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Sensitive Cloud Point Extraction Method for the Determination of Isoxsuprine Hydrochloride in Pharmaceutical forms using Spectrophotometry

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Abstract

A simple and highly sensitive cloud point extraction process was suggested for preconcentration of micrograms amount of isoxsuprine hydrochloride (ISX) in pure and pharmaceutical samples. After diazotization coupling of ISX with diazotized sulfadimidine in alkaline medium, the azo-dye product quantitatively extracted into the Triton X-114 rich phase, dissolved in ethanol and determined spectrophotometrically at 490 nm. The suggested reaction was studied with and without extraction and simple comparison between the batch and CPE methods was achieved. Analytical variables including concentrations of reagent, Triton X-114 and base, incubated temperature, and time were carefully studied. Under the selected optimum conditions, the linearity ranges of calibration curves were 1-9 and 0.5-8 µg/ml with detection limits of 0.26 and 0.09 µg/ml of ISX for batch and CPE methods respectively. A relative standard deviation (RSD %) best than 1.98 and 2.67 % with the percentage recoveries range 100.14 and 99.63 % were obtained for both methods respectively. The proposed methods were successfully used in routine analysis of ISX in pharmaceutical forms with high accuracy and reproducibility.

Keywords: Isoxsuprine hydrochloride, Triton X-114, Cloud point extraction, Sulfadimidine, Diazotization reaction.

التقدير الطيفي لهيدروكلوريد الايزوكسوبرين في الأشكال الصيدلانية بطريقة الاستخلاص بنقطة الغيمة الحساسة

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الخلاصة

تم اقتراح طريقة الاستخلاص بنقطة الغيمة كطريقة بسيطة وعالية الحساسية للتقدير المسبق لكميات بالمايكروغرام من دواء هيدروكلوريد الايزوكسوبرين (ISX) في النماذج النقية والصيدلانية. بعد الازوتة والازدواج لل ISX مع السلفاديميدين المؤزوت في الوسط القاعدي لتتكون صبغة الازو والتي يتم استخلاصها كمياً الى الوسط الغني بعامل الشد السطحي ترايتون X-114 ، يتم اذابتها في الايثانول و تقديرها طيفياً عند الطول الموجي ٤٩٠ نانومتر. تم دراسة التفاعل المقترح باستخدام الاستخلاص وبدونه ومقارنة بسيطة تم عملها بين طريقتي الدفعة والاستخلاص بنقطة الغيمة. اجريت دراسة للمتغيرات التحليلية بعناية متضمنةً تركيز الكاشف، وعامل الشد السطحي ترايتون X-114 وكذلك القاعدة ودرجة الحرارة والوقت. تحت الظروف المحددة المثلى حيث كانت المديات الخطية لمنحنيات المعايرة ١-٩ و ٠,٥-٨ مايكروغرام\مل مع حدود كشف ٠,٢٦ و ٠,٠٩ مايكروغرام\مل لل ISX بطريقتي الدفعة والاستخلاص بنقطة الغيمة عالتوالي. تم الحصول على الانحراف القياسي النسبي (RSD %) اللذي كان افضل من ١,٩٨ و ٢,٦٧٪ مع نسبة استعادية بالمدى ١٠٠,١٤ و ٩٩,٦٣٪ لكلا الطريقتين على التوالي. استخدمت الطرق المقترحة بنجاح في التحليل الروتيني لل ISX في الاشكال الصيدلانية بدقة ومصداقية عاليتين.

الكلمات المفتاحية: هيدروكلوريد الايزوكسوبرين، ترايتون X-114،استخلاص نقطة الغيمة، سلفاديميدين، تفاعل الازوتة.

Introduction

ISX, chemically named 4-Hydroxy-α-[1-[(1-methyl-2-phenoxy-ethyl) amino] ethyl] benzene methanol hydrochloride [(1)], is a pharmaceutical compound that causes relaxation of uterine smooth muscles and dilatation of vessels wall, therefore it used in cases of premature labor [(2)]. Several methods were suggested for assay of ISX in pharmaceutical and biological samples included spectrophotometry [(3)], cyclic voltammetry [(4)], high performance liquid chromatography (HPLC) [(5, 6)], sequential injection spectrophotometry [(7)], and ultra-HPLC-tandem mass spectrometry [(8, 9)]. Spectrophotometric methods are still wieldy used for routine analysis of drugs, because of its simplicity and low cost compared with other analytical techniques. Cloud point extraction (CPE) technique, is a simple extraction process based on used of micelles systems for preconcentration traces amount of different organic and inorganic compounds.

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In aqueous solutions, surfactants molecules tend to form micelles and at a suitable temperature named "cloud point temperature", the solution become turbid. Above this temperature, the turbid solution separates into a surfactant-rich phase and aqueous phase. CPE method has several characteristics feature over other extraction methods, such as high efficiency, sensitivity and enrichment factor, in addition of use safe aqueous medium rather than toxic organic solvents [10]. In the present work, ISX drug was extracted and then estimated by diazotization-coupling reaction with diazotized sulfadimidine (also a drug) as a safe and low cost reagent in alkaline medium. The azo dye product was extracted by CPE process, dissolved and measured spectrophotometrically. A simple comparison was performed between batch and CPE methods (with and without extraction).

Experimental

Equipment

All the absorption spectra and absorbance measurements of the samples were performed by digital single beam spectrophotometer (Shimadzu UV–VIS 1260/Japan) equipped with matched quartz cells (50 µL). For CPE process, a thermostatic water bath expert (England) was used for proving range of incubation temperature, in addition a centrifuge (Hettich, EBA 21) supplied with calibrated centrifuge tubes (50 ml) were used for separation the two phases.

Reagents and materials

All the materials used in this work were of analytical grade. ISX (purity 99.9%) was supplied from the state company for drug industries (SDI/Samara-Iraq). Pharmaceutical tablets containing ISX (Duvilane- Isoxsuprine® 10 mg, Asia Pharmaceutical-Aleppo, Syria) were purchased from local pharmacies and subjected to the method of analysis.

- *Isoxsuprine solution (1000 µg/ml):* Stock standard solution of ISX was prepared by dissolving 0.1 g of pure ISX in distilled water, and completed the volume of 100 ml calibrated flask with the same solvent.
- *Sulfadimidine sodium (0.02 M):* The reagent solution was prepared by transferred 1.8 ml of standard solution of 333 mg/ml of sulfadimidine sodium to 100 ml calibrated flask and completed with distilled water.
- *Sodium nitrite (0.02 M):* A 0.3450 g of $NaNO_2$ was dissolved in 250 ml calibrated flask with distilled water.
- *Triton X-114 (10% v/v):* A 10 ml of Triton X-114 (99.9%, Fluka) was dissolved and diluted with 100 ml of distilled water.

Solutions of pharmaceutical tablets

Crush thirty tablets of pharmaceutical applications containing commercially available ISX. A quantity of the tablets powder equivalent to 100 mg of ISX was accurately weighed and dissolved in 100 ml distilled water. Later the solution was shaked well and filtered to produce a stock solution (1000 µg/ml). More diluted solutions of medical tablets were prepared after dilution with distilled water.

General procedure of batch method (without extraction)

Into 10 ml calibrated flasks, equimolar of 1 ml $NaNO_2$ solution (0.02 M) and 1 ml of sulfadimidine sodium (0.02 M) with 1 ml of 1M hydrochloric acid solution were transferred and in ice-bath with cooling to 10 ºC. Then increasing volumes ranged from 0.1-0.9 ml of 100 µg/ml of ISX (covered the ranged of concentrations 1-9 µg/ml) and 1 ml of 2M of sodium hydrixide were added. The contents of flasks were shaken well and diluted with distilled water, after that left for 10 min for detection spectrophotometrically at 430 nm.

General procedure of extraction method (CPE)

Into 10 ml calibrated flasks, equimolar (1 ml of 0.02 M) of $NaNO_2$ solution and sulfadimidine sodium with 1 ml of 1M hydrochloric acid solution were transferred and in ice-bath (cooling to 10 ºC). Then increasing volumes ranged from 0.05-0.8 ml of 100 µg/ml of ISX (covered the ranged of concentrations 0.5-8 µg/ml), 1 ml of 2M of NaOH and 1ml of Triton X-114 (10% v/v) were added. The contents of flasks were mixed and diluted with distilled water and transferred to a centrifuging tube (10 ml) which equilibrated at 60° C for 30 min in the thermostatic bath. The tubes were then centrifuged for 10 minutes at 3000 rpm to isolate the two phases. After that the tubes were cooled (using ice bath) to assisted the separation process and the aqueous phase was decanted while the surfactant-rich phase (micelles surrounding the azo-dye) was dissolved with 1 ml ethanol and estimated spectrophotometrically at 490 nm.

Results and Discussion

Absorption spectra and the mechanism of reaction

In order to investigate the maximum wavelength for the coloured product, the absorption spectra of azo-dye resultant from diazotization coupling of diazotized sulfadimidine (DSD) with ISX in alkaline medium was shown in Figure 1. Absorption spectra for dye product and the blank were documented between 300 and 700 nm and the maximum absorption band situated at 490 nm demonstrating the formation of a complex between the reagent and drug. The molar ratio of the reactants (DSD and ISX) was assessed by continuous variation method (Job's method) [11] using equimolar concentrations (0.0029 M) of ISX and DSD, and the results indicated the 2:1 ratio product (DSD: ISX) was formed (Fig. 2). The primary step in reaction mechanism involved diazotization process of

sulfadimidine (also drug compound) using sodium nitrite/hydrochloric. In alkaline medium ISX, phenolic compound, converted to more reactive form "phenoxide" which is easy coupling with DSD and formed azo-dye product as shown in Scheme 1.

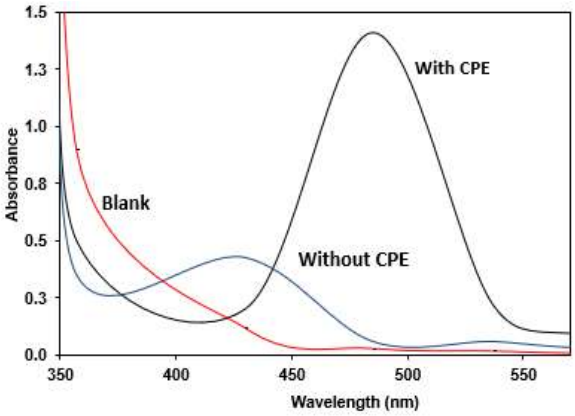


Figure. 1. Absorption spectra of 8 µg/ml of ISX treated DSD with and without CPE recorded against reagent blank, and the blank.

Dependent on Job's method data, the conditional stability constant of the azo-dye product was estimated by the following equation [12]:

$$K_f = \frac{A/A_m}{(1 - A/A_m)^{n+1} C^n n^n}$$

Where ***A*** and A_m are demonstrated to the maximum value of absorbance and the absorbance analogous to the intersection of the two tangents of the Job's method curve respectively (Fig. 2), **n:** stoichiometric constant, and ***C*** is the ISX concentration at the highest absorbance. The obtained value of $K_{f\ was}$ equal to 6.7×10^8 $L^2/mole^2$, point to the stability of the product. Gibbs free energy (ΔG) was calculated using equation: $\Delta G = -2.303\ RT\ \log K_f$ (R, 8.314 J/mol deg and T, 298 K). The negative value of calculated ΔG (-50.36 kJ/mole) indicated the spontaneously of the reaction.

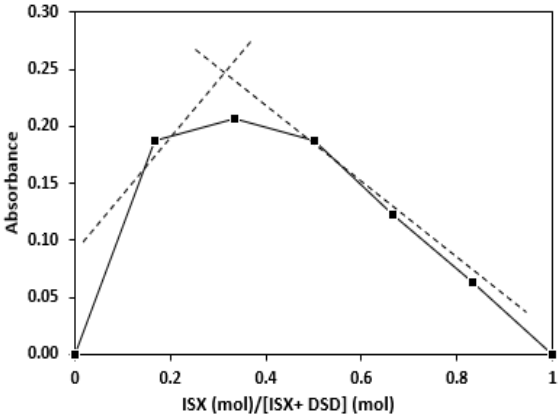
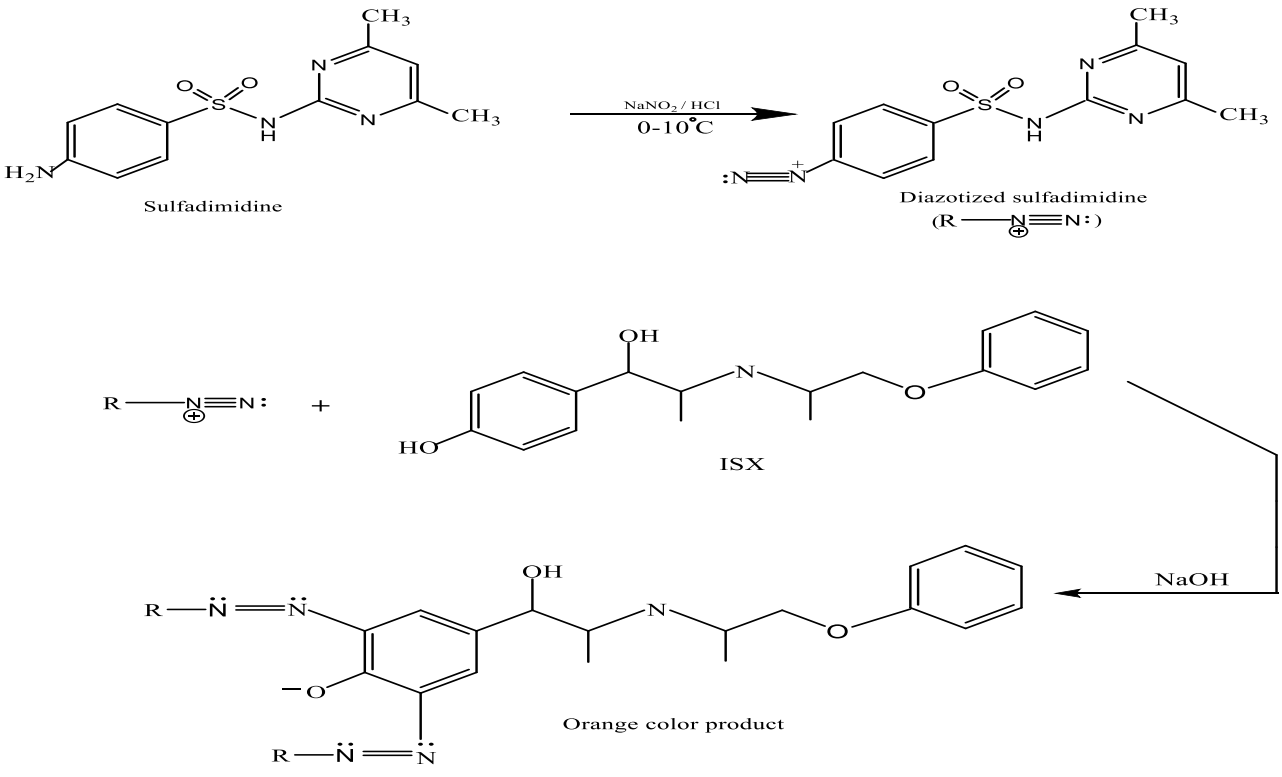


Figure. 2: Job's method of the reaction between ISX and DSD



Scheme 1. Coupling reaction between ISX and DSD

Selected optimum variables for batch and CPE methods

In order to enhance the sensitivity of the azo-dye product, the variables that affect the reaction product were carefully studied for batch and CPE methods. Different conditions such as the sulfadimidine, sodium hydroxide, and Triton-X114 concentrations and extraction conditions like incubation temperature and time were studied by changing one variable with the time, and keeping the others constant. A 8 µg/ml of ISX was used in all optimization experiments, with measuring absorbance at 430 and 490 nm against the blank for both methods respectively.

Study of the chemical variable

Influence of concentration of acid

According to the previous published researches, the diazotization reaction usually carried out by using sodium nitrite and acid to produce nitrous acid which is converted the amino group of reagent to diazonium salt. Different types of acids used for diazotization reaction were examined but only hydrochloric acid give a good result. The influence of different concentrations of HCl (0.05-0.3 M) using different volumes of 1M of acid ranged 1 to 3 ml was examined. The results indicated that 0.1 M of the acid (1 ml of 1 M in 10 ml total volume) gave the best response for both methods (Figure 3).

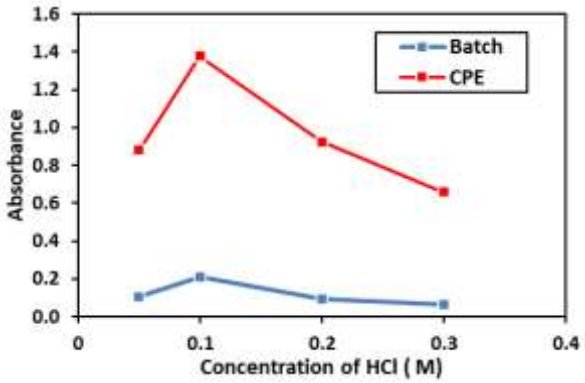


Figure. 3. Influence of concentration of hydrochloric acid for assay 8 µg/ml of ISX (conditions for CPE: incubation time, 30 min; temperature 60 ºC; surfactant volume (10%v/v), 1 ml; sulfadimidine and nitrite, 1ml of 0.02 M)

Influence of concentration of sulfadimidine

Sulfadimidine as a sulfa drug, it is used as new and safe reagent in diazotization coupling reactions. Amino group of sulfadimidine is easy to covert to daizonium salt under diazotization conditions. Various concentrations of reagent (equimolar with nitrite) ranged from 1-6 mM were studied to investigate its effect on the sensitivity of the azo-dye product. The results shown in Figure 4 indicated that maximum analytical signal was obtained when use 2 mM of sulfadimidine (i.e. 1 ml of 0.02 M in 10 ml final volume) for both methods. Therefore, this concentration of reagent and nitrite was designated as the optimum and used in further work.

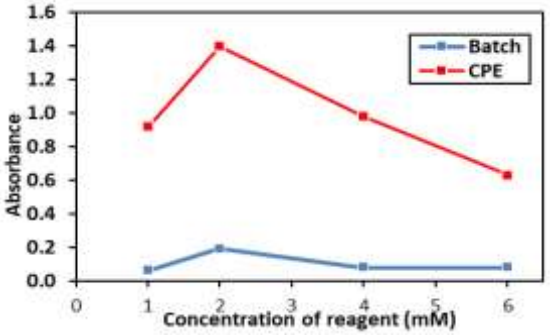


Figure. 4. Effect of concentration of sulfadimidine (conditions for CPE: incubation time, 30 min; volume of surfactant (10%v/v), 1 ml; HCl, 0.1 M)

Influence of type and concentration of base solution

The preliminary experiments indicated that the suggested coupling reaction must be carried out in alkaline medium. In order to activate the phenolic group of ISX, the alkaline medium improves and promotes the coupling reaction by transforming ISX to phenoxide species. Various alkalies (NH_4OH, NaOH, and Na_2CO_3) were considered and the results displayed that sodium hydroxide gave the best analytical response (Fig.5a). The influence of series of NaOH concentrations ranged from 0.06-0.4 M on absorbance of azo-dye was examined. Maximum response was obtained when used 0.1 M of NaOH (i.e. 0.5 ml of 2 M in 10 ml final volume) with and without extraction (Fig.5b).

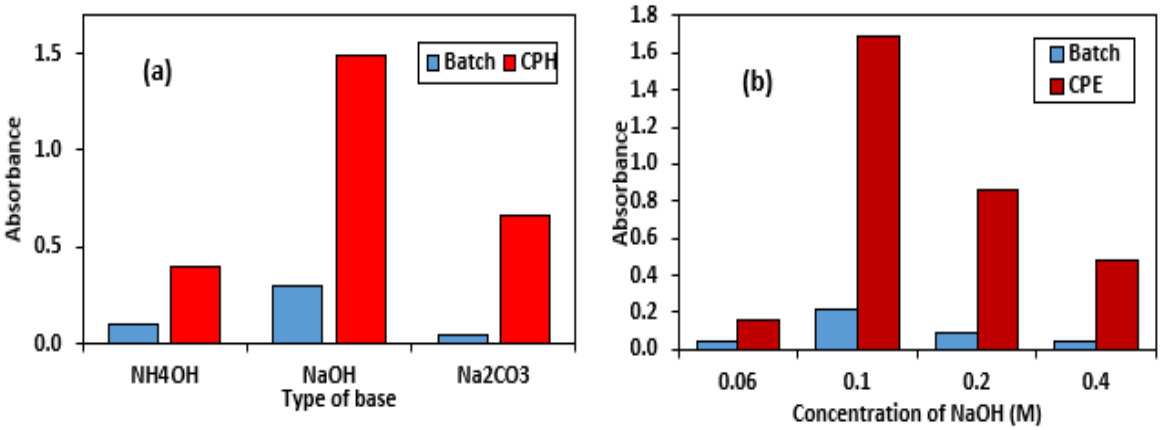


Figure. 5. (a) Effect of type of base, (b) Influence of the concentration of sodium hydroxide solution

Study and optimized CPE parameters

Influence of surfactant concentration and extraction temperature

Among different types of surfactant, Triton-X114 considered to be the most reactive and efficient surfactant that used for extraction different organic and inorganic species. The surfactant amount is typically affected the efficiency of separation process. To study this effect, different volumes of 0.5-2 ml of 10% (v/v) surfactant was examined by extraction different samples of 4 µg/ml of ISX. Figure 6a showed that maximum sensitivity was attained with 1 ml of surfactant and was chosen for further use. More amount of surfactant would not significantly change the response; this may be due to complete separation process. To attain complete extraction and separation, optimum incubation temperature must be investigated. The temperature effect on the extraction of the azo-dye was estimated in the range 40-80 ºC during the 30 min incubation time. The best analytical signal was obtained at 60 ºC (Fig. 6b), so this temperature was selected as optimum.

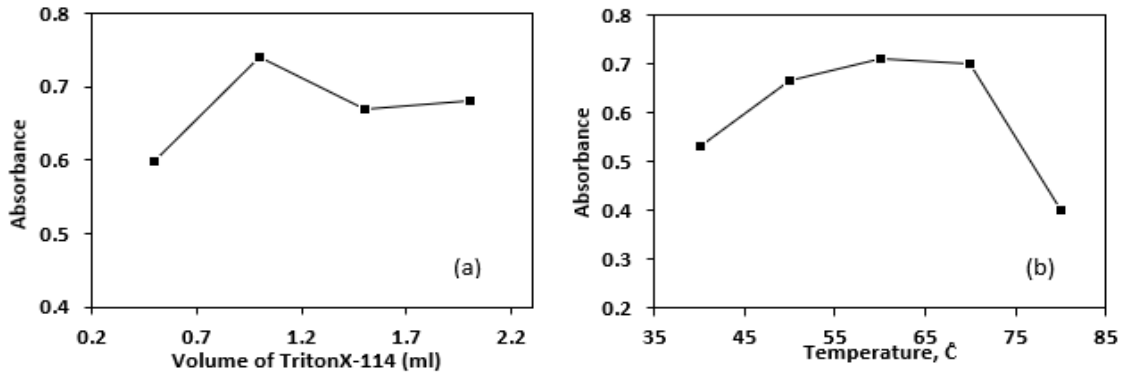


Figure. 6. Study the effect of (a) volume of Triton X-114 (b) incubation temperature (CPE conditions: 4 µg/ml of ISX; incubation time, 30 min; sulfadimidine, 2 mM; NaOH, 0.1 M

Influence of incubation time

The extraction efficiency and the equilibrium between two phases is mainly affected by the incubation time. So the incubation time need for completing separations was studied in the range of 10-40 min at 60 ºC. The results (Fig. 7) showed that the absorbance of extracted azo-dye was enhanced with elevating the incubation time up to 30 min then slightly decreased, so 30 min was selected as the best time to achieve quantitative extraction. The centrifugation time was examined from 2-10 min, and completed separation was performed at 5.0 min, so it was chosen for further use.

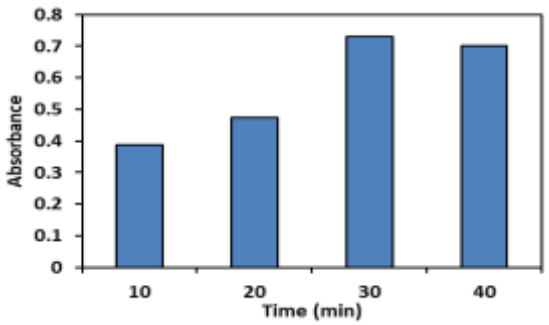


Figure. 7. Study of the incubation time (CPE conditions: 4 µg/ml of ISX; sulfadimidine, 2 mM; NaOH, 0.1 M; Triton X-114, 1% (v/v); Temp., 60 ºC)

Selected optimum variables

All the investigated chemical and physical conditions that may affected the extraction efficiency and sensitivity of azo-dye product are listed in Table 1 for batch and CPE methods.

Table 1. Summary of studied variables with optimum values for assay of ISX using batch and CPE methods

Variable	Studied range	Selected value	
		Batch	CPE
Concentration of HCl (M)	0.05-0.3	0.1	0.1
Concentration of sulfadimidine (mM)	1-6	2	2
Concentration of NaOH (M)	0.06-0.4	0.1	0.1
Volume of 10%(v/v)surfactant (ml)	0.5 –2	---	1.0
Incubation temperature (ºC)	40-80	---	60
Incubation time (min)	10-40	---	30

Methods validation

Using the previous optimum variables for batch and CPE methods summarized in Table 1, which were used for estimation of ISX, the calibration curves for both methods were constructed. For batch and CPE the linearity ranges were 1-9 and 0.5-8 µg/ml of ISX respectively. In addition, the detection limit was estimated from LOD=3SD/b (where SD is standard deviation of 10 replicate of the blank and b the slope of calibration curve) were of 0.26 and 0.09 µg/ml for suggested reaction with and without extraction respectively. The low values of LOD especially for CPE method indicated the sensitivity of the methods. The small values of the analytical characteristics (standard deviation of the residual ($S_{y/x}$), the intercept (S_a) and the slope (S_b)) also showed a small scattering of the calibration points and the accuracy of the present methods. The enrichment factor value "the ratio of the slope of the calibration curve of method with extraction to that of without extraction" was calculated to be 3.4.

Accuracy and repeatability

The accuracy and repeatability of both suggested methods were investigated. Two different concentrations of ISX solutions were assay using batch and CPE methods (with and without extraction) in five replicates. The low values of error (good recoveries values) with acceptable values of relative standard deviation documented in Table 3 indicated the high accuracy and repeatability for both suggested methods.

Table 2. Analytical characteristics for suggested method with and without extraction

Parameter	Value	
	Batch method	CPE method
λ_{max} (nm)	430	490
Regression equation	y = 0.0515x + 0.0077	y = 0.1754x + 0.0068
Correlation coefficient, r	0.9982	0.9996
Range of linearity (µg/ml)	1-9	0.5-8
Detection limit (µg/ml)	0.26	0.09
Quantification limit (µg/ml)	0.86	0.29
Molar absorptivity (L/mol cm)	1.74×10^4	5.93×10^4
Sandell's sensitivity (µg/cm^2)	0.0194	0.0057
Slope (ml/µg)	0.0515	0.1754
Intercept	0.0077	0.0068
Preconcentration factor	10	--
Enrichment factor	3.4	--
$S_{y/x}$	0.0092	0.0152
S_b	0.0012	0.0020
S_a	0.0067	0.0096

Table 3. Accuracy and repeatability for batch and CPE methods

Method	Conc. of ISX (µg/ml)		Recovery%	E_{rel}%	RSD% (n=5)
	Present	Found			
Batch	3	3.02	100.67	0.67	1.94
	4	3.98	99.50	-0.50	1.32
CPE	2	1.98	99.00	-1.00	2.68
	4	4.01	100.25	0.25	1.56

Effect of interferences

With the aim of assessment, the selectivity and efficiently of the suggested methods in routine assay of ISX drug in commercial tablets, the interference of some excipients (lactose, magnesium stearate, polyvinylpyrolidone, and starch) which may be added to the drug compound for manufacturing requirements, were tested. Twentyfold of each interference (40 µg/ml) was added individually to the 2 µg/ml of ISX solution and analysed according to batch and CPE procedures. The ranged of Rec.% of 98-101% referred to insignificant effect of excipients in the analysis of ISX in medical forms.

Assay of ISX in pharmaceutical samples

Both suggested methods were applied efficiently for estimation ISX with and without extraction in commercial medical tablets (Table 4). Good recoveries and variation values obtained from both methods indicated the competence and applicability of these methods in routine analysis of ISX in pharmaceutical forms. In addition, the achieved recoveries were compared with those estimated by applying the HPLC standard method [(1)]. Two common tests were used to perform a statistical comparison between methods (t-and F-tests at a confidence level of 95 percent) [(13)]. The t- and F values measured were lower than the tabulated values, suggesting that there was no major variance in accuracy and applicability between the methods used in the ISX assay of its pharmaceutical tablets.

Table 4. Estimation of ISX in tablets using batch and CPE methods compared with standard method

Pharmaceutical form	Proposed methods										Standard method
	Batch					CPE					
	Conc.(µg/ml)		Rec. (%)*	Mean Rec.(%)	RSD (%)*	Conc.(µg/ml)		Rec. (%)*	Mean Rec.(%)	RSD (%)*	Mean Rec.(%)
	Taken	Found				Taken	Found				
Duvilane (Isoxsuprine hydrochloride® Tablet	4	4.02	100.50	101.00	1.29	4	4.03	100.75	101.38	3.38	100.20
	6	6.09	101.50		3.25	6	6.12	102.00		2.93	
Pure ISX				100.14					99.63		99.50
t (4.303)[c]	1.299					0.695					
F (161.4)[c]	1.509					6.250					

* Average of four determinations; ** theoretical value; conc., concentration; RSD = relative standard deviation. $(n_1 - 1) = 1, (n_2 - 1) = 1, (n_1 + n_2 - 2) = 2$

Conclusion

The present work involves rapid, highly sensitive and green methods for estimation and extraction a micrograms amount of ISX in pharmaceutical applications. A diazotization reaction based on use another drug compound (sulfadimidine) as a green reagent instead of poisonous and expensive reagents. Using Triton X-114, the cloud point extraction method was used to extract the azo-dye resulting from the diazotization reaction. Without requiring poisonous solvents or advanced techniques, a combination of CPE with spectrophotometry provided an easy and low-cost method for ISX estimation. A simple comparison established between batch and cloud point extraction methods indicated the accuracy and repeatability of methods. The presented methods have been successfully implemented with acceptable accuracy for the assay of ISX in pharmaceutical tablets.

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Conflict of Interest: None to declare.

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