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SPECTROPHOTOMETRIC DETERMINATION OF DRUGS BY USING CHLORAMINE-T AND METHYL ORANGE

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ABSTRACT

Simple and sensitive validated spectrophotometric methods has been developed for the assay of five drugs *viz.*, Gemifloxacin (GEM), Ondensetron (OND), Alfuzocin (ALF), Dulaxetine (DUL) and Torsemide (TOR) in pure and pharmaceutical formulations. The proposed method was based on the oxidation of drug by Chloramine-T (excess) and subsequent determination of unreacted Chloramine-T using methyl orange as analytical tool which absorbs at 510 nm. Beer's law is obeyed in the concentration of 2-14 , 4-28 , 3-24 , 3-21 and 1.5-8.5 µg mL-1 for GEM, OND, ALF, DUL and TOR respectively. Different variables affecting the reaction were studied and optimized. The proposed methods were applied successfully to the determination of the examined drugs in pure and pharmaceutical dosage forms with good accuracy and precision.

KEYWORDS: spectrophotometry, Chloramine-T, methyl orange and formulations.

INTRODUCTION

1. Gemifloxacin (GEM) "Fig.1a" is chemically known as 7-[(4Z)-3-(Aminomethyl)-4-methoxyimino-pyrrolidin-1-yl]-1 -cyclopropyl-6-fluoro-4-oxo-1, 8-naphthyridine-3-carboxylic acid. It is a used to treat a variety of bacterial infections. This medication belongs to a class of drugs called quinolone antibiotics. It works by stopping the growth of bacteria. This antibiotic treats only bacterial infections.[1] It will not work for virus infections. Because of its physiological significance the drug has been quantitatively analyzed by different methods. A few analytical methods like HPLC[2-5], Spectrophotometry[6,7], Spectrofluorimetry

[8, 9], LC-MS[10], and Chemiluminisence method[11] developed for the estimation of GEM are mention worthy.

2. Ondensetron (OND) "Fig.1b" is chemically known as (RS)-9-methyl-3-[(2-methyl-1H-imidazol-1-yl) methyl]-2,3-dihydro-1H-carbazol-4(9H)-one. It is a serotonin 5-HT3 receptor antagonist used to prevent nausea and vomiting caused by cancer chemotherapy, radiation therapy, and surgery. It has little effect on vomiting caused by motion sickness,[12] and does not have any effect on dopamine receptors or muscarinic receptors. It is on the World Health Organization's List of Essential Medicines, the most important medications needed in a basic health system. . An extensive literature survey revealed that spectrophotometry[13-16], HPLC [17-21] and LC[22] have been applied for the analysis of OND in bulk and in formulations.

3. Alfuzocin (ALF) "Fig.1c" is chemically known as N-[3-[(4-amino-6, 7-dimethoxy-quinazolin-2-yl)- methyl-amino]propyl] tetrahydrofuran- 2-carboxamide. It is a α1 receptor antagonist used to treat benign prostatic hyperplasia (BPH). It works by relaxing the muscles in the prostate and bladder neck, making it easier to urinate.[23] Analytical methods available for the determination of ALF include spectrophotometry[24-28], HPLC[29-30], LC [31], Conductometry [32], Colorimetry.[33]

4. Duloxetine (DUL) "Fig.1d" is chemically known as (+)-(S)-N-Methyl-3-(naphthalen-1-yloxy)-3-(thiophen-2-yl) propan-1-amine. The main uses of duloxetine are in major depressive disorder, general anxiety disorder, urinary incontinence, painful peripheral neuropathy, fibromyalgia, and chronic musculoskeletal pain associated with osteoarthritis and chronic lower back pain.[34] Literature review reveals that a few methods have been published for analysis of DUL in the bulk form and in pharmaceutical preparations. Methods available include HPLC [35-37], UPLC[38] and Spectrophotometry.[39, 40]

5. Torsemide (TOR) "Fig.1e" is chemically known as N-[(isopropyl amino) carbonyl]-4-[(3-methylphenyl) amino]pyridine-3-sulfonamide.It is mainly used in the management of edema associated with congestive heart failure.[41] It is also used at low doses for the management of hypertension. . Several techniques have been reported in the literature for the determination of TOR in pharmaceuticals and in biological samples include HPLC[42-46], Spectrophotometry [47-52] in pharmaceuticals and in biological samples.

MATERIALS AND METHODS

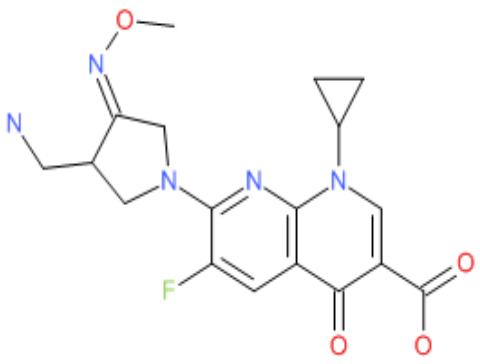
Instrumentation : Spectral and absorbance measurements were made on a Elico 210 double beam spectrophotometer , Systronics 117 spectrophotometer and also on ELICO 159 UV-VIS single beam spectrophotometers using quartz cells of 10 mm path length. A Dhona 200 single pan electrical balance is used for weighing the samples.

Reagents: Gemifloxacine, Ondansetron, Alfuzocin, Dulaxetine and Torsemide drug samples were procured from Hetero drugs pvt limited, Hyderabad as gift samples. The reagents Chloramine-T, methyl orange (AR grade) and HCl supplied by SD Fine chemicals Ltd. Mumbai, are used without any further purification.

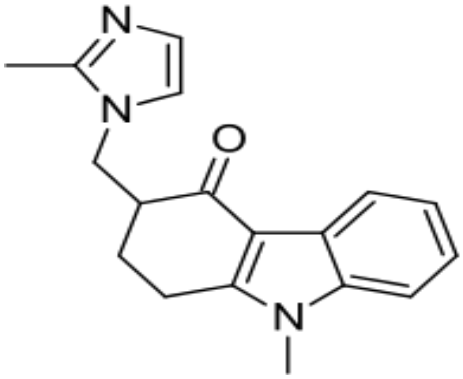
Drug solutions: A stock solution of each drug is prepared in doubly distilled water by dissolving 25mg ofdrugin25mlofwaterand the stock is diluted to the required concentrations. A 0.01 *M* of Chloramine-T and $5x10^{-4}$ *M* Methyl orange are prepared in distilled water and 0.5*M* HCl is prepared from stock. The concentrations of the Chloramine-T and Methyl Orange are set such that 1ml of Chloramine-T in the presence of 1ml of acid exactly neutralize 1ml of Methyl Orange and shows zero absorbance.

RESULT AND DISCUSSION

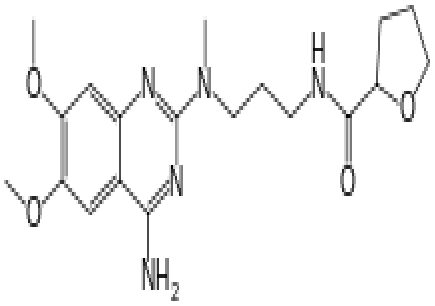
Procedure for Calibration: Different aliquots of drug solution (1-7 ml) were taken into a 10ml standard flask to which 1ml of acid and 1ml of Chloramine-T were added. The contents were occasionally shaken for 15 minutes and finally 1ml of methyl orange is added, the volume is made up to mark using distilled water. Absorbance was measured at 510nm against the blank prepared similarly. The same procedure of analysis is followed either for assay of pure drug or for dosage form. The calibration graphs “Fig. 2” are linear over the concentration ranges are within the permissible range. The optical characteristics and statistical data for the regression equation of the proposed methods are presented in [Table 1.] Six replicate experiments performed and the relative response i.e., absorbance / concentration (µg mL^{-1}) was calculated. The points falling between 95% and 105% of average only are considered for the construction of calibration. The standard deviation of six residual intercepts of the plots is used for calculating LOD and LOQ.



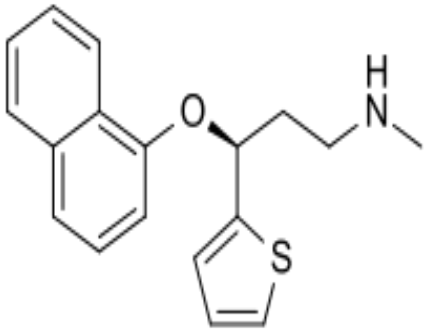
a. Gemifloxacin



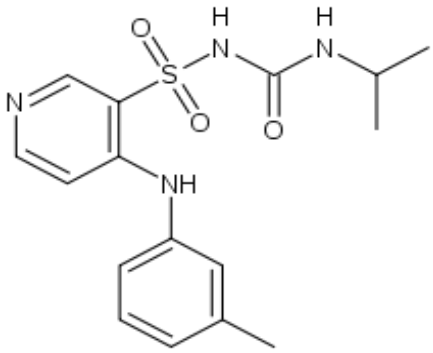
b. Ondensetron



c. Alfuzocin



d. Duloxetine



e. Torsemide

Fig.1.Structures Of The Drugs

Procedure for Assay of Pure Drugs

To test the accuracy and precision of the methods developed, pure sample solutions containing drug in Beer's law limit were chosen. For this study 2.5, 3.0, 3.5 and 4.0µg mL-1 of GEM ; 4.0, 10.0, 12.0 and 16.0 µg mL-1 of OND; 3.0, 5.5, 9.0 and 12.5 µg mL-1 of ALF; 3.0, 3.5, 4.0 and 4.5 µg mL-1 of DUL; 2.0, 3.0, 4.0 and 5.0 µg mL-1 of TOR have been taken and the recovery experiments were performed. The recoveries and their relative standard deviations are tabulated in [Table. 2]

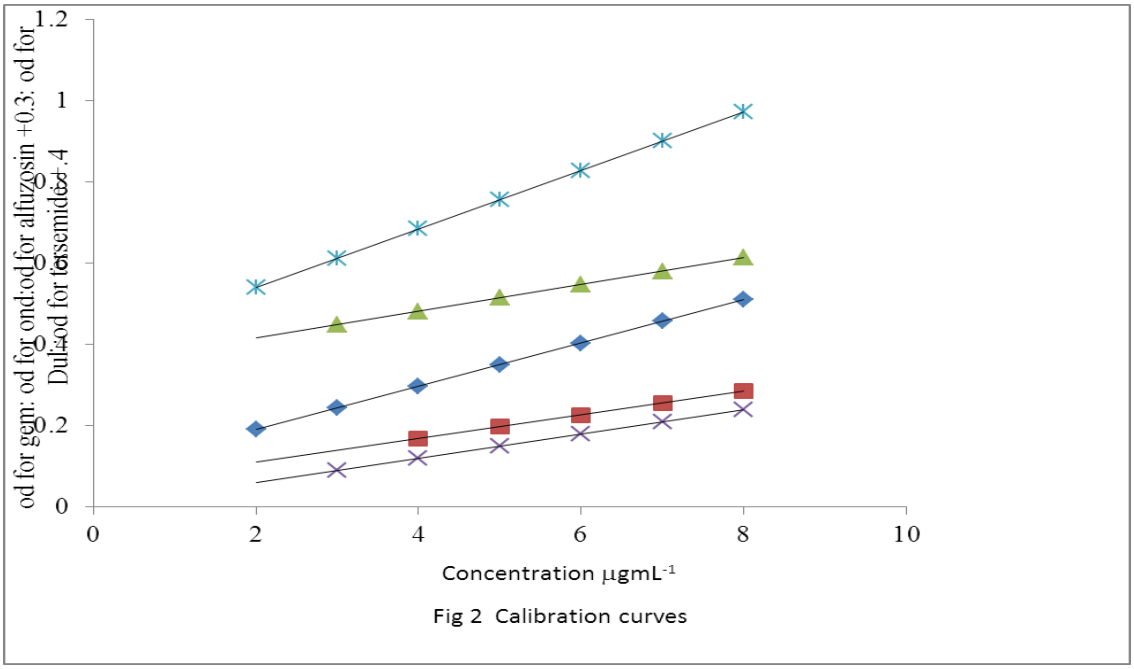


Fig.2 Calibration curves for the determination of drugs.

Method Validation

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 equation for each drug are tabulated in [Table 1]. To assess the precision each experiment was repeated at least 5 times and accuracy is estimated in terms of % recovery and % RSD. Excellent % recovery and RSD being less than 2 for each drug demonstrates accuracy and precision of the methods. Further t-test and F- test values have also been calculated using a standard reference method for each drug. The t-test and F-test values are less than their permissible range indicating high accuracy and precision of the methods [Table 2].

Analysis of Pharmaceuticals

Gemifloxacin

Twenty capsules of EG 1 were weighed accurately and crushed to fine powder. Quantity of tablet powder equivalent to 50mg of analyte was weighed and transferred to 100ml volumetric flask and dissolved in 40 ml of distilled water by using 0.2M HCl. This solution was then filtered through Whatmann filter paper No.41. The volume was made up to the mark of 100ml volumetric flask with distilled water.

Ondensetron

Twenty tablets of Ondem were finely grinded and mixed. An accurately weighed 50 mg of OND was taken into a 100 ml volumetric flask, sonicated and remaining volume is made up with distilled water.

Alfuzosin

Ten tablets of Alfoo 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 mints and the solution was filtered using whatmann No.41 filter paper and further diluted with water to obtain working standard solution.

Duloxetine

Twenty tablets of Diligo were weighed and ground into a fine powder. An amount of tablet powder equivalent to 50 mg of DUL was weighed and transferred into 100 ml beaker containing 50 ml of 0.2 M HCl. After shaking the contents for 20 mins, filtered through Whatmann No. 41 filter paper into a clean 100 ml volumetric flask and the volume was brought up to the mark with the distilled water.

Torsemide

Twenty tablets of Dytor were finely grounded and mixed. An accurately weighed 50 mg of TOR was transferred into a100 ml volumetric flask and dissolved in HCl. Then the solution is was filtered using Whatmann No.41 filter paper and further diluted with distilled water and made volume up to the mark.

To test the applicability of the method developed, pharmaceutical tablet solutions containing drug in the Beer's Law limit were chosen. For this study 2.5,3.0 ,3.5 and 4.0 µg mL -1 of GEM ; 4.0, 10.0, 12.0 and 16.0 µg mL -1 of OND ;3.0, 5.5, 9.0 and 12.5 µg mL -1 of ALF ; 3.0, 3.5, 4.0 and 4.5 µg mL -1 of DUL and 2.0, 3.0, 4.0 and 5.0 µg mL -1 of TOR ; were chosen [Table.3]

Effect of acid concentration: HCl was the medium of choice for estimation of drugs Chloramine-T and Methyl Orange. The absorbance of Methyl orange was not affected in 0.125-1.25 M HCl concentration. A 0.5 M HCl concentration was found optimum for the estimation of drugs in a reasonable time of 5-10 mins and hence the same concentration was employed for the determination of drugs by using Chloramine-T and Methyl Orange.

Table 1: Analytical and regression parameters of spectrophotometric study

Parameter	GEM	OND	ALF	DUL	TOR
λmax, nm	510	510	510	510	510
Beer's law limits µg mL^{-1}	2-14	4-28	3-24	3-21	1.5-8.5
Molar absorptivity, L mol^{-1} cm^{-1}	$5.5x10^{-3}$	$1.17x10^{-3}$	$1.77x10^{-3}$	$1.0x10^{-3}$	$2.51x10^{-3}$
Sandell sensitivity* µg cm^{-2}	0.0188	0.034483	0.0303	0.03333	0.013889
Limit of detection µg mL^{-1}	2.4561	2.035593	0.258844	0.004803	0.01244
Limit of quantification µg mL^{-1}	7.442816	6.168463	0.784374	0.014555	0.037697
Regression equation, Y**=a+bX	0.086 +0.053X	0.053 +0.029X	0.050 +0.033X	0.03X	-0.003 +0.072X
Slope, (b)	0.053	0.029	0.033	0.03	0.072
Intercept, (a)	0.086	0.053	0.050	0.00	-0.003
Correlation coefficient, (r)	0.998	0.993	0.994	1.00	0.999
Regression equation, Y**=a+bX	0.086 +0.053X	0.053 +0.029X	0.050 +0.033X	0.03X	-0.003 +0.072X

*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 drug (µg $mL^{-1)}$

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

Drug	Amount Taken (µgmL^{-1})	Amount Found (µgmL^{-1})	(%) ER	%Recovery	%RSD	Proposed method Mean± SD
GEM	2.5 3.0 3.5 4.0	2.48 3.05 3.48 3.97	0.8 1.6 0.57 0.75	99.2 101.67 99.43 99.25	1.192	99.89±1.201
OND	4.0 10.0 12.0 16.0	3.98 9.96 11.98 15.97	0.5 0.4 0.167 0.19	99.5 99.6 99.83 99.81	0.164	99.69±0.162
ALF	3.0 5.5 9.0 12.5	2.98 5.51 9.01 12.48	0.67 0.18 0.11 0.16	99.33 100.18 100.11 99.84	0.386	99.87±0.390
DUL	3.0 3.5 4.0 4.5	2.95 3.46 3.98 4.49	1.67 1.14 0.5 0.22	98.33 98.86 99.5 99.78	0.655	99.12±0.649
TOR	2.0 3.0 4.0 5.0	2.03 2.98 4.01 4.95	1.5 0.67 0.25 1.0	101.5 99.33 100.25 99	1.119	100.02±1.11

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 mean±SD	Student's t-test	F-test
GEM (Eg1)	1.5 3.0 4.5 6.0	1.0 1.0 1.5 1.0	2.48 3.98 5.97 7.01	0.8 0.5 0.5 0.14	99.2 99.5 99.5 100.14	0.399	98.28 ±0.34	99.59 ±0.397	0.267	1.363
OND (Ondem)	4.0 6.0 8.0 10	2 2 2 2	5.96 7.98 9.99 11.98	0.67 0.25 0.1 0.17	99.33 99.75 99.9 99.83	0.256	100.6 ±0.85	99.70 ±0.254	1.646	0.089
ALF (Alfoo)	3.0 6.0 9.0 12	1.0 1.0 1.0 1.0	3.98 6.98 9.99 13.0	0.5 0.29 0.1 0.0	99.5 99.71 99.9 100	0.220	100.6 ±0.6	99.78 ±0.220	1.457	0.134
DUL (Diligo)	3.0 5.0 7.0 9.0	1.0 1.0 1.0 1.0	4.01 5.01 7.99 9.98	0.25 16.5 0.125 0.2	100.25 100.17 99.88 99.8	0.219	100.20 ±1.126	100.02 ±0.218	1.939	0.037
TOR (Dytor)	1.5 3.0 4.5 7.0	1.5 1.0 1.5 1.0	2.98 3.99 6.01 7.98	0.67 0.25 0.17 0.25	99.33 99.75 100.17 99.75	0.341	99.16 ±0.59	99.75 ±0.340	0.899	0.332

CONCLUSION

This method is simple, rapid and offers the advantages of high sensitivity and a wide range of determination without the need for heating or extracting. The other advantages of the present method over the previously described methods include low detection limit with high accuracy and precision. Therefore this method was chosen for the routine analysis of the above drugs in pharmaceuticals and in bulk drug industries.

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