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Research Article

TWO WAVELENGTHS DEPENDENT SPECTROPHOTOMETRIC METHODS FOR QUANTITATIVE ESTIMATION AND VALIDATION OF OXFENDAZOLE IN PRESENCE OF ITS ALKALI-INDUCED DEGRADATION PRODUCT: A COMPARATIVE STUDY

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ABSTRACT

Four simple, accurate, selective, reproducible and sensitive spectrophotometric methods, each method based on the selection of two wavelengths, have been developed and validated for the determination of oxfendazole in the presence of its alkali-induced degradation product without preliminary separation. These methods include (A) dual wavelength method at 291.6 and 304.9 nm, (B) Q-analysis method at 275.5 and 292 nm, (C) simultaneous equation method at 292 and 299 nm and (D) absorbance ratio method at 275.5 and 292 nm. These methods were validated and successfully applied for the 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.

Keywords: oxfendazole; dual wavelength; Q-analysis; simultaneous equation; absorbance ratio.

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 [2].

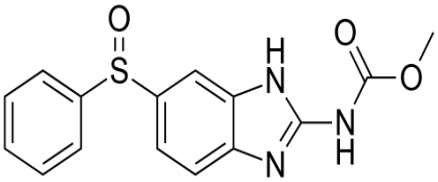


Fig.1: chemical structure of oxfendazole

Different analytical methods were applied for the determination of oxfendazole including potentiometric titration [3, 4], radio immunoassay [5], spectrophotometric methods [6] and HPLC methods either alone or in the presence of other compounds [7-17].

Oxfendazole is an amide group containing a drug 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 the 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 the presence of its alkali-induced degradation product (2-amino-5-Phenylsulfinyl benzimidazole) without preliminary separation.

EXPERIMENTAL

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

Standard solutions

(a) *A 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.

(b) *Working standard solution of oxfendazole (100 µgmL^{-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.

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.

(c) *A stock standard solution of oxfendazole degradation product (1mgmL^{-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.

(d) *Working standard solution of oxfendazole degradation product (100 µgmL^{-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.

PROCEDURE

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 working solutions (100µgmL^{-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.

Dual wavelength method

In zero order absorption spectra of oxfendazole in the range of (1-10µgmL^{-1}), the difference in the absorbance was measured at 291.6 and 304.9 nm. The measured differences in absorbance versus the final drug concentrations in µgmL^{-1} were plotted to get the calibration graph, and the regression equation was derived.

Q-analysis method

Individually zero order spectra of oxfendazole and its degradation product in the range of (1-10µgmL^{-1}) were obtained as before. The absorbance values for both oxfendazole and its alkali-induced degradation product were measured at292nm (λmax of oxfendazole) and 275.5nm (λiso at the iso-absorptive point). The measured absorbance versus the final concentrations in µgmL^{-1} was plotted to get the calibration graph, and the regression equations were derived.

Simultaneous equation method

The absorbance values of oxfendazole and its alkali-induced degradation product solutions were measured at 292nm (λmax of oxfendazole) and 299 nm (λmax of oxfendazole alkali-induced degradation product). The measured absorbance versus the final concentrations in µgmL^{-1} was plotted to get the calibration graphs, and the regression equations were derived.

Absorbance ratio method

Aliquots equivalent to (10–80 µgmL^{-1}) of oxfendazole were transferred from its working solution (100µgmL^{-1}) into a series of 10-mL volumetric flasks containing an aliquot equivalent to (50 µgmL^{-1}) of the alkali-induced degradation product and the volume completed with methanol. The absorbance of each solution was measured at 292nm (λmax of oxfendazole) and 275.5 nm (λiso at the iso-absorptive point), the relationship between the relative absorbance (Q = A1 /A iso) and the relative concentration (C1 / C1 + C2) were plotted, and the regression equation was derived.

Application to laboratory prepared mixtures

Aliquots of oxfendazole and its alkali-induced 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.

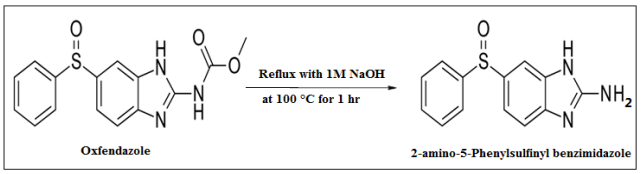
Application to pharmaceutical formulation

The volume of Unifendazole® suspension (22.5w/v oxfendazole) equivalent to 250 mg oxfendazole was accurately taken and dissolved in 10 mL 0.1M methanolic hydrochloric acid in 250 mL flask, and volume was completed with methanol, shacked, and then filtered. 10 mL of the filtrate was accurately transferred into 100-mL volumetric flask and volume was completed with methanol to obtain a solution labeled to contain (100 µgmL^{-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 the disappearance of the peaks at 2741 cm^{-1} of the methyl group and 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, **Figures (4, 5)**.

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

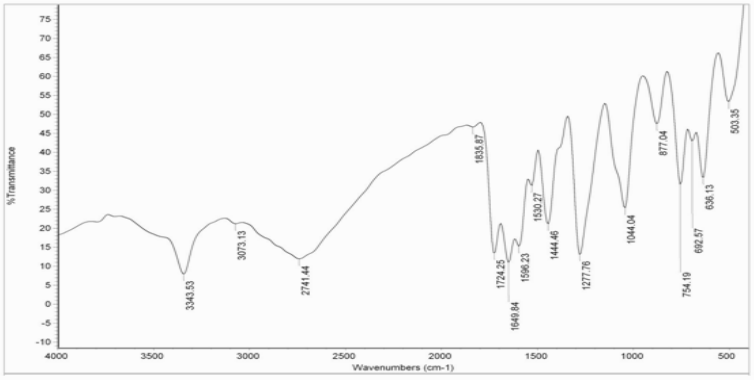


Figure 2: IR spectrum of intact oxfendazole on KBr disc.

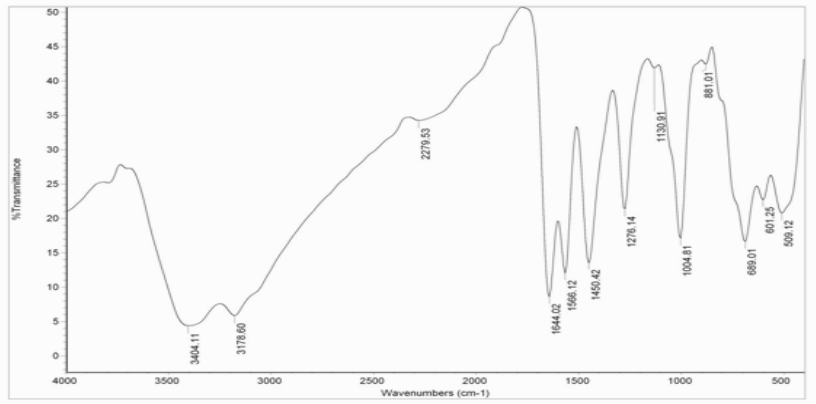


Figure 3: IR spectrum of oxfendazole degradation product on KBr disc.

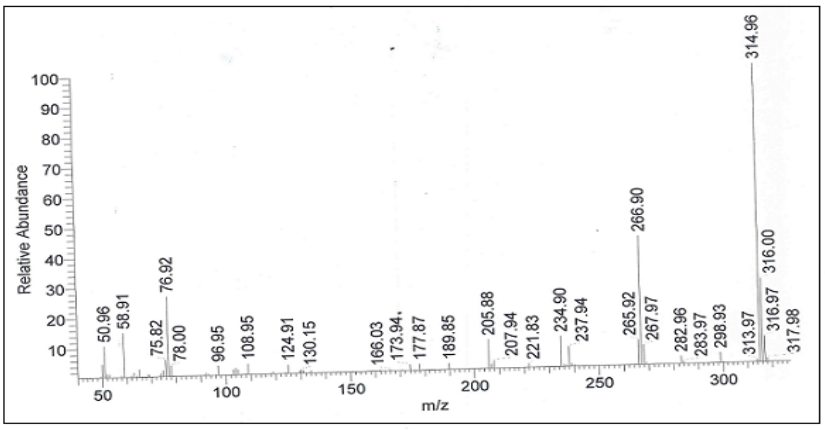


Figure 4: Mass spectrum of intact oxfendazole

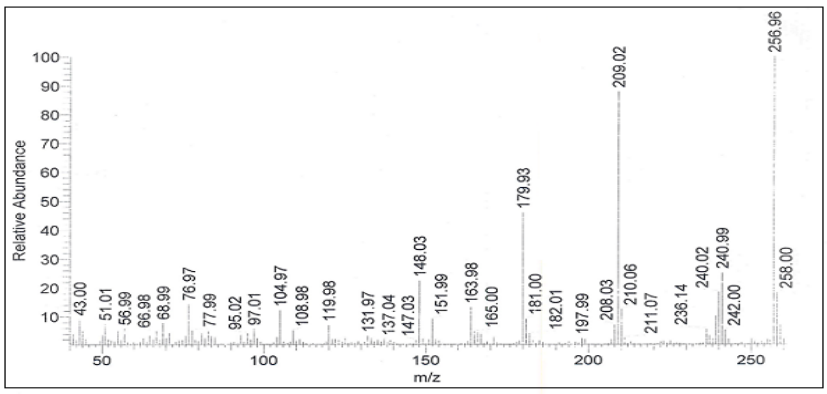


Figure 5: Mass spectrum of oxfendazole degradation product.

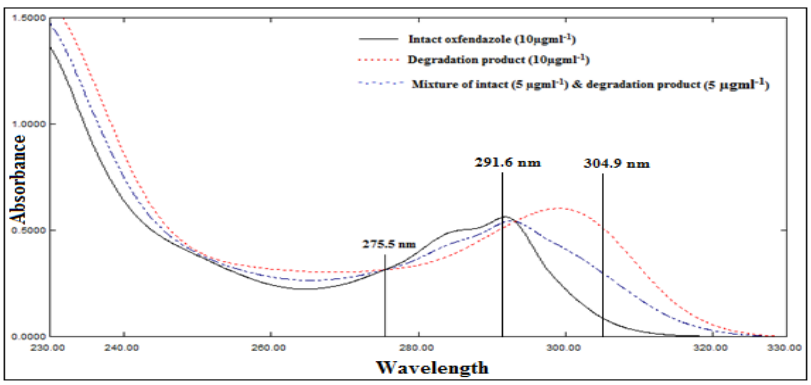


Figure 6: Absorption spectra of oxfendazole, its degradation product and mixture of them with isoabsorptive point at (275.5 nm)

Spectral Characteristics

The zero-order absorption spectra of oxfendazole and its alkali-induced degradation product show severe overlap with isoabsorptive point at 275.5nm as shown in **Figure (6)**. This overlap does not permit direct determination of oxfendazole in the presence of its alkali-induced 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.

Dual wavelength method

In this method, the interference from degradation product can be removed by measuring the difference in absorbance between 291.6 nm and 304.9 nm in the range of (1- 10µgmL^{-1}). This difference is zero for degradation product, while it is directly proportional to the concentration of intact oxfendazole [18, 19].

Q-analysis method

The absorbance values were measured at 292 nm (λ_{max}) of oxfendazole and 275.5 nm (λ_{iso}) in the range of 1- 10µgmL^{-1} for both oxfendazole and its alkali-induced degradation product. Absorptivity coefficients were determined for both oxfendazole and its degradation product and the average values were taken. Absorptivity values and the absorbance ratio were used to develop the next equation from which the concentration of oxfendazole in the sample can be calculated.

$$C_x = \{(Q_M - Q_y)/(Q_x - Q_y)\} \times (A_1/a_{x1})$$

where C_x is the concentrations of oxfendazole in µgmL^{-1}, Q_m is the absorbance of sample mixture at λ_{max}/absorbance of sample mixture at λ_{iso} , Q_x is the mean of absorptivity of the drug at λ_{max} /mean of absorptivity of the drug at λ_{iso}, Q_y is the mean of absorptivity of degradation product at λ_{max} /mean of absorptivity of degradation product at λ_{iso} , a_{iso} is the mean of absorptivity of oxfendazole at λ_{iso}, and A_{iso} is the absorbance of the sample at λ_{iso}. [20, 21].

Simultaneous equation method

In this method, absorbance values were measured at λ_1 (292 nm) and λ_2 (299 nm) for both oxfendazole and its alkali-induced degradation product. The absorptivity coefficients of each component at both wavelengths were determined by dividing each absorbance over each corresponding concentration. The concentration of oxfendazole in laboratory prepared mixtures and pharmaceutical formulation was determined by substituting the absorbance and absorptivity coefficient in the following equation:

$$C_X = \frac{A_2 a_{Y1} - A_1 a_{Y2}}{a_{X2} a_{Y1} - a_{X1} a_{Y2}}$$

Where: C_x is the concentration of oxfendazole, A_1 and A_2 are absorbance of sample at 292 and 299 nm respectively, a_{x1} and a_{x2} are absorptivity of oxfendazole at 292 and 299 nm respectively, a_{Y1} and a_{Y2} are absorptivity of its degradation product at 292 and 299 nm respectively [22, 23]

Absorbance ratio method

The absorbance values were measured at 292 nm (λ_{max} for oxfendazole) and 275.5nm (λ_{iso}) in the original spectra of mixed working standard solutions, then the linear relationship between the absorbance ratio values of oxfendazole and its relative concentration in a mixture was established, (Q_1 vs.$\frac{C_1}{C_1 + C_2}$), from this regression equation the slope (a) and intercept (b) were obtained and used to analyze oxfendazole from next equation:

$$C_x = \frac{Q_1 - b_1}{a_1} \text{x} \frac{A_{iso}}{a_{iso}}$$

Where, C_x is the concentration of oxfendazole, $Q_1 = \frac{A_1}{A_{iso}}$ for oxfendazole, A_{iso} is the absorbance at isoabsorptive point, a_{iso} is the absorptivity at isoabsorptive point which equal to ($\frac{A_{iso}}{C_1 + C_2}$), a_1 is the slope of regression equation (Q_1 vs.$\frac{C_1}{C_1 + C_2}$), b_1 is the intercept value of this regression equation and A_1 denotes the absorbance value of the mixture solution measured at λ_1. [24]

Methods validation

Validations of the proposed methods were assessed as per the ICH guidelines [25] of accuracy, precision, repeatability, interday precision, linearity. Good results were obtained as illustrated in **Table 1**. **Table 2** shows the specificity; recovery percentages of the laboratory prepared mixture of the drug with its alkali-induced degradation product. 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.**

Table1. Assay validation sheet of the proposed methods.

Parameter	Dual wavelength		Q-analysis	Simultaneous equation		Absorbance ratio	
Accuracy (mean ± RSD)[a]	99.46 ± 0.478		99.98 ± 0.421	99.53± 0.374		99.83 ± 0.330	
Precision							
Repeatability (RSD) [b]	0.797	0.501		0.450		0.711	
Intermediate precision (RSD) [c]	0.775	0.651		0.314		0.641	
Wavelength	291.6&304.9 nm	275.5 nm	292 nm	292nm	299nm	275.5 nm	292 nm
Linearity range	(1-10µgmL^{-1})	(1-10µgmL^{-1})		(1-10µgmL^{-1})		(1-8µgmL^{-1})	
Slope	0.0474	0.0312	0.0563	0.0563	0.0259	0.1298	
Intercept	-0.0018	0.0015	-0.0004	-0.0004	-0.0003	1.6565	
coefficient of determination (r^2)	0.9997	0.9997	0.9998	0.9998	0.9996	0.9996	
LOD (µgmL^{-1})	0.112	0.038	0.107	0.107	0.092	0.084	
LOQ (µgmL^{-1})	0.339	0.115	0.324	0.324	0.278	0.254	

[a] Average of three determinations for three concentrations (3, 6 and 9 µgmL^{-1})for dual wavelength, Q-analysis and simultaneous equation and (3 ,5 and 7 µgmL^{-1}) for absorbance ratio method, for oxfendazole repeated three times.[b] The intraday (n=3), average of three concentrations (3, 6 and 9 µgmL^{-1})for dual wavelength, Q-analysis and simultaneous equation and (3 ,5 and 7 µgmL^{-1}) for absorbance ratio method, for oxfendazole repeated three times within the day.[c] The interday (n=3), average of three concentrations 3, 6 and 9 µgmL^{-1})for dual wavelength, Q-alalysis and simultaneous equation and (3 ,5 and 7 µgmL^{-1}) for absorbance ratio method, for oxfendazole repeated three times in three days.

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

Method	Intact in (μgmL^{-1})	Degradate in (μgmL^{-1})	Percent of degradate	Intact found in (μgmL^{-1})	Recovery % of intact
Dual wavelength	9	1	10	8.92	99.11
	7	3	30	6.97	99.69
	5	5	50	5.03	100.76
	3	7	70	3.03	101.12
	2	8	80	2.02	101.05
	Mean ± SD%				**100.17±0.755**
Q-analysis	9	1	10	9.03	100.34
	7	3	30	6.97	99.66
	5	5	50	4.98	99.75
	3	7	70	2.99	99.98
	2	8	80	1.97	98.81
	Mean ± SD%				**99.90 ±0.531**
Simultaneous equation	9	1	10	9.07	100.83
	7	3	30	7.07	101.12
	5	5	50	5.04	100.92
	3	7	70	2.98	99.38
	2	8	80	1.97	98.84
	Mean ± SD%				**100.13±0.970**
Absorbance ratio	8	5	38.46	8.01	100.15
	7	6	46.15	7.03	100.44
	5	8	61.54	5.05	100.96
	4	9	69.23	4.06	101.41
	3	10	76.92	3.05	101.82
	Mean ± SD%				**100.96±0.611**

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 %
Dual wavelength	3	4	3.98	99.47
		5	4.97	99.41
		6	6.02	100.42
		7	6.99	99.94
	Mean ± SD%			**99.81± 0.471**
Q-analysis	3	4	3.95	98.80
		5	4.98	99.64
		6	5.97	99.61
		7	6.99	99.85
	Mean ± SD%			**99.47 ±0.462**
Simultaneous equation	3	4	3.97	99.24
		5	5	100
		6	5.96	99.32
		7	7.02	100.35
	Mean ± SD%			**99.73 ± 0.537**
Absorbance ratio	2	3	2.98	99.34
		4	3.95	98.93
		5	4.97	99.39
		6	5.94	99.11
	Mean ± SD%			**99.19 ±0.215**

All the above methods were applied on the zero-order spectra of oxfendazole and its degradation product without any more processes or spectral manipulation. In each method the choice of pair of wavelengths is the only step of data handling, indicating that they are simple, time-saving and easy in application.

Statistical analysis

To compare the ability of the proposed methods for the determination of oxfendazole in its 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 4: Statistical comparison of the results obtained by the proposed methods and the reported method for the analysis of oxfendazole in Unifendazole ® (22.5% w/v) suspension:

	Dual wavelength	Q-analysis	Simultaneous equation	Absorbance ratio	Reported method[6]
N*	5	5	5	5	5
$\bar{X}$	99.80	100.08	99.97	99.96	99.85
SD	0.669	0.868	0.428	0.588	0.478
RSD%	0.670	0.867	0.428	0.588	0.479
t****	0.151 (2.306)	0.508(2.306)	0.403 (2.306)	0.324(2.306)	——
F**	1.954(6.388)	3.290(6.388)	1.261(6.388)	1.500(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.	4	0.242	0.060	0.154
	Within exp.	20	7.868	0.393	(2.866)

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, dual wavelength, Q-analysis, simultaneous equation and absorbance ratio 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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