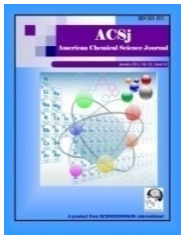

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Spectrophotometric Method for the Determination of Cefotaxime Sodiumand Cefoperazone Sodium in Pure and Pharmaceutical Dosage Forms

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Authors' contributions

This work was carried out in collaboration between all authors. Author RAS collaborated with the experiments and wrote the paper. Authors WSH and MYE performed the statistical analysis. Author AS managed the analyses of the study and revised the paper. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Accuratespectrophotometric method was developed for the estimation of cefotaxime sodium (I) andcefoperazone sodium (II) in both pure and pharmaceutical dosage forms. The method is based on the reaction of the amino groups of the cited drugs with ninhydrin reagent producing a colored product which absorbs maximally at 568 nm. Beer's law was obeyed in the concentration range of (10-90) and (60-350) µg.mL^{-1} for drug (I) and (II) respectively .The correlation coefficient (r^2),molar absorptivity (ε), sandell sensitivity, detection (LOD) and quantitation limits (LOQ) for the studied drugs were calculated. The proposed method was successfullyapplied for the determination of certain pharmaceutical dosage forms containing the studied drugs.

Keywords: Cefotaxime sodium; cefoperazone sodium; spectrophotometric; condensation; ninhydrin.

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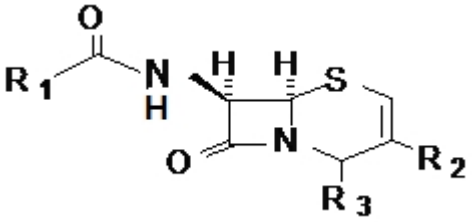
1. INTRODUCTION

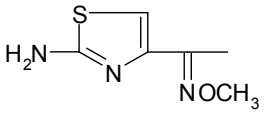
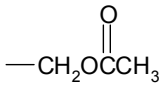
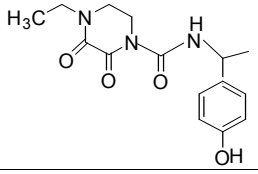
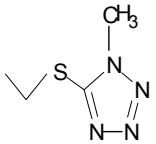
Cephalosporins are one of the most important types of antibiotics used. Cephalosporins are penicillinase-resistant antibiotics .The β-lactam ring structure of cephalosporins interferes with the synthesis of the bacterial cell wall. They are effective against Gram (+) and Gram (−) bacteria. They are one of the safest and the most effective broad-spectrum bactericidal antimicrobial agents and therefore, they are the most frequently prescribed class of antibiotics [1].

Cefotaxime sodium and Cefoperazone sodium are two of third- generation cephalosporins. They have broad spectrum activity against Gram positive and Gram negative bacteria.The official monograph of the cited drugs in British Pharmacopoeia (2010) is high performance liquid chromatography (HPLC) method [2]. In the literature, several methods have been reported for their determination [3-10].

Ninhydrin,awell-known carbonyl reagent, was applied in the pharmaceutical assay of different nitrogenous compounds.Amino acids, amines, amides, hydrazines, piprazines and cyanide, were all assayed using ninhydrin, as it was known to form a condensation product of a distinctive purple color that could be measured spectrophotometrically. Ninhydrin was used for spectrophotometric determination of cephaloridine, cefoxitin sodium and cephalothin sodium [11], some penicillins[12], cyanide [13], tranexamic acid [14],lisinopril[15], Topiramate [16] were all determined spectrophotometrically using ninhydrin. A modified approach for ninhydrin green use was developed for lisinopril determination [17].Spectrofluorimetric procedures were also reported for determination of cyclophosphamide, its isomer ifosphamide [18] and panthenol[19] using ninhydrin.

In this paper, a new, simple, sensitive and precise method for the estimation of cefotaxime sodium (I) and cefoperazone sodium (II) in both pure and pharmaceutical dosage forms was developed. The method was based on is based on the reaction of the amino groups of the cited drugs with ninhydrin reagent producing a colored product which absorbs maximally at 568 nm.



Name	Chemical structure[2]		
	R1	R2	R3
Cefotaxime Sodium(I)			COONa
Cefoperazone sodium(II)			CO ONa

Scheme 1.Chemical structures of the investigated cephalosporin antibiotics

2. EXPERIMENTAL

2.1 Apparatus

Shimadzu recording spectrophotometer UV 1201 (Japan)equipped with 10 mm matched quartz cells. Digital analyzer pH meter (USA) was used.

MLV type thermostatically controlled water bath (Salvis AG Emmenbruck, Luzern, Germany).

2.2 Materials and Reagents

Chemicals of analytical grade and double distilled water were used throughout the work.

1- Cefotaxime sodium, cefotax vials labelled to contain 1000 mg cefotaxime sodium per vial (EGYPTIAN INT. PHARMACEUTICAL INDUSTRIES CO. E.P.I.C.O. Egypt)
2- Cefoperzone sodium, cefozon vials labelled to contain 1000 mg cefoperzone sodium per vial (EGYPTIAN INT. PHARMACEUTICAL INDUSTRIES CO. E.P.I.C.O. Egypt)
3- Ninhydrin (Aldrich Chemical Co. Ltd) 2% w/vand 10-2 M solutions were freshly prepared in methyl alcohol.
4- Borate buffer pH (8) and acetate buffer pH (5) [2].
5- Methanol (El-Nasr pharm. Chem. Co., Egypt).

2.3 Standard Drug Solutions

Preparation of cefotaxime sodium and cefoperazone sodium standard solutions: Stock solution was prepared to contain 1 mg/mL, by dissolving 100 mg of the pure drug in 100mL double distilled water.

Standard solutions of both drugs are stable for 24 hours at below 25°C.

2.4 General Procedure

Into two sets of 10 – mL volumetric flasks, different aliquots of standard solutions containing (0.1 –0.9) and (0.6 – 3.5) mg for (I) and (II) respectively followed by 2 and 1.5 mL of 2% ninhydrin for (I) and (II) respectively were transferred .Then 1.5 mL acetate buffer pH (5) and 2 mL borate buffer pH (8) were added., the mixtures were heated in a boiling water bath for the appropriate time, then cooled to room temperature, the flasks were diluted to volume with double distilled water, the absorbance was measured at 568 nm against a reagent blank treated similarly (Fig.1).

2.5 Procedure for Pharmaceutical Formulations

For cefotax and cefozon vials : Accurate amount of vial equivalent to 100 mg of cefotaxime sodium and cefoperazone sodium was transferred to 100 mL volumetric flasks, completed to 100 mL with double distilled water and the procedure was completed as under general procedure.

2.6 Stoichiometric Relationship

To study the stoichiometry of reaction, Job's method of continuous variation [20] was employed using equimolar (10^{-2})M standard solutions of both drugs with ninhydrin (10^{-2}) M. A series of solutions were prepared in which the total volume of drugs and ninhydrin was kept at 3 mLso,different molar ratios were employed then the procedure was completed as under theabove mentioned procedure.

3. RESULTS AND DISCUSSION

Primary amines and amides can react with ninhydrin giving a condensation product.This product is formed via oxidative deamination and then condensation with two molecules of ninhydrin giving Ruhemann's purple with λmax at 550 – 590 nm [21,22]. In this paper, the primary amino group of cefotaxime sodium and the amide group of cefoperazone sodium react with ninhydrin giving purple color at 568 nm (Fig.1).The suggested reaction mechanism is presented in the following scheme.

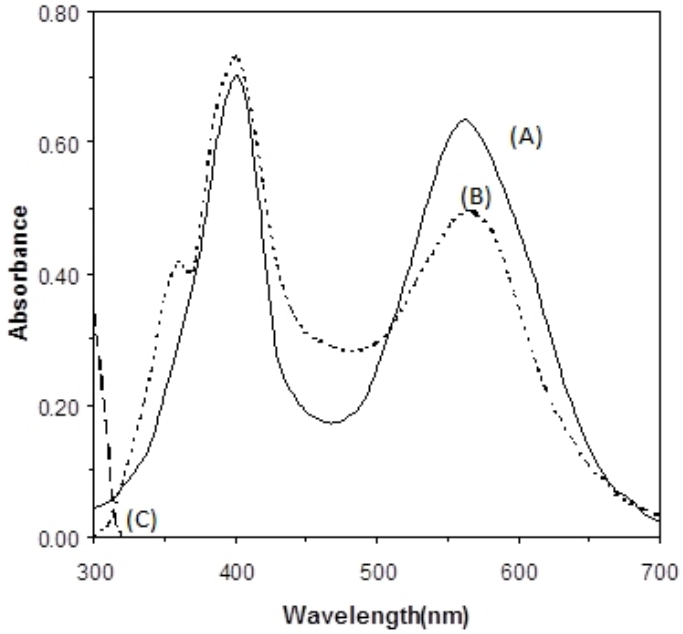
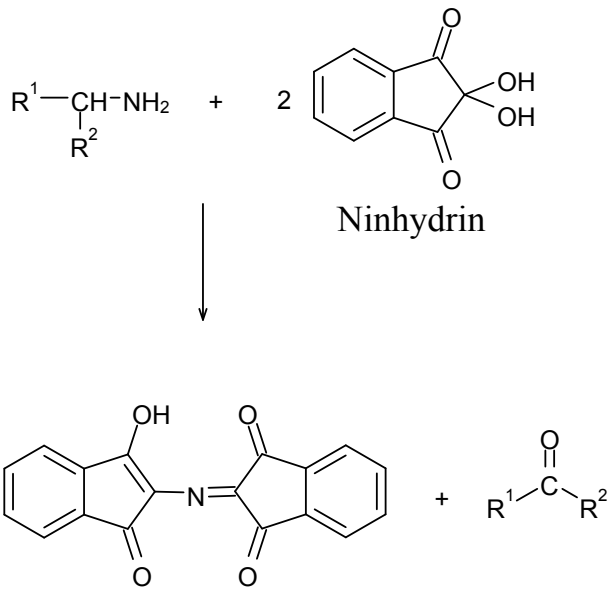


Fig. 1. Absorption spectra of the reaction between 2% w/v ninhydrin and:
(A) 55 μg/mL cefotaxime sodium
(B) 230 μg/mL cefoperazone sodium
(C) Blank solution



Scheme 2.Suggested scheme for the reaction ofninhydrin with the cited drugs

The major disadvantage of colorimetric reactions is the lack of specify due to its dependence on the presence of a certain functional group.However, the proposed method is inexpensive,simple and available especially in developing countries and they do not require complex equipment and intensive sample preparation as chromatographic techniques. So, the proposed method can be used as alternative methods to the reported ones for the routine determination of cited drugs.

3.1 Optimization of the Reaction Conditions

3.1.1 Effect of heating time

The effect of heating time on the absorption intensity was studied .Different heating times in a boiling water bath (at 100°C) from 5 min until one hour were tried.It was found that heating for about 40 and 20 minutes for (I) and (II) respectively (at 100°C) gave maximum absorption intensity (Fig. 2). The produced color was stable for at least 50 and 30 min for (I) and (II) respectively (Fig. 3).

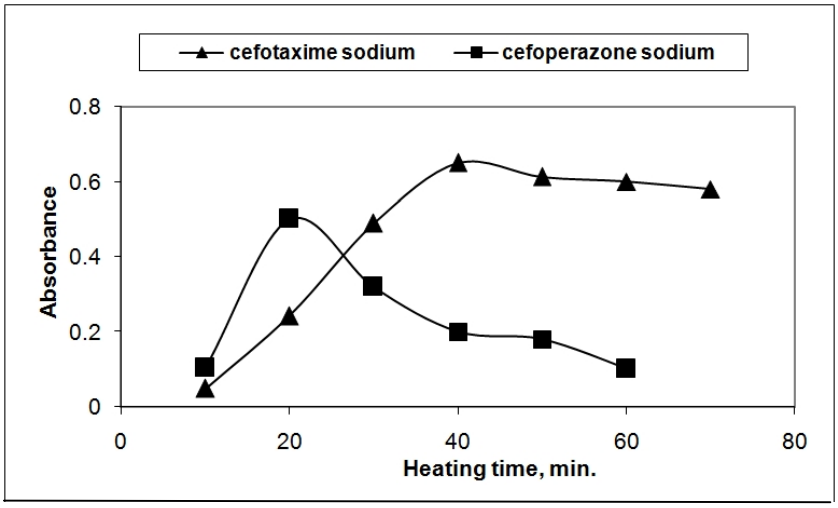


Fig. 2. Effect of heating time on on the reaction of ninhydrin with: - 70 μg/mL cefotaxime sodium - 290μg/mL cefoperazone sodium

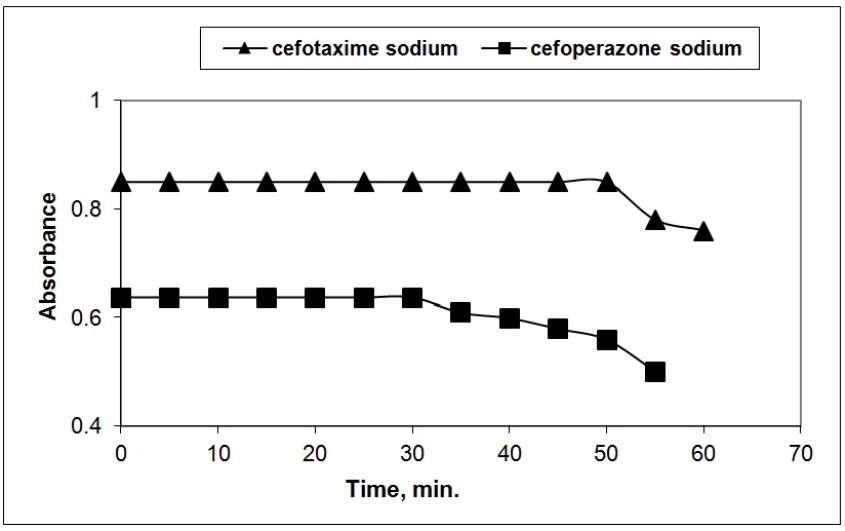


Fig. 3. Stability of the reaction product of ninhydrin with - 70 μg/mL cefotaxime sodium- 290μg/mL cefoperazone sodium

3.1.2 Effect of ninhydrinconcentration

Different volumes of ninhydrin ranging from 0.5 mL to 3 mL were tried.Maximum color intensity was obtained using 2and 1.5 mL of 2% w/v ninhydrinfor (I) and (II) respectively (Fig.4).

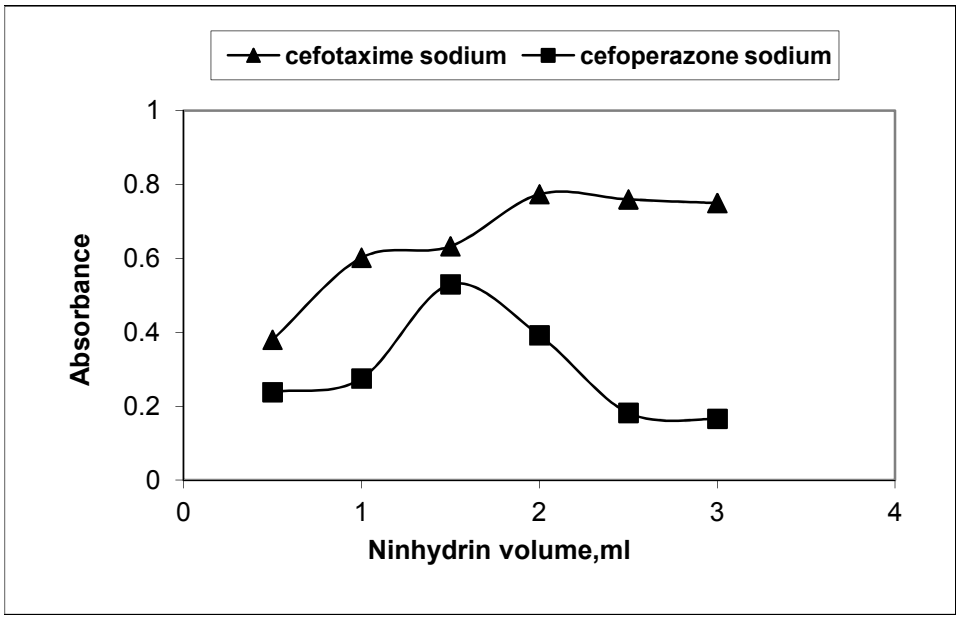


Fig. 4. Effect of ninhydrin volume on the reaction of ninhydrin with:
- 70 μg/mL cefotaxime sodium - 290μg/mL cefoperazone sodium

3.1.3 Effect of pH:

The effect of pH on the reaction was studied over the pH range 4-10. It was found that acetate buffer of pH (5) and borate buffer of pH (8) was the optimum for (I) and (II) respectively. Different volumes of buffer ranging from 0.5 mL to 3 mL were tried.It was found that 1.5 and 2 mL were the optimum for (I) and (II) respectively(Fig.5).

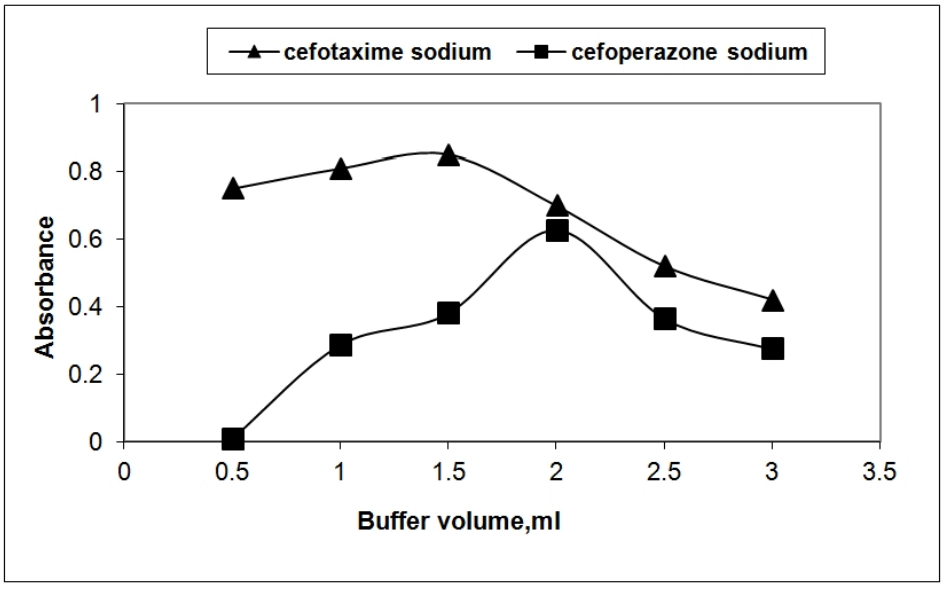


Fig. 5. Effect of buffer volume on the reaction of ninhydrin with:
- 70 μg/mL cefotaxime sodium - 290μg/mL cefoperazone sodium

3.1.4 Effect of solvent

Water, ethanol, methanol, acetone and acetonitrile were tried. Water gave the best results for both drugs.

3.2 Stoichiometric Relationship

The stoichiometry of the rection product formed between the cited drugs andninhydrin was investigated by applying the continuous variation [20]method .The molar ratio of ninhydrin to both drugs (2:1) (reagent:drug) (Fig.6).

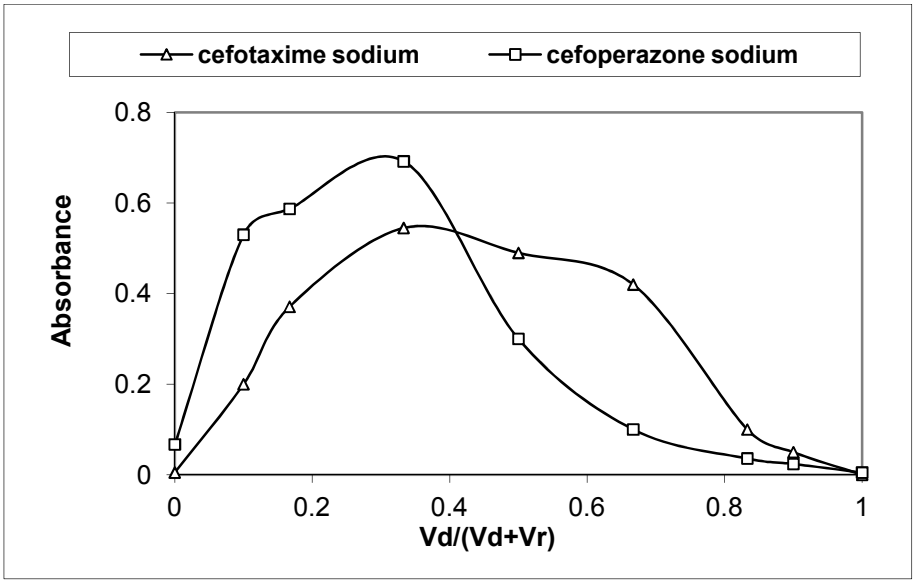


Fig. 6. Continuous variation plot for cefotaxime sodium (10^{-2} M) and cefoperazone sodium (10^{-2} M) with ninhydrin (10^{-2}M)

3.3 Method Validation

3.3.1 Linearity

Standard calibration curves were constructed by plotting absorbances against concentrations for both drugs. Beer's law limits, molar absorptivity,linear regression equations and sandell sensitivity were calculated for each drug (Table 1). The correlation coefficients were found to be 1for both drugs indicating excellent linearity over beer's law limits (Table 1).

3.3.2 Accuracy and precision

Solutions containing one concentration of each drug was analysed in seven replicates. The relative standard deviation (RSD%) and percentage relative error (Er %) were estimated at 95% confidence levels (Table 2). The results showed that the proposed methods have good repeatability and reproducibility.

Table 1. Characteristic parameters for the reaction of cefotaxime sodium and cefoperazone sodium using ninhydrin*

Parameter	Cefotaxime sodium	cefoperazone sodium
λ max (nm)	568 nm	568 nm
Beers law limits (µg/mL)	10-90	60-350
Heating time (min)	40	20
Ninhydrin volume and concentration	2mL (2% w/v)	1.5mL (2% w/v)
Optimum pH	1.5 mL acetate buffer pH (5)	2 mL borate buffer pH (8)
Regression equation**		
Slope (b)	0.011	0.0023
Intercept (a)	0.0788	- 0.0297
Correlation coefficient (r2)	1	1
LOD µg/mL	14.41	22.45
LOQ µg/mL	43.67	68.04
Sandell sensitivity µg.cm-2	0.73	4.77
ε (×104) L.mol-1.cm-1	0.07	0.01

*Average of three experiments
**A=a + bc

Table 2. The intra-day and inter-day accuracy and precision data cefoperazone sodium and cefotaxime sodium using ninhydrin

	Intra-day Taken (µg/mL)	Found (µg/mL)[a]	Recovery %	Precision RSD% [b]	Accuracy Er% [c]	Inter-day Taken (µg/mL)	Found (µg/mL)[a]	Recovery %	Precision RSD% [b]	Accuracy Er% [c]
cefotaxime sodium	50	50.54	101.08	0.65	1.08	50	50.38	100.76	0.68	0.76
Cefotperazne sodium	230	227.76	99.03	0.87	-0.97	230	231.24	100.54	0.60	0.54

a. *Average of seven determinations*.
b RSD%, percentage relative standard deviation
cEr%, percentage relative error

3.3.3 Sensitivity

The detection limit (LOD) and the limits of quantitation, LOQ, were calculated using the The detection limit (LOD) and the limits of quantitation, LOQ, were calculated using the following equation according to the ICH [23] (Table 1).

$$LOD = 3.3\ \sigma/S$$
$$LOQ = 10\ \sigma/S$$

Where σ = the standard deviation of replicate blank responses (under the same conditions as for sample analysis)
S = the slope of the calibration curve

3.4 Analytical Applications

The proposed method was successfully applied to determine the cited drugs in its pharmaceutical dosage forms. Standard addition techniquewas used for studying the recovery of the drugs (Table 3). The results were compared with the reported methods [3,4] using Student t-test and Variance ratio F-test at 95% confidence level (Table4).No significant differences between the proposed method and reported method [3,4].

Table 3. Application of the standard addition technique to the spectrophotometric determination of cefoperazone sodium and cefotaxime sodium using ninhydrinin pharmaceutical dosage forms*

Cefotax vial				Cefozon vial			
Claimed taken µg/mL	**Authentic added µg/mL**	**Found conc. µg/mL**	**Recovery %**	**Claimed taken µg/mL**	**Authentic added µg/mL**	**Found conc. µg/mL**	**Recovery %**
10	-	10.02	100.18	60	-	60.30	100.51
	10	10.02	100.18		60	59.00	98.33
	20	20.29	101.45		110	109.00	99.09
	30	30.20	100.67		170	169.87	99.92
	35	34.84	99.53		190	191.61	100.85
	50	51.11	102.22		210	209.00	99.52
	60	60.20	100.33		240	241.61	100.67
	70	70.20	100.29		290	287.26	99.06
	80	80.20	100.25				
Mean			100.57				99.74
Variance			0.64				0.80
SD			0.80				0.90
SE			0.28				0.34

** Average of three experiments*

Table 4. Determination of cefotaxime sodium and cefoperazone sodium using ninhydrin compared with reported methods [3,4]

Drug		Ninhydrinmethod	Reported method
Cefotaxime sodium	**Mean ±S.D**	10018±0.56	100.02±0.437[3]
	N	7	6
	Variance	0.31	0.192
	Student-t-test	0.56 (2.201)*	
	F-test	1.62 (4.59)*	
Cefoperazone sodium	**Mean ±S.D**	99.97±0.25	99.94±0.42[4]
	N	7	9
	Variance	0.06	0.18
	Student-t-test	0.17 (2.145)*	
	F-test	3.00 (3.59)*	

**The Figures in parenthesis are the theoretical values for t- and F-tests (P < .05).*

4. CONCLUSIONS

The proposed method is accurate in determining the cited drugs in their pharmaceutical formulations without interference from common excipients. One can do the analysis at low cost without losing accuracy. Also, In comparison with the chromatographic methods , the proposed method is time consuming and not very precise as HPLC and GLC methods but these techniques are not available especially in developing countries and they require complex and expensive equipment, intensive sample preparation and personnel skilled in chromatographic techniques. So, the proposed method can be used as alternative methods to the reported ones for the routine determination of cited drugs.

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COMPETING INTERESTS

Authors have declared that no competing interests.

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