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UV Spectrophotometric methods for simultaneous determination of Cefixime-Trihydrate (CFX) and Dexamethasone Sodium Phosphate (DXP)

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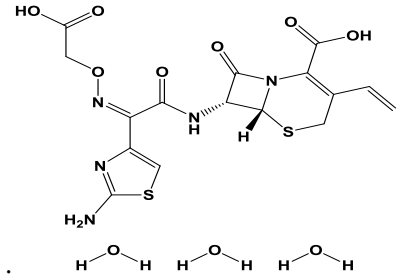
Abstract

Two precise, accurate, selective and sensitive spectrophotometric methods were introduced for simultaneous determination of Cefixime trihydrate [CFX] and dexamethasone sodium phosphate [DXP] in their pure forms and pharmaceutical dosages. The first method, ratio difference method (RD), depends on the difference in amplitudes of the ratio spectra at Δp 290-360 nm and Δp 250-265 nm for determination of CFX and DXP, respectively. The second method, first derivative spectrophotometric method (1D), depends on measuring the peak amplitude of the first derivative selectiely at 311 and 255.6 nm for determination of CFX and DXP, respectively. The proposed methods were validated according to ICH guidelines. Satisfactory results were obtained for for determination of both drugs in laboratory prepared mixture and pharmaceutical dosage forms. The developed methods were compared with official ones.

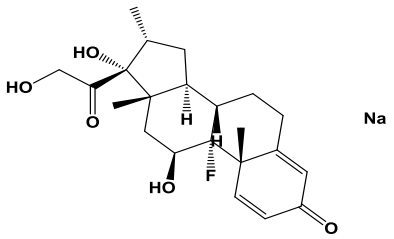
Keywords: Cefiximetrihydrate (CFX), Dexamethasone sodium phosphate (DXP), Ratio difference (RD), First derivative (1D).

1. Introduction

Cefixime trihydrate is chemically knowon as (6R, 7R)-7-[2-(2-amino-4- thiazolyl) glyoxyl-amido]- 8-oxo-3-vinyl-5-1 –azabicyclo [4.2.0] oct-2- ene2-carboxylicacid,7-9z)-[o carboxymethyl)-oxime] trihydrate with molecular formula of $C_{16}H_{21}N_5O_{10}S_2$
and structural formula as shown in Fig.(1a), [1]. Cefixime trihydrate [CFX] is a broad-spectrum, third-generation cephalosporin antibiotic. It is derived semisynthetically from the marine fungus Cephalosporium acremonium with antibacterial activity. Like penicillin, the beta-lactam antibiotic Cefixime inhibits bacterial cell wall synthesis by disrupting peptidoglycan synthesis, resulting in a reduction of bacterial cell wall stability and bacterial cell lysis. This agent is more active against gram-negative bacteria and less active against gram-positive bacteria compared to second-generation cephalosporins [1]. Dexamethasone sodium phosphate, is chemically known as 9α-fluoro-16α-methyl-11β, 17α, 21-trihydroxy-1,4-pregnadiene-3,20-dione.It is a synthetic derivative of the glucocorticoid hydrocortisone that has a long history of use in humans [2]. Dexamethasone sodium phosphate (DXP), is the sodium salt of dexamethasone with empirical formula of $C_{22}H_{28}FNa_2O_8P$ and its structural formula is represented in Fig.(1b).[3]



(a)



(b)

Fig. (1): structural formula of Cefiximetrihydrate (a) and Dexamethasone sodium phosphate (b).

Dexamethasone is widely used in clinical purposes due to its anti-inflammatory and immunosuppressive activities [4], being employed on the treatment of arthritis, asthma, eye inflammations and illnesses of collagen, as well as on the pre-

vention of undesirable immune reactions [5]. Literature survey revealed that various techniques have been utilized for determination of Cefixime trihydrate in body fluids and dosage forms, including spectrophotometric [6,7], spectrofluorometric [8], chemometric [9], chromatographic [10,11], and electrochemical [12, 13] methods. Various reports have been found for quantitative determination of dexamethasone in real samples with different matrices. They are including spectrophotometry [14], liquid chromatography [15, 16], liquid chromatography-mass spectrometry [17,18], and electrochemical methods [19].

No methods have been published for the determination of CFX in combination with DXP. In our work, we aim to introduce spectrophotometric methods for simultaneous determination of CFX and DXP with advantages of being easier, economic, rapid, and selective. Two methods were used in our study which are ratio difference method (RD) and first derivative (1D) UV spectroscopy.

2. Experimental

2.1. Materials and Reagents

Pure Cefixime tri hydrate and dexamethasone sodium phosphate were kindly supplied by NODCAR. Methanol (99.99%), 0.1N HCl and distilled water were purchased. Pharmaceutical formulations: Cefixime capsule labeled to contain 223.8 mg of CFX and Dexamethasone sodium phosphate injectable solution, labeled to contain 10.52 mg/2mL of DXP.They were purchased from local Egyptian pharmacy.

2.2. Apparatus

SHIMADZU UV-1800 PC Series Spectrophotometer (Tokyo, Japan) with two matched 1 cm quartz cells. The scan speed was fast with 0.2 nm spectral band width, 1 nm slit width and 0.1 nm sampling interval. In this study; the first order spectra were manipulated with scaling factor (SF) = 1 and delta lambda (Δλ) = 1 nm.

2.3. Standard solutions

100 µg/mL stock solutions of both CFX and DXP were prepared separately by dissolving 10 mg of each drug separately in 100mL of methanol as a solvent.

2.4. Method optimization

To improve the developed RD and 1D methods, several parameters were evaluated.

2.4.1. Selection of the smoothing and scaling factors for the 1D spectra

In case of 1D method, different smoothing factors (Δλ) were tested including 2, 4 and 8, but Δλ = 4 exhibited the optimum signal to noise ratio with the best resolution. Additionally, different scaling factors were tested including 10 and 100, but the scaling factor = 10 was the most convenient one; as it caused enlargement of the signals and facilitated its measurement, and reduced the reading error.

2.4.2. Selection of the solvent

Methanol, ethanol, distilled water, 0.1 N HCl and 0.1 N NaOH were tried as solvents, where methanol was found to be the best solvent regarding the selectivity and sensitivity.

2.4.3. Selection of the wavelength ranges

For (RD) method, the selected wavelengths, in the ratio spectra should have distinct difference in amplitudes. Various ranges were examined including (200–400), (210–291), (220–291), (221–370), (265–370) and (221–330) nm. The best results of accuracy and precision were obtained using the ranges of (290 ‑ 360) nm for CFX and (250 – 265) nm for DXP. For (1D) method, 311 nm and 255.6 nm were selected as analytical wavelengths for determination of CFX and DXP, respectively

3. Procedures

3.1. Linearity

Standard serial diluations of both CFX and DXP were prepared separately from their standard stock solutions using methanol as a solvent and scanned at 200-400 nm using methanol as blank to obtain the range of concentrations suitable for determination methods. The zero order absorption spectra were recorded and stored in computer.

3.1.1. Construction of calibration curves

3.1.1.a Ratio Difference Spectrophotometric Method (RD).

The stored zero order spectrum for each concentration of CFX was divided by 10µg/mL of DXP as divisor and for each concentration of DXP was divided by 5 µg /ml of CFX as divisor**.** The obtained ratio spectra were used to develop RD calibration curves for CFX and DXP by plotting

the amplitude difference of ratio spectra between 290 and 360 nm for CFX and at 250 and 265 nm for DXP against their corresponding concentrations in µg/mL as shown in Figs. (2,3) and the regression equations were then computed.

3.1.1.b First-Derivative Spectrophotometric Method

The spectral data mentioned previously were processed to obtain first order derivative spectrum at wavelength interval of 4 nm. The two spectra were overlain (Fig. 4). It is appeared that CFX showed zero crossing at 255.6 nm while DXP showed zero crossing at wavelength range from 297 nm to 335 nm. At zero crossing point of DXP (311 nm), CFX showed a measurable dA/dλ whereas at the zero crossing point of CFX (255.6 nm), DXP showed appreciable dA/dλ. Hence, the wavelengths 311 nm and 255.6 nm were selected as analytical wavelengths for determination of CFX and DXP, respectively

3.2. Application to Laboratory Prepared Mixtures

In two series of 10-mL volumetric flasks, aliquots of CFX and DXP standard solutions were transferred to prepare mixtures with different ratios of the two drugs then, the volume was completed with methanol. The spectra of the prepared mixture were recorded. The concentrations of CFX and DXP were obtained using the corresponding regression equations for each method.

3.3. Application to Pharmaceutical Preparations

Content of one Cefixime tri hydrate capsule was weighed and 10 mg of this powder was accurately weighed and dissolved in 100 mL methanol. 1.6mL of dexamethasone sodium phosphate was accurately transferred into 100 mL measuring flask, then the volume was completed to the mark with methanol. In a 10 mL volumetric flask, aliquots of CFX and DXP pharmaceutical solutions were transferred to prepare a mixture with different ratio of the two drugs then, the volume was completed with methanol. The procedures under linearity were followed to determine the concentrations of both drugs from the corresponding regression equation of each method.

4. Results and Discussion

The developed methods were applied to resolve the spectral overlap of CFX and DXP in their binary mixture, as shown in Fig. (5) without previous separation steps.

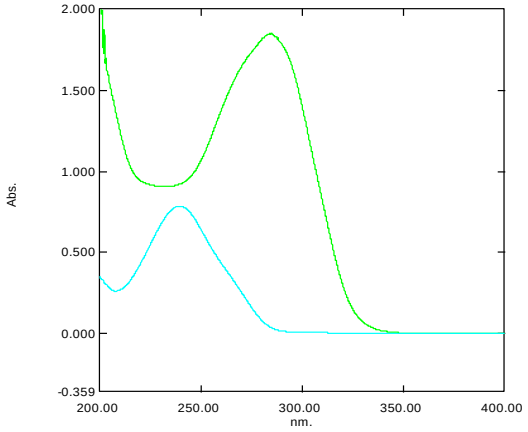


Fig. (4): Zero order absorption spectra of CFX (30 µg/ml) and DXP (30µg/ml) in methanol

4.1. Method development and optimization.

4.1.1. Ratio Difference Method

This method is simple, accurate including two main steps; the first one is to select an appropriate divisor. After many trials, the best results in according to accuracy and precision showed that the divisor of choice was 10µg/mL of DXP and 5µg/mL of CFX. The absorption spectra of each drug were divided by the chosen divisor. The second important step is how to choose the most suitable wavelengths at which the measurements will be recorded [21] . The selected wavelengths, in the ratio spectra should have distinct difference in amplitudes as shown in Fig. (5) and Fig. (6). To obtain a good linearity, many pairs of wavelengths were tried, The most suitable wave length ranges were 290 and 360 nm for CFX, and wave lengths of 250 and 265 nm were selected for DXP.

Linear correlations were obtained for Δp_{CFX} against the corresponding concentration and ΔP_{DXP} against its corresponding concentration as shown in Figs. (7,8).

Their linear regression equations were then computed.

$\Delta P_{CFX} = 3.537\ C + 3.3088$, $R^2 = 0.9992$

$\Delta P_{DXP} = 0.0439C + 0.0244$, $R^2 = 0.9996$

Where ΔP express the difference of peak amplitude of the ratio difference spectrum curve, C is concentration of drug in µg/mL and r is the correlation coefficient.

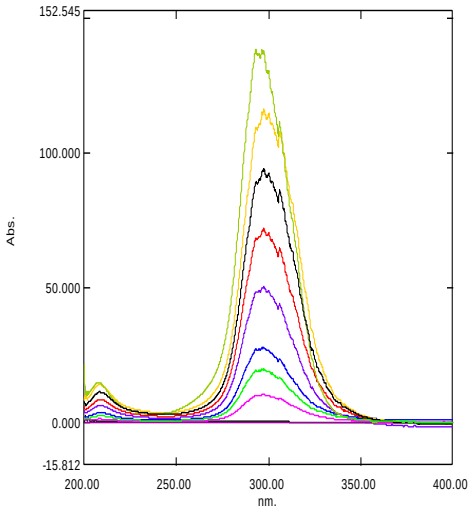


Fig. (5): Ratio spectra of (2-50µg/mL) CFX using DXP (10µg /mL) as divisor.

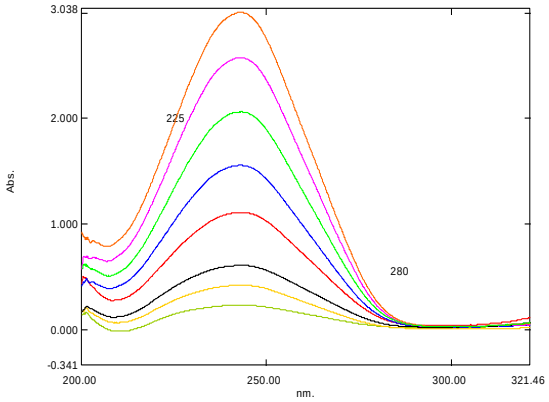


Fig. (6): Ratio spectra of (2-50µg/mL) DXP using CFX (5µg /mL) as divisor.

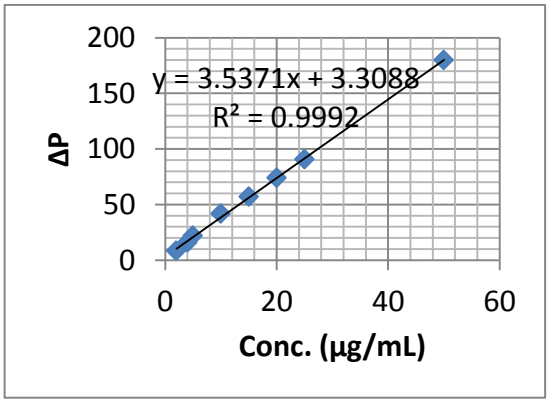


Fig. (7): Calibration curve of RD of CFX using 10µg/mL of DXP as divisor at ΔP(290-360) nm.

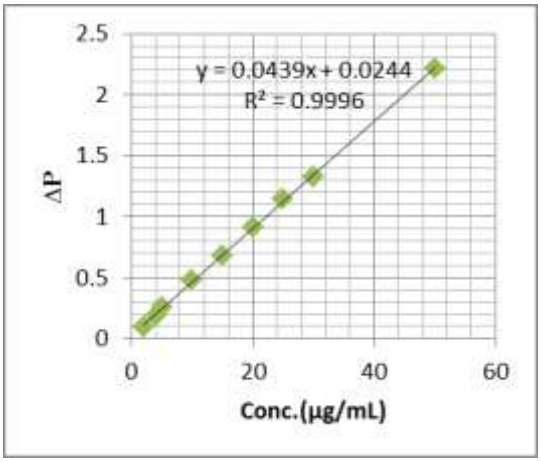


Fig. (8): Calibration curve of RD of DXP using 5µg/mL of CFX as divisor at ΔP(250-265) nm.

4.1.2. First derivative uv spectroscopy:

For the linearity study, aliquots of the drug solutions were further diluted with methanol to get the final working standards of concentration range as 2-50 µg/mL for CFX and 2-50µg/mL for DXP. The first derivative spectra were taken (Figs. 10 and 11) and the derivative absorbances at 311 nm and 255.6 nm for CFX and DXP were measured, respectively. The calibration graphs of both drugs were plotted at 311 nm and 255.6 nm (Figs. 12 and 13). The following regression equations for both the drugs were used for the quantitative estimation of samples.

$dA/d\lambda_{(CFX)}$=-0.0019C-0.0014,R^2= 0.9991

$dA/d\lambda_{(DXP)}$=-0.0007C–0.0002, R^2= 0.999 Where, $dA/d\lambda$ is absorbance of the first derivative absorbance, C is the concentration of drug in µg/mL and r is the correlation coefficient.

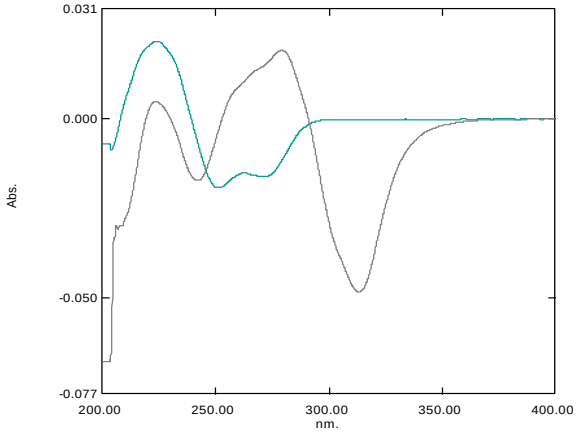


Fig. (9): First derivative UV spectra of 25 µg/mL CFX and 25 µg/mL DXP.

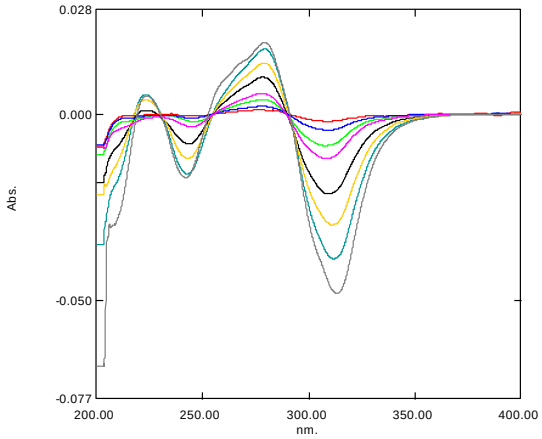


Fig. (10): First drevative UV spectra of CFX.

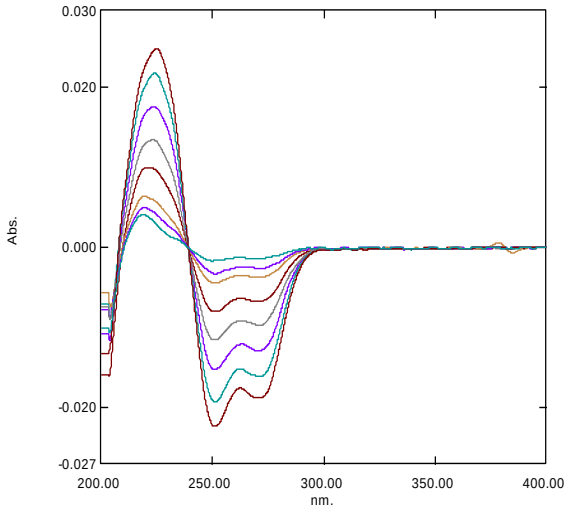


Fig. (11): First derivative uv spectra of DXP.

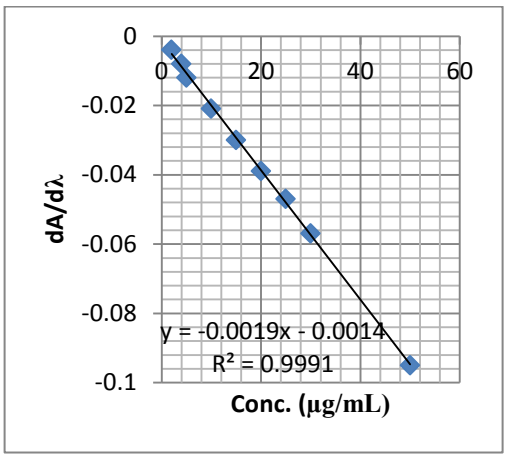


Fig. (12): calibration curve of CFX at ٣١١ nm.

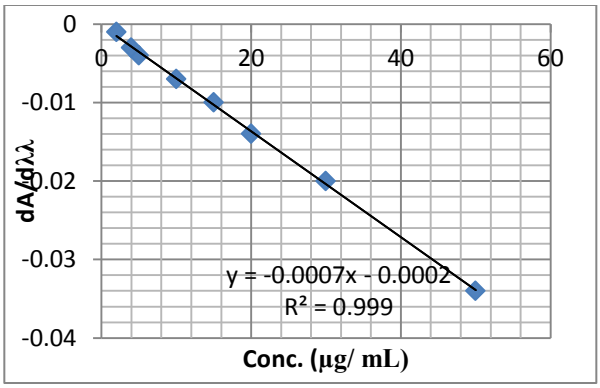


Fig. (13): calibration curve of DXP at 25٥.6 nm

4.2. Validation of the Methods

The developed methods were validated according to ICH recommendations [20].

4.2.1. Linearity and range

Linearity ranges, regression equations, LOD, LOQ and accuracy were determined. Satisfactory results were obtained as shown in Table 1.

4.2.2. Accuracy/Recovery

The accuracies of the developed methods were estimated by considering the percentage recoveries at three concentration levels (10, 15 and 20 µg/ml), for both CFX and DXP, each in triplicate. The mean recoveries and the relative standard deviations were calculated. The results showed that the percentage recovery values were within the range of 100 ± 2% with low standard deviations indicating high accuracy of the suggested analytical methods as mentioned in Table 1.

4.2.3. Precision

The repeatabilities of the developed methods were estimated by applying intra-day precision by assaying freshly prepared solutions in triplicates within one day in the concentration range of (2 – 50) µg/ml for both CFX and DXP. Inter-day precision was evaluated by assaying triplicates of freshly prepared solutions for three successive days using the same concentration range for both drugs. The percentage relative standard deviations (R.S.D.) did not exceed 1.25% (Table1) indicating a high reproducibility of the results and the precision of the proposed methods.

4.2.4. Selectivity

The selectivities of the proposed methods were evaluated by analysis of laboratory prepared mixtures of CFX and DXP in different ratios with concentrations inside the linearity range. Satisfactory results were obtained as shown in Table 2.

4.2.5. Robustness

The robustnesses of the developed methods were tested by performing the spectral measurements under small changes in parameters such as temperature by ± 1.0 and wavelength interval ± 0.2 nm showing no significant effect on absorbance or amplitude.

Applications of the methods in assay of pharmaceutical formulations

The suggested methods were applied for the determination of CFX and DXP in their pharmaceutical formulations. Satisfactory results indicate that there is no interference from dosage form excipients (Table 3).

4.3. Statistical comparison between the developed methods and the reported HPLC method

The results obtained from the two developed methods and those from the reported HPLC method [21] for the determination of CFX and DXP in pharmaceuticals were statistically compared to each other and the results presented in Table 4. This is achieved by performing an ANOVA test and plotting for t- and F-values that are less than theoretical values, indicating that there is no significant difference between them.

5. Conclusion

The previous mentioned spectrophotometric methods were introduced for simultaneous determination of CFX and DXP in their pure forms or in their pharmaceutical preparations . These methods are considered to be simple, accurate, convenient, economic, time saving and show high sensitivity, specificity because they enabled us to determine CFX and DXP without any interference of additives and excipients. The adopted methods can be easily applied without preliminary separation. The suggested methods were completely validated showing satisfactory data for all the method validation parameters tested. Recovery studies indicated that practically there was no interference from dosage form excipients, so these methods can be easily and conveniently adopted for routine content determination of CFX and DXP.

Table (1) Regression parameters and results of determination of pure samples of CFX and DXP by the developed UV spectrophotometric methods.

Parameter	RD		1D	
	CFX	DXP	CFX	DXP
Wavelength Selected(nm)	**Δp= 290-360**	**Δp=250-265**	**311**	**255.6**
Linearity Range (µg/mL)	2-50	2-50	2-50	2-50
Correlation coefficient	0.9992	0.9996	0.9991	0.999
Slope	3.537	0.0439	-0.0019	-0.0007
Intercept	3.3088	o.o244	-0.00014	-0.0002
LOD (µg/mL)	0.8555	0.608	0.869	0.953
LOQ (µg/mL)	2.59	1.845	2.63	2.98
Accuracy [a] (mean±RSD)	99.6 ±1.493	99 ±1.00	98.1 ±1.432	99.5 ±1.722
Repeatability (RSD) [b]	1.298	0.899	1.337	1.450
Intermediate precision (RSD) [c]	0.996	1.197	1.431	1.335

a average of three determinations.

b The intraday, average of three concentrations repeated three times within the day**.**

c The interday, average of three concentrations repeated three times in three day.

Table (2) Determination of CFX and DXP in the laboratory prepared mixtures.

Conc. (µg/mL)		**CFX**		**DXP**	
		Recovery %		**Recovery %**	
CFX	DXP	RD	1D	RD	1D
10	10	100.8	99.1	99.6	100.6
10	20	98.8	97.7	101.6	101.1
10	30	101.6	99.2	101.5	99.2
20	10	103.2	98	102.3	100.1
25	15	102.7	98.3	102.6	99.1
Mean		101.42	98.46	101.52	100.02
SD		1.738	0.665	1.169	0.87
RSD		1.713	0.675	1.151	0.869

Table (3) Determination of CFX and DXP in a binary mixture prepared from Cefixime capsules and Dexamethasone sodium phosphate injectable solution by the developed UV spectrophotometric methods.

Applied methods	**Pharmaceutical preparations**			
	CFX		**DXP**	
	(Recovery %± SD)	**RSD**	**(Recovery %± SD)**	**RSD**
RD	99.266 ± 2.3	2.393	98.2± 1.356	1.383
1D	101.33± 0.577	0.569	98.23± 1.329	1.353

Table 4. Statistical comparison of the results obtained by applying the two proposed methods and the reported HPLC method for determination of CFX and DXP in their dosage form.

Item	**CFX**		**DXP**		**Reported HPLC method [21][c]**	
	RD	**1D**	**RD**	**1D**	**CFX**	**DXP**
Mean	100.32	99.95	100.2	99.83	100.04	100.12
SD	0.724	0.106	0.815	0.922	1.15	1.22
RSD%[a]	0.962	1.25	1.14	0.928	1.11	0.954
Variance	0.718	4.2	1.15	1.15	1.17	2.15
n	5	5	5	5	5	5
Student's t-test (2.228)[b]	1.15	0.85	0.72	0.37		
F-test (5.050)[b]	2.15	1.85	1.56	1.744		

[a]Average of 3 determinations.

[b]Number between parenthesis represent the corresponding tabulated values of t and F at $p = 0.05$.

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