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DISPERSIVE LIQUID-LIQUID MICROEXTRACTION AND SPECTROPHOTOMETRIC DETERMINATION OF CEFAZONE AND PHENYLEPHRINE HYDROCHLORIDE IN THEIR PURE FORMS AND PHARMACEUTICAL PREPARATIONS

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Abstract

A simple, accurate and precise approach which has been based upon the coupling of the Dispersive Liquid-liquid Microextraction (DLLME) with spectrophotometry, was developed for analyzing the Cefazone (CEZ) and Phenylephrine hydrochloride (PEH) in pure forms and pharmaceutical preparations. The method involves DLLME of the formed azo-dyes upon the coupling of the studied drugs with the diazotized 2-aminothiazole. Experimental factors affecting the formation of the azo-dyes and their DLLME were optimized. Under optimum recommended conditions, CEZ and PEH were spectrophotometrically determined after DLLME and Beer's law was conformed at 485nm and 495nm with molar absorptivity values of 1.67×10^{5} $L.mol^{-1}.cm^{-1}$ ($r = 0.9995$) and 5.60×10^{5} $L.mol^{-1}.cm^{-1}$ ($r = 0.9996$), limit of detections of 0.0252 mg/L and 0.0236 mg/L and Limit of quantification of 0.0831 mg/L and 0.0708 mg/L respectively with concentration rang of (0.2-6.0) mg/L for both drugs. Excellent criteria for CEZ and PEH DLLME were obtained with the distribution coefficient of (235 and 216), enrichment factors of (23.172 and 24.043) and preconcentration factor of (25 for both) respectively. The analysis of both drugs in pharmaceutical dosages shows a good agreement with the real amounts.

Key words: Microextraction, Cefazone, phenylephrine hydrochloride, Dispersive

Introduction

Cefazone (CEZ) is a third-generation of cephalosporin, chemically known 7amino-cephalosporanic acid (7-ACA), (6-R,7-R)-3-[(acetyloxy)methyl]-7amino-8-oxo-5thia-1-azabicyclo[4.2.0]oct-2-ene-2carboxylic acid (Neu *et al*., 1979). CEZ is widely used β-lactam antibiotics for treating the bacterial illnesses (Hung *et al*., 2018). The most commonly used broad-spectrum of activity against bacteria which is responsible for resulting in urinary and respiratory tract infections, skin and the female genital tract (Lin *et al*., 2019). A literature review displayed several methods for the determination of cefazone HPLC (Tsou *et al*., 2007; Abdelaleem *et al*., 2016) liquid chromatography-tandem mass spectrometric (LC-MS/MS)] (Wu *et al*., 2018; Zhou *et al*., 2010), classical least squares (Naguib *et al*., 2015). Phenylephrine hydrochloride (PEH) chemically referred to as the (1R)-1-(3hydroxyphenyl)-2-(methylamino) ethanol hydrochloride (Mahdi *et al*., 2019). PEH is a sympathomimetic with essentially direct works on alpha-adrenergic receptors (Habibi *et al*., 2017). It is used for the treat nasal congestion and is often included in the preparations which are intended for relieving the cough and the symptoms of cold (Cox and Wise, 2018). In ophthalmology, used as mydriatic and conjunctival decongestant (Meltzer *et al*., 2015). PEH was reported these methods involve spectrophotometric (Elfatatry *et al*., 2016). High Performance Thin-Layer Chromatographic (El-Yazbi *et al*., 2016), RP-HPLC (Dewani *et al*., 2015), spectrofluorimetry (Maher *et al*., 2015). Through the literature survey, there are many spectrophotometric methods for determining the pharmaceutical formulations such as oxidative coupling reactions (Abood *et al*., 2019), oxidization reactions (Hassan and Al-hraishawi, 2017; Mohammed *et al*., 2019) flow injection coupled with Uv-Vis detectors (Abood *et al*., 2019), dispersive and charge transfer methods (Unsal *et al*., 2015). The chemical structures of 2-aminothiazole, CEZ and PEH are shown in pic. 1.

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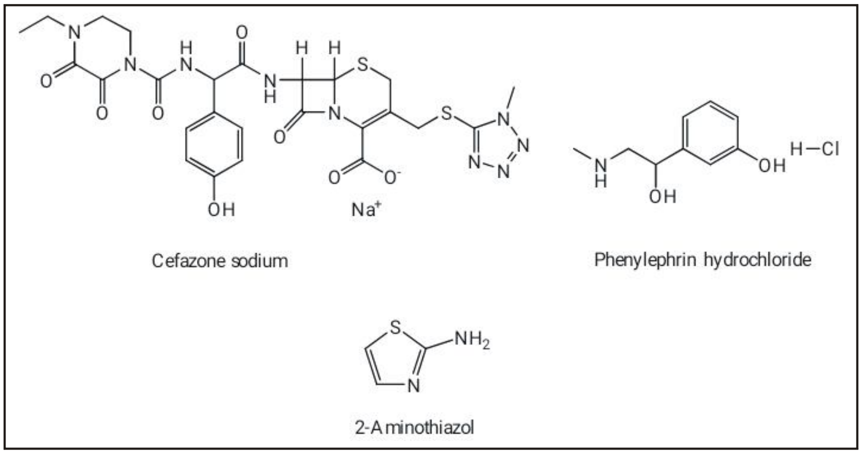


Pic. 1: The chemical structures of cefazone, Phenylephrine hydrochloride and 2-aminothiazole.

Materials and Methods

All Pure standards and reagents have been obtained from the Sigma-Aldrich. Other chemicals and solvents utilized were of analytical grade and high purity grade Distilled water was used for all reagents and sample solutions preparation.

Apparatuses

All spectral measurements were made using a Shimadzu UV-Vis double beam spectrophotometer, UV-160 (Kyoto, Japan), equipped with matched 1cm path-length quartz cuvettes. A centrifuge (Hermle Labortechnik GmbH centrifuge model Z-206-A, Germany) has been utilized in order to increase the speed of the DLLME phase separation

Standard Solution Preparations

1,000 mg/L standard solutions of (CEZ) and (PEH) were prepared through dissolving 100mg of each in separate 100mL volumetric flask with distilled water. Working solutions of cited drugs have been obtained freshly by the serial dilutions. 100 mL of 2-aminothiazole solution (1000 mg/L) has been prepared through the dissolution of 100mg of the reagent in the distilled water. On the other hand, the solutions of other reagents were prepared by dissolving the required amounts in distilled water

Recommended procedure

• Diazotization of 2-aminothiazole and coupling with CEZ or PEH: 0.5 mL aliquots of 9.98 × 10^{-3} M working 2-aminothiazole were transferred into 20 mL calibrated flasks and placed in an ice-bath. After the addition of 1mL of 1mol/L HCl solution to each flask, 1.0 mL or 0.5 mL aliquot of 1.0 % (w/v) sodium nitrite solution, for the case of CEZ or PEH respectively, was added dropwise with gently swaying the flask in the ice-bath so that temperature may not exceed above 5^{o}C. The mixture was then left in an ice-bath for 20 min to ensure the formation of the corresponding diazonium salt. The formed diazonium salt was coupled with 1 mL containing (500-3000 µg) of CEZ or PEH solution in the presence of 2mL of 25% sodium hydroxide. The formed azo-dye in every case has been diluted to the mark with the distilled water.

• Dispersive liquid-liquid microextraction method (DLLME): 1 mL aliquot of the formed azo-dye (containing 2.5-75 µg of CEZ or phenylephrine hydrochloride) is diluted to 10 with distilled water in a calibrated flask and the content of the flask was transferred quantitatively to a 15mL centrifuge tube, 2 mL of organic solvents mixture $CHCl_3$ (as extraction solvent): C_2H_5OH (as dispersive solvent) (1:3, v/v) was quickly injected into the aqueous phase of the azo-dye solution by a syringe. The processes of injection enhance the formation of a cloudy sample that facilitates the separation of the solution. After

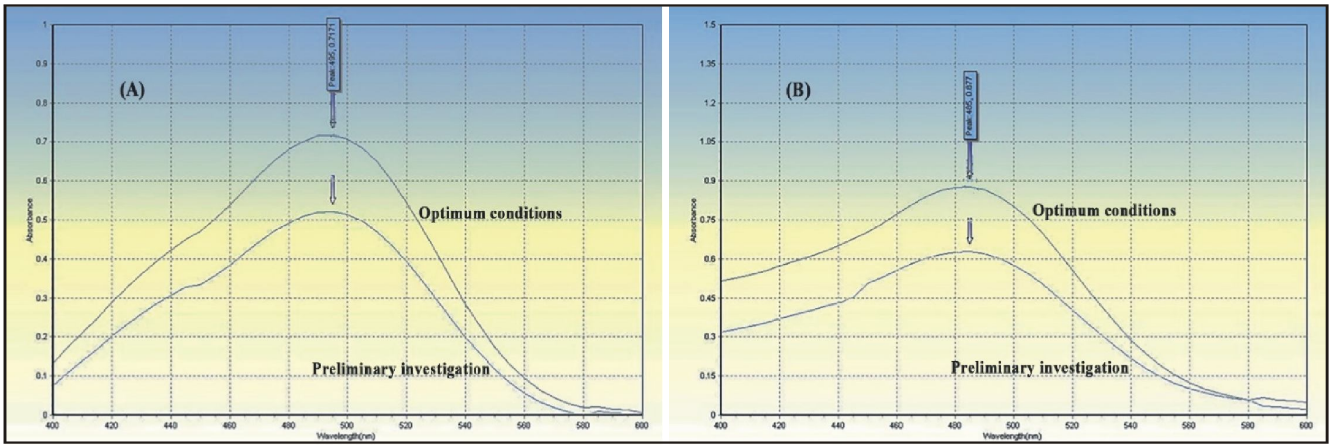


Fig. 1: (A) and (B), The absorption spectra of 2-aminothaizole -PEH azo-dye (ëmax=495 nm) and 2-aminothaizole-CEZ azo-dye (ëmax=485 nm) under preliminary and optimum conditions against corresponding reagent blank respectively.

centrifuging the mixture for 4 min at 3000 rpm for the case of CEZ and 4000 rpm for case PEH and in 4 minutes, the absorbance of the collected organic phase has been measured at 485 nm and 495 nm for CEZ or PEH respectively.

Result and Discussion

Absorption spectra

The first step based upon the formation of yellowish-orange colored azo-dyes by after coupling the studied drugs with diazotized 2-aminothiazole *via* the phenolic group of CEZ and PEH respectively. Fig. 1A and 1B show the recorded spectra of the coupling products under preliminary experimental conditions with maximum absorptions at 485 and 495 for CEZ and PEH respectively.

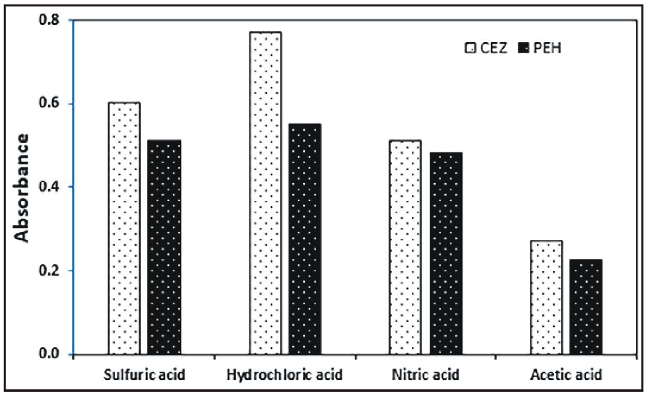


Fig. 2: Effect of acid type.

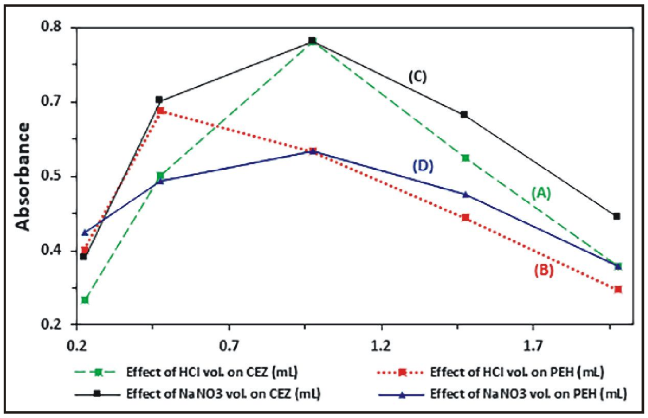


Fig. 3: Effect of HCl and $NaNO_2$.

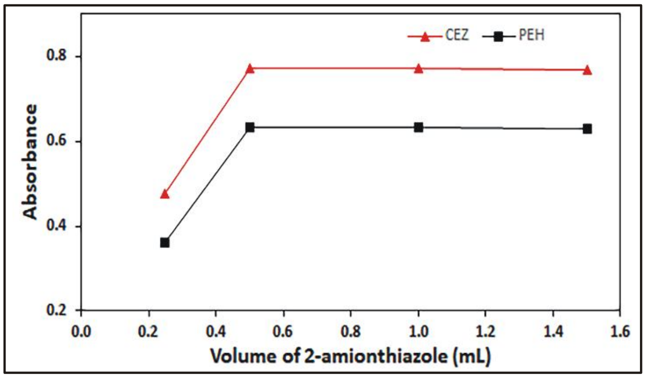


Fig. 4: Effect of reagent.

The conditions for diazotization and coupling reactions were developed by studying various conditions affecting both reactions (viz: the type and concentration of acid, concentration of sodium nitrite, volume of reagent, type and concentration of base) to achieve quantitative colored product formation that gives the highest absorptivity as shown in recorded spectra, fig. 1.

Among the studied acids, the best results were obtained when 1 mL of 1mol/L HCl together with 1mL of 1.0 % (w/v) sodium nitrite was used (Fig. 2 and 3). The effect of volume of 9.98 $\times 10^{-3}$ M of 2-aminothiazole solutions as diazotizing reagent was examined. 0.5 mL of this solution gave the maximum color intensity of the formed azo-dye (Fig. 4). Both the pH and the temperature of the reaction medium are the main factors for controlling diazotization and coupling reaction and need to be arranged to favor coupling rather than diazo decomposition (Akwi and Watts, 2016). Moreover, the time required for the synthesis of diazonium salt was also investigated. The results indicate that carrying out the diazotization reaction in an ice bath at (0-5^{o}C) for 20 min was the optimum (Fig. 5). On the other hand, the type and the volume of different alkali solutions were examined. The results indicated that using of 2.0 mL of 25% sodium hydroxide is optimum (Fig. 6 and 7). The order of addition of the

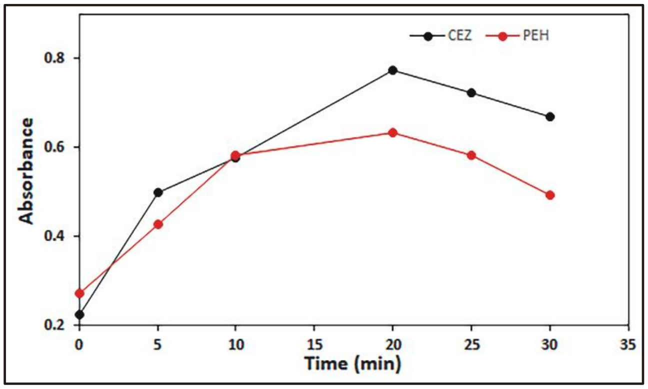


Fig. 5: Effect of time of the diazotization of the reagent.

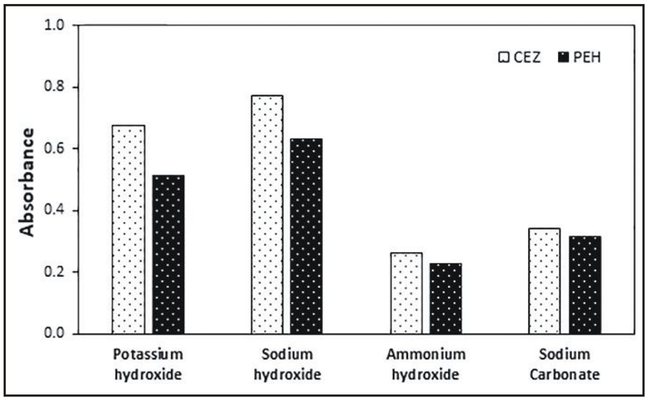


Fig. 6: Effect of the base type.

reactants should be followed, as mentioned in the recommended procedure.

The experimental conditions to achieve complete extraction of the formed drugs azo-dye utilizing dispersive liquid-liquid microextraction were established. The investigation involves the type of organic solvent, the volume of extraction and disperser solvents, extraction time, the ionic strength and the speed and duration of centrifugation. The study shows that neither the time of extraction nor the ionic strength of the aqueous phase plays a significant role in the extraction of studied drugs.

In the DLLME technique, the selection of extraction solvent and disperser solvent is an important step. Generally, the suitable extraction should have a high affinity to analysts, low solubility in water, higher density and lower miscibility than the aqueous sample (Konieczna *et al*., 2016; Abbas and Rasheed, 2018). Ethanol proves that it possess the best characteristics to serve as a dispersive solvent among the other tested solvents (viz; methanol, acetonitrile and acetone). The preconcentration factor and sensitivity of the DLLME are highly dependent on the volume of organic phase (Poormoghadam *et al*., 2015) therefore, the type and the volume of organic solvent used for extraction were studied. The results depicted in fig. 8 and 9 indicate that chloroform shows the greatest extraction ability among the studied solvents when 2 mL if its mixture, with ethanol (as dispersive solvent) in a ratio of 1:3, was used to perform the extraction. The results depicted in fig. 8 and 9 indicate that chloroform shows the greatest extraction ability among the studied solvents when 2 mL if its mixture, with ethanol (as dispersive solvent) in a ratio of 1:3. Increasing the volume of chloroform in the ratio yields in decreasing the absorbance value of the extracted dye. This is due to the dilution effect that helps to decrease the concentration of an analyte in the sediment phase. On the other hand, it was found that absorbance increased with increasing the volume of ethanol Up to 1.5mL, but it decreases after that and this may be due to increased solubility for analysis in a water sample (Kalhor *et al*., 2016).

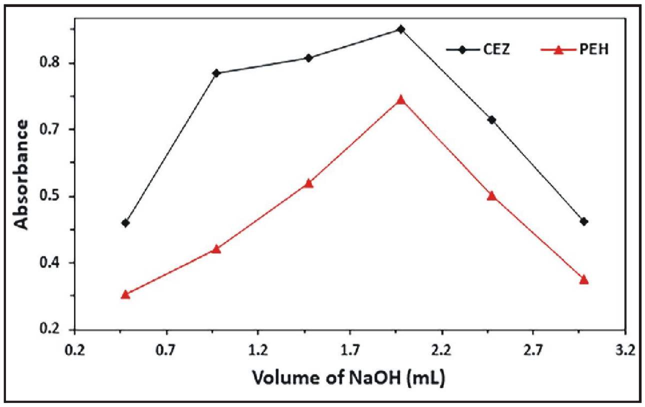


Fig. 7: Effect of NaOH volume.

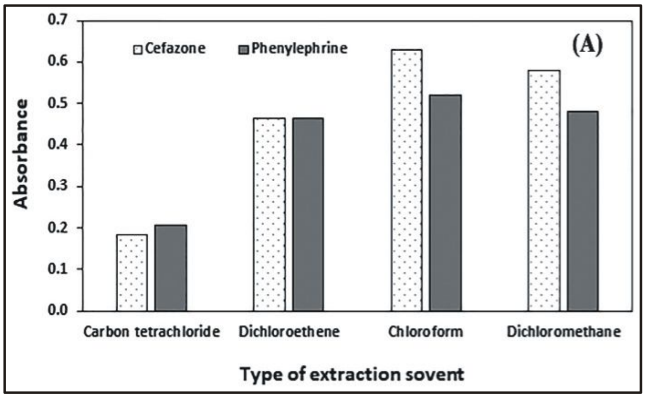


Fig. 8: Effect the type of extraction solvent.

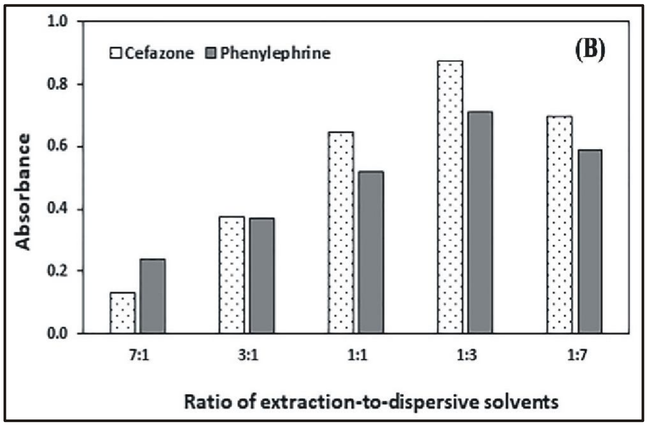


Fig. 9: Effect of extraction-to-dispersive solvents ratio.

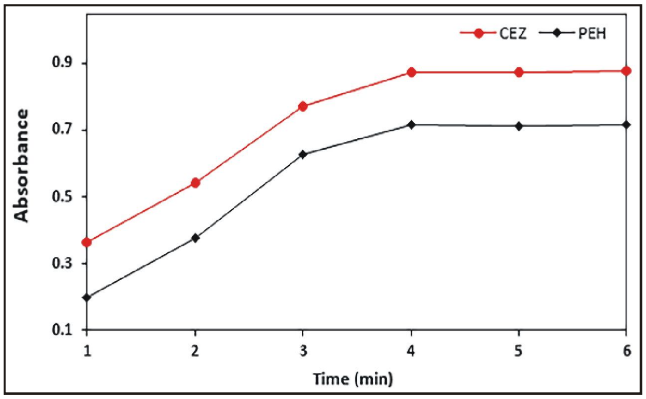


Fig. 10: Effect centrifugation time.

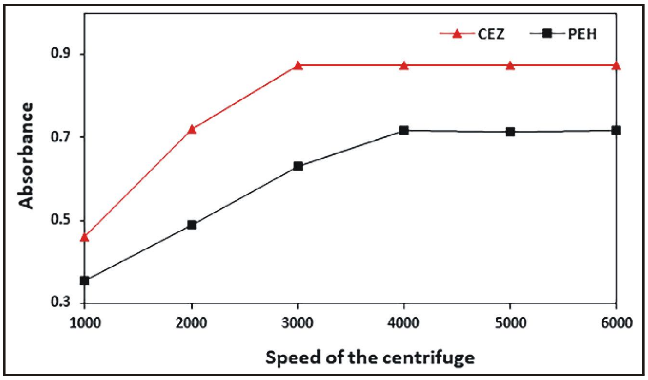
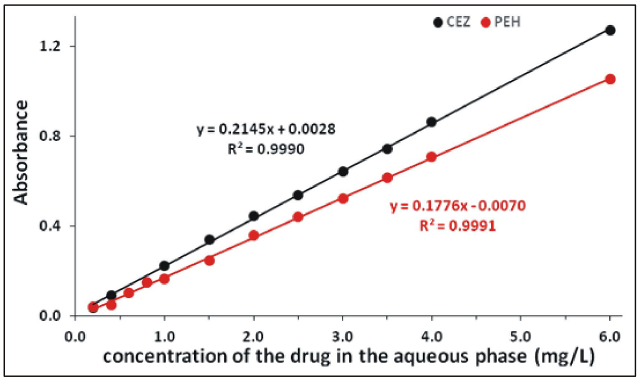


Fig. 11: Effect of centrifugation speed (rpm).

Different centrifugation speeds and durations were studied and the results are illustrated in fig. 10 and 11. 4 min at 3000 rpm for the case of CEZ and 4000 rpm for case PEH in 4 minutes was optimum.

Calibration curves and analytical data

Under the established optimal experimental conditions, for diazotization and DLLME mentioned in the recommended procedures for cefazone and phenylephrine hydrochloride, calibration curves were constructed via the least-squares regression method. Fig. 8 shows the plots of the values of absorbance against the corresponding reagent blank measured at 485mn and 495 after diazotization and DLLME of a set of the standard solutions which contain various amounts of for each drug for CEZ and PEH respectively. The values of correlation coefficient (r) for the regression equations indicate excellent linearity of the plotted curves. Limits of detection and quantification were calculated according to the ICH guide (ICH, 2005). The high values of the molar absorptivity and the low values of detection limits indicate that both procedures are sensitive.



The graphs were linear in the concentration range of (0.2 -6.0) mg/L. Some of the optical and statistical parameters of the determination of CEZ and PHE are tabulated in table 1.

Table 1: Some statistical data and optical characteristics for the determination of CEZ and PEH via the recommended procedures.

Parameters	CEZ	PEH
Linearity range (mg/L)	0.2-6.0	0.2-6.0
Molar absorptivity (ε) L / mole.cm	1.67×10^5	5.6×10^5
Slope	0.2145	0.1776
Intercept	0.0028	0.0070
Sandell's sensitivity (S) μ/cm^2	0.0046	0.0056
Correlation coefficient (r)	0.9995	0.9996
limit of detection, LOD (mg/L)	0.0252	0.0236
Limit of quantification LOQ (mg/L)	0.0831	0.0708
Distribution coefficient (D)	235	216
Enrichment factor (EF)	23.172	24.043
Preconcentration factor	25	25

Table 2: Accuracy and precision for the estimation of CEZ and PEH by the recommended method.

Drug	Conc. of the drug (mg/L)		*RE%	*RSD %
	Taken	Found		
Cefazone	1.000	0.998	-0.200	0.074
	3.000	3.021	0.700	0.128
	5.000	5.087	1.740	0.181
Phenylephrine hydrochloride	1.000	1.003	0.300	0.098
	3.000	3.043	1.433	0.146
	5.000	4.978	-0.440	0.184

The values of correlation coefficient (r) for the regression equations indicate excellent linearity of the plotted curves. Limits of detection and quantification were calculated according to the ICH guide (ICH, 2005). The high values of the molar absorptivity and the low values of detection limits indicate that both procedures are sensitive.

Accuracy and precision

The accuracy of the suggested approach for determining the CEZ and PEH in terms of the relative percentage of the error and the precision in terms of the relative standard deviation percent were assessed by analyzing five replicates of the pure drug at three concentration levels. Table 2 shows that the obtained results were accurate (the values of RE% ±1.740% for CEZ and 1.433% for PEH) and precise (the values of

Table 3: Application of the recommended procedures for the determination of CEZ and PEH in commercial pharmaceutical preparations.

Pharmaceutical preparation	Concentration (mg/L)		Recovery %	*RSD %
	Taken	Found		
Zinol 1g vial Pharco B international Egypt	1.000	1.013	101.300	0.216
	3.000	2.994	99.800	
	5.000	4.988	99.760	
Cefrone 1 g vial Medical Union Pharmaceuticals Egypt	1.000	0.996	99.600	0.221
	3.000	3.040	101.333	
	5.000	5.079	101.580	
Phenylalanine hydrochloride 100 mg/10 mL injection Germany	1.000	1.011	101.100	0.182
	3.000	2.969	98.967	
	5.000	5.081	101.620	
Phenylephrine hydrochloride 10 mg/mL injection EMP / Chicago	1.000	0.998	99.800	0.203
	3.000	3.003	100.100	
	5.000	5.004	100.080	
* Average for 15 replicates.				

RSD % were in the range of 0.074%-0.1.81% for CEZ and 0.098%-0.184% for PHE).

Application of the method

To investigating the suitability of the proposed diazotization- DLLME method, the content of CEZ and PEH in commercial pharmaceuticals formulation were determined under the recommended procedures. The results in table 3 indicate that the recovery percent values are ranged between (99.600-100.580) and those for RSD% do not exceed 0.221 which proves that the proposed method is satisfactory.

Conclusion

In the current work, a new analytical approach for determining the CEZ and PEH has been developed. The method based upon the coupling of the cited drugs with the diazotized 2-aminothiazole and the formed azo-dye was depressively micro-extracted into chloroform and spectrophotometrically determined. Although the proposed method is sensitive (shows low LOD) selective (suffers no interference from excipients in commercial pharmaceutical) since it depends on DLLME, it is simple and needs no sophisticated equipment. Finally, carrying out the analysis under the recommended procedure is not costly, efficient and environment-friendly.

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