Original Article

Solid Phase Extraction-Spectrophotometric Determination Of Isoxsuprine Hydrochloride In Different Samples Using Modified Magnetic Nanoparticales

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Abstract

A green, simple and sensitive spectrophotometric method was proposed for the determination of isoxsuprine hydrochloride (ISX), based on the preconcentration with cetyltrimethylammonium bromide (CTAB) modified magnetic iron oxide nanoparticales (MNPs). ISX was coupled with diazotized metoclopramide drug via diazotization-coupling reaction in alkaline medium to form yellow-orange color product which was extracted and measured spectrophotometrically at λ_{max}444 nm. Various parameters affecting the azo-dye removal by adsorption on modified magnetic nanoparticales (Fe_3O_4@CTAB) such as pH of solution, volume of diazotized reagent, volume of surfactant, amount of adsorbent and type of desorbing solvent were studied. The linear calibration graph were obtained in the concentration range of 0.3-7.0 µg/mL of ISX, the limit of detection and the limit of quantification calculated to be 0.29and 0.99µg/mL, respectively. Accuracy and precision described by relative standard deviation and relative recovery percentages. The results obtained show that the CTAB-coated MNPs could be employed in the extraction of the drug in the azo dye form from different samples. The ISX was extracted successfully in pharmaceutical forms and spiked samples of river water.

1. INTRODUCTION

Nanotechnology has been developed rapidly in recent years. Magnetic nanoparticles (MNPs) possess a high performance in adsorption and separation process due to their interesting properties such as small size, chemical stability, high surface area to volume ratio, high magnetic properties, biocompatibility, and effective ability for binding [Utkan et al., 2011]. Magnetic iron oxide nanoparticles have a wide range of applications with attractive property that magnetic particles can readily be separated from sample solutions by the application of an external magnetic field [Chang et al., 2005 and Liao et al., 2003]. Modification of the surface of magnetic nanoparticles help to introduce these nanoparticles in different applications. Several methods used for this purpose included adsorption of organic compounds from environmental samples using organic vapor condensation, surfactant adsorption, polymer coating, and silanation [Zargar et al., 2009]. A surfactants adsorption is used in many works to modify the surface of MNPs and used in solid phase extraction and determination of target molecules [Takafuji et al., 2004].

ISX (chemically known as 4-Hydroxy-α-[1-[(1-methyl-2-phenoxy-ethyl) amino] ethyl] benzenemethanol hydrochloride) is drug compound causing dilatation of vessels wall and causes relaxation of uterine smooth muscles so it used for premature labor treatment. The literature reported several methods for determination of ISX in different samples included cyclic voltammetry [Shahrokhian et al., 2018], spectrophotometry [Tharpa et al., 2010], sequential injection spectrophotometry [Negussie et al., 2005], high performance liquid chromatography (HPLC)[hashem et al., 1991 and Khalil ,. 2014] and ultra-high-performance liquid chromatography-tandem mass spectrometry [Suo et al., 2017 and Bozzolino et al., 2018].Because of simplicity and low cost of spectrophotometric methods, these methods continue to be the preferred among other techniques for the analysis of drugs and other environmental contaminants in different samples. The phenolic group present within the structure of ISX molecule have capable sites for coupling reaction, therefore ISX drug suggested tobe estimated by diazotization-coupling reaction using metoclopramide (also a drug) as reagent in alkaline medium. The produced azo dye was measured spectro photometricaly after preconcentrated with magnetic nanoparticles (Fe_3O_4) modified with CTAB surfactant. This new procedure is green, highly sensitive and low cost.

2. EXPERIMENTAL

2.1. Reagents and solutions

CTAB, $FeCl_2.2H_2O$ (99.9% m/m) and $FeCl_3.6H_2O$ (96% m/m) were purchased from Merck (Darmstadt, Germany). NaOH (99.9%m/m) and HCl (36%m/m) were purchased from BDH (England). ISX (99.9%m/m) was purchase from sigma and pharmaceutical grade metoclopramide(99.9% m/m)was received from state company for Drug Industries and Medical Appliance, SDI, Samara, Iraq. All solutions were prepared with analytical grade chemicals and deionized water.

- Isoxuprine solution (200 µg/mL): A stock standard solution of ISX was prepared by dissolving20 mg of pure ISX in water, and made up to volume in a 100 mL calibrated flask with the same solvent.
- Hydrochloric acid (1M): was prepared by diluting 8.6 mL of concentrated hydrochloric acid (11.64 M) with distilled water in 100 mL volumetric flask
- Sodium hydroxide (1M): A 4g amount of NaOH was dissolved in a 100mL volumetric flask with distilled water. 0.05M of sodium hydroxide was prepared by dilution with distilled water.
- Diazotized metoclopramide (DMCP) (2×10^{-3}M) reagent solution: 0.0708g amount of pure metoclopramide was dissolved in an amount of distilled water in a 100mL volumetric flask, 3mL of hydrochloric acid (1M) was add and was placed on ice bath, then a 0.0134g of sodium nitrite was added, after 1 minute the volume was completed to mark with distilled water and used as stock solution.
- CTAB (0.2% w/v): A 0.5g amount of CTAB was dissolved in a 250mL volumetric flask with distilled water with few heating.

2.2. Solutions of pharmaceutical forms and river water samples

A twenty tablets of commercial ISX (Isoxuprine® Cairo industry, Egypt) were accurately weighted and finely powdered. A portion of the powder equivalent to 20mg of ISX was accurately weighed and dissolved in distilled water with well shaking into 100mL volumetric flask. The solution then filtered and used as stock solution (200 µg/mL). Further appropriate solutions of pharmaceutical tablets were made using distilled water.

River water was collected from Tigris river from Baghdad in January 2019; the sample was filtered to remove suspended particulate. The working solution was prepared by spiking the drug with river water and estimation of ISX as described under general procedure.

2.3. Apparatus

All UV–Vis spectra and absorbance measurements were performed using a double beam spectrophotometer Shimadzu model UV- visible 260digital recording (Tokyo-Japan) equipped with a 50 µL quartz cell with 1cm path length. PH–meter model from Metrohm (Swiss) the stirrer from IKA (USA). For magnetic separations a strong magnet 1.4 T magnetic field (1×5×4 cm) was used.

2.4. Synthesis of MNPs Fe_3O_4

A chemical (co-precipitation) method was used in the present work for preparation magnatic iron oxide nanoparticles [Faraji et al., 2010]. In 50 mL of deionized and degassed water a10.4 g of $FeCl_3\cdot6H_2O$, 3.27g of $FeCl_2\cdot2H_2O$,and 1.7mL of HCl (12M) were dissolved to prepare a stock solution. A 500mL of 1.5 M of NaOH solution was heated to 80°C in a beaker, the stock solution was added drop wise by a dropping funnel during30min under vigorous stirring by astirrer (1000 rpm).The temperature of the solution was constant at 80°C during the preparation steps. After completion of the reaction, the obtained Fe_3O_4 MNPs were isolated from the reaction medium by strong magnetic field and then were washed with 500 mL deionized water three times and resuspended in 500 mL of deionized water. The produced suspension of MNPs (pH=11 and concentration about 10 mg/mL) was stable up to one month under this conditions (Fig. 1).

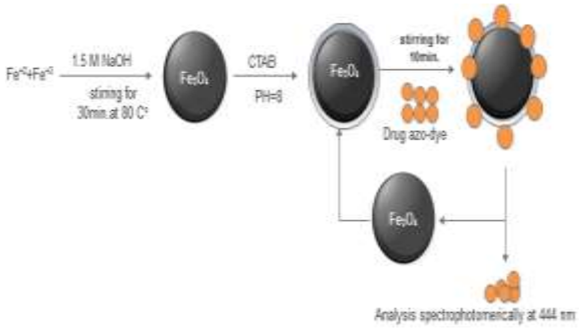


Fig. 1: Preparation of MNPs and determination procedures

2.4. Magnetic solid phase extraction procedure

Extraction procedure and calibration graph were established under the following steps: from a stock solution, 50 mL aqueous solution containing ISX (0.3-7.0 µg/mL) was prepared in a 100 mL beaker,1.0 mL of the DMCP reagent was added, then 2.0 mL of NaOH (0.05M) was added. A yellow color of azo- dye was appeared immediately, thena 1.0mL of MNPs suspension and 2.0 mL of 0.2%w/v of CTAB solution was added to the azo dye solution for extraction process. The mixture solutions were stirred for 10 min and afterazo-dye adsorption, the MNPs were quickly separated (1min) from the sample solution using very strong magnet. The supernatant water was decanted and magnet was removed. Finally theazo dye was desorbed with 2.0mL ethanol and stirring for 2.0 min.The magnet was used again to separate the MNPs and the eluent (colored azo dye) was decanted into smell beaker; then 1.0 mL of the solution was taken for analysis spectrophotometricallyat 444nm.

The remained drug concentration was determined using a calibration graph of batch method (without extraction) and the followed equation was used to calculate recovery percent of extraction or removal percent [Niazi et al., 2015].

$$\text{ER\%}=\frac{C_\circ - C_r}{C_\circ}\times 100 \text{ …………….. } (1)$$

Where $C_\circ$ and $C_r$are the initial and residual concentrations of the isoxsuprine drug in the solution (µg/mL), respectively. All the experiments were carried out at the room temperature, for the optimization of conditions an all subsequent experiments were carried out using 4.0µg/mLof ISX (Fig. 2 and 3).

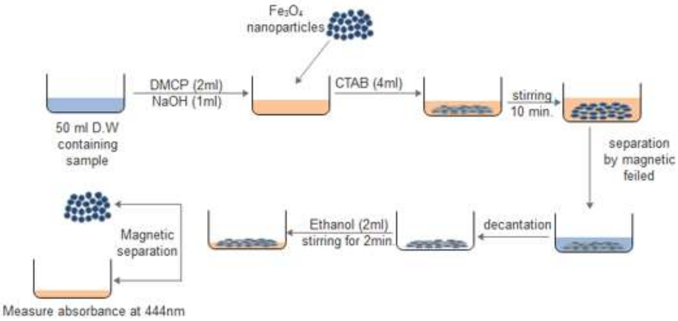


Fig. 2: Extraction steps

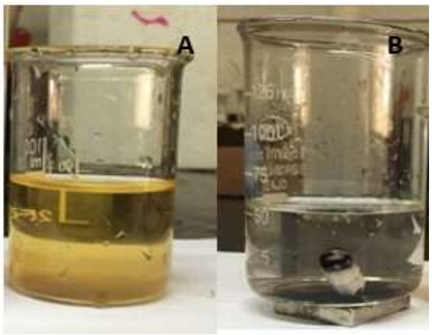


Fig. 3: Photographs of magnetic solid phase extraction steps: (A) before extraction, (B) magnetic separation

3. RESULTS AND DISCUSSION

3.1. Characterization of MNPs

IR, AFM and SEM instruments were used for characterization the synthesized Fe_3O_4 NPs. IR spectra of pure Fe_3O_4 and coated with CTAB surfactant are shown in Fig.(4a and 4b), which obtain that the peaks at 574 cm^{-1} and 576 cm^{-1} in both pure and coated MNPs respectively, these bands were attributed to stretching vibration for Fe-O bonds, the characteristic bands for coated CTAB cationic surfactant that observed in coated MNPs IR spectra and not present in pure Fe_3O_4 spectra obtained at 2920$cm^{-1}$and 2850 cm^{-1}attributed to stretching vibration of methylene group asymmetric and symmetric [Viana et al., 2012] respectively, and then for ($N\text{-}CH_3$) band were appeared at 2485 cm^{-1} and 1398 $cm^{-1}$for stretching vibration asymmetric and symmetric respectively, also band at frequency 910 cm^{-1} was attributed to vibration of (N-C) bond [Venkataraman et al., 2001]. Also, the spectrum of CTAB-coated MNPs shown a small shift in the position of main peaks in comparison with pure MNPs, these changes were done due to the alterations in surface environment of the particles after modifications and proved the coating [Khoshnevisan et al., 2012].

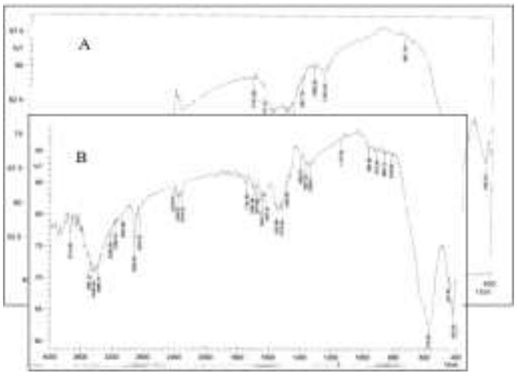


Fig. 4: (A) IR spectrum for pure Fe_3O_4MNPs, (B) IR spectrum for Fe_3O_4 MNPs coated with CTAB surfactant

In addition, the AFM and SEM images of the CTAB-coated Fe_3O_4NPs, as shown in Fig. 5, indicated that the diameter of synthesized nanoparticles were around 90 nm. We claim the size of the preparation nanoparticles are smaller than the size which obtained from in AFM and SEM instruments because the agglomeration of ultrafine particles have been explored on the Fe_3O_4 surface morphology analysis according to the evaluation of AFM and SEM images.

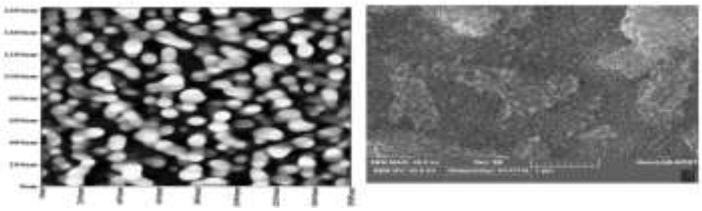
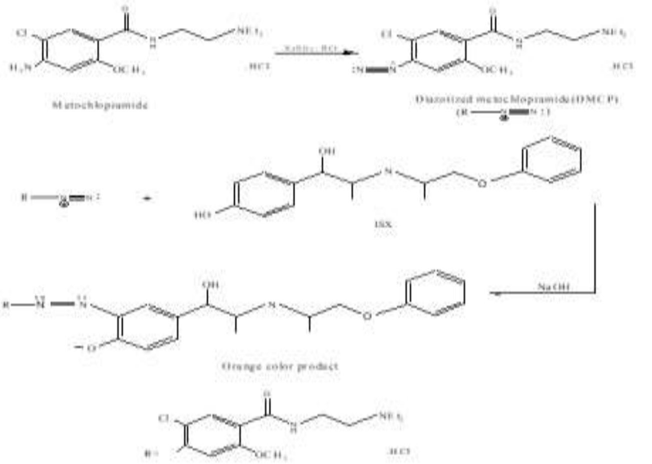


Fig.5: AFM and SEM image of synthesized MNPs



Scheme 1: Proposed mechanism of reaction

3.2. Optimization of the azo dye extraction

In order to achieve highest recovery and successful extraction, different factors that affected on percentage removal of azo dye and absorbance value of colored product were studied.

3.2.1. Effect of CTAB amount

Previous studies indicated that at basic pH the surface of the iron oxide MNPs is negative. Azodye (results from phenolic drug coupling with DMCP) is difficult to be extracted in absence of CTAB, because of repulsion forces between the negative group of the dye with a negative surface charge of Fe_3O_4MNPs in alkaline medium. Positively charged of CTAB made it easy adsorb on negatively charged surfaces of MNPs in alkaline solutions. Influence of various amounts of 0-6 mL of 0.2% w/v of CTAB (below its critical micellar concentration) on the adsorption and removal of dye through Fe_3O_4MNPs substrate was studied.

The adsorption of the azodye increased with increase the amount of CTAB up to 4 mL and then decreased gradually. The increase of efficiency of adsorption in the beginning may be due to the formation of hydrophobic hemi-micelles and admicelles together on the surface of nanoparticles. In the other hand a decrease in extraction amounts of azo dye was occurred when micelles were formed in the bulk aqueous solution because of redistribution of dye between the micellar bulk aqueous solution and MNPs, therefore, 4 mL of 0.2% (w/v) CTAB was chosen as optimum amount.

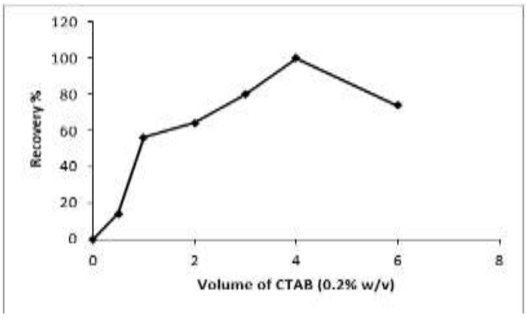


Fig.6: Effect of CTAB amount on extraction efficiency[Conditions: 2 mL of MNPs; 1 mL of 0.05 M NaOH; 2 mL of 2 mM DMCP; 50 mL of 4 µg/mL of ISX; temperature: 25ºC]

3.2.2. Effect of NaOH concentration

The alkaline medium is necessary for diazotization coupling reaction between ISX and DMCP,in addition the charge density of MNPs surface play an important role in adsorption of azo dye and it varies with pH.For iron oxide MNPs the isoelectric point at pH= 6.5 (pH_{zpc}) was reported previously [Zhao et al., 2008]. In alkaline medium ($pH>pH_{zpc}$), the negative charge density on the surface of the MNPsis increased, resulting increase in the adsorption of cationic surfactant (CTAB) on the surface of nanoparticles and extraction efficiency is also increased. The influence of different concentration of NaOHon adsorption performance of azo dye (complex of drug with reagent) was studied in the range 0-10 mLof 0.05M of NaOH (pH range of ≈ 7–12). The results reveal that the absorbance gave the highest absorbance when 1mL of NaOH was added. Addition NaOH more than 1 mL causes a decrease in absorbance, thus 1mL of 0.05M of NaOH was used as optimum value.

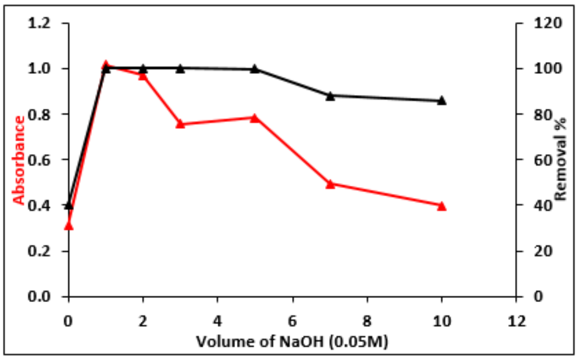


Fig. 7: Effect of NaOH volume on azodye color and removal[Conditions: 2 mL of MNPs; 4 mL of 0.2% (w/v) CTAB; 2 mL of 2 mMDMCP; 50 mL of 4 µg/mL of ISX; temperature: 25ºC]

3.3.3. Effect of the amount of Fe_3O_4MNPs

The amount of Fe_3O_4MNPs for complete removal of azo dye in a 50 mL solution was investigated by varying volume of MNPs suspension (10 mg/mL) in the range of 0.5–4.0 mL. The obtained results in Fig. 8 showed that by increasing the amount of Fe_3O_4MNPs that are modified with CTAB, extraction efficiency was increased up to 2 mL and then decreased. The increasing of removal percent with increase the amount of particles may be due to increasing of accessible sites; therefore, 2 mL of the Fe_3O_4MNPs was selected for all subsequent experiments.

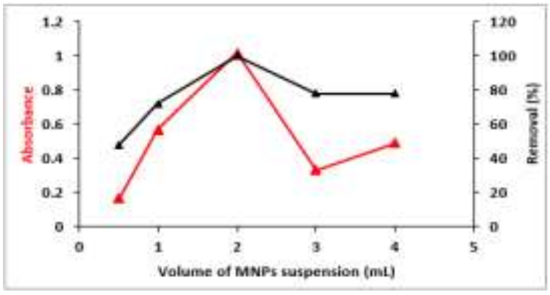


Fig. 8: Effect of MNPs amount on extraction efficiency [conditions: 2 mL of 2 mM of DMCP; 4 mL of 0.2% (w/v) CTAB; 1 mL of 0.05 M NaOH; 50 mL of 4 µg/mL of ISX; temperature: 25ºC]

3.3.4. Effect of diazotized reagent volume

To evaluate the effect of diazotized reagent on the sensitivity of the proposed method, different volumes of 2 mM of DMCP in the range of 0.5-5.0 mL was studied. The result on Fig. 9showed that the extracted azo dye gave maximum absorbance when 2mL of reagent was used, therefore this volume was chosen for furthered experiments.

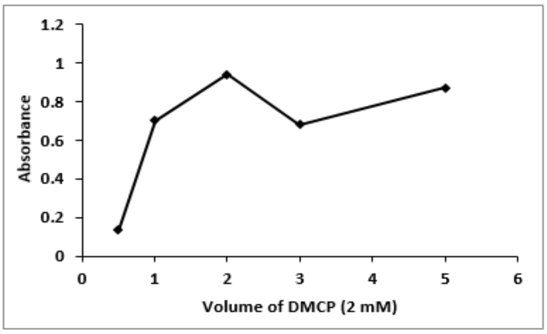


Fig. 9: Effect of diazotized metoclopramide volume on the azo dye absorbance [Conditions: 2 mL of MNPs; 4 mL of 0.2% (w/v) CTAB; 1 mL of 0.05 M NaOH; 50 mL of 4 µg/mL of ISX; temperature: 25ºC; extraction time:5 min]

3.3.3. Effect of the extraction time

In order to increase extraction efficiency and sensitivity of the method, a study of contact time between the adsorbent (MNPs) and the dye in the range of 2-15 min were established under all previous optimum conditions. The results showed that maximum removal of the dye was increased with extraction time up to 10 min after that a maximum removal was decreased slightly, therefore to achieve the maximum adsorption, a 10 min extraction was sufficient and selected in the followed experiments (Fig. 10).

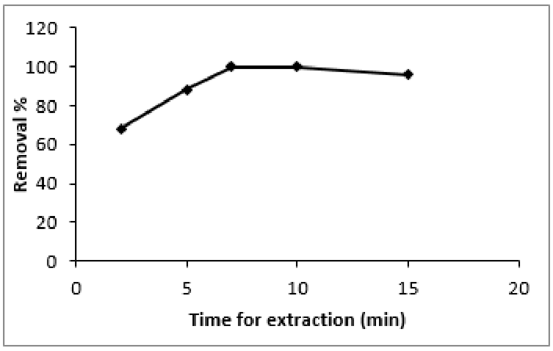


Fig. 10: Effect of extraction time on the efficiency [Conditions: 2 mL of MNPs; 4 mL of 0.2% (w/v) CTAB; 1 mL of 0.05 M NaOH; 50 mL of 4 µg/mL of ISX; temperature: 25ºC]

3.4. Optimization of the desorption conditions

In order to desorption of the adsorpant dye, an organic solvents (eluents) are used for disrupt the hemimicelles structures. Different organic solvents such as methanol, acetic acid, acetone and ethanol were experimented and the obtained results indicated that maximum absorbance was showed when methanol was used in comparison with other solvents. To make the suggested method more biocompatibility, the ethanol solvent was selected rather than methanol for desorption of the dye from the surface of the MNPs (Fig.11a).The volume of the eluent (ethanol) was also studied in the range of1–4mL. The best results were obtained with 1mL of ethanol as showed in Fig.11b. The desorption times were studied and it was found that2 min. is best time for complete desorption of the azo dye from MNPs as appeared in Fig.12.

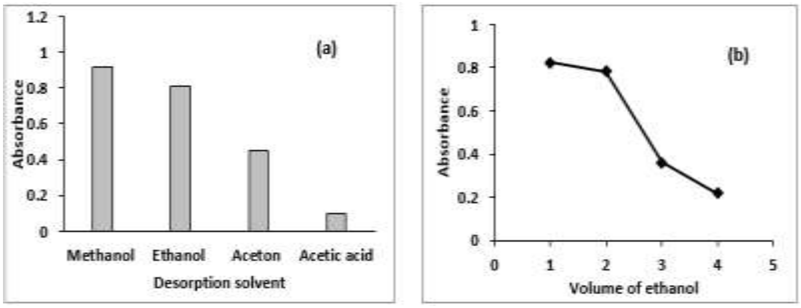


Fig. 11: (a) Studying the type of solvent in desorption, (b)Effect of volume of solvent [Conditions: 2 mL of MNPs; 4 mL of 0.2% (w/v) CTAB; 1 mL of 0.05 M NaOH; 50 mL of 4 µg/mL of ISX; temperature: 25ºC; time of extraction 10 min]

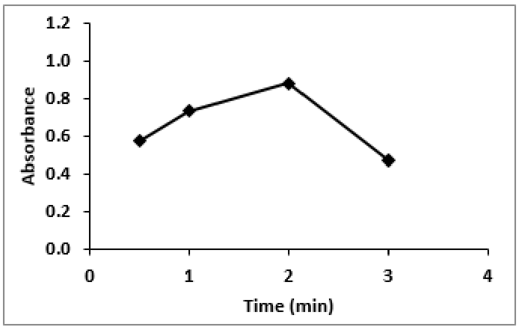


Fig. 12: Effect of the time of desorption

3.5. Enrichment capability of the method

In order to improve higher enrichment factors, a larger volume of sample solution is preferred. The effect of sample solution volume on the extraction of azo dye was studied by using different volumes (10–150 mL) of aqueous solution of ISX under the optimal conditions. It was observed that the best quantitative recovery was showed when the sample volume was 50mL.the extraction recovery decreased when in the larger volume of sample because of the analyte loss from the MNPs surface. Thus, 50 mL of sample volume was selected as suitable volume for analysis of ISX drug in water samples.

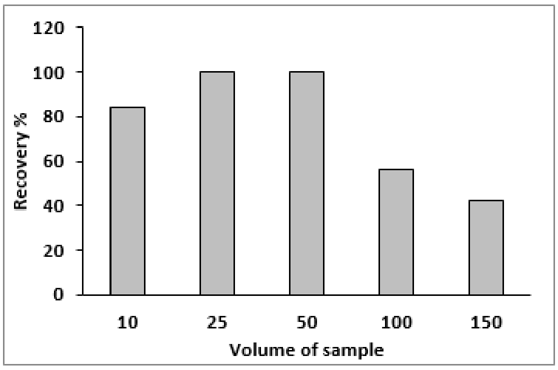


Fig. 13: Studying the enrichment capacity

3.6 Analytical performance

Under the optimal conditions, a linear calibration curve was established in the concentration range of 0.3-7.0 µg/mL. The quantitative parameters such as linear range, correlation coefficient, detection and quantification limits, given in Table1. A series of working standard solutions were prepared by diluting the stock solution of ISX with distilled water. The precision and accuracy of the method were also examined, various ISX concentrations were also analyzed by the proposed method and the analytical absorbance were manipulated by the calibration obtained. There are three concentrations in five replicate for each concentration of ISX were examined and their recoveries (Rec.%) and relative standard deviations (RSD%) were calculated as showed in Table 2.

Table 1: Analytical values of statistical treatments for the calibration graph

Parameter	**Value**
Regression equation	$y = 0.159x + 0.1759$
Correlation coefficient, r	0.9988
Linear range(µg/mL)	0.3-7.0
Limit of detection, LOD(µg/mL)	0.29
Limit of quantification, LOQ(µg/mL)	0.99
Average of recovery %	98.13
RSD (%)	4.39
Molar absorptivity (L/mol.cm)	5.37×10^{4}
Sandell's sensitivity, S (µg/cm^{2})	6.29×10^{-3}
Slope, b (mL/µg)	0.1589
Intercept, a	0.1759
$S_{y/x}$	1.92×10^{-2}
S_{b}	3.01×10^{-3}
S_{a}	1.03×10^{-2}

Table 2: The accuracy and precision of the proposed method

Concentration, µg/mL		**E %**	**Rec.%**	**RSD%**
Present	**Found**			
0.50	0.51	2.01	102.01	2.93
2.00	1.97	-1.50	98.50	4.89
4.00	4.01	0.25	100.25	2.19

3.7. Analytical applications

In order to evaluate the applicability of the newly extraction method for the analysis of IXH in tablets and the spiked river water samples were extracted and analyzed using modified Fe_3O_4MNPs under optimum conditions. Two concentrations for tablets and two spiked concentration in river samples were analysis under the recommended procedure after their solutions were prepared as recommended previously. Table 3and 4shows that the results of five replicate analyses of each sample resulted by the proposed method were in acceptable agreement with the spiking amounts. The results obtained (recoveries values) are summarized in Table 3 and also statistically compared with those of standard method. Two tests (Student's t-and F-test) [Miller et al., 1984], were used to compare the performance of the current method with standard method.

Table 3: Applications of the proposed method in pharmaceutical samples

Dosage form	proposed method						Official method [UV]				
	Taken conc. (μg/mL)	Spiked conc. (μg/mL)	Found conc. (μg/mL)	Rec. $(\%)^a$	Mean Rec. (%)	RSD $(\%)^a$	Taken conc. (μg/mL)	Found conc. (μg/mL)	Rec. $(\%)^b$	Mean Rec. (%)	RSD $(\%)^b$
Isoxsuprine hydrochloride® Tablet (20 mg)	1.00	---	1.01	101.01	99.18	3.57	8.00	7.86	98.29	97.24	2.31
		1.00	1.99	99.50		3.76	12.00	11.54	96.19		1.45
		2.00	2.84	94.67		1.92					
	3.00	---	2.95	98.33		4.33					
		1.00	3.99	99.75		3.08					
		2.00	5.09	101.80		3.57					
Pure ISX					100.25					99.65	
t (4.303)^c **F (161.4)**^{c}	0.68 5.07	$(n_1-1) = 1, (n_2-1) = 1, (n_1+n_2-2) = 2$									

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

Table 4: Applications of the proposed method in river water samples

Sample	Conc., μg/mL		Rec.%*	RSD%*
	Spiked	Found		
River water	1.00	1.01	101.01	3.76
	3.00	2.94	98.00	4.96

*** Average of five determinations.**

4. CONCLUSION

A new SPE method based on the CTAB-coated MNPs was proposed for the extraction of ISX in tablets and water samples. The azo dye resulted from diazotization coupling reaction between ISX and another drug (used as reagent), was successfully extracted with modified magnetic nanoparticles (Fe_3O_4MNPs). The present methodissensitive and low cost method for determination and extraction of IXH in different samples. Preparation of CTAB-coated MNPs is very easily and the experiments have proved that the extraction process can be performed within 10 min with high extraction efficiency, rapid collection of MNPs from the sample, easy elution with more environmental solvent, and good precision and high accuracy. The optimal conditions for detection and extraction of ISX are established and validated in tablets and river water samples through the recovery experiments.

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