Sensitive Extractive Spectrophotometric Methods for Determination of Trovafloxacin Mesylate Using Bromothymol Blue and Eriochrome Black-T

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Two sensitive and selective extractive spectrophotometric methods have been developed for determination of trovafloxacin mesylate either in pure form or its pharmaceutical dosage forms as well as biological fluids. The developed methods are based on the reaction of the drug with bromothymol blue (method I) and eriochrome black-T (method II) in acidic medium. The formed ion-pair coloured complexes at pH 3.5 and 3.8 were extracted quantitatively using chloroform and the absorbance maxima of the organic layer were measured at λ_{max} 415 and 530 nm for methods I and II, respectively. Beer's law was obeyed under optimum experimental conditions and revealed that the linear concentration ranges were 2-100 and 5-130 $\mu\text{g mL}^{-1}$ with bromothymol blue and eriochrome-black T, respectively. The correlation coefficients were ($r = 0.9991$ and 0.9993) with lower detection limits 0.87 and 1.84 $\mu\text{g mL}^{-1}$ and quantification limits 2 and 5 $\mu\text{g mL}^{-1}$ for both methods, respectively. The proposed methods have been successfully applied for determination of trovafloxacin mesylate in pharmaceutical formulations and biological fluids. The interference of some common pharmaceutical additives was investigated and no interference was observed. The reliability and the performance of the proposed methods were validated according to ICH guidelines and the obtained results were statistically treated and compared with those obtained from a reported method.

Keywords: Trovafloxacin mesylate, Extractive spectrophotometry, Bromothymol blue, Eriochrome black-T, Biological fluids.

INTRODUCTION

Bromothymol blue (BTB, Fig. 1a), 4,4'-(1,1-dioxido-3H-2,1-benzoxathiole-3,3-diyl)bis(2-bromo-6-isopropyl-3-methyl-phenol) is a commonly pH sensitive dye that has been used as an indicator in many analytical reactions¹. The literature survey clarified that several methods have been reported for determination of many drugs by ion pair complex formation with bromothymol blue such as ketocanazole, clotrimazole and fluconazole², antiallergic drugs³, diltiazem hydrochloride⁴, anti-parkinsonian drug amantadine⁵, gabapentin⁶, nicardipine⁷, rupatadine fumarate⁸, ketotifen⁹ and mirtazapine¹⁰.

Eriochrome black-T (EBT), is chemically known as 3-hydroxy-4-[1-hydroxy-2-naphthalenyl]azo]-nitro-1-naphthylene sulfonic acid mono sodium salt (Fig. 1b). It is a complexometric indicator which commonly used in complexometric titrations. Several methods have been reported for determination of metals such as nickel¹¹, calcium¹², magnesium, zinc, cadmium and copper¹³. Also, eriochrome black-T was used for determination of nucleic acid¹⁴, pharmaceutical species such as ribavirin¹⁵, gatifloxacin mesylate¹⁶, antihypertensive

drug nifedipine¹⁷, antispasmodic drug drotaverine¹⁸ and epinephrine¹⁹.

Trovafloxacin (TRX), Fig. 1c, is a synthetic broad-spectrum antibacterial agent used to treat serious infections including pneumonia, complicated abdominal infections, gynecologic and pelvic infections. It is also used in the treatment of skin infections²⁰. It is chemically known as (1 α ,5 α ,6 α)-7-((6-amino-3-azabicyclo[3.1.0]hex-3-yl)-1-(2,4-difluorophenyl)-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid, monomethane sulfonate. Few analytical techniques were reported for determination of trovafloxacin in its pharmaceutical formulations and biological fluids. These include high performance liquid chromatography²¹⁻²⁵, chemiluminescence²⁶, voltammetry²⁷, spectrofluorimetry^{28,29} and capillary zone electrophoresis³⁰.

In the present study, simple and accurate spectrophotometric methods are developed for determination of trovafloxacin mesylate. The developed methods involve the formation of coloured chloroform extractable ion-pair complexes of the drug with bromothymol blue and eriochrome black-T in acidic medium. The developed methods will employed for determination of the investigated drug in pure

Fig. 1. Chemical structures of (a) bromothymol blue, (b) eriochrome black-T and (c) trovafloxacin mesylate

bulk powder, dosage forms and biological fluids such as human urine and serum.

EXPERIMENTAL

All chemicals and reagents were of analytical grade and distilled water was used throughout the experimental procedures. Pure grade of trovafloxacin and its pharmaceutical dosage forms (Trovan® 100 mg/ tablet) were supplied from Pfizer Co. Egypt. Anhydrous sodium sulfate, acetic acid (purity 99.7 %) and sodium acetate were purchased from Sigma-Aldrich (Germany). Chloroform (purity 99 %), benzene (purity 99 %), methanol (purity 99.8 %), diethyl ether (purity 99.7 %) and dichloromethane (purity 99.9 %) were purchased from BDH laboratory supplies (England). Bromothymol blue sodium salt and eriochrome black-T were obtained from fine LTD (India). Urine samples were obtained from healthy volunteers and serum samples (Multi-Serum Normal, Randox Laboratories, UK) were obtained from commercial sources.

Ultrospec-2100 pro, ultra-visible spectrophotometer with matched 1 cm quartz cells was used for all spectrophotometric measurements. Shimadzu recording spectrophotometer UV 1201 equipped with 10 mm matched quartz cells. HANNA pH meter (Romania) was used for pH adjustments.

Standard drug solution: Stock solution of trovafloxacin mesylate was prepared by dissolving 10 mg of the drug in 100 mL of methanol: water (20:80 v/v). Further dilutions 2.0-150 $\mu\text{g mL}^{-1}$ were done with the same solvent. The standard solution was stable for one week when kept in refrigerator.

Standard bromothymol blue and eriochrome black-T solutions: Standard bromothymol blue sodium salt 0.04 % (w/v) solution was prepared by dissolving 0.04 g of the dye in 100 mL distilled water. While, for eriochrome black-T 0.2 % (w/v) was prepared in 10 mL methanol then completed to 100 mL using distilled water.

Procedure for calibration graph

Method I: Aliquots of trovafloxacin mesylate standard solution (equivalent to 2-100 $\mu\text{g mL}^{-1}$ of the drug) were transferred into a series of 50 mL separating funnels. 2 mL of 0.04 % (w/v) bromothymol blue was added followed by 2 mL of acetate buffer solution of pH 3.5. The separating funnels were vigorously shaking and the ion pair complex was extracted with 3 $\times$ 3 mL chloroform. The organic layer was separated and dried over anhydrous sodium sulfate then transferred into a series of 10 mL volumetric flasks and completed to volume

using distilled water. The absorbances were measured against a blank solution treated under the same conditions at 415 nm.

Method II: Aliquots of trovafloxacin mesylate standard solution (equivalent to 5-130 $\mu\text{g mL}^{-1}$ of the drug) were transferred into a series of 50 mL separating funnels. 1 mL of 0.2 % (w/v) eriochrome black-T was added followed by 2 mL of acetate buffer solution of pH 3.8. The separating funnels were vigorously shaking and the ion pair complex was extracted with 3 $\times$ 3 mL chloroform. The organic layer was separated and dried over anhydrous sodium sulfate and transferred into a series of 10 mL volumetric flasks. The volumes were completed using distilled water and the absorbances were measured against a blank solution treated similarly at 530 nm.

Stoichiometric studies: To study the stoichiometric relationship of the ion pairs formed from the investigated drug and the reagents, Job's method of continuous variations method³¹ was employed. Master equimolar solutions 1 $\times$ 10⁻⁵ mol L⁻¹ of bromothymol blue, eriochrome black-T and trovafloxacin mesylate were prepared in methanol: water (20:80 v/v). A series of solutions was prepared in which the total volume of the drug and each reagent were kept at 10 mL. The prepared solutions were comprised different complementary proportions (0:10, 1:9 ... 9:1, 10:0) in 10 mL volumetric flasks. The absorbances of the resulting solutions were measured at 415 and 530 nm against a reagent blank treated similarly for both bromothymol blue and eriochrome black-T, respectively.

Analytical applications

Determination of trovafloxacin mesylate in Trovan® tablets: Not less than ten tablets (Trovan® 100 mg per tablet) were finally powdered and mixed well. An accurate weight of the powder equivalent to 10 mg of trovafloxacin mesylate was transferred into a 100 mL volumetric flask, about 20 mL of methanol was added and the flask was sonicated for 10 min. The solution was diluted to volume with distilled water, mixed and filtered. Serial dilutions covering the working concentration ranges of 2-100 and 5-130 $\mu\text{g mL}^{-1}$ were transferred into 10 mL volumetric flasks. Procedure described earlier, was then followed for each reagent. The content of the tablets was calculated using the calibration graph or the corresponding regression equation.

Determination of trovafloxacin mesylate in human urine and serum: The developed extractable spectrophotometric methods were applied for determination of trovafloxacin mesylate in human urine and serum using bromothymol

blue and eriochrome black-T in acidic medium. About 1 mL of human serum or 5 mL of human urine collected from healthy volunteer was spiked with accurately measured trovafloxacin mesylate. Then the spiked solutions were left aside for 5 min. 1 mL acetonitrile, 0.1 mL of NaOH (0.1 mol L^{-1}), 1 mL of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (5 % w/v) were added, where most of the interfering species (mainly proteins) were removed by precipitation³². The solutions were centrifuged for 0.5 h at 3500 rpm and the clear supernatant layer was filtered through $0.2 \mu\text{m}$ Milli-pore filter. Working solutions were then prepared by serial dilution to obtain trovafloxacin mesylate concentration ranges of 2-100 and 5-150 $\mu\text{g mL}^{-1}$. The procedure in section 2.5 for each method was employed and the absorbance was recorded at 415 and 530 nm for trovafloxacin complexes with bromothymol blue and eriochrome black-T, respectively.

RESULTS AND DISCUSSION

Absorption spectra and pH adjustment: To study the effect of acidity on the absorbance spectra of the developed methods, it is noticed that the investigated trovafloxacin mesylate drug is containing amino group in acidic medium undergoes protonation and forms with bromothymol blue and eriochrome black-T a yellow and red ion-pair complexes, respectively. The influence of pH on the absorption spectra was studied using 0.5-3.0 mL of acetate buffer at pH ranges from 2.5-6.0. The calibration plots clarified that the highest absorbance and the most stable reading was obtained in the presence of 2 mL of acetate buffer at pH 3.5 and 3.8 for trovafloxacin complexes with bromothymol blue and eriochrome black-T, respectively as shown in Figs. 2 and 3.

Fig. 2. Effect of pH of acetate buffer solution on formation of ion-pair complex of trovafloxacin with 0.04 % bromothymol blue and 0.2 % eriochrome black-T

Effect of bromothymol blue and eriochrome black-T concentrations: Various concentrations ranges (0.01-1.0) % (w/v) of each of bromothymol blue and eriochrome black-T were used to investigate their effect on the absorbance of the coloured ion-pair complexes formed by the reaction between the investigated drug with each reagent. The procedure described in 2.5. was followed and the obtained absorbance readings were plotted against bromothymol blue and eriochrome black-T concentrations. The most preferred concentrations were found to be 0.04 and 0.2 % for the previously mentioned reagents, respectively as shown in Fig. 4.

Fig. 3. Effect of volume of acetate buffer solution on ion-pair complex formation absorbance of trovafloxacin with 0.04 % bromothymol blue and 0.2 % eriochrome black-T

Fig. 4. Effect of concentration of bromothymol blue and eriochrome black-T on ion-pair complex formation absorbance with trovafloxacin

Effect of bromothymol blue and eriochrome black-T

volume: The volume of the reagents was carefully studied using various amounts (0.2-4.0 mL) of 0.04 % (w/v) bromothymol blue and 0.2 % (w/v) eriochrome black-T solutions. The procedure described earlier was followed and the obtained absorbance readings were plotted against bromothymol blue and eriochrome black-T volumes. The most preferred volume was found to be 2 and 1 mL of bromothymol blue and eriochrome black-T, respectively as shown in Fig. 5.

Fig. 5. Effect of volumes of 0.04 % bromothymol blue and 0.2 % eriochrome black-T solution on ion-pair complex formation absorbance with trovafloxacin

Effect of solvent types: The influence of solvent type on the efficiency of extraction and absorbance intensity was investigated by studying various kinds of solvents such as

(chloroform, dichloromethane, benzene and diethyl ether). The obtained results (Figs. 6 and 7) clarified that chloroform is the appropriate solvent for extraction of the studied drug. It was found that complete extraction was reacted using 3×3 mL of chloroform and then complete to 10 mL with the same solvent.

Fig. 6. Effect of different types of solvents on the extraction of trovafloxacin complex with bromothymol blue complex

Fig. 7. Effect of different types of solvents on the extraction of trovafloxacin complex with eriochrome black-T complex

Effect of extraction time and temperature: The effect of extraction time on the ion-pair complexes was examined. TFX-BTB and TFX-EBT complexes were vigorously shaking for few minutes from 0.5 to 5.0 min with chloroform. It was found that complete colour intensity was attained after 4 and 2 min for the complexes TFX-BTB and TFX-EBT, respectively. On the other hand the effect of temperature on coloured complexes was investigated by measuring the absorbance values at different temperatures. The obtained coloured complexes were stable up to 30 °C while at higher temperatures the investigated drug was concentrated due to the volatile nature of the chloroform. The absorbances of the coloured complexes TFX-BTB and TFX-EBT were found to be stable for at least 8 h at room temperature (25 ± 2 °C).

Stoichiometric ratio of the ion-pair complexes: The stoichiometric ratio of the investigated drug and each reagent in the ion-pair complexes was tested by applying the continuous variations method (Fig. 8). Equimolar solutions 1×10^{-5} mol L⁻¹ of trovafloxacin mesylate and each reagent (bromothymol blue and eriochrome black-T) were employed. The solutions were prepared as a series, in which the total volume

Fig. 8. Job's method of continuous variations graph for the reaction of trovafloxacin with bromothymol blue, and eriochrome black-T; [Drug] = [dye] = 1×10^{-5} mol L⁻¹

of the drug and reagent was kept at 10 mL for both bromothymol blue and eriochrome black-T. The results obtained indicated that the stoichiometric ratio 1:1 of the ion-pairs (TFX: BTB) and (TFX: EBT) were formed through the electrostatic attraction between positive protonated trovafloxacin mesylate and bromothymol blue and eriochrome black-T.

Method validation: To confirm that the analytical procedure is suitable for a specific test and intended uses, method validation was employed. In the present study method validation was carried out with respect to linearity, lower limit of detection, quantification limit, sensitivity, accuracy, precision, robustness and ruggedness according to ICH guidelines³³.

Linearity: At optimum experimental conditions for determination of trovafloxacin mesylate the standard calibration graph of trovafloxacin mesylate was constructed and the absorbance readings were plotted against the concentrations of the investigated drug. Beer's law was obeyed over concentration ranges of 2-100 and 5-150 $\mu\text{g mL}^{-1}$ for TFX-BTB and TFX-EBT complexes, respectively. The linear regression equations and correlation coefficients were listed in Table-1.

Lower limit of detection and quantification: The lower limit of detection (LOD) and limit of quantification (LOQ) for the proposed methods were calculated according to ICH guide-lines³³. The following equations were used:

$$\text{LOD} = 3s/k \quad \text{LOQ} = 10s/k \quad (1)$$

where s is the standard deviation of the response of the blank solution and k is the sensitivity, namely, the slope of the calibration graph. The calculated LOD were found to be 0.87 and $1.84 \mu\text{g mL}^{-1}$ and LOQ were found to be 2 and $5 \mu\text{g mL}^{-1}$ for both methods I and II, respectively.

Robustness and ruggedness of the proposed methods:

To evaluate the robustness of the proposed methods small variations of the methods variables such as concentration, volume of reagents and reaction time were done. The results obtained revealed that there is no significant difference between the results obtained by the proposed methods and those after small variations (Table-1). While, for methods ruggedness % RSD was calculated by applying the same procedure for each method using two different instruments on different days. The results obtained were found to be 0.7 and 0.4 % for TFX-BTB and TFX-EBT methods, respectively. The %RSD was less than 2 % which indicated that the developed methods were robust and rugged.

TABLE-1
CRITICAL RESPONSE CHARACTERISTICS OF TFX-BTB AND TFX-EBT COMPLEXES

Parameter	TFX-BTB	TFX-EBT
$\lambda_{\max}$ (nm)	415	530
*Beer's law limits ($\mu\text{g mL}^{-1}$)	2.0-100	5.0-130
Volume of acetate buffer (mL)	2.0	2.0
Volume of reagent (mL)	2.0	1.0
Concentration (w/v) of reagent	0.04 %	0.2 %
Extracting solvent	Chloroform	Chloroform
Extracting time (min)	4.0	2.0
**Regression equation	$Y = 0.0077x - 0.0019$	$Y = 0.0058x + 0.1841$
Correlation coefficient (r^2)	0.9991	0.9993
LOD ($\mu\text{g mL}^{-1}$)	0.87	1.84
LOQ ($\mu\text{g mL}^{-1}$)	2.0	5.0
Sandell's sensitivity ($\mu\text{g cm}^{-2}$)	0.0043	0.0065
Molar absorptivity (ϵ) $\text{dm}^3 \text{mol}^{-1} \text{cm}^{-1}$	2.97×10^3	4.57×10^3
Robustness	99.52 ± 0.8	99.14 ± 0.3
Ruggedness as %RSD	0.7 %	0.4 %

*Average of three experiments

** $Y = a + bx$

Sensitivity: Standard addition method was used to investigate the validity of the proposed methods for the determination of trovafloxacin mesylate in pure form and its pharmaceutical dosage form. The results obtained (Table-2) clarified high sensitivity of the proposed method for routine determination of trovafloxacin mesylate drug in bulk powder and its dosage form.

Accuracy and precision: Statistical analysis³⁴ of the results obtained by the proposed and reported method³⁰ (capillary zone electrophoresis using 35 mmol L⁻¹ borate buffer

and 35 mmol L⁻¹ phosphate buffer at pH 8.6 and detection at 262 nm) using student's t-test and variance ratio F-test showed no significant differences between the two methods regarding accuracy and precision, respectively. The intra-day precision was evaluated by determination of nine concentrations of pure trovafloxacin mesylate drug. The inter-day precision was also evaluated through replicate analysis of nine concentrations for a period of 3 successive days. The results of intra-day and inter-day precision are summarized in Table-3.

TABLE-2
DETERMINATION OF TROVAFLOXACIN MESYLATE IN PURE FORM AND PHARMACEUTICAL DOSAGE FORM USING BROMOTHYMOLO BLUE AND ERIOCHROME BLACK-T EXTRACTIVE SPECTROPHOTOMETRIC METHODS IN COMPARISON WITH A REPORTED METHOD [Ref. 30]

	TFX-BTB			TFX-EBT			
	Taken ($\mu\text{g mL}^{-1}$)	Found ($\mu\text{g mL}^{-1}$)	Recovery (%)	Taken ($\mu\text{g mL}^{-1}$)	Found ($\mu\text{g mL}^{-1}$)	Recovery (%)	
Pure form	2	1.95	97.5	5.0	4.89	97.8	Reported method ³⁰
	10	9.98	99.8	10.0	10.01	100.1	
	20	19.86	99.3	50.0	49.74	99.4	
	40	39.75	99.4	80.0	80.00	100.0	
	60	59.99	99.9	100.0	99.84	99.8	
	80	79.87	99.8	120.0	118.98	99.2	
	100	99.98	99.9	130.0	130.00	100.0	
Mean $\pm$ SD		99.4 ± 0.9			99.5 ± 0.8		99.0 ± 1.3
n		7			7		6
Variance		0.81			0.64		1.69
*%SE		0.34			0.30		0.53
%RSD		0.91			0.80		1.3
t-test		0.64(2.20)**			0.82(2.20)**		
F-test		2.08(4.95)**			2.64(4.95)**		
Trovan® (100 mg/tablet)	2	1.96	98.0	5.0	4.99	99.8	
	10	10.00	100.0	10.0	9.89	98.9	
	20	19.78	98.9	50.0	49.77	99.5	
	40	39.99	99.9	80.0	80.00	100.0	
	60	59.95	99.9	100.0	99.93	99.9	
	80	80.00	100.0	120.0	119.89	99.9	
	100	98.95	98.9	130.0	128.69	98.9	
Mean $\pm$ SD		99.4 ± 0.8			99.6 ± 0.5		99.5 ± 0.6
n		7			7		6
Variance		0.64			0.25		0.36
*%SE		0.30			0.19		0.24
%RSD		0.80			0.50		0.60
t-test		0.26(2.20)**			0.33(2.20)**		
F-test		1.78(4.95)**			1.44(4.95)**		

*%SE = $SD/\sqrt{n}$ **Fig. in parentheses are the tabulated t- and F- values at confidence limit 95 % [Ref. 34]

TABLE-3
VALIDATION OF THE PROPOSED METHODS FOR DETERMINATION OF TROVAFLOXACIN IN PURE FORM

TFX-BTB				TFX-EBT		
	Taken ($\mu\text{g mL}^{-1}$)	Found ($\mu\text{g mL}^{-1}$)	Recovery %	Taken ($\mu\text{g mL}^{-1}$)	Found ($\mu\text{g mL}^{-1}$)	Recovery %
Intra-day precision	2.0	1.96	98.0	5.0	4.98	99.6
	4.0	3.99	99.8	10.0	9.92	99.2
	6.0	5.97	99.5	20.0	19.93	99.7
	8.0	7.82	97.8	40.0	39.99	99.9
	10.0	9.92	99.2	60.0	59.89	99.8
	20.0	20.0	100.0	80.0	79.91	99.9
	50.0	49.89	99.8	100.0	99.96	99.9
	80.0	80.00	100.0	120.0	119.87	99.8
	100.0	99.98	99.9	130.0	130.00	100.0
Mean $\pm$ SD	99.3 $\pm$ 0.9			99.8 $\pm$ 0.2		
n	9			9		
Variance	0.81			0.04		
*%SE	0.30			0.07		
%RSD	0.90			0.20		
Inter-day precision	2.0	2.00	100.0	5.0	4.98	99.6
	4.0	3.95	98.8	10.0	9.83	98.2
	6.0	5.97	99.5	20.0	20.00	100.0
	8.0	7.88	98.5	40.0	39.74	99.4
	10.0	9.99	99.9	60.0	59.50	99.2
	20.0	20.00	100.0	80.0	79.72	99.6
	50.0	49.87	99.7	100.0	99.90	99.9
	80.0	80.00	100.0	120.0	119.92	99.9
	100.0	99.58	99.6	130.0	129.82	99.8
Mean $\pm$ SD	99.6 $\pm$ 0.6			99.5 $\pm$ 0.5		
n	9			9		
Variance	0.36			0.25		
*%SE	0.20			0.16		
%RSD	0.60			0.50		
*% SE = SD/ $\sqrt{n}$						

*% SE = SD/ $\sqrt{n}$

TABLE-4
DETERMINATION OF TROVAFLOXACIN MESYLATE IN HUMAN URINE AND SERUM USING BROMOTHYMOLO BLUE AND ERIOCHROME BLACK-T EXTRACTIVE SPECTROPHOTOMETRIC METHODS IN COMPARISON WITH A REPORTED METHOD [REF. 30]

TFX-BTB			TFX-EBT			Reported method ³⁰
Taken ($\mu\text{g mL}^{-1}$)	Found ($\mu\text{g mL}^{-1}$)	Recovery (%)	Taken ($\mu\text{g mL}^{-1}$)	Found ($\mu\text{g mL}^{-1}$)	Recovery (%)	
Urine sample	2.0	1.98	99.0	5.0	4.85	97.0
	20.0	19.72	98.6	50.0	49.56	99.1
	40.0	39.65	99.1	80.0	79.99	99.9
	60.0	59.99	99.9	100.0	99.69	99.7
	80.0	79.93	99.9	120.0	119.98	99.9
	100.0	98.92	98.9	130.0	129.92	99.9
Mean $\pm$ SD	99.2 $\pm$ 0.5		99.3 $\pm$ 1.1			99.5 $\pm$ 0.9
n	6		6			6
Variance	0.25		1.21			0.81
%SE	0.20		0.44			0.36
%RSD	0.50		1.11			0.90
t-test	0.73(2.23)*		0.35(2.20)*			
F-test	3.24(5.05)*		1.49(5.05)*			
Serum sample	2.0	1.99	99.5	5.0	4.86	97.2
	20.0	19.65	98.3	50.0	49.50	99.0
	40.0	39.86	99.7	80.0	79.96	99.9
	60.0	59.63	99.4	100.0	99.99	99.9
	80.0	80.00	100.0	120.0	119.89	99.9
	100.0	99.98	99.9	130.0	129.63	99.7
Mean $\pm$ SD	99.5 $\pm$ 0.6		99.2 $\pm$ 1.1			99.3 $\pm$ 0.7
n	6		7			6
Variance	0.36		1.21			0.49
%SE	0.24		0.44			0.28
%RSD	0.60		1.10			0.70
t-test	0.54(2.23)*		0.19(2.23)*			
F-test	1.36(5.05)*		2.46(5.05)*			

*Fig. in parentheses are the tabulated t- and F-values at confidence limit 95 % [Ref. 34]

Determination of trovafloxacin mesylate in human urine and serum: Due to the high sensitivity of the developed methods, we tried to check their applicability to determine trovafloxacin mesylate in biological fluids such as human urine and serum. The obtained results (Table-4) indicated good accuracy and precision as the recovery values in human urine were 99.2 ± 0.5 and 99.3 ± 1.1 for methods I and II, respectively. While, for human serum recovery values were 99.5 ± 0.6 and 99.2 ± 1.1 for methods I and II, respectively, with %RSD of 0.5 and 1.1, 0.6 and 1.1 % for human urine and serum for methods I and II, respectively.

Conclusion

New sensitive and selective extractive spectrophotometric methods for the determination of trovafloxacin mesylate were developed. The developed methods were based on the formation of extractable coloured ion-pair complexes from the reaction of trovafloxacin mesylate with bromothymol blue and eriochrome black-T in acidic medium. It can be evident that beside their sensitivity and simplicity both methods can be employed for determination of trovafloxacin mesylate without elaborated treatment of the samples. No critical reagents or expensive instruments are required. The accuracy and reproducibility of the proposed methods introduce great value and encourage the analytical application of trovafloxacin in pure bulk drug, its pharmaceutical dosage forms and biological fluids.

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REFERENCES

1. R.W. Sabnis, Handbook of Acid-Base Indicators, CRC Press, ISBN: 0-8493-8218-1 (2007).
2. N. Alizadeh and Z. Rezakhani, *J. Chil. Chem. Soc.*, **57**, 1104 (2012).
3. K.C. Ramesh, B.G. Gowda, M.B. Melwanki, J. Seetharamappa and J. Keshavayya, *Anal. Sci.*, **17**, 1101 (2001).
4. N. Rahman and S.N. Hejaz-Azmi, *J. Pharm. Biomed. Anal.*, **24**, 33 (2000).
5. H.A. Omara and A.S. Amin, *J. Saud. Chem. Soc.*, **16**, 75 (2012).
6. B. Patel, J. Patel, H. Singh and B. Patel, *Int. J. Pharm. Sci. Drug Res.*, **3**, 197 (2011).
7. Y. Duneetha and N.V.S. Naidu, *Int. J. Sci. Res.*, **3**, 25 (2014).
8. R.V. Rele, P.D. Desai and S.A. Sawant, *Int. J. Chem. Sci.*, **8**, 22 (2010).
9. A.K. Mahmood, *J. Al-Nah. Univ.*, **16**, 1 (2013).
10. M. Ravi, T.L.G. Veeraiah and C.V.R. Reddy, *Orient. J. Chem.*, **30**, 723 (2014).
11. O. Cakir, E. Coskun, E. Bicer and S. Cakir, *Turk. J. Chem.*, **25**, 33 (2001).
12. A. Young and T.R. Sweet, *Anal. Chem.*, **27**, 418 (1955).
13. S.B. Dabhade and S.P. Sangal, *Microchem. J.*, **13**, 193 (1968).
14. H. Zhou, X. Wu, F. Meng, J. Yang and M. Wang, *Spectrochim. Acta A*, **78**, 681 (2011).
15. N.A. Alarfaj, R.A. Ammar and M.F. El-Tohammy, *Asian J. Chem.*, **26**, 2992 (2014).
16. R.A. Sayed, W.S. Hassan, M.Y. El-Mamml and A.A. Shalaby, *Orient. J. Chem.*, **28**, 639 (2012).
17. N. Rahman, N. Ahmad Khan and S.N. Hejaz Azmi, *IL Farmaco*, **59**, 47 (2004).
18. G. Tuljarani, D. Gowrisankar, P. Kadgapathi, R. Suthakaran and B. Satyanarayana, *Int. J. Pharm. Tech. Res.*, **2**, 1787 (2010).
19. H. Yao, Y. Sun, X. Lin, Y. Tang, A. Liu, G. Li, W. Li and S. Zhang, *Anal. Sci.*, **23**, 677 (2007).
20. National Committee for Clinical Laboratory Standards, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically "C Fourth Edition; Approved Standard, NCCLS Document M7-A4, Vol. 17, No. 2, NCCLS, Wayne, PA, January, (1997).
21. A.H. Mahmood, G.A. Medley, J.E. Grice, X. Liu and M.S. Roberts, *J. Pharm. Biomed. Anal.*, **62**, 220 (2012).
22. J. Sousa, G. Alves, G. Campos, A. Fortuna and A. Falcão, *J. Chromatogr. B*, **930**, 104 (2013).
23. H. Liang, M.B. Kays and K.M. Sowinski, *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, **772**, 53 (2002).
24. R. Teng, T.G. Tensfeldt, T.E. Liston and G. Foulds, *J. Chromatogr. B Biomed. Appl.*, **675**, 53 (1996).
25. K. Borner, H. Hartwig and H. Lode, *J. Chromatogr. A*, **846**, 175 (1999).
26. J.A. Ocana, M. Callejon and F.J. Barragan, *Eur. J. Pharm. Sci.*, **13**, 297 (2001).
27. J.L. Vilchez, J. Taoufiki, L. Araujo and A. Navalón, *J. Pharm. Biomed. Anal.*, **31**, 465 (2003).
28. J.L. Vilchez, J. Taoufiki, O. Ballesteros and A.M. Navalon, *Mikrochim. Acta*, **150**, 247 (2005).
29. J.L. Vilchez, J. Taoufiki and A. Navalón, *Anal. Lett.*, **35**, 257 (2002).
30. J.L. Vilchez, L. Araujo, A. Prieto and A. Navalon, *J. Chromatogr. A*, **56**, 351 (2002).
31. A.M. El-Didamony and M.A. Moustafa, *Arab. J. Chem.*, **3**, 265 (2010).
32. A.F. Al-Ghamdi, M.M. Hefnawy, A.A. Al-Majed and F.F. Belal, *Chem. Cent. J.*, **6**, 15 (2012).
33. ICH Technical Requirements for Registration of Pharmaceuticals for Human Use, Complementary Guidelines on Methodology, Washington, DC, vol. 13 (1996).
34. J.C. Miller and J.N. Miller, Statistics for Analytical Chemistry, Ellis Horwood-Prentice Hall, Chichester, edn 3 (1993).