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Simultaneous Quantitative Determination of Ciprofloxacin and Hydrocortisone by H-Point Standard Addition Method

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Abstract

Ciprofloxacin (Cip) and hydrocortisone (Hyd) were simultaneously measured as hydrochloride and sodium succinate, respectively, using the H-point standard addition method (HPSAM). The approach can precisely identify Cip in the presence of Hyd with various analyte-to-interference ratios (5:5, 5:10, 10:5, 10:10) µg.mL^{-1}, in mixed samples containing (1-5µg.ml^{-1}) of Cip, at the wavelengths of (236 and 257) nm. In the same way, Hyd was analyzed in the presence of Cip in different analytes with an interference ratio of (5:5, 5:10, 10:5, 10:10) µg.mL^{-1}, in mixed samples containing (1-5 µg.mL^{-1}) of Hyd, at wavelengths of (266 and 278) nm. The satisfactory results show good reproducibility of the developed method (RSD equals 0.9735-1.6825 and 0.9692-1.7671 for Cip and Hyd, respectively). The results also show that the excipients had no influence on the assaying of the above drugs (Recovery, 98.87–101.73). The recommended technique has successfully been used to determine the Cip and Hyd in pharmaceutical composites simultaneously with an RSD range of (0.972 to 1.671) and (0.898 to 1.820) for Cip and Hyd, respectively.

Keywords: H-Point, Ciprofloxacin, Hydrocortisone, Standard addition, Spectrophotometric methods.

1. Introduction

Ciprofloxacin, $C_{17}H_{18}FN_3O_3$ [1], is frequently sold as a fluoroquinolone [2]. At very low doses, they exert potent bactericidal actions and a broad antibacterial spectrum [3]. Numerous illnesses, including endocarditis, otorrhea, lower respiratory tract, tissue, gastrointestinal, and urinary disease, have been treated with it. The primary effect is preventing (DNA) replication by inhibiting the gyrase subunit and having an additional impact on chemicals found in cell walls [4]. The Cip's structure is described in **Figure 1**.

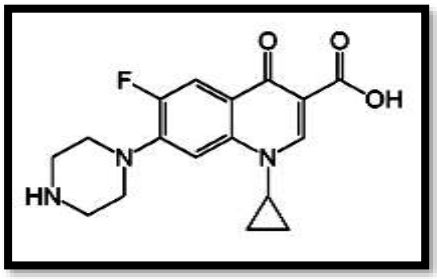

Figure 1. The chemical structure of ciprofloxacin

Corticosteroids like hydrocortisone are frequently used as anti-inflammatory medications [5]. The adrenal cortex produces the hormone hydrocortisone, $C_{21}H_{30}O_5$, which is essential for the immunological and circulatory systems to operate [6]. One of the inexpensive corticosteroids gives available therapy for hospitalized corona patients for respiratory support [7]. It is also used for its anti-inflammatory properties to treat other conditions such as arthritis and colitis [8]. The chemical structure of (Hyd) is provided in **Figure 2**.

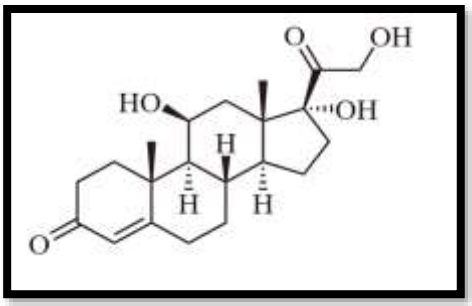

Figure 2. The chemical structure of hydrocortisone

Some spectrophotometric methods have been reported to determine ciprofloxacin [9–16] and hydrocortisone [17–23]. Due to their intrinsic simplicity, sufficient sensitivity, affordability, and widespread presence in all quality control laboratories, these procedures are the most practical ones.

An amendment to the standard addition method called (HPSAM) enables the alteration of an indeterminate error brought on by the existence of an interference in the assaying of an analyte that can be assessed without error [24]. The base of the method lets you find species with spectra that overlap a lot and fix mistakes that happen constantly and proportionally because of interferences and the sample matrix [25]. Also, it can be used in liquid chromatography [26] and spectrophotometry [27]. In addition, time is a variable in the study of kinetic data [28,29]. This research aims to study the effectiveness of the proposed method to assay Cip and Hyd in their pure forms and medicinal composites.

2. Materials and Methods

2.1 Apparatus

- Shimadzu 1800 UV-vis spectrophotometer (Japan).
- Cip and Hyd pure powders (purity 99.9%) were obtained from (SDI, Iraq).

2.2 Preparations and general procedure

Ciprofloxacin (as hydrochloride) standard solution (250 µg.mL^{-1}) was made by combining 25 mg of Cip with 10 mL of distilled water, then diluting the mixture to 100 mL in a volumetric flask. By adding more dilutions, fresh working solutions were created.

To make hydrocortisone (as a sodium succinate) standard solution (250 µg.mL^{-1}), 25 mg of Hyd was carefully weighed and then dissolved in 10 mL of distilled water to 100 ml in a volumetric flask. By adding more dilutions, fresh working solutions were created.

2.3 Determination of Ciprofloxacin

Cip and Hyd were combined in a series of 2.5 mL aliquots with a ratio of (10:10, 10:20, 20:10, 20:20) µg.mL^{-1}, and 1 mL of various concentrations (5-25) µg.mL^{-1} of Cip solution was added. Each resultant combination was diluted in a volumetric flask using distilled water to a concentration of 5 mL. The absorbance at (236 and 257) nm was measured against a blank for the reagent using a portion of the solution above that was transferred into a quartz cell. After dilution, the mixture's final ratio is (5:5, 5:10, 10:5, 10:10) µg.mL^{-1}.

2.4 Determination of Hydrocortisone

Hyd and Cip were combined in a series of 2.5 mL aliquots with a ratio of (10:10, 10:20, 20:10, 20:20) µg.mL^{-1}, and 1 mL of various concentrations (5-25) µg.mL^{-1} of Hyd solution was added. Each of the resultant combinations was diluted with distilled water to a concentration of 5ml in a volumetric flask, and the absorbance at (266 and 278) nm was measured in comparison to a blank for the reagent. After dilution, the mixture's final ratio is (5:5, 5:10, 10:5, 10:10) µg.mL^{-1}.

3. Results and Discussion

The absorption spectra for Cip and Hyd are shown in Figure 3. Each material inhibits the analytical determination of the other. Therefore, HPSAM was used to determine Cip and Hyd simultaneously.

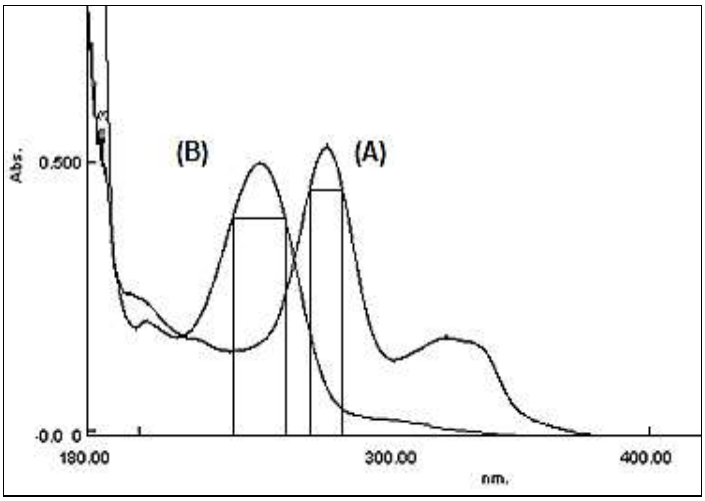

Figure 3. Absorption spectra of (A) 5 µg.mL^{-1} of (Cip) and (B) 12 µg.mL^{-1} of (Hyd)

3.1 H-Point standard addition method (HPSAM)

The following guidelines were used to choose the proper wavelengths to apply the H-Point Standard Addition Method [27–30]:

i. The sample's signal should be linear with the analyte concentration at the two chosen wavelengths. In contrast, the interference signal should stay constant regardless of changes in the analyte concentration.

ii. The analytical signals for the analyte and interference combined in the mixture are equal to the total signals of the two species.
iii. The selected wavelengths increment steep slopes to attain the best sensitivity.

In this work, for the simultaneous determination of Cip and Hyd, a wavelength pair between (236-257) and (266-278) nm was used, respectively, as shown in Figures 4 and 5. When one is considered the analyte (Cip) and the other as interference (Hyd), and vers versa, the calibration line plots for the H-Point Standard Addition are shown in (A and B). The concentration of the analyte (C_H) was calculated directly from the cross of the two lines. In contrast, the interference concentration was determined by the H-point (A_H) coordinate value for a standard of the analyte solution.

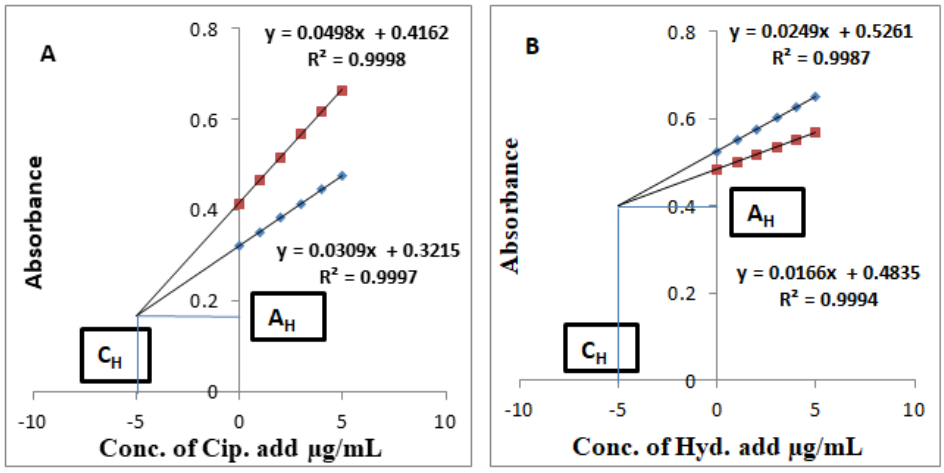

Figure 4. Graphs of HPSAM at constant concentration of (5µg.mL^{-1}) for both Cip and Hyd, where (A) Cip added to Hyd, (B) Hyd added to Cip.

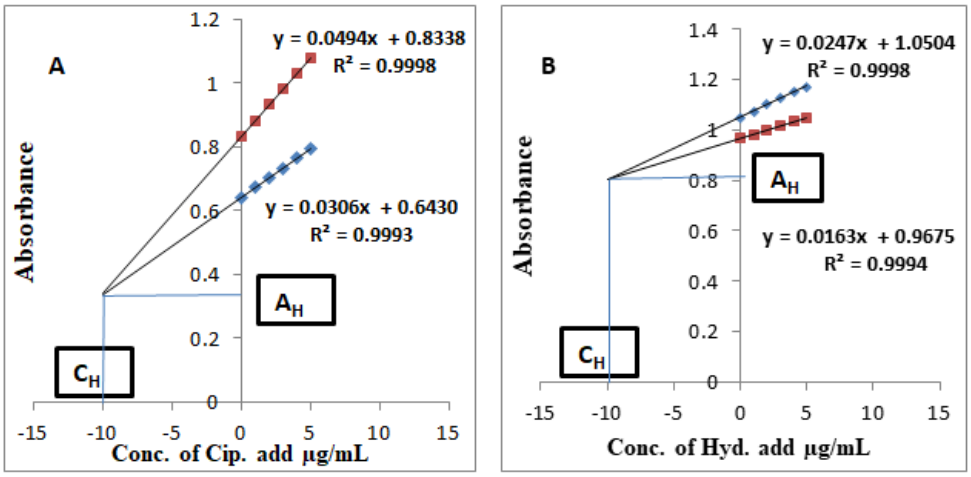

Figure 5. Graphs of HPSAM at constant concentration of (10µg.mL^{-1}) for both Cip and Hyd, where (A) Cip added to Hyd, (B) Hyd added to Cip

3. 2 Application of HPSAM

The applicability of the suggested method to estimate Cip and Hyd was clarified after using it on several samples and demonstrating that the analyte concentration (C_H) is independent of (A_H), which is also independent of (C_H), as shown in **Figures 6 and 7**.

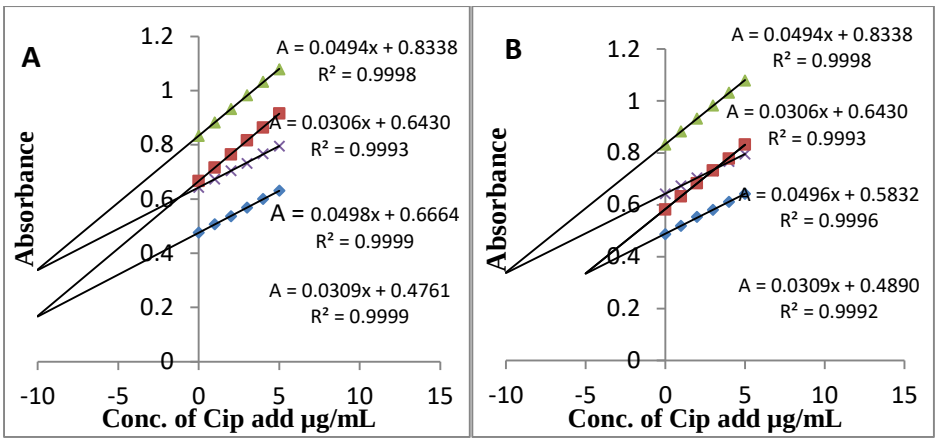

Figure 6. Graphs of HPSAM at (A) constant Conc. of Cip (5 µg.mL^{-1}) and changing Conc. of Hyd (5 and 10 µg.mL^{-1}), (B) constant Conc. of Hyd (5 µg.mL^{-1}) and changing Conc. of Cip (5 and 10 µg.mL^{-1})

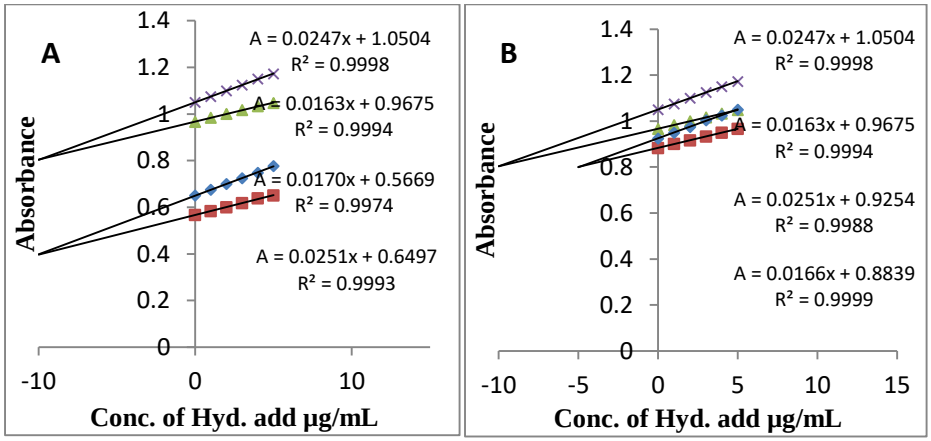

Figure 7. Graphs of HPSAM at (A) constant Conc. of Cip (10 µg.mL^{-1}) and changing Conc. of Hyd (5 and 10 µg.mL^{-1}), (B) constant Conc. of Hyd (10 µg.mL^{-1}) and changing Conc. of Cip (5 and 10 µg.mL^{-1})

3.3 Accuracy and precision

Using the suggested technique, several synthetically mixed samples with various Cip and Hyd concentration ratios were examined. The proposed method was checked for accuracy and precision by comparing the Cip and Hyd analysis results with the actual values found in the sample. **Tables 1 and 2** demonstrate that the method's accuracy for determining Cip and Hyd is acceptable. On the other hand, the standard deviation value for three duplicate trials was used to measure the approaches' repeatability.

Table 1. Accuracy and precision for the analysis of mixture contain Cip (as analyte) and Hyd (as interference) at various concentration ratios

Exp. No.	C_H	A_H	Analyte (Cip)*				Interference (Hyd)*			
			Taken	Found	Rec.%	R.S.D	Taken	Found	Rec.%	R.S.D
1	5.0106	0.1667	5	5.0106	100.21	1.5981	5	5.1172	102.34	1.7671
2	10.069	0.2848	10	10.069	100.69	0.9735	5	5.0381	100.76	1.8344
3	10.149	0.3788	10	10.149	101.49	1.4817	10	9.9659	99.659	0.9692

* Average of three measurements.
* Conc. (µg.mL^{-1}).

Table 2. Accuracy and precision for the analysis of mixture contain Hyd (as analyte) and Cip (as interference) at various concentration ratios

Exp. No.	C_H	A_H	Analyte (Hyd)*				Interference (Cip)*			
			Taken	Found	Rec.%	R.S.D	Taken	Found	Rec.%	R.S.D
1	5.1325	0.3983	5	5.1325	102.65	1.7266	5	5.0841	101.08	0.9868
2	10.222	0.3931	10	10.222	102.22	1.5038	5	4.9692	99.384	1.6825
3	9.8690	0.8066	10	9.8690	98.690	1.7298	10	10.1860	101.86	1.4393

* Average of three measurements.
* Conc. ($\mu g.mL^{-1}$).

The proposed method was compared statistically with other methods found in the literature [18,31], and the results are shown in **Table 3** below.

Table 3. Analytical parameters for simultaneous determination of Cip and Hyd by the Proposed and other methods

Methods	λ_{max} (nm)	Linearity ($\mu g.mL^{-1}$)	Slope	R^2	Rec.%	RSD
Spectrophotometric	278 244	2.0-14 1.0-14	0.0512- 6.3589	0.9999- 1.0000	99.92-100.57	0.32-1.65
HPLC	243 278.6	7.44-52.9 29.7-222	191.14 272.13	0.9988- 0.9995	98.00-101.1	1.20-1.40
Proposed method	236-257 266-278	1.0-5.0 1.0-5.0	0.0166- 0.0486	0.9974- 0.9999	98.690-102.34	0.9692-1.7671

3. 4 Interferences study

The findings demonstrated no interferences (Recovery, 98.87-101.73) in determining 10 $\mu g.mL^{-1}$ of Cip and Hyd in the presence of 250 $\mu g.mL^{-1}$of the investigated excipients, as shown in **Table 4.**

Table 4. Percent recovery for determination of 10 $\mu g.mL^{-1}$ of Cip and 10 $\mu g.mL^{-1}$of Hyd in the presence of 250 $\mu g.mL^{-1}$of Excipients

Excipients	Cip		Hyd	
	Found*	Rec. %	Found*	Rec. %
Starch	9.943	99.43	10.129	101.29
Glucose	10.123	101.23	9.924	99.24
Lactose	9.982	99.82	9.887	98.87
Sucrose	9.889	98.89	9.952	99.52
Sodium Citrate	10.173	101.73	10.122	101.22

* Average of three measurements.
* Conc. ($\mu g.mL^{-1}$).

3.5 Analysis of dosage forms

The abovementioned findings suggest the strategy runs well with the tested medications. As a result, the proposed approach was used to analyze the pharmaceutical dosage forms' active component content (HPSAM). The results in **Tables 5 and 6** were in line with expectations.

Table 5. Determination of Cip in the presence of Hyd in some pharmaceutical preparations

Sample	Analyte (Cip)*				Interference (Hyd)*			
	Taken	Found	Rec.%	R.S.D	Taken	Found	Rec.%	R.S.D
Bactiflox™ Neo 750 mg Ciprofloxacin as Hydrochloride Tablets, acino Switzerland	5	4.952	99.040	1.628	5	4.944	98.880	1.521
TYFLOX 500 mg Ciprofloxacin as Hydrochloride Tablets, ajanta pharma limited,India	10	10.204	102.05	1.013	5	5.162	103.24	0.898
CIPRODAR Sterile Eye Drops 0.3% Ciprofloxacin as Hydrochloride, Dar Al Dawa, Jordan	10	4.915	98.300	0.972	10	10.197	101.97	1.033

* Average of three measurements.
* Conc. (µg.mL^{-1}).

Table 6. Determination of Hyd in the presence of Cip in some pharmaceutical preparations

Sample	Analyte (Hyd)*				Interference (Cip)*			
	Taken	Found	Rec.%	R.S.D	Taken	Found	Rec.%	R.S.D
Hydrocortisone Roussel Tablets 10 mg SANOFI, France	5	5.066	101.32	1.607	5	5.079	101.40	0.975
Hidrkortizone Vial 100mg, Hydrocortisone as sodium succinate, Hemofarm, Serbi	10	9.873	98.730	1.601	5	5.142	102.84	1.492
Hydrocortisone Vial 100mg Hydrocortisone as sodium succinate, EIPICO, Egypt	10	10.262	102.62	1.820	10	10.111	101.11	1.671

*Average of three measurements.
* Conc. (µg.mL^{-1}).

4. Conclusion

Cip and Hyd were simultaneously measured using the H-point standard addition method in mixed samples containing different analytes with different interference ratios (5:5, 5:10, 10:5, 10:10) µg.mL^{-1}. A wavelength pair between (236-257) and (266-278) nm was used, respectively. The results show that the recommended method is rapid, straightforward, and it is successfully used to determine Cip and Hyd in medicinal composites, with RSD ranging from 0.972 to 1.671 and from 0.898 to 1.820, respectively. The results show that the excipients did not influence the simultaneous assay of the above drugs (Recovery, 98.87-101.73).

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Conflict of Interest

The authors declare that they do not have any competing interests.

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Ethical Clearance

This work has been approved by the Scientific Committee at the University of Baghdad/ College of Education for Pure Sciences Ibn Al-Haitham.

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