CERIMETRIC DETERMINATION OF FOUR ANTIHYPERTENSIVE DRUGS IN PHARMACEUTICAL PREPARATIONS

A.M. EL-DIDAMONY† AND E.A.H. ERFAN

Chemistry Department, Faculty of Science, Zagazig University, Zagazig, Egypt
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ABSTRACT

A sensitive spectrophotometric method is described for the determination of atenolol (ATE), timolol maleate (TIM), captopril (CPL) and diltiazem hydrochloride (DIL.HCl) in bulk drugs and in pharmaceutical preparations. The method is based on the oxidation of the studied drugs by a known excess of cerric (IV) in acid medium followed by determination of unreacted oxidant by adding a fixed amount of methyl orange dye (MO) and the increasing in absorbance is measured at 510 nm. In this method the amount of cerium (IV) reacted corresponds to drugs concentration. The experimental conditions were optimized. Regression analysis of a Beer's plot showed good correlation in the concentration ranges of 3.2-6.4, 8.0-18, 3.4-5.2 and 3.6-5.2 µg/ml for ATE, TIM, CPL and DIL.HCl, respectively. The calculated molar absorptivity values are 5.28×10^4, 3.27×10^4, 6.43×10^4 and 1.12×10^5 L /mol cm, respectively and the corresponding Sandell's sensitivity values are 5.043, 13.2, 3.381 and 4.024 ng/cm^2, respectively. The limit of detection (LOD) and quantification (LOQ) are reported. No interference was observed from the additives and the applicability of the method was tested by analyzing the pharmaceutical preparations containing the investigated drugs. Statistical comparison of the results with those of official methods shows excellent agreement and indicates no significant difference in precision.

Key words: Cerimetry, Antihypertensive drugs, Pharmaceutical preparations.

INTRODUCTION

Anti-hypertensive drugs are used to help control blood pressure in people whose blood pressure is too high. Blood pressure is a measurement of the force with which blood moves through the body's system of blood vessels. Although everyone's blood pressure goes up and down in the course of a typical day-getting higher when they are active and going down when they sleep. Some people have blood pressure that stays high all the time. This condition is known as hypertension [1]. There are many classes of medications for treating hypertension, called anti-hypertensive. Beta blockers are a common class of prescription drugs that counteract the stimulatory effects of adrenaline (epinephrine) on what are called the beta receptors. These receptors are found in many tissues of the body including the nervous system and heart. When beta receptors are stimulated, the heart beats faster and harder and the blood vessels constrict, resulting in an elevation of blood pressure. Atenolol and timolol belong to β- blockers that block activity of β-adrenergic receptors and are of wide spectrum of pharmacological action; used in the treatment of ischemic heart disease, coronary failure, or as illegal doping agents [2-4].

Atenolol, 4-(2-hydroxy-3-isopropylaminopropoxy) phenyl acetamide (Scheme 1), is official in the Indian Pharmacopoeia [5], which describes a UV-spectrophotometric method for its assay in tablets. The drug is also official in the British Pharmacopoeia, which describes high-performance liquid chromatographic which is two-stage processes [6]. Several procedures for atenolol determination have been employed, for application in pharmaceutical and biological samples. These procedures include capillary electrophoresis [7-11], HPLC [12-17], liquid chromatography [18-21], gas-liquid chromatography [22], and voltammetric [23-25]. Other techniques include UV, Visible and kinetic spectrophotometry [26-32], and chemluminescence [33,34], were reported.

Timolol, (–)-(S)-1- *tert* -butylamino-3-(4-morpholino-1,2,5- thiadiazol-3-yloxy)propan-2-ol (Scheme. 1), is used as an antihypertensive and an antiglucoma agent. Several procedures have been reported in the literature for the analysis of timolol such as electrophoresis [35,36], high-performance liquid chromatography [37-39], liquid chromatography [40-42], voltammetric [43,44], and UV-visible spectrophotometry [45-47]. Timolol is officially recognized in the USP [48], and BP [49]. The method of analysis for the bulk drug was non-aqueous titration detecting the end point potentiometrically.

Angiotensin-converting enzyme (ACE) inhibitors; drugs in this group work by preventing a chemical in the blood angiotensin I, from being converted into a substance that increases salt and water retention in the body. These drugs also make blood vessels relax, which further reduces blood pressure. Captopril belongs to ACE and it was the first orally active ACE applied to the treatment of hypertension, coronary heart disease and congestive heart failure in clinical medicine. Determination of captopril 1-[(2S)-3-mercapto-2-methylpropionyl]-Lproline (Scheme 1) has previously been reported by capillary electrophoresis [50,51], high performance liquid chromatography [52-58], liquid chromatography [59], voltammetry [63-67], spectrophotometry and kinetic spectrophotometry [68-72], spectrofluorimetry [73], and chemiluminescence.[74-80] The drug is listed in United States Pharmacopoeia, which recommends a HPLC method for its assay in bulk and tablet formulations [81].

Calcium channel blockers; drugs in this group slow the movement of calcium into the cells of blood vessels. This, in turn, relaxes blood vessels, increases the supply of oxygen-rich blood to the heart and reduces the heart's work load diltiazem hydrochloride is a member of this group. The assay procedure of DIL.HCl (1, 5-Benzothiazepin-4(5H) one, 3-(acetyloxy) -5-[2-(dimethylamino) ethyl]-2, 3-dihydro-2-(4-methoxyphenyl)-monohydrochloride,(+)-cis, (Scheme 1) listed in British Pharmacopoeia [82], where in the drug and its formulations are estimated by an HPLC (Id) method. The other methods for its estimation include capillary electrophoresis [83,84], HPLC [85-89], and spectrophotometry [90-94].

In this paper, an attempt for the determination of four important antihypertensive drugs, viz., ATE, TIM, CPL, and DIL.HCl has been made. The method is based on the oxidation of the investigated drugs with slight excess of cerric (IV) in acidic medium. The unconsumed of Cerric (IV) is then estimated by adding a fixed amount of methyl orange dye to form colored species which absorbs maximally at 510 nm. The proposed procedure is found to provide a simple and accurate method for the determination of ATE, TIM, CPL, and DIL.HCl.

EXPERIMENTAL

4Apparatus

All the absorbance spectral measurements were made using spectroscan 80 D double-beam UV/Visible spectrophotometer (Biotech Engineering Ltd. (UK), with wavelength range 190 nm ~ 1100 nm, spectral bandwidth 2.0 nm, with 10 mm matched quartz cells. An Orion Research Model 601 A/digital analyzer, pH-meter with a combined saturated calomel glass electrode was used for pH measurements.

Reagents and Materials

All reagents and chemicals used were of analytical or pharmaceutical grade and all solutions were prepared fresh daily.

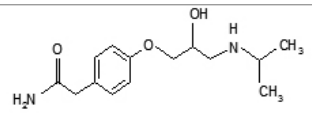
Standard Solution of Pure Drugs

Atenolol (ATE) and timolol maleate (TIM) were supplied from Egyptian Pharmaceutical Industries (EIPICO) 10th of Ramadan City, Egypt; captopril (CPL) that supplied from Mina Pharma, El-Obour City, Egypt and diltiazem hydrochloride (DIL.HCl) were supplied from Glaxowellcome, Egypt, which were reported to be 99.8% purity, as gift and were used as received. Stock solutions of ATE, CPL, and DIL.HCl (100 µg/ml) were prepared by dissolving 10 mg of pure drugs in 20 ml of distilled water in 100 ml calibrated flask and then diluted stepwise to the mark with distilled water.

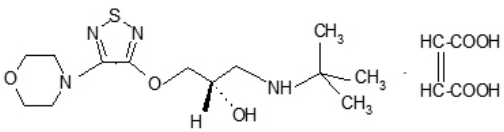
TIM stock solution (500 µg/ml) was prepared by dissolving accurately

e-mail address: ak_eldidamony@yahoo.com

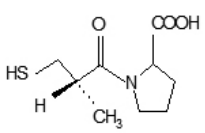
weighed 50 mg of pure drug in the least amount of distilled water and then diluted stepwise to 100 ml in calibrated flask to obtain working concentration of 500 µg/ml.



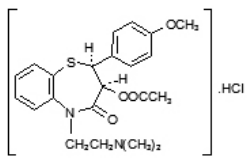
Atenolol



Timolol Maleate



Captopril



Diltiazem HCl

Scheme 1. Chemical structure of atenolol, timolol maleate, captopril and diltiazem hydrochloride drugs

Cerric Ammonium Sulphate

A stock solution of 5×10^{-3} M Cerric ammonium sulphate (CAS) (E-Merck, Darmstadt, Germany) was freshly prepared by dissolving 316.2 mg from [$CeN_4H_{20}S_4O_{18}$, M. Wt. 632.55 g mol^{-1}] of the salt in the least amount of 1.0 M H_2SO_4 and diluted to 100 ml in calibrated flask with the same acid.

Methyl Orange Dye

A stock solution of 1×10^{-3}M of methyl orange dye (MO), was prepared by dissolving 32.7 mg of dye (E- Merck, Darmstadt, Germany 99% purity) in distilled water and diluting to 100 ml in a calibrated flask with distilled water.

Sulphuric Acid

A stock solution of 1.0 M H_2SO_4 was prepared by diluting the concentrated acid (Sp. gr. 1.84, 98.0%) with distilled water.

Recommended Procedures and Calibration Curves

Aliquots solution of pure ATE (3.2 – 6.4 µg/ml), TIM (8 – 18 µg/ml), CPL (3.4 – 5.2 µg/ml) and DIL.HCl (3.6 – 5.2 µg/ml) were transferred into a series of 25 ml volumetric flask. For each drug, add 1.0 ml of 1.0 M H_2SO_4 and followed by 1.0 ml of 5×10^{-3} M CAS. The contents were mixed well and then heat in boiling water bath for 15, 25, 20, and 15 min for ATE, TIM, CPL and DIL.HCl drugs, respectively and then left for cooling. Finally, 1.0 ml of 1×10^{-3} M MO dye were added; diluted to the mark with distilled water and mixed well. After 5 min absorbance was measured at 510 nm against reagent blank treated similarly omitting the drugs. The concentration of unknown was read from calibration graphs or calculated using the regression equation obtained by using Beer's law.

Assay of Pharmaceutical Formulations

a- For tablets

Tablets containing the drugs were obtained from the local market. ATE pharmaceutical preparations were tenormin tablets, 50 mg/tab (Astra Zeneca, Egypt) and Atelol tablets, 50 mg/tab (Pharco, Egypt). CPL commercial tablets were capoten 25 mg/tab (Bristol-Myers Squibb, Egypt) and capozide, 50 mg/tab (EIPICO, Cairo, Egypt). DIL.HCl pharmaceutical preparations were delay-tiazem SR capsules, 90 mg/cap (GlaxoSmithKline S.A.E., El Salam City, Cairo, A.R.E.) and altiazem tablets, 60 mg/tab (EIPICO, 10th of Ramadan City, Egypt). Ten tablets or capsules of each commercial pharmaceutical formulation for ATE, CPL or DIL.HCl were accurately weighed and the average weight of tablet or capsule was calculated. The tablets or capsules were crushed well to a fine powder. A portion of the powder equivalent to 10 mg of ATE, CPL or DIL.HCl were dissolved in the least amount in distilled water and then filtered using a Whatman no. 42 filter paper in 100 ml calibrated flask. The volume was completed to the mark with distilled water and analyzed as described above.

b- For Eye Drops

TIM pharmaceutical preparations were cusimolol eye drops and sterile ophthalmic solution, 5 mg/ml (Rameda, 10th of Ramadan City, Egypt). The content of five bottles of cusimolol eye drops (Sterile ophthalmic solution 5 mg/ml) of TIM pharmaceutical formulations, were mixed and the average volume of one bottle was determined. An accurate measured volume equivalent to 50 mg of drug was transferred into a 100 ml calibrated flask; diluted to the mark with distilled water and analyzed as described above.

RESULTS AND DISCUSSION

Oxidation-reduction reactions have been used as the basis for the development of simple and sensitive spectrophotometric methods for the determination of many pharmaceutical compounds [95-99]. Cerric (IV) ammonium sulphate, because of its high oxidation potential and excellent solution stability, has been widely used as an effective analytical reagent in these methods [95]. The proposed spectrophotometric method is indirect and is based on determination of the residual CAS after bringing the reaction between the investigated drugs and CAS to completion. The residual CAS was determined by reacting with a fixed amount of methyl orange dye and measuring the increase in absorbance at 510 nm (Scheme 2).

The possible reaction scheme is:

Step 1:

Drugs + known excess of Ce^{4+} $\xrightarrow{H+}$ Oxidation product of drugs + Ce^{3+} + Unreacted Ce^{4+}

Step 2:

Unreacted Ce^{4+} + MO dye $\xrightarrow{H+}$ Oxidation product of MO dye + Unreacted MO dye measured spectrophotometrically at 510 nm.

Scheme 2. The suggested reaction pathway between Ce^{4+} and MO dye.

Optimization of the reaction conditions

The optimum conditions for development and stability of the color were 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 temperature and heating time

A solution of cerric ammonium sulphate is remarkably stable over long periods at room temperature, so to enhance the oxidation process of ATE, TIM, CPL and DIL.HCl drugs, the effect of temperature was studied by heating a series of sample and the blank solutions at different temperature ranging from 40 to 100 °C in water bath, the time required for complete oxidation of ATE, TIM, CPL and DIL.HCl drugs was also studied by measuring the absorbance of sample against blank solution at various time intervals to obtain constant absorbance value. The results indicated that the oxidation process is accelerated by raising temperature and took place completely at 100 °C for 15, 25, 20 and 15 min for ATE, TIM, CPL, and DIL.HCl, respectively and any delay up to 30 min in the determination of unreacted Ce^{4+} had no effect on the absorbance (Fig. 1).

Selection of acid type and acid concentration

In order to investigate the optimum acid concentration facilitating the oxidation process, different types of acids were examined (HCl, H_2SO_4, H_3PO_4, CH_3COOH and HNO_3) to achieve maximum yield of redox reactions. The most suitable acid was found to be used is 1.0 M sulphuric acid. The optimum conditions were established by varying H_2SO_4 concentration and observing its effect on the absorbance of the colored product. The results indicated that a constant absorbance was obtained with 0.5 - 2 ml of 1.0 M H_2SO_4, so subsequent studies were performed with 1.0 ml of 1.0 M H_2SO_4 for each drug.

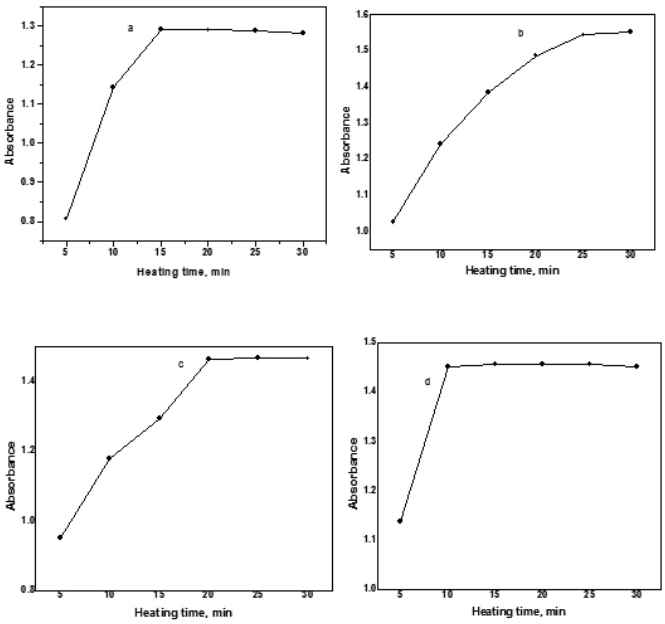


Fig. 1. Effect of heating time on oxidation product of a- ATE (6.4 µg/ml), b- TIM (18 µg/ml), c- CPL(4.8 µg/ml) and d- DIL.HCl (5.2 µg/ml).

Sequence of addition

The sequence of addition of CAS, H_2SO_4, and drugs solution were studied *via* the formation of the colored complexes. There was no appreciable change in the absorbance of oxidation product when the sequence of these reactants was altered.

Effect of the concentration of cerric ammonium sulphate

The influence of the concentration of cerric (IV) ammonium sulphate on the absorbance of the colored products was investigated using different volumes of 5×10^{-3} M from 0.1 to 2 ml. The results indicate that the highest intensity and reproducible results are obtained on using 1.0 ml of 5×10^{-3} M cerric (IV) ammonium sulphate and the color intensity decreased above the upper limits. Therefore, 1.0 ml of 5×10^{-3} M cerric ammonium sulphate was taken as the optimum concentration for all measurements (Fig. 2).

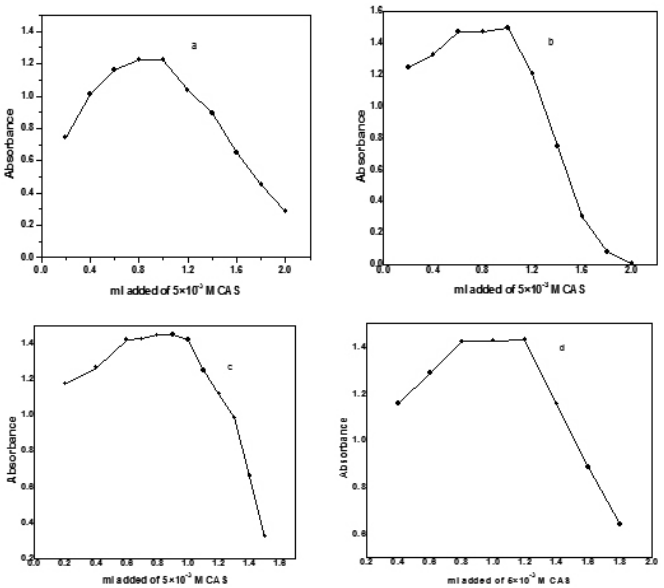


Fig. 2. Effect of ml added of $5'10^{-3}$ M CAS on development of reaction product of a- ATE (6.4 µg/ml), b- TIM (18 µg/ml), c- CPL (4.8 µg/ml) and d- DIL.HCl (5.2 µg/ml).

Effect of dye concentration

The effect of methyl orange concentration required for bleaching by unreacted CAS was studied. One milliliter of 1×10^{-3} M methyl orange solution is recommended as optimum concentration to have maximum absorbance at 510 nm in an overall volume of 25 ml. The effect of time after the addition of dye indicated that shaking for 1.0 min is sufficient to give reliable results. The color remains constant for at least 24 h.

Validation of the proposed method

Linearity, detection and quantification limit

The linearity of the calibration graphs is apparent from the correlation coefficient (r), obtained by determining the best-fit line via linear least-squares treatment. The linearity based on the Beer's law is obeyed up to 3.2, 8, 3.4 and 3.6 µg/ml for ATE, TIM, CPL and DIL.HCl drugs, respectively (Fig. 3). The correlation coefficient (r), the slope (b) and the intercept (a) of the regression equation A = a + bC (A = absorbance, C = ATE, TIM, CPL and DIL.HCl concentration in µg/ml are summarized in Table 1. The apparent molar absorptivity (ε), Sandell's sensitivity, limit of detection (LOD) and limit of quantification (LOQ) are also given in Table 1. Results listed in Table 1, indicate high sensitivity and low background effect of the methods. Limits of detection (LOD) and limits of quantification (LOQ) were calculated as follows [100]:

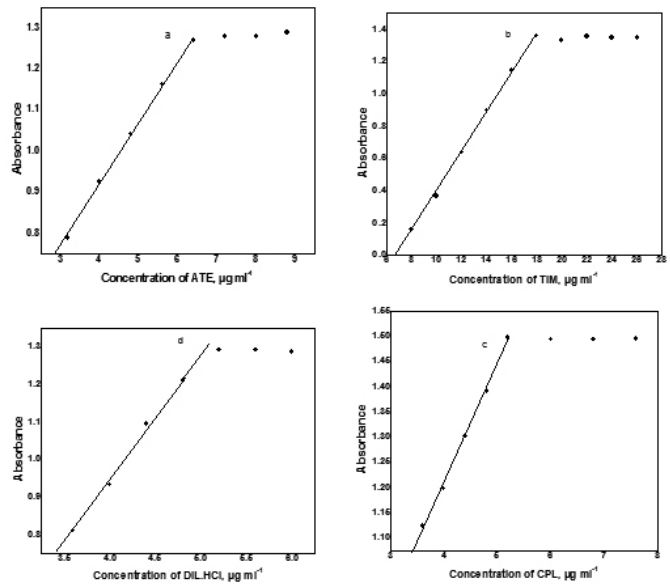


Fig. 3. Calibration curves for determination of a- ATE (3.2 – 6.4 µg/ml), b- TIM (8-18 µg/ml), c- CPL (3.4 – 5.2 µg/ml) and d- DIL.HCl (3.6 – 5.2 µg/ml).

$LOD = 3.3\ s/k$; $LOQ = 10\ s/k$

Whereas *s,* is the standard deviation of the absorbance of ten reagent blank determinations and *k,* is the sensitivity, namely the slope of the related calibration graphs.

Accuracy and precision

To evaluate the accuracy and precision of the proposed method, solutions containing four different concentrations of the studied drugs within the linearity range were analyzed, each measurement being repeated five times. Intraday precision was measured by calculating the relative standard deviation and the accuracy of the proposed method was measured by calculating relative error and found that the small values of them indicate the high accuracy and high precision of the proposed spectrophotometric method. The results of the study are compiled in Table 2.

Robustness

Robustness was examined by evaluating the influence of a small variation of the methods variables including the concentration of analytical reagents and reaction time on the performance of the proposed method. In these experiments, one parameter was changed whereas the others were kept unchanged and the recovery percentage was calculated for each time. It was found that small

variations in these variables did not affect the method significantly. This was an indication of the reliability of the proposed method during its routine application for analysis of the investigated drugs and so the proposed spectrophotometric method is considered robust.

Table 1. Analytical parameters and optical characteristics of the proposed method using Ce^{4+} and MO.

Parameters	Drugs			
	ATE	TIM	CPL	DIL.HCl
λ_{max} (nm)	510	510	510	510
Beer's law limit, µg/ml	3.2-6.4	8-18	3.4-5.2	3.6-5.2
Molar absorptivity, L/mol cm	5.28×10^4	3.27×10^4	6.43×10^4	1.12×10^5
Sandell's sensitivity, ng/cm^2	5.0433	13.2	3.3818	4.0242
Correlation coefficient (r)	0.9985	0.9994	0.9989	0.9975
Linear regression equation*				
$S_{y/x}$	0.0327	0.0152	0.0199	0.0189
Intercept (a)	0.3418	-0.8608	0.2941	-0.2996
Slope (b)	0.1448	0.1251	0.2277	0.3097
S.D. of slope (S_b)	0.0129	2.40×10^{-3}	0.0157	0.0149
S.D. of intercept (S_a)	0.1387	0.0644	0.1547	0.1470
LOD, µg/ml	0.0912	0.0467	0.1306	0.0303
LOQ, µg/ml	0.3037	0.1556	0.4348	0.1012

*A = a + bC, where A is the absorbance and C is the concentration of drug in µg/ml

Table 2. Evaluation of accuracy and precision of the proposed method using Ce^{4+} and MO.

Drugs	Taken, µg/ml	Recovery, %	Precision, RSD[a] %	Accuracy, Er[b] %	SE
ATE	3.2	100.28	2.57	0.28	0.0104
	3.6	100.04	2.45	0.04	0.0212
	4	100.05	1.68	0.05	0.0204
	4.4	100.04	2.11	0.04	0.0137
TIM	10	99.99	2.34	-0.01	8.6×10^{-3}
	12	100.12	0.978	0.12	3.1×10^{-3}
	14	100.01	1.46	0.01	6.5×10^{-3}
	16	100.01	0.652	0.01	3.8×10^{-3}
CPL	3.6	100.21	1.11	0.21	0.0154
	4	99.99	2.60	-0.01	5.8×10^{-3}
	4.4	100.02	2.41	0.02	0.0213
	4.8	100.06	1.98	0.06	0.0170
DIL.HCl	4	100.42	1.69	0.42	7.9×10^{-3}
	4.4	100.04	0.847	0.04	1.9×10^{-3}
	4.8	100.04	2.94	0.04	0.0246
	5.2	100.06	1.02	0.06	0.0114

[a]Relative standard deviation for five determinations.
[b]Er, Relative error.

Interference studies

The effects of common excipients and fillers added in pharmaceutical preparations were tested for their possible interferences in the assay of cited drugs. It was observed that the talc, glucose, starch, lactose, dextrose and magnesium stearate did not interfere in the determination at the levels found in dosage forms. This is clear from the results obtained for pharmaceutical formulations, which are presented in Table 3.

Applications

The proposed method was successfully applied for the determination of investigated drugs in pharmaceutical formulations. The performance of the proposed method was judged by calculating the student's *t*- and *F*- values. At 95% confidence level, the calculated *t*- and *F*- values did not exceed the theoretical values as evident from Table 3. Hence, it was concluded that there is no significant difference between the proposed methods and the official methods. Moreover, the spectrophotometric method for determination of the investigated in pharmaceutical formulations reported in this paper are simple, fast, inexpensive, precise, accurate and it may be suitable for routine analysis.

CONCLUSION

The proposed method has the advantages of simplicity and rapidity for the determination of atenolol, timolol maleate, captopril and diltiazem hydrochloride drugs in both pure and in pharmaceutical preparations. The reagents utilized in the proposed methods are cheaper, readily available and the procedures do not involve any tedious sample preparation. These advantages encourage the application of the proposed method in routine quality control analysis of atenolol, timolol maleate,

Table 3. Results of analysis of pharmaceutical preparations containing the studied drugs using Ce^{4+} and MO.

Drugs	Drug formulations	Labeled mg content	Found, mg	Recovery[a], %	t- and F-test	Official methods
ATE	Tenormine tablet[b]	50	50.987	101.97	t = 0.587 F = 1.079	99.92
	Atelol tablet[c]	50	49.531	99.06	t = 0.304 F = 1.102	98.95
TIM	Cusimolol eye drops[h] solution	5	5.087	101.74	t = 1.250 F = 0.926	100.95
CPL	Capoten tablet[d]	25	25.145	100.58	t = 0.786 F = 1.038	100.32
	Cpozide tablet[e]	50	50.780	101.56	t = 1.361 F = 1.180	100.96
DIL. HCl	Delay-tiazime SR capsule[f]	90	91.005	102.01	t = 1.059 F = 1.034	101.24
	Altiazem tablet[g]	60	59.921	99.87	t = 0.823 F = 1.155	99.97

[a] average of five determinations.
[b] AstraZeneca, Egypt.
[c] Pharco, Egypt.
[d]Bristol-Myers Squibb, Egypt.
[e]EIPICO, Cairo, Egypt.
[f] GlaxoSmithKline S.A.E., El Salam City, Cairo, A.R.E.
[g]EIPICO, 10th of Ramadan City, Egypt.
[h] Rameda, 10th of Ramadan City, Egypt.
Theoretical value for t- and F-values for five degrees of freedom and 95% confidence limits are 2.57 and 5.05, respectively.

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