IOSR Journal of Applied Chemistry (IOSR-JAC)
e-ISSN: 2278-5736.Volume 9, Issue 2 Ver. I (Feb. 2016), PP 62-68
www.iosrjournals.org

Spectrophotometric Determination Of Drugs Based On Oxidation By Using Bromate-Bromide Mixture And Methyl Orange Dye

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Abstract*: A novel, simple, sensitive and rapid spectrophotometric method has been developed for the estimation of Gemifloxacin (GEM), Moxifloxacin (MOX), Tramadol (TRA) and Trimetazidine (TRI). The present spectrophotometric method is based on the bromination of the drug with a known excess of the bromate–bromide mixture under acidic conditions followed by the estimation of the surplus bromine by reaction with Methyl Orange dye, and measuring the absorbance at 508 nm. Beer's law was obeyed in the concentration range of 5-35μg/ml, 1.5-10.5 μg/ml, 3-30 μg/ml and 2.5-10 μg/ml for GEM, MOX, TRA and TRI respectively. The method was validated for accuracy, precision and recovery studies. Statistical analysis proved the method was Precise, reproducible, selective, specific, and accurate for analysis of GEM, MOX, TRA and TRI. The wide linearity range, sensitivity, accuracy, and simple procedure imply that the proposed technique demonstrated to be appropriate for routine analysis and quality control assays of tablets.*

Key Words*: Bromate–bromide mixture, Gemifloxacin, Methyl Orange, Moxifloxacin, Spectrophotometric, Tramadol,Trimetazidine.*

I. Introduction

Gemifloxacin mesylate (GEM) [Fig. 1] chemically R,S-7-(3 amino methyl 4- syn methoxyimino-1pyrrolidinyl)-1cyclopropyl-6-flouro1,4,dihydro 4- oxo-1,8 napthyridine-3-carboxylic acid methane-sulphonate1 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 [1, 2].

The physiological importance of Gemifloxacin mesylate initiated several reports on its determination, both in pharmaceuticals and in biological fluids. Several methods have been employed such as uv spectrophotometry [3-6], colorimetry [7], spectroflourometry [8] and TLC [9] for estimation of this drug.

Moxifloxacin hydrochloride (MOX) [Fig.2] chemically known as l-cyclopropyl-6-fluoro-l, 4-dihydro-8-methoxy- 7-[(4aS, 7aS)-octahydro-6H-pyrrolo-[3, 4-b] pyridin-6-yl]-4-oxo- 3-quinolinecarboxylic acid hydrochloride is a new generation, 8-methoxyquinolone derivative of fluoroquinolone antibacterial agent, synthetic, active against a broad spectrum of pathogens, encompassing Gram-negative and Gram-positive bacteria. However, most of fluoroquinolones show miner side effect one of these is skin reaction including photosensitivity. This response is inhibited by co-administration with H2 receptor antagonist [10,11].

Due to its importance of physiological activity, the drug has been quantified by almost all available physical and chemical methods. A number of assay methods have been reported for the analysis of Moxifloxacin *viz.*,UV spectrophotometry [12-15], HPLC[16], Atomic apsorption[17] and Voltametry[18].

Tramadol hydrochloride (TRA) [Fig.3] is a monoamine uptake inhibitor and centrally acting analgesic, used for treating moderate to severe pain. Tramadol hydrochloride is chemical (+/-) cis-2-(Dimethylamino) methyl)-1-(3-methoxy phenyl) cyclohexanol hydrochloride [19,20]. Because of the therapeutic importance of Tramadol there is much interest in its determination for the purpose of pharmaceutical quality control. The important and recent references reported for quantitative determination of this drug included a vast number of references thus far reported for the assay of the drug and pharmaceuticals like UV spectrophotometry[21-22]. Spectroflouromtry[23], Atomic absorpstion [24], conductometry[25] and HPLC [26].

Trimetazidine dihydrochoride (TRI) [Fig.4] 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 [27,28]. The antianginal efficacy of TRI is comparable to propranolol but it does not reduce cardiac rate–pressure product or coronary blood flow. The therapeutic importance of Trimetazidine required the development of sensitive, simple and reliable methods for industrial quality control of pharmaceutical preparations and clinical monitoring of the drug in toxicological studies. It is important to emphasize that there are a large number of analytical procedures

reported and these references cited almost all earlier methods of analysis of this drug such as RP-HPLC[29-30],UV [31-32], LC-MS[33] and HPLC with Spectroflourometry[34].

Through survey of literature revealed that the above mentioned four drugs have not been quantified by oxidation with using bromate bromide mixture in acidic media.

II. Experimental

2.1. 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.

2.2. Reagents And Chemicals:

Bromate–bromide mixture, Prepared by dissolving 100 mg of $KBrO_3$ (S. d. Fine Chem., India) and 1 g of KBr (S. d. Fine Chem., India) in water and diluting to one liter in a valumetric flask . The solution was appropriately diluted to obtain 10 μgml^{-1} of solution, with reference to potassium bromate.

Metyl Orange (500 μgml^{-1}), A 500 μgml^{-1} stock solution was prepared by dissolving 58.82 mg of the dye (S. d. Fine Chem. Mumbai, India, 85 % dye content) in water and diluting to the mark in a 100 ml valumetric flask. This was appropriately diluted to obtain a 50 μgml^{-1} solution

Hydrochloric acid solution (5M), Prepared by diluting the appropriate volume of concentrated acid (Quligens Fine Chemicals, India) with water.

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 20 mg of pure drug in water and diluting to 100 mL in a calibrated flask with double distilled water. these drug sloutions further diluted to get working concentraion.

All the employed chemicals were of Analytical grade and double distilled water was used throughout the study.

III. Assay Method

Different aliquots of a standard solutions of 1– 7 ml of 5μgml^{-1} Gem, 1- 7 ml of 1.5 μgml^{-1} Mox, 1-4 ml of 2.5μgml^{-1} Tri and 0.5-5 ml of 6μgml^{-1} Tra were accurately weighed and transferred into a different series of 10 ml volumetric flasks and to each flask was then added 1ml of 5Mhydrochloric acid followed by 1 ml of the bromate–bromide reagent (10 $\mu g\ ml^{-1}$ w. r. to $KBrO_3$). The flasks were stoppered, the contets mixed well and allowed to stand for 5 min with occasional shaking. Finally, 1 ml of 50 $\mu g\ ml^{-1}$ Methyl Orange solution was added to each flask, diluted to the mark with water, mixed well and the absorbance of each solution was measured at 508 nm against a water blank after 5 minutes. calibration graphs were prepared by plotting the absorbance *versus* the concentration of drug (Fig. 5 - Fig. 8). The concentration of the unknown was read from the calibration graph or calculated from the regression equation derived from Beer's law [Table 1].

IV. Procedure For The Tablets

4.1. Gemifloxacin:

One tablet of Eg 1 containing 320 mg of Gemifloxcin was weighed and powder equivalent to 50mg was taken in a 50ml volumetric flask and it was dissolved and diluted up to the mark with doubled distilled water. The resultant solution was sonicated for 5 minutes. The solution was then filtered using Whatman filter paper No.41. From the filtrate, appropriate dilutions were made in distilled water to obtain the desired concentration.

4.2. Moxifloxacin:

One tablet (Moxicip-400mg) was powdered, weighed and average weight of the tablets was determined. An amount of powder equivalent to 100 mg of Moxifloxacin was taken into a 100ml volumetric flask. The residue was filtered on Whatman No. 41 filter paper and washed with doubly distilled water. This solution was further diluted as necessary to complete the analysis following the recommended procedures

4.3. Tramadol:

Ten tablets of tramadol HCl (Dolotram: 20mg) were weighed and finely powdered in a glass mortar. Tablet powder equivalent to 100 mg of TH was weighe d accurately and taken into 100 mL volumetric flask. The drug was dissolved in distilled water and the volume was made up to the mark. The solution was suitably diluted and assayed as under the respective assay procedure described for the preparation of calibration curves for both the methods.

4.4.Trimetazidine:

Five tablets of Carvidon, each contains 20mg were crushed and finely powdered. A portion of the powder, equivalent to the average weight of 100mg of Trimetazidine was transferred to a 100 ml volumetric flask and dissolved in 50 ml doubly distilled water. The content was shaken for 15–20 min; the volume was diluted to the mark with water, mixed well and filtered using a Whatmann No. 42 filter paper. The first 10 mL portion of the filtrate was discarded and a suitable aliquot of the filtrate was diluted appropriately with water to get required concentration for the assay.

V. Results And Discussion

Bromate-bromide mixture in acid medium shows as an equivalent solution of bromine and has been widely used for the assay of several organic and bio active pharmaceutical compounds [35-40]. The proposed method describes the in situ generation of bromine by the action of the hydrochloric acid on bromate-bromide mixture. In the present method varying concentrations of drug solutions were reacted with a fixed and known excess amount of bromate-bromide mixture in hydrochloride acid medium and after a predetermined time, then the un reacted bromine is determined by treating with a known fixed amount of methyl orange and measuring the absorbance at 508nm. A linear relation has been found between absorbance and concentration of drugs which formed the basis for quantification of the drug.

A preliminary showed that 1 ml of 50 ugml methyl orange acid form in a total volume of 10 ml produced a maximum absorbance at 508nm.This colour was completely and irreversibly bleached by 1 ml of 10 μgml^{-1} of $KBrO_3$ in the presence of of excess of bromide in hydrochloric acid medium hence different amounts of drugs were reacted with 1 ml of $KBrO_3$ (plus excess of KBr) and after the bromination was judged complete, the surplus bromine (in situ generated) was determined by treating with 1 ml of 50 μgml^{-1} of methyl orange and measuring the absorbance at 508 nm this enabled to determine the concentration of drug which could be quantified.

VI. Optimization Of Experimental Variables

6.1.Effect Of Acid Concentration:

The effect of acid concentration on the measured species was investigated by following the assay procedures. The effect of 1 mL of HCl of different concentrations (1.0, 2.0, 3.0, 4.0, 5.0 and 10.0 M) was studied by measuring the absorbance of the colored product using a fixed concentration of dugs 10.0 $\mu g\ mL^{-1}$ of GEM, 5 $\mu g\ mL^{-1}$ of MOX, 25 $\mu g\ mL^{-1}$of TRA and 4 $\mu g\ mL^{-1}$of TRI, it is clear that the absorbance of the colored product remained constant with 1.0 mL of 3 to 10 M HCl. Therefore, 1.0 mL of 5.0 M HCl was selected for method.

6.2. Reaction Time And Color Stability:

The effect of time on the reaction between drugs and bromate-bromide mixture in the presence of HCl was studied by keeping all other reaction conditions unchanged. The absorbance of the colored species was measured after different reaction times (5.0-45.0 min) and the results showed that the reaction was complete within 15 minutes and remained stable for at least 1 h.

VII. Analytical Parameters

The standard calibration curves under the optimum experimental conditions were constructed by plotting the absorbance vs. concentration. A linear correlation was found between absorbance at € max and concentration of drugs in the concentration ranges given in Table 2. The graphs are described by the regression equation:

$Y = a + bX$

(where Y = absorbance; a = intercept; b = slope and X = concentration in µg/mL). The regression parameters such as slope (b), intercept (a) and correlation coefficient (r) are presented in Table 2. The molar absorptivity (€), Sandell's sensitivity, limits of detection (LOD) and quantitation (LOQ) of method is also given in Table 2. The high values of €, low values ofSandell's sensitivity, LOD and LOQ values indicate the high sensitivity of the proposed methods

The accuracy and precision of the methods were established by analyzing the pure drug solution at 6 different levels (with working limits). The relative error (%) which is a measure of accuracy & RSD (%) a measure of precision are summarized in Table 2 and reveal the high accuracy and precision of the methods.

VIII. Robustness And Ruggedness

The robustness of the methods was evaluated by making small incremental changes in the volume of acid and reaction time, and the effect of the changes on the absorbance of the measured species was studied. The

changes had negligible influence on the results as revealed by small intermediate precision values expressed as %RSD .

Method ruggedness was expressed as the %RSD of the same procedure applied by four different analysts as well as using three different cuvettes. . Further t-test and F-test values have also been calculated using a standard reference method. The t-test and F-test values are less than their permissible range indicating high accuracy and precision of the method(Table 2).

IX. Application To Formulations

The proposed method was applied to the determination of pharmaceutical tablets and purchased from local stores. The tablet solution containing drug in the Beer's law limits were chosen. To assess the precision each tablet analysis 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 applicability of the methods for pharmaceutical analysis, the obtained results (Table 3) were statistically compared with the reference method.

The results obtained by the proposed methods agreed well with those of the reference method. When the results were statistically compared with those of hthe reference method by applying the Student's t-test for accuracy and F-test for precision, the calculated Student's t-value and F-value at 95% confidence level did not exceed the tabulated values. Hence, no significant difference exists between the proposed methods and the reference method with respect to accuracy and precision

X. Conclusion

The useful method for the micro determination of drugs in bulk drug as well as in pharmaceutical formulations have been developed and validated as per the current ICH guidelines [41]. The methods use bromate-bromide mixture as a green brominating reagent instead of hazardous liquid bromine.Some of the reported methods even though more sensitive, suffer from the disadvantages of a heating step. The proposed methods have the advantages of selectivity and easily adaptable to routine analysis. Moreover, they are free from complicated analytical procedures such as heating or extraction steps and are cost effective when compared to several non-spectrophotometric methods .

Aknowledgement

We are grateful to Head, Department of Chemistry,Osmania University for providing facilities. one of the authors B.Vijayakumar is thanksful to CSIR.

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Table 1. Analytical Parameters for determination of drugs by oxidation with bromate-bromide Mixture

Name of Drug Property	Gem	Mox	Tra	Tri
λ max	508	508	508	508
Beer's law limits (μgmL^{-1})	1-10	0.3-2.1	0.4-2.8	0.6-4.8
Sandell's sensitivity(μgcm^{-2})	0.0147	0.015	0.0435	0.0149
Molar absorptivity L mol^{-1} cm^{-1}	9400	29651	6177	22798
Std. Dev.of intercepts	0.0055	0.008	0.0054	0.0125
LOD (μgmL^{-1})	0.265	0.396	0.771	0.615
LOQ (μgmL^{-1})	2.88	1.208	2.33	1.864
Slope, b	0.019	0.067	0.023	0.067
Intercept, a	0.086	0.075	0.111	-0.057
Correlation coefficient	0.981	0.991	0.980	0.995
Regression equation$Y=a+bx^{*}$	0.086+ 0.019X	0.075+ 0.067X	0.111+ 0.023X	-0.057+ 0.067X

*X=concentration of the drug, (μgmL^{-1})

Table 2. Recovery studies to evaluate accuracy and precision for determination of drugs by oxidation with bromate-bromide mixture

Name of the Drug	Amount Taken($\mu g ml^{-1}$)	Amount Found($\mu g ml^{-1}$)	Relative error%	% Recovery	RSD %	Proposedmethod Mean ± SD	Ref method Mean± SD	t-test(*)	F-test(**)
Gem	8 12 20 25	8.100 12.07 19.91 24.91	1.23 0.58 0.45 0.36	101.26 100.63 99.57 99.64	0.814	100.278 ±0.816	100.17 ±0.947	-0.199 (2.45)	0.742 (4.28)
Mox	3 5 7 9	2.978 5.024 7.025 8.973	0.74 0.48 0.36 0.30	99.28 100.49 100.32 99.71	0.571	99.958 ±0.5711	99.66 ±1.204	-1.449 (2.57)	0.225 (4.95)
Tra	10 15 20 25	10.05 14.90 20.07 25.03	0.50 0.67 0.35 0.12	100.59 99.34 100.35 100.15	0.539	100.106 ±0.54	99.82 ±0.302	0.799 (2.57)	3.287 (4.95)
Tri	3 4 6 9	3.020 4.013 5.985 9.016	0.66 0.32 0.25 0.18	100.69 100.33 99.77 100.18	0.384	100.246 ±0.385	100.31 ±0.218	0.782 (2.45)	3.114 (4.28)

*t- test and **F-test values from literature

Table 3. Application of praposed method for the analysis of studied drugs in pharmaceutical formulation by oxidation with bromate-bromide mixture

Name of theDrug	Amount Taken(µg ml^{-1})	Amount Found(µg ml^{-1})	Relative error %	% Recovery	RSD %	ProposedmethodMean ± SD	Refmethod Mean± SD	t-test (*)	F-test (**)
Gem	6 10 15 20	6.06 10.07 14.88 20.09	0.99 0.71 0.81 0.43	101 100.72 99.191 100.42	0.798	100.34 ±0.798	99.65 ±0.636	0.316 (2.45)	1.574 (4.95)
Mox	2 4 6 8	2.019 3.978 5.912 8.026	0.94 0.55 1.49 0.32	100.96 99.417 98.532 100.32	1.062	99.81 ±1.059	97.3 ±0.837	-0.326 (2.57)	1.603 (4.95)
Tra	8 12 16 20	7.946 11.91 16.07 20.07	0.68 0.78 0.42 0.35	99.319 99.223 100.42 100.35	0.646	99.828 ±0.645	99.89 ±0.401	0.658 (2.45)	3.036 (4.95)
Tri	3 5 7 9	3.021 5.018 7.017 9.017	0.70 0.36 0.24 0.19	100.69 100.35 100.24 100.18	0.227	100.37 ±0.228	99.82 ±0.153	0.629 (2.57)	2.228 (4.95)

*t- test and **F-test values from literature

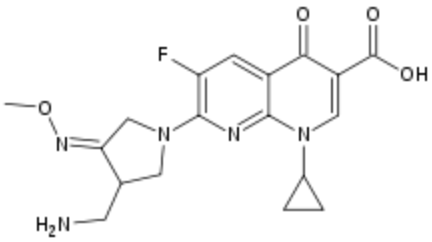

Fig.1. Gemifloxacin

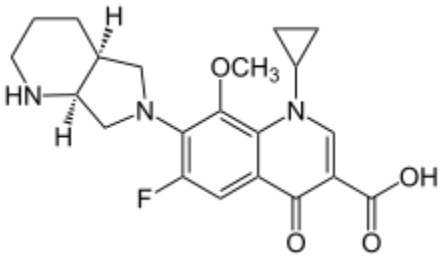

Fig.2. Moxifloxacin

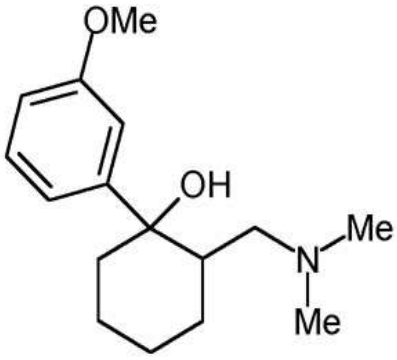

Fig.3. Tramadol

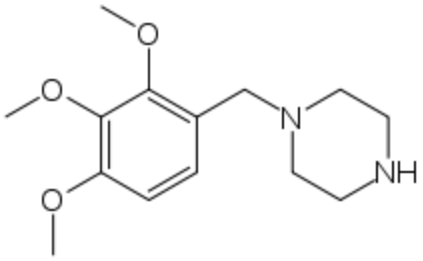

Fig.4. Trimetazidine

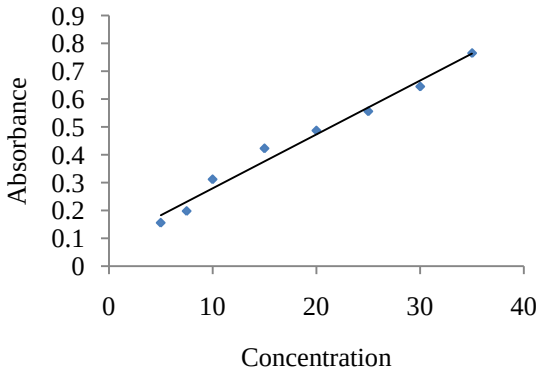

Fig.5 Calibration graph of Gemifloxacin

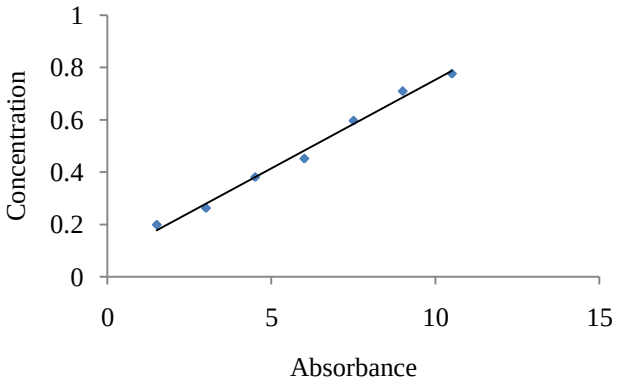

Fig. 6 Calibration graph of Moxifloxacin

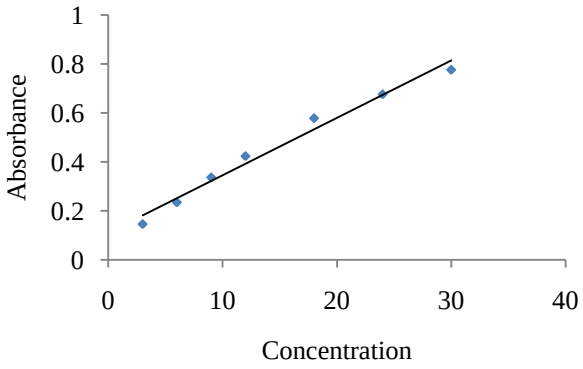

Fig.7. Calibration graph of Tramadol

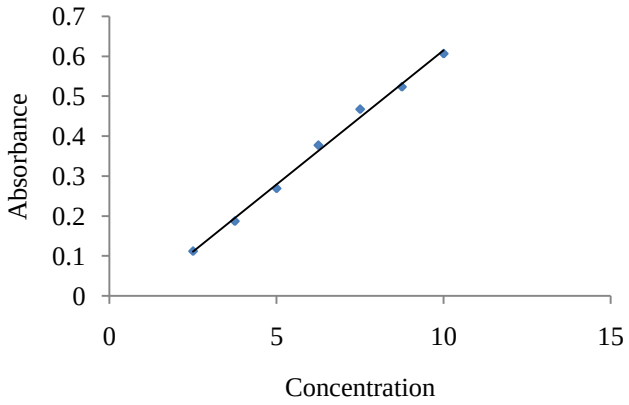

Fig.8. Calibration graph of Trimetazidine