first derivative of ratio spectra (^{1}DD) method at 295.4 nm and (C) area under the curve (AUC) method at wavelength ranges of (290-294 nm) and (297-301 nm). All methods were applied in the range of (1-10µg mL^{-1}). **Results:** These methods were validated and successfully applied for determination of oxfendazole in Unifendazole® suspension. The obtained results were statistically compared with those of the reported method by applying t-test and F-test at 95% confidence level and no significant difference was observed regarding accuracy and precision. **Conclusion:** The proposed methods are simple, rapid, economic, accurate and precise to determine oxfendazole in the presence of its alkali-induced degradation product without previous separation steps.

Keywords: *Alkaline degradation; Area under curve; Derivative ratio spectra; Derivative spectrophotometry; Oxfendazole*

INTRODUCTION

Oxfendazole is chemically known as: [5-(Phenylsulfinyl)-1*H*-benzimidazol-2-yl] carbamic acid methyl ester[1] (**Figure 1**). It is a benzimidazole carbamate anthelmintic used in veterinary medicine, for protecting livestock against roundworm, strongyles, and pinworms.[2]

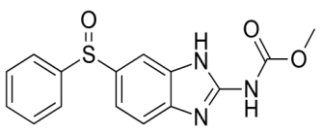


Figure 1. Chemical structure of oxfendazole

Different analytical techniques were applied for quantitative estimation of oxfendazole including potentiometric titration[3,4], radio immunoassay[5], colorimetric (based on the measurement of green colored manganate obtained due to oxidation of oxfendazole by permanganate in an alkaline medium at 610 nm) and direct measurement (at 290 nm in the acetic acid–water (1:1) solvent system) spectrophotometric methods[6] and HPLC methods either alone or in presence of other compounds[7-17].

Oxfendazole is an amide group containing compound that made it highly sensitive to hydrolytic degradation in basic conditions with the production of the degradation product (2-amino-5-Phenylsulfinyl benzimidazole).

There is no stability indicating analytical methods were reported for determination of oxfendazole in presence of its degradation product.

The aim of this work is to develop and validate simple, sensitive and selective spectrophotometric methods for the determination of oxfendazole in presence of its alkali-induced degradation product (2-amino-5-phenylsulfinyl benzimidazole) without preliminary separation.

MATERIALS AND METHODS

Instruments

Shimadzu UV-Vis. 1650 Spectrophotometer, (Japan), equipped with 10 mm matched quartz cells. Hot plate (Torrey pines Scientific, USA). Jenway, 3510 pH meter (Jenway, USA). Rotatory evaporator (Scilogex-RE 100-pro, USA). Pye-Unicam SP-3-300 infrared spectro-photometer (potassium bromide dicks). GCMS-QP-1000EX mass spectrometer at 70 ev (Shimadzu, Tokyo, Japan).

Materials and Reagents

Pure standard

Standard oxfendazole powder was kindly supplied by Unipharma Co. for pharmaceutical industries, Al Obour city, Cairo, Egypt. (B. NO: 60414005).

Pharmaceutical preparation

Unifendazole® suspension, the product of Unipharma Co. for pharmaceutical industries, Al Obour city, Cairo, Egypt. (B.NO: 390215), which labeled to contain 22.5% w/v oxfendazole.

Reagents and solvents

Hydrochloric acid, (El-Nasr Co., Egypt), prepared as 0.1M methanolic solution and 1M aqueous solution. Sodium hydroxide, (El-Nasr Co., Egypt), prepared as 1M aqueous solution. Methanol.

Degraded sample

One gram of oxfendazole was dissolved in 25 mL 0.1M methanolic hydrochloric acid, and then transferred into 250 mL conical flask, then 50 mL of 1 M NaOH solution was added. The flask was heated to boiling under reflux for 1hr, cooled and neutralized with 1M HCl. Subsequently, the solution was dried under vacuum then the degradation product was extracted with methanol and re-crystallized after evaporating methanol.

Standard solutions

Stock standard solution of oxfendazole (1mg mL^{-1}) was prepared by dissolving 0.1g of oxfendazole in 10 mL 0.1 M methanolic hydrochloric acid and complete to 100 mL with methanol.

Working standard solution of oxfendazole (100 µg mL^{-1}) was prepared by accurate transferring 10 mL of oxfendazole from its stock standard solution into 100 mL volumetric flask, then the volume was completed to the mark with methanol.

Stock standard solution of oxfendazole degradation product (1mg mL^{-1}) was prepared by dissolving 0.1g of (2-amino-5-phenylsulfinyl benzimidazole) in 50 mL methanol and complete to 100 mL with the same solvent.

Working standard solution of oxfendazole degradation product (100 µg mL^{-1}) was prepared by accurate transferring 10 mL of oxfendazole degradation product from its stock standard solution into 100 mL volumetric flask, then the volume was completed to the mark with methanol.

Methods

Construction of calibration curves

Different aliquots equivalent to (10 – 100 µg) of both oxfendazole and its alkali-induced degradation product were accurately transferred from their standard solutions (100 µg mL^{-1}) into two separate series of 10-mL volumetric flasks and completed to volume with methanol. The absorption spectra (from 200 to 400 nm) of these solutions were recorded using methanol as a blank.

First derivative method (^{1}D)

For the determination of oxfendazole in presence of its degradation product, the first derivative (^{1}D) of the zero-order spectra of oxfendazole and its degradation product were obtained. Then the amplitudes of the derivatized spectra of oxfendazole measured at 298.9nm, at which no interference from the degradation product, and plotted against corresponding drug concentrations, and the regression equation was derived.

First derivative of ratio spectra method (^{1}DD)

For the determination of oxfendazole in presence of its degradation product, the zero-order spectra of oxfendazole were divided by the spectrum of 10 µg mL^{-1} of its degradation product to obtain ratio spectra, and then the first derivative of ratio spectra (^{1}DD) were obtained. Then the amplitudes of the derivatized ratio spectra measured at 295.4 nm were plotted against corresponding drug concentrations, and the regression equation was derived.

Area under the curve method (AUC)

The area under the curve (**AUC**) of oxfendazole and its degradation product were measured at both wavelength ranges of (290-294 nm) and (297-

301nm) and calibration curves were obtained by plotting the area under curve values against the corresponding concentrations in µg mL^{-1}, and the regression equations were derived.

Application to laboratory prepared mixtures

Aliquots of oxfendazole and its degradation product were mixed to prepare different mixtures containing different ratios of both. The procedures mentioned under construction of calibration curves were followed and the concentrations of oxfendazole were calculated.

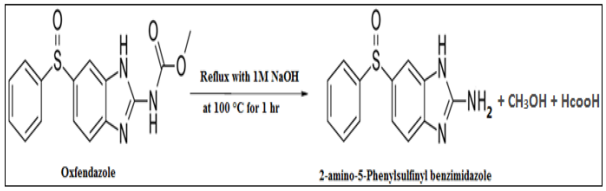
Application to pharmaceutical formulation

Volume of Unifendazole® suspension (22.5w/v oxfendazole) equivalent to 250 mg oxfendazole was accurately taken and dissolved in 10 mL 0.1 M methanolic hydrochloric acid in 250 mL flask and volume was completed with methanol, shacked, and then filtered. 10 mL of the filtrate were accurately transferred into 100 mL volumetric flask and volume was completed with methanol to obtain a solution labeled to contain 100 µg mL^{-1} of oxfendazole. The solution was analyzed using the procedure described previously.

RESULTS AND DISCUSSION

Degradation of oxfendazole

It is found that complete alkaline degradation of oxfendazole was obtained after refluxing the drug with 1M sodium hydroxide at 100 °C for 1 hr, and the degradation was confirmed by TLC method using chloroform: methanol: acetic acid (90:8:2, by volume) as a developing system, where one spot of the degradation product obtained with significant separation from that of intact oxfendazole, where a suggested degradation pathway is shown as follows:



Scheme 1. Suggested degradation pathway of oxfendazole

Identification of the degradation product:

Infrared (IR) spectrum of the degradation product showed abroad peak at 3404 cm^{-1} which may be assigned to the primary amine group, also disappearance of the peaks at 2741 cm^{-1} of the methyl group and at 1724 cm^{-1} of the carbonyl group, **Figures 2, 3**. Mass spectrometry showed that the compound has a molar mass of 256.96 indicating the presence of the degradation product, **Figure 4 a, b**.

In conclusion, all the above evidences indicate that the degradation product could be 2-amino-5-Phenylsulfinyl benzimidazole (**Scheme 1**).

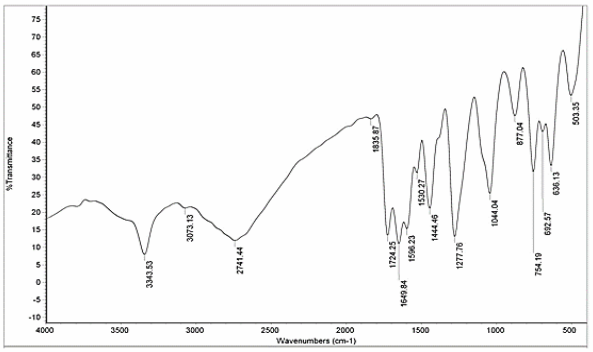


Figure (2): IR spectrum of intact oxfendazole on KBr disc.

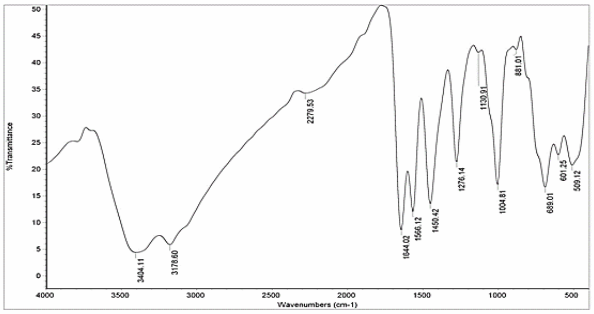


Figure (3): IR spectrum of oxfendazole degradation product on KBr disc.

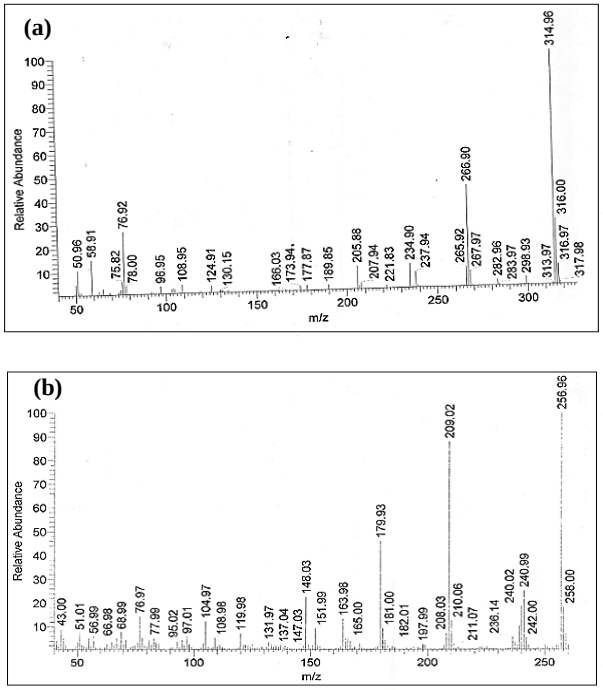


Figure (4): (a) Mass spectrum of intact oxfendazole. (b) Mass spectrum of oxfendazole degradation product.

Spectral characteristics

The zero-order absorption spectra of oxfendazole and its alkali-induced degradation product show severe overlap, as shown in **Figure 5**. This overlap does not permit direct determination of oxfendazole in the presence of its degradation product. To overcome this problem, different spectrophotometric methods were developed and validated to allow the determination of oxfendazole in the presence of its alkali-induced degradation product without previous separation.

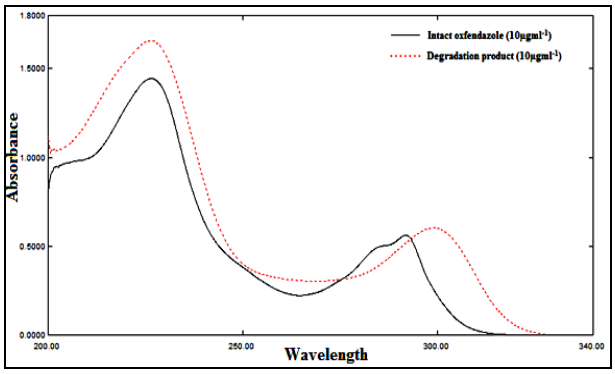


Figure (5): Absorption spectra of intact oxfendazole and its degradation product.

First derivative method[18,19]

Derivative spectrophotometry offers a powerful tool for a better resolution enhancement when signal overlap or interference exists. The first derivatives of the absorption spectra were obtained to allow quantitative determination of oxfendazole in the presence of its degradation product, where the amplitudes at 298.9 nm are proportional to the concentrations of oxfendazole without interference from its degradation product, as shown in **Figures 6, 7**.

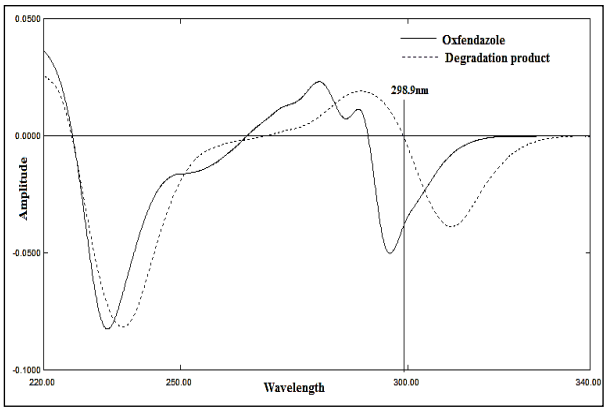


Figure (6): First derivative of the absorption spectra of intact oxfendazole (10 µg mL^{-1}) and of its degradation product (10 µg mL^{-1}).

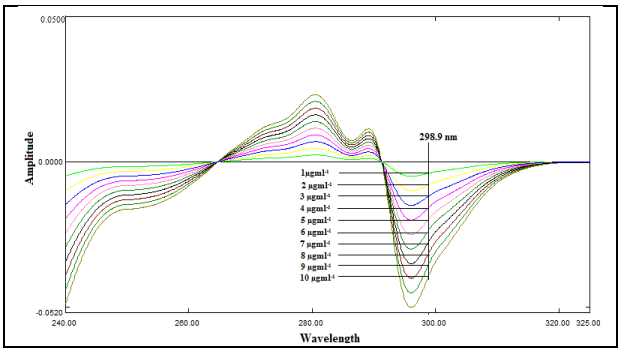


Figure (7): First derivative of the absorption spectra of oxfendazole at various concentrations (1- 10µg mL^{-1}).

First derivative of ratio spectra [20,21]

First derivative of ratio spectra method is a simple method, capable of determining oxfendazole in presence of its alkali-induced degradation product with minimal data processing. In this method, the absorption spectra of the drug were divided by the absorption spectrum of the degradation product (10 µg mL^{-1}), as a divisor, to get the ratio spectra, as shown in **Figure 8**. The amplitudes of the first derivative of the ratio spectra at 295.4 nm are proportional to the concentrations of the drug without interference from its degradation product (divisor), as shown in **Figures 9, 10**.

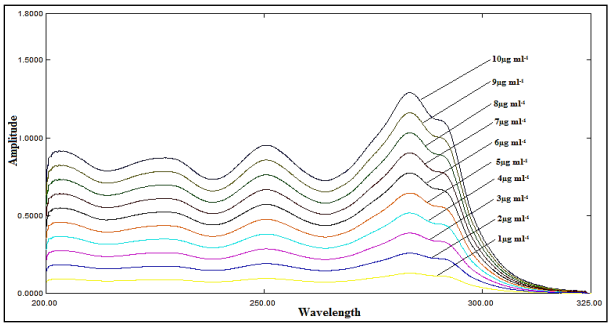


Figure (8): Ratio spectra of oxfendazole (1-10 µg mL^{-1}) using (10 µg mL^{-1}) of its degradation product as a divisor.

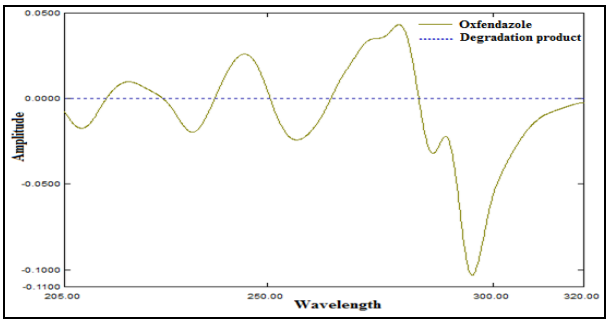


Figure (9): First derivative of the ratio spectra of oxfendazole (10 µg mL^{-1}) and its degradation product (10 µg mL^{-1}) using (10 µg mL^{-1}) of the degradation product as a divisor

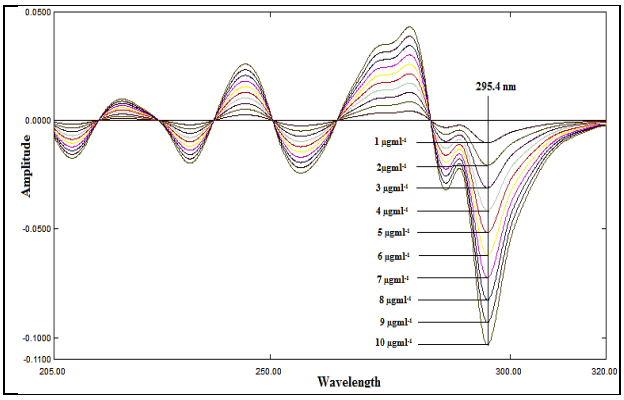


Figure (10): First derivative of ratio spectra of oxfendazole (1-10 µg mL^{-1}) using (10 µg mL^{-1}) of its degradation product as a divisor.

Area under the curve method[22, 23]

In this method, the area under the curve for oxfendazole and its degradation product were recorded over wavelength ranges of (290–294 nm) and (297–301 nm), **Figures 11, 12**. The absorptivity 'a' values of oxfendazole and its degradation product were determined at each wavelength range. The concentrations of oxfendazole in presence of its degradation product can be obtained from the following equation:

$$C_{(x)} = (A_{m1}\, a_{y2} - A_{m2}\, a_{y1}) / (a_{x1}\, a_{y2} - a_{x2}\, a_{y1})$$

Where $C_{(x)}$ is oxfendazole concentration, A_{m1} and A_{m2} are the area under the curve of the mixture at the wavelength range (290 - 294) nm and (297 – 301) nm, respectively. Where a_{x1} = A_{x1}/conc. in µg mL^{-1}, a_{x2} = A_{x2}/conc. in µg mL-1 and a_{y1} = A_{y1}/conc. in µg mL^{-1}, a_{y2} = A_{y2}/conc. in µg mL^{-1} for oxfendazole and its degradation product, respectively.

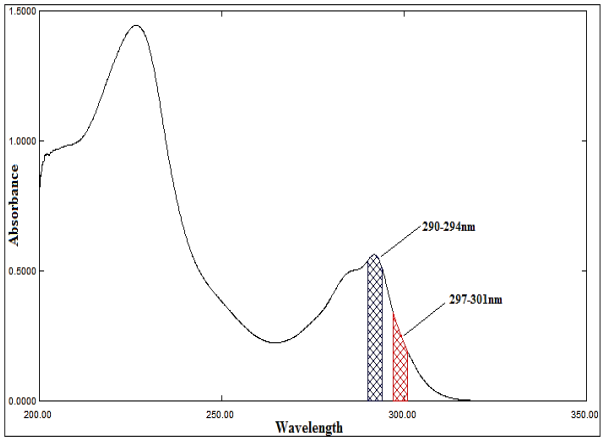


Figure (11): Zero-order absorption spectra of oxfendazole (10 µg mL^{-1}) showing area under curve over the ranges of (290–294 nm) and (297–301 nm.

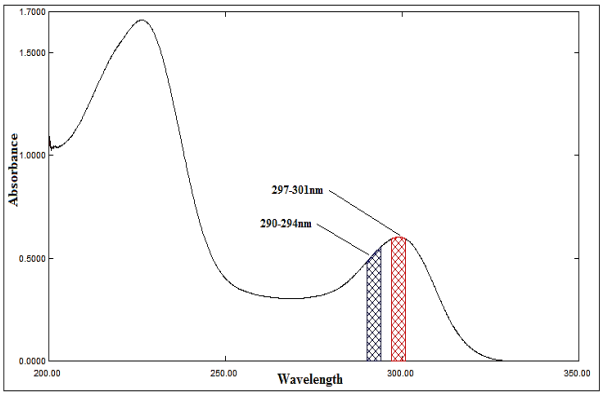


Figure (12): Zero-order absorption spectra of oxfendazole degradation product (10 µg ml^{-1}) showing area under the curve over the ranges of (290–294 nm and (297–301 nm).

Methods of validation

Validations of the proposed methods were assessed as per the ICH guidelines [24] of accuracy, precision, repeatability, inter day precision, linearity.

Linearity and range

Calibration graphs were constructed by plotting the amplitudes of the first derivative and first derivative of ratio spectra methods or area under curve values for area under curve method at the selected wavelengths versus drug concentrations in µg mL^{-1}. The regression plot was found to be linear over the range of 1-10 µg mL^{-1}. The linear regression equations for the graphs were:

y = 0.0039 x - 0.0003 (r^2 = 0.9997)
for first derivative method
y = 0.0104 x - 0.0004 (r^2 = 0.9998)
for first derivative of ratio spectra method
y = 0.2209 x - 0.0086 (r^2 = 0.9998)
or AUC method in the range of 290-294 nm
y = 0.1053 x - 0.0064 (r^2 = 0.9996)
for AUC method in the range of 297-301 nm

Where y is the amplitude or area under curve values, x is the drug concentration in µg mL^{-1} and r^2 is the determination coefficient. Linearity range, regression equation, intercept, slope and determination coefficient for the calibration data were presented in **Table 1**.

Limits of detection and quantitation

The limits of detection (LOD) and the limits of quantitation (LOQ) were calculated according to ICH guidelines from the following equations:

LOD = 3.3 σ / S LOQ = 10 σ / S

Where σ is the standard deviation of y-intercepts of regression lines and S is the slope of the calibration curve. LOD and LOQ values were mentioned in **Table 1**.

Table 1. Assay validation sheet of the proposed methods

Parameter	First derivative	First derivative of ratio spectra	Area under the curve	
Accuracy (mean ± RSD)[a]	99.46 ± 0.910	99.38 ± 0.364	99.57± 0.156	
Precision				
Repeatability (RSD) [b]	0.858	0.618	0.457	
Intermediate precision (RSD) [c]	0.953	0.612	0.623	
Wavelength	298.9 nm	295.4 nm	290-294 nm	297-301 nm
Linearity range	(1-10 µg mL^{-1})	(1-10 µg mL^{-1})	(1-10 µg mL^{-1})	
Slope	0.0039	0.0104	0.2209	0.1053
Intercept	- 0.0003	-0.0004	- 0.0086	-0.0064
coefficient of determination (r^2)	0.9997	0.9998	0.9998	0.9996
LOD (µgmL^{-1})	0.169	0.079	0.043	0.088
LOQ (µgmL^{-1})	0.512	0.242	0.130	0.267

[a] *Average of three determinations for three concentrations (3, 6 and 9 µgmL^{-1}), for Oxfendazole repeated three times.*
[b] *The intraday (n=3), average of three concentrations (3, 6 and 9 µgmL^{-1}), for Oxfendazole repeated three times within the day.*
[c] *The interday (n=3), average of three concentrations (3, 6 and 9 µgmL^{-1}), for Oxfendazole repeated three times in three days.*

Accuracy

The proposed methods were applied for measuring different concentrations of oxfendazole within their linearity range and the concentrations were calculated each from their corresponding regression equations. The accuracy of the proposed methods was calculated and RSD% was obtained. Good results were obtained as shown in **Table 1.**

Precision

Precision was evaluated by calculating intraday (repeatability) and interday (Intermediate precision) precision after repeating measuring of the three different concentrations three times in the same day and assaying the sample in triplicate on three successive days using the proposed methods. The calculated RSD% values were listed as shown in **Table 1** indicating satisfying precision of the proposed methods.

Specificity

The specificity of the proposed methods was assured by applying them to laboratory prepared mixtures of the intact oxfendazole together with its degradation product. The proposed procedures were adopted for the selective determination of intact oxfendazole in presence of up to 80% of its degradation product for first derivative and first derivative of ratio spectra methods and up to 70% for area under curve method.

The mean percentage recovery ±SD % was shown in **Table 2.** The validity of the proposed procedures is further assessed by applying the standard addition technique showing no interference from excipients. The results obtained were shown in **Table 3**. Although area under the curve method was applied on zero-order absorption spectra and first derivative method needs only one data processing step, first derivative of ratio spectra method show the lowest LOD and LOQ values. All three methods are simple, time saving and easy in application.

Statistical analysis

In order to compare the ability of the proposed methods for the determination of oxfendazole in pharmaceutical preparation, the results obtained by applying each of the proposed methods and the reported spectrophotometric method[6] were subjected to statistical analysis **Table 4**. The calculated *t* and *F* values were less than the theoretical ones indicating that there were no significant differences between the proposed and the reported methods. Another statistical comparison of the results obtained by the proposed methods and the reported method for determination of oxfendazole in pharmaceutical product using one-way ANOVA test was shown in **Table 5**. The results obtained by applying these methods show no significant differences between all of them.

Table 2: Determination of oxfendazole in presence of its alkali- degradation product in their laboratory prepared mixtures by the proposed methods

Method	Intact (µg mL^{-1})	Degradate (µg mL^{-1})	Percent of degradate	Intact found (µg mL^{-1})	Recovery % of intact
First derivative	9	1	10	8.97	99.71
	7	3	30	7.02	100.36
	5	5	50	5.05	101.02
	3	7	70	3.02	100.85
	2	8	80	2.02	101.28
	Mean ± SD%				**100.47 ± 0.604**
First derivative of ratio spectra	9	1	10	8.97	99.68
	7	3	30	6.96	99.45
	5	5	50	5	100
	3	7	70	3.01	100.32
	2	8	80	2.02	100.96
	Mean ± SD%				**99.86 ± 0.567**
Area under the curve	9	1	10	9.01	100.11
	7	3	30	6.99	99.86
	5	5	50	5.02	100.40
	4	6	60	3.99	99.75
	3	7	70	3.04	101.33
	Mean ± SD%				**99.96±0.773**

Table 3. Application of standard addition technique to the analysis of Unifendazole® suspension by applying the proposed methods

Method	Pharmaceutical Taken (µg/mL)	Pure added (µgmL^{-1})	Pure found (µgmL^{-1})	Recovery %
First derivative	3	4	3.92	98.07
		5	4.97	99.48
		6	5.92	98.71
		7	6.94	99.26
	Mean ± SD%		**98.88± 0.629**	
First derivative of ratio spectra	3	4	3.93	98.31
		5	4.96	99.23
		6	5.93	98.87
		7	6.99	99.86
	Mean ± SD%		**99.07 ±0.647**	
Area under the curve	3	4	3.94	98.56
		5	4.95	99.13
		6	5.99	99.88
		7	6.96	99.52
	Mean ± SD%		**99.27 ± 0.566**	

Table 4. Statistical comparison for the results obtained by the proposed methods and the reported method for the analysis of oxfendazole in Unifendazole® (22.5% w/v) suspension

	First derivative	First derivative of ratio spectra	Area under the curve	Reported method[6]
N*	5	5	5	5
$\bar{X}$	99.26	99.42	99.31	99.85
SD	0.374	0.264	0.345	0.478
RSD%	0.377	0.266	0.348	0.479
t***	2.187 (2.306)	1.756 (2.306)	2.032 (2.306)	____
F**	1.635 (6.388)	3.196 (6.388)	1.917 (6.388)	____

** No. of experiment.*
*** The values in the parenthesis are tabulated values of t and F at p= 0.05 level of significance.*

Table 5. One-way ANOVA testing for the different proposed methods used for the determination of oxfendazole in Unifendazole® suspension

Drug	Source	DF	Sum of squares	Mean square	*F* value
Oxfendazole	Between exp.	3	1.091	0.363	2.581
	Within exp.	16	2.253	0.141	(3.238)

The values between parentheses are the theoretical F value at p = 0.05 level of significance). The population means are not significantly different.

CONCLUSION

In this research simple, rapid, accurate, reproducible, precise and sensitive methods namely, first derivative, first derivative of ratio spectra and area under the curve were described and applied for quantitative determination of oxfendazole in pure form or in the presence of its alkali-induced degradation product without any preliminary separation step. The proposed methods do not need any sophisticated apparatus or a special program and could be easily applied in quality control laboratories. Moreover, the proposed methods were successfully applied to Unifendazole® suspension and no interference from pharmaceutical formulation excipients was found.

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