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QUANTITATIVE DETERMINATION OF DRUGS AND PHARMACEUTICALS USING CHLORAMINE-T AND METHYL ORANGE AS DYE

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ABSTRACT

New sensitive and rapid spectrophotometric methods for the determination of five drugs *viz.*, Fosinopril (FOS), Mebeverine Hydrochloride (MEB), Phenylephrine Hydrochloride (PHE), Terazosin Hydrochloride (TER) and Voriconazole (VOR) in pure and pharmaceutical formulations. The proposed methods involve the oxidation of drugs by addition of excess amount of Chloramine-T in acidic medium and subsequent determination of unreacted Chloramine-T using a fixed amount of methyl orange as a dye which absorbance at 510 nm. Beer's law is obeyed in the concentration of 24-168, 5-35, 12-84, 2-14 and 20-140 µg mL^{-1} for FOS, MEB, PHE, TER and VOR respectively. Different variables affecting the reaction were studied and optimized. The limits of detection and quantification were also reported for this method.

KEYWORDS: Quantification, Chloramine-T, Methyl Orange, Formulations and Spectrophotometric Determination.

INTRODUCTION

1. Fosinopril

Fosinopril (FOS) is chemically known as (2S, 4S) -4-cyclohexyl-1-[2-[[2-methyl 1 (propanoyloxy) propoxy]-(4-phenylbutyl) phosphoryl] acetyl) pyrrolidine-2-carboxylic acid (Fig.1). It is an angiotensin converting enzyme (ACE) inhibitor[1] used for the treatment of hypertension, alport syndrome, diabetic kidney disease and left ventricular dysfunction. Fosinopril is the only phosphinate-containing ACE inhibitor. Fosinopril is de-esterified by the

liver or gastrointestinal mucosa and converted to its active form, Fosinoprilat competitively binds to ACE, preventing ACE from binding to and converting angiotensin I to angiotensin II which lowers tangential vascular resistance and decreases blood pressure, therefore helping to alleviate the negative effects of all on cardiac performance.[2]

Because of its physiological significance, the drug has been quantified by almost all physical and chemical methods. A few recent papers have been published such as Spectrometry.[3,4] and HPLC.[5]

2. Mebeverine Hydrochloride

Mebeverine Hydrochloride (MEB) is chemically known as (RS)-4-(ethyl [1-(4methoxyphenyl) propan-2-yl] amino) butyl 3, 4-dimethoxybenzoate hydrochloride (Fig.2). Mebeverine belongs to a category of anti-spasmodics known as musculotropic drugs and is used largely in treatment of irritable bowel syndrome and gastrointestinal spasm secondary to organic disorder.[6,7] Several methods have been reported in literature for the determination of this drug either per se or in formulations.

In this concern, the following techniques have been described: Spectrophotometric methods[8,9], electrochemical methods[10,11] and Chromatographic methods.[12,13]

3. Phenylephrine hydrochloride

Phenylephrine hydrochloride (PHE), chemically (R)-1-(3- hydroxyphenyl)-2-thylaminoethanol hydrochloride[14] (Fig.3) is a direct sympathomimetic agent, a selective α1 agonist, causing vasoconstriction. It is also a frequent constituent of orally administered nasal decongestant preparations.

A few analytical methods viz; RP-HPLC[15], HPTLC[16], and Spectrophotometry[17] have been developed for the determination of Phenylephrine hydrochloride.

4. Terazosin hydrochloride

Terazosin hydrochloride dihydrate (TER) is known chemically as 2-[4-(2-tetrahydrofuranyl) carbonyl]-1-piperazinyl-6, 7-dimethoxy-4-quinazolinamine mono hydrochloride dehydrate. (Fig.4) TRZ is a highly selective potent α-1 adrenoreceptor antagonist. It is an effective drug for hypertension (high blood pressure) and benign prostatic hyperplasia (enlarged prostate). It causes the blood vessels (veins and arteries) to relax and expand, improving blood flow.

Terazosin hydrochloride dehydrate also relaxes muscles in the prostate and bladder neck, making it easier to urinate.

Terazosin has been determined by many analytical methods, such as HPLC with Fluorescence detection[18,20], RP-HPLC[21], Mass spectrometric detection.[22] HPTLC[23], Spectrophotometry[24] and Fiuorimetry.[25]

5. Voriconazole

Voriconazole (VOR) is a triazole antifungal that is a derivative of fluconazole. It is chemically (2R, 3S)-2-(2, 4-difluorophenyl)-3-(5-fluoro pyrimidin-4-yl) -1- (1, 2, 4-triazol-1-yl) butan- 2-ol (Fig. 5).[26] Like all azole antifungal, its mechanism of action is the inhibition of a cytochrome P-450-dependent enzyme, 14-á-sterol demethylase that is essential to the synthesis of ergo sterol for the fungal cell membrane. This inhibition is more selective for fungal than for mammalian enzyme systems. The accumulation of 14-alphamethyl sterols results in a decrease in ergo sterol, which is an essential component of fungal cell wall formation. The resulting cell wall abnormalities are thought to be responsible for VOR's antifungal activity.

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 Voriconazole such as UV Spetrophotometry[27], RP-HPLC[28], HPTLC[29] and Titrimetry.[30]

Fig. I: Structures of the drugs.

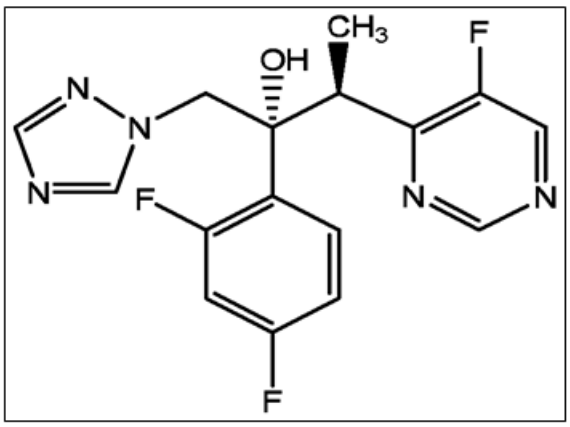

Fig. 1: Fosinopril.

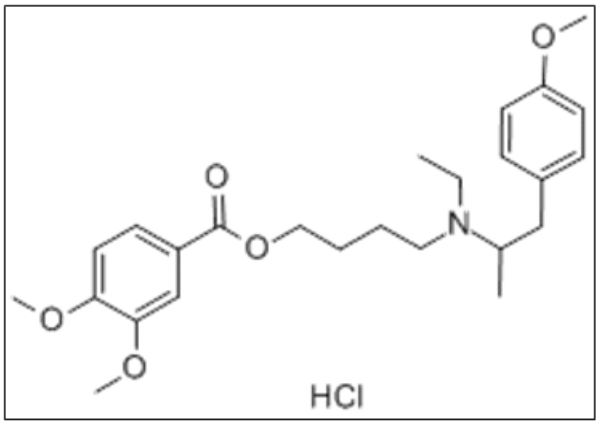

Fig. 2: Mebeverine Hydrochloride.

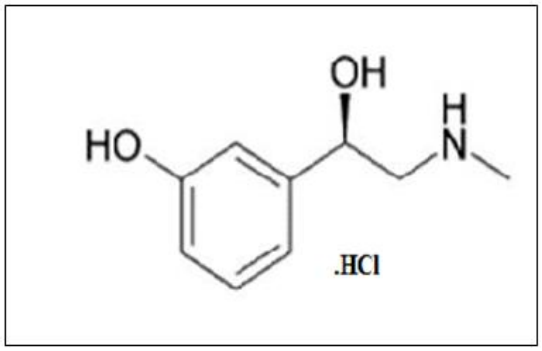

Fig. 3: Phenylephrine hydrochloride.

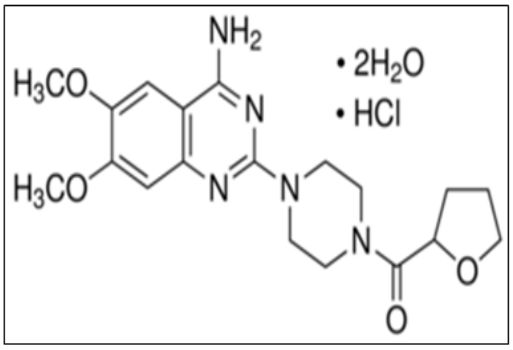

Fig. 4: Terazosine.

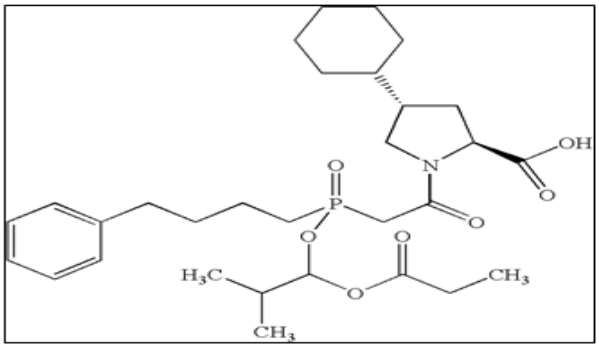

Fig. 5: Voriconazole.

MATERIALS AND METHODS

Instrumentation

All the Spectral absorbance measurements were made using an Elico 210 double beam spectrophotometer and 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

Fosinopril, Mebeverine HCl, Phenylephirine HCl, Terazosin HCl and Voricanazole 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 standard solution of drugs are prepared first in doubly distilled water by dissolving 25mg of drug in 25mL of distilled water and are further diluted to the requirement analysis. 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.

RESULT AND DISCUSSION

Procedure for Calibration

Aliquot solution of pure drugs (1-7 mL) was transferred into a 10mL volumetric flask to which 1mL of acid and 1mL of Chloramine-T were added. The contents were mixed and occasionally shaken for 15 minutes and finally 1mL of methyl orange is added, the volume is made up to mark using distilled water. The Absorbance of the solution was measured at 510nm against the blank solution prepared similarly. The same procedure of analysis is followed either for assay of pure drug or for dosage form. The calibration graphs "Fig. II" 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 standard deviation of six residual intercepts of the plots is used for calculating LOD and LOQ. The concentration of the unknown was read from the calibration graph by using Beer's law.

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 24.0, 48.0, 72.0 and 96.0µg mL-1 of FOS; 4.0, 10.0, 15.0 and 20.0 µg mL-1 of MEB; 12.0, 24.0, 36.0 and 48.0 µg mL-1 of PHE; 2.0, 4.0, 6.0, and 8.0 µg mL-1 of TER; 20.0, 40.0, 60.0 and 80.0 µg mL-1 of VOR have been taken and the recovery experiments were performed. The recoveries and their relative standard deviations are tabulated in [Table. 2].

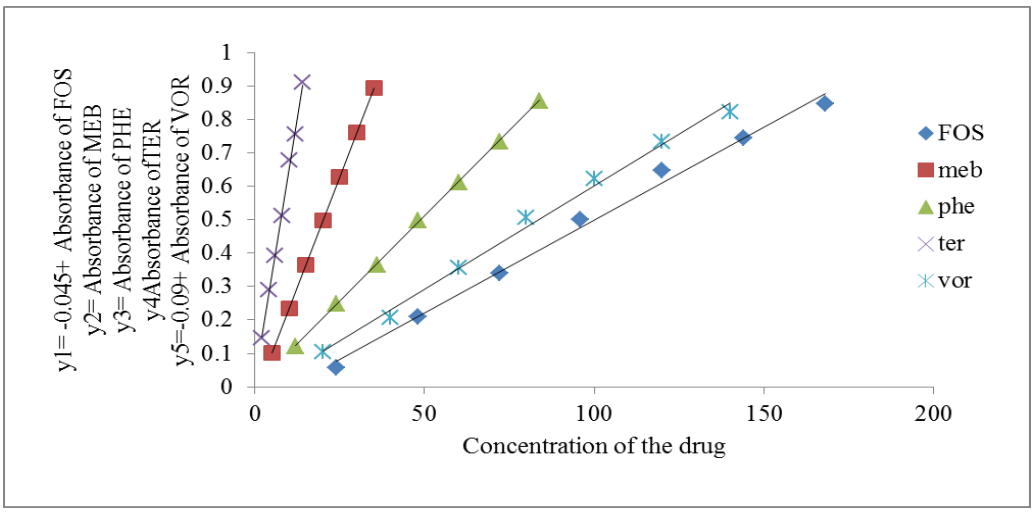

Fig. II: Calibration curves for the determination of drugs Method.

Method Validation

A linear correlation was found between absorbance at lambda max and concentration of the studied drugs. Beer's law data using 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 optical characteristics such as Beer's law limits, Slope, Intercept, Correlation coefficient, Sandell's sensitivity and Regression equation for each drug are tabulated in [Table 1]. The LOD and LOQ calculated according to ICH guidelines are also 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 formulations

Fosinopril

Twenty capsules of Fosinopril were weighed accurately and crushed to fine powder. Quantity of tablet powder equivalent to 50mg of FOS 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 Whitman filter paper No.41. The volume was made up to the mark of 100mL volumetric flask with distilled water. This solution was further diluted to get working concentration.

Mebeverine HCl

Twenty tablets of MEVA sr were finely grinded and mixed. An accurately weighed 50 mg of MEB was taken into a 100 mL volumetric flask, sonicated and remaining volume is made up with distilled water. This solution was further diluted to get working concentration.

Phenylephrine HCl

Ten tablets of Dolgencorp were weighed accurately and powdered. The powder equivalent to 50 mg of PHE 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.

Terazosin HCl

Twenty tablets of Terazen were weighed and ground into a fine powder. An amount of tablet powder equivalent to 50 mg of TER 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 Whitman No. 41 filter paper into a clean 100 mL volumetric flask and the volume was brought up to the mark with the distilled water. This solution was further diluted to get working concentration.

Voriconazole

Twenty tablets of Vfend were finely grounded and mixed. An accurately weighed 50 mg of VOR 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. This solution was further diluted to get working concentration.

To test the applicability of the method developed, pharmaceutical tablet solutions containing drug in the Beer's Law limit were chosen. For this study 26.0,36.0,56.0 and 76.0 µg mL-1 of FOS; 6.0,8.0, 12.0 and 16.0 µg mL-1 of MEB; 14.0, 20.0, 32.0 and 42.0 µg mL-1 of PHE; 2.0, 3.0, 5.0 and 10.0 µg mL-1 of TER and 24.0, 36.0, 56.0 and 70.0 µg mL-1 of VOR; 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 sprctrophotometric study.

Parameter	FOS	MEB	PHE	TER	VOR
λmax, nm	510	510	510	510	510
Beer's law limits µg mL^{-1}	24-168	5-35	12-84	2-14	20-140
Molar absorptivity, L mol^{-1} cm^{-1}	$2.66x10^3$	$9.5x10^3$	$1.8x10^3$	$2.8x10^3$	$3.3x10^3$
Sandell sensitivity* µg cm^{-2}	0.219	0.0384	0.09	0.16	0.016
Limit of detection µg mL^{-1}	11.507	39.113	1.091	15.05	11.01
Limit of quantification µg mL^{-1}	34.871	118.52	3.30	45.61	33.3
Regression equation, Y**=a+bX	0.025 +0.005X	-0.03 +0.026X	0.057 +0.011X	0.023 +0.062X	0.006 +0.066X
Slope, (b)	0.005	0.026	0.011	0.062	0.066
Intercept, (a)	0.025	-0.03	0.057	0.023	0.006
Correlation coefficient, (r)	0.988	1.00	0.957	0.966	0.992

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	Taken (µg/mL)	Found (µg/mL)	er (%)	Recovery (%)	RSD (%)	Proposed method Mean ± SD
FOS	24	23.98	0.08	99.92	0.042	99.95 ± 0.04
	48	47.96	0.08	99.92		
	72	72.00	0.00	100		
	96	95.98	0.02	99.98		
MEB	5	4.96	0.80	99.20	0.34	99.7 ± 0.34
	10	9.98	0.20	99.80		
	15	14.97	0.20	99.80		
	20	20.00	0.00	100		
PHE	12	11.96	0.33	99.60	0.14	99.8 ± 0.1401
	24	23.98	0.83	99.91		
	36	36.00	0.00	100		
	48	47.98	0.04	99.96		
TER	2	1.98	1.00	99.00	0.051	99.96 ± 0.05
	4	4.01	0.25	100.25		
	6	5.96	0.66	99.33		
	8	7.98	0.25	99.75		
VOR	20	19.98	0.10	99.90	0.05	99.96 ± 0.051
	40	40.01	0.02	100.02		
	60	59.98	0.03	99.96		
	80	79.98	0.02	99.97		

Table 3: Results of assay of tablets by the proposed methods by recovery experiments and statistical evaluation method.

Drug	Taken (µg/mL)	Found (µg/mL)	er (%)	Recovery (%)	RSD (%)	Proposed method Mean ± SD	Reference method Mean ± SD	t-test	F-test
FOS	26 36 56 76	25.98 35.96 56 76.01	0.076 0.111 0 0.013	99.92 99.88 100.00 100.013	0.060	99.95 ± 0.059	99.42 ±0.7718	2.225	0.005
MEB	6 8 12 16	5.98 8.01 11.96 15.98	0.33 0.125 0.33 0.125	99.66 100.125 99.66 99.875	0.218	99.83 ±0.21	99.96 ±0.732	1.679	0.080
PHE	14 20 32 42	13.98 20.01 32 41.98	0.142 0.05 0 0.04	99.86 100.05 100.00 99.96	0.082	99.83 ±0.0821	100.94 ±1.042	2.240	0.006
TER	2 3 5 10	1.98 2.98 5.01 9.98	1 0.66 0.2 0.25	99.00 99.33 100.2 99.80	0.520	99.58 ±0.521	99.90 ±0.470	0.174	1.220
VOR	24 36 56 70	24.01 35.98 55.96 69.96	0.04 0.05 0.07 0.05	100.041 99.95 99.93 99.95	0.052	99.96 ± 0.051	99.90 ±0.730	2.270	0.004

CONCLUSION

This method is simple, rapid and selective 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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