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Research Article

ASSAY OF EZETIMIBE IN BULK AND IN ITS PHARMACEUTICAL FORMULATIONS BY SPECTROPHOTOMETRY

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ABSTRACT

Two simple, sensitive, precise and accurate visible spectrophotometric methods have been developed for the assay of ezetimibe in bulk drug and in pharmaceutical formulations. The first method, i.e. the 2, 2′-bipyridyl method, is based on the oxidation of the drug with Fe (III) and the estimation of Fe (II) produced after chelation with 2, 2′-bipyridyl at 530 nm. The second method, i.e. the potassium dichromate method, is based on oxidation of the drug by $K_2Cr_2O_7$ in acidic medium and the determination of chromium (III) ion produced at 600 nm. Reaction conditions were optimized to achieve the maximum color intensity. The absorbance was found to increase linearly with increase in concentration of ezetimibe, which was corroborated by the calculated regression coefficient value (0.9984–0.9991). Beer's law is obeyed in the concentration range of 2–40 and 10–200 µg/ml for 2, 2′-bipyridyl and $K_2Cr_2O_7$, respectively. The molar absorptivity, Sandell's sensitivity, limit of detection and limit of quantification have been evaluated. The proposed methods were applied to the quantification of ezetimibe in pharmaceutical formulations. The results obtained by the proposed methods were statistically compared by means of student t-test and by the variance ratio F-test with those of the reported (UV spectrophotometry) method. Common excipients used in pharmaceutical formulations do not interfere in the proposed methods.

Keywords: Ezetimibe, 2, 2′-bipyridyl, Potassium dichromate, Spectrophotometric analysis.

INTRODUCTION

Ezetimibe (EZT)[1-5], chemically known as (3*R*, 4*S*)-1-(4-fluorophenyl)-3-[(3*S*)-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 works by preventing cholesterol and other plant sterols from being absorbed into the bloodstream. The overall effect is a reduction in cholesterol level in the blood. In conjunction with any of the statins such as simvastatin, atorvastatin etc., and a cholesterol-lowering diet, EZT is used to lower cholesterol in people with inherited familial hypercholesterolaemia.

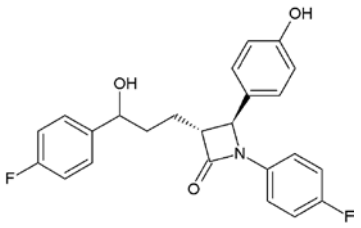

Fig. 1: Structure of Ezetimibe

The literature is enriched with several techniques for determination of EZT in pharmaceutical dosage forms and/or biological fluids, including high performance liquid chromatography (HLC)[6-11], thin layer chromatography (TLC)[6], liquid chromatography-mass spectrophotometry (LC-MS)[12-15], voltametry[16], gas chromatography-mass spectrophotometry (GC-MS)[17]. The above reported techniques for EZT determination are tedious, dedicated to sophisticated and expensive analytical instruments.

Spectrophotometry is by far the instrumental technique of choice in the laboratories of underdeveloped and developing nations for the quantification of drugs, owing mainly to its simplicity, high sensitivity & selectivity and often demanding lowcost equipment. Few visible[18,19], UV[20] and derivative[20,6] spectrophotometric methods are reported in the literature. The reported spectrophotometric methods suffer from one or more disadvantages such as narrow linear response[18-20,6], lack of selectivity & sensitivity [18-20,6] and usage of expensive reagent[19]. The need for a sensitive, cost effective and reliable spectrophotometric method for the estimation of EZT is thus obviously recognized.

Our present paper describes two optimized and validated visible spectrophotometric methods (2,2′-bipyridyl and potassium dichromate) for the determination of EZT in bulk and commercial dosage forms. The 2, 2′-bipyridyl method is based on the oxidation of EZT with $FeCl_3$ and subsequent complexation of resulting Fe (II) with 2, 2′-bipyridyl. The resulting red colored chromogen due to the formation of Fe (II)-bipyridyl complex absorbs maximally at 530 nm. Potassium dichromate method is based on the oxidation of the drug by $K_2Cr_2O_7$ and consequently the reduction of dichromate ions (VI) to the corresponding green colored chromium ions (III) of λ_{max} at 600 nm in acidic medium. In both methods, the absorbance of red colored Fe (II)-bipyridyl complex and green colored chromium ions (III) at their corresponding λ_{max} increases linearly with an increasing concentration of the EZT.

MATERIALS AND METHODS

Instrumentation

- All spectrophotometric measurements were carried out using an Elico (Hyderabad, India) double beam model SL 159 digital spectrophotometer. The cells used for absorbance measurements were 1-cm matched quartz cells.
- Kemi KWB 220 model water bath (Ernakulam, India) was used to control the temperature for color development.
- Samples were weighed by using Essae-Teraoka electronic weighing balance (Goa, India) PG1000 model.

Reagents

All the chemicals used were of analytical reagent grade and used as received. Double distilled water was used in the preparation of all solutions. All the solutions were prepared afresh daily.

- 0.2 M Orthophosphoric acid: The solution was prepared by diluting 1.31 ml of 85% orthophosphoric acid (Merck, Mumbai, India) to 100 ml with water.
- 0.2% 2, 2′-Bipyridyl: The solution was prepared by dissolving 200 mg of 2, 2′-bipyridyl (Merck, Mumbai, India) in 100 ml of water.
- 0.5% Ferric chloride: The solution was prepared by dissolving 500 mg of $FeCl_3$ (Sdfine-Chem limited, Mumbai, India) in 100 ml of water.

- 0.1% Potassium dichromate: The solution was prepared by dissolving 100 mg of $K_2Cr_2O_7$ (Sdfine-Chem limited, Mumbai, India) in 100 ml of water.
- N Sodium hydroxide: The solution was prepared by dissolving 400 mg of NaOH (Merck, Mumbai, India) in 100 ml of water
- Commercially available 36.8 N Sulphuric acid (Fisher Scientific, Mumbai, India) was, as such used.

EZT Standard Solutions

Pharmaceutical grade EZT was graciously donated by a local pharmaceutical company. The EZT stock solution (1 mg/ml) was prepared by dissolving 100 mg of the drug in 20 ml of 0.1 N NaOH and then diluted to 100 ml with water. Working standard solution containing 200 µg/ml of EZT was prepared by further dilution of the stock solution for 2, 2'-Bipyridyl method. For $K_2Cr_2O_7$ method the stock solution was used as such.

Commercial Tablet Dosage Forms of EZT

Ezentia (Sun Pharma, Mumbai, India), Zetica (Torrent Pharma., Ahemdabad, India), Ezzicad (Glenmark Pharmaceuticals Ltd., Mumbai, India) and Ezetib (Unisearch Labs Ltd., Mumbai, India) tablets containing 10 mg of EZT were purchased from the local pharmacy market.

Recommended Procedure for the Determination of EZT

2, 2'-Bipyridyl Method

Aliquots (0.1-2.0 ml) of 0.2 % EZT solution to obtain the final concentrations in the range 2–40 µg/ml were pipetted into a series of boiling test tubes. Subsequently, 1.5 ml of 0.5% $FeCl_3$, 1.5 ml of 0.2% 2, 2'-bipyridyl and 1 ml of 0.2 M orthophosphoric acid were added. The contents of the tubes were mixed well and heated on a water bath at 65°C for 15 minutes. The tubes were cooled at room temperature. The contents of the tubes were transferred to 10 ml volumetric flasks and diluted to volume with distilled water. The absorbance of the red colored chromogen was recorded at 530 nm against a reagent blank treated likewise except without drug. The calibration curve was constructed by plotting the absorbance at 530 nm against the final concentration of EZT in µg/ml. The amount of EZT in unknown samples was calculated from either the calibration curve or the corresponding regression equation.

Potassium Dichromate Method

Aliquots (0.1-2.0 ml) of 1 % EZT solution, corresponding to 10–200 µg/ml, were transferred into a series of 10 ml volumetric flasks. Then 1 ml of 0.1% $K_2Cr_2O_7$ was added, followed by 2 ml of H_2SO_4. The above contents were mixed thoroughly. The reaction was allowed to proceed for 5 minutes at room temperature (25±5 ºC). After the completion of the reaction, the solutions were diluted to volume with distilled water. The absorbance of the green colored solution was measured at 600 nm against the reagent blank treated similarly except without the drug. The calibration curve was constructed by plotting the absorbance at 600 nm against the final concentration of EZT in µg/ml. The amount of EZT in unknown samples was calculated from either the calibration curve or the corresponding regression equation.

Procedure for the Determination of EZT in Pharmaceutical Formulations (Tablets):

Ten tablets were accurately weighed and finely powdered. An amount of tablet powder equivalent to 50 mg of EZT was weighed and dissolved in 25 ml of methanol (Merck, Mumbai, India). The mixture was shaken for 20 minutes and filtered through Whatman No. 1 filter paper. The filtrate was evaporated to dryness on a water bath. The residue was dissolved in 5 ml of 0.1 N NaOH. The solution was then transferred into a 50 ml volumetric flask, made up to the mark with distilled water. Suitable aliquots of the EZT solution (20 and 100 µg/ml) were used for analysis and treated as described in the above procedures 2, 2'-bipyridyl and potassium dichromate. The recovery of EZT was calculated from either the corresponding linear regression equation or the calibration curve.

RESULTS AND DISCUSSION

Chemistry of the Colored Species

Ferric salts (ferric chloride) play a prominent role in the colorimetric determination of organic compounds. Acting as an oxidant, the ferric salt is converted into ferrous salt which can easily be detected by the usual reagent for divalent iron, potassium ferricyanide[21], 1,10-phenanthroline[22], 2,2'-bipyridyl[23], 2,2',2'-Terpyridyl[23] or triazine[24]. 2,2'-Bipyridyl forms a complex of low tinctorial value with Fe (III) which in turn functions as a better oxidant than Fe(III) itself. The reduction product is tris complex of Fe (II). Based on its complexing tendency and oxidizing properties, ferric salt was recommended in the quantification of indapamide[25], imipramine hydrochloride[26], desipramine hydrochloride[26], clomipramine hydrochloride[26], trimipramine maleate[26], opipramol[26], pipazethate hydrochloride[27], domperidone[28], metoclopramide[28], amoxycillin[29], ciprofloxacin[29] piroxicam[29], cinitapride hydrogen tartarate[30] and formoterol[31]. The proposed 2, 2'-bipyridyl method was based on the oxidation of EZT by Fe (III) in $FeCl_3$. The resulting Fe (II) complexes with unshared pair of electrons on each of the two nitrogen atoms of 2,2'-bipyridyl to produce red colored chromogen having maximum absorption at 530 nm against the corresponding reagent blank (Fig 2). The corresponding reagent blank has almost negligible absorbance at 530 nm. The absorbance of the chromogen formed remained stable for at least 9 hours. Fe (III) interferes to a little extent (especially in the lower range of Beer's law limits) in the determination of Fe (II). The reactivity of the interfering entity (Fe (III)) has been made insignificant by complexing it with orthophosphoric acid. The possible reaction mechanism is given in Fig 3.

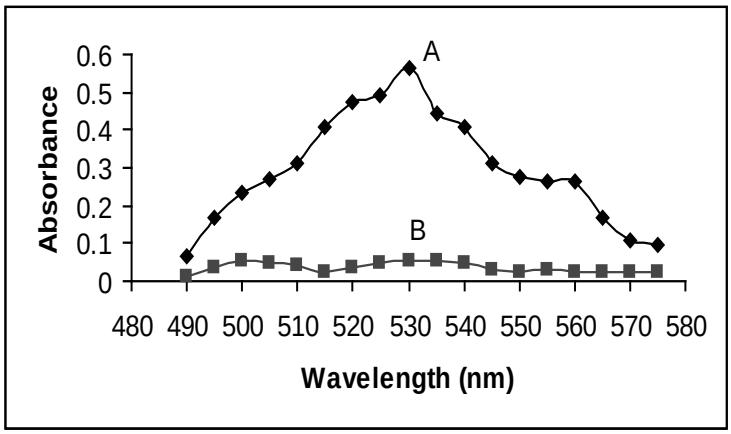

Fig. 2: Absorption spectra of A) Fe (II)-2, 2'-Bipyridyl complex against reagent blank B) Reagent blank against water

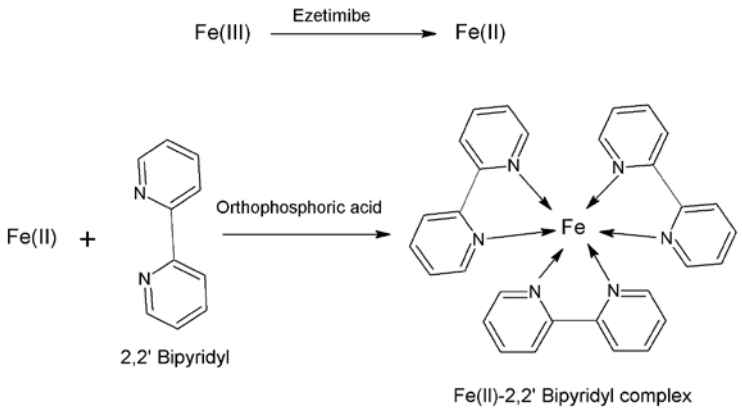

Fig. 3: Proposed reaction scheme of formation of Fe (II)-2, 2'-Bipyridyl complex

Potassium dichromate solution in the presence of sulphuric acid is commonly used as an oxidizing agent in organic chemistry. The oxidizing property of $K_2Cr_2O_7$ is applied for the quantification of the compounds having pharmaceutical significance by spectrophotometric method [32-36]. The results obtained in the $K_2Cr_2O_7$ method were due to the oxidation of the drug with dichromate ions (VI) in concentrated H_2SO_4 medium at room temperature. The absorbance of the green colored chromium (III) ions produced is measured at 600 nm. The absorption spectrum of the chromium (III) ions formed is shown in the Fig 4. The corresponding reagent blank has almost negligible absorbance at this wavelength.

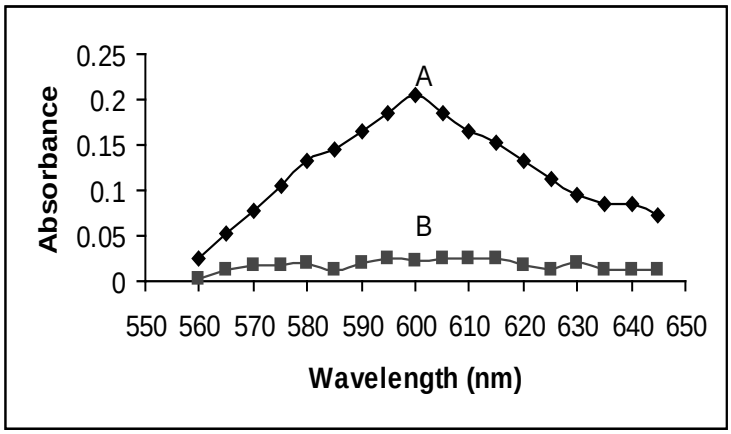

Fig. 4: Absorption spectra of A) Chromium (III) ions against reagent blank B) Reagent blank against water

Optimization of Reaction Conditions

The optimum conditions affecting the formation of Fe(II)-bipyridyl complex (2, 2'-bipyridyl method) and chromium (III) ions ($K_2Cr_2O_7$ method) were studied and maintained throughout the experiments.

2, 2'-Bipyridyl Method

The effect of the concentration of $FeCl_3$ was studied by treating 20 µg/ml EZT with 1 ml of 0.2% 2, 2'-bipyridyl, 1 ml of 0.2 M orthophosphoric acid and varying volumes (0.5–2.5 ml) of 0.5% $FeCl_3$. The absorbance of red colored chromogen at 530 nm was increased with increasing volume of $FeCl_3$ and became constant at 1.5 ml; above this volume, the absorbance remained unchanged. Therefore, 1.5 ml of 0.5% $FeCl_3$ was used in all the measurements.

The influence of the concentration of 2, 2'-bipyridyl on the absorbance at 530 nm was investigated by treating 20 µg/ml EZT with 1.5 ml of 0.5% $FeCl_3$, 1 ml of 0.2 M orthophosphoric acid and varying volumes (0.5–2.5 ml) of 0.2% 2, 2'-bipyridyl. The absorbance was increased with increasing volume of 2, 2'-bipyridyl and became constant at 1.5 ml; beyond this volume, the absorbance remained constant. Therefore 1.5 ml of 0.2% 2, 2'-bipyridyl was suggested for the determination procedures.

The orthophosphoric acid was essential to increase the stability of the developed red colored complex by maintaining the desired pH. The effect of adding various volumes of orthophosphoric acid on absorbance at 530 nm was examined. The concentration of orthophosphoric acid was varied between 0.2-2.0 ml of 0.2 M orthophosphoric acid. The maximum absorbance was obtained with 1 ml of orthophosphoric acid. Therefore, 1 ml of 0.2 M orthophosphoric acid was adequate to increase the stability of the colored complex.

The influence of temperature on the formation of Fe (II)-bipyridyl complex was studied at different temperatures (40-80 ^{0}C). The optimum temperature was established as 65 °C. However, no significant improvements occurred above 65°C.

To investigate the optimum heating time for the color development, the contents of the reaction mixture were heated for 5-30 min on a water bath at 65°C. The maximum intensity of color was obtained at 15 min of heating. Therefore, 15 min of heating time was used throughout the experiment.

Potassium dichromate method

The influence of the concentration of $K_2Cr_2O_7$ was observed during the formation of green colored chromium (III) ions. To study this, an aliquot of EZT containing 100 µg/ml was pipetted followed by varying volumes (0.2-2.0 ml) of 0.1 % $K_2Cr_2O_7$ and 2.0 ml of H_2SO_4. The maximum absorbance was attained with 1.0 ml of 0.1 % $K_2Cr_2O_7$; above this volume the absorbance remained constant. Therefore, 1.0 ml of 0.1 % $K_2Cr_2O_7$ was used in all the further measurements.

To investigate the effect of volume of sulphuric acid for green colored chromium (III) ions development, different volumes (0.5-4.0 ml) of H_2SO_4 were mixed with 1 ml of EZT (100 µg) and 1.0 ml of 0.1 % $K_2Cr_2O_7$. The results reveal that the addition of 2.0 ml of H_2SO_4 gave the highest absorbance, which remained constant up to 4.0 ml. Therefore, 2.0 ml of the H_2SO_4 was taken for the determination of the EZT throughout the experiment.

It was observed that the absorbance of the chromium (III) ions at 600 nm became constant after 5 minutes, so for all the measurements 5 minutes reaction time was selected. The absorbance of the chromium (III) ions remained constant for 18 hours.

Method Validation

Linearity

At described experimental conditions for EZT determination, standard calibration curves for EZT with 2, 2'-bipyridyl and $K_2Cr_2O_7$ reagents were constructed by plotting an increase in absorbencies *vs* concentrations (Fig 5 and Fig 6). A linear correlation was found between absorbance and concentration of EZT in the ranges given in Table 1. The statistical parameters given in the regression equation were calculated from the calibration graphs. The high values of the regression coefficient (r^2) and low values y-intercepts of the regression equations, proved the linearity of the calibration curves (Table 1). The optical characteristics such as molar absorptivity and Sandell's sensitivity values were calculated for both the methods and are presented in Table 1.

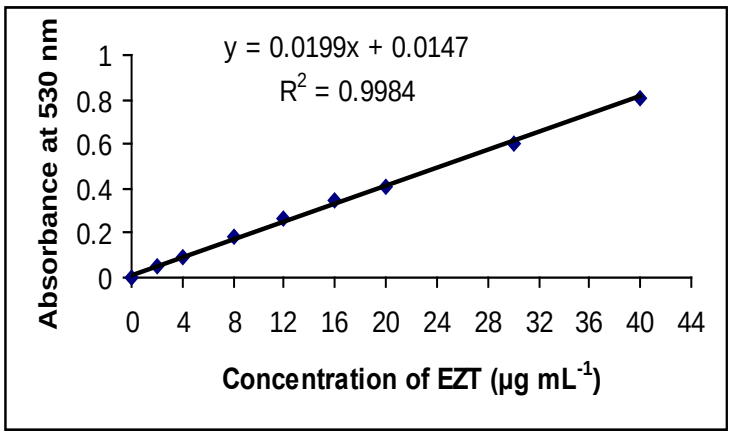

Fig. 5: Linearity curve for 2, 2'-Bipyridyl method

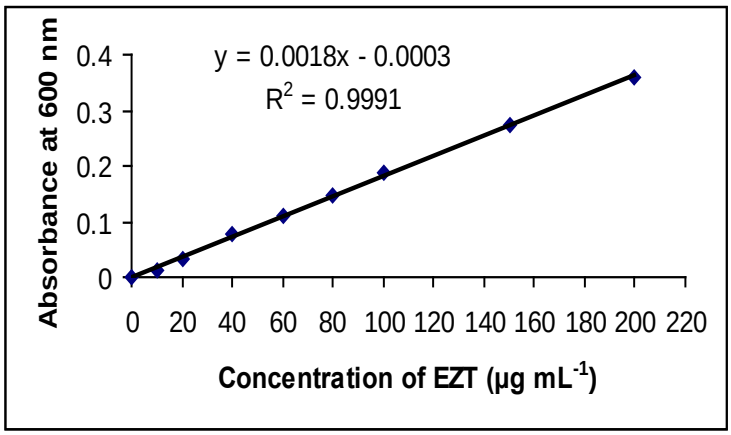

Fig. 6: Linearity curve for Potassium dichromate method

Table 1: Optical and statistical characteristics of the proposed methods

Parameters	2, 2'-Bipyridyl Method	Potassium dichromate Method
Beer's Limit (µg/ml)	2-40	10-200
Molar Absorptivity (L/mole/cm)	1.003 x 10^4	5.322 x 10^3
Sandell's sensitivity (µg cm^{-2}/0.001 Absorbance unit)	0.0408	0.0769
Stability of colored products (hours)	9	18
LOD (µg/ml)	0.217	0.322
LOQ (µg/ml)	0.658	0.977
Standard deviation$	0.00131	0.00154
Relative standard deviation (%)	0.318	0.759
Range of error (%) (Confidence Limits)		
0.05 level	± 0.256	± 0.634
0.01 level	± 0.393	± 0.938

$Average of six determinations

Limit of Detection and Limit of Quantification

The limit of detection (LOD) and limit of quantitation (LOQ) was determined by taking the ratio of standard deviation of the reagent blank with respect to water and slope of calibration curve multiplied by a factor of 3.3 and 10, respectively. The results presented in Table 1 reveal the very high sensitivity of the proposed methods.

Accuracy and Precision

The precision and accuracy of the proposed methods were assessed by determining the relative standard deviation (RSD) and percentage relative error of six replicate analyses on the same solution containing fixed concentration of EZT (within Beer's law limit). The results of the intra-assay RSD and percentage relative error, which is the measure of precision and accuracy respectively, are shown in Table 1. The low values of RSD and percentage relative error in solution reveal the high precision and accuracy of the proposed methods.

Standard Addition Method for Validation (Recovery Studies)

The validity and accuracy of the proposed methods were further assessed by recovery studies using the standard addition technique. For this purpose, a known amount of pure drug at three different levels was spiked to the fixed and known quantity of pre analyzed tablets and the nominal value of drug was estimated by the proposed methods. The results (Table 2) were reproducible with low SD and RSD. No interference from the commonly encountered tablet excipients was observed and thereby establishes some degree of selectivity.

Table 2: Results of recovery study by standard addition method

EZT in the tablet (mg)	Pure EZT added (mg)	Total Found (mg) ± SD (n = 5)	Recovery (%)	RSD (%)	Error (%)
2,2'-Bipyridyl method					
10	5	15.06 ± 0.238	100.40	1.580	0.40
10	10	19.94 ± 0.162	99.70	0.812	0.30
10	15	25.10 ± 0.183	100.40	0.729	0.40
Potassium dichromate method					
10	5	14.95 ± 0.227	99.66	1.518	0.34
10	10	20.06 ± 0.269	100.30	1.340	0.30
10	15	25.04± 0.137	100.16	0.547	0.16

Application of the Proposed Methods

To evaluate the proposed methods, they were applied to the determination of EZT in four commercial tablet dosage forms. The recoveries are close to 100%, indicating that there is no serious interference in samples. The good agreement between these results and known values indicate the successful applicability of the proposed methods for the determination of EZT in tablets. The results of the proposed methods were compared statistically with those of the reference method[20]. The results are given in Table 3. A statistical analysis of the results by student t-test and by the variance ratio F-test showed no significant difference in accuracy and precision between the proposed methods and the reference method.

Table 3: Results of analysis of tablets by the proposed and reference methods

Brand name of tablet	Labeled amount (mg)	Found (mg) ± SD (n = 5)	Recovery (%)	RSD (%)	t value*	F value@
Reference method						
Ezentia	10	10.03 ± 0.058	100.30	0.578	-	-
Zetica	10	9.96 ± 0.096	99.60	0.963	-	-
Ezzicad	10	9.98 ± 0.072	99.80	0.721	-	-
Ezetib	10	10.07 ± 0.064	100.70	0.635	-	-
2,2'-Bipyridyl method						
Ezentia	10	9.92 ± 0.093	99.20	0.937	0.329	2.934
Zetica	10	9.95 ± 0.083	99.50	0.834	1.296	2.672
Ezzicad	10	10.05 ± 0.067	100.50	0.646	0.937	1.702
Ezetib	10	9.95 ± 0.091	99.50	0.914	0.638	1.349
Potassium dichromate method						
Ezentia	10	10.03 ± 0.069	100.30	0.687	1.336	2.296
Zetica	10	9.93 ± 0.081	99.30	0.845	1.283	1.672
Ezzicad	10	10.05 ± 0.053	100.50	0.527	0.935	1.638
Ezetib	10	9.93 ± 0.041	99.30	0.412	0.342	1.348

* Tabulated t-value at 95% confidence level is 2.306; @Tabulated F- value at 95 % confidence level is 6.390

CONCLUSIONS

Two new, cost effective, simple and sensitive visible spectrophotometric methods, using 2, 2'-bipyridyl and $K_2Cr_2O_7$ as reagents, were developed for the determination of EZT in bulk and in pharmaceutical formulations. The developed methods were also validated. From the statistical data, it was found that the proposed methods were accurate, precise, reproducible and can be successfully applied to the analysis of EZT in pharmaceutical formulation without interference of excipients.

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