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Determination of Flucloxacillin-sodium in Binary Mixture with Ampicillin-trihydrate Using Univariate and Multivariate Spectrophotometric Methods: A Comparative Study

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ABSTRACT

Objectives: The aim of this study was to develop four simple and accurate spectrophotometric methods for determination of flucloxacillin-sodium in binary mixture with ampicillin-trihydrate without separation. **Methods:** One of them was a univariate constant center method and the other three methods were multivariate chemometric methods named; Savitsky-Golay filters, continuous wavelet transform of ratio spectra and wavelet transform of first derivative of the ratio spectra. **Results:** The proposed methods adopted for selective determination of flucloxacillin-sodium and obey Beer's law in the range (2-20 µg mL^{-1}). **Conclusion:** The proposed methods were simple, rapid, economic, accurate and precise; they were successfully applied for the determination of flucloxacillin-sodium in pure form and in pharmaceutical preparations.

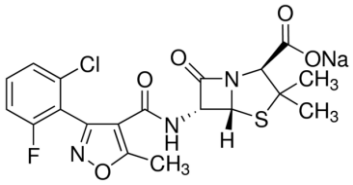
Keywords: *Constant center; Continuous wavelet transform; Flucloxacillin-sodium; Multivariate chemometric; Savitsky-Golay filters*

INTRODUCTION

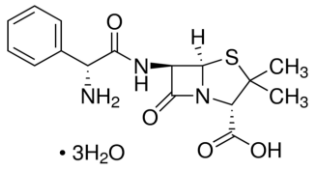
Flucloxacillin-sodium is a semisynthetic *penicillins*, chemically known as; (6*R*)-6-[3-(2-chloro-6-fluorophenyl)-5-methylisoxazole-4-carboxamido] penicillanic sodium[1,2] (**Figure 1a**). It contains a large phenyl-substituted isoxazolyl moiety which protects the *β*-lactam bond (N1-C7) by steric hindrance from hydrolysis by a variety of β-lactamases, including cephalosporinases, penicillinases, and extended spectrum beta-lactamases[3]. It is used primarily to treat infections caused by bacteria that are resistant to other penicillin-type antibiotics, but when flucloxacillin-sodium prescribed on its own, it is less effective than more commonly used penicillin-type antibiotics, so it is often prescribed in combination with other penicillins to ensure an extended spectrum of efficacy in treatment of joint infections, pneumonia, and toxic shock syndrome[4,5].

Many spectrophotometric methods have been reported for the individual determination of flucloxacillin-sodium[6-8], also, colorimetric determination of flucloxacillin-sodium[9-13] and some HPLC methods were reported for its individual determination[14-16]. Different univariate spectrophotometric and multivariate chemometric methods were reported for determination of flucloxacillin-sodium in combination with amoxicillin by Khalid et al, [17, 18].

But few methods have been reported for the determination of flucloxacillin-sodium in binary mixture with ampicillin-trihydrate (**Figure 1b**); including spectrophotometry[19], LC[20] and UPLC–MS/MS[21].



a) Flucloxacillin-sodium



(b) Ampicillin-trihydrate

Figure 1. Chemical structure of flucloxacillin-sodium (a) and ampicillin-trihydrate (b).

In this paper, four different spectrophotometric methods were applied for determination of flucloxacillin-sodium in binary mixture with ampicillin-trihydrate; univariate spectrophotometric constant center method [22-26], and three multivariate chemometric methods named; Savitsky-Golay filters[27-32], continuous wavelet transform of ratio spectra[27,29-33] and wavelet transform of first derivative of ratio spectra [27,34-38].

MATERIAL AND METHODS

Experimental

Instruments

SHIMADZU dual beam UV–visible spectrophotometer (Kyoto/Japan), model UV-1650 PC connected to IBM compatible and aHP1020 Laser jet printer. The spectral band was 2 nm and the scanning speed was 2800 nm/min and a 1nm data interval.

Software

- Constant center method was done with the bundled software, UV-Probe personal spectroscopy software version 2.43 (SHIMADZU).
- Savitsky-Golay filters; was done with our own written code in Matlab 8.2.0.701 (R2013b).
- Continuous wavelet transform and derivative wavelet were done with our own written code in Matlab 8.2.0.701 (R2013b) in conjugation with wavelet toolbox.
- The *t*-test and *F*-test were performed using Microsoft® Excel

Chemicals and reagents

- Ampicillin-trihydrate (99.86 %) was kindly supplied by was kindly supplied by EIPICO Pharmaceutical Company, Cairo, Egypt.
- Flucloxacillin-sodium (99.73 %) was kindly supplied by EIPICO Pharmaceutical Company, Cairo, Egypt.
- **Ampiflux®** Capsules labeled to contain 250 mg of each per capsule, Batch No. (601015), the product of Pharco B International Co., Egypt, were purchased from local pharmacies.
- Sodium hydroxide (El-Nasr Company, Egypt), prepared as 0.01 N aqueous solution
- Water used throughout the procedures was freshly double distilled.

Standard solutions

Standard solution of ampicillin-trihydrate or flucloxacillin-sodium (0.1 mg mL^{-1}) was prepared by dissolving 10 mg of the drug powder in 50 mL of 0.01 N sodium hydroxide and the volume was completed to 100 mL with the same solvent.

Procedure

Linearity and range

Different aliquots of flucloxacillin-sodium or ampicillin-trihydrate standard solution ranging from (2–20 µg mL^{-1}) were separately transferred into a series of 10-mL volumetric flasks and completed to volume with 0.01N NaOH. The absorption spectra were scanned and stored in the computer over the range (200–400 nm) using the same solvent as a blank.

Determination of flucloxacillin-sodium in binary mixture with ampicillin-trihydrate using univariate constant center spectrophotometric (CCSM) Method

(i) Flucloxacillin-sodium:

The calibration curve relating the absorbance of the zero order spectra of flucloxacillin-sodium at λ_{max} 208 nm versus the corresponding concentrations was constructed, the regression equation was computed.

(ii) Ampicillin-trihydrate:

The stored absorption spectra of ampicillin-trihydrate were divided by the absorption spectrum of flucloxacillin-sodium (10 µg mL^{-1}), where the obtained ratio spectra were recorded. The calibration curve relating the difference between the amplitudes of the obtained ratio spectra at (211 and 240 nm) and amplitudes at 211 nm was constructed, the regression equation was computed.

Determination of flucloxacillin-sodium in binary mixture with ampicillin-trihydrate using ratio derivative by Savitsky-Golay filters.

The absorption spectra of flucloxacillin-sodium were divided by the spectrum of 10 µg mL^{-1} ampicillin-trihydrate to obtain the ratio spectra, those ratio spectra were transferred to Matlab (R2013b) for

signal processing and analysis where; the first derivative of the obtained ratio spectra was employed according to the SGF method through the use of 7-point window size and a cubic model filter. The amplitudes of the first derivative of the ratio spectra that calculated by SGF were measured at 234 nm and were plotted against concentrations in µg mL^{-1} to construct the calibration curve and regression equation was derived.

Determination of flucloxacillin-sodium in binary mixture with ampicillin-trihydrate using continuous wavelet transform of ratio spectra method

The ratio spectra obtained as described above were transferred to Matlab (R2013b) where; the wavelet domain and the wavelet coefficients were calculated using *bior 1.1* family and [scale value (a) =40]. The amplitudes of the transformed signals at 250 nm were measured. The calibration curve was constructed by plotting the amplitude of the transformed signals at 250 nm versus the final concentrations in µg mL^{-1} and the regression equation was derived.

Determination of flucloxacillin-sodium in binary mixture with ampicillin-trihydrate using wavelet transform of first derivative of the ratio spectra method

The ratio spectra obtained as described above, then the first derivative of the ratio spectra using $\Delta\lambda$ = 10 nm and a scaling factor of 20 were calculated, the first derivatives of ratio spectra were transferred to Matlab (R2013b) where; the wavelet domain and the wavelet coefficients were calculated using *bior 1.1* family and [scale value (a) = 40]. The amplitudes of the transformed signals at 243 nm were measured and plotted against corresponding concentration to construct the calibration curve.

Application to laboratory prepared mixtures

Different aliquots of flucloxacillin-sodium together with ampicillin-trihydrate were transferred from their working solutions into a series of 10-mL volumetric flasks to prepare mixtures containing different ratios of both. The volumes were completed with the solvent. The spectra of the prepared series from 200 to 400 nm were recorded and stored. The general procedure was followed for each method. The concentrations of flucloxacillin-sodium were calculated from the corresponding regression equation for each proposed method.

Application to pharmaceutical preparation

The content of twelve capsules of **Ampiflux®** capsules was weighed and mixed. Appropriate weight of powder equivalent to 10 mg of flucloxacillin-sodium was accurately transferred to a 100-mL flask and the volume was made up to 80 mL with 0.01 N NaOH. The solution was shaken for 15 min then sonicated for 30 min after that filtered through Whatman filter paper. The volume was completed to 100 ml with the same solvent to obtain a solution labeled to contain 0.1 mg mL^{-1} of flucloxacillin-sodium. Necessary dilutions were done with the same solvent to obtain different concentrations of flucloxacillin-sodium then analyzed by the corresponding regression equation for each method. To assess the accuracy of the proposed methods, standard addition technique was applied.

Statistical analysis

Statistical comparison between results obtained by applying the proposed methods and those obtained by applying the reported method [19] showed less calculated t and F values than the tabulated ones revealing no significant difference in accuracy and precision, **Table 4.**

RESULTS AND DISCUSSION

The proposed methods have advantages over the reported derivative spectrophotometric methods, as it were more sensitive (linearity range for the proposed methods 2-20 µg mL^{-1}, while for the reported methods 50-300 µg mL^{-1}) with lowest LOD and LOQ values. Also, the proposed chemometric methods (SGF, CWT, and DWT) has advantages over the reported PCR and CLS methods as it can be performed using a simple absorption spectrum and no need to build of experimental design.

Spectral characteristics

The zero-order absorption spectra of flucloxacillin-sodium and ampicillin-trihydrate showed sever overlap which does not permit direct spectrophotometric determination of flucloxacillin-sodium, as shown in (**Figure 2**).

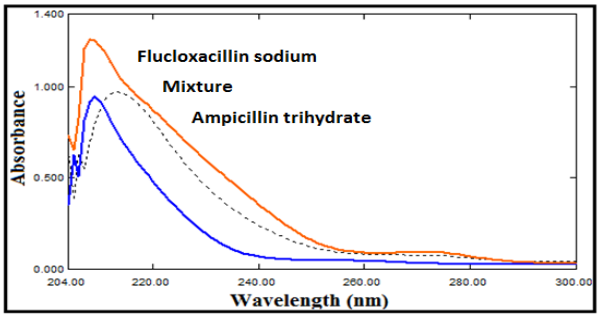


Figure 2. Overlain absorption spectra of ampicillin-trihydrate (——, 20 µg mL^{-1}), flucloxacillin-sodium (——, 20 µg mL^{-1}) and their mixture (1:1) containing 10 µg mL^{-1} of each (- - - -) showing λ_{max} of respective drugs using 0.01N NaOH as a solvent.

Table 1. Regression and validation data for the determination of flucloxacillin-sodium by the proposed methods

Parameters	CCSM	SGF	CWT	DWT
Accuracy (mean ± SD)[a]	100.80 ± 0.759	100.03 ± 0.815	100.01 ± 0.708	100.52 ± 0.501
Precision				
Repeatability (RSD)[b]	0.653	0.569	0.835	0.509
Intermediate precision (RSD)[c]	0.560	0.665	0.780	0.556
Linearity range	2-20 µg mL^{-1}			
Wavelength (nm)	208	234	250	243
Slope ± SD	0.0668 ± 0.008	0.0308 ± 0.001	0.2458 ± 0.002	0.7306 ± 0.003
Intercept ± SD	0.0724 ± 0.011	0.0299 ± 0.002	0.0055 ± 0.011	0.3689 ± 0.025
LOD	0.4757	0.1419	0.1344	0.1024
LOQ	1.5856	0.4731	0.4480	0.3412
Determination coefficient (r^2)	0.9998	0.9999	0.9999	0.9998

[a]Average of three determinations for three concentrations (8, 10 and 16 µg mL^{-1}) for CCSM (8,12, and 16 µg mL^{-1}) for SGF, CWT and DWT repeated three times.
[b]The intraday (n = 3), an average of three concentrations (8, 10 and 16 µg mL^{-1}) for CCSM, (8,12, and 16 µg mL^{-1}) for SGF, CWT and DWT` repeated three times within the day.
[c]The interday (n = 3), an average of three concentrations (8, 10 and 16 µg mL^{-1}) for the constant center method, (8,12, and 16 µg mL^{-1}) for SGF, CWT and DWT methods repeated three times in three days.

Constant Center (CCSM) Method:

The main character of this method is that; the zero order absorption spectra of the target drug recovered using smart mathematical calculations. **T**he absorption spectra of ampicillin-trihydrate were scanned and divided by the absorption spectrum of (10 µg mL^{-1}) of flucloxacillin-sodium, as shown in **(Figure 3)**, where the ratio spectra obtained represented by:

$$\left(\frac{\textbf{Ampicillin-trihydrate}}{\textbf{Flucloxacillin-sodium divisor}} + \textbf{Constant}\right)$$

- Ratio difference at the two selected wavelengths was represented by:

$$\left(\frac{\textbf{Ampicillin-trihydrate}}{\textbf{Flucloxacillin-sodium divisor}}\right) \textbf{ at } \lambda_{211} - \left(\frac{\textbf{Ampicillin-trihydrate}}{\textbf{Flucloxacillin-sodium divisor}}\right) \textbf{ at } \lambda_{240}$$

- The linear relation between the difference of the ratio spectra (at 211 and 240 nm) and the amplitude of the ratio spectra at 211 nm over the range (2-20 µg mL^{-1}) were obtained, the regression equation was computed, and was represented by:

P_1-P_2 = Slope P_1 ± intercept Eq.. (1)

P_1: is the amplitude of the ratio spectra & equal to $\left(\frac{\textbf{Ampicillin}}{\textbf{Flucloxacillin divisor}}\right)$ at λ_{211} nm.
P_2 : is the amplitude of the ratio spectra at λ_{240} nm.
$P_{postulated}$: is the calculated amplitude & equal to $\left(\frac{\textbf{Ampicillin}}{\textbf{Flucloxacillin divisor}}\right)$ at λ_{211} nm.

For the determination of flucloxacillin-sodium in binary mixture with ampicillin-trihydrate, the zero order spectra of the mixture were scanned and the ratio spectra of the mixture were obtained using (10 µg mL^{-1}) of flucloxacillin-sodium as a divisor. The amplitude of the ratio spectra of the mixture was recorded at 211 and 240 nm and substituted in equation (1) to obtain the postulated value ($p_{postulated}$).

P_{211}-P_{240}= 0.7134 (ampicillin / flucloxacillin divisor) + 0.0530 Eq. …….. (1)

The constant value (C.V) representing the amplitude corresponding to $\left(\frac{\textbf{Flucloxacillin}}{\textbf{Flucloxacillin divisor}}\right)$ at λ_{211} nm and was obtained by subtracting the $P_{postulated}$ value from the recorded amplitude ($P_{recorded}$) of the ratio spectra of the laboratory-prepared mixture at λ_{211}nm.

C.V= $P_{recorded}$ –$P_{postulated}$ at λ_{211}nm **Eq. (2)**

Table 2. Determination of intact flucloxacillin-sodium in laboratory prepared mixtures with ampicillin-trihydrate by the proposed methods

Method	Flucloxacillin added (µg mL^{-1})	Ampicillin (µg mL^{-1})	Flucloxacillin found (µg mL^{-1})	% R
CCSM	6	14	6.13	102.17
	8	12	8.13	101.63
	10	10	10.09	100.90
	12	8	12.18	101.50
	14	6	13.83	98.79
Mean ± SD				**100.05 ± 1.755**
SGF	4	16	4.07	101.75
	6	14	6.09	101.50
	8	12	8.07	100.88
	10	10	10.14	100.40
	12	8	12.07	100.58
	14	6	13.92	99.43
Mean ± SD				**100. 41 ± 1.917**
CWT	4	16	4.08	102.00
	6	14	5.95	99.17
	8	12	7.87	98.38
	10	10	9.95	99.50
	12	8	11.83	98.58
	14	6	14.19	101.36
Mean ± SD				**98.68 ± 0.862**
DWT	4	16	4.06	101.50
	6	14	5.99	99.83
	8	12	7.88	98.50
	10	10	9.90	99.00
	12	8	11.86	98.83
	14	6	13.98	99.86
Mean ± SD				**99.47 ± 0.921**

Then original spectra of flucloxacillin-sodium in the mixture were obtained by multiplying the obtained *constant value* ($\frac{\text{Flucloxacillin-sodium}}{\text{Flucloxacillin-sodium divisor}}$) of the laboratory mixture by the spectrum of flucloxacillin-sodium divisor, as shown in (**Figure 4)**, then concentration of flucloxacillin-sodium was determined from the regression equation relating the absorbance of the zero order spectra of flucloxacillin-sodium at 208 nm to the corresponding concentrations.

Optimization of experimental conditions

CCSM involves two complementary steps; The first step; the choice of the divisor, which should compromise between minimal noise and maximum sensitivity. The divisor concentrations of 10 µg mL^{-1} gave the best results. The second step; the choice of the two wavelengths at which measurements were performed; where the ratio spectrum of interfering substance has the same value while; the component of

interest has a significant difference in these two ratio values with concentrations. The selected wavelengths were 211 and 240 nm ($\Delta P_{211-240}$) which gave the best results. CCSM not affected by background noise in lower concentration, $P_{postulated}$ was obtained from a regression equation and the constant value was obtained from subtraction of [$P_{recorded}$ - $P_{postulated}$] not through a plateau.

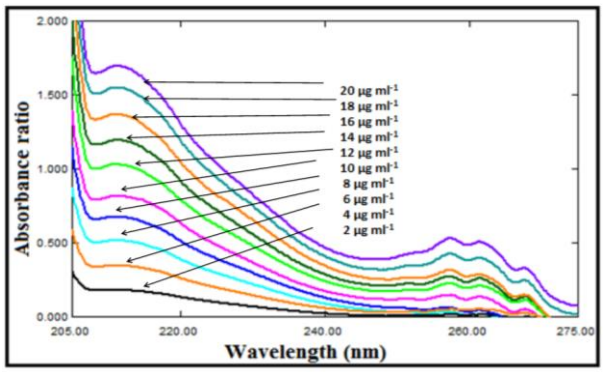


Figure 3. Ratio spectra of ampicillin-trihydrate (2-20 µg mL^{-1}) using (10 µg mL^{-1}) of flucloxacillin-sodium as a divisor.

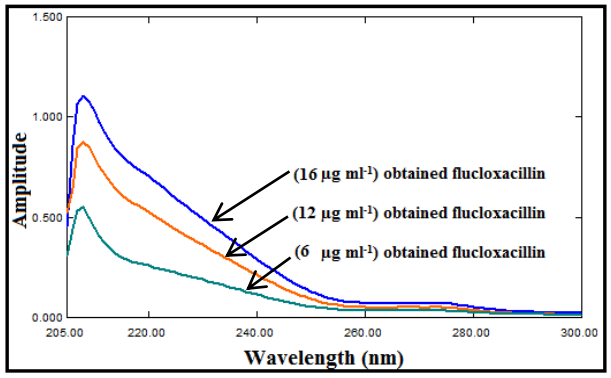


Figure 4. The final spectra of flucloxacillin-sodium at various concentrations (2 – 20 µg mL^{-1}) after multiplication by the spectrum of 10 µg mL^{-1} of flucloxacillin-sodium.

Ratio derivative by Savitsky-Golay filters (SGF) Method

In this method, the ratio spectra of flucloxacillin-sodium were obtained using absorption spectrum of ampicillin-trihydrate (10 µg mL^{-1}) as a divisor, (**Figure 5**). The first derivative of the obtained ratio spectra was employed according to the SGF method by the use of 7-point window size and a cubic model filter. The amplitude of the first derivative of the ratio spectra was calculated by SGF at 234 nm for flucloxacillin-sodium, which proportional to the concentrations of the drug without interference from the divisor, (**Figure 6**).

Optimization of experimental conditions

The different parameters related to the calculation of the Savitsky-Golay coefficients were optimized including the selection of the devisor concentration, function order, the number of points (window size 5,7, 9), model filters (quadratic and cubic) and wavelength for quantitation (234, 248, 268 and 280 nm, it was found that the divisor of concentration 10 µg mL^{-1} was suitable to obtain the ratio spectra and then the first order derivative was applied. 7-point window size and a cubic model filter were selected for processing the signals of the ratio spectra as they give best results.

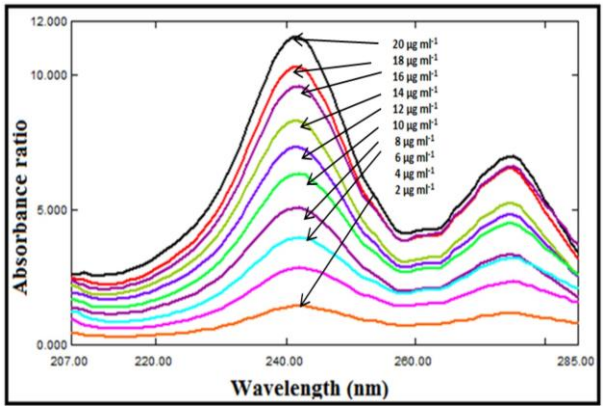


Figure 5. Ratio spectra of flucloxacillin-sodium (2-20 µg mL^{-1}) using (10 µg mL^{-1}) of ampicillin-trihydrate as a divisor.

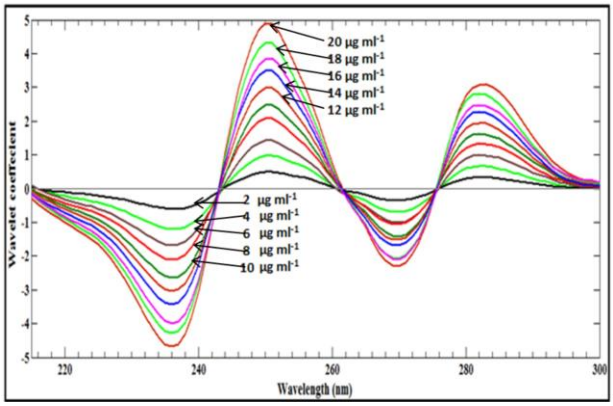


Figure 6. Savitzky-Golay application on the ratio spectra of flucloxacillin-sodium (2-20 µg mL^{-1}) using (10 µg mL^{-1}) of ampicillin-trihydrate as a divisor.

Continuous wavelet (CWT) transform of ratio spectra method

In this method, the ratio spectra obtained (as under 5.3. as shown in **Figure 5**). The obtained ratio spectra were employed using *bior 1.1* family with [scale

Table 3. Application of standard addition technique to the analysis of Ampiflux® capsules by applying the proposed methods

Pharmaceutical taken (8 µg mL^{-1})	Added standard (µg mL^{-1})	CCSM*	SGF*	CWT*	DWT*
Ampiflux® capsules Batch No. (601015)	6	101.33	99.84	101.00	100.50
	8	100.87	99.63	99.63	101.13
	10	99.40	98.90	100.40	100.80
Mean ± RSD%		100.53 ± 1.003	99.46 ± 0.496	100.34 ± 0.684	100.81 ± 0.313

**Average of three determinations*

Table 4. Statistical comparison between the results obtained by applying the proposed spectrophotometric methods and reported method for determination of flucloxacillin-sodium in Ampiflux® capsules

Parameter	CCSM	SGF	CWT	DWT	Reported method[d]
Mean[a]	100.44	100.22	99.66	100.75	100.79
S.D.	1.319	0.846	1.123	0.840	0.939
n[b]	5	5	5	5	5
Variance	1.740	0.716	1.261	0.706	0.882
***t*-test** (2.306)[c]	1.894	1.860	1.860	1.860	____
***F*-value** (6.388)[c]	1.974	1.231	1.430	1.252	____

[a] *The mean of percent recovery of pharmaceutical preparation.*
[b] *Number of experiments.*
[c] *The values in parenthesis are tabulated values of "t "and "F" at (P = 0.05)*
[d] *First derivatives of the ratio spectra were calculated using 70 µg mL^{-1} ampicillin as a divisor, Δλ = 3 nm. The amplitude values were measured at 273.5 nm [19].*

value (a) = 40] to get the wavelet coefficients. The amplitudes of these coefficients as calculated by CWT at 250 nm were proportional to the concentrations of flucloxacillin-sodium without interference from ampicillin-trihydrate, **(Figure 7)**.

Optimization of experimental condition

Wavelet transform enhances the signal-to-noise ratio, so higher sensitivity was obtained thus consider as a solution for many chemistry problems by choosing the suitable scaling parameter. The only limitation is the need for special software (Matlab) to transform the signals. The different parameters associated with the calculation of the continuous wavelet transform were optimized, included the selection of the divisor concentration. The selection of continuous wavelet family is the most important step to get the best signal with higher peak amplitudes and by using an appropriate scale parameter, both resolution and SNR of the signal can be improved, thus, wavelet type such as (biorthogonal, coiflets, Symlets wavelets) were tried and the scaling value.

The divisor concentration (10 µg mL^{-1}) was applied to obtain the ratio spectra; it was found that the optimum transform to be biorthogonal CWT because it gave the highest sensitivity with the property of SNR improvement. Thus *bior 1.1* family with [scale value (a) =40] were applied for their calculations. The transformed signals were measured at the maximum points so λ_{250} nm was chosen.

Wavelet transform of first derivative of the ratio spectra (DWT) method

In this method, the ratio spectra obtained (as under 5.3. as shown in **Figure 5)**, then the first derivative of the ratio spectra using Δλ = 10 nm and a scaling factor of 20 were calculated **(Figure 8).** In an effort to enhance the sensitivity and possibility of analyzing minor components in mixtures, the first derivatives of ratio spectra were transferred to the wavelet domain and the wavelet coefficients were calculated using *bior 1.1* family and [scale value (a) = 40]. The amplitudes of the transformed signals at 243 nm were measured, **(Figure 9)**.

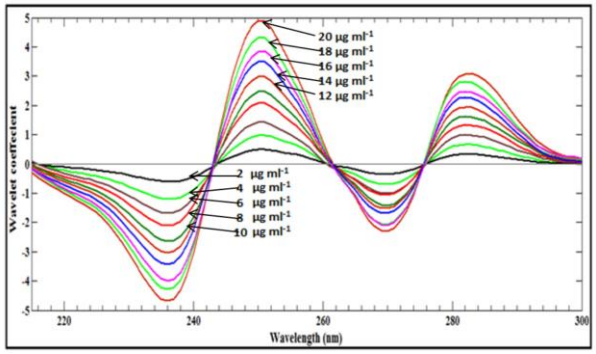


Figure 7. CWT of the ratio spectra of flucloxacillin-sodium at various concentrations (2-20 µg mL^{-1}) using 10 µg mL^{-1} of ampicillin-trihydrate as a divisor.

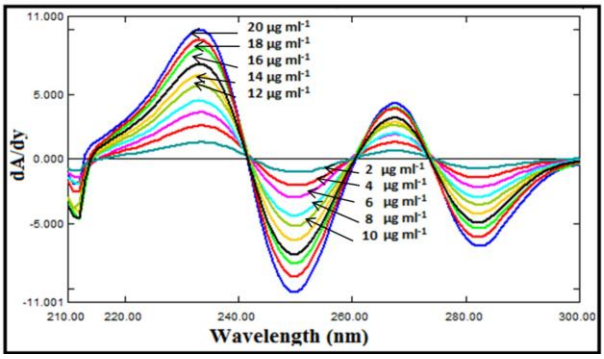


Figure 8. First-derivative spectra of flucloxacillin-sodium using (10 µg mL^{-1}) of ampicillin-trihydrate as a divisor.

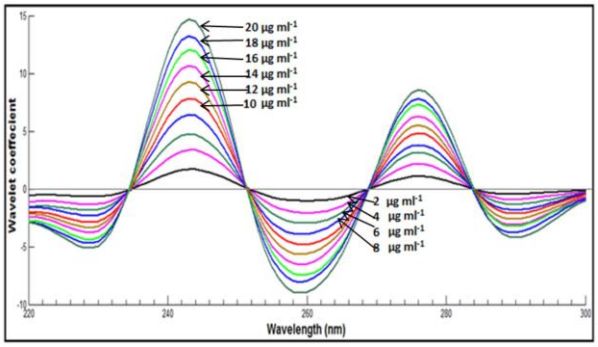


Figure 9. Wavelet transform of first derivative of the ratio spectra for flucloxacillin-sodium at various concentrations (2-20 µg mL^{-1}) using 10 µg mL^{-1} of ampicillin-trihydrate as a divisor.

Optimization of experimental condition

The well-known advantages of derivative spectroscopy were; presence of a lot of maxima and minima give an opportunity for the determination of active compounds in the presence of interfering substances, it eliminates the baseline shift effect arising from instrument or sample handling, moreover it enhances resolution permitting identification of substances with close λ_{max}) The combination of derivative and wavelet transform was performed in an effort to increase the number of zero-crossing points as well as to obtain a higher sensitivity and selectivity as compared to the original derivative or wavelet spectra.

Different **scaling factors** and different **smoothing factors** (**Δλ**) values were tested also, different parameters associated with the calculation of the continuous wavelet transform were optimized including; the selection of the divisor concentration, wavelet type and the scaling value, the divisor concentration (10 µg mL^{-1}) was applied to obtain the ratio spectra, then first derivative of the ratio spectra using Δλ = 10 nm and a scaling factor of 20 were calculated, and *bior 1.1* family with [scale value (a) =40] were applied for their calculations, the amplitudes of the transformed signals at 243 nm were measured.

Methods validation[39]

The methods were validated as per ICH guidelines

- **Linearity**

Under the described experimental conditions, the calibration graphs were rectilinear over the concentration range of 2-20 µg mL^{-1} for all methods. The regression parameters were listed in **Table 1.**

- **Limits of detection and quantitation:**

The limit of detection (LOD) and the limit of quantitation (LOQ) were calculated in accordance with ICH guidelines from the following equations:

$$LOD = 3.3\ \sigma / S$$
$$LOQ = 10\ \sigma / S$$

Where **σ:** is the standard deviation of y-intercepts of regression lines

S: is the slope of the calibration curve.

LOD was found to be 0.4757, 0.1419, 0.1344 and 0.1024µg mL^{-1}, while LOQ was found to be 1.5856, 0.4731, 0.4480 and 0.3412 µg mL^{-1} for CCSM, SGF, CWT and DWT method, respectively, **Table (1).** It was found that the DWT method was the most sensitive as indicated with lowest LOD and LOQ values.

- **Accuracy and precision**

Accuracy and precision were tested; the accuracy was represented as percentage recovery **(%R)**, while precision was represented as percentage relative standard deviation **(% RSD)**, **Table 1.**

- **Specificity**

The specificity of the proposed methods was assured by analysis of laboratory mixtures of

flucloxacillin-sodium with ampicillin-trihydrate. The proposed constant center method able to detect flucloxacillin-sodium in presence of up to 70 % of ampicillin-trihydrate, while the other three chemometric methods able to determine the drug in presence of up to 80% of ampicillin-trihydrate. The results were listed in **Table 2.**

- **Recovery study by standard addition technique**

The validity of the proposed was performed by adopting standard addition technique. Results were listed in **Table 3.**

CONCLUSION

In this paper; a comparative study was developed between four, simple, sensitive, selective, accurate, rapid and economical spectrophotometric methods which were applied for the determination of flucloxacillin-sodium in binary mixture with ampicillin-trihydrate. The proposed methods were validated according to ICH guidelines and good results obtained conforming high sensitivity of the proposed methods in comparison with the reported methods, thus, the proposed methods valuable for application in quality control laboratories for analysis of flucloxacillin-sodium in pure form and capsules.

It was found that the DWT method was the most sensitive as indicated with lowest LOD and LOQ values. It combined the advantages of derivatives in and wavelet. Moreover, the transformed signals are measured at the maximum points, in the same time; the presence of a lot of maxima and minima give an opportunity for the determination of active compounds in the presence of interfering substances and helps to find the best points.

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Conflict of Interest

The authors declare that they don't have any conflict of interest.

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