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ORIGINAL PAPER

Rapid and Sensitive Online Determination of Some Selective α_1-Blockers by Flow Injection Analysis with Micelle-Enhanced Fluorescence Detection

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Abstract A rapid, sensitive and selective flow injection analysis (FIA) method was developed for the determination of some selective α_1-blockers including; terazosin (TER), doxazosin (DOX), prazosin (PRZ), and alfuzosin (ALF). The method was based on enhancement of the native fluorescence of the studied drugs in the presence of sodium dodecyl sulfate (SDS). The method was optimized for the buffer type, concentration and pH, surfactant type and concentration, flow rate and detection wavelengths in order to achieve the maximum sensitivity. The results showed that the best sensitivity was obtained by using SDS (10 mM) in phosphate buffer (20 mM, pH=3), flow rate was 0.5 ml/min and the detector was set at λ_{ex}=250 and λ_{em}=389. Under these optimum conditions there was a linear relationship between the concentration and the fluorescence intensity in the range from 5–400 ng ml^{-} with correlation coefficient of more than 0.998. The detection and quantitation limits for the studied drugs by the proposed method were 3.2–11.9 ng ml^{-1} and 10.8–39.7 ng ml^{-1}, respectively. The method was validated in accordance with the requirements of ICH guidelines and shown to be suitable for intended applications. Moreover, the binding constants for α_1–blockers –SDS system were determined using the adduct model. The proposed method has been applied successfully for the analysis of the pure forms for studied drugs and also their pharmaceutical formulations and the results were compared with official methods.

Keywords Selective α_1—blockers · Flow injection analysis · Fluorescence · SDS · Pharmaceutical formulations

Introduction

α_1-Adrenoceptor antagonists are now well established as the most common treatment for lower urinary tract symptoms associated with benign prostatic hyperplasia although initially introduced for the management of hypertension [1]. However, these agents have the potential to produce orthostatic hypotension and other blood pressure-related adverse effects in normotensive patients and in those receiving concurrent treatment with other antihypertensive agents as a result, more "uroselective", less vasoactive α-blockers have been developed [2, 3]. In an order to minimize these side effects, selective α_1-antagonists, e.g. prazosin (PRZ), were subsequently developed. More recently, agents such as alfuzosin(ALF), doxazosin(DOX), and terazosin(TER) introduced and claimed for "uroselectivity" and "prostate" selectivity have emerged [4, 5]. Chemically, selective α_1-adrenergic receptor antagonists are 6,7-dimethoxyquinazolin-4-amine derivatives including; prazosin(PRZ), terazosin(TER), doxazosin(DOX) and alfuzosin(ALF)as shown in Fig. (1) [6].

To date, several analytical methods were reported for the determination of selective α_1-antagonists in pure forms as well as in their pharmaceutical formulations. These methods included; spectrophotometry [7–15], high performance thin layer chromatography [16–18], and high performance liquid chromatography (HPLC) [19–33]. However; most of these reported methods have some practical complications for routine laboratory use; spectrophotometric methods were not selective and sensitive enough, chromatographic methods require long analysis time, and LC-MS methods are relatively expensive and require technical experiences. Additionally, some of these methods caused significant environmental pollutions. In this sense, green chemistry discipline is to assure the sustainable development of direct monitoring methods

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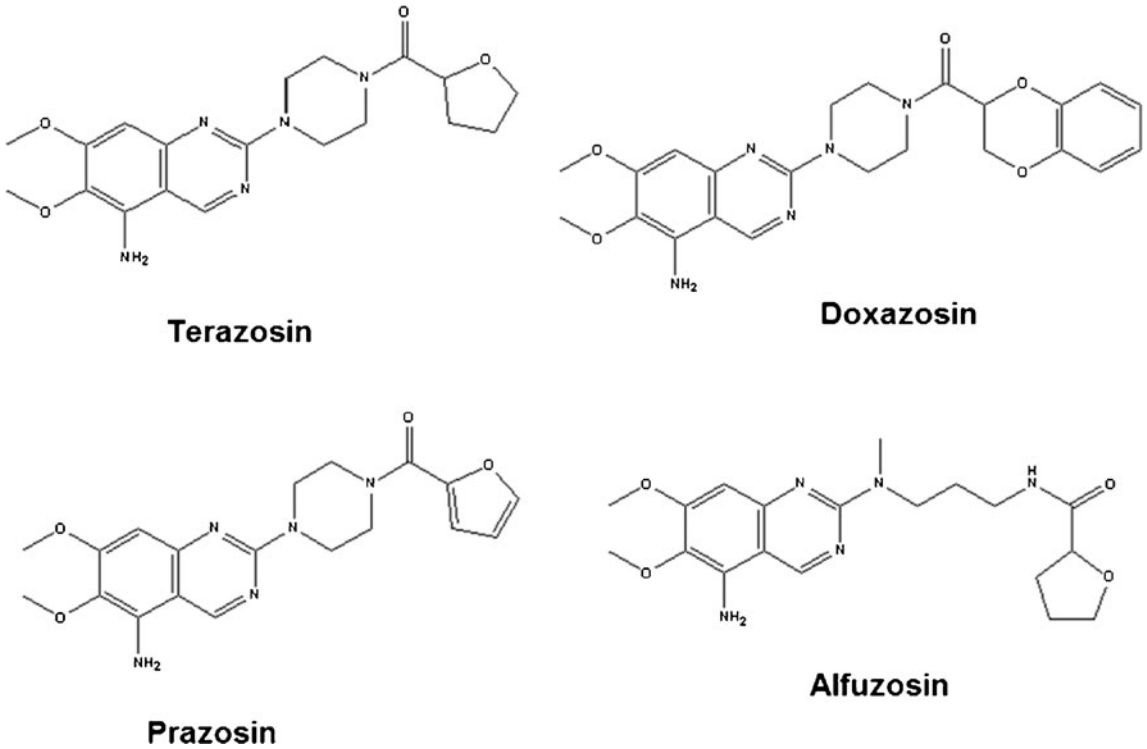


Fig. 1 Chemical structures of selective α_1-blockers

involving the use of non-toxic reagents without sample preparation. Therefore, we intended in this study to develop a fast and environmentally safe method for the selective determination of selective α_1-blockers using online flow injection analysis (FIA) technique. The loss in sensitivity produced in flow injection systems by dispersing the analyte into the carrier stream without enhancer led us to design a system to enhance the detection signals. The method was based upon enhancement of their native fluorescence of the selective α_1-blockers by employing an anionic surfactant sodium dodecyl sulfate (SDS). The aggregation process of SDS and α_1-blockers was investigated at the critical micelle concentration (CMC). Under established optimal conditions, the system was adapted to the quantitative determination of the studied drugs in their pure forms as well as their pharmaceutical formulations. The method was validated according to ICH guidelines [34].

Experimental

Instrumentation

For FIA measurements, a Younglin Autochro-3000 system (Younglin, Korea) with fluorescence detector (FP, Jasco, Japan) and without column was used. A Rheodyne injection valve with a 20-μL loop was used. The fluorescence detector was set at λ_{ex} 250 nm and λ_{em} 389 nm. The sample or standard solutions containing α_1-blockers was injected and combined with the carrier stream. The carrier solution was prepared with SDS (10 mM) in 20 mM phosphate buffer of pH 3.0. The carrier solution was prepared daily then filtered and degassed by sonication before use. All measurements were carried out at a flow rate 0.5 mL min^{-1}. A Shimadzu RF-5301PC spectrofluorimeter (Shimadzu Corporation, Kyoto, Japan), equipped with a Xenon discharge lamp and 1 cm quartz cells were used for measurements of the fluorescence spectra in batch method. A pH meter (Orion Expandable Ion Analyzer, Orion Research, Cambridge, MA, USA), model EA 940 with combined glass electrode was used for pH measurements.

Reagents and Chemicals

PRZ, TER, DOX and ALF were purchased from European Egyptian Pharmaceuticals Industries (Cairo, Egypt). Methanol (HPLC grade) was purchased from Sigma Aldrich (Seelze, Germany). SDS, Carboxy methyl cellulose, Cetrimide, and Tween 60 and 80 were from Novartis Pharma AG (Basle, Switzerland). Sodium dihydrogen phosphate, sodium hydroxide, and phosphoric acid for pH adjustment were from Cairo Pharmaceuticals Co. (Cairo, Egypt). Double distilled water was obtained through WSC-4D water purification system (Hamilton Laboratory Milton Glass Ltd., Kent, USA). Pharmaceutical preparations as Itrin®tablets (2 mg TER) obtained from Kahira/Abbot Co. (Cairo, Egypt), Terazin®tablets (5 mg TER) from(Pharaonia Co., (Alexandria, Egypt), Cardura®tablets (1 mg DOX) from Pfizer Co., (Cairo, Egypt), Dosin®tablets (1 mg DOX) from Eipico Co., (Cairo, Egypt), Doxacor®tablets (2 mg DOX) from Hexal Co., (Cairo, Egypt), Minipress®tablets

(1 mg PRZ) from Pfizer Co. (Cairo, Egypt),and Xatral S.R®tablets (5 mg ALF) from Amriya/Synthelabo Co. (Alexandria,Egypt)

Preparation of the Standard Solutions

Stock solution of selective α_1-blockers standards solutions (100 μg ml^{-1}) were prepared by transferring an accurately weighed α_1-blockers salt solution, equivalent to about 10 mg in 100 mL volumetric flask, diluted with ultra-pure methanol, mixed and completed to the volume then stored in the fridge at 4 °C in well-closed light resistant containers. The working solutions were prepared by further dilution of the stock solution with methanol immediately before use. SDS solution (10 mM of SDS in 100 ml Ultra pure water),and 20 mM phosphate buffer solution from sodium dihydrogen phosphate in pure water and adjust pH to 3 by phosphoric acid.

FIA Configuration

The FIA system used for the online micelle-enhanced fluorescence determination of α_1-blockers was consisted of a pump, which was used to propel the carrier stream through a narrow tube; an injection port, through which a well-defined volume of a sample solution was injected into the carrier stream in a reproducible manner; and a microreactor in which the sample zone dispersed and reacted with the components of the carrier stream, forming a species which was sensed by a flow through detector and recorded. A bypass loop allowed passage of carrier when the injection valve is in the load position. A stream of sample or standard solutions containing α_1-blockers was combined with the carrier stream. The carrier stream consisted of a solution prepared with SDS (10 mM), and phosphate buffer (pH 3) to obtain the optimal conditions for α_1-blockers fluorescent emission at flow rate 0.5 ml min^{-1}. The drugs sample and the carrier stream interacted in the reactor and flowed to the fluorescence detector measured at λ_{ex} 250 nm and λ_{em} 389 nm. The valve was switched in such a manner that in one position it allowed the flow of the carrier stream and in the second position the flow of the sample or standards and the carrier solution. The used FIA system is presented in suppl. (Fig. 1).

Method Validation

The validation was performed according to International Conference on Harmonization (ICH) guidelines [34].

Linearity

The linearity of the method was checked by analyzing six solutions in the range 5–80 ng mL^{-1} for TER (5, 10, 20, 40, 60, and 80 ng mL^{-1}), 20–200 ng mL^{-1} for DOX (20, 40, 60, 80, 100 and 200 ng mL^{-1}), 20–400 ng mL^{-1} for PRZ (20, 40, 80, 100, 200 and 400 ng mL^{-1}),and 20–200 ng mL^{-1} for ALF (20, 40, 80, 100, 150 and 200 ng mL^{-1}). Each solution was prepared in triplicate. Calibration curves were constructed as fluorescence intensity on Y-axis versus the concentrations on X-axis and the linear relationship calibration parameters were determined.

Limits of Detection and Quantification

The limit of detection (LOD) was defined as the least amount of the analyte that can be readily detected but not necessarily quantified. It is usually regarded as the amount which the signal-to-noise ratio (S/N) is 3:1. The limit of quantitation (LOQ) was defined as the least amount of the analyte that can be quantified with good accuracy and precision. It is usually regarded as the amount which the S/N is 10:1. Samples with known concentrations of each analyte were prepared and analyzed by the proposed FIA method. LOD and LOQ were then established experimentally by evaluating the minimum levels at which the analyte could be readily detected or accurately quantified are 3.24–11.91 ng ml^{-1} and 10.80–39.70 ng ml^{-1}, respectively.

Accuracy and Precision

Intra-day precision was checked by repeated analysis (n=6) of standard solutions at three different concentration; low, medium, and high concentration levels (5, 20 and 80 ng mL^{-1}) for TER, (20, 80 and 200 ng mL^{-1}) for DOX, (20, 80 and 400 ng mL^{-1}) for PRZ, and for ALF (20, 80 and 200 ng mL^{-1}). The inter-day precision was tested by repeating the analysis over a period of three consecutive working days. The overall precision of the method was expressed as relative standard deviations (RSD). Method accuracy was determined by analyzing known amounts of standard TER, DOX, PRZ and ALF to a sample solution of known concentration and comparing measured and calculated values. The accuracy was expressed as percent to the true value,

Robustness

The robustness of an analytical method is a measure of its ability to be unaffected by small, but deliberate variations in method conditions. Robustness test gives an indication of the proposed method reliability during normal usage. For the determination of a method's robustness, the method parameters such as buffer pH, mobile phase composition, surfactant concentration, flow rate and emission wavelength, were varied within the optimum range, and the quantitative influence of the variables was determined.

Study of SDS Micelle Formation with α_1-Blockers

The study of SDS micelle formation with selective α_1-blockers was conducted by Molecular Operating Environment (MOE®)

software [35]. All the molecular modeling calculations were performed using MOE® version 10.2010,1 Chemical Computing Group Inc., Montreal, Canada. The computational software operated under "Windows XP". All the interaction energies and different calculations were automatically calculated. Target compounds optimization; the target compounds were constructed into a 3D model using the builder interface of the MOE program. They were subjected to energy minimization, all the minimizations were performed with MOE until a RMSD gradient of 0.01 Kcal/mole and RMS distance of 0.1 Å with MMFF94X force-field and the partial charges were automatically calculated [35].

Analysis of Dosage Forms

Ten tablets were weighed and finely powdered. An accurately weighed quantity of the powder equivalent to 5, 10 or 25 mg of α_1-blockers was transferred into a 50 ml calibrated flask, and dissolved in about 20 ml of methanol. The contents of the flask were swirled, sonicated for 5 min, and then completed to volume with methanol. The contents were mixed well and filtered rejecting the first portion of the filtrate. The prepared solution was diluted quantitatively with methanol to obtain a suitable concentration for the analysis for assay. An aliquot of 20-µL was injected to FIA system.

Results and Discussion

Fluorescence Characteristics of α_1-Blockers

Fluorescence characteristics of selective α_1–blockers in aqueous media in the presence and absence of SDS were studied. TER, DOX, PRZ and ALF exist in solution as ionized species, neutral form, and/or molecular aggregates, depending on pH, solvent, and concentration [36]. The excitations and emissions of spectra of the studied α_1– blockers PRZ (5 µg ml^{-1}), DOX (8 µg ml^{-1}), TER (5 µg ml^{-1}) and ALF (3 µg ml^{-1}) in aqueous media in the presence and absence of SDS in pH=3 were shown in Fig. 2. The emission maxima for different α1—blockers with SDS were ranged from 387–396 nm using a slit width of±5 nm in the spectrofluorimeter. Therefore, ± 5 nm variations were expected in the measured emission maxima. Moreover, variations in emission maxima were studied between 379 and 398 nm and no significance differences were observed in the measured values for all the studied drugs. Hence, the detection emission wavelength was fixed at 389 nm for the emission maxima for all the studied drugs.

Experimental data showed that the enhancement factor for α_1 blockers –SDS system (5–6 folds respect to fluorescence intensity in aqueous medium). The enhancement of the studied drugs fluorescence intensity by addition of SDS was associated

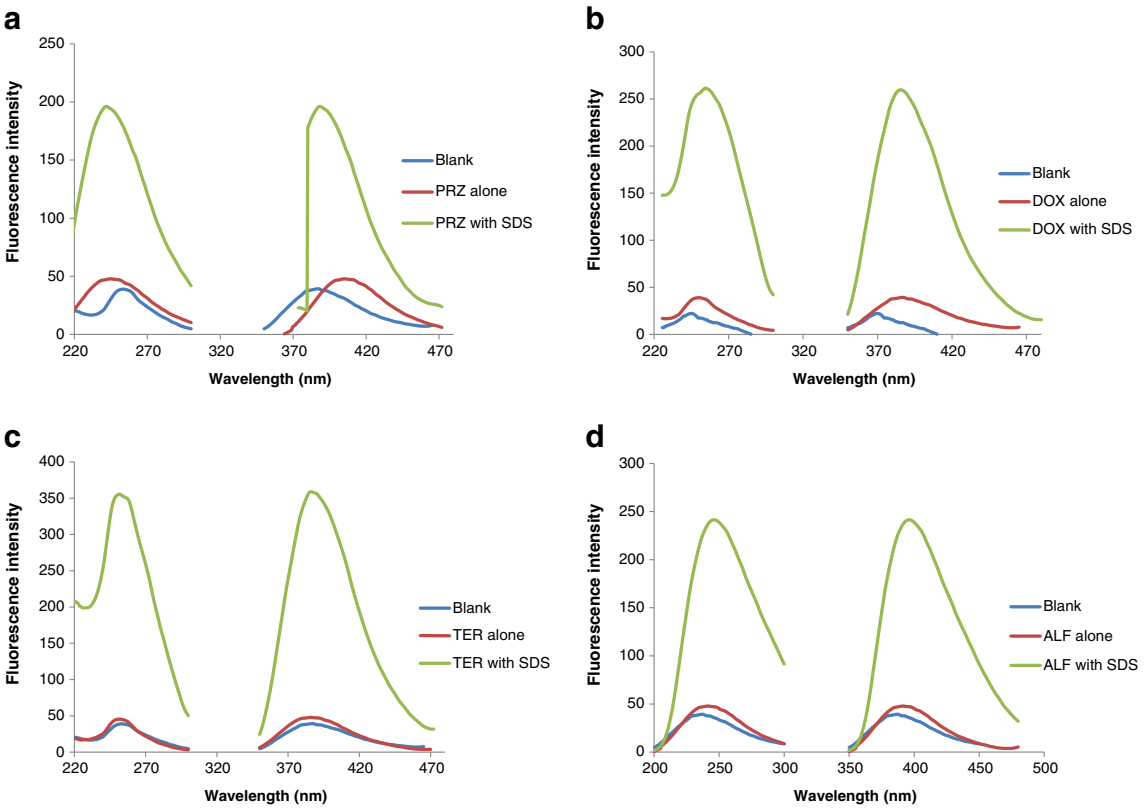


Fig. 2 Fluorescence spectra of (**a**) PRZ,5 µg ml^{-1} (**b**) DOX,8 µg ml^{-1} (**c**) TER, 5 µg ml^{-1} (**d**)ALF, 3 µg ml^{-1} in the optimal working conditions (phosphate buffer 20 mM, pH 3 and SDS 10 mM). Where; *a*: blank, *b*:drug alone, and *c*:drug with SDS

Table 1 Optimization of FIA variables for selective α_1—blockers SDS system

Compound	Calibration curve[a] (n=3)				Detection limit (ng ml^{-1}) without SDS	Detection limit (ng ml^{-1}) with SDS	Quantitation limit (ng ml^{-1}) with SDS	λex (nm)	λem without SDS (nm)	λem with SDS (nm)
	Range (ng mL^{-1})	Slope[b](±SD)	Intercept[b](±SD)	r						
TRZ	5–80	1.05±0.02	1.22±1.14	0.9987	3.2	39.3	10.8	250	377	387
DOX	20–200	0.30±0.01	−1.91±0.82	0.9990	8.2	103.0	27.2	254	370	389
PRZ	20–400	0.13±0.01	−0.18±0.18	0.9999	4.2	326.5	13.8	250	387	390
ALF	20–200	0.11±0.01	2.42±0.45	0.9977	11.9	207.8	39.7	248	392	396

[a] Relative Fluorescence Intensity versus concentration (ng mL^{-1})

[b] Data presented as mean ± SD of three experiment

to a slight bathochtomic shift (red shift) of the maximum λem. Excitation and emission wavelengths of the studied α_1 blockers in the presence and absence of SDS were presented in Table 1. Traditionally α_1 – blockers have large molar absorptivity in the visible region of the electromagnetic spectrum, which is attributed to a $\pi \rightarrow \pi^*$ transition. The fluorescence intensities were influenced by the substituent of the amino groups of the quinazoline base [36]. Figure 3 shows the three–dimensional (3D) excitation–emission plot for the excitation and emission spectra of TER solution (5 μg mL^{-1}) in the presence of 10 mM SDS and phosphate buffer (20 mM, pH 3) in batch method.

It was found that these characteristic spectra contain rich information, such as the peak positions and peak relative intensities. Moreover, the fluorescence properties of the studied α1–blockers could be identify by these characteristic spectra. These results indicated that the fluorescent product(s) can be generated inside the anionic micellar core and the nature of the fluorescence intensity enhancement was probably due to the formation of the spherical micelles. The methoxy groups and benzene rings of quinazoline incorporated within the hydrophobic micelle interior and the cationic charge on amino group of quinazoline ring represented the polar part to outside [37]. The fluorescence increase in micellar media was attributed to a stabilization/protection of the excited state singlet that hinders decay by quenching and other non-radiative deactivation processes [38, 39].

Optimization of FIA Conditions

Nature and Concentration of Surfactant Agents

Addition of a surfactant above its critical micelle concentration (CMC) increases the fluorescence intensity of many fluorophores [40]. This fact has been used to develop improved methods for spectrofluorimetric determination of many fluorophores [40]. The fluorescence properties of α_1 – blockers in various surfactant media were studied using different surfactant types including; anionic surfactants (SDS and CMC; 5 mM), cationic surfactant (Cetrimide, 5 mM) and non-ionic surfactant (tween 60 and 80 5 mM). It was found that

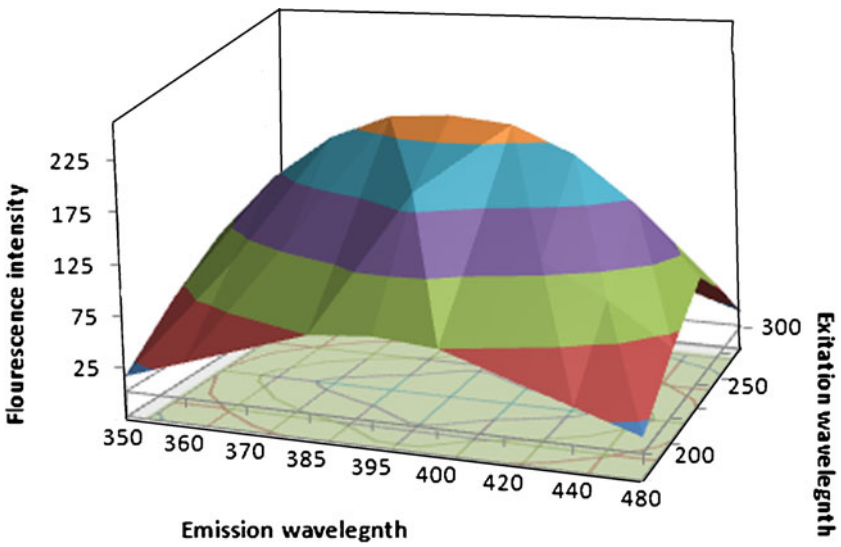


Fig. 3 Three–dimensional excitation–emission plot for the excitation and emission spectrum of TRZ

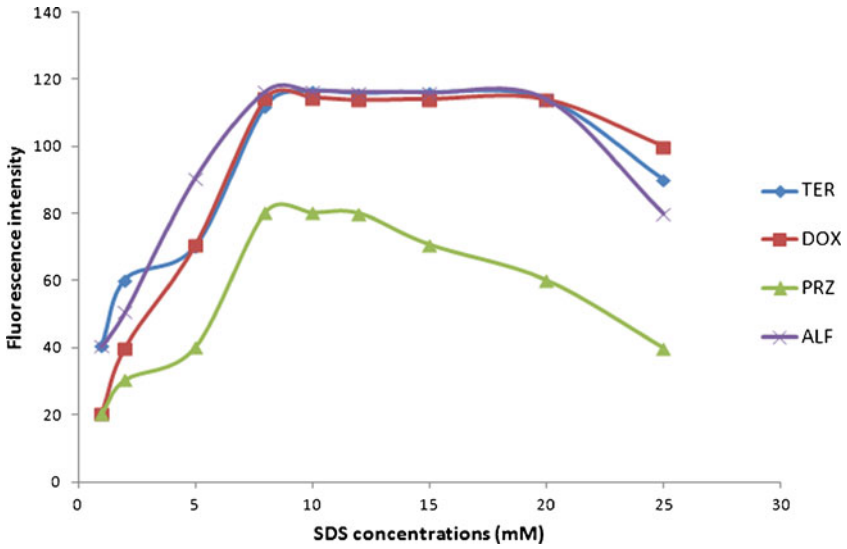


Fig. 4 Effect of the SDS concentration on the fluorescence intensities of selective $\alpha_1$blockers (8 μg ml^{-1})

SDS when used as a surfactant for micelle formation gave maximum enhancement of the fluorescence intensities for all the studied α_1 – blockers. Therefore, SDS surfactant was used for subsequent work. The modification of the features of the fluorescence spectra of α_1 – blockers occurred due to the change in the environment of the drugs by micellar formations with SDS. Furthermore, the effect of SDS concentration in the range 1–25 mM on the fluorescence intensities of the studied α_1 – blockers were investigated (Fig. 4). The intensity was enhanced as the SDS concentration increased and reached maximum at 10 mM, then remained constant in a wide interval above the critical micellar concentration (CMC). The fluorescence intensity decreased slightly at concentration above 20 mM. The decrease in the fluorescence intensity suggested the formation of mixed aggregates at concentrations above the CMC [41]. Therefore 10 mM of SDS was chosen for subsequent work.

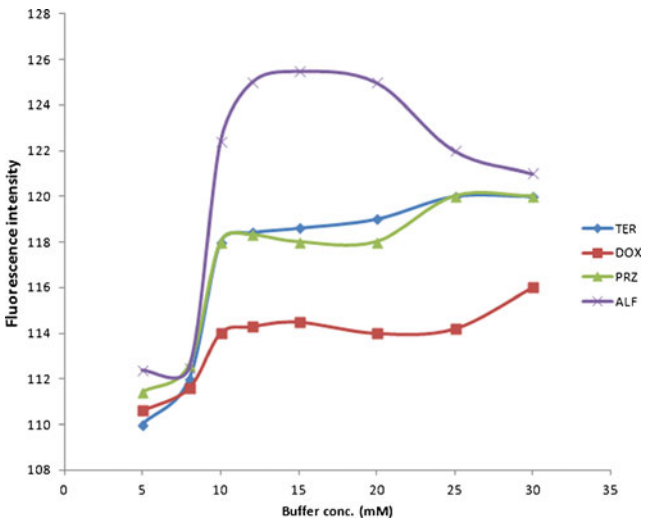


Fig. 5 Effect of the buffer concentration on the fluorescence intensities of selective $\alpha_1$blockers (8 μg ml^{-1})

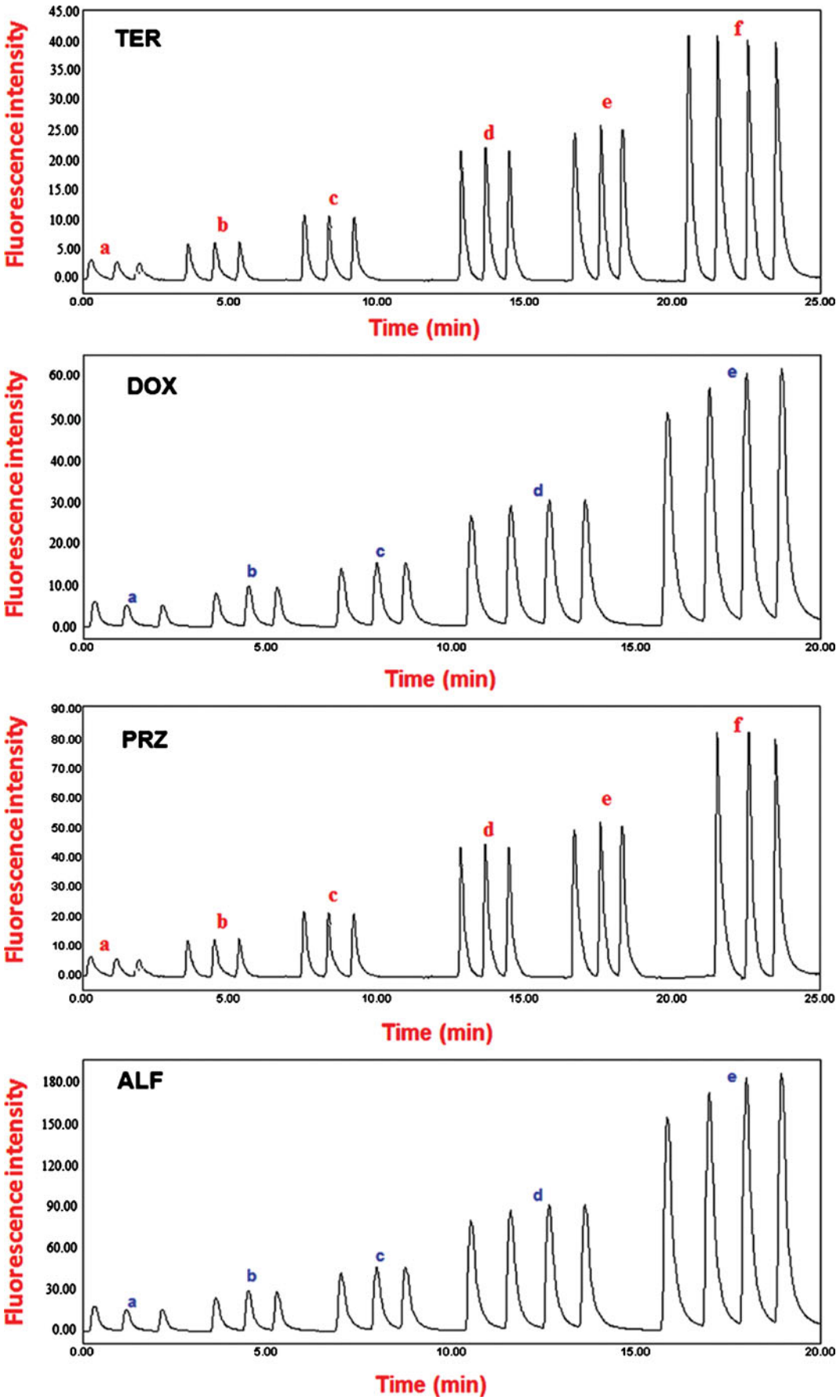
Influence of Buffer Concentrations and pH

Different buffer solutions including; acetate, phosphate and Britton Robinson buffers were tested for their effect on the enhancement of the fluorescence intensity of α_1 – blockers drugs. The phosphate buffer solution was found to be most suitable for the fluorescence enhancement of these drugs. In addition, the effect of phosphate buffer concentration and pH on the enhanced fluorescence of studied drugs in micellar medium were studied. Phosphate buffer solutions in concentration ranges 5–30 mM were tested for their effects on the fluorescence intensity (Fig. 5). It is seen that a phosphate buffer concentration of 20 mM gave an adequate buffer capacity without an excessive loss of sensitivity. Further changes in the buffer concentration produced little change in fluorescence intensity. Furthermore, the effect of pH on the enhancement of the fluorescence intensities was tested in the range 1–12. The results showed that the fluorescent intensities of α_1–blockers in SDS solution reached a maximum value between pH 2.5– 4. A phosphate buffer of pH 3 in surfactant medium showed an increase in fluorescence intensities of almost 5–6 times with respect to that of α_1-blockers in aqueous medium; hence, pH 3.0 was selected as optimum with phosphate buffer for further assays.

Effect of FIA System Flow Rate

The effect of the flow rate of the FIA system on the fluorescence intensities of the studied α_1 – blockers was carried out

Fig. 6 FIA chromatograms for PRZ (*a*: 20 ng ml^{-1} *b*: 40 ng ml^{-1} *c*: 80 ng ml^{-1} *d*: 200 ng ml^{-1} *e*: 250 ng ml^{-1} *f*: 400 ng ml^{-1}); DOX (*a*: 20 ng ml^{-1} *b*: 40 ng ml^{-1} *c*: 80 ng ml^{-1} *d*: 100 ng ml^{-1} *e*: 200 ng ml^{-1}); TER (*a*: 5 ng ml^{-1} *b*: 10 ng ml^{-1} *c*: 20 ng ml^{-1} *d*: 40 ng ml^{-1} *e*: 60 ng ml^{-1} *f*: 80 ng ml^{-1}); and ALF (*a*: 20 ng ml^{-1} *b*: 40 ng ml^{-1} *c*: 80 ng ml^{-1} *d*: 100 ng ml^{-1} *e*: 200 ng ml^{-1} ▶


TER
Fluorescence intensity
Time (min)
a
b
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DOX
Fluorescence intensity
Time (min)
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PRZ
Fluorescence intensity
Time (min)
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ALF
Fluorescence intensity
Time (min)
a
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Table 2 Accuracy and precision of the developed method

Sample	Concentration (ng mL^{-1})	Intra-day assay(n=6)		Inter-day assay(n=3)	
		Accuracy (%)	Precision (RSD%)	Accuracy (%)	Precision (RSD%)
TER	5	99.1	1.29	98.1	1.93
	20	100.9	0.69	100.9	2.12
	80	99.8	0.24	99.6	0.98
DOX	20	99.9	0.49	99.3	0.98
	80	100.1	0.16	100.2	0.33
	200	99.6	0.13	99.8	0.25
PRZ	20	99.6	0.52	99.2	1.04
	80	100.2	0.24	100.3	1.62
	400	99.9	0.09	99.8	0.32
ALF	20	101.7	0.82	101.1	1.65
	80	99.4	0.24	99.6	0.48
	200	99.1	0.45	99.3	0.91

due to its impacts on the contact time between surfactant and tested drugs. The results showed that the fluorescent signal increases with the increase of rate till 0.5 mL min^{-1}. For higher flow rates >2 mL min–1 great turbulence was observed, due to the introduction of bubbles into the flow system, with consequent fluctuation in the fluorescent values. Therefore, a flow rate of 0.5 mL min^{-1} was selected as optimum. Under these optimal conditions the sampling rate was 60 samples h^{-1} indicating the high throughput of the proposed method.

Table 4 calculation of Binding constant for the SDS–α_1–blockers system

Drug	Iα	I_O	I_s	$\frac{I\infty - I\circ}{I\alpha - Is}$	K_B
TER	358.47	47.43	112.15	−4.81	5.34
DOX	259.51	22.15	114.25	−2.57	8.66
PRZ	196.07	47.43	80.56	−4.49	5.64
ALF	241.29	47.48	116.85	−2.81	8.13

Method Validation

Linearity

Calibration curves of α1–blockers were conducted under optimal working conditions. FIA chromatograms of different concentrations of TER, DOX, PRZ and ALF were shown in Fig. 6. The calibration graphs were fitted by least-squares regression analysis, employing the areas of α_1 – blockers standards and fluorescent signals The calibration equations were; F=a+b C(r=0.9977−0.9999), where **F** is the fluorescence intensity (average of three measurement for each), **C** is the concentration of $\boldsymbol{\alpha}_1$–blockers expressed in ng ml^{-1}, **a** is the intercept and **b** is the slope. The slope of the calibration graph is the calibration sensitivity according to IUPAC definition for quantification of tested $\boldsymbol{\alpha}_1$ – blockers. The linearity was obtained in range 5 to 80 ng mL-1 for TER; 20 to 200 ng mL-1 for DOX and ALF; and (20 to 400 ng mL-1 for PRZ. Results were shown in Table (1).

Table 3 Robustness of the developed FIA method

Sample	% Recovery ± SD			
	TER	DOX	PRZ	ALF
No variations	99.95±0.06	99.67±0.38	99.24±0.73	99.87±0.37
Buffer pH				
pH 2.8	98.46±0.68	97.45±0.03	97.18±1.78	99.85±0.27
pH 3.2	97.48±0.53	98,15±0.17	98.68±0.56	98.95±1.04
Buffer conc.				
0.015 mole/L	99.76±0.15	98.54±0.36	100.76 ±1.63	99.43±0.64
0.025 mole/L	100.68±0.08	99.03±0.95	97.46±0.74	100.73±0.48
SDS conc.				
9 mole/L	98.56±0.13	99.06±0.21	98.26±0.59	99.76±0.66
11 mole/L	103.61±0.11	99.68±0.21	98.3±0.32	98.88±0.38
Flow rate				
0.7 ml/min	100.03±1.37	100.15±0.26	100.75±0.21	100.17±1.54
0.4 ml/min	98.65±0.97	97.46±1.69	98.54±0.05	99.02±1.90
Detection λ_{em}				
379 nm	99.88±1.26	99.94±0.58	98.73±0.16	99.59±o.48
398 nm	99.04±0.03	98.62±0.86	99.27±1.65	99.17±0.83

Table 5 Determination of pharmaceutical formulations by the developed FIA method and official methods

Product	Ingredient (content, mg)	% Recovery [a] ± SD		*f*-test [b]	*t*-test [b]
		This method	Official method [c]		
Itrin	TER, 2	102.05±0.279	100.78±0.573	3.33	0.81
Terazin	TER, 5	101.66±0.343	99.22 ±1.488	1.74	0.53
Cardura®	DOX, 1	97.96 ±1.119	103.63±1.306	5.81	0.45
Dosin	DOX, 1	100.11±0.031	96.49 ±1.991	1.83	0.78
Doxacor	DOX, 2	98.95 ±0.270	96.78 ±0.702	2.93	0.93
Minipress®	PRZ, 1	100.05±1.041	101.53±1.969	1.43	0.77
Xatral S.R.	ALF, 5	100.14±1.233	104.41±1.864	1.02	0.66

[a] Average of five determination ± SD

[b] Theoretical values at 95 % confidence limit; t=2.306, f=6.388

[c] Reported methods [7–9] and official method BP [45]

Limit of Detections and Limit of Quantitations

LOD and LOQ for the studied α_1 – blockers were established experimentally by evaluating the minimum levels at which the analyte could be readily detected (LOD) or accurately quantified (LOQ) in the presence and absence of SDS. The obtained LOD and LOQ for the studied α_1 – blockers in the presence of SDS were 3.2–11.9 ng mL^{-1} and 10.8–39.7 ng mL^{-1}, respectively. While the obtained LOD for the studied α1 – blockers in the absence of SDS were 39.3 – 326.5 ng mL-1. LOD and LOQ values confirmed the high sensitivity of the proposed method. Results were shown in Table (1). The sensitivities for the detection were 10–30 times in the presence of SDS compared to without SDS surfactant. Moreover, compared to reported methods for determination of α_1 – blockers, The proposed FIA method was found to be 3–500 times more sensitive than most of the reported methods [7–25]. Moreover, the proposed method was faster than all the reported methods as about 60 samples $hours^{-1}$ could be analyzed by the proposed methodology indicating the high throughput of the proposed method.

Accuracy and Precision

The intra-day and inter-day precision and accuracy of the method were determined by repeating injections (n=6) of standard solutions prepared at three different concentration levels over a three consecutive days. Relative standard deviations (RSD)<2.12 % were obtained in all cases. The intra-day and inter-day accuracy were in the range 98.1–101.7 %. The obtained results are shown in Table 2. These data indicated that reproducible and reliable results were obtained.

Robustness

Method's robustness was examined by evaluating the influence of small variations of method variables on the method's suitability and sensitivity. Results were shown in Table 3. It was found that none of these variables significantly affect the analytical parameters of method. This provides an indication of the reliability of the proposed method during normal usage, and so the proposed FIA method can be considered robust.

Binding Constants for the SDS–α_1–Blockers System

The binding constant values (KB) for the SDS–α_1–blockers system were obtained using the adduct model from fluorescence data of α_1–blockers as a function of SDS surfactant concentration using Eq.(1) [42]:

$$\mathbf{I}_\alpha - \mathbf{I}_0 / \mathbf{I}_\alpha - \mathbf{I}_\mathbf{S} = 1 + 1/\mathrm{K_B}[\mathrm{M}] \quad (1)$$

Where $\mathbf{I_\alpha}$ is the emission intensity at infinite micellar concentration; $\mathbf{I_0}$ the emission intensity without micelles; $\mathbf{I_S}$ the emission intensity at inter media micellar concentration; $\mathbf{K_B}$ the binding constant; and **[M]** the micellar concentration in mol L^{-1}. The concentration of the micelles [M] can be determined using the relation below [43]:

$$[\mathbf{M}] = ([\text{surfactant}] - \mathbf{CMC})/\mathrm{N_{av}} \quad (2)$$

Where **[surfactant]** = total surfactant concentration; $\mathbf{N_{av}}$ = aggregation number. N_{av} is ca. 62 [44]. According to this model, the solubilization process is considered as an addition reaction of solute molecules **(S)** in the micellar aggregations **(M)**, giving $\mathbf{MS_i}$ adducts (a micelle containing i molecules of solute). From the slope of the plot of $\mathbf{(I_\alpha - I_0)/(I_S - I_0)}$ versus inverse micellar concentration, the binding constants $\mathbf{K_B}$ were determined, and values were shown in Table 4.

Application for Analysis of Dosage Forms

The developed method was applied for the determination of α_1–blockers in commercial pharmaceutical samples and the results were shown in Table 5. The obtained results were compared with those obtained by official [45] and reported methods [7–9] as shown in Table 5. It is clear from the table that there is no significant difference between results obtained by the developed FIA method or official methods, as indicated by t- and F-tests. The results in Table 5 indicate that the extraction method was convenient for all the investigated

drugs with good recoveries and there is no interference from the frequently encountered excipients. The proposed method is fast, sensitive, accurate and precise. It is suitable for the determination of the studied drugs in their dosage forms and application in quality control laboratories.

Conclusions

The proposed FIA method for the determination of α_1–blockers in pharmaceuticals samples had the advantages of simplicity, sensitivity, speed, accuracy and the use of environmentally safe reagents. An important point of novelty of this work arises from the small volumes of sample and reagents employed and their nontoxic characteristics. The fluorescent detection gives a special selectivity without interferences from the common excipients found in commercial pharmaceutical forms. The use of SDS micellar system provided a simple means to enhance the fluorescence of α_1–blockers. The binding constants for α_1–blockers –SDS system were determined using the adduct model. The addition of SDS/phosphate buffer gave 10-30-fold increase in sensitivity and improved the limit of detection without further sample manipulation. Additionally, the remarkable wide linearity range with the high sensitivity and the high sampling rate made the proposed FIA system more suitable for routine analysis of α_1–blockers in quality control laboratories.

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