



Development of Simple Green Spectrophotometric and Conductometric Methods for Determination of Cephalosporins in Pure, Pharmaceutical Dosage forms and Human Urine.

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ABSTRACT

Five Simple, accurate and rapid spectrophotometric and conductometric methods were developed for the determination of four third generation cephalosporins, namely, cefotaxime sodium (I) , cefoperazone sodium (II), ceftazidime pentahydrate (III) and cefdinir (IV) in pure active ingredient, pharmaceutical dosage forms and human urine. *Method A:* is based on the reaction of the sulphide ions produced from the alkaline hydrolysis of the cited four drugs with P- aminophenol (PAP). This reaction results in a thionine dye (phenothiazine derivative) formation which exhibits maximum absorbance at 545 nm. *Method B:* is based on oxidation of drug (I and III) with a known excess of n-bromosuccinimide (NBS) in acidic medium followed by the determination of unreacted amount of n-bromosuccinimide with metol and sulphanilic acid. The purple-red reaction product exhibits maximum absorbance at 520 nm. *Method C:* is based on the formation of yellow chelate between drug (IV) and palladium (II) chloride in buffered medium (pH 3.5) with an absorption maximum at 314 nm. *Method D:* is based on the reaction of drug (IV) with aqueous ninhydrin to give yellow colored product in the presence of bicarbonate with an absorption maximum at 433 nm . *Method E:* A conductometric method is based on the reaction of the four cited drugs with phosphotungstic acid (PTA) forming an ion associate in aqueous medium. Validation of the proposed methods was carried out. All proposed methods were successfully applied for the commercial dosage forms of the cited drugs. Method C was successfully applied for the determination of cefdinir in human urine.

Keywords

Cephalosporins; green; spectrophotometric; conductometric; pharmaceutical preparations and human urine.

Council for Innovative Research

Peer Review Research Publishing System

Journal: Journal of Advances in Chemistry

Vol. 4, No. 3

editor@cirworld.com

www.cirworld.com, member.cirworld.com



1. INTRODUCTION

Cephalosporins are one of the most important type of antibiotics used vary widely. Analysis of these antibiotics is challenge due to their sensitivity and instability to different conditions. Cephalosporins are related to the penicillins in their structures and pharmacology. Like the penicillins, cephalosporins have a β -lactam ring in their structures that interferes with bacterial cell wall synthesis. They are used for the treatment of infections caused by Gram (+) and Gram (-) bacteria. They are among the safest and the most effective broad-spectrum bactericidal antibiotics and therefore, they are the most frequently prescribed class of antibiotics [1]. Cephalosporins are divided into first-, second-, third-, and fourth-generation agents. The studied drugs are four of third- generation agents.

Several methods have been reported for cephalosporins determination. The official procedures for the cited drugs (I ,II ,III and IV) utilize liquid chromatography [2,3] which is expensive .The literature reveals that many methods were developed for cephalosporins determinations [4,5,6].

The majority of the reported methods were HPLC methods which require expensive equipment, chemicals and intensive sample preparation. Use of organic solvents and hazardous materials is the common feature in most previous methods. So the aim of this work is to develop five simple, accurate, precise, and rapid green spectrophotometric and conductometric methods for the estimation of the cited drugs in pure, pharmaceutical dosage forms and human urine. The proposed methods have higher sensitivity than other methods. With these methods, chemist can carry out cefotaxime sodium, cefoperazone sodium, ceftazidime pentahydrate and cefdinir analysis at low cost without interference from excipients or biofluid matrix.

2. EXPERIMENTAL

2.1. Instruments

Shimadzu recording spectrophotometer UV 1201 equipped with 10 mm matched quartz cells and conductometer model 470 portable conductivity / TDS meter, 25 DEG.C-C10 dip-type cell with a cell constant, K_{cell} of 1.09 were used. Digital analyzer pH meter (USA) was employed for pH measurement.

2.2. Reagents and materials

All chemicals and materials were of high analytical grade, and double distilled water was used through 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- Ceftazidime pentahydrate, cefidime vials labelled to contain (ceftazidime pentahydrate – sodium carbonate mixture equivalent to 500 mg ceftazidime per vial).
- 4- Cefdinir (Bristol - Myers Squibb Pharmaceutical Co., Cairo, Egypt). Dinar capsules labelled to contain 300 mg cefdinir per capsule, Dinar suspension labelled to contain 125 mg cefdinir per 5 mL (Kahira Pharma Co for ADWIA Co. S.A.E 10th of Ramadan City , Egypt) .
- 5- P-Aminophenol (Acros Belgium) , was prepared as 10^{-2} M as by dissolving 0.27 g PAP in 200 mL distilled water, 5 mL concentrated sulphuric acid were added and the volume was completed to 250 mL with distilled water.
- 6- Zinc acetate (0.5 M in 0.1 M sulphuric acid), potassium chloride (2 M aqueous solution), were obtained from Adwic Chemical Company, (Egypt).
- 7- Ammonium iron(III) sulphate (SD Fine – Chem Limited (SDFCL), India), 0.5 M solution was prepared in 0.5 M sulphuric acid.
- 8- N-bromosuccinimide (Sigma Aldrich,Germany) (0.1 g%, w/v) aqueous solution.
- 9- Metol (Sigma Aldrich ,Germany) (0.2 g%, w/v) aqueous solution.
- 10- Sulphanilic acid (Sigma Aldrich,Germany) (0.2 g%,w/v) aqueous solution.
- 11- Palladium(II) chloride (Sigma, Milwaukee, WI,USA) was prepared as 2×10^{-3} M by dissolving 35.5 mg of palladium(II) chloride in 1 mL of concentrated hydrochloric acid and diluting to 50 mL with distilled water, with the aid of heat and then the solution was cooled and diluted to 100 mL with distilled water.
- 12- Ninhydrin (Sigma, Milwaukee, WI,USA) was prepared as 0.5% aqueous solution.
- 13- Phosphotungstic acid (Winlab , UK) was prepared as 10^{-2} M aqueous solution.
- 14- Sulphuric acid , hydrochloric acid and sodium hydroxide were obtained from El-Nasr Chemical Company, (Egypt).
- 15- Walpole's acetate buffer was prepared by adding different volumes of 0.2 M acetic acid to different volumes of 0.2 M sodium acetate (El-Nasr Chemical Company, Egypt) .
- 16- Sodium Bicarbonate (Saturated Solution) (El-Nasr Chemical Company, Egypt) was prepared by dissolving 25 g NaHCO_3 in 100 mL of water . Stirring for twenty minutes with a magnetic stirrer was carried out then the clear supernatant was taken by filtration.

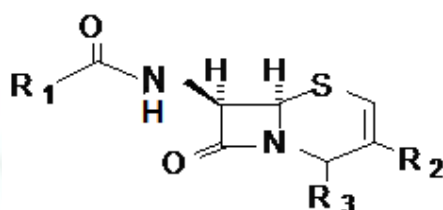
2.3. Preparation of standard solutions

1 - For the method A, stock working solutions of drugs (I,II,III) were prepared to contain 6 mg/mL, dissolved in double distilled water. For drug (IV), solution of 3 mg / mL dissolved in 1 mL 1 M NaOH and the volume was completed with double distilled water to the mark

2 - For the method B , Stock working solutions of 0.2 mg / mL dissolved in 0.2 M and 0.05M HCl for drug (I and III) respectively.

3 - For the methods C and D, solution of 0.2 mg / mL dissolved in 1 mL 1M NaOH and the volume was completed with double distilled water to the mark.

4- For the method E, stock working solutions of all drugs were prepared to contain 3 mg/mL.



Name	Chemical structures [2,3]		
	R ₁	R ₂	R ₃
Cefotaxime Sodium(I)			COONa
Cefoperazone sodium(II)			COONa
Ceftazidime Pentahydrate(III)			COOH
Cefdinir (IV)			COOH

Scheme (1): Chemical structures of the investigated cephalosporin antibiotics.

2.4. CALIBRATION PROCEDURES

2.4.1. Method A

Into four sets of 10 mL volumetric flasks, accurate volumes of the standard solution of each drug containing (0.6-7.2), (1.8-13.8), (1.2-10.8) and (0.3 - 3.6) mg of drug (I, II, III and IV) respectively were transferred and the solutions were completed with 1M NaOH to the mark. The flasks were heated in a boiling water bath for 50 minute for drug (I and II) and 60 minute for drug (III and IV) and then cooled to room temperature. One mL of each of these solutions was transferred into another three sets of 10 mL volumetric flasks. Two milliliters of zinc acetate solution then the specified volume of p-aminophenol and Ammonium iron (III) sulphate solutions were added to each flask. The flasks were shaken for 30 seconds and allowed to stand for the specified time. The volumes were completed to 10 mL with distilled water. Absorbance was measured at 545 nm against blank solution (Figure 1).

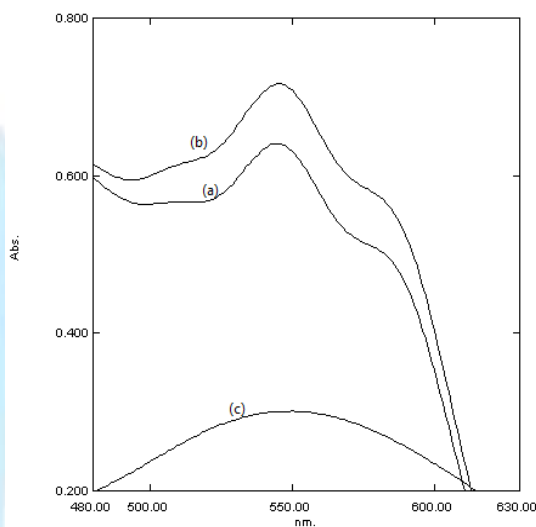


Figure 1: Absorption spectra of :

- (a) The thionine dye formed through the reaction with 48 µg/mL cefotaxime sodium.
- (b) The thionine dye formed through the reaction with 108 µg/mL cefoperazone sodium.
- (c) Blank solution.

2.4.2. Method B

Into two sets of 10 mL volumetric flasks, accurate volumes of the standard solution of each drug containing (0.02-0.32) and (0.04-0.32) mg of drug (I and III) respectively were transferred. To each flask 0.8 and 2 mL of NBS were added for drug (I and III) respectively. The content was mixed well. The flasks were kept for 15 and 10 min for drug (I and III) respectively with intermittent shaking. Then, the specified volume of metal was added. After 1 minute, the specified volume of sulphanic acid was added to each flask, The flasks were kept aside for 3 minutes. Then, the volume was diluted to the mark with bidistilled water and mixed well. The absorbances were measured at 520 nm (Figure 2). Calibration curves were prepared by plotting decreasing values of absorbance against drug concentrations.

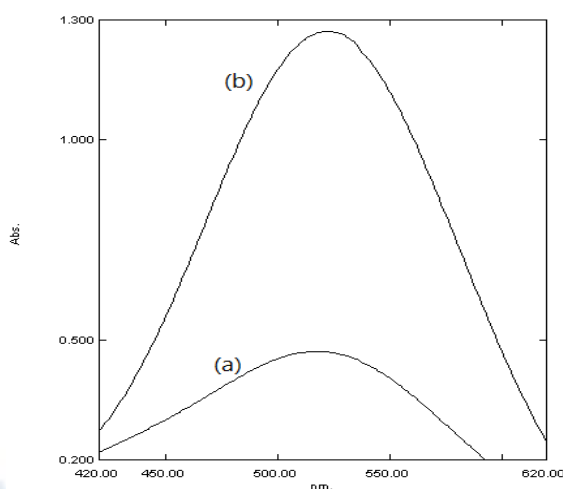


Figure 2: Absorption spectra of:
(a) The reaction between NBS solution and of 17 $\mu\text{g/mL}$ cefotaxime sodium.
(b) Blank solution.

2.4.3. Method C

To a set of 10 mL volumetric flasks, accurately measured aliquots of standard drug (IV) solution in the range of (0.03 - 0.26) mg were transferred. 2 mL buffer solution of pH 3.5 were then added, then 0.5 mL of 2 M potassium chloride and 1 mL Pd(II) chloride solution. The solutions were allowed to stand at room temperature (25 °C) for 10 minutes, then diluted to volume with bidistilled water, absorbance was measured at 314 nm against blank solution (Figure 3).

2.4.4. Method D

To a set of 10 mL volumetric flasks, accurately measured aliquots of standard drug (IV) solution in the range of (0.04 - 0.3) mg were transferred and completed to 5 mL with bidistilled water. 1 mL of ninhydrin solution followed by 1 mL of saturated solution of sodium bicarbonate were added to each flask. The flasks were heated in a boiling water bath for 15 minutes, cooled and then diluted to volume with bidistilled water. Absorbance was measured at 433 nm versus blank solution (Figure 3).

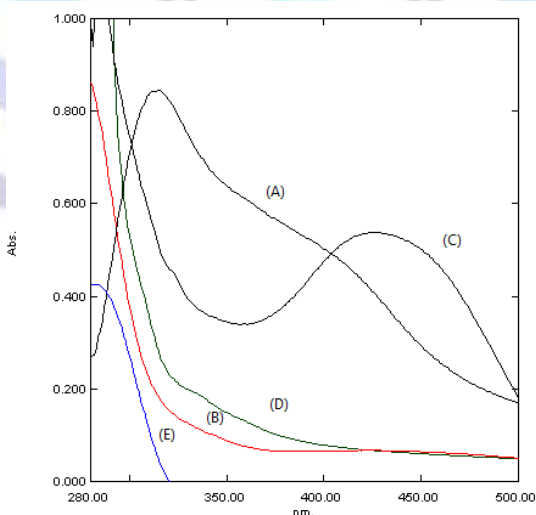


Figure 3: Absorption spectra for:
(A) The complex formed through reaction of of 24 $\mu\text{g/mL}$ cefdinir with Pd(II) chloride against reagent blank.
(B) 2 mL buffer solution of pH 3.5, 0.5 mL of 2 M KCl and 1 mL Pd(II) chloride solution .
(C) The reaction product of 17 $\mu\text{g/mL}$ cefdinir with ninhydrin against reagent blank.
(D) 1mL of ninhydrin solution and 1 mL of saturated solution of NaHCO_3 .
(E) 24 $\mu\text{g/mL}$ cefdinir against water.

2.4.5. Method E

Aliquots of drug solution containing (3 - 30 mg) were transferred to a 50 mL calibrated flasks. Volumes were made up to the mark using bidistilled water and transferred to a beaker. Titration with 10^{-2} M phosphotungstic acid was performed. The conductance was measured subsequent to each addition of phosphotungstic acid solution and after stirring for 2 minutes, the conductance was corrected for dilution [7] using the following equation.

$$\Omega^{-1}_{\text{correct}} = \Omega^{-1}_{\text{obs}} [(v_1 + v_2) / v_1]$$

Where Ω^{-1}_{obs} is the observed electrolytic conductivity, v_1 is the initial volume and v_2 is the volume of phosphotungstic acid solution added. A graph of corrected conductivity versus the volume of added phosphotungstic acid solution was constructed and end-point was estimated (Figure 4).

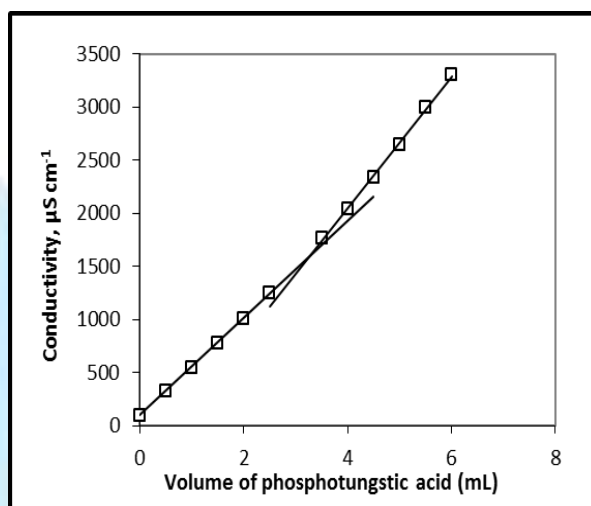


Figure 4: Conductometric titration curve of 15 mg cefoperazone sodium versus 10^{-2} M PTA.

2.4.6. Preparation and analysis of pharmaceutical preparations

2.4.6.1. For drugs (I, II and III)

2.4.6.1.1. Method A and E :

The content of one vial was transferred into 100 mL volumetric flask and the volume was completed with distilled water. Accurate volume of vial equivalent to 300 and 150 mg for method A and C respectively was transferred to 50 mL volumetric flask. The volume was completed with double distilled water and the procedure was completed as under general procedure.

2.4.6.1.2. For Method B:

The content of one vial was transferred into 100 mL volumetric flask and the volume was completed with 0.2 M and 0.05 M HCl for drug (I and III) respectively. Accurate volume of vial equivalent to 10 mg for drug (I and III) was transferred to 50 mL volumetric flask. The volume was completed with 0.2 M and 0.05M HCl for drug (I and III) respectively and the procedure was completed as under general procedure.

2.4.6.2. For drug (IV)

2.4.6.2.1. For cefdinir capsules:

The contents of 10 capsules were removed and their weight was determined accurately. The combined contents were mixed and a quantity equivalent to 10 mg for method C and D and equivalent to 150 mg for method A and E was transferred into 50 mL volumetric flasks, extracted with 1 mL 1 M NaOH and shaken with 10 mL bidistilled water, then filtered and diluted to 50 mL with bidistilled water. The assay was completed as under general procedure.

2.4.6.2.2. For cefdinir suspension:

An accurately measured volume of the freshly reconstituted oral suspension equivalent to 10 mg for method C and D and equivalent to 150 mg for method A and E was transferred into 50 mL volumetric flasks, extracted with 1 mL 1 M NaOH and shaken with 10 mL distilled water, then filtered and diluted to 50 mL with distilled water. The assay was completed as under general procedure.

2.4.6.2.3. Preparation and analysis of human urine samples (for method C)

Human urine samples were collected freshly from healthy volunteers. Blank urine pool was diluted 1:1 with double distilled water, then spiked with the appropriate amounts of stock solution to prepare samples. The assay was completed as described above.

3. RESULTS AND DISCUSSION

Many of the reported methods suffered from poor sensitivity, use of expensive organic solvents and extraction step. The use of organic solvent as the reaction medium is undesirable. Green or environmentally- friendly analytical methods are promising in recent years. Modern analytical methods need to be green without losing accuracy and sensitivity. The aim of the present work was to develop five new sensitive, cost effective methods for the determination of cephalosporins in pure drug, in pharmaceutical preparations and human urine. Method (A) is based on the use of PAP in sulphuric acid and aqueous medium. Method (B) is based on redox reaction in acidic and aqueous medium. Method (C) is based on use of the palladium (II) chloride in walpole acetate buffer pH 3.5, unlike other methods which are based on the use of palladium (II) chloride in DMF [6]. No spectrophotometric method was reported for cefdinir determination in human urine, so method (C) is advantageous in its determining in human urine. Method (D) is based on use of aqueous ninhydrin in bicarbonate medium, unlike other methods which are based on the use of methanolic or ethanolic ninhydrin [8]. Method (E) is based on direct titration of the cited drugs with PTA in aqueous medium. So, all proposed methods are free from usage of hazardous and expensive chemicals. Since inexpensive and easily available chemicals are used, the developed methods are green low cost analytical methods for cefdinir.

3.1. Method development (Method A)

Para amino phenol (PAP) was widely used in many analytical methods for pharmaceutical compounds determination. It was used for determination of cimetidine, famotidine, nizatidine, ranitidine hydrochloride [9] and cephalixin [10].

Method A is based on the alkaline hydrolysis of the cited drugs producing the sulphide ions which react with PAP and ferric ions by ring closure redox reaction giving red thionine dye (phenothiazine derivative).



Scheme (2): Ring closure redox reaction of PAP with the alkaline hydrolysis product of the cited drugs.

3.1.1. Optimization of the reaction conditions

The effect of hydrolysis time, effect of PAP volume, ammonium iron (III) sulphate volume and effect of reaction time were studied. It was found that 50 minutes hydrolysis time for (I and II) and 60 minutes for (III and IV), 4.5 mL of PAP for (I and II), 3 mL for (III) and 4 mL for (IV) (Figure 5), 1.5 mL of ammonium iron (III) sulphate for (I, II and III) and 2 mL for (IV) and 1 minute for (I and II) and 3 minutes for (III and IV) were sufficient to give maximum absorbance. The color produced was stable for 1 hour.

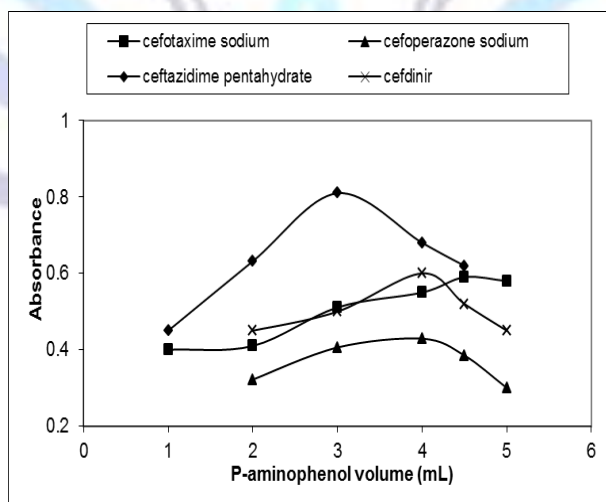


Figure 5: Effect of p-aminophenol volume on thionine dye formation using:

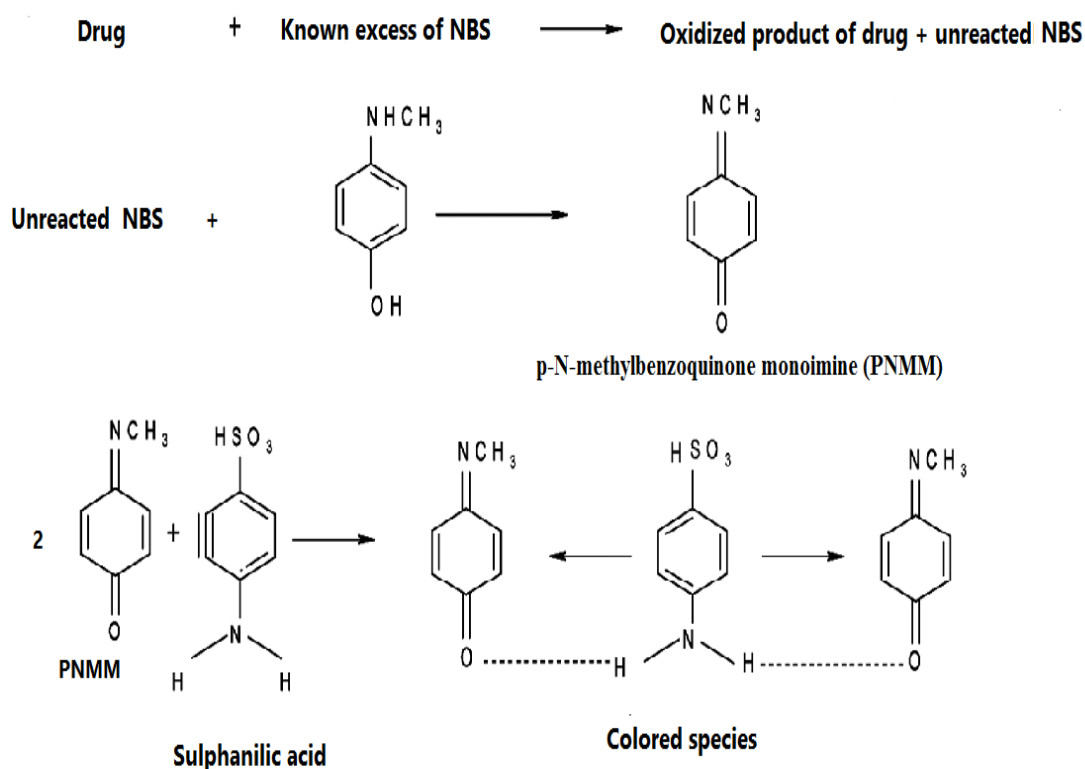
- 48 µg/mL cefotaxime sodium
- 108 µg/mL cefoperazone sodium
- 102 µg/mL ceftazidime pentahydrate
- 24 µg/mL cefdinir

3.2. Method developemt (Method B)

NBS- metol- primary arylamine combination was used for the determination of the oxidant, thereby permitting the indirect assay of many oxidisable substances including drugs in which the drug is oxidized with a known excess of NBS and, after the reaction, the unreacted NBS reacts with metol and primary arylamine and the purple color formed is measured and correlated to drug concentration.

Many pharmaceuticals have been estimated by this approach using NBS as oxidant and sulphanilic acid as primary arylamine .e.g. aspartame [11] and pioglitazone hydrochloride [12].

In this method, the application of NBS-metol-primary amine combination to the determination of (I and III) is described. The method is based on the oxidation of the drug by a known excess of NBS in acidic medium and subsequent determination of the unreacted NBS by interacting with metol and the primary aromatic amine, sulphanilic acid. The studied drugs when added in increasing amounts to a fixed amount of NBS, consume NBS and consequently, there will be a concomitant fall in the NBS concentration. This is observed as a proportional decrease in the absorbance of the reaction mixture on increasing the concentration of drugs .The following scheme illustrates the proposed reaction mechanism.



Scheme (3): Proposed reaction mechanism of the studied drugs and NBS-metol-primary amine combination.

3.2.1. Optimization of the reaction conditions

The effect of acid type, molarity of hydrochloric acid, NBS volume, metol volume, sulphanilic acid volume, reaction time between drug and NBS, waiting time after addition of sulphanilic acid and time after dilution were studied. It was found that 0.2 M and 0.05 M hydrochloric acid, 0.8 and 2 mL of NBS solution, 0.5 and 1 mL of metol solution (Figure 6), 0.5 and 1 mL of sulphanilic acid solution, 15 and 10 minutes reaction time with NBS and 3 minutes waiting time after addition of sulphanilic acid were sufficient to give maximum absorbance. Solutions of cefotaxime sodium can be measured immediately after dilution with water giving stable absorbances. For cefotaxime sodium, the solution should be stand for 5 minutes after dilution then measured due to increasing the absorbances in the first five minutes after dilution. All absorbances for both drugs are stable for 30 minutes.

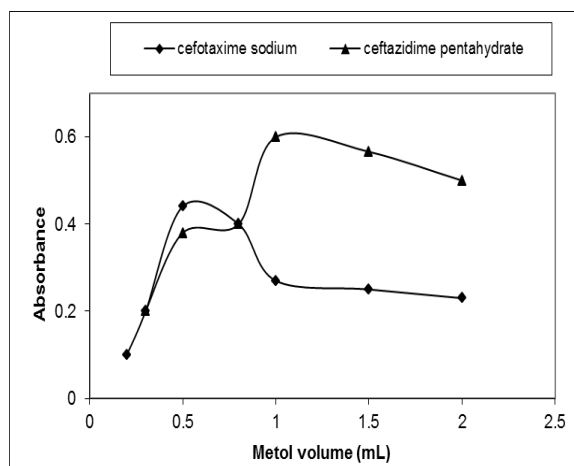


Figure 6: Effect of metal volume on the reaction of NBS-metal-primary amine combination with
 - 17 µg/mL cefotaxime sodium - 27 µg/mL ceftazidime pentahydrate

3.3. Method development (Method C)

The use of palladium (II) chloride as a complexing agent for drugs quantitation is very wide. Palladium(II) chloride was found to form complexes of square or 5-co-ordinate shape [13]. The chelate complex of palladium (II) ions is water-soluble and does not need extraction procedure. Several drugs were determined spectrophotometrically by measuring the color intensity of their complexes with palladium (II) ions, e.g. metoclopramide, promazine [14] and cefotaxime, cefuroxime and ceftazolin [6].

Reaction of Pd(II) chloride with drug (IV) produced yellow complex which was soluble in walpole acetate buffer pH 3.5. The absorption spectra showed a maximum absorbance at 314 nm.

3.3.1. Optimization of the reaction conditions

Effect pH, effect of KCl, effect of reagent volume, effect of reaction time, effect of temperature and effect of order of addition were studied. It was found that 2 mL of Walpole acetate buffer pH 3.5, 0.5 mL of 2 M KCl, 1 mL 2×10^{-3} M Pd(II) chloride (Figure 7), 10 minutes were sufficient to give maximum absorbance with cefdinir. Temperatures higher than room temperature caused absorbance decrease. The most suitable order of addition was found to be drug, buffer, KCl and Pd(II) chloride. The color produced was stable for 30 minutes.

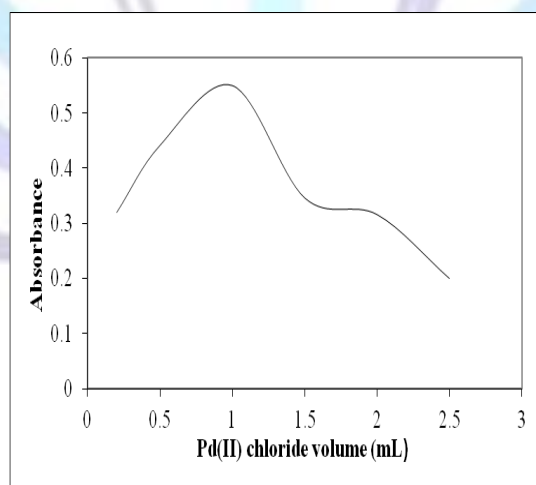


Figure 7: Effect of Pd(II) chloride volume on the absorbance of the complex formed with 24 µg/mL cefdinir.

3.3.2. Composition of the complex

The stoichiometry of the complex between cefdinir and Pd(II) chloride was studied by applying Job's method of continuous variation [15] using an equimolar (5×10^{-4} M) solutions of cefdinir and Pd(II) chloride. The total volume of drug and Pd(II) chloride was kept at 2 mL then the procedure was completed as under the above mentioned procedure. The results obtained showed that the stoichiometric ratio of the complex was (1 : 1) (reagent:drug) (Figure 8).

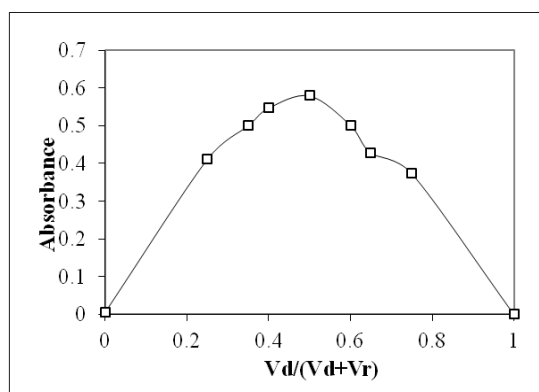


Figure 8: Continuous variation plot for cefdinir ($5 \times 10^{-4} \text{ M}$) with pd(II) chloride ($5 \times 10^{-4} \text{ M}$).

Vd is the volume taken from drug molar solution. Vr is the volume taken from Pd (II) chloride molar solution.

3.3.3. Formation Constant of the Reaction Product

The formation constant (K_f) of the complex was calculated using the following formula [16]:

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

Where; K_f the formation constant of the complex between cefdinir and Pd(II) chloride .

A = Maximum absorbance value of the continuous variation curve (Figure 8).

A_m = Absorbance value corresponding to the intersection of the two tangents of the continuous variation curve (Figure 8).

n = Number of Pd(II) chloride molecules in the complex.

C = Molar concentration of cefdinir corresponding to maximum absorbance.

The formation constant (K_f) of complex was found to be $3.5 \times 10^4 \text{ L.mol}^{-1}$ indicating high stability of the complex. The Gibbs free energy change of the reaction (ΔG) was also calculated using the following equation:

$$\Delta G = - 2.303 RT \log K_f$$

Where; ΔG = Gibbs free energy change of the reaction (K.J./mole).

R = Universal gas constant (8.314 joules).

T = Absolute temperature (273 + 25 °C).

The Gibbs free energy change of the reaction (ΔG) was found to be $- 2.6 \times 10^4 \text{ K.J./mole}$. It has a negative value which indicates the spontaneous nature of the reaction [16].

3.4. Method developemt (Method D)

Ninhydrin (triketohydrindane hydrate) is a carbonyl reagent which can form a purple condensation product which can be measured spectrophotometrically .So, it was applied in the pharmaceutical assay of different nitrogenous compounds such as some penicillins [17] and tranexamic acid [8]. A modified approach for ninhydrin green use was developed for lisinopril determination based on the formation of a yellow colour product with aqueous ninhydrin in the presence of bicarbonate with an absorption maximum at 420 nm [18]. So the aim of this work is to develop simple green method for cefdinir determination by the reaction with aqueous ninhydrin in bicarbonate medium giving yellow color measured at 433 nm. The green use of ninhydrin leads to short heating time (15 minutes) and avoidance of organic solvent use, so reduces cost.

3.4.1. Optimization of the reaction conditions

The effect of pH, volume of NaHCO_3 , concentration of ninhydrin and heating time were studied. Different molarities of NaOH were used to study the effect of pH on the reaction. No color product was formed in NaOH medium, so the reaction is specific in bicarbonate medium .1 mL of NaHCO_3 , 1 mL of ninhydrin (Figure 9) and heating for 15 minutes were sufficient to give maximum absorbance and stable yellow color product. The developed yellow color was stable for 1 hour.

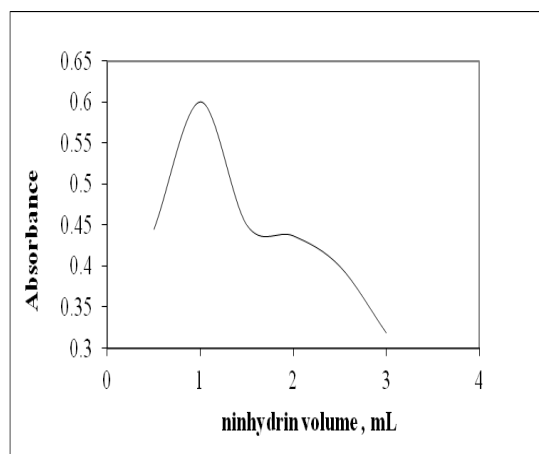


Figure 9: Effect of ninhydrin volume on the absorbance of the reaction product of 17 µg / mL cefdinir.

3.5. Method E (conductometric method)

Conductometric titration is one of the simplest analytical techniques used in drug standardization laboratories. Precipitometric conductometric titrations using phosphotungstic acid as a titrant are commonly used for the quantitative determination of different compounds eg. reprotolol HCl , pipazethate HCl , salbutamol sulphate [19].

The present method aims to introduce new conductometric method for the determination of the cited drugs which is very simple in application and of low expenses but as the same time having a high degree of accuracy and precision when compared to the reported methods .

The conductance measured before any addition of the titrant (volume of phosphotungstic acid equals zero) is due to the formation of RNHx^+ and OH^- by hydrolysis. During titration, replacement of the RNHx^+ ions by mobile H^+ occurs resulting in ion associate formation and the conductivity increases. The conductivity continues to increase rapidly after the end-point. Curve break is observed at molar ratio of 3 : 2 (drug-reagent) for all drugs except for ceftazidime pentahydrate which has a curve break at drug-reagent molar ratio of 1:1.

Representative titration curve is shown in (Figure 4) indicating two straight lines intersecting at the end point. After the end point, sudden change in the slope occurs.

3.5.1. Optimization of the reaction conditions

The optimum conditions for performing the titration in a quantitative manner were elucidated as described below.

3.5.1.1. Titration medium.

Preliminary experiments in aqueous solutions of both drug and reagent, drug and reagent solutions in ethanol–water (50%, v/v) mixture, methanolic solutions of both drug and reagent, drug and reagent solutions in methanol-water (50% v/v) mixture and drug and reagent solution in acetone–water (50% v/v) mixture. Aqueous medium led to higher conductance and most sharp end point for all drugs.

3.5.1.2. Reagent's concentration.

The optimum concentration of phosphotungstic acid was found to be 10^{-2} M to give a constant and stable conductance for all drugs. Concentrations less than 10^{-2} M give unstable readings.

3.5.2. Composition of the complex:

In 50 mL volumetric flask , 6 millilitres of 10^{-2} M drug solution were transferred , completed to 50 mL with bidistilled water , transferred to a beaker and titrated by 10^{-2} M PTA. The conductance was measured after 2 minutes stirring from each addition of reagent solution. A graph of corrected conductivity versus PTA volume was constructed indicating curve break at a molar ratio of 3 : 2 (drug-reagent) except for ceftazidime pentahydrate which has a curve break at drug-reagent molar ratio of 1:1.

3.6. Method validation

3.6.1. For the methods (A , B, C and D)

Standard calibration curves for the cited drugs were constructed by plotting absorbances against concentrations. Beer's law limits, linear regression equations, molar absorptivity and sandell sensitivity were estimated for each method (Table 1). The correlation coefficients were found to be 0.9999 indicating excellent linearity over beer's law limits for all methods (Table 1). The detection limit (LOD) and limits of quantitation for the proposed methods were estimated according to ICH



[20] and listed in (Table 1). Their values indicate the high sensitivity of the proposed methods. Accuracy and precision were determined by analyzing one concentration of each drug 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 reproducibility.

Table 1: Spectral characteristics and statistical data of the regression equations for product formed through reactions of cefdinir in methods A, C and D *.

Parameter	Method (A)	Method(C)	Method (D)
λ max (nm)	545 nm	314 nm	433 nm
Beers law limits ($\mu\text{g} / \text{mL}$)	3 - 36	3 - 26	4 - 30
Regression equation**			
Slope (b)	0.024	0.0316	0.033
Intercept (a)	0.0337	0.0795	- 0.0233
Correlation coefficient (r^2)	0.9999	0.9999	0.9999
LOD $\mu\text{g} / \text{mL}$	2.16	2.36	6.34
LOQ $\mu\text{g} / \text{mL}$	6.55	7.15	19.23
Sandell sensitivity $\mu\text{g}.\text{cm}^{-2}$	0.04	0.02	0.33
ϵ ($\times 10^4$) $\text{L}.\text{mol}^{-1}.\text{cm}^{-1}$	1.09	1.65	0.12

*Average of 3 experiments

** $A = a + bc$

Table 2: The intra-day and inter-day accuracy and precision data for cefoperazone sodium using the proposed methods (A and B).

	Intra-day					Inter-day				
	Taken $\mu\text{g}/\text{mL}$	Found $(\mu\text{g}/\text{mL})^a$	Recovery %	Precision RSD% ^b	Accuracy Er% ^c	Taken $(\mu\text{g}/\text{mL})$	Found $(\mu\text{g}/\text{mL})^a$	Recovery %	Precision RSD% ^b	Accuracy Er% ^c
ethod(A)	90	89.81	99.79	0.83	- 0.21	90	89.96	99.96	0.89	- 0.04
ethod(B)	26	25.79	99.19	0.64	- 0.81	26	25.74	99	0.53	- 1

a Average of seven determinations.

b RSD%, percentage relative standard deviation

c Er %, percentage relative error.

3.6.2. For Method E:

Recovery study of the cited drugs content in their commercial preparations was performed using the proposed method. (Table 3) showed that the proposed method is accurate and reproducible over a concentration range of 3 - 30 mg. Table 4 showed comparison of the results obtained using method D with the reported method [21] using Student t-test and Variance ratio F-test at 95% confidence level .



Table 3: Application of method (E) to the determination of cefdinir in its dosage forms*

Dinar® suspension			Dinar® capsules		
Taken (mg)	Found (mg)	Recovery%	Taken (mg)	Found (mg)	Recovery%
3	3.05	101.58	2	2	100
4	4	100	4	4.05	101.19
6	6.07	101.19	7	7.05	100.68
8	8.1	101.19	8	8.05	100.60
10	10	100	12	11.90	99.20
12	11.91	99.21	14	13.95	99.66
14	14.05	100.34			
mean		100.50			100.22
S.D.		0.85			0.73
S.E.		0.32			0.30
R.S.D.		0.84			0.73
Variance		0.72			0.54

*Average of 3 determinations

Table (4): Statistical data for determination of ceftazidime pentahydrate through the proposed methods compared with the reported method [21].

	Method(A)	Method(B)	Method(E)	Reported method [21]
Mean ± S.D	99.73 ± 0.46	100.09 ± 0.40	100.05±0.62	99.97±0.288
N	9	9	6	8
Variance	0.21	0.16	0.37	0.08
Student-t-test	1.27 (2.131)*	0.71 (2.131)*	0.33 (2.17)*	-
F-test	2.63 (3.60)*	2 (3.60)*	4.625 (4.88)*	-

*Theoretical values of t and F at p = 0.05

3.7. Analytical applications

The proposed methods were successfully applied to determine the cited drugs in their commercial dosage forms. Recovery studies were performed (Tables 3 and 5). The results validation was determined by comparison with the reported method [21] using Student t-test and Variance ratio F-test at 95% confidence level (Table 4). It was found that no significant differences between the proposed methods and reported method [21].

**Table 5: Application of standard addition technique for determination of cefotaxime sodium in its pharmaceutical dosage forms in methods A and B*.**

Method (A)				Method (B)			
Claimed taken $\mu\text{g/mL}$	Authentic added $\mu\text{g/mL}$	Found conc. $\mu\text{g/mL}$	Recovery %	Claimed taken $\mu\text{g/mL}$	Authentic added $\mu\text{g/mL}$	Found conc. $\mu\text{g/mL}$	Recovery %
6	---	6.10	101.71	2	---	2.04	102.07
	6	6.10	101.71		2	2	100
	12	12.09	100.72		4	4.07	101.65
	18	18.15	100.83		8	7.99	99.90
	24	23.98	99.90		10	9.93	99.34
	30	30.28	100.92		12	12.12	101.03
	36	36.02	100.07		14	14.19	101.36
	42	41.93	99.83		16	16.26	101.60
	48	48.15	100.31		18	18.32	101.79
	54	54.21	100.39		22	22.04	100.19
	60	59.49	99.15		24	24.40	101.65
Mean			100.50				100.96
Variance			0.61				0.87
SD			0.78				0.93
SE			0.25				0.30

*Average of three experiments

3.8. Human urine (for method C)

Method C was successfully applied to determine cefdinir in spiked human urine samples with excellent precision and accuracy (Table 6). No interference was found from the biological urine matrix. Moreover, the linearity of the proposed method was checked over a concentration range of 4 - 26 $\mu\text{g}\cdot\text{mL}^{-1}$. Excellent linearity was observed. The regression equation was found to be $y = 0.0347x + 0.0726$. The correlation coefficient (r^2) was found to be 0.9993. The detection (LOD) and quantitation limits (LOQ) were found to be 1.64 and 4.97 $\mu\text{g}\cdot\text{mL}^{-1}$ respectively. Their values confirm the sensitivity of the proposed method in human urine.

So, The proposed methods are simple, green, accurate and precise in determining the cited drugs in its pharmaceutical formulations and human urine without interference from common excipients or biological matrix. The proposed methods have higher sensitivity than many of the reported methods. All methods are green analytical methods so, they are inexpensive and ecofriendly. Moreover, they are less time-consuming and do not require difficult extraction procedures. So, the proposed methods are suitable for routine analysis of the cited drugs in control laboratories.

**Table 6: Application of method (C) for cefdinir determination in spiked human urine.**

Added ($\mu\text{g} / \text{mL}$)	Found ($\mu\text{g} / \text{mL}$)	Recovery%*
4	4.07	101.66
6	6.03	100.47
10	10.05	100.47
14	14.22	101.60
16	16.34	102.16
18	18.21	101.18
20	20.55	102.77
24	24.70	102.91
26	26.60	102.30
Mean \pm SD		101.73 \pm 0.90

*Average of 3 experiments

4. ACKNOWLEDGMENTS

Finally, Authors express their great thanks to Dr. Omnia Ahmed Emam Ismaiel in the Analytical Chemistry Department, Zagazig University for their great advices through this work. Also, authors would like to ensure that they do not have any conflict of interest with any companies.

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