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Optimized and validated spectrophotometric methods for the determination of ezetimibein pharmaceutical formulations using potassium permanganate

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ABSTRACT

Three sensitive, accurate and validated spectrophotometric methods has been developed for estimation of ezetimibe (EZT)in pure and dosage forms. The proposed methods is based on oxidation reaction of EZT with a known excess potassium permanganate ($KMnO_4$) as an oxidimetric reagent in acid medium followed by determination of unreacted oxidant by adding a fixed amount of amaranth (AM), orange G (OG) andmethylene blue (MB) dyes followed by measuring the absorbance at 520, 480and664 nm, respectively.The experimental conditions affecting the reaction were studied and optimized. The beer's law was obeyed in the concentration ranges of 2.0-15, 2.0-10, and 2.0-12 μg mL^{-1} using AM, OG and MB dye, respectively with a correlation coefficient ≥ 0.9992. The calculated molar absorptivity values are 1.7012 × 10^4, 3.0117 × $10^4$and 1.5321 × 10^4L mol^{-1} cm^{-1} using AM, MB and OG dyes, respectively. The limits of detection and quantification were reported. Intra-day and inter-day accuracy and precision of the methods have been evaluated. No interference was observed from the additives and the applicability of the method was tested by analyzing the pharmaceutical preparations containing the investigated drug. The proposed methods were successfully applied to the assay of EZT in tablet preparations and the results were statistically compared with those of the reported method by applying Student's t-test and F-test. The reliability of the methods was further ascertained by performing recovery studies using the standard addition method.

Keywords: Spectrophotometry; Ezetimibe; Potassium permanganate; Oxidation reactions; Tablets.

INTRODUCTION

Ezetimibe (EZT), chemically known as (3R, 4S)-1-(4-fluorophenyl)-3-[(3S)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl) azetidin-2-one (Fig. 1), is a cholesterol absorption inhibitor and is used to lower high cholesterol level in people with primary hypercholesterolaemia. It prevents transport of cholesterol through the intestinal wall by selectively blocking the absorption of cholesterol from dietary and biliary sources. The overall effect is a reduction in cholesterol level in the blood. This reduces the overall delivery of cholesterol to the liver, thereby promoting the synthesis of LDL receptors and a subsequent reduction in the serum LDL-C (1,2).

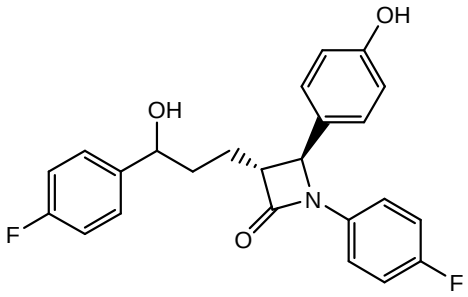


Fig.1. The chemical structure of EZT.

Few reported methods for the determination of EZT as single drug or in combination in bulk, pharmaceutical formulations and/or biological fluids have been developed using high performance liquid chromatographic methods (HPLC) (3-11), spectrofluorimetric methods (12-14), spectrodensitometric method (15) and electrochemical methods (16-18**).** These methods were time-consuming, tedious, and/or dedicated to sophisticated and expensive analytical instruments. All the above methods developed for the quantification of EZT employed complex analytical instruments for its estimation mainly in bulk drug powders and dosage forms. However, most of these methods are complex, require expensive experimental setup and skilled personnel, suffer from time-consuming procedures, and are inaccessible to many laboratories in developing and under developed nations. In contrast, spectrophotometry is considered as the most convenient analytical technique in most quality control and clinical laboratories, hospitals and pharmaceutical industries for the assay of different classes of drugs in pure, pharmaceutical formulations and biological samples, due to its simplicity and reasonable sensitivity with significant economic advantages.

Few spectrophotometric methods have been reported for the quantification of EZT in pharmaceutical formulations(19-40) (Table 1). However, these previously reported methods suffer from one or the other disadvantage such as poor sensitivity, depending on critical experimental variables, few methods require a rigid pH control and tedious and time-consuming liquid–liquid extraction step; some other methods have a relatively narrow dynamic linear range, involve a heating step, and/or use of expensive reagent or large amounts of organic solvents. For these reasons, it was worthwhile to develop a new, simple, cost effective and selective spectrophotometric method for the determination of in pharmaceutical dosage forms.

Table 1. Comparison between the report spectrophotometric methods for determination of EZT

Method	Wavelength (nm)	Beer's law ($\mu g\ mL^{-1}$)	Molar absorptivity ($L\ mol^{-1}cm^{-1}$)	Detection limit ($\mu g\ mL^{-1}$)	References
UV-spectroscopy	324	5.0-30	$4.327x\ 10^4$	0.126	30
Potassium permanganate / Alkaline medium	610	2.0-16	2.415×10^4	0.106	31
Potassium permanganate / Acidic medium	550	2.0-40	1.617×10^4	0.204	
(Para dimethyl amino benzaldehyde	420	10-50	NA	NA	32
Picric acid	400	10-30	NA	NA	
2,2'-bipyridyl/ $FeCl_3$	530	2-40	1.003×10^4	0.217	33
Potassium dichromate	600	10-200	5.322×10^3	0.322	
1,10-phenanthroline / $FeCl_3$	510	2.0-12	1.45×10^4	2.0	34
Hexacyanoferrate (III) / $FeCl_3$	740	5.5-28	5.51×10^4	5.5	
Folin-Ciocalteu/ alkaline medium	760	4.0-22	NA	NA	35
N-1-napthyl-ethylene diaminedihydrochloride / nitrous acid	550	2.5-12.5	2.1×10^4	NA	36
Patent blue-V / HCl	232 & 257	20-50	NA	NA	37
2, 4- dinitrophenylhydrazine	457	4.0-10	409.43 x 103	0.06	38
3-methyl-2-benzthiazolinone hydrochloride	633	2.0-8.0	409.43 x 103	0.093	39
Bromophenol blue	250	1.0-50	8.9×10^4	NA	40
Bromocresol green	250	1.0-50	8.5×10^4	NA	
$KMnO_4$ / AM	520	2.0-15	$1.7012x\ 10^4$	0.50	
$KMnO_4$ / OG	480	2.0-10	3.0117×10^4	0.47	Proposed work
$KMnO_4$ / MB	664	2.0-12	1.5321×10^4	0.57	

NA: Not detected

From the foregoing paragraphs, it is clear that $KMnO_4$despite its strong oxidizing power, versatility, and high oxidation potential and stability in solution has not been applied for the assay of EZT in pure form and tablets. The three dyes, AM, OG and MB are well known for their high absorptivity and have been utilized for estimation of excess oxidant. The present work aims to develop a simple, rapid, sensitive, accurate, precise, cost-effective and validated spectrophotometric method for the estimation of EZT in pure and dosage forms. The method is based on the oxidation of EZT with slight excess of potassium permanganate ($KMnO_4$) in acidic medium. The unconsumed of $KMnO_4$ is then estimated by adding a fixed amount of AM, OG and MB dyes and measuring the absorbance at 520, 480 and 664 nm, respectively.

MATERIALS AND METHODS

Apparatus

All absorption spectra were made using Varian UV–Vis spectrophotometer (Cary 100 Conc., Australia) equipped with 10 mm quartz cell was used for absorbance measurements. This spectrophotometer has a wavelength accuracy of ±0.2 nm with a scanning speed of 200 nm/min and a bandwidth of 2.0 nm in the wavelength range of 200–900 nm.

Materials and reagents

All chemicals, solvents and reagents used in this work were of analytical reagent or pharmaceutical grade and all solutions were prepared fresh daily. Bidistilled water was used throughout the investigation. Pure sample of EZT was kindly supplied by the Egyptian Company for Chemicals and Pharmaceuticals (ADWIA), 10^{th} of Ramadan City, Egypt. All pharmaceutical preparations were obtained from commercial sources in the local markets. Zetamibe tablet labeled to contain (10 mg EZT per tablet) were purchased from the Egyptian Company for Chemicals and Pharmaceuticals (ADWIA) (10^{th} of Ramadan City, Egypt). Choletimb tablets labeled to contain (10 mg EZT per tablet) were purchased from Marcyrl Pharmaceutical Industries, El Obour City, Cairo, Egypt Global Napi Pharmaceuticals Company (GNP), Egypt. Ezetrol tablets labeled to contain (10 mg EZT per tablet) were purchased from Global Napi Pharmaceuticals Company (GNP), Egypt.

Standard solution

A stock standard solution (200 µg mL^{-1}) of EZT was prepared by dissolving 20 mg of pure EZT in methanol further diluted to 100 mL with methanol in a 100 mL measuring flask. The standard solution were found stable for at least one week without alteration when kept in an amber colored bottle and stored in a refrigerator when not in use.

Potassium permanganate ($KMnO_4$) (5.0×10^{-4} mol L^{-1})

A stock solution of 5.0×10^{-4}mol L^{-1} $KMnO_4$ was freshly prepared by dissolving 0.079 g of $KMnO_4$ (Sigma-Aldrich) in 10 mL of warm bidistilled water then completed to the mark in a 100 mL calibrated flask and standardized using sodium oxalate(41) and kept in a dark bottle and a refrigerator when not in use.

Sulfuric acid (H_2SO_4) (2.0 mol L^{-1})

A 2.0 mol L^{-1} of H_2SO_4 was prepared by adding 10.8 mL of concentrated acid (Merck, Darmstadt, Germany, 98%) to bidistilled water, cooled to room temperature, transfer to 100 mL with measuring flask, diluted to the mark and standardized as recorded(42).

Dyes (1000 µg mL^{-1})

A stock solutions (1000 µg mL^{-1}) AM, OG and MB were first prepared by dissolving accurately weighed 112 mg of each dye (Sigma-aldrish, 90 % dye content) in bidistilled water and diluting to volume in a 100 mL calibrated flask. The solution was then diluted 5.0-fold to get the working concentration of 200 µg mL^{-1} each dye.

Recommended procedures

Different aliquots (0.1-0.75 mL), (0.1-0.5 mL) and (0.1-0.6 mL) of a standard 200 µg mL^{-1} EZT solution using AM, OG and MB methods, respectively, were transferred into a series of 10 mL calibrated flasks followed by adding 1.0 mL 2.0 mol $L^{-1}H_2SO_4$ and 2.0 mL of $KMnO_4$solution (5.0×10^{-4}mol L^{-1}) were added successively. The flasks were stoppered, content mixed and the flasks were kept aside for 5.0 min with occasional shaking. Finally, 1.5 and 1.0 mL of (200 µg mL^{-1}) (AM or OG) and MB solution was added to each flask and mixed well, and then the volume was diluted to the mark with water. The decrease in color intensity of dyes were measured spectrophotometrically after 3.0 min against a blank solution containing the same constituent except drug treated similarly, at their corresponding

λmax 520, 480and 664 nm for AM, OG and MB methods, respectively. The concentration range was determined in each case by plotting the concentration of EZT against absorbance at the corresponding maximum wavelengths.

Procedure for tablet formulations

The contents of twenty tablets of each drug were weighed accurately and ground into a fine powder. An accurate weight of the powdered tablets equivalent to 10 mg EZT was dissolved in methanol with shaking for 5.0 min and filtered using a Whatman No. 42 filter paper. The filtrate was diluted to the mark with methanol for EZT in a 50 mL measuring flask to give and 200 μg mL^{-1} stock solution of EZT for analysis by the proposed spectrophotometric methods. A convenient aliquot was then subjected to analysis by the spectrophotometric procedures described above. Determine the nominal content of the tablets using the corresponding regression equation of the appropriate calibration graph.

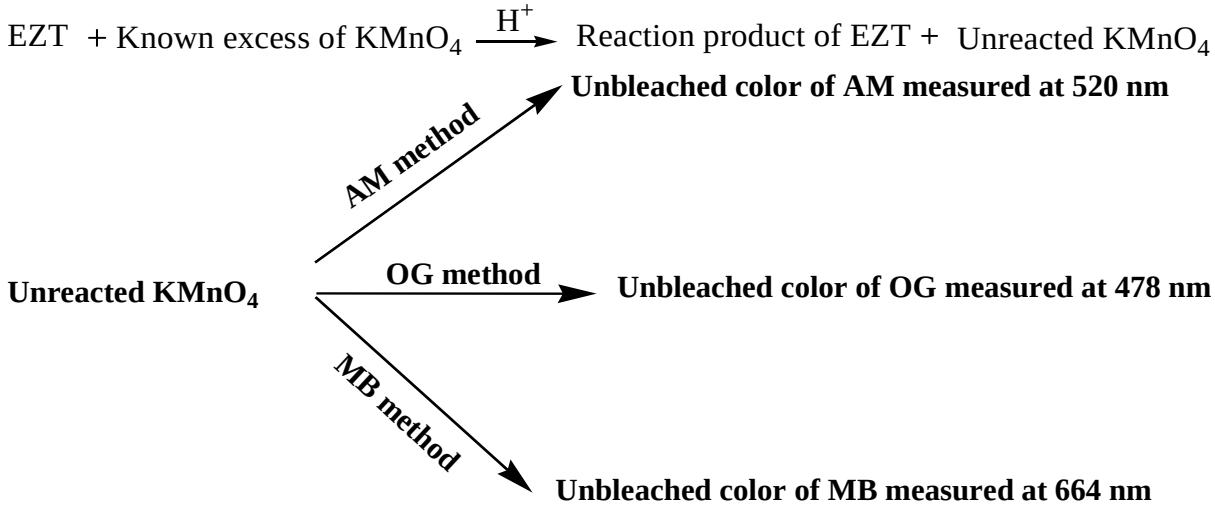
RESULTS AND DISCUSSION

Absorption spectra

The spectrophotometric method for the determination of EZT involves two steps namely:

1. Oxidation of the studied drugs with a known excess of $KMnO_4$ in acidic medium at room temperature (25 ±2 °C).
2. Determination of the residual $KMnO_4$ by reacting it with a fixed amount of amaranth, orange G and methylene blue dyes and measuring the absorbance of dyes at λ_{max} 520, 480 and 664 nm for AM, OG and MB methods, respectively.

These methods make use of the bleaching action of $KMnO_4$on the dyes, the decolorization being caused by the oxidative destruction of the dyes. EZT when added in increasing concentrations to a fixed concentration of $KMnO_4$consumes the latter proportionally and there will be a concomitant decrease in the concentration of $KMnO_4$. When a fixed concentration of dye is added to decreasing concentrations of $KMnO_4$, a concomitant increase in the concentration of dye is obtained. Consequently, a proportional increase in the absorbance at the respective λ_{max} is observed with increasing concentrations of EZT. The tentative reaction scheme of spectrophotometric methods is shown in Scheme 1.



Scheme 1. Tentative reaction scheme for the proposed spectrophotometric methods

Optimization of the reaction conditions

The optimum conditions for the assay procedures and color development for each method have been established by varying the parameters one at a time, keeping the others fixed and observing the effect produced on the absorbance of the colored species.

Effect of acid type and concentration

To study the effect of acid concentration, different types of acids were examined (H_2SO_4, H_3PO_4 and CH_3COOH) to achieve maximum yield of redox reaction. The results indicated that H_2SO_4 was the most suitable acid with $KMnO_4$ as oxidant. Moreover, different volumes (0.2–3.0 mL) of 2.0 mol L^{-1} H_2SO_4 were tested and found to be 1.0 mL of

2.0 mol L^{-1} H_2SO_4 was ideal for the oxidation step in three methods and the same quantity of acid was employed for the estimation of the dye.

Effect of $KMnO_4$concentration

The influence of the volume of 5.0×10^{-4}mol L^{-1} $KMnO_4$ on the reaction has been studied. It is apparent from Fig.2 that the absorbance increased with increasing volume of 5.0×10^{-4}mol L^{-1} $KMnO_4$ solution from (0.25-3.0 mL) and reached maximum when 2.0 mL of $KMnO_4$ was added to a total volume of 10 mL in case of the three dyes. Therefore, it was found that maximum color intensity of the products was achieved with 2.0 mL of $KMnO_4$(5.0×10^{-4}mol L^{-1})for all measurements.

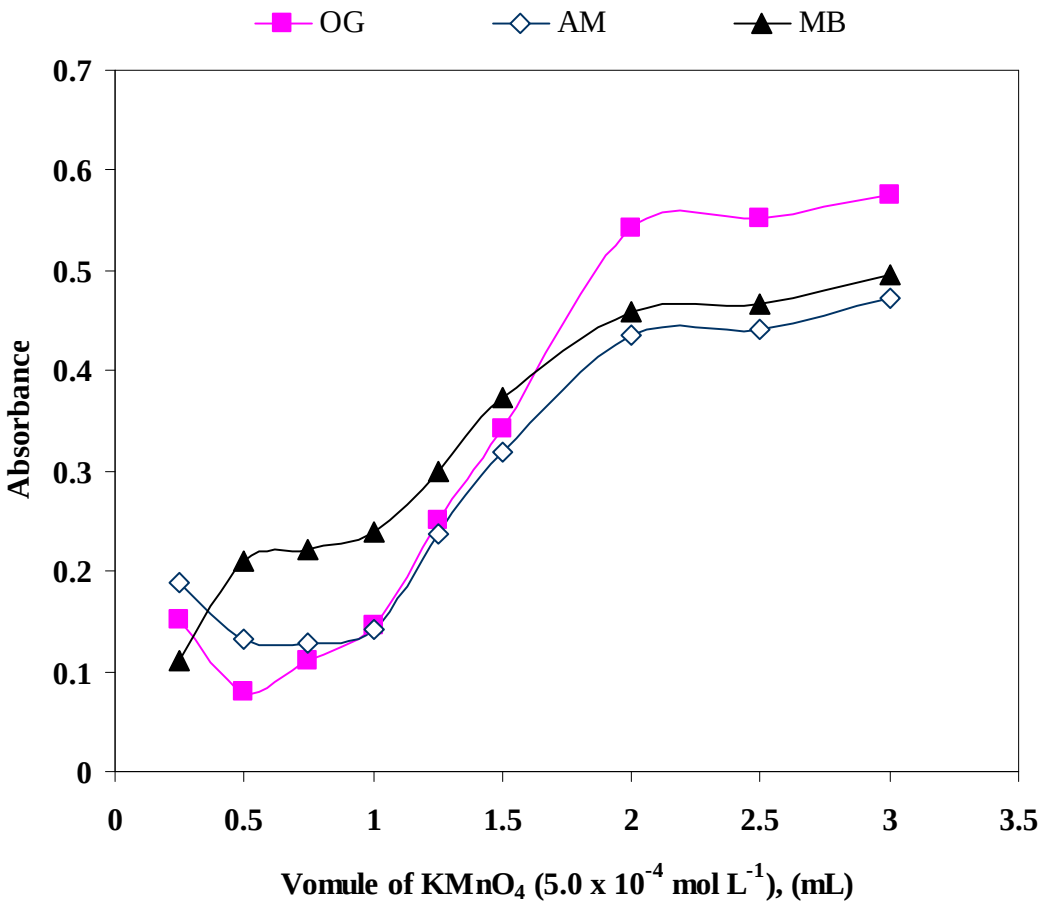


Figure2. Effect of volume of $KMnO_4$ (5.0×10^{-4}molL^{-1}) of the oxidation product of EZT with $KMnO_4$ and three dyes in H_2SO_4 medium

Effect of dye concentration

The effect of AM, OG and MB concentration on the intensity of the color developed was carried out to obtain the optimum concentration of dyes that produces the maximum and reproducible color intensity by reducing the residual of $KMnO_4$. The effect dye concentration was studied in the range of 0.25–3.0 mL of each dye (200 µg mL^{-1}). It was found that maximum color intensity was achieved with 1.5 and 1.0 mL of (AM or OG) and MB dye solutions, respectively (Fig.3). The color was found to be stable up to 8.0 h.

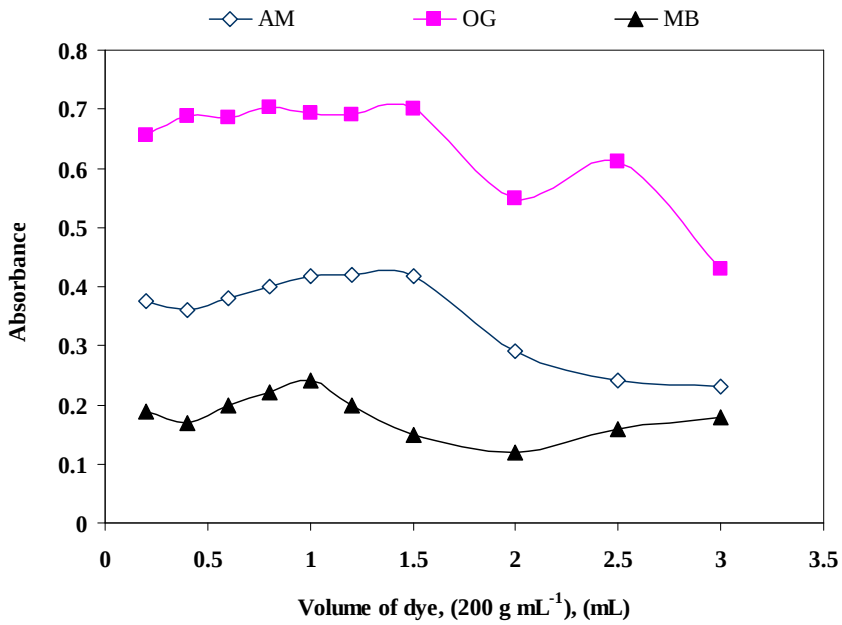


Figure3. Effect of volume of dyes (200 µg mL^{-1}) of the oxidation product of EZT with $KMnO_4$ and dyes in H_2SO_4 medium

Effect of temperature and mixing time

The effect of temperature was studied by heating a series of sample and blank solutions at different temperatures ranging from 25 to 60 °C in water bath. It was found that raising the temperature does not accelerate the oxidation process and does not give reproducible results, so maximum color intensity was obtained at room temperature (25±2 °C). The effect of mixing time required completing oxidation of EZT and for reducing the excess oxidant was studied by measuring the absorbance of sample solution against blank solution prepared similarly at various time intervals 2.0–20 min. It was found that the contact times gave constant and reproducible absorbance values at 5.0 min at room temperature (25 ± 2 °C). The time required for complete oxidation of EZT is not critical and any delay up to 10 min in the determination of unreacted $KMnO_4$ had no effect on the absorbance. After oxidation process, 3.0 min standing time was found necessary for the complete bleaching of the dye color by the residual $KMnO_4$and the absorbance of the unreacted dye was stable for at least 8.0 h, thereafter.

Effect of sequence of addition

The optimum sequence of addition was $KMnO_4$–H_2SO_4–EZT–dye. Other sequences gave lower absorbance values under the same experimental conditions.

Stoichiometric ratio

The molar ratio method described by Yoe and Jones (43) was employed to determine the stoichiometry of drug, oxidant and dyes. The molar ratio between oxidant and dye [Dye]/[$KMnO_4$] at the selected conditions was carried out, by keeping the concentration of the oxidant constant (2.0 mL of 5.0 x 10^{-4}mol L^{-1}) $KMnO_4$, and EZT (10 µg mL^{-1}) and variable volumes (0.2-3.0 mL) of dye (5.0 x 10^{-4}mol L^{-1}) were added. The absorbance was measured atthe suitable wavelength against blank solution prepared by the same manner. The absorbance values were then plotted against the molar ratio [Dye]/[$KMnO_4$].

The molar ratio between the drug (EZT) and oxidant ($KMnO_4$) [EZT]/[$KMnO_4$] at the selected conditions was carried out, by keeping the concentration of the oxidant constant (2.0 mL of 5 x 10^{-4}mol L^{-1}) $KMnO_4$, and (2.0 mL of 5.0 x 10^{-4}mol L^{-1}) dye and different volumes (0.2-3.0 mL) of the drug (5.0 x 10^{-4}mol L^{-1}) were added. The absorbance was measured at the suitable wavelength against blank solution prepared by the same manner. The absorbance values were then plotted against the molar ratio [EZT]/[$KMnO_4$]. Experimental results showed that the inflection of the lines at stoichiometric ratio (1:1) for [Dye]/[$KMnO_4$]; (1.0:2.0)[EZT]/[$KMnO_4$] and (1.0:2.0) [EZT]/[Dye] as shown in Table 2.

Method validation
The proposed methods have been validated for linearity, sensitivity, precision, accuracy, selectivity and recovery.

Linearity and sensitivity
Under the optimum conditions a linear correlation was found between absorbance at λ_{max} and the concentration of EZT in the ranges of 2.0-15, 2.0-10 and 2.0-12 µg mL^{-1} using AM, OG and MB methods, respectively. The calibration graph is described by the equation*:*

$$A = a + b\,C \qquad (1)$$

Where A= absorbance, a= intercept, b= slope and C= concentration in µg mL^{-1}, obtained by the method of least squares. Correlation coefficient, intercept and slope of the calibration data are summarized in Table 2. For accurate determination, Ringbom concentration range (44) was calculated by plotting log concentration of drug in µg mL^{-1} against transmittance % from which the linear portion of the curve gives an accurate range of microdetermination of EZT and represented in Table 2. Sensitivity parameters such as apparent molar absorptivity and Sandell's sensitivity values, as well as the limits of detection and quantification, were calculated as per the current ICH guidelines (45) and illustrated in Table 2. The high molar absorptivity and lower Sandell sensitivity values reflect the good and high sensitivity of the proposed methods. The validity of the proposed methods was evaluated by statistical analysis (46) between the results achieved from the proposed methods and that of the reported method. Regarding the calculated Student's *t*-test and variance ratio *F*-test (Table 2), there is no significant difference between the proposed and reported method(36) regarding accuracy and precision.

The limits of detection (LOD) and quantification (LOQ) were calculated according to the same guidelines using the formulas(45, 46):

$$LOD=3.3\sigma/s \text{ and } LOQ=10\sigma/s \qquad (2)$$

Where σ is the standard deviation of five reagent blank determinations, and s is the slope of the calibration curve.

Table 2. Analytical and regression parameters of proposed oxidation spectrophotometric methods for determination of EZT

Parameters	AM	OG	MB
Wavelength, nm	520	480	664
Beer's law limits, µg mL^{-1}	2.0-15	2.0-10	2.0-12
Ringboom limits, µg mL^{-1}	4.0-12	4.0-8	4.0-10
Molar absorptivity, x 10^4 L mol^{-1} cm^{-1}	1.7012	3.0117	1.5321
Sandell sensitivity, ng cm^{-2}	24.07	13.59	26.72
Regression equation[a]			
Intercept (a)	0.038	0.0165	0.0185
Standard deviation of intercept (S_a)	0.007	0.011	0.009
Slope (b)	0.0207	0.069	0.0334
Standard deviation of slope (S_b)	0.006	0.009	0.01
Correlation coefficient, (r)	0.9992	0.9994	0.9994
Mean ± SD	99.70±1.30	99.15±1.40	99.20±1.05
RSD%	1.30	1.39	1.04
RE%	1.36	1.46	1.09
Limit of detection, µg mL^{-1}	0.50	0.47	0.57
Limit of quantification, µg mL^{-1}	1.67	1.57	1.90
Calculated *t*-value [b]	0.32	0.37	0.36
Calculated *F*-value [b]	1.32	1.53	1.16
[Dye]/[$KMnO_4$]	1:1	1:1	1:1
[EZT]/[$KMnO_4$]	1:2	1:2	1:2
[EZT]/[Dye]	1:2	1:2	1:2

[a] A = a + bC, where C is the concentration in µg mL^{-1}, A is the absorbance units, a is the intercept, b is the slope.
[b] The theoretical values of t and F are 2.57 and 5.05, respectively at confidence limit at 95% confidence level and five degrees of freedom (p= 0.05).

Accuracy and precision
In order to evaluate the precision of the proposed methods, solutions containing three different concentrations of EZT were prepared and analyzed in six replicates. The analytical results obtained from this investigation are summarized in Table 3. Lower values of the relative standard deviation (R.S.D%) and percentage relative error

(R.E%) indicate the precision and accuracy of the proposed methods. The percentage relative error is calculated using the following equation:

$$\% R.E. = \left[\frac{found - taken}{taken} \right] \times 100 \qquad (3)$$

The assay procedure was repeated six times, and percentage relative standard deviation (R.S.D%) values were obtained within the same day to evaluate repeatability (intra-day precision) and over five different days to evaluate intermediate precision (inter-day precision).For the same concentrations of EZT inter- and intra-day accuracy of the methods was also evaluated. The percentage recovery values with respect to found concentrations of EZT were evaluated to ascertain the accuracy of the methods. The recovery values close to 100% as compiled in Tables 3 shows that the proposed methods are very accurate.

Table 3. Results of intra-day and inter-day accuracy and precision study for EZT obtained by the proposed $KMnO_4$method

Method	Taken (µg mL^{-1})	Recovery %	Precision RSD % [a]	Accuracy RE %	Confidence Limit [b]
			Intra-day		
AM	5.0	99.30	0.60	-0.90	4.965 ± 0.031
	10	99.10	0.88	-0.90	9.91 ± 0.092
	15	99.50	1.15	-0.50	14.925 ± 0.18
MB	3.0	100.40	0.85	0.40	3.012 ± 0.027
	6.0	99.60	1.20	-0.40	5.976 ±0.075
	9.0	99.40	1.68	-0.60	8.946 ± 0.158
OG	4.0	99.70	0.80	-0.30	3.988 ± 0.033
	8.0	99.10	1.10	-0.90	7.928 ± 0.092
	12	100.50	1.50	0.50	12.06 ± 0.19
			Inter-day		
AM	5.0	99.00	0.90	-1.0	4.95 ± 0.047
	10	99.30	1.05	-0.70	9.93 ± 0.109
	15	99.80	1.40	-0.20	14.97 ± 0.22
MB	3.0	100.40	0.70	0.40	3.012 ± 0.022
	6.0	99.20	0.95	-0.80	5.952 ± 0.059
	9.0	99.10	1.30	-0.90	8.919 ± 0.122
OG	4.0	99.50	0.87	-0.50	3.98 ± 0.036
	8.0	99.60	1.25	-0.40	7.968 ± 0.105
	12	100.30	1.70	0.30	12.036 ± 0.215

[a] RSD%, percentage relative standard deviation; RE%, percentage relative error.
[b] Mean ± standard error.

Table 4. Results of method robustness and ruggedness studies

Methods	Nominal amount concentration (µg mL^{-1})	RSD%			
		Robustness		Ruggedness	
		Variable alerted [a]			
		Acid volume (n=3)	Reaction time (n=3)	Different analysts (n=3)	Different instruments (n=3)
AM	5.0	1.30	1.15	0.86	0.95
	10	1.80	1.48	1.80	1.70
	15	2.15	2.20	2.50	2.25
OG	3.0	1.50	1.20	1.60	1.40
	6.0	1.90	1.60	1.85	1.90
	9.0	2.80	2.70	2.50	2.50
MB	4.0	1.10	0.90	0.89	1.10
	8.0	1.70	1.90	2.10	1.90
	12	2.20	2.40	2.60	2.30

Robustness and ruggedness

For the evaluation of method robustness, volume of H_2SO_4 was slightly altered (1.0±0.2 mL) and the reaction time was slightly varied deliberately (5.0±1.0 min) (after adding $KMnO_4$) in the three methods. The analysis was performed with altered conditions by taking three different concentrations of EZT and the methods were found to

remain unaffected as shown by the RSD% values in the ranges of 1.10-2.80% and 0.90-2.70% for acid volume and time, respectively. Methods ruggedness was expressed as the RSD of the same procedure applied by three different analysts as well as using three different instruments (spectrophotometers). The inter-analysts RSD% were in the ranges 0.86-2.60%, whereas the inter-instruments RSD% ranged from 0.95-2.50% suggesting that the developed methods were rugged. The results are shown in Table 4.

Recovery studies

To ascertain the accuracy, reliability and validity of the proposed methods, recovery experiment was performed through standard addition technique. This study was performed by spiking three different levels of pure EZT (50, 100 and 150% of the level present in the tablet) to a fixed amount of EZT in tablet powder (pre-analysed) and the total concentration was found by the proposed methods. The determination with each level was repeated three times and the percent recovery of the added standard was calculated from:

$$\textbf{\% Recovery} = \frac{[C_F - C_T]}{C_p} \times 100 \qquad (4)$$

Where $C_F$is the total concentration of the analyte found, C_T is a concentration of the analyte present in the tablet preparation; C_P is a concentration of analyte (pure drugs) added to tablets preparations. The results of this study presented in Table 5 revealed that the accuracy of the proposed methods was unaffected by the various excipients present in tablets which did not interfere in the assay.

Table 5. Results of recovery experiments by standard addition method for the determination of EZT using $KMnO_4$methods in tablets using the proposed methods

Samples	Taken drug in tablet (µg mL^{-1})	Pure drug Added (µg mL^{-1})	Total found (µg mL^{-1})	Recovery [a] (%) ± SD	Total found (µg mL^{-1})	Recovery [a] (%) ± SD	Total found (µg mL^{-1})	Recovery [a] (%) ± SD
NBS			**AM**		**OG**		**MB**	
Zetamibe tablets (10 mg)	4.0	2.0	5.958	99.30±0.60	5.952	99.20±0.40	5.976	99.60±0.60
	4.0	4.0	7.928	99.10±1.10	8.016	100.20±0.70	7.96	99.50±1.20
	4.0	6.0	10.05	100.50±1.70	9.98	99.80±0.90	10.0	99.00±1.50
Choletimb tablets (10 mg)	4.0	2.0	5.952	99.20±0.68	6.024	100.40±0.80	5.964	99.40±0.96
	4.0	4.0	8.064	100.80±0.85	8.048	100.60±1.30	8.04	100.50±1.18
	4.0	6.0	10.03	100.30±1.30	9.95	99.50±1.70	9.93	99.30±1.60
Ezetrol tablets (10 mg)	4.0	2.0	6.012	100.20±0.75	5.952	99.20±0.55	6.042	100.70±0.80
	4.0	4.0	7.96	99.50±1.10	7.992	99.90±1.0	7.952	99.40±1.10
	4.0	6.0	9.91	99.10±1.80	9.90	99.00±1.40	10.03	100.30±1.60

Application of pharmaceutical formulations (tablets)

The proposed methods were applied to the determination of EZT in pharmaceutical formulations (tablets). The results in Table 6 showed that the methods are successful for the determination of EZT and that the excipients in the dosage forms do not interfere. A statistical comparison of the results obtained from the assay of EZT by the proposed methods and the reported method (36) for the same batch of material is presented in Table 6. The results agree well with the label claim and also were in agreement with the results obtained by the reported method[36]. When the results were statistically compared with those of the reported method by applying the Student's t-test for accuracy and F-test for precision, the calculated t-value and F-value at 95% confidence level did not exceed the tabulated values for five degrees of freedom [46]. Hence, no significant difference between the proposed methods and the reported method at the 95 % confidence level with respect to accuracy and precision.

Table 6. Results of analysis of tablets by the proposed methods for the determination of EZT and statistical comparison with the reported method

Samples	Proposed method recovery [a] (%) ± SD			Reported method[36]
	AM	OG	MB	
Zetamibe tablets (10 mg)	99.30 ± 1.20	99.20 ± 0.98	100.20 ± 1.06	99.77 ± 1.40
t-value [b]	0.56	0.74	0.54	
F-value [b]	1.36	2.04	1.74	
Choletimb tablets (10 mg)	99.10 ± 0.70	99.90 ± 1.25	99.30 ± 0.70	99.64 ± 0.92
t-value [b]	1.04	0.37	0.32	
F-value [b]	1.2	1.84	1.2	
Ezetrol tablets (10 mg)	99.70 ± 0.85	99.60 ± 0.35	99.80 ± 0.89	99.33 ± 0.62
t-value [b]	0.78	0.84	0.96	
F-value [b]	1.87	3.13	2.06	

[a]*Average of six determinations.*

CONCLUSION

Three new, useful simple, rapid, and cost-effective spectrophotometric methods have been developed for determination of EZT in bulk drugs and in their tablets using $KMnO_4$ as oxidizing agent and validated as per the current ICH guidelines. The present spectrophotometric methods are characterized by simplicity of operation, high selectivity, comparable sensitivity, low-cost instrument, they do not involve any critical experimental variable and are free from tedious and time-consuming extraction steps and use of organic solvents unlike many of the previous methods reported for EZT . The assay methods have some additional advantages involve less stringent control of experimental parameters such as the stability of the colored system, accuracy, reproducibility, time of analysis, temperature independence and cheaper chemicals. These advantages encourage the application of the proposed methods in routine quality control analysis of EZT in pure and dosage forms.

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