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Stability-indicating spectrophotometric methods for determination of ceftriaxone in presence of its alkaline degradation product

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ABSTRACT

Three simple, sensitive, accurate and precise spectrophotometric methods are used for the determination of Ceftriaxone (CTRX) in presence of its alkaline degradation product (DCTRX), without preliminary separation. The first method is the dual wavelength method, the second is the bivariate method and the third is the Savitzy-Golay filter. The accuracy, precision, and linearity ranges of the proposed methods are determined. The specificity is assessed by analyzing synthetic mixtures containing the drug and its degradate. The methods are validated according to the ICH guidelines and accuracy, precision, repeatability and robustness are found to be within the acceptable limit. The mathematical explanation of the procedures is illustrated. The methods are used for the determination of Ceftriaxone in vials and the obtained results are statistically compared with each other and with the reported method. The comparison showed that there is no significant difference between the proposed methods and the reported method.

High lights

- Simple, accurate, selective and precise spectrophotometric methods.
- These methods can be applied for severely overlapped mixtures.
- The limitations and advantages of each aspect were explained.
- These approaches can be applied for the analysis of the dosage form without need for sophisticated instruments or expensive solvents.
- The methods were validated according to ICH guidelines and the parameters were found to be within the limits.

KEYWORDS

Spectrophotometry;
Ceftriaxone;
Savitzky-golay;
Bivariate;
Dual wavelength.

INTRODUCTION

Ceftriaxone (Beta-lactam antibiotic) is a third generation cephalosporin characterized by a broad antibacterial spectrum and a resistance to Beta-lactamase-producing organisms. In addition to its antimicrobial activity (streptococci, staphylococci, pneumococci, etc.[1]), Ceftriaxone exhibits a long elimination half-life and diffuses well into cerebrospinal fluid. These characteristics are of considerable clinical and, hence, analytical interest[2].

Many studies have been reported for the determination of CTRX including HPLC[3], high-performance capillary electrophoresis[4], chemilumines-

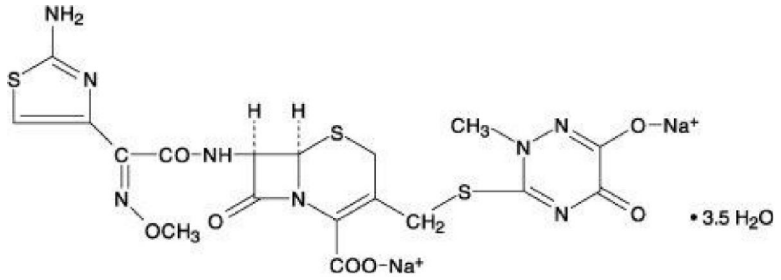

Figure 1 : Structure of ceftriaxone sodium

cence[5-9]. Ceftriaxone Sodium (Figure 1) is chemically known as 5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylicacid,7-[[2-amino-4-thiazolyl) (methoxyimino)acetyl]amino]-8 oxo-3[[(1,2,5,6-tetrahydro-2-methyl-5-,6-diaxo-1,2,4–triazin-3-yl)thio]methyl]-, disodium salt, [6R-[6α,7β (Z)]]-, hydrate, 2:7.

The aim of the work described in this paper was to develop and validate an assay for determination of Ceftriaxone sodium in pure and pharmaceutical dosage forms to make it simple, robust, and sensitive.

Theoretical background

Dual wavelength method

Several binary mixtures were resolved by dual wavelength method[10, 11].The principle of dual wavelength method is that the absorbance difference at two points on the spectra is directly proportional to the component of interest, independent of the interfering component. The pre-requisite for dual wavelength method is the selection of two such wavelengths where the interfering component shows the same absorbance while the component of interest shows significant difference in absorbance with concentration[10, 11]

The Bivariate method

This method is based on the simple mathematic algorithm[12], in which data are used from four linear regression equations, two calibrations for each component at two selected wavelengths using the method of Kaiser[13]. The principle of Bivariate calibration is in the measurements of binary mixtures (A, B) at the two selected wavelengths (1, 2) then two equations are obtained:

$$A_{AB1}=m_{A1}C_A+m_{B1}C_B+e_{AB1}$$

$$A_{AB2}=m_{A2}C_A+m_{B2}C_B+e_{AB2}$$

Where e_{AB1}, e_{AB2} are the sum of the intercepts of the linear calibration at two wavelengths ($e_{AB1}=e_{A1}+e_{B1}$), m_A, m_B are the slopes of linear regression and c_A and c_B are the concentrations of the analytes. The resolution of such equations set allows the evaluation of C_A and C_B values:

$$C_B = \frac{(A_{AB1} - e_{AB1})m_{A2} + (e_{AB2} - A_{AB2})m_{A1}}{m_{A2}m_{B1} - m_{A1}m_{B2}}$$

$$C_A = \frac{A_{AB1} - e_{AB1} - m_{B1}C_B}{m_{A1}}$$

This simple mathematic algorithm allows the resolution of the binary mixture by measuring the absorbance of the mixture at two wavelengths and using the parameters of the linear regression functions evaluated individually for each component at these same wavelengths. The method of Kaiser[13] was used for the selection of optimum wavelength set which assured the best sensitivity for the determination. A series of sensitivity matrices, K, was created for each binary mixture and for every pair of preselected wavelengths:

$$K = \begin{vmatrix} m_{A1} & m_{B1} \\ m_{A2} & m_{B2} \end{vmatrix}$$

Where m_{A1}, $_2$, $m_{B1,2}$ are the sensitivity parameters of the components A,B at two selected wavelengths(1,2). It was decided to use the values of the linear regression calibration slopes as the sensitivity factor. The determinants of these matrices were calculated and the wavelength set selected for which the highest matrix determinant value was obtained.

The savitzky - golay method

This method determines a derivative spectrum by moving a spectral window comprising 2π+1 measurement points over an absorbance spectrum. Then a polynomial of order m is fitted to the measurement points inside the spectral window.

$$P(\lambda) = a_0 + a_1\lambda + a_2\lambda^2 + \text{———} + a_m\lambda^m$$

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This fit polynomial introduces smoothing, which is dependent on the user selectable parameters n and m. From the resulting fit parameters $a_0 \ldots . a_m$ the derivatives at the window center λ_0 can be derived easily:

$$\left(\frac{dA}{d\lambda}\right)_{\lambda 0 = 0} = a_1 + 2\,a_2\,\lambda + \ldots\ldots + m\,a_m\,\lambda^{m-1} = a_1$$

$$\left(\frac{d^2A}{d\lambda^2}\right)_{\lambda 0 = 0} = 2a_2 + \ldots\ldots + m\,(m-1)\,a_m\,\lambda^{m-2} = 2a_2$$

$$\left(\frac{d^3A}{d\lambda^3}\right)_{\lambda 0 = 0} = 6a_3 + \ldots\ldots + m\,(m-1)\,(m-2)\,a_m\,\lambda^{m-3} = 6a_3$$

Once the derivatives are determined at λ_0, the window is moved one measurement point to the right followed by a polynomial fit inside this new window until it reaches the end of the spectrum[14].

EXPERIMENTAL

Material and reagents

All chemicals and reagents used were of analytical grade and all solutions were prepared in doubly distilled water.

(a) Ceftriaxone disodium was kindly supplied by the Egyptian International Pharmaceutical Industries Company (EIPICO) under the license from Roche (Switzerland), 10th of Ramadan City, Egypt; its purity was certified to be 99.9± 0.5. Stock solution was prepared by accurately weighing 100 mg of the drug into a100mL calibrated flask, dissolved in water and kept in the dark to avoid any degradation of the drug.

(b) Pharmaceutical Preparations: "Ceftriaxone" vial: (batch number 1307553) containing 1000 mg of Ceftriaxone disodium per vial.

(c) Solvent: distilled water.

Instruments

SHIMADZU dual beam UV–visible spectrophotometer (Kyoto/ Japan), model UV-1650 PC connected to IBM compatible and aHP1020 laser jet printer. The bundled software, UV-Probe personal spectroscopy software version 2.1 (SHIMADZU) was used. The spectral band was 2 nm and scanning speed is 2800 nm/min and 1 nm data interval

Software

The calculations used for SG filters were carried out using PLS toolbox software version 2.1. The t-test and F-test were performed using Microsoft Excel.

Procedures

Standard solutions

(a) Standard stock solution of Ceftriaxone disodium 1mg/mL in distilled water.

(b) Standard working solutions of Ceftriaxone disodium were prepared from stock solution by appropriate dilutions with distilled water.

(c) Preparation of the degradation product (DCTRX): Stock solution was prepared by treating 0.01gm of Ceftriaxone disodium with 10 mL phosphate buffer solution (pH10) and heated at 60°C in a water bath for 30 min. The solution was cooled and diluted to volume with phosphate buffer solution. Then neutralized with 0.1 N HCL and evaporated to dryness. The residue was dissolved in methanol, filtered into 100 mL measuring flask and completed to volume with the distilled water to obtain stock solution of alkaline degradate derived from 0.1 mg/mL[15]. Complete degradation of the antibiotic was confirmed by using TLC. Aliquots of different concentrations of Ceftriaxone degradation product (DCTRX) were accurately transferred into series of 10 mL volumetric flasks and the volumes were completed to the mark with water. These solutions were scanned over a range of 200–400 nm and stored in the computer.

Spectral characteristics of ceftriaxone disodium and its degradate

The zero order (D^0) absorption spectra were recorded against distilled water as a blank over a range of 200- 400 nm.

Linearity and construction of calibration curves

Dual wavelength method

Different aliquots from CTRX stock standard solution were accurately measured, transferred into a set of 10 mL volumetric flasks and completed to volume with water to give (6 – 24μg/mL). The prepared solutions were scanned in the range of 200-400nm. Absorbance values at 220nm and 247nm were measured. CTRX was determined by plotting the difference in absorbance values at 220 and 247nm

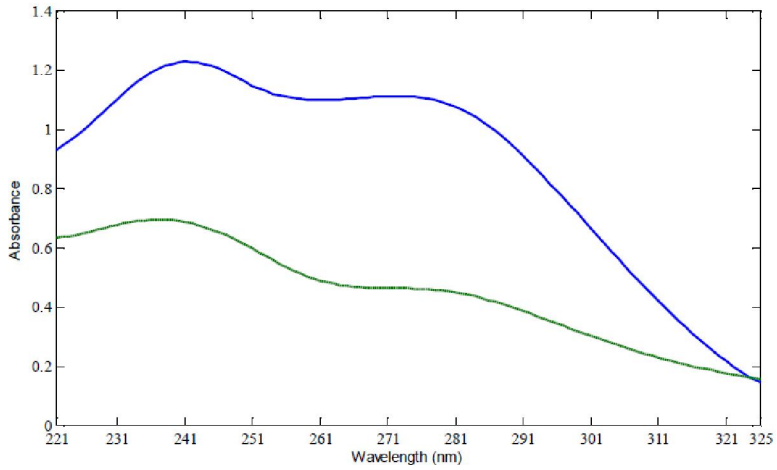

Figure 2 : Zero order spectra of (30µg / mL) Ceftriaxone () and (30µg/ mL) degradate (……).

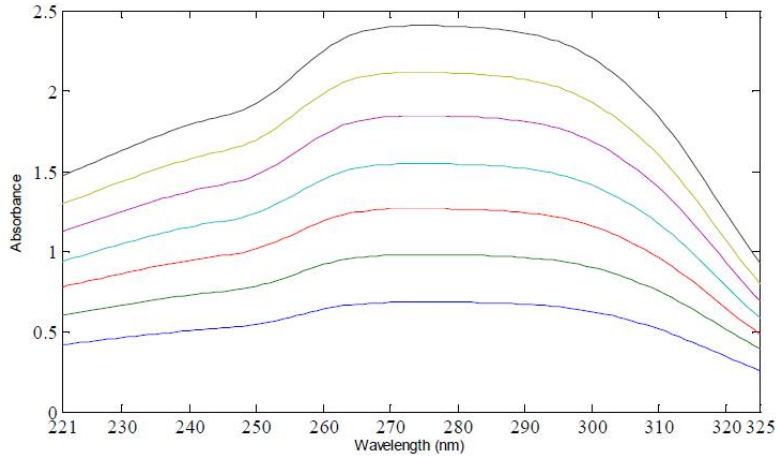

Figure 3 : Ratio spectra of ceftriaxone (6-24µg/mL) using (24µg/mL) of degradate as a divisor

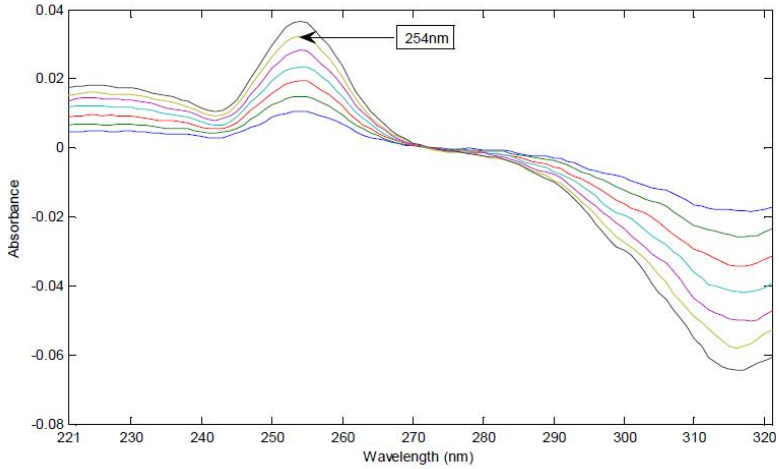

Figure 4 : Savitsky- Golay (1st derivative) application on the ratio spectra of Ceftriaxone (6-24µg/mL) using (24µg/ mL) of degradate as a divisor

(difference is zero for DCTRX) against its corresponding concentrations.

The Bivariate method

Aliquots of CTRX stock solution were accurately measured, transferred into a series of 10 mL volumetric flasks, and the volume was completed with distilled deionized water to give (6 – 24 µg/mL).. The zero-order spectra were recorded using distilled deionized

water as a blank. The absorbance of pure CTRX was measured at 250 and 270nm and plotted against the corresponding concentrations, and then the regression equations were computed at the selected wavelengths.

The derivative ratio method using Savitsky–Golay filter (SG)

Aliquots from CTRX stock standard solution were accurately measured, transferred into a set of 10 mL volumetric flasks and completed to volume with water to give (3 – 24 μg/mL). The zero order absorption spectrum of each solution was recorded versus water as a blank, divided by the spectrum of the degradation product (24μg/mL) used as a divisor for all concentrations. The first derivative of the obtained ratio spectra was employed according to the SG method using Matlab software through the use of 5-point window size and a cubic model filter. Calibration curve was constructed by plotting the amplitude of the first derivative of the ratio spectra as calculated by SG at 254 nm against their corresponding concentrations.

Analysis of artificial mixtures

Laboratory prepared mixtures containing CTRX and different percentages of its degradation product were prepared and analyzed using the same procedure described under (Linearity and Construction of Calibration Graphs).

Application of the proposed methods to the analysis of CTRX in pharmaceutical preparation

An accurately weighed amount equivalent to 10mg of the drug was transferred into a 100mL volumetric flask, dissolved in about 50mL distilled water, sonicated for 5 min, diluted to the mark with distilled water mixed well and filtered; the first portion of the filtrate was rejected. Necessary dilutions were made with distilled water to obtain different concentrations of the sample solution. The spectra of these solutions were scanned from 200 to 400nm, stored in the computer and analyzed by the proposed methods. Standard addition technique was carried out to assess the accuracy of the proposed methods.

RESULTS AND DISCUSSION

The zero-order absorption spectra of CTRX and its alkaline degradate showed severe overlapping. This overlapping hinders direct spectrophotometric measurements (Figure 2). The aim of the present work is to develop accurate, specific, reproducible, and sensitive stability indicating spectrophotometric methods for the determination of CTRX in pure form or in pharmaceutical formulations in the presence of its alkaline degradation product.

Bivariate method

Eight wavelengths were taken and the slope values of the linear regression equations were estimated for the respective components at the selected wavelengths. Using the obtained data, the sensitivity matrices were created and the respective determinants were calculated (TABLE 6).

For Bivariate determination of CFTX and its degradate, the wavelengths 250 and 270 were used. At these selected wavelengths, the calibration curves were obtained in the range of 6-24 μg/mL for CFTX. The linear regression equations were computed (TABLE 1). CFTX could be determined in presence of up to 87.5% of its alkaline degradation product, with mean percentage recovery of 100.38±0.804 (TABLE 3).

Dual wavelength

Linear correlation was obtained between the differences absorbance values at the selected wavelengths in amplitude at 220–247 nm for CFTX in the range of 6–24 μg/mL, and the regression equations were computed (TABLE 1). The method was checked by the analysis of laboratory prepared mixtures of CFTX and its alkaline degradate in different ratios as presented in TABLE 2. CFTX could be determined in presence of up to 87.5% of its alkaline degradate, with mean percentage recovery of 100.82±1.38% (TABLE 3).

The derivative ratio method using savitsky–golay filter

Following the general rules for the use of SG function in processing the obtained ratio spectra, the different parameters associated with the calculation of the SG coefficients were optimized. These include the selection of function order, number of points (window size) and wavelength for quantitation. The optimum parameters were selected for Cefoperazone when the coeffi-

TABLE 1 : Linearity studies and regression equations of the proposed methods

Parameters	Dual wavelength	Bivariate		Savitzky- Golay
wavelength	220 – 247 nm	250nm	270nm	254nm
Calibration range	(6 – 24 µg/mL)	(6 – 24 µg/mL)		(3 - 24µg/mL)
slope	0.0108	0.0453	0.0437	0.0014
intercept	0.0064	0.061	0.0604	0.0021
The square correlation coefficient (r^2)	0.9995	0.9998	0.9998	0.9996

TABLE 2 : Method validation obtained by applying the proposed methods

Method	Conc (µg/mL)	Intraday*		Interday*	
		Accuracy (R%) ± SD	Precision (RSD%)	Accuracy (R%) ± SD	Precision (RSD%)
DW	15	101.56 0.356	0.351	101.15±1.426	1.409
	18	100.93±0.514	0.510	99.55±1.485	1.497
	21	99.74 ± 0.441	0.442	99.00±0.674	0.680
BV	15	100.32±0.695	0.693	99.83±0.225	0.225
	18	101.14±0.071	0.070	100.89±0.394	0.391
	21	100.60±0.546	0.543	100.25±0.579	0.578
SG	15	100.40±1.730	1.723	98.94±0.606	0.613
	18	101.27±1.755	1.733	101.72±0.039	0.039
	21	101.71±0.847	0.832	101.37±0.273	0.269

***Average of three determinations**

TABLE 3 : Determination of intact Ceftriaxone in laboratory prepared mixtures with its alkaline degradate by the proposed methods

Conc. Of CFTX (Mg/mL)	Conc. Of DCFTX (Mg/mL	% of DCFTX	DW[a]	BV[a]	SG[a]
21	3	25	100.18	99.27	100.24
18	6	37.5	101.95	100.18	98.81
15	9	50	101.98	100.13	98.41
12	12	62.5	99.69	101.11	101.79
9	15	75	98.97	99.27	99.52
6	18	87.5	102.16	101.23	98.81
Mean			100.82	100.38	99.60
SD			1.380	0.804	1.253

[a] % Recovery

TABLE 4 : Application of standard addition technique to the analysis of Ceftriaxone vial by applying the proposed methods

Pharmaceutical Conc. µg/mL	Added standard µg/mL	DW[a]	BV[a]	SG[a]
5	12	98.92	101.82	100.60
	15	99.51	99.41	100.95
	18	98.87	99.28	99.21
	Mean RSD%	99.1±0.359	100.17±1.429	100.25±0.920

[a] % Recovery

cients calculated for the drug provide smoothed derivative spectra that give precise and accurate results for the estimated analyte. Consequently, a first order derivative was applied. 5 Point window size and a cu-

bic model filter were selected for processing the signals of the ratio spectra as they give better results. In the application of this method, the derivative ratio spectra by SG of a pure compound and its laboratory mixture would coincide in the spectral region corresponding to a maximum point or a minimum point of the wavelength as shown in Figure 4. These coinciding points of the SG derivative spectra were selected as working wavelengths.

CFTX concentrations were determined by measuring the signal amplitude at 254 nm. Linear correlation was obtained between the amplitude at 254 nm, against the corresponding concentrations of Ceftriaxone. The yielded statistical results are summarized in TABLE 1.

Methods Validation

Validation was done according to ICH recommendations[47].

Linearity

The linearity of the methods was evaluated by analyzing concentrations of CFTX between 3-24 µg/mL. Each concentration was repeated three times. The assay was performed according to the experimental conditions previously mentioned. The linear equations were summarized in TABLE 1.

Range

The calibration range was established through considerations of the practical range necessary according to adherence to Beer's law to give accurate precise and linear results as shown in TABLE 1.

Accuracy

The accuracy of the results was checked by applying the proposed methods for determination of different samples of CFTX. The concentrations were obtained from the corresponding regression equations. From which the percentage recoveries suggested good accuracy of the proposed methods were calculated with mean percentage recovery shown in TABLE 2.

Repeatability

Three concentrations of CFTX (15, 18, 21µg/mL) were separately analyzed three times intraday using the proposed methods. The relative standard deviations were calculated as shown in TABLE 2.

Reproducibility (intermediate precision)

TABLE 5 : Statistical comparison between the results obtained by applying the proposed spectrophotometric methods and the reported methods for determination of ceftriaxone in ceftriaxone® vial

Parameter	DW	BV	SV	Reported method
Mean	100.32	100.53	100.05	99.94
S.D.	1.808	1.367	1.151	1.884
N	5	5	5	5
Variance	3.269	1.869	1.326	3.549
t-test	0.326 (2.306)	0.563 (2.306)	0.11531 (2.306)	
F-Value	1.086 (6.388)	1.899 (6.388)	2.677 (6.388)	

The values in the parenthesis are the corresponding theoretical values of t and F at (P = 0.05)

TABLE 6 : Application of kaiser method for selection of the wavelength set for CFTX and DCFTX

λ/λ	240	245	250	255	260	265	270
240	0	-65.44	-99.91	-196.91	-292.55	-336.69	-375.99
245		0	-37.01	-134.57	-229.17	-272.31	-310.69
250			0	-867.58	-950.84	-995.28	-1035.19
255				0	-87.98	-127.02	-161.69
260					0	-38.28	-72.23
265						0	-33.93
270							0

The previous procedures were repeated inter-day on three different days for the analysis of the three chosen concentrations. The relative standard deviations were calculated as shown in TABLE 2.

Specificity

Specificity of the methods was achieved by the analysis of different laboratory prepared mixtures of CFTX and DCFTX within the linearity range. Satisfactory results were shown in TABLE 3.

The validity of the proposed procedures is further assessed by applying the standard addition technique showing no excipients interference. The results obtained were shown in TABLE 4

Statistical analysis

Statistical comparison of the results obtained by the proposed methods and reported method was shown in TABLE 5. The calculated t and F values were less than the theoretical ones indicating that there were no significant differences between the proposed methods and the reported first derivative method[17] with respect to accuracy and precision.

CONCLUSION

The proposed methods are simple, sensitive, accurate, and rapid stability-indicating assay methods for the determination of CFTX in presence of its degradation product. The methods are suitable and valid for application in laboratories lacking liquid chromatographic instruments. These methods were applicable for assay and purity testing of CFTX in bulk and pharmaceutical formulations without interference of additives in the pharmaceutical preparation. These methods are also less time consuming and economic stability indicating methods.

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