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Quantification of Drugs and Pharmaceuticals Using N-Bromosuccinimide and Methyl Orange Dye: A Spectrophotometric Study

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ABSTRACT

Simple, sensitive and selective methods are developed for the spectrophotometric determination of drugs, viz., Dobutamine hydrochloride, Lomefloxacin hydrochloride, Sildenafil citrate, Ramipril, Telmisartan based on their reactivity towards N- bromosuccinamide (NBS). The method of each drug depends upon oxidation of drugs by NBS (Excess) and estimating the amount of un-reacted NBS by Methyl Orange dye at ëmax 508nm. The calibration curves obeyed Beer's law over the concentration range of 5-38 µg mL^{-1} (DOB), 5-50 µg mL^{-1} (LOM), 4-28 µg mL^{-1} (SIL), 2-14 µg mL^{-1} (RAM) & 6-42 µg mL^{-1} (TEL). This method has been applied for the determination of drugs in their pure form as well as in tablet formulations. The method has been validated in terms of guidelines of ICH.

Key words: Methyl orange, NBS, Quantification, Spectrophotometry, Validation.

INTRODUCTION

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 myocardial 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.

Lomefloxacin (Fig. 1 b) chemically known as (RS)-1-ethyl-6,8-difluoro- 7-(3- methylpiperazin-1-yl)- 4-oxo-quinoline-3- carboxylic acid is one of the third generation fluoroquinolones with some specific activity in upper respiratory tract infections and community acquiredpneumonia. It

is also used in meningitis, osteomyclitis, urinary tract infections, sexually transmitted diseases, bacteraemia, nosocomially acquired infections, gastrointestinal infections and in combination with other agents in the treatment of tuberculosis.[9] Because of its physiological significance several methods have been developed for its quantitative determination viz.,Spectrofluorimetry [10], Spectrophotometry [11] and HPLC .[12,13]

Sildenafil citrate (SIL) is designated chemically as 1- h[3- (6,7-dihydro-1-methyl-7-oxo-3-propyl-1-Hpyrazolo[4,3-d]pyrimidin-5-yl)-4-ethoxyphenyl]sulfonylj-4-methylpiperazine citrate and has the structural formula shown in [Fig. 1(c)]. It is used in oral therapy for erectile dysfunction, is a selective inhibitor of cyclic guanosine mono phosphate (cGMP) specific phosphodiesterase type 5 (PDE5) .[14] Some techniques have been developed for quantitative determination of SIL in pharmaceutical formulations. Some important ones are HPLC [15,16,17], flow-injection analysis with multiple pulse amperometric detection [18], atomic emission spectrometry [19], spectrophotometry[20,21] and LC-MS. [22]

Ramipril chemically is (2S,3aS,6aS)-1-[(2S)-2-{[(2S)-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(d)]. It effectively reduces both supine alterations in the pulse rate. It is indicated for Hypertension and cardiac failure. [23] Some methods for the analysis of ramipril are Spectrophotometry [24,25,26], High-performance liquid chromatographic and chemometric based spectrophotometry [27]and simple colorimetry[28] have been employed.

Telmisartan or (2-(4-{[4-methyl-6-(1-methyl-1H-1,3-benzodiazol-2-yl)-2-propyl-1H-1,3-benzodiazol-1-yl]methyl}phenyl)benzoic acid or [Fig.1(e)] is a cardiovascular drug, indicated for hypertension.[29] Because of its physiological

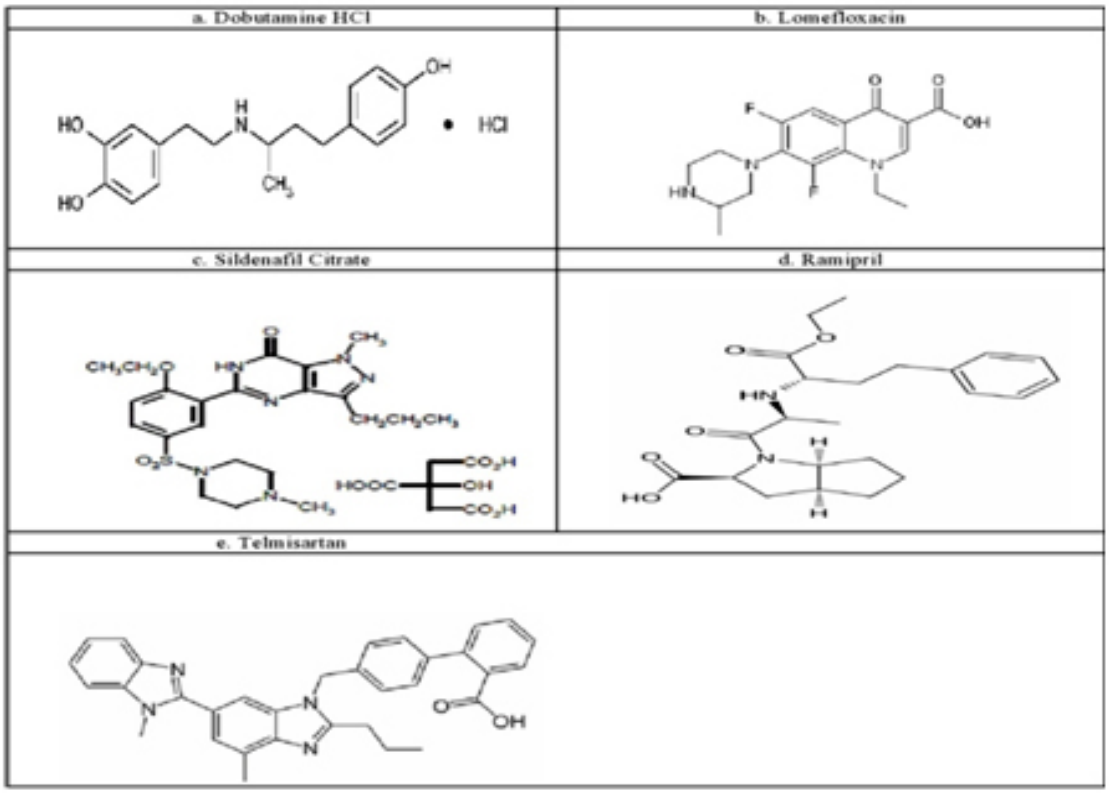

Fig. 1: Structures of the Drugs

importance many physical and instrumental techniques have been developed for the quantification of TEL like HPLC[30-36], Spectrophotometry[37-40], LC-MS/MS [41] and HPTLC[42, 43].

EXPERIMENTAL

Instrument

All absorbance measurements were recorded on Shimadzu 140 double beam spectrophotometer as well as on Thermo Nicolet 100 & Elico double beam SL210 UV- Visible spectrophotometers using matched pair of Quartz cells of 10mm path length.

Materials and reagents

All the reagents used were of analytical-reagent grade and distilled water was used throughout the investigation. NBS solution (0.01%) was prepared by dissolving N-bromosuccinimide (Himedia Laboratories pvt.Ltd, Mumbai) in water with the aid of heat and standardized. The solution was kept in an amber colored bottle and was diluted with distilled water appropriately to get 70 ìg mL^{-1} NBS for use in spectrophotometric method.

A stock solution of Methyl Orange ($5x10^{-4}$ M) 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 50 µg 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 10 mg of pure drug in water and diluting to 100 mL in a calibrated flask with water. The solution was diluted stepwise to get working concentrations.

Assay procedure

Aliquots containing 2-50 µg mL^{-1} of drug were transferred into a series of 10 mL standard flasks using a micro burette. To this, 1 mL of NBS was followed by 1 mL of 1M HCl and contents were shaken well. After 30 minutes, 1 mL of Methyl Orange dye added to the content. Then contents were shaken well and diluted up to the mark. The absorbance of each solution was measured at 508 nm against the corresponding reagent blank.

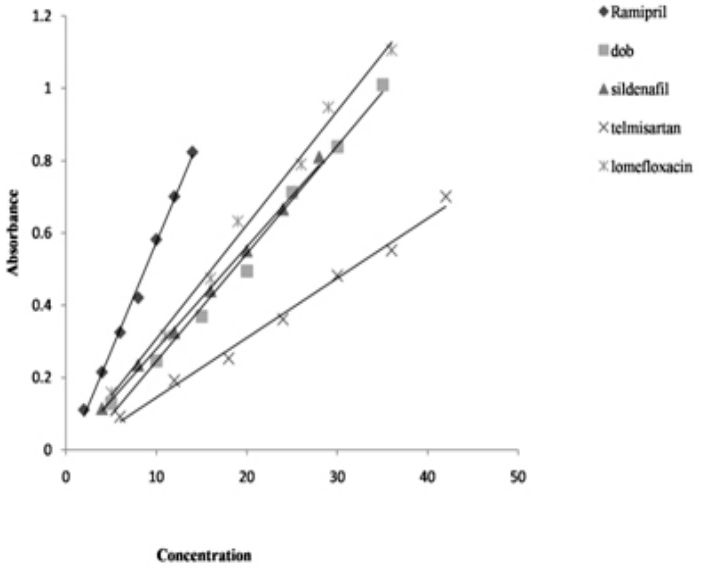

Fig. 2: Calibration curves of the Drugs

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 2].

Procdure foe 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 [Table 1].

Procedure for tablets

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 5µgmL^{-1}. Quantification was performed using 5,8,11,14&17 ìg mL^{-1} of Dobutamine hydrochloride.

Table 1: 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
DOB	5	4.96	0.8	99.20	0.386	99.90±0.38
	8	7.98	0.25	99.75		
	11	11.01	0.09	100.09		
	14	14.02	0.14	100.14		
	17	16.93	0.41	99.59		
LOM	5	5.01	0.20	100.20	0.323	99.96±0.319
	10	9.96	0.40	99.60		
	15	14.92	0.53	99.47		
	20	20.01	0.05	100.05		
	25	25.02	0.08	100.08		
SIL	4	3.98	0.50	99.50	0.353	99.82±0.349
	6	6.01	0.17	100.17		
	10	9.94	0.60	99.40		
	16	16.02	0.13	100.13		
	20	19.95	0.25	99.75		
RAM	2	2.01	0.50	100.50	0.662	99.81±0.71
	4	3.95	1.25	98.75		
	6	6.01	0.17	100.16		
	10	9.96	0.40	99.60		
	12	11.97	0.25	99.75		
TEL	6	5.89	1.80	98.17	0.529	98.91±0.531
	10	9.92	0.80	99.20		
	14	13.91	0.64	99.36		
	18	17.88	0.67	99.33		
	20	19.89	0.55	99.45		

Lomefloxacin Hydrochloride

Ten tablets of LOMEBACT were weighed accurately, and powdered. The powder equivalent to 50 mg was transferred into a 100 mL volumetric flask, containing a mixture of distilled water (10.0 mL) and HCl (2.0 mL). The flask was shaken for 5 min and the solution was filtered using whatmann No: 41 filter paper and further diluted with water to obtain working standard solution.

Sildenafil citrate

Four tablets of ALISIGA containing 25 mg each amounting about 100 mg of SIL was accurately weighed, dissolved in water and diluted to volume 100 mL calibrated flask. This solution was diluted stepwise to give a series of concentrations suitable for the construction of the calibration graph.

Ramipril

To determine the content of Ramipril in pharmaceutical preparations, 20 tablets of Corpil (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.

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 6 µg mL^{-1} of Telmisartan.

RESULTS AND DISCUSSION

Each method developed for quantification of drugs has been validated in terms of precision, accuracy, Limit of detection, Limit of quantification, Linearity, Selectivity and Ruggedness. The Beer's law limits, Slope, Intercept, Correlation coefficient, Sandell's sensitivity and Regression equations for each drug are tabulated in [table 2]. To assess the precision each experiment 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 accuracy and precision of the methods.

Factors effecting absorbance

Effect of acid concentration

To study the effect of acid concentration,

Table 2: Analytical and regression parameters of Spectrophotometric method

Parameter	DOB	LOM	SIL	RAM	TEL
λmax(nm)	508	508	508	508	508
Beer's law limits(µg mL^{-1})	5-35	5-50	4-28	2-14	6-42
Molar absorptivity(L moL^{-1} cm^{-1})	8.5 $x10^4$	1.2$x10^6$	1.9$x10^4$	2.5$x10^4$	5.2$x10^4$
Sandell sensitivity*(µg cm^{-2})	0.035	0.167	0.036	0.013	0.067
Limit of detection(µg mL^{-1})	0.623	1.37	0.885	0.166	0.286
Limit of quantification(µg mL^{-1})	1.931	4.138	2.681	0.503	0.866
Regression equation (Y**=a+bX)	0.031 +0.053X	0.031 +0.006X	0.004 +0.028X	0.069 +0.081X	0.02 +0.016X
Intercept, (a)	0.031	0.031	0.004	0.069	0.02
Slope, (b)	0.053	0.006	0.028	0.081	0.016
Correlation coefficient, (r^2)	0.993	0.993	0.997	0.995	0.991
Standard deviationof intercept (S_a)	0.010	0.002	0.008	0.005	0.0014
Standard deviationof slope (S_b)	0.002	0.001	0.001	0.002	0.00133

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(CARDIJECT)	4.0	1.0	4.96	0.80	99.20	0.366	99.75±0.425	99.80±0.365
	6.0	1.5	7.47	0.13	99.6			
	10.0	0.0	10.01	0.10	100.1			
	15.0	5.0	19.98	0.10	99.9			
	20.0	5.0	25.0	0.00	100			
LOM(LOMEBACT)	5.0	0.5	5.48	0.36	99.64	0.176	99.97± 0.203	100.0±0.176
	9.0	1.0	10.01	0.10	100.1			
	15.0	0.0	15.0	0.0	100			
	20.0	5.0	24.99	0.04	99.96			
	30.0	0.0	30.0	0.0	100			
SIL(ALISIGA)	3.0	1.0	3.96	1.0	99.0	0.412	100.01±0.402	99.61±0.411
	6.0	2.0	7.95	0.63	99.38			
	11.0	1.0	11.98	0.17	99.83			
	20.0	0.00	20.0	0.0	100			
	22.0	2.0	23.96	0.17	99.83			
RAM(CORPIL)	1.0	1.0	1.98	1.0	99.0	0.415	99.95±0.396	100.01±0.414
	3.5	0.5	4.0	0.0	100.0			
	6.0	0.0	6.0	0.0	100			
	9.0	1.0	9.98	0.20	99.8			
	12.5	1.5	13.97	0.21	99.79			
TEL(TELI)	5.0	1.00	5.96	0.67	99.33	0.322	100.0±0.298	99.92±0.322
	11.0	1.00	12.02	0.17	100.17			
	18.0	0.00	17.97	0.17	99.83			
	20.0	5.0	24.98	0.08	99.92			
	25.0	3.0	28.01	0.04	100.04			

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

Pharmaceuticals/ tablets/ injection	DOB	LOM	SIL	RAM	TEL
Student's t-test	0.262 (3.182)	0.246 (3.182)	0.038 (3.182)	0.077 (3.182)	0.134 (3.182)
f-test	0.738 (4.75)	0.752 (4.75)	1.045 (4.75)	1.093 (4.75)	1.168 (4.75)

different types of acids were examined (H_2SO_4, HCl, and $H_3PO_4$and CH_3COOH) to achieve maximum yield of Redox reaction. The results indicated that the hydrochloric acid was the preferable acid with NBS as oxidant. The reaction was performed in a series of 10 mL volumetric flask containing 8.0 ìgmL^{-1} 0f the cited drugs, different volumes (0.5–2.5 mL) of 1M HCl and 1 mL of NBS (0.01%) 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 mL of Methyl Orange 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 1M HCl. Above this volume, the absorbance decreased. Therefore, a volume of 1 mL of 1M HCl 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 ± 2°C for the periods ranging for 5-10 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 NBS on color development was performed, it was observed that in both cases the absorbance increased with increase in the volume of NBS. It reached maximum when 1 mL of 70 µg mL^{-1} NBS 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 70 µg mL^{-1} NBS was used for all measurements.

Effect of dye concentration

In order to ascertain the linear relationship between the volume of added NBS and the decrease in absorbance of Methyl Orange dye, experiments were performed using 1 mL of 1M HCl with varying volumes of NBS. The decrease in absorbance was found to be linear up to the 1 mL of NBS with optimum volume 1.0 mL of Methyl Orange dye for fixed concentration drug solution. The color was found to be stable up to 24 hours.

Application to formulations

The proposed methods were 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 recipients in the dosage forms do not interfere.

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 at the 95 % confidence level with respect to accuracy and precision [Table 4].

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, 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 was within the permissible limits showing the absence of interference by the inactive ingredients in the assay.

CONCLUSION

This is simple, rapid, and cost-effective methods for the determination of drugs have been developed and validated. The proposed method is more sensitive and the methods depends 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.

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