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Spectrophotometric determination of isoxsuprine hydrochloride in pharmaceutical tablets Using Reverse flow system with lead dioxide solid-phase reactor

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ABSTRACT

A reversed flow injection system using a solid phase reactor was developed for spectrophotometric determination of isoxsuprine hydrochloride (ISX) in bulk and pharmaceutical tablets. The reactor (4 i.d. and 40 mm length) packed with PbO_2 immobilized in a polymeric matrix (150 mg) was used to oxidized *p*-phenylenediamine to quinone diimine which is transported to the liquid phase and subsequently coupled with ISX to produce a green product whose absorbance was monitored spectrophotometrically at 690 nm. All the physical and chemical parameters that affected the flow injection manifold were studied carefully. In addition, the variables of the reactor involved the chemical composition, degree of packing, the reactor length and particle size were also optimized. The calibration graph for ISX was linear over the range of 25–300 μg/mL with the detection limit of 8.96 of ISX. The solid phase material was stable for more than one month and the relative standard deviation was best than 2% with the sampling frequency of 36 samples/hour. The reaction stoichiometry was evaluated by Job's method and was found to be 1:1 (ISX: PPD). The method was applied for the assay of ISX in pharmaceutical samples and the results obtained are an agreement with those obtained using the standard Pharmacopeia method.

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INTRODUCTION

Isoxsuprine hydrochloride (ISX) (4-Hydroxy-α-[1-[(1-methyl-2-phenoxy-ethyl) amino] ethyl] benzene methanol hydrochloride) is a vasodilator drug causing direct relaxation of vascular and uterine smooth muscle (Reynolds, 1996). It is used for the treatment of premature labor and cerebral vascular insufficiency (Raynaud's phenomenon) (Giorgino and Egan, 2010). Different techniques have been employed for assay of ISX in pharmaceutical dosage forms included spectrophotometry (Tharpa *et al.*, 2010), chemiluminescence spectrometry (Aly *et al.*, 2000), sequential injection spectrophotometry (Beyene *et al.*, 2005), cyclic voltammetry (Shahrokhian and Hafezi-Kahnamouei, 2018), gas chromatography-mass spectrophotometry (Bosken *et al.*, 2004), high-performance liquid chromatography (HPLC) (Belal *et al.*, 2000) and ultra-HPLC-tandem mass spectrometry (Bozzolino *et al.*, 2018). Among these techniques, the flow injection-spectrophotometric methods considered to be the preferred choice, especially for the analysis of drugs in different samples, because of their low cost and simplicity. A combination of the flow injection analysis (FIA) system with solid-phase reactor (SPR) is an inter-

esting strategy especially due to the possibility of to use of insoluble reagents in addition to increased sensitivity of measurement by decreasing the number of lines of flow manifolds. PbO_2 (or any slightly soluble oxidant) is immobilized on the cellulose acetate matrix by a manual procedure which is simple and fairly expeditious. FIA involved using several kinds of SPRs were extensively used for the determination of many organic and inorganic compounds and drugs in different samples (Emara *et al.*, 2012; Sartori *et al.*, 2011; Vicentini *et al.*, 2012). In the present work, an SPR filled with immobilized PbO_2 used for the determination of ISX was confirmed. The method is based on oxidized *p*-phenylenediamine (PPD) to quinone diimine into the prepared reactor and subsequently coupled with ISX to produce green products monitored spectrophotometrically at 690 nm.

EXPERIMENTAL

Equipment

The flow injection-spectrophotometric equipment involved a six-channel peristaltic pump (Ismatec, Labortechnik–Analytik, Switzerland) Model CH–8152 supplied with flexible vinyl tubing (0.5 mm i.d.) was used for pumping the solutions. Under the reverse mode flow system, the reagent solutions were injected manually into the drug stream using an injection valve (Rheodyne, Altex 210, Supelco-USA). The measurements of absorbance were accomplished using a digital single beam recording spectrophotometer (Shimadzu UV-Visible 1240) equipped with a flow cell (50 μL and 1cm bath length). Several lengths of reaction coils (RC) were made using different lengths of Teflon tubes (0.5 mm i.d.) were used for mixing reagents.

Reagents and solutions

A stock standard solution of ISX (1000 μg/mL) was prepared by dissolving 0.05 g of the pure drug obtained from the Iraqi Pharmaceutical Manufacturing Company-Iraq (99.9% purity) in distilled water and made up to volume in a 100 mL calibrated flask with the same solvent. More diluted solutions of the drug were prepared by simple diluting of a standard solution.

A solution of 0.125 M of *p*-phenylenediamine (Merck) was daily prepared by dissolving 0.6759 g of PPD in distilled water and then transferred into 50mL volumetric flask and complete the volume to the mark with distilled water.

The analyzed pharmaceutical tablets of ISX were obtained from commercial sources: Isoxsuprine hydrochloride® 20 mg (Cairo industry, Egypt) and Duvilane®-10 mg (ASIA-Aleppo, Syria). A twenty tablets of both applications weighed and the mean weight of the of a single tablet was estimated. Later the tablets were finely powdered and a portion of the powder equivalent to 50 mg of ISX was dissolved with well shaking in 50 mL distilled water and filtered. Further appropriate solutions to pharmaceutical tablets were made using distilled water.

Preparation SPR and immobilization method

Preparation of oxidant reactor and immobilization of PbO_2 onto inert support was performed according to previously published work (Zhang and Tang, 2005). In 100 mL beaker and under continuous stirring, a 0.5 g of cellulose acetate was wholly dissolved in 0.5 mL of DMF and 3 mL of acetone solvents. Then the powder of PbO_2 (4 g) was added gradually with manual stirring until an obvious increase in viscosity of the mixture was obtained. Later the homogenized product washed with distilled water and the obtained polyester (solid material) was left under air-drying. The particle size was selected by cutting the polymer with scissors and sieving on known mesh sieves. The SPRs were prepared by packing the particles of immobilized PbO_2 into different lengths of glass tubes (2.0 mm i.d.) and hold in the column with small pieces of sponge at extremities.

Flow injection system

A schematic diagram of the flow injection manifold is shown in Figure 1. A single-line manifold was selected for applying the reverse FIA procedure. Through the injection valve, the reagent (PPD) was injected into the stream of the ISX solution, which is then oxidized through the oxidant reactor (containing immobilized PbO_2). The reactants then mixed in reaction coil leading to the formation of a colored product. The absorbance of the product was detected at λ_{max}690 nm using a spectrophotometric detector.

RESULTS AND DISCUSSION

The catalytic oxidation of PPD on the SPR was adopted as a base of the present work. PPD is oxidized by PbO_2 to a desirable reactive coupling species (quinone diimine) (Sastry *et al.*, 1989). which couples with the ISX at their nucleophilic site, (preferably at ortho-position) by the electrophilic attack to give the green product. Although both ortho positions of ISX are available, the attack and coupling occur only at one position according to the result obtained from applying Job's method. Thus depending on the stoichiometry of reaction of 1:1 (ISX: PPD), the proposed reaction mechanism has

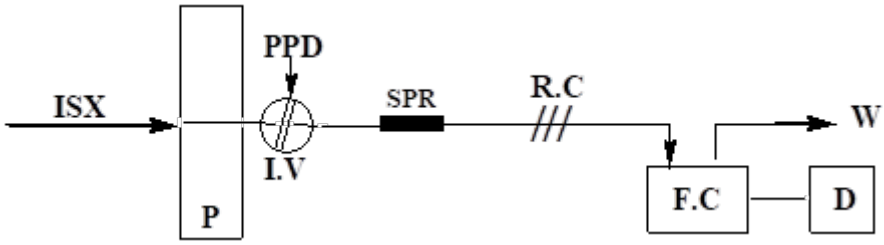


Figure 1: FIA manifold (I.V, Injection valve; ISX,Isoxsuprine HCl; P, Peristaltic pump;R.C, Reaction Coil; F.C, Flow cell; W, Waste; D, Detector; SPR, solid-phase reactor)

been proposed in Scheme 1.

A preliminary investigation step was performed to obtain a maximum wavelength used for carrying out the absorbance measurements. The absorption spectra of the product against the blank were obtained using a manual system and the maximum value of absorption was detected at 690 nm versus the reagent blank. In order to achieve the optimal conditions to promote the oxidative coupling reaction used in the present work, several parameters had to be studied. The packing column must be conditioned by the carrier solution for 10 min before use in order to reduce the effect of particle compaction on signal value. The ISX was pumping by a peristaltic pump and the reagent p-phenylenediamine was injected three times for each experiment.

Optimization of FIA-SPR variables

In order to achieve maximum sensitivity, different experimental parameters of the method were optimized.

Choice of the manifold type

In order to increase the sensitivity of the suggested method and to obtain a long lifetime of the oxidant reactor a reverse mode, a single line FIA manifold was adopted. Different types of FIA manifolds were examined to obtain different reaction paths, but only the manifold in Figure 1gavethe best absorbance and good reproducibility and was thus chosen for further use. The selected manifold worked by injected the DPP inside the stream of the ISX solution using the injection valve, which was then oxidized by SPR-$PbO_2$and then mixed in the RC. The absorbance product was measured at 690 nm through the detector at the end of the manifold.

Optimization of column parameters

Several parameters are affected the performance of SPR, such as the composition ratio (CAC/PbO_2), particle size, the length of reactor and degree of packing. These parameters were carefully studied.

The composition ratio of SPR

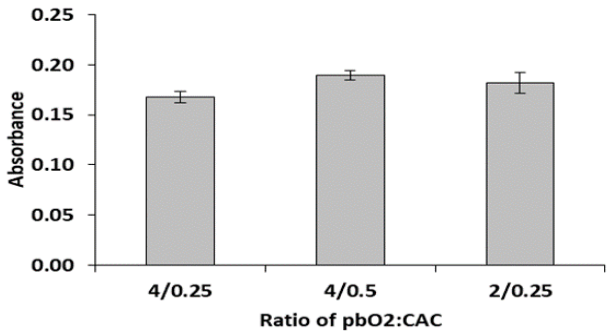


Figure 2: Study the composition ratio of SPR

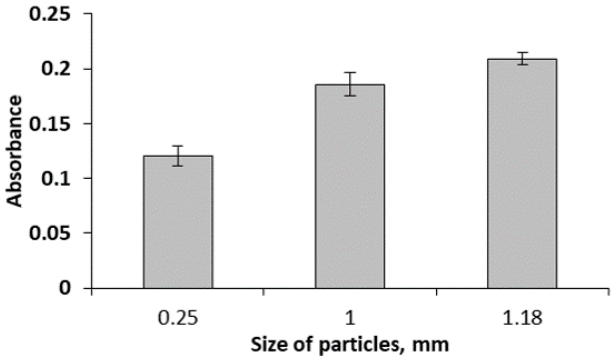


Figure 3: Effect of particles size

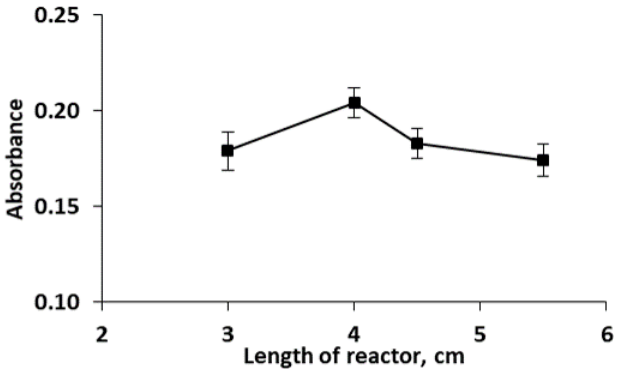
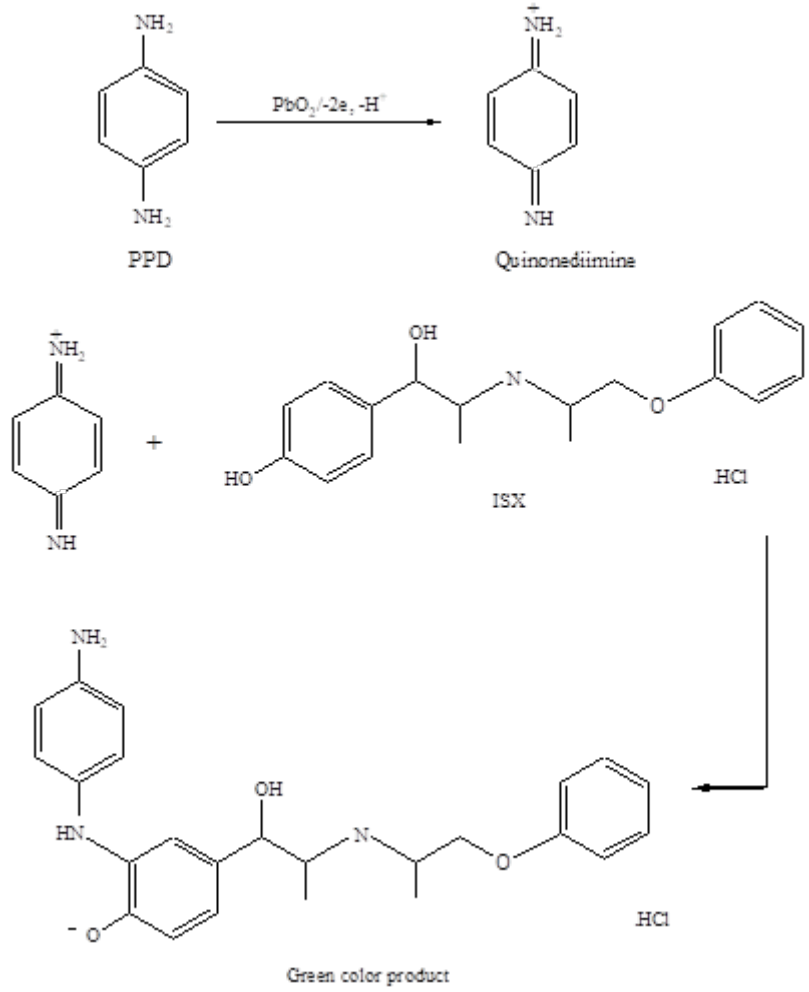


Figure 4: Effect of length of the reactor



Scheme 1: Proposed reaction mechanism

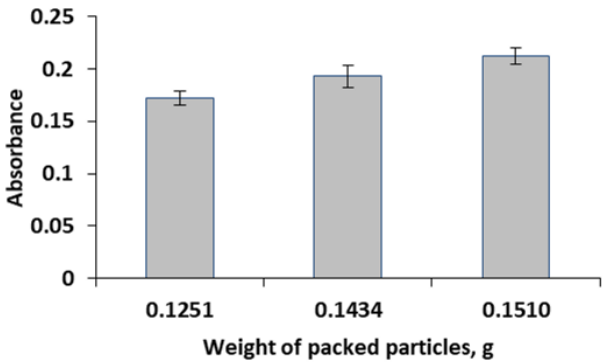


Figure 5: Degree of packing the SPR

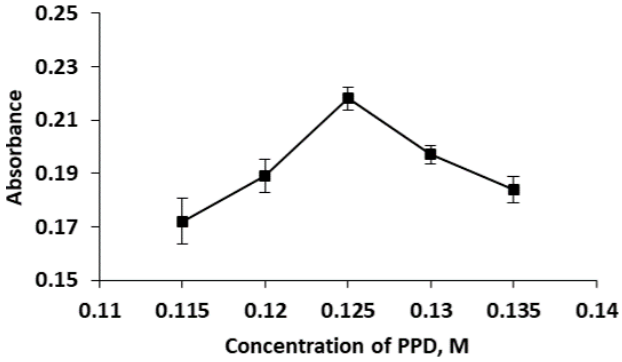


Figure 6: Effect of PPD concentration

The study of the best composition ratio of the SPRs was evaluated for three different ratios of (PbO_2/CAC): 4/0.25, 4/0.5 and 2/0.25(w: w, g). The results (Figure 2) show that the reactor prepared with a ratio of 4:0.5 provided the highest sensitivity and good repeatability and was adopted for further use. Usually, using a very small amount of inert support (CAC) must be avoided to ensure efficient immobilization of PbO_2 particles and to reduce the partial solubility of oxidant during the continuous flow of solutions which reduce the lifespan of the reactor.

Solid-phase particle size

The efficiency of the packing column is affected by the uniformity of particles of SPR. Therefore the effect of uniform particle-size was tested in a range of 0.25–1.18 mm selected using known mesh sieves

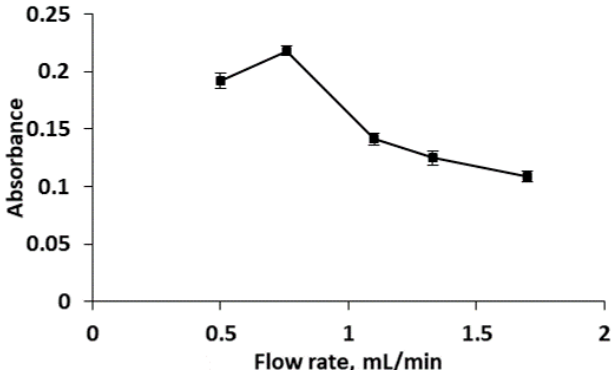


Figure 7: Effect of flow rate

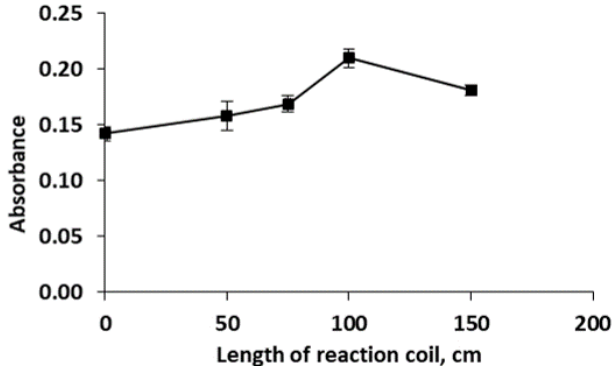


Figure 8: Effect of reaction coil length

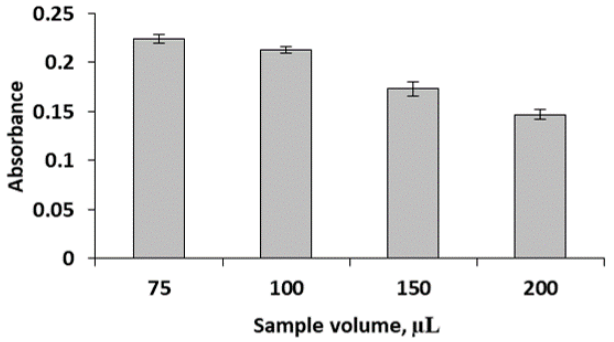


Figure 9: Effect of sample volume

with taking the consideration of operation the FIA manifold at low pressure. According to the results shown in Figure 3, the 1.18 mm particle size was selected as a compromise between sensitivity and repeatability. Particle size less than 0.25 mm would cause a hydrodynamic resistance in the manifold leading to decrease the sampling rate, while the size larger than 1.18 mm would not be used because of the difficulties of packing the column with this volume.

Influence of the reactor length

The length of the reactor was also studied by changing the length of the reactor from 3 to 5.5 cm. The highest absorbance signal was obtained with a 4-cm column length. The absorbances were decreased beyond this length, maybe due to the dispersion of the sample zone (Figure 4). A reactor 4 cm was selected as optimum length and was used in the next experiments.

Degree of packing

For optimum reactor (4 cm length and 1.18 mm size), the amount of particles using for packing this reactor was also studied. Different weights of solid phase material (PbO_2/CAC) were used to study the degree of packing of the reactor. Maximum absorbance with good repeatability was obtained using 0.151 g of the particles (Figure 5). The experiments indicated that the strong packing of material must be avoided (more than 0.151 g in this case) to reduce the resistance against the flowing solution formed by strong packing of the particles.

Optimization of chemical and physical variables

Influence of p-phenylenediamine concentration

The effect of PPD concentration was studied in the range of 0.115- 0.135 M by injecting 75 μL of reagent into the carrier stream of 100 μg/mL of ISX. The results in Figure 6 showed that the absorbance increase with increasing the PPD concentration up to 0.125Mand then decreased. A concentration of 0.125 M of PPD was chosen for all experiments.

Influence of the total flow rate

The effect of the total flow rate of the manifold was examined by varying the total flow rate (ranged of 0.5-1.7 mL/min) with monitoring the change in absorbance. As can be seen from Figure 7, the absorbance signal increased with increased the flow rate up to 0.76 mL/min and then significantly decreased. The decrease in absorbance was expected due to decreased the reaction time between the PPD and the analyte in the sample zone that moving through the SPR. As a result, a flow rate of 0.76 mL/min was selected for the best sensitivity coupled with rapidity.

Influence of reaction coil length

In order to increase the mixing of the reactants and provide a sufficient time for improvement of the colored product, a reaction coil was used in the manifold. The effect of the reactor coil length was studied in the 0–150 cm. Maximum absorbance and best repeatability were obtained at a lengthof100 cm. Therefore, it was selected for further use. Using of longer lengths was offset by the increased in the dispersion of the sample zone (Figure 8).

Influence of injected volume

Different volumes of injector loop ranged between 75 to 200 μL were used to determine the influence of injected sample volume. A 75 μL volume produced

Table 1: The range of studied variables and optimum values

Parameter	Tested range	Optimum value
Conc. of PPD (M)	0.115-0.135	0.125
Reaction coil (cm)	0-150	100
Total flow rate (mL/min)	0.5-1.7	0.76
Sample volume (μL)	75-200	75
The ratio of PbO2 :CA (w:w,g)	Different ratios	4:0.5
The length of the reactor (cm)	3-5.5	4
The weight of particle (g)	0.1251-0.1510	0.1510
The size of the particle (mm)	0.25-1.18	1.18

Table 2: statistics and analytical values of thecalibration graphs

Parameter	Value
λmax (nm)	690
Regression equation	y=0.0017x+0.0525
The correlation coefficient (r)	0.9990
Linearity range (μg/mL)	25-300
Slope, b (mL/μg)	0.0017
Intercept, a	0.0525
Percentage linearity, % r2	99.81
y/x	1.15×10-2
Sb	4.38×10-5
Sa	7.49×10-3
RSD %	< 3
Recovery %	99.90
Sample through-put (hr^{-1})	36
LOD (μg/mL)	8.96
LOQ (μg/mL)	29.87

Table 3: Accuracy and precision of the proposed method

Sample number	Conc. of ISX, (μg/mL)		Error %*	(Recovery ± SD)%*	RSD%*
	Taken	Found			
1	100	97.35	-2.65	97.35 ± 0.36	1.6
2	150	153.68	2.45	102.45 ± 0.64	2.0

Table 4: Effect of tablets interferences

Interference (1000 μg/mL)	Conc. of ISX (μg/mL)		(Recovery ± SD)%*
	Added	Found	
PVP	100	99.22	99.22 ±0.41
Talc		97.52	97.52±0.31
Starch		102.95	102.95±0.42
Magnesium stearate		101.87	101.87±0.40

Table 5: Results obtained for assay of ISX in tabletsusing proposed FIA and official methods.

Dosage form	FIA method					Official method				
	Taken conc. (μg/ml	Found conc. (μg/mL)	Rec. (%)	Mean Rec. (%)	RSD (%)	Taken conc. (μg/ml	Found conc. (μg/mL)	Rec. (%)	Mean Rec. (%)	RSD (%)
Isoxsuprine hydrochloride® (Tab, 20 mg)	50	49.03	98.06	98.40	1.79	5	5.08	101.60	99.40	1.28
	100	98.74	98.74		2.30	10	9.72	97.20		2.02
Duvilane® (Tab.10 mg)	50	49.46	98.92	98.05	2.67	5	5.09	101.80	101.55	1.30
	100	97.17	97.17		1.88	10	10.13	101.30		1.46
Pure ISX				99.90					99.65	
t (2.776)c	1.601	(n1 –1) = 2,(n2 –1) = 2, (n1+ n2 – 2) = 4								
F (19.000)c	1.431									

a, (Average of five determinations); b,(Average of three determinations); c, Theoretical value

a higher peak height and highly sample throughput, therefore being the selected loop size (Figure 9).

Under the previous optimum parameters (summarized in Table 1), the sample throughput was estimated by dependent on response time (the time required to reach the maximum absorbance). A 36 sample per hour was obtained from the optimized system, considerable to the response time of about 1.67 min. The prepared solid phase material is stable for more than one month and the packing reactors gave reproducible results for at least 29 injected samples with a relative standard deviation best than3% (n=20).

Analytical performance

A series of standard solutions of ISX was prepared and propelled into the manifold described in the Figure 1 under the optimum conditions (Table 1). The calibration graph was linear from 25-300 μg/mL ISX, and fitted the equation, y=0.0017x+0.0525, where y denotes the absorbance and x the ISX concentration (μg/mL). The correlation coefficient was 0.9990, with the limit of detection (LOD) of 8.96 μg/mL The statistical treatments for graph and analytical values were summarized in (Table 2). To examine the accuracy of the present method and repeatability of measurements, two concentration solutions of ISX were used and the assay process was applied in five replicates. A small value of RSD% and percentage error of the method referred to good accuracy and precision of the method (Table 3).

Study of interferences

In order to evaluate the selectivity of the proposed method in the determination of ISX in pharmaceutical forms, the possible effect caused by common excipients used in pharmaceutical preparations was studied. A comparison between the absorbance produced by a standard solution of 100 μg/mL ISX, with that produced by a similar solution, spiked with a 10-fold excess concentration of four interferences (talc, magnesium stearate, poly vinylpyrolidone (PVP) and starch). The results (Table 4) indicated that there was no significant difference between the analytical signals, which that mean there was no interference.

Pharmaceutical applications

The present method was successfully used for the assay of ISX in commercial tablets (Table 5). A recovery test was established using three different concentrations of each type of tablets. The obtained results show that mean average recoveries values were 98.40% and 98.04 for both types of tablets, which indicated the accuracy of the suggested method. The results obtained from the suggested method were compared with those obtained by applying the standard British pharmacopeia's method (UV method) (on CD-ROM, 2001). The statistical comparison between proposed and standard methods using the student t- and F-test (Miller, 2005) indicated that the calculated values were less than the theoretical one, which referred to the insignificant difference between both methods in accuracy and repeatability.

CONCLUSIONS

The suggested method showed a fast and sensitive way for ISX assay by incorporation of a SPR packing with immobilized of PbO_2 on an inert support. The present method required non-rigid experimental conditions with a wide dynamic linear range. In addition, it is free from interferences, heating and extraction steps. The results obtained are in agreement with those obtained by the British Pharmacopoeia method. Stability and repeatability of SPR make it an attractive choice for the estimation of ISX and other drugs in pharmaceutical forms using FIA manifold.

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