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QUANTITATIVE DETERMINATION OF DRUGS BY USING CERIUM (IV) AND RHODAMINE B COUPLE: A SPECTROPHOTOMETRIC STUDY

M. Sasikala, B. Tirupati and G.Venkateshwarlu *

Department of Chemistry, University College of science, Osmania University, Hyderabad-500007, India.

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Correspondence to Author:

G. Venkateshwarlu

Department of Chemistry, University college of science, Osmania university, Hyderabad - 500007, India.

E-mail: venkateshwarlugoud@yahoo.com

ABSTRACT: Simple, sensitive, accurate and precise spectrophotometric methods for quantitative determination of five drugs viz., Tramadol Hydrochloride (TDH), Dobutamine (DOB), Trimetazidine (TRMZ), Terazosin (TRZ) and Esmolol (ESM) were developed. The method for each drug depends upon oxidation of drugs by Ce (IV) (Excess) and estimating the amount of un reacted Ce (IV) by Rhodamine-B dye at 557 nm. The calibration curves obeyed Beer's law over the concentration range of 14-130 $\mu\text{g ml}^{-1}$ (TDH), 1-14 $\mu\text{g ml}^{-1}$ (DOB), 8-120 $\mu\text{g ml}^{-1}$ (TRMZ), 16-130 $\mu\text{g ml}^{-1}$ (TRZ), and 12-84 $\mu\text{g ml}^{-1}$ (ESM). The methods have been validated in terms of guidelines of ICH and has been applied to the analysis of pharmaceuticals.

INTRODUCTION: Tramadol Hydrochloride (TDH) (**Fig.1a**) is chemically known as Tramadol [(±) Trans - 2 - (dimethylaminomethyl) - 1 - (3-methoxy- phenyl)-cyclohexanol hydrochloride]. It is a centrally acting opioid analgesic. ¹ Tramadol and its metabolite (+)-Odesmethyl-tramadol (M1) are weak agonists of the μ opioid receptor. (+)-Tramadol inhibits serotonin reuptake and (-)-tramadol inhibits norepinephrine reuptake.

Various methods have been reported for the determination of tramadol in bulk, pharmaceutical preparations, biological fluids and hair including spectrophotometry ²⁻⁷, high performance liquid chromatography (HPLC) ⁸⁻¹¹, potentiometry ¹² and chromatography ¹³.

Dobutamine:

(DOB) (**Fig.1b**) Dobutamine hydrochloride, C₁₈H₂₃NO₃·HCl, chemically: 4-(2-((1-methyl-3-(4-hydroxybenzene) propyl)amido) ethyl)-1,2-dihydroxybenzen hydrochloric salt is an adrenalin receptor concussion medicine indicated obvious curative effect for coronary heart disease, acute myocardial infarction, and expansionary cardiomyopathy ¹⁴. There are various analytical methods for the assay of dobutamine, spectrophotometric analysis ¹⁵⁻¹⁹, HPLC ²⁰, spectrofluorimetry ²¹, chromatography ²² and voltammetry ²³. However, these methods are often costly, tedious, time consuming or suffer from the disadvantages of low sensitivity, narrow linear range, and the use of volatile organic solvents. As an alternative to it, establishment of simple, rapid and sensitive analytical methods are necessary.

Trimetazidine:

(TRMZ) (**Fig.1c**) is chemically known as 1-[(2,3,4-Trimethoxyphenyl) methyl] piperazine dihydrochloride. It is a coronary vasodilator drug that has been used in management and prophylaxis of angina pectoris and in ischaemia of

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neurosensory tissues as in meniere's disease²⁴. It seems to have an antioxidant effect. Several methods have been reported for the determination of Trimetazidine dihydrochloride. These methods include spectrophotometry²⁵⁻²⁹, HPLC³⁰, voltametry³¹ and Chromatographic methods³².

Terazocin:

(TRZ) (**Fig.1d**) is chemically known as RS-1-(4-amino-6, 7-dimethoxy-2-quinazoliny)-4-[(tetrahydro-2-furanyl) carbonyl]- piperazine monohydrochloride. It is a α_1 -adrenoceptor blocker with a long lasting action.³³ It is used in the management of hypertension and in benign prostate hyperplasia to relieve symptoms of urinary obstruction. Terazosin is rapidly and almost completely absorbed from the gastrointestinal tract after oral administration and is extensively metabolized in the liver to yield piperazine and three other inactive metabolites. Literature review reveals that a few methods have been published for analysis of TRZ in the bulk form and in pharmaceutical preparations. Methods available include Spectrophotometry³⁴⁻⁴⁰, potentiometry⁴¹ and HPLC^{42,43}.

Esmolol:

(ESM) (**Fig 1e**) hydrochloride is a class II antiarrhythmic and is chemically known as methyl (RS)-3-{4-[2-hydroxy - 3 - (propan - 2 - yl amino) propoxy] phenyl} propanoate hydrochloride. It is cardio selective beta1 receptor blocker with rapid onset. It is used in the treatment for the rapid control of heart rate⁴⁴. ESM decreases the force and rate of heart contractions by blocking beta-adrenergic receptors of the sympathetic nervous system.

The methods which were reported in the literature for the determination of ESM includes Spectrophotometry⁴⁵⁻⁵⁰, HPLC⁵¹ and chromatography^{52, 53} methods. Since the above-mentioned methods are complex and expensive, there is a need to develop simple, less expensive, and more selective method for the determination of ESM. Hence, in the present investigation, an attempt was made to develop new spectrophotometric methods for determination of ESM in bulk and pharmaceutical formulations.

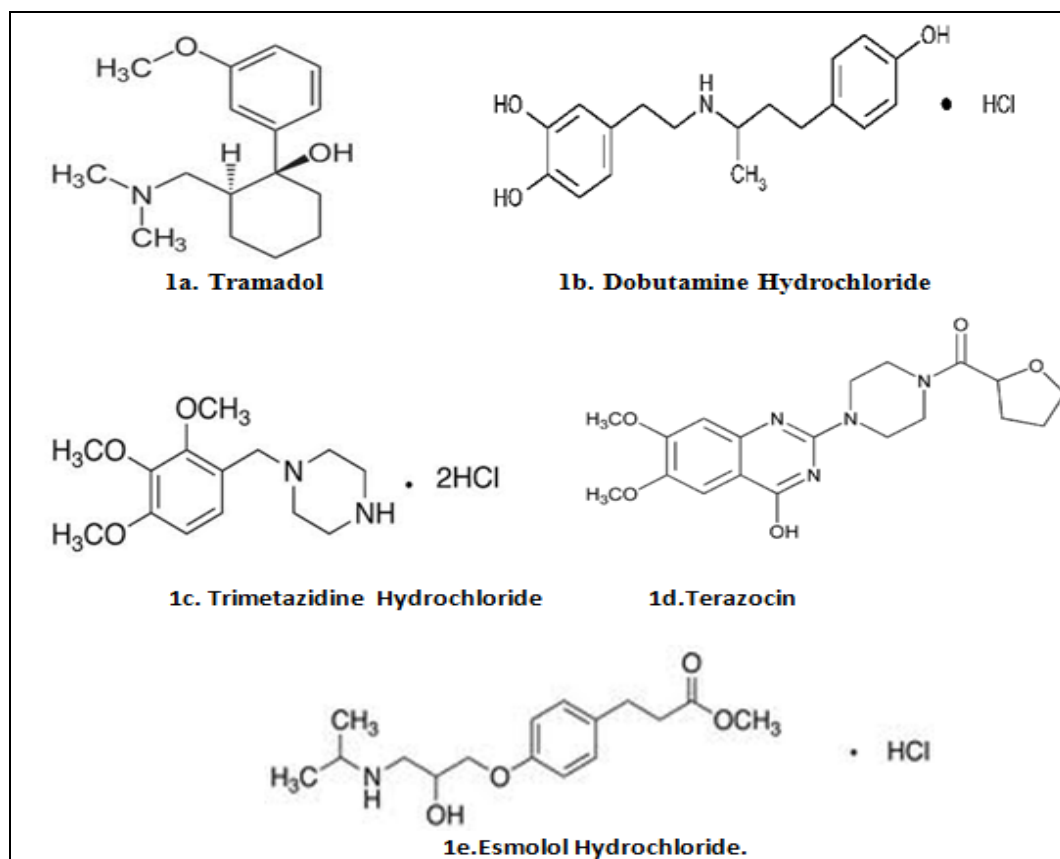


FIG.1: STRUCTURES OF DRUGS

About the method:

Cerium (IV) is a good oxidizing agent like KMnO_4 , $\text{K}_2\text{Cr}_2\text{O}_7$ etc., it has been used for quantitative determination of drugs based on the oxidation of drugs. The spectrophotometric methods involved addition of excess Ce(IV) and unreacted cerium is estimated by suitable dyes, viz., Indigo Carmine, Methyl Orange, Safranin-O and Xylene cyanol. We report Rhodamine-B dye is suitable for estimation of unreacted Ce (IV) absorbance at 557 nm.

Experimental:**Apparatus:**

Spectral and absorbance measurements were made on an Elico 210 double beam spectrophotometer, Systronics 117 spectrophotometer and also on ELICO 159 UV-VIS single beam spectrophotometers using quartz cells of 10 mm path length. A Dhona 200 single pan electrical balance is used for weighing the samples.

Materials and Methods:

All reagents used were of analytical-reagent grade and distilled water was used throughout the investigation.

Cerium (IV) Solution:

Cerium (IV) sulphate ($\text{CeSO}_4 \cdot 2\text{H}_2\text{O}$, 99.9 % pure) was prepared by dissolving 750 mg of chemical (Merck, Mumbai, India) in 2 N H_2SO_4 with the aid of heat and filtered using glass wool and diluted to 250 ml with the same acid and cerium is standardized by Ferrous Ammonium Sulphate uses Ferroin indicator. The solution was then diluted appropriately with 2 N H_2SO_4 to get working concentrations of 4.0×10^{-3} M (0.25%).

Rhodamine-B Dye:

Aqueous solutions of $50 \mu\text{g ml}^{-1}$ of Rhodamine-B dye was prepared by dissolving an appropriate weight of 0.050 grams in 100 ml bi distilled water.

Sulphuric Acid:

Prepared by diluting the concentrated acid (Merck, Mumbai, India, Sp. gr. 1.84, 98.0 %) with water appropriately to get 2 N acid.

Preparation of drug solution:

Standard drug solution ($200 \mu\text{g ml}^{-1}$) was prepared by dissolving 20 mg of drug with distilled water to

the mark in 100 ml standard flask. The stock solution was diluted appropriately to get the working concentration.

Procedure:

Aliquots containing 1.6 - 56.00 $\mu\text{g ml}^{-1}$ of drug were transferred into a series of 10 ml standard flasks using a micro burette. To this, 1 ml of Ceric Ammonium Sulphate followed by 1 ml of 2N H_2SO_4 and contents were shaken well. After 30 minutes, 1 ml of 0.02% Rhodamine-B dye was added to the flask. Then contents were shaken well and diluted up to the mark. The absorbance of each solution was measured at 523 nm against the corresponding reagent blank.

Assay of drug pure sample:

To the test the accuracy and precision of the methods developed pure sample solutions containing drug in the Beer's Law limit were chosen. For this study 14-130 μgml^{-1} of TDH, 1-14 μgml^{-1} of DOB, 8-120 μgml^{-1} of TRMZ, 16-130 μgml^{-1} of TRZ and 12-84 μgml^{-1} ESM have been taken. (Table 1) To each of the solution 1 ml of 250 $\mu\text{g ml}^{-1}$ of cerium, 1 ml of 2 N of H_2SO_4 were added and the unreacted cerium is analyzed as described above using Rhodamine-B dye.

Procedure for analysis of pharmaceuticals:**Tramadol hydrochloride:**

For the analysis of pharmaceutical formulations ten tablets (Cambidol, 50mg) were weighed, powdered and equivalent to 10 mg of tramadol hydrochloride was transferred in to 100 ml volumetric flask. 60.0 ml of distilled water was added and ultrasonicated for 20 min, then made up to the mark with distilled water. The resulting solution was mixed and filtered through Whatmann filter paper no. 42. From the filtrate solution was diluted appropriately with distilled water in order to obtain working concentration of drug used for the analysis.

Dobutamine hydrochloride:

Four tablets (Dobusol, 250 mg) were weighed and grounded. The powder equivalent to 10mg dobutamine hydrochloride was stirred well with methanol, sonicated about 30 minutes. The solution was filtered through Whatmann filter paper in a 100ml volumetric standard flask and the residue was washed well with methanol for complete

recovery of the drug and methanol was evaporated. The residue was dissolved in 100 mL of distilled water and it was further diluted to get required concentration for the analysis of the drug.

Trimetazidine:

About ten tablets (Trivedon, 20 mg) were powdered and equivalent to 10 mg of trimetazidine has been taken in to a 100 ml of volumetric flask and added about 30 ml of methanol, sonicated for 30 min and filtered through Whatmann filter paper No 42. The residue was washed thrice with methanol for complete recovery of drug and methanol was evaporated. The residue was dissolved in 100 mL of distilled water. It was used as stock sample solution. The aliquot portions of this stock solution were further diluted with distilled water to get the final concentration required for the determination of the drug.

Terazosin:

Ten tablets (Terazen, 2mg) were weighed and grounded. A quantity equivalent to 10mg of terazosin was transferred into a 100 mL calibrated flask and the volume was finally diluted to the mark with water, mixed well and filtered using a Whatman No. 42 filter paper. First 10 mL portion of the filtrate was discarded and a suitable aliquot of the subsequent portion was diluted appropriately to get required concentration and the assay was completed according to the procedure described above.

Esmolol hydrochloride:

10 ml of (Mini block, 100 mg mL⁻¹) injection of drug was taken into a 100 mL calibrated flask and added 30 ml of distilled water followed by sonication for 15 minutes. The solution was finally made up to 100 ml. It was used as stock sample solution and was further diluted with the distilled water to get working concentration solution for assay.

Method of validation:

The each method developed quantification of drugs has been validated in terms of precision, accuracy, limit of detection, limit of quantification, linearity, selectivity and ruggedness. Absorbance time curves were drawn, initial rate and fixed time methods were used to assess the recovery of the drug. To

assess the precision each experiment was repeated at least 5 times and accuracy is estimated in terms of percent recovery and percent RSD. Excellent percent recovery and RSD being less than 2 for each drug demonstrates accuracy and precision of the methods. Further t-test and F-test values have also been calculated using a standard reference method. The closeness of t-test and F-test values is less than that they permissible range indicating high accuracy of the methods **Table 2**.

As mentioned earlier limit of detection is the minimum limit that can be detected but not necessarily quantified is determined for each drug. LOD is determined from the standard deviation of y-intercepts of regression lines of replicate determinations.

$$\text{LOD} = 3.3 \text{ s/S}$$

Where s = standard deviation of intercept (n=6)
S = slope of linearity plot

LOQ the minimum concentration of analyst using calibration curve is also determined.

LOQ = 10s/S.

Limits of linearity of calibration curves are mentioned in the **Fig. 2** under the title Beer's law limit. To test the selectivity known excipients of each drug are added to the pure drug sample and recovery experiments were performed. Ruggedness is resistance of method for a small change in variables like instrument, and analyst or both to test the Ruggedness of the method absorbance data was collected using 3 different instrument and 2 analysts no significant changes were observed either by change of instrument or analyst hence the method may be taken as robust.

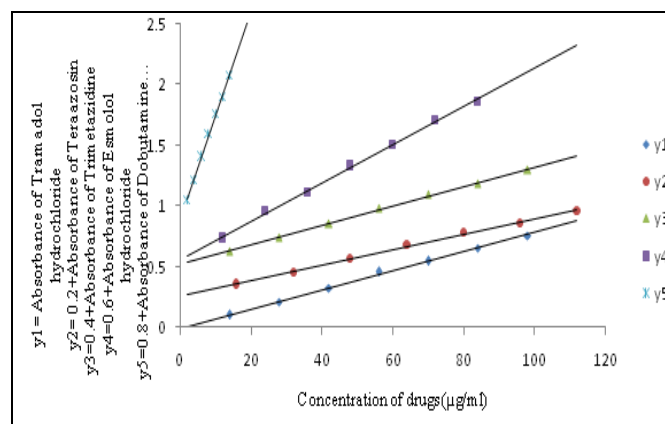


FIG.2: CALIBRATION CURVES

Factors effecting absorbance:**Effect of acid 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 the sulphuric acid was the preferable acid with Ce (IV) as oxidant. The reaction was performed in a series of 10 ml volumetric flask containing $8.0 \mu\text{gml}^{-1}$ of the cited drugs, different volumes (0.5–2.5 ml) of 2.0 N H_2SO_4 and 1 ml of Ce(IV) ($4.0 \times 10^{-3} \text{M}$) were added. After 5.0 min of heating time at $60 \pm 2^\circ\text{C}$ in a water bath, the solution was cooled for about 3.0 min, 1.0 ml of Rhodamine-B dye were added, then complete to 10 ml total volume with water. It was found that the maximum absorbance was obtained at 1 ml of 2 N H_2SO_4 . Above this volume, the absorbance decreased therefore, a volume of 1 ml of 2 N H_2SO_4 was used for all measurements.

Effect of heating time:

In order to obtain the highest and most stable absorbance, the effect of heating time on the oxidation re-action of drugs were catalyzed by heating in a water bath at $60 \pm 2^\circ\text{C}$ for the periods ranging for 2.5-20 min. the time required to complete the reaction and maximum absorbance was obtained after 5.0 min of heating. After oxidation process, the solution must be cooled at least for 3.0 min before addition of dye.

Effect of oxidant concentration:

When a study on the effect of Ce (IV) on color development was performed, it was observed that in both cases the absorbance increased with increase in the volume of Ce (IV). It reached maximum when 1 ml of $200 \mu\text{g ml}^{-1}$ Ce (IV) solution was added to a total volume of 10 ml for drugs solutions. The color intensity decreased above the upper limits. Therefore, 1 ml of $200 \mu\text{g ml}^{-1}$ Ce (IV) was used for all measurements.

Effect of dye concentration:

In order to ascertain the linear relationship between the volume of added Ce (IV) and the decrease in absorbance of Amaranth dye, experiments were performed using 1 ml of 2 N H_2SO_4 with varying volumes of Ce (IV). The decrease in absorbance was found to be linear up to the 1 ml of $200 \mu\text{g ml}^{-1}$ Ce (IV) with optimum

volume 1.0 ml of Rhodamine-B dye for fixed concentration drug solution. The color was found to be stable up to 24 hours.

Analysis of pharmaceuticals:

To the test the applicability of the method developed solution of pharmaceutical tablets solutions containing drug in the Beer's Law limit were chosen. To assess the precision each tablet analysis was repeated at least 6 times and accuracy is estimated in terms of percent recovery and percent RSD. Excellent percent recovery and RSD being less than 2 for each drug demonstrates applicability of the methods for pharmaceutical analysis **Table 2**. Further t-test and F-test values have also been calculated using a standard reference method. The closeness of t-test and F-test values is less than that they permissible range indicating excellent applicability of the methods for pharmaceutical analysis **Table 3**. The excellent recovery studies indicate that methods developed can be applied to pharmaceutical analysis without hesitation.

RESULTS AND DISCUSSION: The ability of cerium (IV) sulphate to oxidize drugs, and bleach the color of amaranth dye is the basis of the indirect spectrophotometric method developed here. In this method the drugs were reacted with a measured excess of cerium (IV) sulphate in acidic medium and the unreacted oxidant was determined by reacting with amaranth followed by absorbance measurement at 523 nm. The absorbance increased linearly with increasing concentration of drug, when increasing amounts of each drug were added to a fixed amount of 0.25% of CAS, consumed the latter and there occurred a concomitant fall in its concentration. When fixed amount of the dye was added to decreasing amount of oxidant, an concomitant increase in the concentration of dye resulted. This was observed as a proportional increase in absorbance at the respective λ_{max} with increasing concentration of each drug. One ml of 2N acid was used in the reaction, as this concentration was found ideal.

$\text{D} + \text{Ce (IV) excess} \rightarrow \text{D oxidation product} + \text{Ce (III)} + \text{Ce (IV) unreacted}$: (1)

$\text{Ce (IV) unreacted} + \text{Rhodamine} \rightarrow \text{oxidation product of rhodamine} + \text{unreacted rhodamine}$: (2)

Measured spectrophotometrically at λ_{\max} =557 nm
Scheme 1: Reaction Scheme of the indirect determination of drug by oxidation with Ce (IV) sulphate

Analytical data:

A linear correlation was found between absorbance at λ_{\max} and concentration ranges, and sensitivity parameters such as molar absorptivity, Sandal's sensitivity, detection limit and quantification limit are presented in **Table 1**. Regression analysis of Beer's law data using the method of least squares

was made to evaluate the slope (b), intercept (a), correlation coefficient (r) and is also given in **Table 1**.

Accuracy and precision:

The accuracy and precision of the methods were established by analyzing the pure drug solution at 6 different levels (with working limits). The relative error (%) which is a measure of accuracy & RSD (%) a measure of precision are summarized in **Table 2** and reveal the high accuracy and precision of the methods.

TABLE 1: ANALYTICAL PARAMETERS FOR DETERMINATION OF DRUGS BY OXIDATION WITH CERIUM (IV) AND RHODAMINE-B

Parameter	TDH	DOB	TRMZ	TRZ	ESM
λ_{\max} , nm	557	557	557	557	557
Beer's law limits $\mu\text{g mL}^{-1}$	14-130	1-14	8-120	16-130	12-84
Molar absorptivity, $\text{L mol}^{-1} \text{cm}^{-1}$	2.40×10^5	2.45×10^5	4.56×10^4	2.67×10^5	2.87×10^5
Sandell sensitivity* $\mu\text{g cm}^{-2}$	0.0045	0.0054	0.0078	0.0063	0.0089
Limit of detection $\mu\text{g mL}^{-1}$	0.0845	0.1025	0.3754	0.4578	0.6341
Limit of quantification $\mu\text{g mL}^{-1}$	0.3412	0.3458	1.897	1.452	2.745
Regression equation, Y**					
Intercept, (a)	-0.003	0.080	0.121	0.062	-0.039
Slope, (b)	0.007	0.085	0.0158	0.006	0.015
Correlation coefficient, (r)	0.998	0.966	0.998	0.999	0.997
Standard deviation of intercept (Sa)	0.0115	0.0152	0.0145	0.062	0.0465
Standard deviation of slope (Sb)	0.0846	0.0423	0.0728	0.0753	0.053

TABLE 2: DETERMINATION OF ACCURACY AND PRECISION OF THE METHODS ON PURE DRUG SAMPLES

Drug	Taken ($\mu\text{g/ml}$)	Found ($\mu\text{g/ml}$)	(%)er	Recovery (%)	RSD (%)	Proposed method Mean \pm SD
TDH	1.5	1.48	1.33	98.66	0.785	99.77
	2.5	2.52	0.80	100.80		± 0.784
	4.5	4.49	0.22	99.77		
DOB	4.0	4.02	0.50	100.50	0.494	100.37
	5.0	5.04	0.80	100.80		± 0.496
	6.0	5.99	0.16	99.83		
TRMZ	2.0	2.01	0.50	100.50	0.490	100.35
	4.0	4.03	0.70	100.75		± 0.492
	5.0	4.99	0.20	99.80		
TRZ	1.5	1.49	0.66	99.33	0.805	99.87
	2.5	2.52	0.80	100.80		± 0.804
	4.0	3.98	0.50	99.50		
ESM	2.0	2.02	1.00	101.00	0.428	100.63
	4.0	4.03	0.75	100.75		± 0.431
	6.0	6.01	0.16	100.16		

TABLE 3: RESULTS OF ASSAY OF TABLETS BY THE PROPOSED METHODS AND STATISTICAL EVALUATION AND RECOVERY EXPERIMENTS BY STANDARD ADDITION METHOD

Tablets	Drug in tablet $\mu\text{g mL}^{-1}$	Drug added $\mu\text{g mL}^{-1}$	Total found $\mu\text{g mL}^{-1}$	er (%)	Recovery (%)	RSD (%)	Reference method ^[1-5] Mean \pm SD	Propose method \pm SD	t-test	F-test
Cambidol (TDH)	1.5	1.0	2.49		99.66					
	1.5	2.0	3.51	1.40	100.28		101.70	100.06	0.145	1.26
	1.5	3.0	4.52	0.28	100.44	1.516	± 1.70	± 1.516	(2.571)	(4.95)
	1.5	0.0	0.98	0.44	98.00					
	1.0	0.0	2.02	1.00	101.00					
	2.0	0.0	3.03	1.00	101.00					
Dobusol (DOB)	3.0			1.00						
	1.0	0.5	1.50	0.00	100.00					
	1.0	1.0	2.04	2.00	102.00		101.00	100.41	0.23	1.29
	1.0	1.5	2.50	0.00	100.00	0.881	± 1.0	± 0.885	(2.571)	(4.95)
	2.0	0.0	1.99	0.50	99.55					
	3.0	0.0	3.02	0.66	100.66					
Trivedon (TRMZ)	4.0	0.0	4.01	0.25	100.25					
	1.0	1.0	2.02	1.00	101.00					
	1.0	2.0	3.02	0.66	100.66		99.80 ± 1.26	100.54	1.754	0.042
	1.0	3.0	4.02	0.50	100.50	0.265		± 0.267	(2.571)	(4.95)
	2.0	0.0	2.01	0.50	100.50					
	4.0	0.0	4.01	0.25	100.25					
Terazen (TRZ)	6.0	0.0	6.02	0.33	100.33					
	1.0	1.5	2.48	1.00	99.20					
	1.0	3.0	4.02	0.50	100.50		99.90	100.55	1.145	0.212
	1.0	4.5	5.51	0.02	100.18	1.022	± 0.470	± 1.028	(2.477)	(4.28)
	1.0	0.0	1.02	2.00	102.00					
	3.0	0.0	3.05	1.66	101.66					
Miniblok (ESM)	5.0	0.0	4.99	0.20	99.80					
	1.0	0.5	1.51	0.66	100.00	0.641				
	1.0	1.0	1.99	0.50	99.50					
	1.0	1.5	2.49	0.40	100.60		100.45	100.30	0.142	1.045
	3.0	0.0	3.02	0.66	100.66		± 0.573	± 0.643	(3.182)	(4.75)
	4.0	0.0	4.04	1.00	101.00					
	5.0	0.0	5.02	0.40	100.40					

CONCLUSION: The present study described the successful development of new, simple, sensitive, selective, accurate and rapid spectrophotometric method for the accurate determination of drugs each one in its pharmaceutical forms Cerium (IV) sulphate as the oxidizing reagent. There is no interference from additives and excipients. The method thus can be used in the determination of these drugs in pure and pharmaceutical formulations. So, it is the good alternative to the reported methods for the determination of these drugs.

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