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OXIDATIVE SPECTROPHOTOMETRIC DETERMINATION OF DRUGS IN PHARMACEUTICAL FORMULATION USING N-BROMOSUCCINAMIDE AS AN OXIDANT AND SAFRANIN-O DYE.

Munnuru Pandurangam and A. Panasareddy*

Department of Chemistry, University College of Science, Osmania University, Hyderabad-500007, India.

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***Corresponding Author**
A. Panasareddy
Department of Chemistry, University College of Science, Osmania University, Hyderabad-500007, India.

ABSTRACT

A simple, sensitive and selective method has been developed for the spectrophotometric determination of drugs, viz., Moxifloxacin, Ramipril, Samaritan, Trimetazidine and Lacosamide. The proposed method involve the addition of excess NBS of known concentration in the presence of acidic medium and the unreacted NBS is determined by the measurement of the decrease in the absorbance of the dye safranin- o blue (λmax 532 nm). The colored species in acidic medium, reactants are allowed to react and the unreacted NBS is estimated by the measurement in the decrease in the absorbance of the safranin-o dye (λ_{max} 532 nm). This method has been validated in terms of guidelines of ICH and applied to the quantification of selected drugs in bulk and dosage forms.

KEYWORDS: Spectrophotometry, Drugs, NBS, Safranin-o, Quantification, Validation.

INTRODUCTION

Moxifloxacin [MOX]

Moxifloxacin (1-cyclopropyl-7-(2, 8- diazobicyclo [4.3.0] nonane)-6-fluoro-8- methoxy-1, 4-dihydro-4- oxo-3-quinoline carboxylic acid) (Figure 1) is a fourth generation fluoroquinolone. The determination of MOX is not yet described in any pharmacopoeias. Therefore, a simple[1,2], accurate method is required for their determination in pharmaceutical formulations. A survey of literature revealed that MOX has been determined in biological fluids or pharmaceutical products by HPLC[3] and potentiometric method.[4]

Ramipril (RAM)

Ramiprilis (2S, 3aS, 6aS)-1-[(S)-2-[[(S)-1- (ethoxycarbonyl)-3-phenylpropyl] amino] propanoyl] octahydrocyclopenta[b]pyrrole-2- carboxylic acid. It is an angiotensin converting enzyme (ACE) inhibitor. ACE inhibitors are used to treat hypertension and congestive heart failure. They act by lowering the production of angiotensin II, thereby relaxing arterial muscles while at the same time enlarging the arteries, allowing the heart to pump blood more easily, and increasing blood flow due to more blood being pumped into and through larger passage ways.[5] The British Pharmacopoeia recommends a liquid chromatographic (LC)[6] method for its determination in raw material and in dosage forms.[7]

Sumatriptan Succinate [SUM]

Sumatriptan succinate is a selective 5-hydroxytryptamine1 receptor subtype agonist is chemically designated as 3-[2-(dimethyl amino) ethyl]-N-methyl-indole-5-methane sulfonamide succinate (1:1). This drug used in the treatment of migraine attacks. Literature survey reveals many methods for estimation of SUM[8,9] and very few methods are available for simultaneous determination by UV[10,11], HPTLC[12] and HPLC.[13,15]

In this communication, a new simple, rapid and precise HPLC method has been reported for simultaneous determination of SUM which can be used for its routine analysis in normal laboratories.

Trimetazidine [TDH]

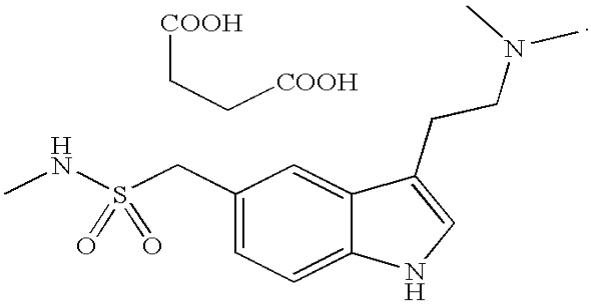
Tramadol Hydrochloride is chemically known as Tramadol [(±) Trans - 2 - (dimethylaminomethyl) – 1 - (3-methoxy- phenyl)-cyclohexanol hydrochloride]. It is a centrally acting opoid analgesic. [1] Tramadol and its metabolite (+)-Odes methyl-tramadol (M1) are weak agonists of the μ opioid receptor. (+)-Tramadol inhibits serotonin reuptake and (–)-tramadol inhibits norepinephrine reuptake.

Various methods have been reported for the determination of tramadol in bulk, pharmaceutical preparations, biological fluids and hair including spectrophotometry[16,19], high performance liquid chromatography (HPLC)[20,22], potentiometry[23] and chromatography.[24]

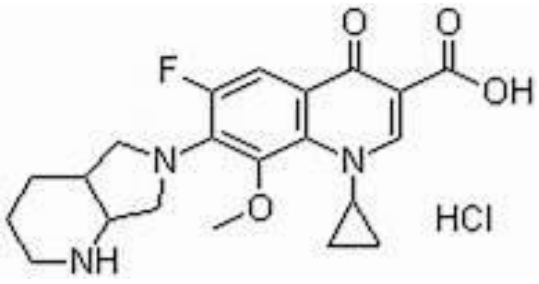
Lacosamide [LAC]

Lacosamide is chemically (R)-2-acetamido-N-benzyl-3methoxy propionamide used to treat partial-onset seizures in people with epilepsy who are at least 17 years old. It selectively enhances slow inactivation of voltage-gated sodium channels and modulates collapsin response mediator protein-2 (CRMP-2) involved in neuronal differentiation and control of axon outgrowth.[1] The literature survey revealed that there were some analytical methods reported for lacosamide like HPLC.[25,27] The review prompted us to develop a simple, precise and economic HPTLC[28] method for the estimation of Lacosamide in bulk and in tablet dosage form.

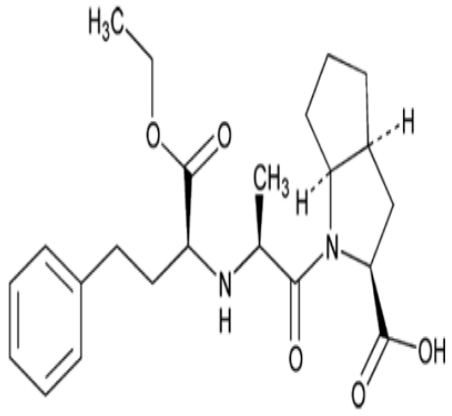
Structures of the drugs



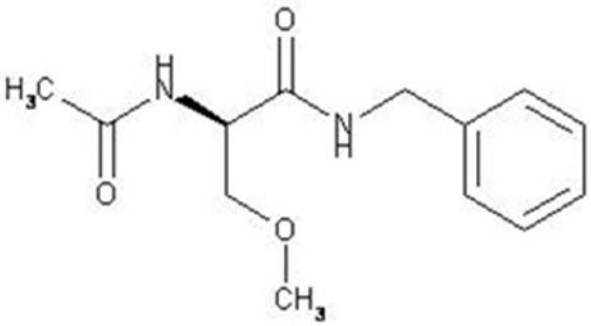
[Fig.1] Sumatriptan succinate.



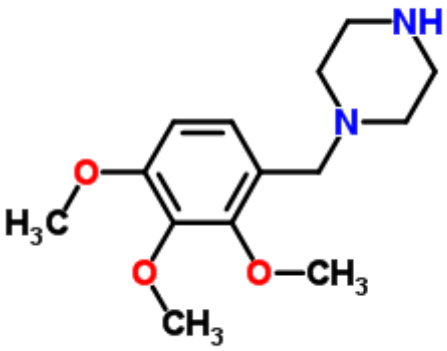
[Fig.2] Moxifloxacin.



[Fig.3] Ramipril.



[Fig.4] Lacosamid.



[Fig.5] Trimetazidine.

MATERIALS AND METHODS

Instrument

The analysis of the drugs were recorded on Shimadzu 140 double beam spectrophotometer as well as on Elico 210 UV- Visible double beam & Elico 159 UV- Visible single beam spectrophotometers using matched pair of Quartz cells of 10mm path length.

Materials

Stock solution of NBS (0.5%) was prepared by dissolving 0.5gm NBS in 100mL standard flask with double distilled water and this Stock solution of NBS was further diluted to get the working concentration.

Safranin-o stock solution (0.02%) was prepared by dissolving in distilled water. The dye solution was further diluted to get the working concentration.

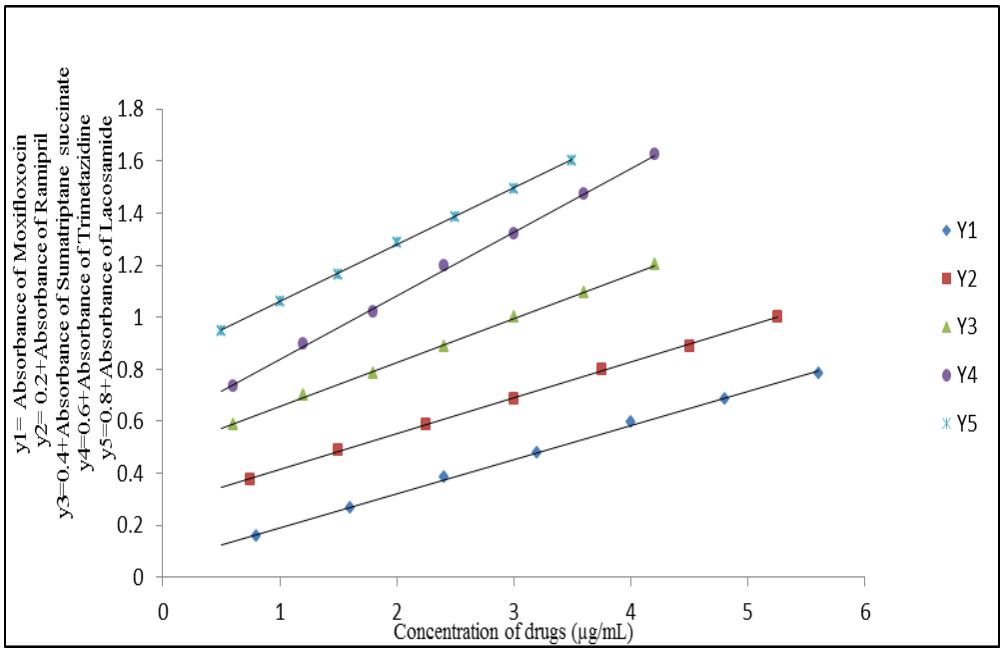
The pharmaceutical grade drugs were supplied by Hetero Drugs Pvt. Limited. Hyderabad. A stock solution of drug was prepared by dissolving accurately weighed 20mg of pure drug in water and diluting to 100mL in a volumetric flask with distilled water. The solution was diluted stepwise to get working concentrations.

Assay Procedure

Aliquots of pure drug solution (1 to 7mL) was transferred to a series of 10mL calibrated flask and to this 1m of $2M$ H_2SO_4 was added, followed by 1mL of NBS solution (0.5%). and the solution was shaked for 15 min. The 1mL of 0.02% safranin-o solution was added to each

flask, diluted to the mark with water and the absorbance of solution was measured at 532 nm against a reagent blank.

Calibration curves [figure. 6] 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.



Procedure for Assay of Pure Drug

To test the accuracy and precision of the methods developed pure sample solutions containing drug in the Beer's law limit were chosen. For this study 20,40 and 60 µg ml-1 of MOX; 10, 20, and 30 µg ml-1 of RAM; 20, 30 and 40 µg ml-1 of SUM; 10, 20 and 30 µg ml-1 of TRI; 2, 4 and 6 µgml-1 of LACO have been taken. The concentration chosen and recovery are tabulated in table 2. For this purpose standard deviation method also adapted.

Procedure For Tablets

1. Sumatriptan Succinate

Ten tablets of sumatriptan succinate [Suminat-25 and Suminat-50)] each containing 25 mg of SUM were weighed and finely powdered in a mortar. A quantity of powder equivalent to 20 mg of SUM was weighed accurately and dissolved in 100 ml of double distilled water, and sonicated for 20 min. It was used as stock sample solution and was further diluted with the same solvent to get working standard solution.

2. Ramipril

Ten tablets of ramipril [Cardiopril 5mg] were weighed and grounded. A quantity equivalent to 20mg of RAM was transferred into a 100mL calibrated flask, mixed well and filtered using a 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.

3. Moxifloxacin

Tentablets of drug [Mahaflox 400mg] wereweighed and grounded and transferred into a 100mLcalibrated flask and added 30mL of distilled water followed by sonication 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.

4. Trimetazidine

Four tablets [Cardimax 60 mg] were weighed and grounded. The powder equivalent to10mg TRI was stirred well with methanol, sonicated about 30 minutes. The solution was filtered through Whitman filter paper in a 100mLvolumetric 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.

5. Lacosamide

Ten tablets [Lacoste 100mg] were powdered and equivalent to about 10mg of LAC had been taken in to a100mL of volumetric flask and added about 30mL of methanol, sonicated For 20 min and filtered through Whitman 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.

RESULTS AND DISCUSSION

Method Development

The proposed spectrophotometric method is indirect and is 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 Safranin-o 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 Safranin-o spectrophotometric ally by measuring the absorbance of their acidic solutions at their respective λmax and the upper limits were found to be 0.02% for safranin-o. NBS concentration of 0.5% was found to bleach the color due to 0.02% of Safranin-0.Hence different amounts of drug reacted with 0.5% NBS in this method before determining the residual NBS as described under the respective procedure.

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 these methods are given in table 1. The limits of detection (LOD) and quantitation (LOQ) calculated according to ICH guidelines[15] 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 Sulphuric acid was slightly altered. The reaction time (after adding NBS, time varied was 15± 2min) and the time after addition of dyeis 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 are 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 excipients in the dosage forms do not interfere. The results are compared to the available validated reported[16,20] 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.

Table 1: Analytical and regression parameters of spectrophotometric method.

Parameter	MOX	RAM	SUM	TRI	LAC
λmax, nm	532	532	532	532	532
Beer's law limits μg mL^{-1}	20-140	10-70	20-80	10-70	2-14
Molar absorptivity, L mol^{-1} cm^{-1}	4.75×10^3	4.86×10^3	3.67×10^3	4.92×10^3	1.55×10^4
Sandell sensitivity μg cm^{-2}	0.2	0.08	0.1	0.07	0.02
Limit of detection μg mL^{-1}	2.286	1.373	1.725	3.081	0.7092
Limit of quantification μg mL^{-1}	6.928	4.16	5.281	9.33	2.14
Intercept, (a)	0.1	-0.005	0.094	0.011	0.022
Slope, (b)	0.005	0.012	0.01	0.013	0.049
Correlation coefficient, (r)	0.997	0.997	0.970	0.995	0.999
Standard deviation of intercept (σ)	0.0034	0.004	0.0052	0.012	0.027
Regression equation, Y	0.005X+0.1	0.012X-0.005	0.010X+0.094	0.013X-0.011	0.049X+0.022

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

Drug	Taken (µg mL^{-1})	Found (µg mL^{-1})	Error (%)	Recovery (%)	RSD (%)	Proposed method Mean ± SD
MOX	20 40 60	19.98 40.02 59.94	0.10 -0.05 0.10	99.9 100.05 99.9	0.0750	99.9375 ± 0.075
RAM	10 20 30	9.98 19.96 30.01	0.20 0.20 0.03	99.8 99.8 100.03	0.1398	99.92 ± 0.1396
SUM	20 30 40	19.98 29.94 39.98	0.10 0.20 0.05	99.9 99.8 99.95	0.100203	99.92 ±0.1001
TRI	10 20 30	9.96 19.98 30.01	0.4 0.1 0.033	99.6 99.9 100.03	0.188	99.87 ±0.1887
LAC	2 4 6	1.96 4.01 6.02	2 0.25 0.33	98 100.25 100.33	1.119	99.39 ±1.112

Table 3: Results of Assay of Tablets by the Proposed Method and Statistical Evaluation.

Tablets	Taken (µg mL^{-1})	Found (µg mL^{-1})	er (%)	Recovery (%)	RSD (%)	Proposed method Mean ± SD	Referance method mean ± SD	Student's t-test	F-test
MOX [Suminat]	15 24 35	14.98 23.95 35	0.133 0.208 0.00	99.566 99.79 100.00	0.090	99.89 ±0.090	100.23 ±0.8926	2.1965	0.0102
RAM [Cardipril]	06 14 26	5.94 13.95 25.98	1.00 0.357 0.076	99.00 99.64 99.92	0.470	99.65 ±0.468	99.98 ±0.8134	0.8994	0.3320
SUM [Mahaflox]	15 25 30	14.96 24.98 30.01	0.266 0.080 0.033	99.73 99.92 100.033	0.144	99.93 ±0.1446	99.86 ±0.2456	0.8680	0.3466
TRI [Cardimax]	8 16 24	7.98 15.98 23.94	0.250 0.125 0.25	99.75 99.87 99.75	0.096	99.82 ±0.0964	100.23 ±0.896	2.173	0.0115
LAC [Lacoste]	3 5 7	2.95 4.98 7.01	1.666 0.4 0.14	98.33 99.6 100.14	0.769	99.40 ±0.0764	100.32 ±0.8936	0.2669	0.7335

CONCLUSION

These are simple, rapid, and cost-effective methods for the determination of drugs have been developed and validated. The proposed methods are 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.

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