

SPECTROPHOTOMETRIC DETERMINATION OF SOME CEPHALOSPORINS USING 2,2'-DIPHENYL-1-PICRYLHYDRAZYL

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تم استخدام طريقة طيف ضوئية لتعيين بعض السيفالوسبورين بواسطة كاشف ٢,٢-ثنائي فينيل-١-بكريل الهيدرازيل والكاشف السابق ينتزع ذرة هيدروجين من الأدوية المستخدمة وهي عملية ازدواج الشق الحر ينتج عنه نقص في درجة اللون البنفسجي للكاشف الذي يقاس عند درجة امتصاص قصوى قدرها ٥٢٠ ن. م. مكوناً ٢,٢-ثنائي فينيل-١-بكريل هيدرازين متناسباً مع تركيز العقار وقد وجد أن التفاعل يتبع قانون بيير في مدى من ٥-٣٠ ميكروجرام لكل مللي لتر من السيفالوسبورين. وقد تم اختبار مدى صحة هذه الطريقة بتحليل الأدوية المذكورة في صورتها النقية وكذلك أشكالها الصيدلانية بدون تداخل من الإضافات العادية وذلك بنسبة استرجاع تتراوح ما بين ٩٧,٩٨ إلى ١٠١,٢٪ وبمعامل انحراف معياري ١,١٥ إلى ٠,٩٨.

A spectrophotometric method was developed for the determination of some cephalosporins using 2,2'-diphenyl-1-picrylhydrazyl (DPPH). The latter employed to abstract a hydrogen atom from the drug thereby promoting a process of radical coupling. This results in a reduction of the violet color (520 nm) of DPPH with the formation of the yellow colored 2,2'-diphenyl-1-picrylhydrazine (DPPH₂). This fading in color of DPPH reagent depends on the concentration of the drug being determined. Beer's law was obeyed in the ranges of; 5-30, 5-25 and 10-30 µg/ml for cephalixin, cefadroxil and cephadrine respectively. The validity of the method was tested by carrying out standard addition procedure analyzing the studied drugs in pure form as well as in their pharmaceutical preparations without interference from common additives. The percentage recovery ranged from (97.98-101.21) ±1.15-0.98.

INTRODUCTION

DPPH is an intense violet-colored stable, free radical that reacts with amines¹ and thiols² by abstracting a hydrogen atom to form the yellow colored (DPPH₂). This decrease in the intensity of the violet color is used as a measure of the quantity of an amine or thiol. The radical DPPH was chosen for the present work because it does not dimerize.³ In addition it is a highly colored and its concentration at any time is estimated by its absorption in the visible range.³

Cephalosporins were determined by titrimetric,⁴⁻⁷ spectrophotometric,⁸⁻¹⁷ fluorimetric,¹⁸⁻²² HPLC,²³⁻²⁷ potentiometric²⁸ and polarographic²⁹⁻³⁰ methods.

The proposed method was applied for the

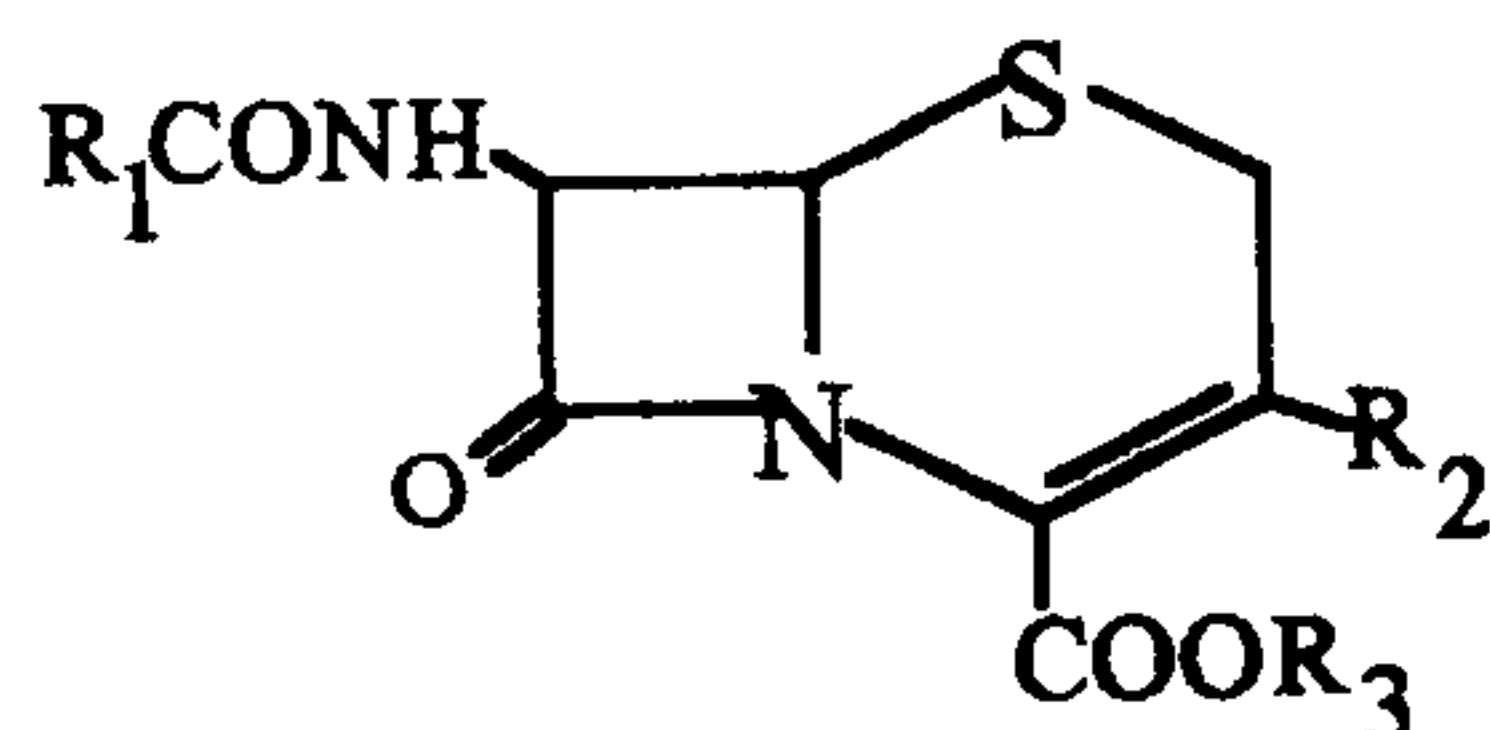
determination of some cephalosporins (cephalexin, cephadrine and cefadroxil) in both pure forms and in pharmaceutical formulations. The results obtained show that the proposed procedure is satisfactory, sensitive, accurate, and precise, in addition to its simplicity. The structures of the determined cephalosporins as well as others to verify the mechanism are shown in Table I.

EXPERIMENTAL

Apparatus

Perkin-Elmer Lambda 3B uv/vis (USA) and A Uvidec-320 (Japan) spectrophotometers with a matched pair of 1 cm Quartz cells, Memert type thermostatically controlled water bath

Table I: Structure of the studied cephalosporins:



	Generic Name	R ₁	R ₂	R ₃
1	Cephalexin		-CH ₃	H
2	Cephradine		-CH ₃	H
3	Cephadroxil		-CH ₃	H
4	Cephalordine			H
5	Cephapirin		-CH ₂ OCOCH ₃	H
6	Cefazolin Sodium			Na
7	Cefotaxime Sodium		-CH ₂ OCONH ₂	Na
8	Cefuroxime Sodium		-CH ₂ OCONH ₂	Na

(Germany) were used. All volumetric measurements were made with standard glassware.

Materials

All solvents and reagents were of analytical reagent grade. The pharmaceutical grade pure drugs; cephalixin monohydrate, cefadroxil monohydrate and cephradine were obtained from Amoun Pharmaceutical Industries Company (APIC, Egypt). Cephaloridine was obtained from Eli Lilly & Co Ltd (Basingstoke, UK), cephalixin and cefazolin sodium were obtained from Bristol-Myers Squibb (Squibb, Egypt), cefotaxime sodium was obtained from Hoechst (Hoechst, Egypt) and cefuroxime sodium was obtained from Glaxo (Glaxo, Egypt). All compounds were complying with requirements recommended by official or other reported methods and used as such without further purification. A standard stock solution of 50 mg/100 ml of each studied cephalosporin was prepared in methanol.

Reagents and solutions

2,2'-Diphenyl-1-picrylhydrazyl (Sigma, St. Louis, Mo, USA). A stock solution of 1.5 mg/ml was prepared by dissolving 0.15 g of DPPH in methanol and then diluted to 100 ml with the same solvent. Ten milliliter of this solution were diluted to 100 ml to give 0.15 mg/ml (working DPPH solution). The stock and working solutions were kept in a refrigerator and protected from light. The solution was found to be stable for at least one week at 4°C. Methanolic solution (30 µg/ml) of 2,2'-diphenyl-1-picrylhydrazine, DPPH₂ (Sigma, St. Louis, Mo, USA) was freshly prepared and protected from light. All Chemicals and solvents were analytical grades.

Pharmaceutical preparations

The commercial dosage forms subjected to analysis were Neocef capsules (labeled to contain 250 mg cephalixin per capsule), El-Nile Co., Egypt; Duracef capsules (labeled to contain 500 mg cefadroxil per capsule), Velosef capsules (labeled to contain 500 mg cephradine per capsule), Velosef vials (labeled to contain 1 gm cephradine per vial), and Velosef suspension

(labeled to contain 125 mg cephradine per 5 ml), Squibb Egypt; Ibdroxil Capsules (labeled to contain 250 mg cefadroxil per capsule), Ibdroxil suspension (labeled to contain 125 mg cefadroxil per 5 ml), Amoun Pharmaceutical Industries Co., Egypt; and Ceporex tablets (labeled to contain 250 mg cephalixin per tablet), Glaxo, Egypt.

General procedure

One ml of the standard cephalosporin solution was added to 2 ml of DPPH solution into 10 ml volumetric flask, mixed well, heated in water bath at 60°C for 15 minutes, cooled, completed to the mark with methanol and the absorbance was measured at 520 nm against a blank solution prepared simultaneously.

Assay of tablets

Twenty tablets were accurately weighed and finely powdered. An amount equivalent to about 50 mg of each drug was weighed accurately. The powder was transferred to 100 ml volumetric flask, extracted successively three times with 10 ml of methanol and completed to the mark with the same solvent. The extract is diluted to obtain 50 µg/ml before carrying out the general procedure.

Assay of capsules and vials

An amount equivalent to 50 mg of the drug from the mixed contents of at least 20 capsules or vial, accurately weighed and transferred into a 100 ml volumetric flask. Thirty milliliter of methanol are added to the flask and shaken for 10 minutes. The methanolic supernatant solution is filtered into a 100 ml volumetric flask. The extraction is repeated twice and the contents of the volumetric flask is diluted to volume with the same solvent. An accurately measured volume of this solution is pipetted into 10 ml volumetric flasks then complete as under general procedure.

Assay of powdered suspension

An accurately weighed amount of powdered suspension equivalent to about 50 mg of the cephalixin and ibdroxil was transferred to 100 ml volumetric flask then complete as under assay of tablets.

RESULTS AND DISCUSSION

The ultraviolet-visible spectra of the assay solution of cephalexin, DPPH and DPPH₂ are shown in Figure I. The reaction is assumed to proceed via abstraction of hydrogen atoms from the cephalosporins by DPPH.³¹ This is accompanied by the change of violet color of DPPH to give the yellow colored DPPH₂ and the corresponding free radical of the drug (Scheme I).

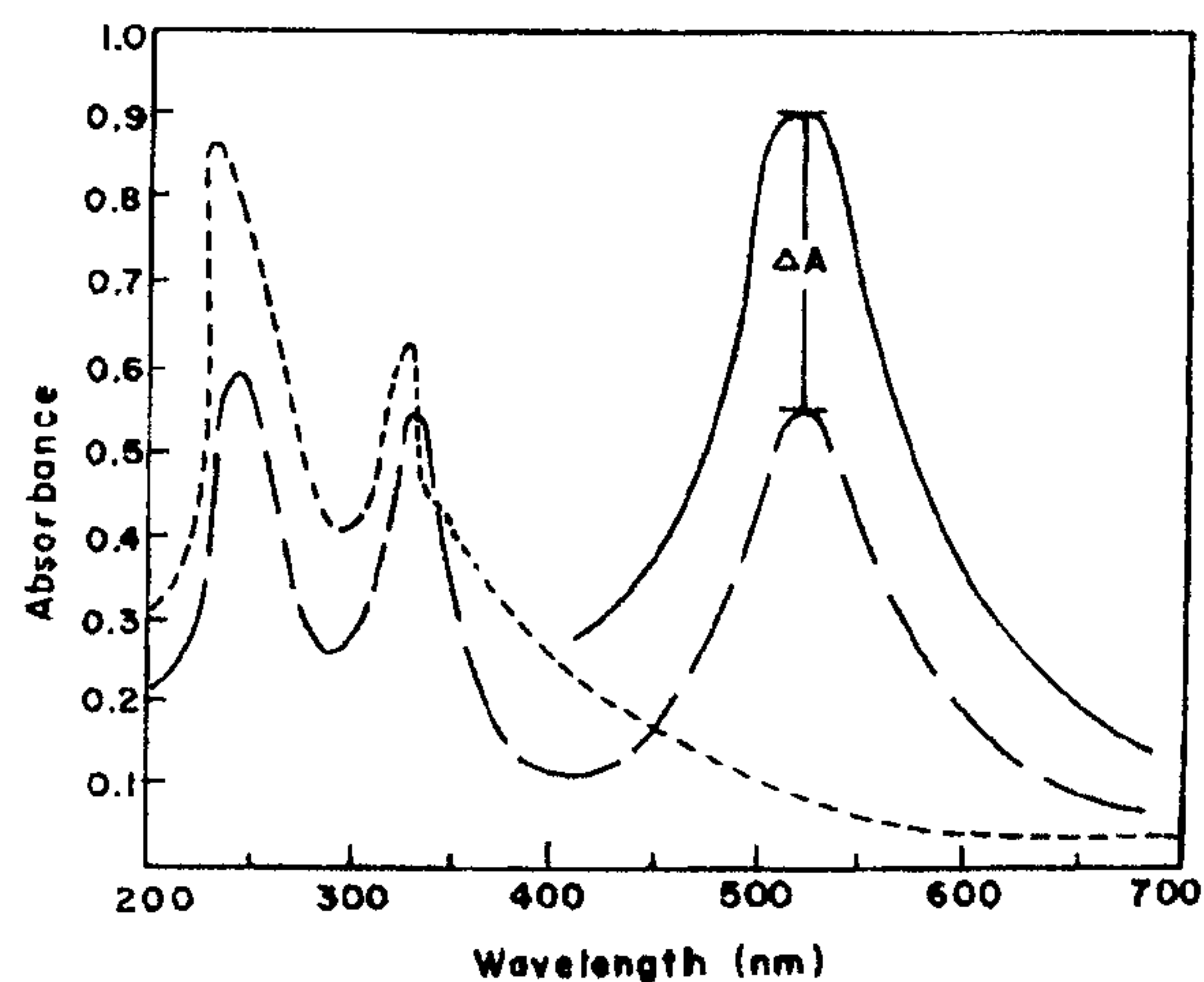


Fig. I: Absorption spectra of (—) DPPH, 30 $\mu\text{g/ml}$, reaction mixture of DPPH and cephalexin (25 $\mu\text{g/ml}$) (---) and DPPH₂, 30 $\mu\text{g/ml}$ (· · ·).

Optimization of variables

a) Reagent concentration

DPPH is added in excess to that required to react with cephalosporins. By measuring the excess reagent, the consumed DPPH would correspond to the amount of the drug. The concentration of the reagent that gives the highest absorption value within the practicable sensitivity range of absorbance was found to be 0.15 mg/ml. Two milliliters of this solution per 10 ml of the reaction mixture was used.

b) Reaction time and temperature

The temperature and reaction time were determined for the studied drugs by following the color reduction of DPPH solution at ambient temperature ($20 \pm 2^\circ\text{C}$) and in a water bath thermostatically controlled at 40, 50 and

$60 \pm 2^\circ\text{C}$ for different times. A gradual color reduction of the reagent was obtained by increasing temperature from 20 - $60 \pm 2^\circ\text{C}$. Higher temperature (more than 60°C) was avoided because of the instability of the reagents. For selection of reaction time, higher value of ΔA , more reproducible results and wider range of stability of ΔA values were considered. Maximum ΔA value was obtained at $60 \pm 2^\circ\text{C}$ after 15 minutes and remained constant for at least one hour. This has provided the advantage of measuring comfortably at any time within that period without any change in ΔA value.

c) Effect of pH

The effect of pH of the added acetate buffer on color reduction was studied for the investigated drugs. The results revealed that the reaction was independent on pH. Because of this independence of the reaction on pH, further investigations were not carried out to establish whether the constituents or pH range of the buffer solutions have or have not any effect on the interaction of DPPH with investigated drugs.

d) Influence of solvents

The effect of dilution of the reaction product by different solvents namely, methanol, ethanol, n-propanol, isopropanol, n-butanol, acetone was studied. The results indicated that all solvents had no effect on the position of maximum absorption while the reactivity (ΔA value) was affected. Methanol was found to be the most suitable solvent.

e) Quantification

Under the above optimal reaction conditions, linear relationship between the concentration of each drug and ΔA at 520 nm was obtained in the concentration range 5-30 $\mu\text{g/ml}$ (Table II).

Interference

Before proceeding with the analysis of cephalosporins in dosage forms, interference abilities from added common excipients (such as lactose, sucrose, starch, magnesium stearate and

gum acacia) were carried out to explore their effect. Samples were prepared by mixing known amount of the investigated drugs with various amounts of the common excipients. The good percentage recoveries of the investigated drugs obtained from those synthetic mixtures show that no interference from these additives takes place with the proposed method.

Assay of dosage forms

It is evident from the results obtained previously that the proposed method gave satisfactory results with the drugs in bulk. Thus different pharmaceutical formulations containing the investigated drugs were analyzed. The quite satisfactory results obtained (Table III) indicate the fair specificity of the method to the analysis of the investigated cephalosporins.

Table II: Spectral detection limits and calibration graph characteristics of the analyst solutions.

Cephalosporins	Linear range μg/ml	$\epsilon \times 10^4$ Lmol ⁻¹ cm ⁻¹	γ Correlation coefficient	Y = a + bx*	
				a	b
Cephalexin monohydrate	5-30	1.6	0.9957	0.0092	0.0203
Cephadrine	10-30	2.1	0.9975	-0.1074	0.0278
Cefadroxil monohydrate	5-25	1.3	0.9957	-0.0089	0.0314

* Regression line equation.

All the values are means of four determinations.

Table III: Analysis of some pharmaceutical formulations containing cephalosporins.

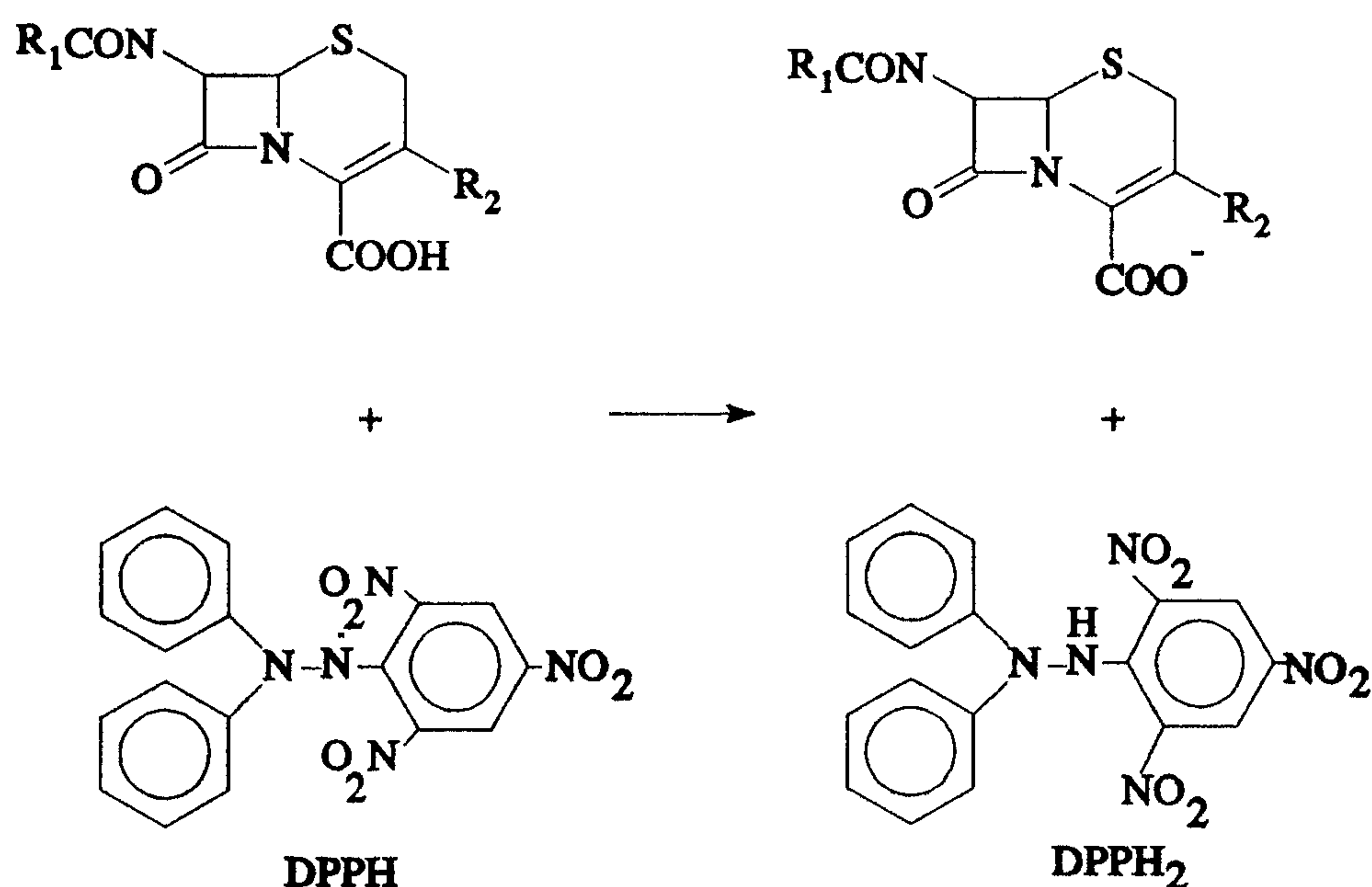
Dosage forms	Lable claim	Found ±SD*	Add mg	Recovery % ±SD*
Neocef capsules	250 mg/cap	98.72 ± 1.32	250	100.10 ± 1.78
Ceporex tablets	250 mg/tab	97.33 ± 1.50	250	100.98 ± 1.78
Velosef capsules	500 mg/cap	96.75 ± 1.69	500	97.98 ± 1.51
Velosef suspension	125 mg/5 ml	101.95 ± 1.06	125	101.21 ± 0.98
Velosef vials	1000 mg/vial	100.00 ± 1.01	1000	99.57 ± 1.63
Duracef capsules	500 mg/cap	95.30 ± 0.87	500	98.79 ± 1.31
Ibidroxil capsules	250 mg/cap	102.78 ± 0.99	250	101.00 ± 1.11
Ibidroxil suspension	125 mg/5 ml	97.90 ± 1.26	125	101.13 ± 0.99

* Average of four determinations.

Mechanism

The analytical importance of the violet colored hydrazyl, DPPH stems from the ease with which it can be reduced to the yellow colored hydrazine, DPPH₂.³¹ The reaction involves the abstraction of a hydrogen atom from the carboxylic group of cephalixin, cephadrine, cephadroxil, Cephaloridine and cephalirin to give diphenylpicrylhydrazine (DPPH₂) and the corresponding carboxylate anion (Scheme I). Neither amidic group or amino group contribute in the reaction. The sodium salts of cephalosporins (Cefazolin, Cefotaxime and Cefuroxime) failed to interact

with DPPH. As a trial to verify the suggested mechanism, uv-vis spectrum of DPPH₂, thirty $\mu\text{g/ml}$ was scanned and compared with the spectrum of the reaction mixture containing cephalixin as an example of the reactive drugs and the free radical reagent, DPPH. Figure I shows that, the reaction proceed via the abstraction of hydrogen atom from the carboxyl group by the hydrazyl and formation of the corresponding hydrazine. However, the free radicals can not be isolated or identified and it may be stabilized according to unknown mechanism.



Scheme I

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