Diazotised Suhphanilic Acid Reagent for the Determination of Trace Amounts of Cephadroxil in Aqueous Solution – Application to Pharmaceutical Preparations

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الخلاصـة

يشمل هذا البحث تطوير طريقة طيفية بسيطة لتقدير السيفادروكسيل في المحلول المائي تعتمد الطريقة على اقتران السيفادروكسيل مع الكاشف المؤزوت حامض السلفانيلك بوجود هيدروكسيد الصوديوم، لتكوين صبغة آزوية ذات لون اصفر التي تكون ذائبة في الماء ومستقرة وتعطي أعلى امتصاص عند الطول الموجي 445 نانوميتر. كانت حدود قانون بير في مدى 10-240 مايكروغرام سيفادروكسيل في حجم التفاعل النهائي 25 مللتر (0.4-9.6جزء/مليون)، وكانت الامتصاصية المولارية 1.73×10^4 لتر. مول$^{-1}$.سم$^{-1}$ ودلالة ساندل 0.0210 مايكروغرام/سم^2، الخطأ النسبي بين 0.42 – و2.22+%، والانحراف القياسي النسبي في مدى1.7 ± إلى 2.4 ± % اعتماداً على مستوى التركيز. كذلك تم دراسة طبيعة الصبغة الآزويه المتكونة. وتم تطبيق الطريقة بنجاح في تقدير السيفادروكسيل في المستحضرات الصيدلانية المختلفة.

ABSTRACT

A simple spectrophotometric method for the determination of cephadroxil in aqueous solution is developed. The method is based on the coupling of cephadroxil with diazotised sulphanilic acid reagent in the presence of sodium hydroxide. The yellow azo-dye formed is water–soluble, stable, and shows maximum absorption at 445 nm. Beer's law is obeyed over the range 10–240 µg/25 ml (0.4 –9.6 ppm) with a molar absorptivity of 1.73×10^4 l.mol^{-1}cm^{-1}, Sandell's sensitivity index of 0.0210 µg/cm^2, a relative error of –0.42 to +2.22 % and a relative standard

deviation of ± 1.7 to ± 2.4 %, depending on the concentration level. The composition of the yellow azo-dye has been evaluated. The proposed method has been successfully applied to the determination of cephadroxil in various pharmaceutical preparations.

Introduction

Cephadroxil is an antibiotic in a class of drugs called cephalosporins. Cephadroxil is used to treat many different types of bacterial infections such as bronchitis, tonsillitis, ear infections, skin infections, and urinary tract infections. Cephadroxil may also be used for purposes other than those cited[1].

A variety of techniques has been used for the determination of cephadroxil: chromatography [2,3], flow injection [4,5], and fluorometry [6,7]. Also, various spectrophotometric methods have been used in determination of cephadroxil as pure and in pharmaceuitical preparations using reagents such as diazotised benzocaine in the presence of triethylamine [8], diazotised *p*-nitroaniline [9], 4-aminoantipyrine in the presence of an alkaline oxidizing agent (hexacyanopherateIII) [10], 3-methyl-2-benzothiazolinone hydrazone hydrochloride in the presence of ceric ammonium sulphate, 4-aminophenazone in the presence of potassium hexacyanopherrate (III), 2,6-dichloroquinone-4-chlorimide [11], Cu(II) and V(V) in sulphuric acid [12], Folin-Ciocalteu reagent in presence of sodium hydroxide and stannous chloride [13], molybdophosphoric acid as an oxidising agent [14], Ce (IV) or Fe (III) [15], after nitration a subsequent complexation with an nucleophilic reagent, nitrosation and subsequent metal chelation, coupling with diazo reagent, and reaction with copper and extraction of the resulting chelate into chloroform [16].

However some of above methods suffer from several disadvantage such as the need for non aqueous medium [8] or extraction of the resulting dye into non- aqueous solvent [16].

The objective of the investigation reported in this paper is to evaluate a simple spectrophotometric method for the determination of cephadroxil; the method is based on coupling with diazotised sulphanilic acid in basic medium. The resulting yellow dye formed proves to be intense, water–soluble and stable.

Materials and Methods

Apparatus

The spectrophotometric measurements are carried out on Shimadzu UV-Visible Recording Spectrophotometric UV-160, using 1-cm silica cells.

Reagents

All chemicals used are of the highest purity available.

Cephadroxil working solution, 100 μg / ml. A 0.01g amount of cephadroxil is dissolved in distilled water with warming, then the volume is completed to 100 ml in a volumetric flask, the solution is stable for one week at least.

Diazotised sulphanilic acid reagent solution, 50 mM. A 0.865 g of sulphanilic acid is dissolved in about 75 ml of distilled water and the mixture is heated until the clear solution is obtained, , then 1 ml of concentrated hydrochloric acid is added, the mixture is then cooled to 0 - 5°C in an ice– bath, and a 0.345g sodium nitrite is added and stirred vigorously. After 5 minutes the solution is made up to volume in 100 ml volumetric flask with cooled distilled water, and is kept in a brown bottle in a refrigerator .This solution is prepared freshly each day [17].

Sodium hydroxide solution, 1N. This solution is prepared by appropriate dilution of the concentrated volumetric (Fluka) solution with distilled water and then transferred to a plastic bottle.

Solution of pharmaceutical preparations

Cephadroxil tablets solution, 100 μg.ml^{-1}. Weigh and finely powder 10 tablets (each one contains 500 mg cephadroxil), an accurately weighed amount of powder equivalent to 0.01g cephadroxil is dissolved in 50 ml distilled water then the solution is warmed with shaking to increase the solubility, filtered into 100-ml calibrated flask, then the solution is completed to the volume with a distilled water.

Cephadroxil capsule solution, 100μg.ml^{-1}. Weigh and mix the contents of five capsules (each one contains 500 mg cephadroxil), an accurately weighed amount of powder equivalent to 0.01g cephadroxil is dissolved in 50 ml distilled water then the solution is warmed with shaking to increase the solubility, filtered into 100-ml calibrated flask, then the solution is completed to the volume with a distilled water.

Cephadroxil suspension solution, 100μg.ml^{-1}. This solution is prepared by dissolving the content of the container in mixture containing 5 ml of

hydrochloric acid (0.1N)and 10 ml of ethanol then the solution is diluted to 60 ml with distilled water (each 5ml contain 250 mg cephadroxil), after filteration of the solution, 2ml which equivalent to 0.1 g cephadroxil is transferred in to a 100-ml calibrated flask and the volume is completed with a distilled water.A 10 ml of the above solution is diluted to 100 ml to prepare 100μg ml^{-1}solution.

Procedure and Calibration graph. To a series of 25- ml volumetric flasks transfere 10 – 280 μg (0.4 – 11.2 ppm) of cephadroxil, 4 ml of diazotised sulphanilic acid (50mM) and 6 ml of 1N NaOH and the volumes are made to the mark with distilled water. The absorbances are read against a reagent blank, prepared in the same manner but without cephadroxil, at 445 nm using 1-cm cells. The calibration graph is linear over the range 10-240 μg/25ml (0.4–9.6 ppm) and higher concentrations show negative deviation (Fig.1). The molar absorptivity, calculated in the region of least photometric error and at the wavelength of maximum absorption, is found to be 1.73×10^4 $l.mol^{-1}.cm^{-1}$, with Sandell sensitivity index of 0.021 μg. cm^{-2}.

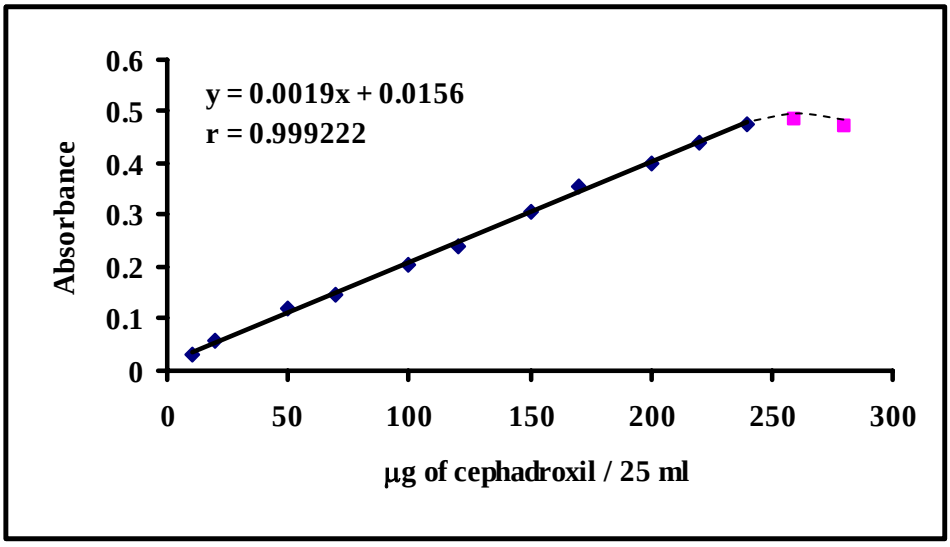

Fig. 1: Calibration graph of cephadroxil determination

Results and Discussion

For the subsequent experiments. 100 μg of cephadroxil is taken and final volumes are brought to 25 ml with distilled water.

Absorption spectra. When cephadroxil in aqueous solution is treated with diazotized sulphanilic acid reagent solution, an absorption peak is obtained showing intense absorption at 445-nm characteristic of the yellow dye. The 445-nm wavelength of maximum absorption has been

used in all subsequent experiments. The reagent blank shows very nill absorption (0.036) at the wavelength of maximum absorption (Fig.2).

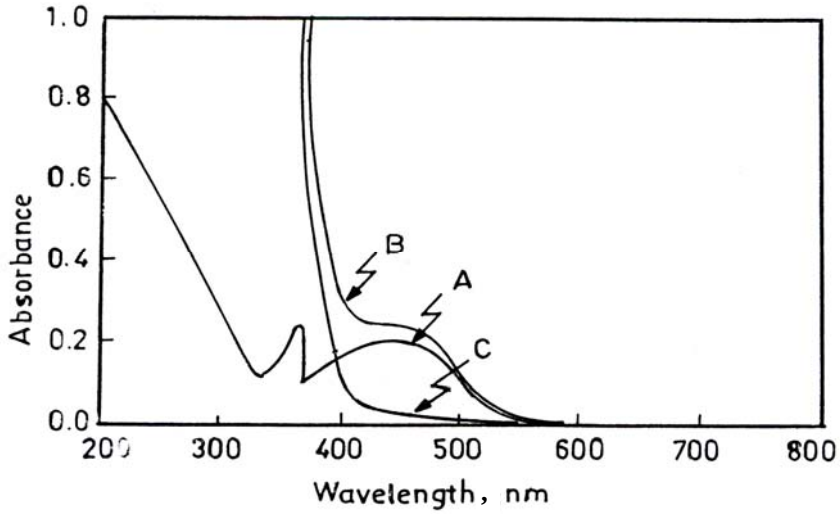

Fig. 2: Absorption spectra of 100μg cephadroxil / 25ml treated according to the recommended procedure and measured against (A) reagent blank, (B) distilled water and (C) reagent blank measured against distilled water

Study of the optimum reaction conditions. The various parameters affecting and related to the yellow azo-dye have been studied and optimum conditions have been selected.

Effect of base. The preliminary experiments have shown that the azo-dye develops only completely in alkaline medium. Different amounts of bases (strong and weak) have been used (Table1).

Table 1: The effect of base on absorbance and colour contrast

Base used (1N) Solution	Variable	Absorbance* / ml of base used					pH range
		1.0	1.5	2.0	2.5	3.0	
NaOH	A	0.153	0.162	0.112	0.102	0.098	11.77-12.06
	Δλ**, nm	141	143	142	141	140	
KOH	A	0.150	0.128	0.111	0.107	0.126	11.84-12.23
	Δλ, nm	141	142	141	140	140	
Na_2CO_3***	A	0.341	0.360	0.378	0.457	0.549	9.70-9.96
	Δλ, nm	42	39	40	47	37	
$NaHCO_3$***	A	0.217	0.247	0.193	0.157	0.169	8.74-9.02
	Δλ, nm	74	74	75	71	72	

* Adding 1 ml diazotised sulphanilic acid

** $\Delta\lambda = \lambda_{max}S - \lambda_{max}B$
where S=The dye, B=Blank

*** Gives unstable azo-dye

The experimental data show that sodium carbonate and sodium bicarbonate give better sensitivity than sodium hydroxide and potassium hydroxide. But the later bases give better colour contrast and the azo-dye formed has good stability compared with weak bases, so that 1.5 ml of 1N NaOH is recommended for the subsequent experiments.

Effect of diazotized sulphanilic acid reagent amount. The effect of the amount of the diazotized sulphanilic acid on the maximum absorbance of the azo-dye formed from different amounts of cephadroxil has been investigated. The results show that 4 ml of diazotized sulphanilic acid (50mM) reagent solution gives the highest intensity with a correlation coefficient (r) of 0.998018 over a range of cephadroxil concentration 20–120 μg/25ml (Table2), therefore 4ml is recommended for the subsequent experiments.

Table 2: The effect of diazotised sulphanilic acid amount on absorbance

ml of Diazotised sulphanilic acid reagent solution (50 mM)	Absorbance / μg of cephadroxil present						r
	20	40	60	80	100	120	
1	0.04	0.067	0.083	0.113	0.158	0.187	0.99027
2	0.043	0.072	0.103	0.125	0.167	0.196	0.997499
3	0.048	0.079	0.117	0.145	0.179	0.200	0.997485
4	0.051	0.088	0.132	0.159	0.205	0.250	0.998018
5	0.055	0.081	0.124	0.151	0.192	0.256	0.989547

Effect of surfactant

Table 3 shows that an addition of surfactants gives no useful effect. Therefore, it has been recommended to eliminate the use of surfactants in the subsequent experiments.

Table 3: Effect of surfactant.

Surfactant solution	Absorbance* / order** of addition					
	I		II		III	
	A	Δλ	A	Δλ	A	Δλ
CPC, 1×10^{-3}M	Turbid	…	Turbid	…	Turbid	…
SDS, 1×10^{-3}M	0.167	142	0.175	140	0.193	140
Triton X-100, 1%	0.180	141	0.177	140	0.190	142

* Absorbance without surfactant = 0.210

** I. Cephadroxil (C) + Surfactant (S) + diazotised Sulphamic acid (R) + NaOH (OH)
II. C + R + S + OH
III. C + R + OH + S

*** Δλ without surfactant = 144 nm

Effect of time on colour development

A study of the time effect on colour development showed that the colour formed practically within about one minute. The azo-dye formed from lower concentrations of cephadroxil gives good stability for at least 1 hour. At higher amounts of cephadroxil (150µg / 25ml), the resulting colo-ured azo-dye becomes unstable above 45 minutes (Table4).

Table 4: The effect of time and cephadroxil amount on absorbance

µg Cephadroxil/ 25ml	Absorbance / minute standing time							
	0	**10**	**20**	**30**	**40**	**45**	**50**	**60**
50	0.115	0.114	0.112	0.111	0.109	0.109	0.107	0.108
100	0.217	0.214	0.210	0.207	0.202	0.200	0.205	0.212
150	0.325	0.323	0.320	0.315	0.312	0.318	0.337	0.388

Accuracy and precision.

To check the accuracy and precision of the method, cephadroxil is determined at three different concentrations. The relative error% and relative standard deviation% results indicate the high accuracy and precision of the proposed method (Table 5).

Table 5: Accuracy and Precision of the method

Amount of cephadroxil taken, µg/25ml	Relative error, %*	Relative standard deviation, %*
50	-0.416	±2.408
100	+1.611	±2.249
150	+2.22	±1.686

* Average of five determinations.

Nature of the dye. Job's and mole – ratio methods indicate that the dye has a composition of 1:1 cephadroxil to diazotized sulphanilic acid reagent Hence the dye may have the following structure (Fig3):

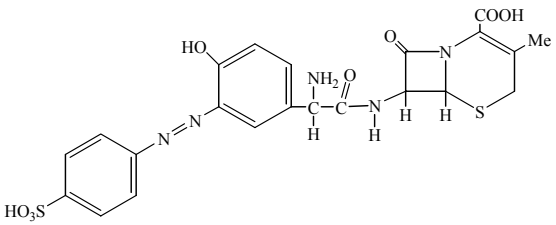

Fig. 3: The possible structure of the yellow azo-dye

Interference

The effect of some foreign compounds which often accompany pharmaceutical preparations are studied by adding three different amounts (100, 500 and 1000μg) to 100μg cephadroxil in a final volume of 25 ml (Table 6).

Table 6: Effect of foreign compounds for assay of cephadroxil

Foreign compound	Recovery (%) of 100μg cephadroxil per μg foreign compound added		
	100	**500**	**1000**
Glucose	101.00	98.50	97.50
Glycerin	102.10	100.52	101.05
Lactose	98.50	99.50	97.00
Starch	100.52	101.57	98.42

The results in Table 6 indicate that the studied foreign compounds do not interfere in the determination of cephadroxil using the proposed method. An error not more than of ±3**%** in the absorbance readings is considered tolerable from that of the cephadroxile alone.

Analytical applications

The proposed method is applied to determine cephadroxil in different pharmaceutical preparations. On applying proposed procedure, good recovery is obtained as shown in Table 7.

Table7. Analytical applications

Pharmaceutical preparation	μg Cephadroxil present/25ml	μg Cephadroxil measured/25ml	Recovery, %
Cephadroxil tablets (500mg/tablet) Ajanta Pharma Limited (India)	100	99.02	99.02
	150	152.40	101.60
	200	201.94	100.97
Cephadroxil capsules (500mg/capsule) Bristol Myerssquibb (Egypt)	100	103.60	103.60
	150	152.53	101.69
	200	204.76	102.38
Cephadroxil oral suspenstion syrup (250mg/5ml) pharmaceutical (India)	100	98.00	98.00
	150	148.99	99.33
	200	197.50	98.75

* Average of three determinations.

Evaluation of the proposed method

Due to the difficulties of using the standard method for determination of cephadroxil in its pharmaceutical preparations [18], instead we used standard addition method in order to prove that the proposed method is applied to the determination of cephadroxil without interferences (Table 8).

Table 8. The results of standard addition method

Pharmaceutical preparation	μg cephadroxil present/25ml	μg cephadroxil measured/25ml	Recovery, %
Cephadroxil tablets(500mg/tablet) Ajanta Pharma Limited (India)	50	51.5	103.0
	100	100.5	100.5
Cephadroxil capsules (500mg/capsule) Bristol-Myerssquibb (Egypt)	50	52.0	104.0
	100	100.0	100.0
Cephadroxil oral suspenstion syrup (250mg/5ml) pharmaceutical (India)	50	50.5	101.0
	100	99.0	99.0

* Average of three determinations

The results in Table 8 are in agreement with certified values of pharmaceutical preparations and with the standard addition procedure.

Comparison of the methods

The results of comparison between the analytical variables of the present method with other spectrophotometric methods show that the present method is sensitive for the determination of cephadroxil and has a wider application part(Table 9).

Table 9. Comparison of the methods

Analytical parameters	Present method	Literature method[9]	Literature method[10]
pH	11.82	…	…
Temperature (C°)	Room temperature	Room temperature	Room temperature
λmax (nm)	445	479	500
Medium of reaction	Aqueous	Aqueous	Aqueous
Type of reaction	Diazo coupling	Diazo coupling	Oxidative coupling
Reagent	Diazotised sulphanilic acid	Diazotised *p*-nitroaniline	4-Amino- antipyrine with potassium hexacyano- pherrate

Beer's law range (ppm)	0.4-9.6	0.4-8	1-28
Molar absorptivity ($l.mol^{-1}.cm^{-1}$)	1.73×10^4	2.22×10^4	1.30×10^4
Colour of the dye	Yellow	Orange	Red
Nature of the dye	1:1	1:1	1:1
Application of the method	Has been applied to the assay of cephadroxil in pharmaceutical preparations (tablets, capsules and suspensions)	Has been applied to the assay of cephadroxil in pharmaceutical preparations (tablets and suspensions)	Has been applied to the assay of cephadroxil in pharmaceutical preparations (capsules and suspensions)

Conclusion

The proposed method is simple and sensitive, do not need any pretreatment of cephadroxil or extraction of the dye formed, has good accuracy and precision and has been successfully applied to the determination of cephadroxil in various pharmaceutical preparations.

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