International Journal of Modern Engineering Research (IJMER)
www.ijmer.com Vol. 3, Issue. 5, Sep - Oct. 2013 pp-3079-3085 ISSN: 2249-6645

Spectrophotometric Determination of Cardiovascular Drugs

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ABSTRACT: *Simple, sensitive, accurate and precise spectrophotometric methods for quantitative determination of cardiovascular drugs viz., Dobutamine Hydrochloride (DOB), Ramipril (RAM), Telmisartan (TEL), Verapamil Hydrochloride (VER) were developed. The method of each drug depends upon oxidation of drugs by Ce (IV) (Excess) and estimating the amount of un reacted Ce (IV) by amaranth dye at 523nm. The calibration curves obeyed Beer's law over the concentration range of 1.6-12µg ml^{-1} (DOB), 8-56 µg ml^{-1} (RAM), 2-14 µg ml^{-1} (TEL) & 2-12 µg ml^{-1} (VER). The method has been validated in terms of guidelines of ICH and has been applied to analysis of pharmaceuticals.*

Keywords: *Cerium (IV), Amaranth dye couple, drugs, Determination, UV-VIS Spectrohotometry*

I. INTRODUCTION

1.1. Dobutamine hydrochloride

Dobutamine hydrochloride, [Fig.1 (a)], is chemically as 4-(2-((1-methyl-3-(4-hydroxybenzene) propyl) amido) ethyl)-1,2-di-hydroxybenzene hydrochloric salt, is an adrenalin receptor concussion medicine indicated obvious curative effect for coronary heart disease, acute miocardial infarction and expansionary cardiomyopathy [1].

The literature survey reveals that several analytical methods such as enzymatic catalytic spectrofluorimetry [2], Spectrophotometry [3,4,5], high performance liquid chromatography [6,7] and flow-injection chemiluminescence method [8] have been developed for determination of Dobutamine hydrochloride

1.2. Ramipril

Ramipril chemically is (2*S*,3*aS*,6*aS*)-1-[(2*S*)-2-{[(2*S*)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino}propanoyl]-octahydrocyclopenta[*b*]pyrrole-2-carboxylic acid, is a highly lipophilic, long acting angiotensin converting enzyme (ACE) inhibitor, [Fig.1(b)]. It effectively reduces both supine alterations in the pulse rate. It is indicated for Hypertension and cardiac failure [9].

Some methods for the analysis of ramipril are Spectrophotometry [10,11,12], High-performance liquid chromatographic and chemometric based spectrophotometry [13] and simple colorimetry [14] have been employed.

1.3. Telmisartan

Telmisartan or (2-(4-{[4-methyl-6-(1-methyl-1*H*-1,3-benzodiazol-2-yl)-2-propyl-1*H*-1,3-benzodiazol-1-yl]methyl}phenyl)benzoic acid or [Fig.1(c)] is a cardiovascular drug, indicated for hypertension [15].

Because of its physiological importance many physical and instrumental techniques have been developed for the quantification of TEL like HPLC [16-22], Spectrophotometry [23-26], LC-MS/MS [27] and HPTLC [28,29].

1.4. Verapamil Hydrochloride

Verapamil Hydrochloride [Fig.1(d)] is chemically known as 5-[N-(3, 4-dimethoxy-phenethyl)-N-methyl-amino]-2-(3,4-dimethoxyphenyl)-2-isopropylvaleronitrile hydrochloride. It is a calcium-channel blocker, has been indicated for anti-arhythmic drug to manage supra ventricular tachyarrhythmias[30] and it is a anti-anginal drug.

Some analytical methods for quantitative determination of VER are described *viz.*, capillary electrophoresis [31], tandem mass spectrometry detection (LC-MS/MS) [32, 33], HPLC [34] and Inverse volt-ampere method [35].

Through survey of literature revealed that oxidative method of quantification of these drugs by Ce (IV) have been not reported yet, although the methods simple sensitive, precise and accurate [36,37].

II. ABOUT THE METHOD

Cerium (IV) is a good oxidizing agent like $KMnO_4$, $K_2Cr_2O_7$ *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 un reacted cerium is estimated by suitable dyes, which should be oxidized by cerium *viz.*, Indigo Carmine, Methyl Orange, Safranin-O and Xylene cyanol.

Amaranth dye is suitable for estimation of unreacted Ce (IV) absorbance at 523 nm.

III. EXPERIMENTAL

3.1. Apparatus

Spectral and absorbance measurements were made on a thermo electron corporation single beam U.V.-Vis spectrophotometer by using 1 cm quartz cells.

3.2. Materials and methods

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

3.2.1. Cerium (IV) solution

Cerium (IV) sulphate ($CeSO_4.2H_2O$, 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 standardized and cerium is standardized by ferrous ammonium sulphate and ferroin indicator. The solution was then diluted appropriately with 2 *N* H_2SO_4 to get working concentrations of $4.0x\ 10^{-3}$ M (0.25%).

3.2.2. Amaranth dye

Aqueous solutions of $0.8x10^{-3}$ *M* of Amaranth dye was prepared by dissolving an appropriate weight of 0.0483 grams in 100 ml bi distilled water.

3.2.3. 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.

3.2.4. Preparation of drug solution

Standard drug solution (200 μgml^{-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.

IV. PROCEDURE

Aliquots containing 1.6 — 56.00 $\mu g\ ml^{-1}$ of drug were transferred into a series of 10 ml standard flasks using a micro burette. To this, 1 ml of CAS was followed by 1 ml of 2N H_2SO_4 and contents were shaken well. After 30 minutes, 1 ml of 0.02% of amaranth added to the content. 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.

V. 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 1.6-12 μgml^{-1} of DOB, 8-56 μgml^{-1} of RAM, 2-14 μgml^{-1} of TEL, 2-12μgml^{-1} of VER have been taken. To each of the solution 1 ml of 250 $\mu g\ ml^{-1}$ of cerium, 1 ml of 2 N of H_2SO_4 were added and the un reacted cerium is analyzed as described above using amaranth dye.

VI. PROCEDURE FOR ANALYSIS OF PHARMACEUTICALS

6.1. Dobutamin Hydrochloride

Two Cardiject injection (50mg/4ml/injection)s of DOB were placed in a boiling tube and worked out to get working standard solutions of 1.6 μgmL^{-1}. Quantification was performed using 1.5, 3.0, 4.5 & 6.0 $\mu g\ ml^{-1}$ of Dobutamine hydrochloride.

6.2. Ramipril

To determine the content of Ramipril in pharmaceutical preparations, 20 tablets of Cosrpil (lable claim : 5 mg/tablet) were weighed and finely powdered. A portion of the powder equivalent to 50mg. ramipril was stirred with 50 ml doubly distilled water and let stand for 10 minutes. The residue was filtered on Whatmann No.42 filter paper and wash with doubly distilled water. This solution was further diluted as necessary to complete the analysis concentration solutions for assay.

6.3. Telmisartan

Four tablets (Teli: 20mg/tablet) were weighed and powdered. Accurately weighed quantity of tablet powder equivalent to about 25 mg of telmisartan was transferred into 50 ml volumetric flask, added 25 ml of acetonitrile and shaken for ten minutes, the volume was then adjusted to mark with acetonitrile and mixed, the solution was filtered through Whatmann filter paper No.42 and the filtrate was then appropriately diluted with acetonitrile to get a final concentration of 2 $\mu g\ ml^{-1}$ of telmisartan.

6.4. Verapamil Hcl

Two tablets of Veramil (themis chemicals:40mg/tablet) were accurately weighed and finely powdered. The powder equivalent to 20 mg of VER was transferred into 100ml volumetric flask and dissolved in 0.2 M H_2SO_4. then the solution was filtered using whatmann No.41 filter paper and further diluted with distilled water to obtain working standard solutions.

VII. 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.

LOD = 3.3 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.

VIII. FACTORS EFFECTING ABSORBANCE

8. 1. 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 μgml^{-1} 0f the cited drugs, different volumes (0.5–2.5 ml) of 2.0 N H_2SO_4 and 1 ml of Ce(IV) ($4.0x\ 10^{-3}M$) were added. After 5.0 min of heating time at 60 ± 2°C in a water bath, the solution was cooled for about 3.0 min, 1.0 ml of amaranth 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.

8.2. 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 ± 2°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.

8.3. 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 µg 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 µg ml^{-1} Ce (IV) was used for all measurements.

8.4. 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 µg ml^{-1} Ce (IV) with optimum volume 1.0 ml of Amaranth dye for fixed concentration drug solution. The color was found to be stable up to 24 hours.

IX. 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 3]. 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 4]. The excellent recovery studies indicate that methods developed can be applied to pharmaceutical analysis without hesitation.

X. 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 (scheme 1). 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.

D + Ce $(IV)_{excess}$ → D oxidation product + Ce (III) + Ce $(IV)_{unreacted}$: (1)

Ce $(IV)_{unreacted}$ + amaranth → oxidation product of amaranth + uncreated amaranth : (2)

Measured spectrophotometrically at λ_{max} =523 nm
Scheme 1: Reaction Scheme of the indirect determination of drug by oxidation with Ce (IV) sulphate

XI. 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].

XII. 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.

XIII. CONCLUSION

The present study described the successful development of new, simple, sensitive, selective, accurate and rapid spectrohotometric 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.

ACKNOWLEDGEMENT

We are grateful to Head, Department of Chemistry and Principal, Nizam College, Osmania University for providing facilities.

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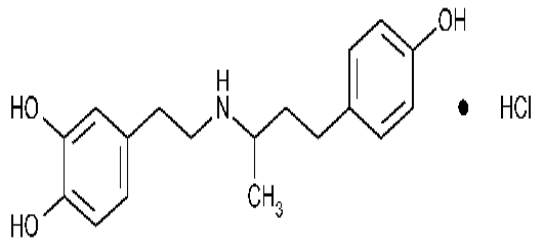
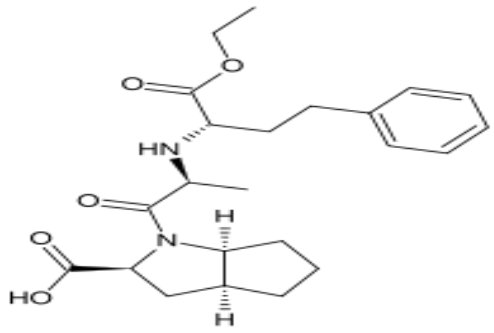
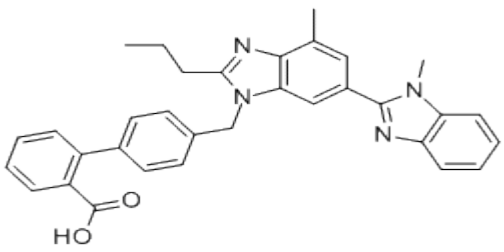
a) DOBUTAMINE HCl



b) RAMIPRIL



c) TELMISARTAN



d) VERAPAMIL HCl

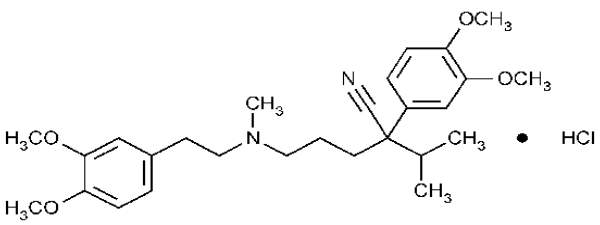


Figure 1 : Structures of Drugs

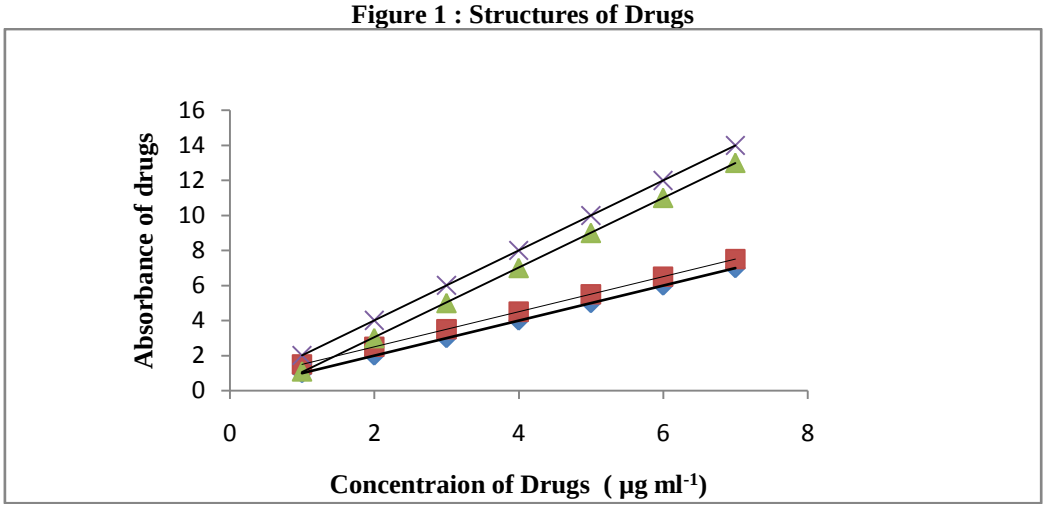


Fig.2 Calibration curves

Table 1: Analytical and Regression parameters of Spectrophotometric method

Name of drug Property	DOB	RAM	TEL	VER
λ_{max}	523	523	523	523
Beer's law limits (µg ml^{-1})	1.6-12	8-56	2-14	2-12
Sandell's sensitivity (µg cm^{-2})	0.0127	0.0833	0.0204	0.0172
Std.Dev. Of intercepts	0.0306	0.0273	0.0204	0.0788
LOD (µg ml^{-1})	1.2763	7.5158	1.3704	4.4823
LOQ (µg ml^{-1})	3.8678	22.775	4.1527	13.5828
Slope (b)	0.079	0.012	0.049	0.058
Intercept (a)	0.089	0.025	0.171	0.061
Correlation coefficient	0.988	0.98	0.99	0.969
Regression equation Y = a + bx	0.215	0.121	0.269	0.177

*Limit of determination as the weight in µg per mL of solution, which corresponds to an absorbance of A = 0.001 measured in a cuvette of cross-sectional area 1 cm 2 and path length of 1 cm. Y** = a+bX, where Y is the absorbance and X=concentration of drug (µg ml^{-1})

Table 2: Determination of Accuracy and Precision of the methods on pure Drug Samples

Name of the Drug	Amount Taken (µg ml^{-1})	***Amount Found*** (µg ml^{-1})	% er	% Recovery	RSD %	Proposed method mean ± SD
DOB	1.5 3.0 4.5 6.0	4.51 2.99 4.46 6.01	0.67 0.33 0.88 0.16	100.67 99.67 99.11 100.17	0.6696	100.01 ± 0.671
RAM	1.0 2.0 4.0 6.0	0.99 2.01 3.98 5.97	1.00 0.50 0.50 0.50	99.00 100.50 99.50 99.50	0.6316	99.59 ± 0.622
TEL	3.0 5.0 7.0 9.0	2.99 4.97 6.98 8.99	0.33 0.60 0.28 0.11	99.67 99.40 99.71 99.89	0.2031	99.70 ± 0.199
VER	3.0 5.0 7.0 9.0	3.02 5.01 6.99 8.96	0.67 0.20 0.14 0.44	100.67 100.20 99.86 99.56	0.4762	100.18 ± 0.469

Table 3: Results of assay of tablets by the proposed methods and statistical evaluation and recovery experiments by standard addition method

Pharmaceuticals/ tablets/ injection	Drug in tablet (µg ml^{-1})	Drug added (µg ml^{-1})	Total found (µg ml^{-1})	er %	Recovery %	RSD%	Reference method mean ±SD	Proposed method ±SD
DOB	2.0 4.0 6.0 8.0	1.0 1.0 0.0 0.0	2.98 4.99 5.98 7.99	0.67 0.20 0.33 0.13	99.33 99.88 99.67 99.88	0.261	101 ± 1.0	99.7 ± 0.257
RAM	08.0 10.0 12.0 14.0	1.0 1.0 0.0 0.0	8.98 11.01 11.99 14.00	0.22 0.10 0.08 0.00	99.98 100.09 99.92 100.00	0.070	100.96 ± 0.672	100.10 ± 0.07
TEL	3.0 5.0 7.0 9.0	1.0 1.0 0.0 0.0	3.98 6.02 6.99 8.98	0.50 0.33 0.43 0.22	99.50 100.33 99.57 99.78	0.377	100.5 ± 0.393	99.80 ± 0.361
VER	2.0 4.0 6.0 8.0	1.0 1.0 0.0 0.0	3.02 5.0 5.98 7.99	0.50 0.0 0.33 0.16	100.67 100.0 99.67 99.88	0.432	98.93 ± 0.37	100 ± 0.396

Table 4: Student's t-test and f-test values for Pharmaceutical analysis

Pharmaceuticals/ tablets/ injection	DOB	RAM	TEL	VER
Student's t-test	1.327 (1.42)	1.396 (1.393)	0.074 (1.45)	0.059 (1.19)
f-test	0.066 (3.15)	0.011 (4.02)	0.841 (3.85)	1.145 (3.24)