WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 8, 1383-1395. Research Article ISSN 2277– 7105

SPECTROPHOTOMETRIC DETERMINATIONOF DRUGS USING N-BROMOSUCCINIMIDE AND RHODAMINE-B DYE AS ANALYTICAL REAGENT

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Article Received on
10 May 2015,

Revised on 05 June 2015,
Accepted on 02 July 2015

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ABSTRACT

Simple, sensitive and selective methods are developed for the spectrophotometric determination of drugs, viz., Tramadol hydrochloride, Dobutamine hydrochloride, Trimetazidine, Terazosin hydrochloride dihydrate and Esmolol hydrochloride, based on their reactivity towards *N*- bromosuccinimide (NBS). The methods involve the addition of excess NBS of known concentration in the presence of 1*M* HCl, reactants are allowed to react and the unreacted NBS is estimated by using Rhodamine-B dye (λ_{max} 557nm). These methods have been applied for the determination of drugs in their pure form as well as in tablet formulations. The effect of excipients has also been studied and found to have no effect. These methods have been validated in terms of guidelines of ICH**.**

KEY WORDS: Spectrophotometry, Drugs, NBS, Rhodamine-B, Quantification, Validation.

INTRODUCTION

Tramadol hydrochloride (TDH) is chemically (±)-*Trans*-2- dimethylaminomethyl-1-(3-methoxyphenyl) cyclohexanol hydrochloride is a centrally acting synthetic opioid analgesic, non-steroidal anti-inflammatory drug.[1-4] used in treating several pains including treatment of Rheumatoid arthritis, restless legs syndrome and fibromyalgia. Its quantification method is reported.[5] in which earlier methods of determination.

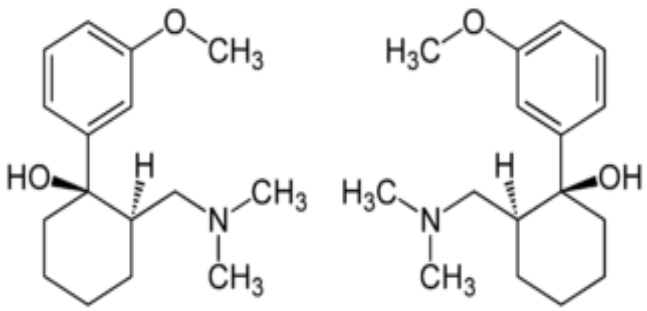
Dobutamine hydrochloride (DOB) is chemically: 4-(2-((1-methyl-3-(4-hydroxybenzene) propyl) amido) ethyl)-1,2-di-hydroxybenzene hydrochloric salt is an adrenalin receptor

concussion medicine indicated obvious curative effect for coronary heart disease, acute miocardial infarction, and expansionary cardiomyopathy.[6] Determination of dobutamine hydrochloride.[7] reported in which past methods are reviewed.

Trimetazidine (TRMZ); 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.[8] The antianginal efficacy of TRMZ is comparable to propranolol but it does not reduce cardiac rate–pressure product or coronary blood flow.[9] Trimetazidine exhibits some cytoprotective effects on myocardial energy metabolism and exerts an antianginal effect in the absence of significant hemodynamic effects.[10] For these clinical successes, TRMZ has become unique among the antianginal agents, and it has been clinically used throughout many countries worldwide.[11,12] Recent determination method.[13] is preceded by many methods cited therein.

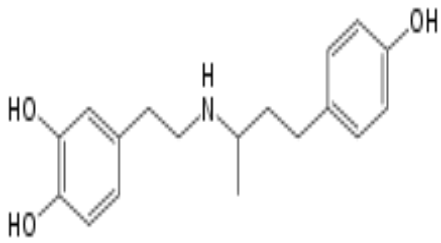
Terazosin hydrochloride dihydrate (TRZ) is known chemically as 2-[4-(2-tetrahydrofuranyl) carbonyl]-1-piperazinyl-6, 7-dimethoxy-4-quinazolinamine mono hydrochloride dihydrate. TRZ is a highly selective potent α-1 adrenoreceptor antagonist. It is an effective drug for hypertension (high blood pressure) and benign prostatic hyperplasia (enlarged prostate). It causes the blood vessels (veins and arteries) to relax and expand, improving blood flow. TRZ also relaxes muscles in the prostate and bladder neck, making it easier to urinate.[14] It is recently quantified by UV spectrophotometric method.[15] The report includes past quantification references on the drug.

Esmolol hydrochloride (ESM) methyl 3-{4-[2-hydroxy-3-(isopropylamino) propoxy] phenyl} propionate hydrochloride is an ultra-short acting _-adrenergic receptor antagonist used for the rapid control of heart rate in patients with atrial fibrillation or atrial flutter. Since ESM is widely used in the rapid control of heart rate,[16] it is important to develop and validate analytical methods for its determination in pharmaceutical dosage form. A recent quantification method.[17] in which various earlier methods of quantification are reviewed.



.HCl

Tramadol hydrochloride



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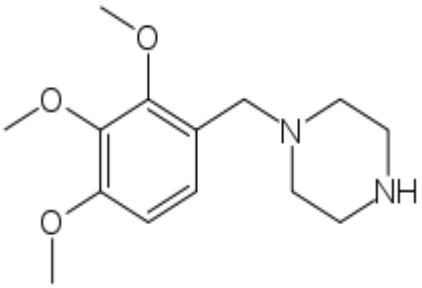
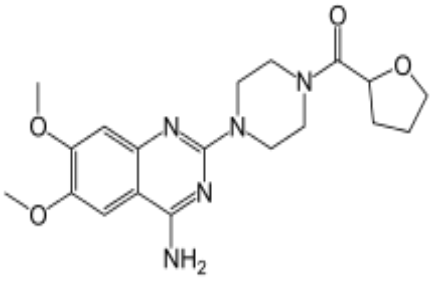
Dobutamine hydrochloride

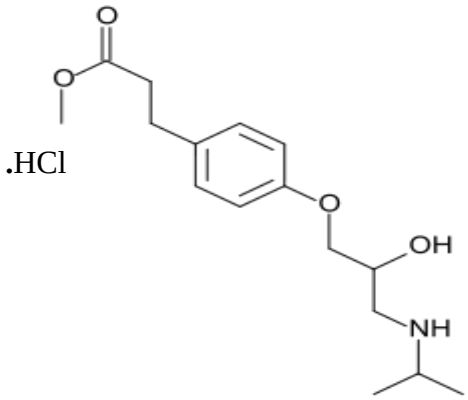


Trimetazidine



Terazosin

Scheme 1 - Structures of the Drugs



.HCl

Esmolol hydrochloride

Thorough survey of literature on the above mentioned drugs revealed that quantification using NBS as oxidizing reagent has not been reported yet although the reagent is common, known to offer simple, sensitive method of quantification for drugs.[18-20] This prompted the

authors to develop quantification methods for the above cited drugs, (Scheme 1), using NBS as an oxidizing agent and Rhodamine-B as analytical reagent.

MATERIALS AND METHODS

Instrument

All absorbance measurements were recorded on Shimadzu 140 double beam spectrophotometer as well as on Thermo Nicolet 100 & Elico 159 UV- Visible single beam spectrophotometers using matched pair of Quartz cells of 10mm path length.

Materials

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

NBS solution (0.01mol L^{-1}) was prepared by dissolving N-bromosuccinimide (Himedia Laboratories pvt.Ltd, Mumbai) in water with the aid of heat and standardized [18, 20]. The solution was kept in an amber colored bottle and was diluted with water appropriately to get 70µg mL^{-1} NBS for use in spectrophotometric method.

A stock solution of Rhodamine- B (500µg mL^{-1}) was prepared by dissolving the dye (s. d. Fine Chem. Ltd., Mumbai) in water and filtered using glass wool. The dye solution was diluted to 50µg mL^{-1}.

Hydrochloric acid (1 *M*): Concentrated hydrochloric acid (S.D. Fine Chem., Mumbai, India; sp. gr. 1.18) was diluted appropriately with water to get 1*M* acid.

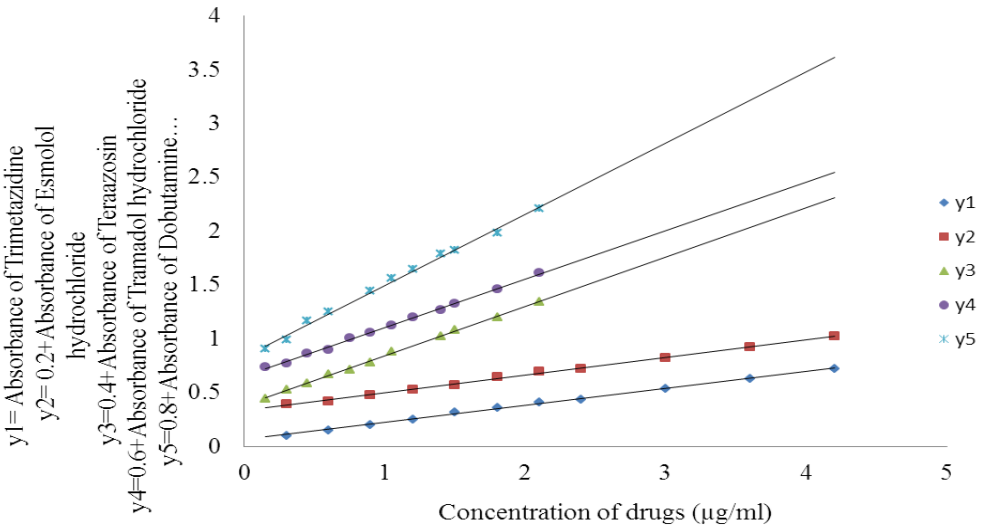
The pharmaceutical grade drugs were supplied by Arabindo pharmaceuticals and hetero drugs Pvt. Lmt Hyderabad. A stock standard solution of drugs were prepared by dissolving accurately weighed 10mg of pure drug in water and diluting to 100mL in a calibrated flask with water. The solution was diluted stepwise to get working concentrations.

Assay procedure

Aliquots of pure drug solution (0.5 to 7mL,) were transferred into a series of 10mL calibrated flask and the total volume was adjusted to 4mL with water. To each flask, 1mL of 1mol L^{-1} hydrochloric acid was added, followed by 1mL of NBS solution (70µg mL^{-1}). The contents were mixed and the flasks were set aside for 10 min under occasional shaking. Finally, 1mL

of 50µg mL^{-1} Rhodamine B solution was added to each flask, diluted to the mark with water and the absorbance of solution was measured at 557 nm against a reagent blank after 10 min.

Calibration curves were constructed for all the drugs by plotting the absorbance versus the concentration of drugs. The absorbance data was collected for six replicate experiments and absorbance to concentration ratio called the relative response was determined. The relative responses between 95% to 105% of average only are considered for construction of the Calibration curves (figure 1).



(Figure 1) Calibration curves

Procdure for assay of pure drug

Sample solutions of each drug in the beer's law limits were chosen and recovery experiments were performed to check the accuracy and precision. The concentration chosen and recovery are tabulated in table 2. For this purpose standard deviation method also adapted. Excellent recovery and %RSD being less than 2 speaks about the precision and accuracy of the method.

Procedure for tablets

1. Tramadol hydrochloride

For the analysis of pharmaceutical formulations ten tablets (Cambidol,50mg) were weigheted, powered and equivalent to 10mg of tramadol hydrochloride was transferred in to 100mL volumetric flask. 60.0mL 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.

2. Dobutamine hydrochloride

Four tablets (Dobusol, 250mg) were weighed and grounded. The powder equivalent to10mg 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 100mL of distilled water and it was further diluted to get required concentration for the analysis of the drug.

3. Trimetazidine

About ten tablets (Trivedon, 20mg) were powdered and equivalent to 10mg of trimetazidine has been taken in to a100mL of volumetric flask and added about 30mL 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 100mL 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**.**

4. Terazosin

Ten tablets (Terazen, 2mg) were weighed and grounded. A quantity equivalent to 10mg of terazosin was transferred into a 100mL 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 10mL 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.

5. Esmolol hydrochloride

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

RESULTS AND DISCUSSION

Method development

The proposed spectrophotometric methods are indirect and are based on the determination of the excess of NBS after allowing the reaction between drug and a measured amount of NBS to be complete. The excess of NBS was determined by reacting it with a fixed amount of Rhodamine-B dye. The methods make use of bleaching action of NBS on the dye, the decolouration being caused by the oxidative destruction of the dyes. Drug when added in increasing concentrations to a fixed concentration of NBS, consumes the latter proportionally and there occurs a concomitant fall in the concentration of NBS. When a fixed concentration of dye is added to decreasing concentrations of NBS, a concomitant increase in the concentration of dye is obtained. Consequently, a proportional increase in the absorbance at the respective λmax is observed with increasing concentration of drug.

Preliminary experiments were conducted to determine the maximum concentrations of Rhodamine-B spectrophotometrically by measuring the absorbance of their acidic solutions at their respective λmax and the upper limits were found to be 5µg mL^{-1} for Rhodamine-B. NBS concentration of 7 µg mL^{-1} was found to bleach the red color due to 5 µg mL^{-1}Rhodamine-B. Hence different amounts of drug reacted with 7µg mL^{-1} NBS in these methods before determining the residual NBS as described under the respective procedure.

Hydrochloric acid was found to be a convenient medium for this method. For a quantitative reaction between drug and NBS, a contact time of 10 min was found sufficient. Constant absorbance readings were obtained when the reaction times were extended up to 15 min and a standing time of 5–10 min was necessary for the bleaching of dye color by the residual NBS. The measured color was stable for several hours even in the presence of the reaction product.

Analytical data

A linear correlation was found between absorbance at λmax and concentration of all drugs in the ranges given in table 1. Regression analysis of the Beer's law data using the method of least squares was made to evaluate the slope (b), intercept (a) and correlation coefficient (r)for each system and the values are presented in table 1. The optical characteristics such as Beer's law limits and Sandell sensitivity values for both methods are given in table 1. The limits of detection (LOD) and quantitation (LOQ) calculated according to ICH guidelines [21] are also presented in table 1 and reveal the very high sensitivity of the methods.

$LOD = 3.3S_a/b$

LOQ = $10S_a/b$.

Where S_a= standard deviation of the intercept (n = 6)

b = slope of Calibration plot.

Precision and Accuracy

Intra-day precision was assessed from the results of six replicate analyses on pure drug solution. The mean values and relative standard deviation (RSD) values for replicate analyses at three different levels (amounts/concentrations) were calculated. To evaluate the inter-day precision, analysis was performed over a period of five days, preparing all solutions afresh each day.

The accuracy of the methods was determined by calculating the percentage deviation observed in the analysis of pure drug solution and expressed as the relative error. Table 2 summarizes the intra-day precision and accuracy data for the assay of pure drugs solution by the proposed methods.

Robustness and Ruggedness

To evaluate the robustness of the methods, volume of Hydrochloric acid was slightly altered. The reaction time (after adding NBS, time varied was 10 ± 2min) and the time after addition of dye is slightly changed. To check the ruggedness, analysis was performed by three different analysts and on three different spectrophotometers by the same analyst.

Application to formulations

The proposed method is applied to the determination of drugs in tablets. The results in Table 3 showed that the methods are successful for the determination of drugs and that the excipients in the dosage forms do not interfere. The results are compared to the available validated reported.[18, 22-25] methods on each drug and the results agree well with the claim and also are in agreement with the results obtained by the literature method. Statistical analysis of the results using Student's t-test for accuracy and F-test for precision revealed no significant difference between the proposed methods and the literature method with respect to accuracy and precision.

Recovery experiment was performed via standard addition technique to ascertain the accuracy and validity of the proposed methods. To a fixed and known amount / concentration of drug in tablet powder (pre-analyzed), pure drug was added at three levels (50, 100 and 150

% of the level present in the tablet) and the total was found by the proposed methods. Each experiment was repeated six times and the percent recovery of pure drugs added and the percent recovery of the added standard was calculated. Results of this study presented in Table 3 indicate that the commonly excipients present in the formulations did not interfere in the assay.

Table 1 Analytical and Regression Parameters of Spectrophotometric Methods.

Parameter	TDH	DOB	TRMZ	TRZ	ESM
λmax, nm	557	557	557	557	557
Beer's law limits µg mL^{-1}	0.15-4.0	0.15-7.0	0.3-6.5	0.15-2.1	03-4.2
Molar absorptivity, L mol^{-1} cm^{-1}	1.50×10^5	1.86×10^5	8.90×10^4	1.68×10^5	2.11×10^5
Sandell sensitivity* µg cm^{-2}	0.0022	0.0015	0.0063	0.0021	0.0060
Limit of detection µg mL^{-1}	0.0914	0.1096	0.4386	0.0864	0.7143
Limit of quantification µg mL^{-1}	0.2771	0.3323	1.329	0.2622	2.1646
Regression equation, Y**					
Intercept, (a)	0.051	0.032	0.061	0.016	0.133
Slope, (b)	0.451	0.662	0.158	0.458	0.164
Correlation coefficient, (r)	0.997	0.955	0.997	0.998	0.997
Standard deviation of intercept (Sa)	0.0125	0.022	0.021	0.012	0.0355
Standard deviation of slope (Sb)	0.0948	0.0306	0.0819	0.0875	0.065

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 drugs in µg per mL.*

Table 2 Determination of Accuracy and Precision of the Methods on Pure Drug Samples.

Drug	Taken (µg/ml)	Found (µg/ml)	er (%)	Recovery (%)	RSD(%)	Proposed method Mean ± SD
TDH	1.5 2.5 3.5	1.49 2.51 3.49	0.66 0.40 0.28	99.33 100.40 99.71	0.543	99.81 ± 0.542
DOB	4.0 5.0 6.0	4.05 5.03 5.98	0.75 0.60 0.33	100.75 100.60 99.66	0.5905	99.91 ± 0.590
TRMZ	3.0	3.01	0.33	100.33		99.70

	4.0 5.0	4.02 4.99	0.50 0.20	100.50 99.80	0.366	±0.3651
TRZ	1.5 3.5 4.0	1.49 3.51 3.98	0.66 0.28 0.50	99.33 100.28 99.50	0.504	100.21 ±0.506
ESM	2.0 4.0 6.0	1.99 4.01 6.00	0.50 0.28 0.00	99.50 100.25 100.00	0.397	100.33 ±0.381

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 drugs in µg per mL.*

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 µg mL^{-1}	Drug added µg mL^{-1}	Total found µg mL^{-1}	er (%)	Recovery (%)	RSD (%)	Reference method Mean± SD	Propose method ± SD	t-test	F-test
Cambidol (TDH)	1.0 1.0 1.0 1.0 2.0 3.0	1.0 2.0 3.0 0.0 0.0 0.0	2.03 2.99 4.02 0.99 2.01 3.02	1.50 0.33 0.50 1.00 0.50 0.66	101.50 99.66 100.50 99.00 100.5 100.66	0.761	101.70 ±1.70	100.29 ±0.764	1.176 (2.571)	0.201 (4.95)
Dobusol (DOB)	1.0 1.0 1.0 2.0 3.0 4.0	0.5 1.0 1.5 0.0 0.0 0.0	1.49 2.02 2.51 1.98 3.01 3.99	0.66 1.00 0.40 1.00 0.33 0.25	99.33 101.00 99.00 100.33 100.33 99.75	0.738	101.00± 1.0	99.95 ±0.737	0.16 (2.571)	0.041 (4.95)
Trivedon (TRMZ)	1.0 1.0 1.0 2.0 4.0 6.0	1.0 2.0 3.0 0.0 0.0 0.0	2.01 2.99 3.98 2.00 3.99 6.01	0.50 0.33 0.50 0.00 0.25 0.16	100.50 99.666 99.50 100.00 99.75 100.16	0.367	99.80 ±1.26	99.92 ±0.366	1.536 (2.571)	0.084 (4.95)
Terazen (TRZ)	1.0 1.0 1.0 1.0 3.0 5.0	1.5 3.0 4.5 0.0 0.0 0.0	2.49 4.01 5.49 1.01 3.00 5.01	0.40 0.25 0.18 1.00 0.00 0.20	99.60 100.25 99.81 101.00 100.00 100.20	0.478	99.90±0.470	101.14 ±0.484	0.044 (2.477)	1.060 (4.28)
Miniblock (ESM)	1.0 1.0 1.0 3.0 4.0 5.0	0.5 1.0 1.5 0.0 0.0 0.0	1.51 1.99 2.48 2.99 4.02 5.01	0.66 0.50 0.80 0.33 0.50 0.20	100.66 99.50 99.20 99.66 100.50 100.20	0.586	100.45 ±0.573	99.95 ±0.5861	0.038 (3.182)	1.045 (4.75)

CONCLUSION

These are simple, rapid, and cost-effective methods for the determination of drugs have been developed and validated. The proposed method is more sensitive methods and the methods rely on the use of simple and cheap chemicals and techniques but provide sensitivity comparable to that achieved by sophisticated and expensive technique like HPLC. Thus, they can be used as alternatives for rapid and routine determination of bulk sample and tablets.

ACKNOWLEDGEMENTS

The authors are thankful to the Head, Department of Chemistry, Osmania University, Hyderabad-500007 for providing facilities. One of the authors TIRUPATHI B is thankful to CSIR for SRF.

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