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SPECTROPHOTOMETRIC ESTIMATION OF DRUGS USING N-BROMO SUCCINAMIDE AND AMARNTH DYE COUPLE

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ABSTRACT

Simple, specific, accurate and precise UV–visible spectrophotometric methods have been developed for the estimation of five drugs *viz.*, Dobutamine hydrochloride, Duloxetine hydrochloride, Ondansetron hydrochloride, Sumatriptan succinate and Verapamil hydrochloride. These methods involve the addition of a known excess of NBS to the drugs in acid medium followed by estimation of residual NBS by reacting with a fixed amount of Amaranth and measuring the absorbance at 520nm. The proposed methods were found to be successful for the estimation of these drugs in bulk and their formulations. The results of analysis have been validated statistically for linearity, accuracy, precision, LOD and LOQ.

Keywords: UV-visible spectrophotometry, Validation, Amaranth, Dobutamine HCl, Duloxetine HCl, Ondansetron HCl, Sumatriptan succinate, Verapamil HCl.

INTRODUCTION

Dobutamine hydrochloride (DOB) [Fig.1a] is a sympathomimetic with direct effects on β_1-adrenergic receptors, giving it a prominent inotropic action on the heart. It also has some alpha and β_2 agonist properties. DOB is chemically known as 4-(2-{[3-(p-hydroxyphenyl)-1methylpropyl] amino} ethyl pyrocatechol hydrochloride. DOB is used to increase the contractility of the heart in acute heart failure, as occurs in cordiogenic shock and myocardial infarction. It is also used in septic shock. Literature survey revealed that several analytical methods have been reported for determination of Dobutamine hydrochloride in pure drug, pharmaceutical dosage forms and in biological samples using spectrophotometry [1-2], gas chromatography [3], liquid chromatography [4] and absorptive stripping voltametry [5].

Duloxetine hydrochloride (DUL) [Fig.1b] is a selective serotonin and norepinephrine reuptake inhibitor (SSNRI) for oral administration. DUL is an anti-depressant and is chemically (+)-(S)-N-methyl-3-(naphthalene -1-yloxy)-3-(thiophene-2-yl)propan-1-amine hydrochloride. The antidepressant and pain inhibitory actions of DUL are believed to be related to its potentiation of seratonergic and noradrenergic activity in the CNS. A literature survey indicated few methods for the determination of DUL *viz*., HPLC [6-8], direct UV spectrophotometry [9,10], Extractive spectrophotometry [11], Visible spectrophotometry [12] and UPLC [13].

Ondansetron hydrochloride dehydrate (OND) [Fig.1c] is used as a selective serotonin 5-HT_3 receptor antagonist, is well established in patients with nausea and vomiting associated with cancer chemotherapy, radiotherapy or anesthesia and surgery. Preliminary data have shown OND to have clinical benefit in patients with nausea and vomiting associated with drug over dosage or poisoning, anti-infective or antidepressant therapies, neurological trauma. A number of spectrophotometric methods [14,15] and HPLC [16,17] are available in the literature for the determination of Ondansetron in pharmaceutical formulations.

Sumatriptan succinate (SUM) [Fig.1d] is most frequently prescribed anti-migraine drug of triptan class. It is chemically known as 3-[2-(Dimethylamino) ethyl]-N-methyl-1H indole-5-methane sulphonamide succinate (1:1) base. SUM is a specific and selective 5-hydroxy tryptamine receptor [$5HT_{1D}$] agonist with no effect on the other 5HT receptor [$5HT_2$-$5HT_7$] sub types.

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It is widely used for acute relief of migraine attack. SUM undergoes an extensive biotransformation mainly through Mono amino oxidase-A. Several analytical techniques like spectrophotometric methods [18,19], Extractive spectrophotometry [20,21], HPLC [22,23] and voltametry [24] have been reported for SUM in combination with other drugs.

Verapamil hydrochloride (VEH) [Fig.1e] i.e 5-[N-(3,4-dimethoxy-phenethyl)-N-methyl-amino]-2-(3,4 dimethoxyphenyl)-2-isopropylvaleronitrile hydrochloride or Isoptin is clinically a very useful member of the calcium channel blocker. It is used in the treatment of supraventricular arrhythmias, angina and hypertension. The different analytical methods that are reported for its determination include HPLC [25], potentiometry [26], conductometry [27], liquid chromatography- gas chromatography [28]' atomic emission spectrophotometry [29], adsorptive stripping voltametry [30].

A comparision of various techniques used for estimation of above drugs in terms of sensitivity and reproducibility are presented in Table-1.

Thorough survey of literature revealed that simple spectrophotometric methods are not yet reported for the above drugs.In this communication we present simple, accurate, precise methods for the quantification of above drugs.

MATERIALS AND METHODS

The pharmaceutical grade drugs were supplied by Arabindo Pharmaceuticals and Heterodrugs Pvt. Ltd, Hyderabad. Amaranth, HCl were purchased from S.d fine chem. Pvt. Ltd, Mumbai, India. N-bromosuccinamide (NBS) is purchased from SRL chemicals, Mumbai, India. Whatman filter paper no.42 was used for filtration purpose. All the reagents used were of analytical-reagent grade and distilled water was used throughout the investigation. Tablets were purchased from the local market.

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. A high precision Analytical balance was used for weighing the reagents.

Preparation of Standard stock solutions:

N-bromosuccinamide (NBS): An approximately 0.01M solution was prepared by dissolving about 0.1779 g of NBS in 100 ml distilled water. It is diluted appropriately to get 124µg ml^{-1}.

Amaranth [$0.8x10^{-3}$M]: stock solution was prepared by dissolving 0.0484g of Amaranth in 100 ml distilled water. From this stock solution, 353µg /ml test solution was prepared.

Drugs: A standard solutions of drugs were prepared by dissolving accurately weighed 10 mg of pure drug in water and diluted to the mark in 100 ml calibrated volumetric flasks. The stock solutions of DOB, DUL, OND, SUM and VEH were diluted with water to obtain 3.5µg/ml, 3µg/ml,4µg/ml,8µg/ml and 16µg/ml respectively. Conc. Hydrochloric acid (HCl): conc HCl is diluted appropriately with distilled water to get 1 M HCl solution.

Construction of calibration curve:

Aliquots of pure drug solution (1.0-7.0ml) were transferred into a series of 10ml calibrated flasks. To each flask 1ml of 1M HCl acid was added followed by 1.0ml of NBS solution. The flasks are stoppered and contents were mixed and the flasks are set aside for 15 min under occasional shaking. Finally, 1.0 ml of Amaranth solution was added to each flask and the volume was adjusted to the mark with water and mixed well. The absorbance of each solution was measured at 520 nm after 5 min.

A standard graph was prepared by plotting the absorbance versus the concentration of drugs. The concentration of the unknown were read from the calibration graph or computed from the regression equation derived using Beer's law data. Calibration curve for each drug was drawn in (Fig.2).

Analysis of commercial Dosage forms:

A quantity of finely ground tablets powder equivalent to 10 mg of drug DOB(Candiforce-250), DUL (Ulozet-40), OND (Ondem-4), SUM (Sumitrex-25 mg), VEH (Calaptin-40) were accurately weighed and taken in 60 ml distilled water in 100 ml volumetric flask and left for 10 min for complete dispersion and then filtered through Whatman No.42 filter paper.First 10 ml portion of the filtrate was rejected and a convenient aliquot of filtrate was further diluted for the analysis within the limits of Beer's law.

Drug + known excess of NBS—---------»Reaction product of the drug + Unreacted NBS

Unreacted NBS+ Fixed amount of Amaranth----------» Absorbance measured at 520nm.

RESULTS AND DISCUSSION

N-bromosuccinamide (NBS) has been used widely as a brominating and oxidizing agent for organic compounds. The proposed methods are indirect and are based on the oxidation and bromination reaction between drug and NBS and determination of residual NBS after allowing the reaction between drug and measured amount of NBS to be complete. The amount of NBS reacted corresponds to the drug content in all the methods.

The calibration curves for DOB, DUL, OND, SUM and VEH, over a concentration range of 0.35-

2.45 µg/ml, 0.3-2.1µg/ml, 0.4-2.8µg/ml, 0.8-4.82 µg/ml, 1.6-11.2µg/ml and respectively, were plotted and molar absorptivity for drugs were calculated at the wavelength of 520nm. The regression characteristics were reported in Table-2.The result of assay is reported in Table-3.The accuracy of the proposed method was evaluated by percentage recovery studies of the drugs. The %RSD was less than 2% , showing high degree of precision of the proposed method. The results of the method lie within the prescribed limit, showing that method is free from interference from excipients.

Method development:

Many dyes are irreversibly destroyed to colorless species by oxidizing agents in acidic medium. The proposed spectroscopic methods are based on the reaction between the drug and measured excess of NBS and subsequent determination of the latter by reacting it with a fixed amount of amaranth and measuring the absorbance at 520 nm. These methods make use of the bleaching action of NBS on the dyes, the decolourization being caused by the oxidative destruction of the dyes. The drug when added in increasing concentrations to a fixed concentration of NBS consumes the later and there will be a concomitant decrease in the concentration of NBS. When a fixed concentration of amaranth 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 performed to fix the upper concentrations of the dyes that could be determined spectrophotometrically in acidic medium and these were found to be 35.3 µg/ml. A NBS concentration of 12.4 µg/ml was found to irreversibly destroy the red colour of 35.3 µg/ml amaranth in acid medium. Hydrochloric acid was found to be a convenient medium for these methods. However, since 1 M acid concentration was found optimum for the oxidation and bromination reaction in a reasonable time of 15 min. The same concentration was maintained for the determination of unreacted NBS with dye, and even this reaction time is not critical. Any delay up to 30 min had no effect on the absorbance. A contact time of 5 min is necessary for the complete destruction of dye by the residual NBS.

Method validation:

The proposed methods were validated according to guidelines of International Conference on Harmonization (ICH). Under the described experimental conditions, standard experimental conditions, standard calibration curves for the studied drugs were constructed by plotting absorbance versus concentration. Conformity with Beer's law was evident in the concentration range cited in Table-2.The linear regression equations, molar absorptivity, Sandell's sensitivity, limits of detection (LOD) and limits of quantification (LOQ) were listed in the same Table. Standard deviation, relative standard deviation, variance and standard error were calculated.

The accuracy of the method was established by analyzing the pure drug at three levels (within working limits) and the precision was ascertained by calculating the relative standard deviation of six replicate determinations on the same solution containing the drug at three levels in Table-3. The analytical results for accuracy and precision showed that the proposed methods have good repeatability and reproducibility.

The percentage recoveries of the pure drugs using the proposed methods compared with that given by reference methods are illustrated in Table-4. The validity of the proposed method in literature is evaluated by statistical analysis between the results obtained and that of reference methods. student's t-test and variance ratio F-test are chosen for the comparison of the results. Values are within the permissible range reported in literature

Table 1.Comparisionof various techniques

Drug	Method	Sensitivity	Recovery
DOB	1)Liquid chromatography	10-30µg/ml	99.54-100.6%
	2)RPHPLC	10^{-3}-0.5µg/ml	99.2%
DUL	1)Spectrofluorimetry	0.02-0.400µg/ml	98.71-99.17%
	2)Visible spectrophotometry	10-50 µg/ml	98.79%
	a)oxidation of DUL with brucine ($NaIO_3$)	4-20 µg/ml	99.17.%
	b)C.T absorption	1-25 µg/ml	99.8-101.3%
	3)RPHPLC		
	4)Extractive spectrophotometry		
	a)BTB		
	b)BPB	2.5-25 µg/ml	99.8-100.2%
	c)BCG	2.5-25 µg/ml	99.3%
	5)Direct UV Spectrophotometry	3.0-25 µg/ml	
		5-25 µg/ml	
OND	1)RPHPLC	10-50 µg/ml	99.96-100.36%
	2)UV spectrophotometry	5-25 µg/ml	99%

SUM	1)HPLC 2)HPTLC 3)UV spectrophotometry 4)Ion association method 5)Visible spectrophotometry	10-100 µg/ml 100-1000 µg/ml 0.5-3.5 µg/ml 2-10 µg/ml 4-20 µg/ml	99.79% 99.94-100.3% 99.30% 99.304%
VH	1)Conductometry 2)Potentiometry 3)Voltametry 4)Atomic emission spectrophotometry	4190 µg/ml 4.19µg/ml 0.0049-4.19µg/ml 1.96-62.86 µg/ml	97.1-102.8% 100.3%

Table 2.Analytical and regression parameters of spectrophotometric methods

Parameters	**DOB**	**DUL**	**OND**	**SUM**	**VEH**
λmax nm	520	520	520	520	520
Beer's law limit(µg/ml)	0.35-2.45	0.3-2.1	0.4-2.8	0.8-5.6	1.6-11.2
Molar absorptivity(L/mol/cm)	$24.92x10^3$	$2.281x10^3$	$32.84x10^3$	$6.407x10^3$	$5.842x10^3$
Sandell sensivity*(µg/cm^2)	0.00763	0.00735	0.00793	0.0053	0.0066
Limit of detection(µg/ml)	1.839	18.27	7.381	16.01	15.852
Limit of quantification(µg/ml)	5.714	55.517	22.36	48.52	48.62
Regression equation**					
Intercept(a)	0.140	0.005	0.252	0.054	0.060
Slope(b)	0.375	0.455	0.081	0.187	0.379
Correlation coefficient(r)	0.991	0.996	0.995	0.991	0.994
Standard deviation of intercept(S_a)	0.016	0.0322	0.0085	0.0330	0.0446
Variance(S_a^2)	$2.56x10^{-4}$	$1.03x10^{-3}$	$7.22x10^{-5}$	$1.089x10^{-3}$	$1.98x10^{-3}$
Standard deviation of slope(S_b)	0.0007	0.0018	0.00408	0.0068	0.0102

*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. Determination of accuracy and precision of the methods on pure drug samples

Drug	**Taken (µg/ml)**	**Found (µg/ml)**	**% error**	**% Recovery**	**% RSD**	**Proposed method mean±S.D**
DOB	2.0 4.0 6.0	2.01 4.04 5.9	0.5 1.0 1.6	100.5 101 98.3	1.439	99.33±1.43
DUL	1.0 4.0 7.0	1.02 3.98 7.01	2.0 0.5 0.14	102 99.5 101	1.248	100.8±1.25
OND	1.5 4.0 5.5	1.49 4.02 5.48	0.66 0.5 0.36	99.33 100.5 99.63	0.608	99.82±0.607
SUM	3.0 5.0 6.5	3.01 4.9 6.6	0.33 2.0 1.53	100.3 98 101.5	1.778	99.93±1.779
VEH	1.5 3.0 4.5	1.49 3.02 4.49	0.66 0.66 0.22	99.33 100.6 99.77	0.644	99.9±0.644

*Three replicate determinations

Table 4. Results of assay of tablets by the proposed methods and statistical evaluation and recovery experiments by standard addition method

Tablet	Drug taken (µg/ml)	Drug found (µg/ml)	Error (%)	Recovery (%)	RSD (%)	Reference Method mean±SD	Proposed Method mean±SD	t-test	F-test
Candiforce-250 (DOB)	0.35 0.5 0.7	0.351 0.499 0.71	0.28 0.2 1.42	100.2 99.8 101.4	0.828	99.84±0.91	100.4±0.832	0.018 (3.18)	0.835 (5.28)
Ulozet-40 (DUL)	0.3 0.6 0.9	0.295 0.61 0.89	1.66 1.66 1.11	98.33 101.6 98.8	1.78	98.79±1.29	99.06±1.77	0.167 (2.47)	1.885 (5.31)
Ondem-4 (OND)	0.4 0.6 1.2	0.41 0.61 1.19	2.5 1.66 0.83	102.5 101.6 99.1	1.72	99.4±0.763	101.8±1.76	0.881 (3.18)	5.3 (5.28)
Sumitrex-25 (SUM)	1.6 3.2 4.8	1.61 3.19 4.78	0.625 0.31 0.41	100.6 99.68 99.58	0.562	99.3±0.314	99.95±0.562	0.508 (3.18)	3.2 (5.28)
Calaptin-40 (VEH)	0.8 1.2 2.4	0.81 1.19 2.42	1.25 0.83 0.83	101.25 99.16 100.8	1.09	80.5±2.34	100.4±1.100	1.031 (2.47)	0.22 (5.31)

Fig 1. Structure of drugs

Fig1a: Dobutamine hydrochloride

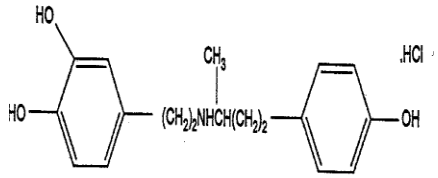


Fig.1b: Duloxetine hydrochloride

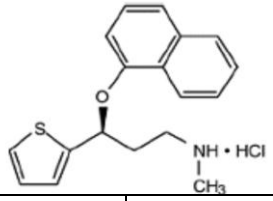


Fig.1c: Ondosetron hydrochloride dehydrate

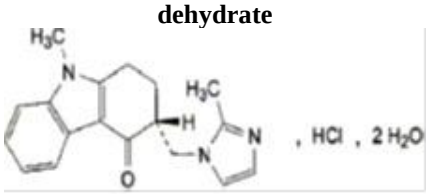


Fig.1d: Sumatriptan succinate

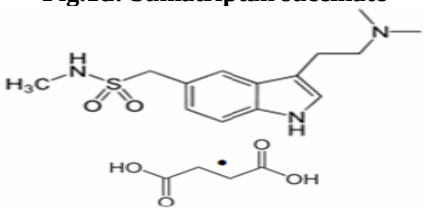


Fig.1e:Verapamil hydrochloride

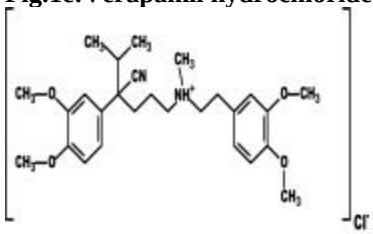
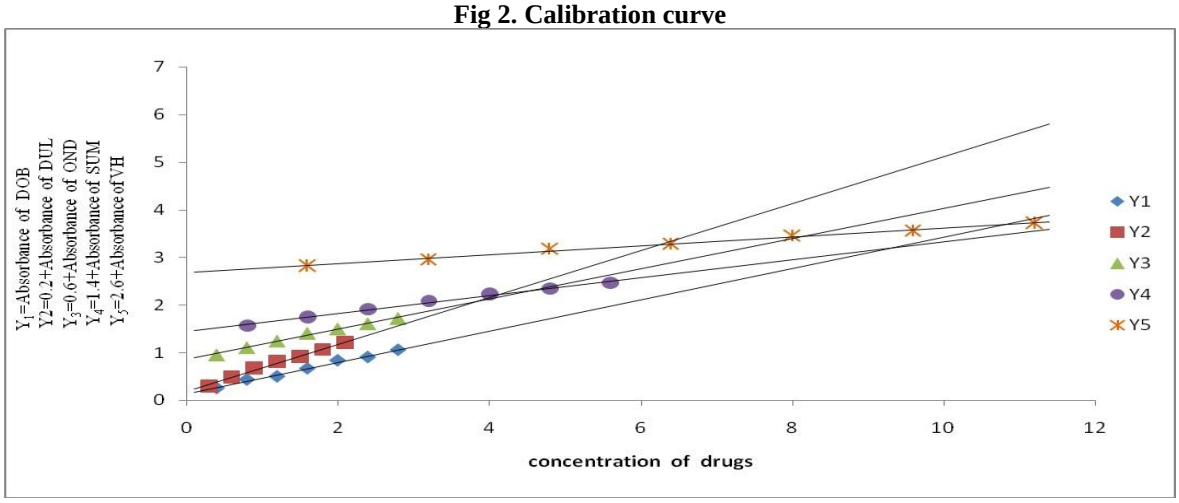


Fig 2. Calibration curve



CONCLUSION

The obtained results from the method for the determination of mentioned drugs indicates that method is simple, accurate and precise. The method is economical compared to other sophisticated analytical instruments. Hence can be used for routine analysis of commercially available formulations. The method is suitable for the determination of these drugs in tablet formulation without interference from commonly used excipients. The solvents used for the method are inexpensive and simple to prepare, and could be used in a quality control laboratory for routine drug analysis.

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