2024, 21(4): 1286-1295
https://doi.org/10.21123/bsj.2023.8332
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

Simultaneous spectrophotometric method for determination of both ciprofloxacin and cephalexin by using H-point standard addition method

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Received 06/01/2023, Revised 02/04/2023, Accepted 10/04/2023, Published Online First 20/09/2023, Published 01/04/2024

Abstract

An easy, simple and accurate method for estimating ciprofloxacin in the presence of cephalexin or vice versa in a mixture of the two. It has been possible to successfully apply the proposed method H-point standard addition method (H-PSAM) in the estimation of ciprofloxacin in the presence of cephalexin interfering at wavelengths 240-272.3 nm and with different concentrations of ciprofloxacin 4-18 µg.ml^{-1}. As well as the estimation of the cephalexin in the presence of ciprofloxacin interfering at wavelengths 262-285.7 nm and with different concentrations of cephalexin 6-18 µg.ml^{-1} in a mixture of them. The results showed the absence of any interferences by the additives that the drugs may contain on these compounds and within the detection limits of ciprofloxacin equal to 0.1732 µg.ml^{-1} and drug cephalexin equal to 0.4620 µg.ml^{-1}. RSD% less than 2%. The method has been successfully applied for the determination of the two drugs in some pharmaceutical preparations. The proposed method is considered one of the low-cost methods and it does not need to introduce the properties in a series of reactions and fixation of the reaction conditions to estimate them. Rather, it is done by estimating the property after dissolving it directly in distilled water in the presence of another drug with it in a mixture. It is considered one of the successful methods in the assessment, especially for properties that are close in spectrum absorption for which it is not possible to find ways to separate the mixture more accurately than this proposed method without overlapping and the effects of one on the other. The proposed method in this research was successful in the estimation of both drugs ciprofloxacin and cefalexin in a mixture without drug interaction with the other.

Keywords: Drug, Estimate, Method, Sample, Spectrophotometric.

Introduction

Ciprofloxacin (1-cyclopropyl-6-fluoro-4-oxo7-(piperazin-1-yl)-quinolone-3-carboxylic acid), its structure is shown in Fig. 1, is an antimicrobial agent for the fluoroquinolone the second type of obstetrics together the spectrum of activity is wide against gram-positive and gram-negative of the

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bacteria, inclusive pseudomonas aeruginosa. Mechanism efficacy of anti-bacterial quinolones, such as ciprofloxacin, is imagined to share the repression for bacterial topoisomerase II and topoisomerase IV, key enzymes in the replication process, copy, reassemble and correction DNA of bacterial, impersonation during bactericidal action. Ciprofloxacin salt is utilized in the production of ointments that are used for the eyes, which is prescribed for eye infections, such as corneal ulcers and bacterial conjunctivitis, caused by susceptible microorganism, such as the microorganisms staphylococcus epidermidis. Ciprofloxacin is considered one of the most used antibiotics of the second generation of quinolone since the late 1980 and early 1990. It is considered one of the favorite medicines in the treatment of urinary tract infections, sharp uncomplicated cystitis in females, chronic bacterial prostatitis, lower respiratory tract infections, sharp sinusitis, skin structure infections, bone and joint infections, infectious diarrhea, typhoid fever, uncomplicated cervical and urethral gonorrhea[1-4].

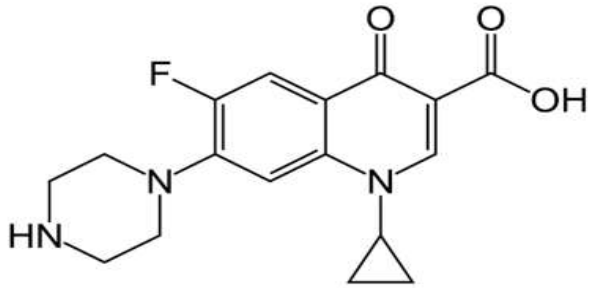

Figure 1. Structure of ciprofloxacin

Several methods have been developed for the estimation of ciprofloxacin using voltammetry [1], LC [2], UV-Vis and back titration [3], HPLC/MS [4], HPLC [5,6], RP-HPLC [7,8], Fluorescence [9], UFLC [10], UV-Vis [11-14], Manufactured membrane selective electrodes [15,16], Cloud Point Extraction [17] and Capillary electrophoresis [18,19].

Cephalexin Fig. 2, is a derivate for amynodesacetoxy cephalosporin acid belonging to antibiotics from β- lactams of the I obstetrics together with a range of activity from pharmacological. It has cefalexin a large gram-negative and gram-positive, and it has antimicrobial like properties. It is produced in the form of a gelatin capsule or the form of suspension. Cephalexin was listed among the top 200 drugs, it is often described in the USA according to National Data Corporation Health1. It is a type of antibiotic that kills bacteria and is used for infections of the skin and various organs in humans or animals [20-22].

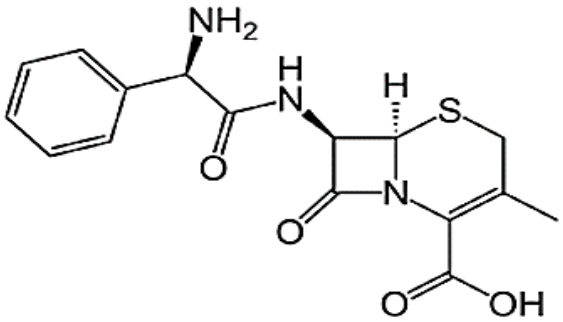

Figure 2. Structure of cephalexin

Several analytical methods have been used for the determination of cephalexin, including UV-Vis [21-24], voltammetry [20, 25], RP-HPLC [26, 27], HPLC/MS [28] and Potentiometric method [29]. The H-point method has recently been used in the estimation of some drugs, as in the following research [30-32].

In this work, a selective H- point standard audition method was used for the determination of both drugs ciprofloxacin and cephalexin in their mix, and the method was successfully applied to the estimation of assays in pharmaceutical preparations. It is considered one of the proposed direct methods for estimation using UV-Vis spectrophotometer.

Device

The device used a UV-Vis spectrophotometer model 1800 (Kyoto, Shimadzu, Japan) two beams and uses a cell made of quartz. It measures a range of wavelengths from 190 nm to 1100 nm.

Materials and Methods

All materials used are analytical materials of high purity from the Samara Company of Iraq (SDI) and dissolved in distilled water. Pharmaceuticals that have been used in applications ciprofloxacin injection 2 mg. ml^{-1} from two companies Micro in India and Samarra in Iraq but cephalexin capsule

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containing 500 mg from Samara in Iraq and Micro in India.

Standard solution

A stock solution from both drugs ciprofloxacin and cephalexin was prepared with melted 0.01 gm in a volumetric flask 100 ml and it is diluted with distilled water (100 $\mu g.ml^{-1}$). Several solutions were prepared from it and diluted using the same solvent.

Preparation of pharmaceutical solutions

Ciprofloxacin is prepared by withdrawing 5 ml of ciprofloxacin injection 2 $mg.ml^{-1}$, transferring it to a volumetric flask of 100 ml, diluting it with distilled water and several concentration solution were prepared from it and dilute using the same solvent.

Cephalexin the content of 10 capsules is weighed and the weight of the average content of one capsule is taken. It is dissolved in distilled water and transferred quantitatively to a volumetric flask of 100 ml and diluted to the mark with distilled water. After that, it is filtered using filter paper No.40 to get rid of the insoluble additives. The filtrate is taken and used in preparation of different concentrations.

Results and Discussion

Fig. 3 shows the absorption spectra of each drug ciprofloxacin and cephalexin, and it is clear that each drug overlaps in the analytical estimation with the other. Therefore, the simultaneous estimation method can be used H-point (H-PSAM).

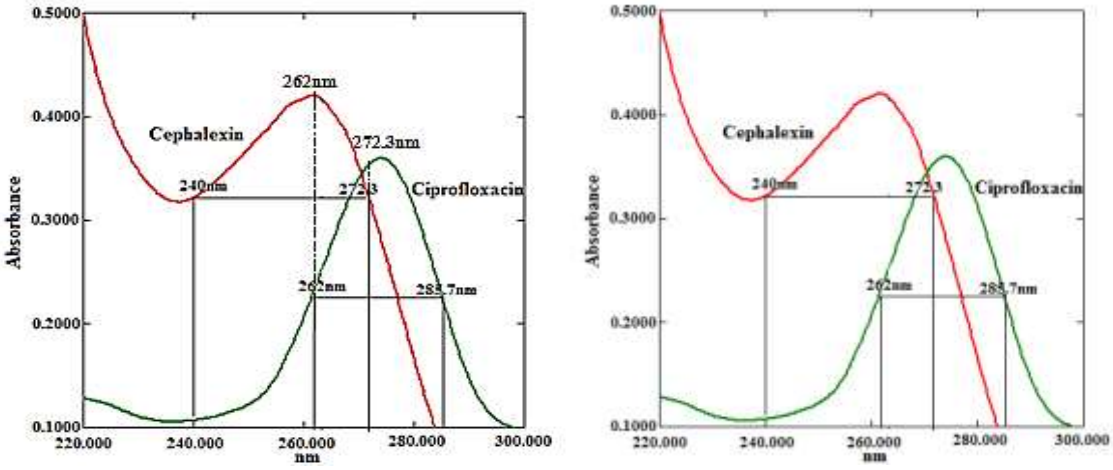

Figure 3. Absorption spectra of 16 $\mu g.ml^{-1}$ of Cephalexin against blank (water) and 4 $\mu g.ml^{-1}$ of ciprofloxacin against blank (water).

H-Point standard addition method (H-PSAM)

The following principles were followed in selecting the appropriate wavelengths for the H-PSAM application.

1. The two wavelengths selected must remain the analysis signal linear with focus. While the overlapping signal remains equal even if the analyte concentration are changed.

2. Analytical signals of the mixture consisting of the substance to be analyzed and the interfering substance are equal for all the individual signs of each type.

3. The different slope of the two straight lines is obtained at two specific wavelengths λ_1 and λ_2, it must be large to obtain high accuracy, so the wavelength pair is determined by the values that give the largest increase in slope.

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The calculation method used was based on the consideration of ciprofloxacin (x) as the one to be estimated and the interfering one being cephalexin (y), then the method was reversed so that the cephalexin (x) is what is required to be estimated and the interfering is the ciprofloxacin (y). The x is estimated by selecting two wavelengths located on both sides of the maximum absorption of y, where the absorption peaks of y are equal and there is the largest difference between the absorption peaks of x. when drawing calibration curves, two drawings will come out. We will get two Eqs. 1, 2.

$A_{262\ nm}$=0.0159X+ 0.0939 1

$A_{285.7nm}$=0.0064X+ 0.0552 2

The slopes and intercept are subtracted from the two Eqs. 1, 2 as in Eqs. 3, 4

Slope 3= slope 1 - slope 2=0.0095 ……3

Intercept 3=intercept 1 - intercept 2 = 0.0387 …….4

The intercept 3 is divided by slope 3 to extract the concentration of the unknown C_H as in the Eq. 5.

C_H=intercept 3 / slope 3= 4.07 µg.ml^{-1} …….5

The interferometric absorbance is calculated as A_H at the intersection point of the interferometric axis with the x-axis. The concentration obtained in one of the two Eqs. 1, 2 from the drawing is applied with the signal inverted as in Eq.6

$A_{262\ nm}$=0.0159 x 4.0 - 0.0939 …….6

=- 0.0303 without signal

$A_{262\ nm}$=0.0303

The calculation method is described by theia' A N et al [33].

Pairs are chosen for the wavelengths 240-272.3 nm and 262-285.7 nm for the simultaneous estimation of the ciprofloxacin in a mixture with the cephalexin, Fig. 4 shows the drawing of the measurement addition line for the ciprofloxacin, which is considered analytical and cephalexin as overlapping. The concentration of the analyte is calculated directly from the parametric point of intersection of the two lines, while the concentration of the interfering is calculated by the calibration method with a single standard using H-point.

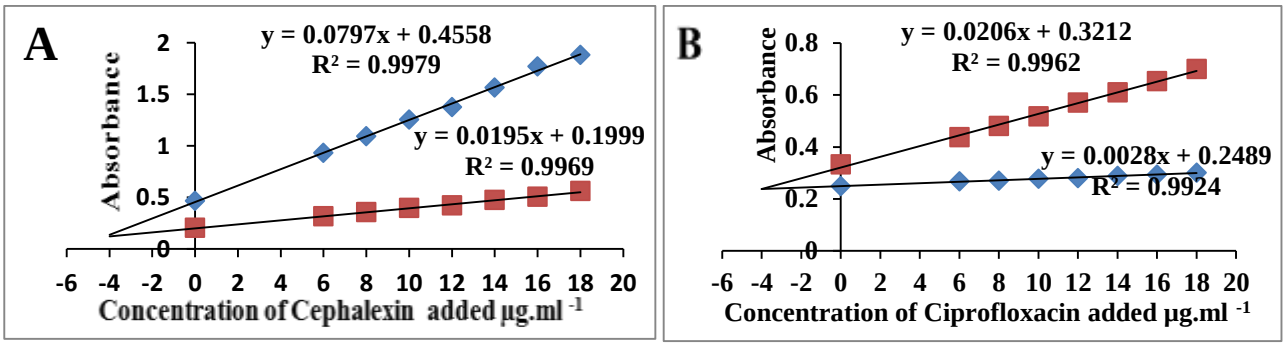

Figure 4. Plots of H-PSAM for a mixture of (A) fixed concentration of cephalexin (4 µg .ml^{-1}) and fixed concentration of ciprofloxacin (4 µg .ml^{-1}), (B) fixed concentration of ciprofloxacin (4 µg .ml^{-1}) and fixed concentration of cephalexin (4 µg .ml^{-1}).

The possibility of applying the H-Point (H-PSAM) method

Using the proposed method for several concentrations of the substance to be analyzed independently of the overlap between the absorbance versus the concentration is also independent of the analyte, so the proposed method can be applied to remove the interference. Figs. 5 and 6 show the results for both the drug ciprofloxacin and cephalexin.

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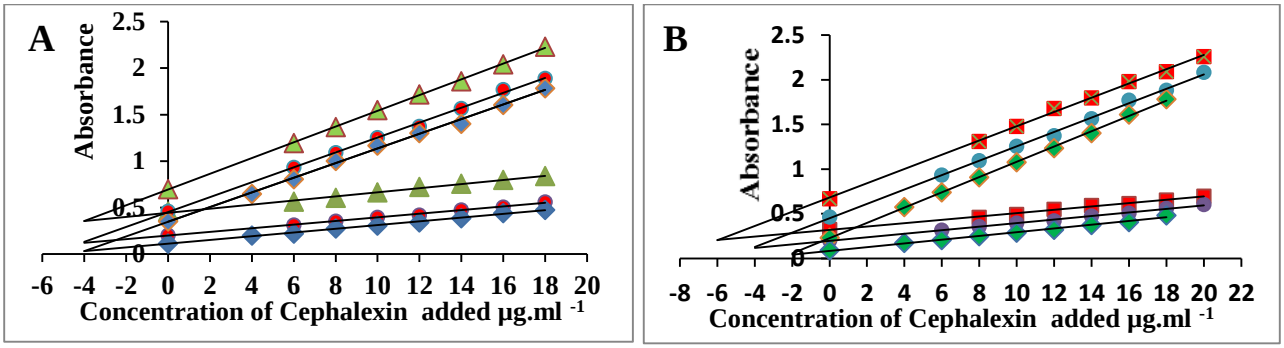

Figure 5. Plots of H-PSAM for: (A) different concentrations of cephalexin (2, 4 and 16 µg.ml^{-1}) and fixed concentration of ciprofloxacin (4 µg .ml^{-1}) (B) fixed concentration of cephalexin (4 µg.ml^{-1}) and different concentration ciprofloxacin (2, 4 and 6 µg.ml^{-1}).

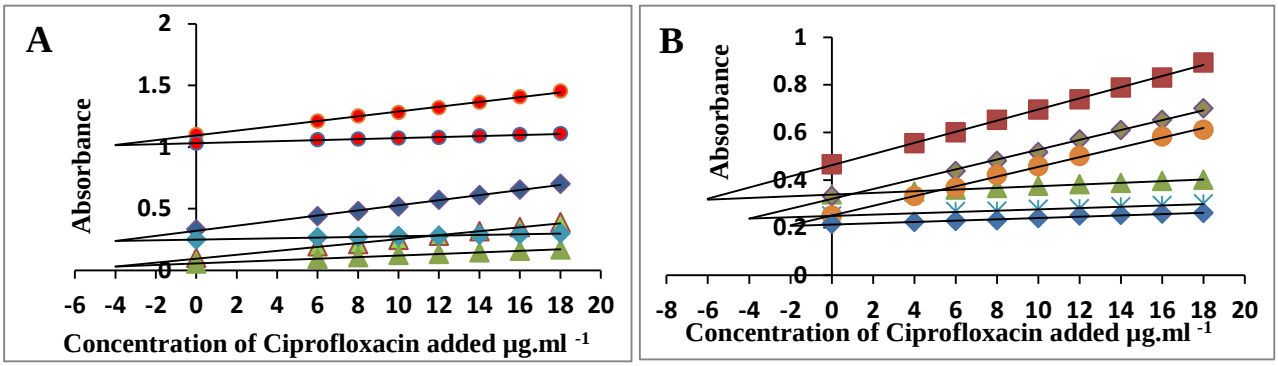

Figure 6. Plots of H-PSAM for: (A) different concentrations of ciprofloxacin (2, 4 and 16 µg.ml^{-1}) and fixed concentration of cephalexin (4 µg .ml^{-1}) (B) fixed concentration of ciprofloxacin (4 µg.ml^{-1}) and different concentration cephalexin (2, 4 and 6 µg.ml^{-1}).

Limit of Detection (LOD)

LOD has been calculated using theoretical (slope method) in Eq.7

L.O.D. =3S_B / slope ….. 7

$S_B = \sigma_{n-1}$ standard deviation of blank for n=13

LOD for the drug ciprofloxacin equal to 0.1732 µg.ml^{-1} and drug cephalexin equal to 0.4620 µg.ml^{-1}. The results obtained from the proposed method were compared with sensitive methods such as the spectrum derivative method to use the suggested method to identify these compounds in pharmaceutical preparations. Maha et al have determined the ciprofloxacin in tablets using the derivative spectrophotometric method with values for LOD found equal to 0.45 µg.ml^{-1} with RSD% less than 2% [13]. It is also encouraging to compare the validation of the method proposed in this paper that used to estimate cephalexin with that found by Neda found that the LOD 0.808 µg.ml^{-1} with RSD% less than 2% via using a zero-order method and LOD 0.781 µg.ml^{-1}with RSD% less than 2% for the area under curve method [21].

Precision and accuracy

Several samples were used with different concentrations of ciprofloxacin and with the presence of cephalexin as an interfering factor to estimate the ciprofloxacin, and different concentrations of cephalexin were used and with the presence of ciprofloxacin as an interfering factor for the estimation of cephalexin. The experiment was repeated five times, the results are shown in (Table 1).

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Table 1. Results of a series of different concentrations of cephalexin and ciprofloxacin by H-PSAM.

Exp. No.	Equation**	R^2	Analyst to Interference Ratio (µg.ml^{-1})		C_H	A_H	Analyst Conc.(µg.ml^{-1}) Cephalexin			
							taken	found	Rec. %	RSD%
1	A_{262nm}=0.0159X+0.0939	0.9950	4	2	4.0	0.0303	4	4.073	101.82	0.2455
	$A_{285.7nm}$=0.0064X+0.0552	0.9955								
2	A_{262nm}=0.0234X+0.4623	0.9987	6	4	6.0	0.3219	6	6.055	100.91	0.0761
	$A_{285.7nm}$=0.0035X+0.3383	0.9973								
3	A_{262nm}=0.0194X+1.0942	0.9965	4	16	4.1	1.0146	4	4.177	104.42	1.3047
	$A_{285.7nm}$=0.0042X+1.0307	0.9925								
Exp. No.	Equation**	R^2	Analyst to Interference Ratio (µg.ml^{-1})		C_H	A_H	Analyst Conc.(µg.ml^{-1}) Ciprofloxacin			
							taken	found	Rec. %	RSD%
1	$A_{272.3nm}$=0.0788X+0.3487	0.9971	4	2	4.0	0.0321	4	4.017	100.42	0.1503
	A_{240nm} = 0.02X + 0.1125	0.9961								
2	$A_{272.3nm}$=0.0797X+0.6822	0.9981	6	4	5.9	0.2073	6	5.958	99.30	0.9093
	A_{240nm}=0.0188X+0.3193	0.9955								
3	$A_{272.3nm}$=0.0846X+0.695	0.9995	4	16	3.9	0.3579	4	3.984	99.60	1.4662
	A_{240nm} = 0.0221X+0.446	0.9929								

****Equation of Standard addition line, the subscript numbers on A values show the wavelength of absorbance measurement**

Applications

From the results that were reviewed above, it is clear that the proposed method gave acceptable results with the drugs that were estimated in this way. The same proposed method was used in analyzing the shapes of pharmaceutical samples to estimate their content of the active substance. The results are shown in Table 2.

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Table 2. Determination of cephalexin and ciprofloxacin in a mixture in some pharmaceutical compounds by (H-PSAM) for n=3.

Sample Ciprofloxacin	Equation**	Analyte Interference Ratio		A_H	C_H	Analyte Ciprofloxacin					
						Labeled Amount µg.ml^{-1}	Found Amount µg.ml^{-1}	Conc.* Taken µg.ml^{-1}	Conc.* Found µg.ml^{-1}	Rec.* %	RSD %
Injection USP (2mg/ml) Micro – India	$A_{272.3nm}$= 0.1428x+0.5425 A_{240nm}= 0.0435x+0.1362	4	4	0.0418	4.0	2	2.036	4	4.092	102.290	0.1222
Injection USP (2mg/ml) Samarra-Iraq	$A_{272.3nm}$ = 0.1299x+0.572 A_{240nm} = 0.035x+0.1711	4	4	0.0232	4.2	2	2.112	4	4.224	105.600	0.3030
Sample Cephalexin	**Equation****	**Analyte Interference Ratio**		**A_H**	**C_H**	**Analyte Cephalexin**					
						Labeled Amount µg.ml^{-1}	**Found Amount µg.ml^{-1}**	**Conc.* Taken µg.ml^{-1}**	**Conc.* Found µg.ml^{-1}**	**Rec.* %**	**RSD %**
Capsule (500mg) Micro – India	A_{262nm} = 0.0757x+0.6687 $A_{285.7nm}$ = 0.0045x+0.3767	4	4	0.3582	4.1	500	512.625	4	4.101	102.525	0.04876
Capsule (500mg) Samarra-Iraq	A_{262nm} = 0.0711x+0.6584 $A_{285.7nm}$ = 0.0083x+0.4007	4	4	0.3666	4.1	500	512.875	4	4.103	102.575	0.9103

Conclusion

From the results obtained above, it is clear that the proposed H-point standard addition method gave acceptable results with the drugs that were estimated, namely ciprofloxacin and cephalexin, which were estimated in this way. The same proposed method was used in analyzing the shapes of pharmaceutical samples to estimate the active substance content of ciprofloxacin and cephalexin, the results were close to their actual content. This confirms that the proposed method is good, inexpensive and simple in estimating ciprofloxacin and cephalexin in a combination of the two without the need for complex interactions and the study of conditions for these interactions. The method proved effective in obtaining low detection limits equal to 0.1732 µg.ml^{-1} and 0.4620 µg.ml^{-1} for ciprofloxacin and cephalexin respectively with RSD% less than 2% compared to other methods used by other researchers in estimating the two drugs.

Acknowledgment

Thanks and appreciation to everyone who participated in the completion of this research.

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Authors' Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee in University of Baghdad.

Authors' Contribution Statement

K. A. S., E. N. M., M. A. M. and Dh. F. H. contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript.

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طريقة القياس الطيفي المتزامن لتقدير كل من سيبروفلوكساسين وسيفاليكسين باستخدام طريقة H - pointللاضافة المعيارية

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الخلاصة

طريقة سهلة وبسيطة ودقيقة لتقدير السبروفلوكساسين في وجود السيفاليكسين او العكس بالعكس في خليط منهما. طبقت الطريقة المقترحة بطريقة الاضافة القياسية لنقطة بنجاح في تقدير السبروفلوكساسين بوجود السيفاليكسين كمتداخل عند الاطوال الموجية 240-272.3 نانوميتر وبتراكيز مختلفة من السبروفلوكساسين 4-18 مايكروغرام . مل$^{-1}$ وكذلك تقدير السيفاليكسين بوجود السبروفلوكساسين الذي يتداخل باطوال موجية 262-285.7 نانوميتر وبتراكيز مختلفة من السيفاليكسين 6-18 مايكروغرام . مل$^{-1}$ في مزيج لهما. اظهرت النتائج عدم وجود اي تداخلات من قبل المواد المضافة التي تحتويها الادوية على هذه المركبات وضمن حدود كشف السبروفلوكساسين يساوي 0.1732 مايكروغرام . مل$^{-1}$ وعقارالسيفاليكسين يساوي 0.4620 مايكروغرام . مل$^{-1}$. الانحراف القياسي النسبي المئوي اقل من 2% . تم تطبيق الطريقة بنجاح لتقدير العقارين في بعض المستحضرات الصيدلانية. تعتبر الطريقة المقترحة من الطرق القليلة التكلفة وعدم حاجتها الى ادخال الادوية في سلسلة من التفاعلات وتثبيت لظروف التفاعل لغرض تقديرها وانما تتم عن طريق تقدير الدواء بعد اذابته مباشرة في الماء المقطر بوجود العقار الاخر معه في مزيج وتعتبر من الطرق الناجحة في التقدير خاصة للادوية المتقاربة في طيف الامتصاص لها والتي من غير الممكن ايجاد طرق لفصل الدوائين وتقديرهما بصورة ادق من هذه الطريقة المقترحة دون التداخل وتاثير احدهما على الاخر. الطريقة المقترحة في هذا البحث كانت ناجحة في تقدير كل من السبروفلوكساسين والسيفاليكسين في مزيج لهما دون تداخل دواء مع الاخر.

الكلمات المفتاحية: دواء ، تقدير ، طريقة ، نموذج ، طيفي.