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OXIDATIVE SPECTROPHOTOMETRIC DETERMINATION OF DRUGS USING $KMnO_4$ AND RHODAMINE-B

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ABSTRACT

Simple, accurate, and precise, UV-Vis spectrophotometric methods have been developed for the estimation of four drugs *viz*., Verapamil, Dobutamine HCl, Montelukast sodium, Tri metazedine di hydrochloride. The method is based on the oxidation of drugs with acidic $KMnO_4$ (excess) and subsequent estimation of unreacted $KMnO_4$ by using Rhodamine-B as an analytical reagent. The proposed methods were found to be successful for the estimation of these drugs in bulk and its pharmaceutical formulations. Results of analyses were validated statistically. Statistical comparison of the results with the reference method shows excellent agreement and indicates no significant difference in accuracy and precision.

INTRODUCTION

Verapamil

Verapamil Hydrochloride (VRP) 5-[N-(3, 4-dimethoxy - phenethyl) - N- methylamino]-2- (3, 4-dimethoxyphenyl)-2-isopropylvaleronitrile hydrochloride, or (Isoptin) [1], have the chemical structure as shown in (Fig.1). Verapamil major effect is on the slow Ca channel. The inhibition of the action potential inhibits one limb of the reentry circuit believed to underlie most paroxysmal super ventricular tachycardia that uses the AV node as a reentry point. It is categorized as a class IV ant arrhythmic drug. The drugs reduce systemic vascular resistance & mean blood pressure, with minor effect on cardiac output. The different analytical methods that are reported for its determination include high performance liquid chromatography [1], high performance thin layer chromatography [2], liquid chromatography [3], gas chromatography [4], potentiometry conductometry, [5] strippingvoltammetry [6] and atomic emission spectrometry [7]. To our knowledge, there is no simple and accurate UV spectrophotometric method for quantitative determination of Verapamil HCl in its bulk and tablet dosage forms.

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Dobutamin hydrochloride

Dobutaminhydrochloride (DBH) chemically 4-(2-((1—methyl-3-(4hydroxybenzene) propyl)amido)ethyl)-1,2-di-hydroxybenzene hydrochloride salt (Figure-2) is an adrenalin receptor concussion medicine indicated obvious curative effect for coronary heart diseases, acute myocardial infarction and expansionary cardiomyopathy [8]. There are various analytical methods for the assay of dobutamine, such as nonaqueous solution titration [9], spectrophotometric analysis [9], HPLC [10], carbon paste electrode adsorption voltammetric analysis [11], multy wall carbon nanotube modified glassy carbon electrode electrochemical analysis [12].

Montelukast sodium

Montelukast sodium (MTK) is chemically (R-(E))-1-(((1-(3-(2-(7-chloro-2-quinolinyl) ethenyl) phenyl)-3(2-(1-hydroxymethylethyl) phenyl) propyl) thio) methyl) cyclopropaneacetic acid, monosodium salt. (Figure-1). Montelukast sodium primarily used for the treatment of asthma in children and adults. It is a potent selective inhibitor of leukotriene D4 (LTD4) at the cysteinyl leukotriene receptor cysLT1. Only a few methods have been reported for determination of Motelukast including

derivative spectroscopy [13], by colorimetry [14], by fluorimetry [15], by TLC [16], by HPTLC [17], by simultaneous UV determination in combination drug formulation [18], by volttametry [19], by high performance liquid chromatography (HPLC) [20] and by LCMS [21].

Trimetazedine Di Hcl

Trimetazidine (TMZ); 1-[(2, 3, 4-trimethoxyphenyl)methyl]piperazine dihydrochloride is a clinically effective antianginal agent that has been used in the prophylaxis and management of angina pectoris, and in ischemia of neurosensorial tissues as in Meniere's disease. Trimetazidine exhibits some cytoprotective effects on myocardial energy metabolism and exerts an anti angina effect in the absence of significant hemodynamic effects. For these clinical successes, TRMZ has become unique technique. Trimetazidine dihydrochloride have been determined in pharmaceutical formulations and/or biological fluids by high performance thin-layer chromatography [22], liquid chromatography [23-25].

Therefore this method was made to develop a simple spectrophotometric method for the estimation of above mentioned drugs in pharmaceutical formulations.

MATERIALS AND METHODS

Experimental

The pharmaceutical grade drugs were supplied by Arabindo Pharmaceuticals and Hetero drugs Pvt. Ltd Hyderabad. Rhodamine-B, $KMno_4$ and H_2SO_4 were purchased from S.D. Fine chem. Pvt. Ltd., Mumbai, India. Whatman filter paper no.42 was used for filtration purpose. All the reagents used were of AR grade and double distilled water was used throughout the investigation. Tablets were purchased from the local market.

Instrumentation

All absorbance measurements were recorded on shimadzu 140 double beam spectrophotometer as well as Thermo Nicolet 100 & Elico 159 UV-Visible single beam spectrophotometers using matched pair of quartz cells of 10mm path length. A high precision analytical balance was used for weighing the reagents.

Preparation of standard stock solution

$KMnO_4$($7.6X10^{-2}$M) stock solution was prepared by dissolving 0.1209gm of sample in 100ml standard flask with double distilled water. Rhodamine-B ($1x10^{-2}$M) solution was prepared by dissolving 0.0479gm in 100ml standard flask with double distilled water. Stock solution of $KMnO_4$ was further diluted to the concentration of 0.145µg mL^{-1} and Rhodamine-B was further diluted to the 45.48 µg mL^{-1}.

Preparation of Standard Drug Solution

Standard stock solutions of drugs were prepared by dissolving accurately weighed 40mg drug to separate 100ml volumetric flasks. The stock solutions of Calaptin 40, Cardiject, Montair-5, and Trivedon-20 were further diluted with the same solvent i.e. distilled water. Concentrated H_2SO_4 diluted appropriately with distilled water to get 0.2M acid solution.

Preparation of Calibration Curve

Aliquots of pure drug solution (1 to 7ml) were transferred into a series of 10ml calibrated flasks. To each flask 1ml of 0.2M H_2SO_4 acid solution was added followed by 0.5ml of $KMnO_4$ solution. The contents were mixed and the flasks were heated for 10min. These were cooled and 1ml of Rhodamine-B solution was added to each flask, diluted to the mark with water and the absorbance of solution was measured at 557nm. A standard graph was prepared by plotting the absorbance versus the concentration of drugs and computed from the regression equation derived using Beer's law. Calibration curve for each drug drawn in Fig 2.

Analysis of pharmaceutical preparations

An accurately weighed powder equivalent to 10mg of drug Calaptin 40, Cardiject, Montair-5, Trivedon-20 were transferred in separate 100ml volumetric flask. The content was dissolved in sufficient distilled water and the solutions were filtered through whatman filter paper no.42. The volume in each flask was made up to 100ml with distilled water. From these solutions aliquots containing required concentrations of the drugs were taken for analysis and the solutions were then analysed as described under respective calibration curve procedure. The amount of drugs was determined by referring to the calibration curve. The analysis procedure was repeated 6,5,3,3 respectively for each pharmaceutical formulation and the results of analysis of pharmaceutical formulations are reported in Table 3.

METHOD VALIDATION

The proposed methods were validated according to guidelines of international conference on harmonization (ICH) [26].

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample, was studied by analyzing drug at three concentrations and process was repeated for three times each .

Precision

Precision of the system was evaluated by analyzing seven independent sample preparations obtained from homogenous sample and %RSD value obtained was

calculated to determine any intra-day variation. These studies were also repeated on different days to determine inter-day variation.

Limit of Detection and Limit of Quantitation

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantitated as an exact value. Based on the standard deviation of the Y-intercept and the slope, detection limit (DL) may be expressed as:

$$DL = 3.3s/S$$

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy. Based on the standard deviation of the response and slope, the quantitation limit (QL) may be expressed as:

$$QL = 10s/S$$

Where,

s = the standard deviation of the residual intercept

S = the slope of the calibration curve.

RESULTS AND DISCUSSION

The calibration curves for Verapamil, Dobutamine HCl, Montelukast sodium, Tri metazedine din HCl over a concentration range of 0.1-0.7, 0.5-3.5, 0.1-0.7, 1.2-8.4 µg mL^{-1}respectively were plotted and molar absorptivity for drugs were calculated at the wavelength of 557nm. The regression characteristics are reported in Table-1. The results of assay are reported in Table-2. The percent recovery from commercial formulation was shown in Table-3.

Table 1. Analytical and regression parameters of spectrophotometric method

Parameter	verapamil	Dobutamine HCl	Montelukast sodium	Tri metazedine di HCl
λmax, nm	557	557	557	557
Beer's law limits µg mL^{-1}	0.1-0.7	0.5-3.5	0.1-0.7	1.2-8.4
Molar absorptivity $Lmol^{-1}$ cm^{-1}	0.072×10^{7}	0.1304×10^{6}	0.116×10^{6}	0.056×10^{6}
Sandell sensitivity µg mL^{-1}	0.0055	0.007	0.0058	0.0057
Limit of detection µg mL^{-1}	0.3795	0.9785	0.506	0.3903
Limit of quantification µg mL^{-1}	1.150	2.965	1.589	1.182
Regression equation Y**=a+bX				
Intercept, (a)	0.055	0.076	0.0701	0.055
Slope, (b)	0.1803	0.1416	0.1715	0.173
Correlation coefficient, (r)	0.982	0.925	0.9759	0.982
Standard deviation of intercept (Sa)	0.024	0.0489	0.0514	0.216
standard deviation of slope(Sb)	0.1826	0.1649	0.1628	0.182

*Limit of determination as the weight in µg per mL of solution, which corresponce 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 drugs in µg mL^{-}

Table 2. Determination of accuracy and precision of the methods on pure drug Samples

Drug	Taken (µg mL^{-1})	Found (µg mL^{-1})	Er (%)	Recovery (%)	RSD (%)	Proposed method mean ± SD
VRP	4.2 6.5 7.0	6.61 7.01 1.47	0.71 1.69 014	99.33 101.6 100.14	1.146	100.35±1.146
DBH	1.5 2.0 3.9	1.47 2.03 3.9	0.42 1.5 0.00	98 101.5 100	1.75	99.83±1.75
MTK	2.3 6.0 3.5	2.28 6.01 3.51	0.86 0.16 0.28	99.13 100.16 100.28	0.632	99.85±0.632
TMZ	2.0 4.5 7.0	2.0 4.53 7.02	0.00 0.66 0.28	100 100.66 100.28	0.218	100.31±0.218

Table 3. Results of assay of tablets by proposed method and statistical evaluation

Tablet	Taken (µg mL^{-1})	Found (µg mL^{-1})	er (%)	Recovery (%)	RSD (%)	Reference method mean ± SD	Proposed method mean ±SD
Verapamil (calaptin40)	4.2 6.5 7.0	4.23 6.49 7.0	0.71 0.15 0.00	100.71 99.84 100	0.462	100.03 ± 0.33	100.18 ±0.463
Dobutamine HCl (cardiject)	1.5 2.0 3.9	1.48 2.03 3.92	1.33 1.5 0.51	98.66 100.5 100.5	1.063	101 ± 1.0	99.88 ±1.062
Montelukast sodium (montair5)	2.3 6.0 3.5	2.3 6.02 3.48	0.00 0.33 0.57	100 100.33 99.42	0.460	99.61 ± 0.22	99.91 ±0.460
Tri metazedine di HCl (truvedon 20)	2.0 4.5 7.0	2.0 4.49 6.98	0.00 0.22 0.28	100 99.77 99.71	0.153	100.01± 1.25	99.82 ±0.153

Table 4. Student's t-test and F-test values for pharmaceutical analysis

Pharmaceuticals/tablets	VRP	DBH	MTK	TMZ
Student's t-test	0.539 (1.440)	0.095 (1.476)	1.273 (1.638)	0.214 (2.353)
F-test	1.965 (3.52)	1.127 (4.11)	4.371 (9.24)	0.014 (9.29)

Fig1. Verapamil

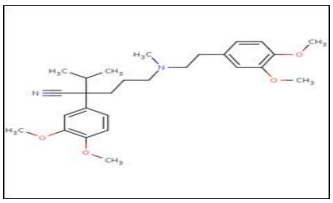


Fig 2. Dobutamine HCl

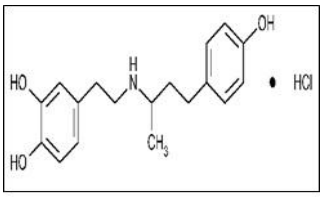


Fig 3. Montelukast sodium

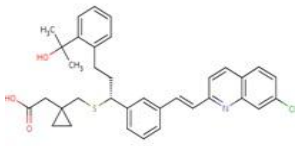


Fig 4. Tri Metezedine di HCl

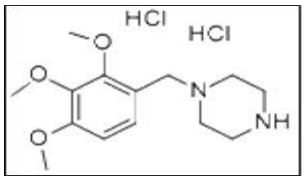
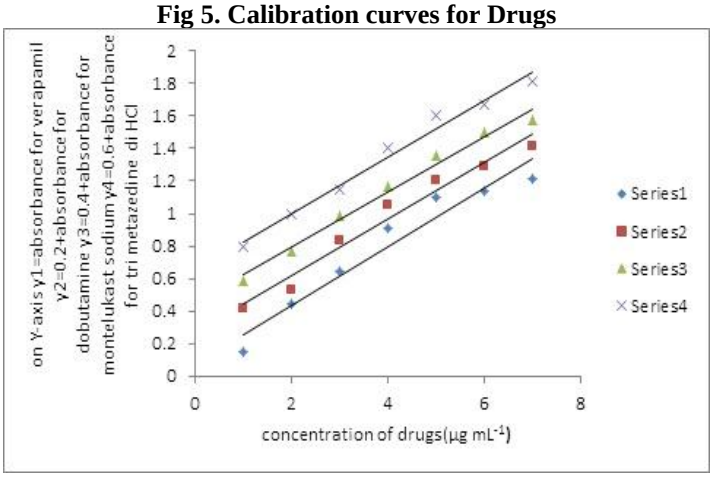


Fig 5. Calibration curves for Drugs



The accuracy of the proposed method was evaluated by percentage recovery studies of the drugs. The %RSD was also less than 2% for intra-day determinations showing high degree of the proposed method. The results of the method lie within the prescribed limit, showing that method is free from interference from excipients.

CONCLUSION

The proposed methods are accurate, simple, rapid and selective for the simultaneous estimation of above mentioned drugs in bulk and tablet dosage form by standard calibration method. The method is economical compared to other sophisticated analytical instruments.

Hence can be used for routine analysis of commercially available formulations. The solvent used for the method are inexpensive and simple to prepare, and could be used in quality control laboratory for routine drug analysis.

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