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Spectrophotometric Determination Of Drugs Using N-Bromosuccinimide And Rhodamine-B Dye

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Abstract: Simple, sensitive and selective methods are developed for the spectrophotometric determination of drugs, *viz.*, Moxifloxacin, Gemifloxacin, Sumatriptan succinate, Phenylephrine hydrochloride and Duloxetine hydrochloride, based on their reactivity towards N- bromosuccinimide (NBS). The method involves the addition of excess NBS of known concentration in the presence of 1M HCl, reactants are allowed to react and the unreacted NBS is estimated by the measurement in the decrease in the absorbance of the Rhodamine-B dye (λ_{max} 557nm). 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: Spectrophotometry, Drugs, NBS, Rhodamine-B, Quantification, Validation.

INTRODUCTION

Moxifloxacin (MOX) [1-Cyclopropyl-b-fluoro-1, 4-dihydro-8-methoxy-7-[(4aS, 7aS)-octahydro-6H-pyrrolo [3, 4–6] pyridine-6-yl]-4-oxo-3-quinoline carboxylic acid] [1] is a broad-spectrum antibiotic that is active against both Gram-positive and Gram-negative bacteria [2]. The bactericidal activity of the drug is mediated by the inhibition of DNA gyrase (topoisomerase II) and topoisomerase IV, essential enzymes involved in bacterial DNA replication, transcription, repair and recombination. Moxifloxacin is prescribed for the bacterial infections of the respiratory tract including sinusitis, community acquired pneumonia and acute exacerbations of chronic bronchitis [3]. Its recent quantification method is reported [4] wherein other methods of ouantification have been mentioned.

Gemifloxacin (GEM) (chemically R,S-7-(3 amino methyl 4- syn methoxyimino-pyrrolidinyl)-1cyclopropyl-6-flouro1,4,dihydro 4- oxo-1,8 napthyridine-3-carboxylic acid methane- sulphonate [5-7] is a new flouroquinolone antibacterial compound with enhanced affinity for bacterial topoisomerase-IV and is being used for the treatment of respiratory and urinary tract infections, light brown powder, freely soluble in water and slightly soluble in Methanol. A recent method of quantification is published in International Journal of PharmTech Research [8] wherein the authors presented earlier studies on assay of the drug.

Sumatriptan succinate (SUM) is the most frequently prescribed anti-migraine drug of triptan class. It is chemicallyknown as 3-[2-(Dimethylamino) ethyl] –N-methyl-1H indole-5- methane sulphonamide succinate (1:1) base [9]. SUM is a specific and selective 5- hydroxyl tryptamine receptor (5-HT1D) agonist with no effect on the other 5HT receptor (5HT2-5 HT7) sub types. It is used widely for prophylaxisand acute relief of migraine attack with or without aura. Recent determination method [10] is preceded by many methods cited therein.

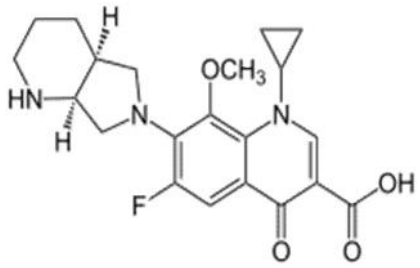
Phenylephrine hydrochloride (PHE), chemically (R)-1-(3- hydroxyphenyl)-2-thylaminoethanol hydrochloride [11] is a direct sympathomimetic agent, a selective α1 agonist, causing vasoconstriction. It is also a frequent constituent of orally administered nasal decongestant preparations. It is recently quantified by UV spectrophotometric method [12].

The report includes past quantification references on the drug.

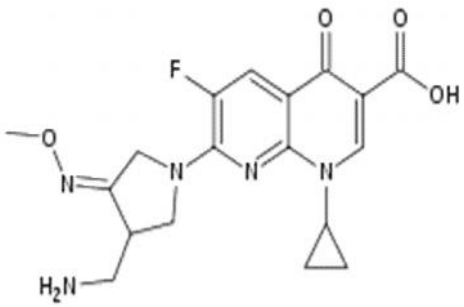
Duloxetine hydrochloride (DUL) – (3S)-N-methyl-3-(naphthalen-1-yloxy)-3-(thiophen-2-yl) propan-1-amine hydrochloride [13]. It is a potent inhibitor of serotonin and nor epinephrine reuptake and thus it is used for major depressive disorders. Furthermore, it provides evidence of an effect on pain in the case of urinary incontinence [14, 15] independent of its effect on depression. Therefore, DUL is an alternative to current therapeutic options in the treatment of the different symptoms of depression [16].A recent method [17] in which various earlier methods of quantification have been reviewed.

(Scheme-1)

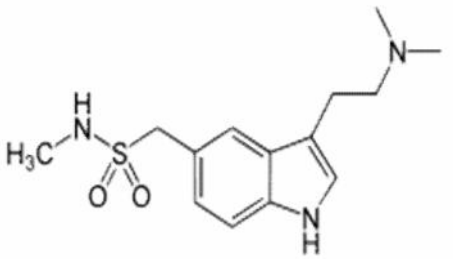
Structures of drugs



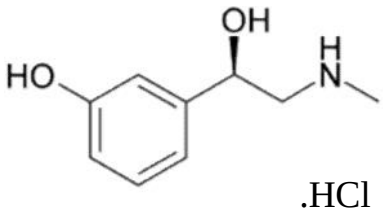
Moxifloxacin



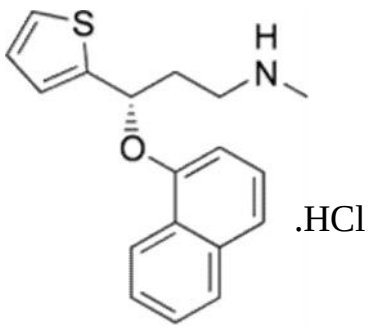
Gemifloxacin.



Sumatriptan succinate



Phenylephrine hydrochloride



Duloxetine 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.

EXPERIMENTAL

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 AND REAGENTS

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

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

A stock solution of Rhodamine B (500 µg 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 50 µg mL^{-1}.

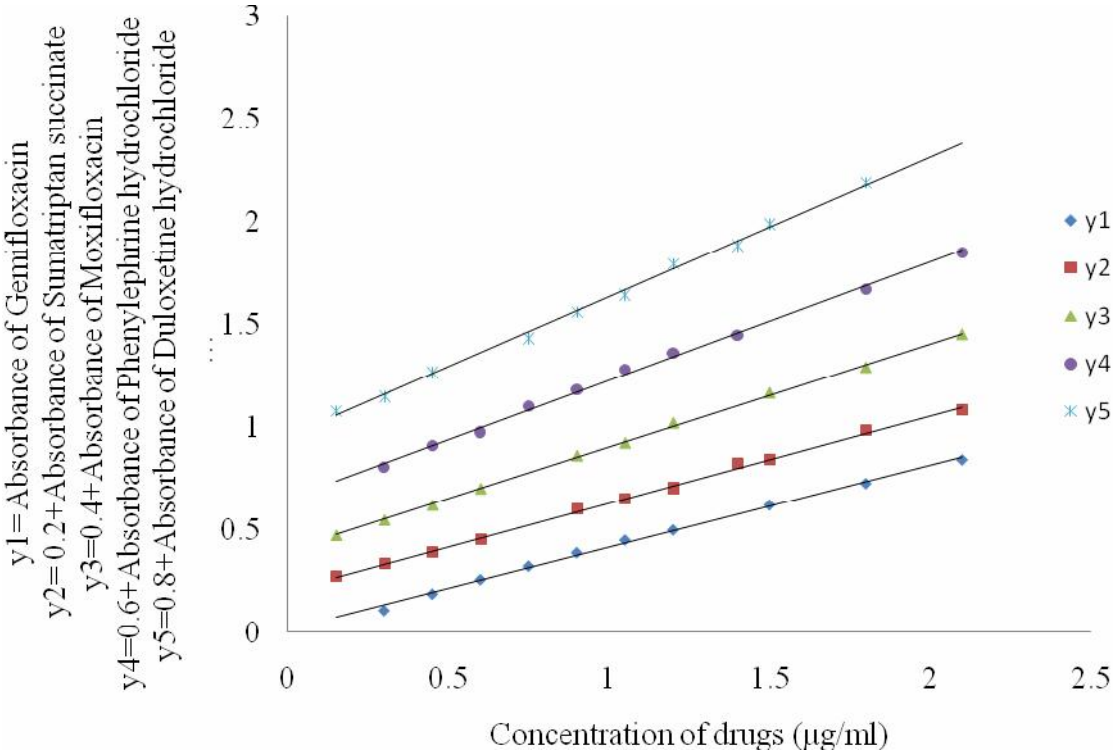
Hydrochloric acid (1 *M*): Concentrated hydrochloric acid (S.D. Fine Chem., Mumbai, India; sp. gr. 1.18) was diluted appropriately with water to get 1 *M* acid.

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 of 3 µg mL^{-1}.

ASSAY PROCEDURE

Aliquots of pure drug solution (0.5 to 7 mL, 3 µg mL^{-1}) were transferred into a series of 10-mL calibrated flask and the total volume was adjusted to 5 mL with water. To each flask, 1 mL of 1 mol L^{-1} hydrochloric acid was added, followed by 1 mL of NBS solution (70 µg mL^{1}). The contents were mixed and the flasks were set aside for 10 min under occasional shaking. Finally, 1 mL of 50 µg 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)

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.

PROCEDURE FOR TABLETS

1. MOXIFLOXACIN

For the analysis of pharmaceutical formulations three tablets (Crosmox – 400mg) were weighed and grounded. A quantity equivalent to 10mg of Moxifloxacin was transferred into a 100 mL 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 10 mL portion of the filtrate was discarded and a suitable aliquot of the subsequent portion was diluted appropriately to get 3 µg mL^{-1} and the assay was completed according to the procedure described above.

2. GEMIFLOXACIN

Five tablets (AdGem, 320 mg) were weighed and grounded. The powder equivalent to10mg gemifloxacin 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 100 mL of distilled water and it was further diluted to get required concentration for the analysis of the drug.

3. SUMATRIPTAN SUCCINATE

Ten tablets (Sumitrex, 25mg) were grounded and the powder equivalent to10 mg of sumatriptan succinate was weighed, dispersed in 25 mL 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 100 mL of distilled water .It was used as stock sample solution and was further diluted with the same solvent to get working standard solution..

4. PHENYLEPHRINE HYDROCHLORIDE

Ten tablets (Dolgencorp,10mg) were weigheted powered and equivalent to about 10 mg of phenylephrine hydrochloride was transferred to 100 ml volumetric flask; 60.0 ml 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 so as to obtain final concentration of drug and the resulting solution was used for the analysis.

5. DULOXETINE HYDROCHLORIDE

About ten to fifteen tablets (Ulozet, 40 mg) were powdered and equivalent to about 10 mg of Duloxetine hydrochloride had been taken in to a100 ml of volumetric flask and added about 30 ml 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 100 mL 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.

RESULTS AND DISCUSSION

METHOD DEVELOPMENT

The proposed spectrophotometric method is indirect and is based on the determination of

The surplus NBS after allowing the reaction between drug and a measured amount of NBS to be complete. The residual NBS was determined by reacting it with a fixed amount of Rhodamine-B dye. The method makes 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 amount of dye is added to decreasing concentrations of NBS, a proportional increase in the concentration of dye leading to a linear increase in the absorbance at the respective λmax (557nm) 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 5 µg 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 7 µg mL^{-1} NBS in this method 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 to15 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 drug 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.

Table 1 Analytical and regression parameters of spectrophotometric method

Parameter	MOX	GEM	SUM	PHE	DUL
λmax, nm	557	557	557	557	557
Beer's law limits µg mL^{-1}	0.15-2.1	0.3-2.1	0.15-2.1	0.15-2.1	0.15-1.8
Molar absorptivity, L mol^{-1} cm^{-1}	1.95×10^5	1.42×10^5	1.31×10^5	1.14×10^5	3.93×10^5
Sandell sensitivity* µg cm^{-2}	0019	0.0025	0.0023	0.0017	0.0014
Limit of detection µg mL^{-1}	0.0276	0.0447	0.3813	0.0691	0.1941
Limit of quantification µg mL^{-1}	0.0836	0.1356	1.1556	0.2094	0.5882
Regression equation, Y**					
Intercept, (a)	-0.001	0.016	0.005	0.052	0.153
Slope, (b)	0.502	0.398	0.424	0.573	0.680
Correlation coefficient, (r)	0.999	0.996	0.998	0.997	0.997
Standard deviation of intercept (Sa)	0.0042	0.0054	0.049	0.012	0.040
Standard deviation of slope (Sb)	0.0184	0.0205	0.0417	0.0246	0.0385

*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 cm2 and path length of 1 cm. Y** = a+bX, where Y is the absorbance and X concentration of drugs in µg per mL

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. Table2 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 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 excipients in the dosage forms do not interfere. The results are compared to the available validated reported [12,22-25] 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 at the 95 % confidence level 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, 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 (Table 3) was within the permissible limits showing the absence of interference by the inactive ingredients in the assay.

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
MOX	1.2 2.0 3.0	1.18 2.0 3.02	1.66 0.00 0.66	98.33 100.00 100.66	1.200	99.66 ±1.204
GEM	1.0 3.0 5.0	1.0 2.98 5.06	0.00 0.66 1.20	100.00 99.33 101.20	0.945	100.17 ±0.947
SUM	2.0 4.0 6.0	2.01 3.9 6.02	0.50 2.50 2.00	100.50 97.50 100.33	1.382	99.44 ±1.375
PHE	2.5 3.5 4.5	2.49 3.5 4.44	0.40 0.00 1.33	99.60 100.00 98.66	0.691	99.42 ±0.687
DUL	3.5 4.0 5.0	3.5 4.11 5.12	0.00 2.70 2.40	100.00 102.75 102.40	1.470	101.71 ±1.496

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 (%)	Recovey (%)	RSD (%)	Reference method Mean **± SD**	Propose method **± SD**	t-test	F-test
Crosmox (MOX)	0.50 0.50 0.50 1.2 2.0 3.0	1.0 2.0 3.0 0.0 0.0 0.0	1.49 2.51 3.49 1.18 2.0 3.02	0.66 0.40 0.28 1.66 0.00 0.66	99.33 100.4 99.71 98.33 100.00 100.66	0.839	98.03 ±0.970	99.73 ±0.837	0.28 (3.182)	0.60 (4.75)
Adgem (GEM)	0.50 0.50 0.50 1.0 3.0 5.0	0.2 0.4 0.6 0.0 0.0 0.0	0.71 0.89 1.09 1.0 2.98 5.01	1.42 1.11 0.90 0.00 0.66 0.20	100.42 98.88 99.09 100.00 99.33 100.2	0.638	101.9 ± 0.34	99.65 ±0.636	0.95 (3.182)	3.43 (4.75)
Sumitrex (SUM)	0.50 0.50 0.50 2.0 4.0 6.0	0.3 0.6 0.9 0.0 0.0 0.0	0.80 1.11 1.41 2.01 3.99 6.03	0.00 0.90 0.71 0.50 0.25 0.50	100.00 100.90 100.71 100.50 99.75 100.50	0.433	99.473 ± 0.339	100.39 ±0.435	0.31 (3.182)	1.64 (4.75)
Dolgen-corp (PHE)	0.50 0.50 0.50 2.5 3.5 4.5	0.4 0.8 1.2 0.0 0.0 0.0	0.89 1.29 1.72 2.49 3.5 4.44	1.11 0.76 1.17 0.40 0.00 1.33	98.88 99.23 101.17 99.60 100.00 98.66	0.915	100.94 ±1.042	99.59 ±0.912	0.23 (2.447)	0.76 (4.28)
Ulozet (DUL)	0.50 0.50 0.50 3.5 4.0 5.0	0.5 1.0 1.5 0.0 0.0 0.0	1.01 1.51 1.99 3.5 4.11 5.12	1.00 0.66 0.50 0.00 2.70 2.40	101.00 100.66 99.50 100.00 102.75 102.40	1.279	99.75 ±2.056	101.05 ±1.293	0.59 (3.182)	0.39 (4.75)

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 table**ts.**

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