Original Article

Simultaneous determination of ofloxacin and cefixime by first and ratio first derivative UV spectrophotometry

Abstract

Aim: Derivative spectrophotometry offers a useful approach for the analysis of drugs in multicomponent mixtures. Objective of the current study was to develop simple and rapid simultaneous methods for the determination of ofloxacin (OFX) and cefixime trihydrate (CEF) in bulk and pharmaceutical formulations. **Materials and Methods:** Two UV spectroscopic methods were developed and assessed for their feasibility in the simultaneous estimation. The first method was based on the first derivative absorption at 282.8 nm for OFX (zero crossing for CEF) and at 318.6 nm for CEF (zero crossing for OFX). The method was applied in the concentration of 2 µg/ml to 20 µg/ml. Alternatively, the ratio derivative spectrophotometry method was developed making use of amplitude in first derivative of corresponding ratio spectra at 337.2 nm (maxima) and 317 nm (maxima) to estimate OFX and CEF, respectively. **Results:** The results showed higher correlation coefficient (~0.999) in both the proposed methods. Further, the methods were validated for precision, accuracy and assessed the drug content in bulk drug and formulation. Moreover, the data observed indicated also suggest that no interference of excipients during the estimation. **Conclusion:** The study concludes that the proposed methods are simple, rapid, sensitive, accurate and reproducible and could be an alternative to the existing chromatographical methods for the simultaneous determination of OFX and CEF in pharmaceutical dosage forms and in dissolution studies.

Key words:
Cefixime, first derivative, ofloxacin, ratio first derivative, spectrophotometry

Introduction

Ultraviolet (UV) spectroscopic method of analysis is extensively used in the analysis of pharmaceutical ingredients probably due to its high sensitivity and cost-effectiveness. Both direct and indirect measurement methods are utilized in this approach to estimate the drug content in formulations. However, indirect measurement methods such as derivative spectrophotometry and ratio-spectra derivative spectrophotometric approaches has capitulated many analytical research scientists in the last three decades. Several promising methods for the estimation of multicomponents in pharmaceutical formulations have been developed using this approach. Literature suggests that the derivative spectrophotometry generally helps in eliminating interference from formulation matrix by using the zero-crossing techniques.[1-5] Moreover, the presence of multiple maxima and minima gives the opportunity for determination of active compounds in presence of other interfering compounds or formulation excipients. The application of the described technique was investigated in a tablet formulation with combination of cefixime trihydrate (CEF) and ofloxacin (OFX), which is available as tablets.

CEF is chemically 5-thia-1-azabicyclo [4.2.0]oct-2-ene-2-carboxylic acid, 7-[(2-amino-4-thiazolyl)[(carboxymethoxy) imino]acetyl]amino]-3-ethenyl-8-oxo-,trihydrate, [Figure 1]

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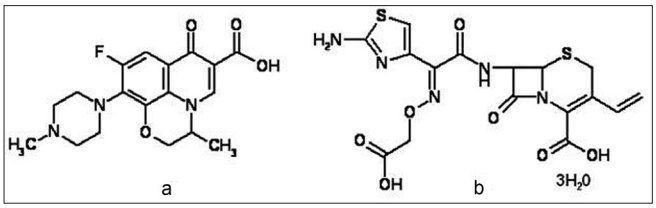

Figure 1: Structure of ofloxacin (1) and cefixime trihydrate (2)

used as broad spectrum, third-generation cephalosporin antibiotic. It acts by interfering in the synthesis of the bacterial cell wall[6] Review on the literature indicated that the analysis of this drug was generally performed by UV spectroscopy,[7,8] spectrofluorometry,[9] HPLC,[10-16] HPLC/MS[17] and HPTLC.[18]

OFX a synthetic fluoroquinolone, anti-infective agent has an expanded spectrum of activity and increased antibacterial potency compared with nonfluorinated quinolones.[19] Chemically it is 9-fluro-2, 3 dihydro-3-methyl-10(4-methyl-1- piperazynyl-7-oxo-7H-pyrido [1, 2, 3-de]-1, 4-benzoxacine-6-carboxilic acid [Figure 1]. It acts by targeting bacterial DNA gyrase and topoisomerase IV. Extensive literature survey reveals that several analytical methods such as spectrophotometric,[20-22] spectrofluorometry,[23] electrophoresis,[24] HPLC[25-27] and LC/MS/MS[27,28] were used to estimate the OFX in its pure form, pharmaceutical dosages, when the drug is alone or in combination with other drugs.

The combination (OFX and CEF) is preferred in the treatments of typhoid fever, urinary and respiratory tract infections, noscomial infections, soft tissue and intra-abdominal infections[29] and is formulated by several pharmaceutical industries. However, there are no simple, rapid and cost effective analytical methods to estimate the drug content in the combined dosage forms. The HPLC and HPTLC methods reported in the literature are quite expensive and time consuming.[30,31] Hence there is an urgent demand to develop a simple and rapid method such as spectroscopic method to assess the drug content in this combination. However, the major concern for these two drugs is the overlapping of absorption bands, which also restricts the direct measurement using UV spectroscopy. The objective of the current study was to develop rapid, accurate, reproducible, validated and economical first derivative and ratio first derivative analytical methods for the simultaneous determination of OFX and CEF from pure and tablet formulation.

Materials and Methods

Experimental

Instruments and chemicals

Double beam UV-visible spectrophotometer (Shimadzu, 1700), connected to computer and loaded with UV-probe software was used for the drug estimation. For all absorbance measurements 1 cm quartz cuvettes were matched and used. Demineralized and double distilled water and Whatmann filter paper (no.41) were used throughout the experimental work. The sample of OFX was procured from Micro Labs, India and CEF from Dr. Reddy's laboratory, India. Multicomponent tablet ZENFLOX Plus (OFX 200 mg and CEF 200 mg per tablet) marketed by Mankind Pharma Ltd, New Delhi, India was purchased from local market.

Preparation of standard stock solution

Stock solutions of OFX and CEF (200 µg/ml) were prepared by accurately weighing and dissolving in required amount of drugs in water, separately. The working standard solutions of the respective drugs were obtained by diluting the stock solutions in water.

Method development

Method – I (First derivative spectrophotometric method)

In the first-order derivative method, OFX and CEF solutions (10 µg/ml) were prepared, separately. Both the solutions were scanned in the spectrum mode from 220 to 400.0 nm. The absorption spectra thus obtained were derivatized from first to fourth order. First order derivative (n=1) was selected for analysis of both the drugs. The zero crossing wavelengths, 282.8 nm and 318.6 nm were selected for OFX and CEF, respectively. The calibration curves were constructed by scanning different concentration solutions and derivatizing the spectrum. The absorbance of OFX and CEF were measured at 282.8 nm and 318.6 nm, respectively, and working calibration curves of both the drugs were plotted separately. The concentration of individual drug present in the mixture was determined against calibration curve in quantitation mode.

Method II (First derivative of the ratio spectra)

For OFX, the absorption spectra of solutions prepared at different concentrations (2-20 µg /ml) in its binary mixture with CEF were recorded against water and divided by the spectrum of the standard solution of CEF (16 µg/ml in water) to get the ratio spectra of OFX. The first derivative of the ratio spectra were than calculated. The amount of OFX was determined by measuring the first derivative signal at 337.2 nm. A similar procedure was followed for different concentrations of CEF (2-20 µg/ml) when OFX was constant (16 µg/ml in water). Similarly, content of CEF was determined by measuring the first derivative signal at 317 nm.

Analysis of pharmaceutical dosage forms

To determine the content of OFX and CEF simultaneously in tablets (label claim: 200 mg OFX and 200 mg CEF, film coated); 20 tablets were weighed; their average weight determined and were finely powdered. The correct amount of powder (equivalent to 20 mg of both the drugs) was dissolved in water by stirring for 30 min. After filtration, appropriate aliquots were subjected to above methods and the amount of OFX and CEF were determined.

Validation of the methods

The methods were validated according to the ICH guidance[32,33] in terms of linearity, accuracy, precision, limits of quantization and selectivity.

Linearity

Linearity of the proposed methods were determined by analyzing the solutions of OFX and CEF in the range of 2-20 µg/ml. The limit of detection (LOD) and limit of quantification (LOQ) was then established by evaluating the minimum level at which drug solution can be readily quantified with accuracy. LOD and LOQ were calculated according to the 3.3σ/s and 10 σ/s criteria, respectively, where σ is the standard deviation of the peak area and s is the slope of the corresponding calibration curve.

Accuracy

Accuracy of the method was determined by performing recovery studies by standard addition method in which preanalyzed samples were taken (5 µg/ml) and standard drug was added at three different levels i.e., 80%, 100% and 120%. The total concentrations were determined by using the proposed methods. Each level was made in triplicate. The % recovery of the added pure drugs were calculated as % recovery = [(Ct-Cs)/Ca] X 100, where Ct is the total drug concentration measured after standard addition; Cs drug concentration in the formulation sample; Ca drug concentration added to formulation.

Precision

System precision was assessed by preparing fresh 10 µg/ml solutions from independent stock solutions and measuring the absorbance at selected wavelengths (n=6). Intraday and interday precision of the method were confirmed by repeating the absorbance at 5 µg/ml, 10 µg/ml, 15 µg/ml three times in a day and also on two different days.

Results and Discussion

In the preliminary stage the aqueous solubility of OFX and CEF were determined (data not shown). It was found that OFX and CEF possess good aqueous solubility and showed good UV absorption, hence water has been selected as solvent in the current investigation. Preliminary studies were carried out to determine the spectrum of both the drugs in aqueous solutions (individual drugs and binary mixtures).The UV spectra of OFX and CEF (10 µg/ml each) and their mixture obtained were presented in Figure 2. It is apparent from the figure that more than 90% of spectra are overlapping each other, indicating the intricacy in measuring these drugs by direct UV absorption measurement in a binary mixture.

First to four-orders derivative spectra were obtained from the zero-order spectra using digital differentiation for both the drugs. Both the first-order derivative spectra present well-defined zones for determination of each analyte and the sensitivities are found to be high. Further, it was also observed that the increase in order of derivation decreased the sensitivity. Hence, the first derivative was selected for the current investigation. For first derivative spectroscopy, the spectra of the OFX and CEF obtained by scanning in water, were changed to first derivative spectra. For the first derivative spectra, different wavelengths (2, 4, 8 and 10 nm) were attempted to obtain the optimum Δλ. The results signified that 4 nm is an optimum Δλ and this wavelength was selected and used. One maxima absorbance (282.8 nm) and one minima wavelength (369.1 nm) were found for OFX where the CEF possess zero crossing [Figures 3 and 4]. In the laboratory mixture mean recovery and standard deviation were found to 100.95±0.48 and 99.81±2.68 at 282.8 nm and 369.1 nm, respectively. Further, one maxima (263.7 nm) and two minima wavelengths (294 nm and 318.6 nm) were found for CEF at zero crossing for OFX [Figures 3 and 5]. The synthetic mixture mean recovery and standard deviation were found to 101±2.63, 101.55±1.82 and 99.48 ±0.89 at 263.7 nm 294 nm and 318.6 nm respectively. The 282.8 nm peak for OFX and 318.6 nm peak for CEF were selected for determination of drug in synthetic mixture and pharmaceutical formulation due to low standard deviation and good mean recovery. Moreover, the method linear response obtained in the current investigation was in the concentration range of 2-20 µg/ml for both OFX and CEF with correlation coefficient more than 0.999.

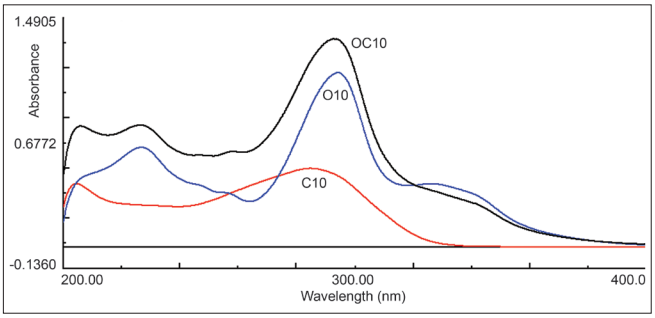

Figure 2: UV absorption spectra of ofloxacin (10 µg/ml , O10), cefixime (10 µg/ml , C10) and combination (10 µg/ml each, OC10)

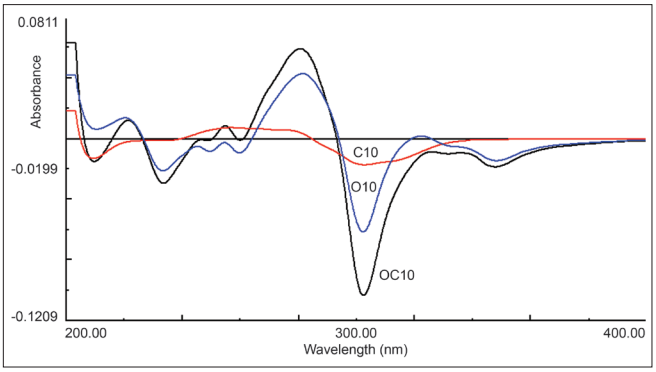

Figure 3: First derivative UV absorption spectra of ofloxacin (10 µg/ml , O10), cefixime (10 µg/ml , C10) and combination (10 µg/ml each OC10)

In the second method for determination of OFX, the UV spectra of OFX standards of increasing concentration in its binary mixture with CEF were divided by the spectrum of 16 µg/ml CEF solution, from the spectra obtained [Figure 6] and their first derivative was calculated. As seen in Figure 7, there exist one maxima at 337.2 nm and one minima at 353.6 nm. It was found that measured signals at these wavelengths are proportional to the concentrations of the drug. In the laboratory mixtures, mean recovery and standard deviation were found to 99.38±0.89, and 101.72±1.12 with peaks at 337.2 and 353.6 nm, respectively. The peak at 337.2 nm was selected for determination of drug in synthetic mixture and formulation due to its low standard deviation value and suitable mean recovery. Similarly, for determination of CEF, UV spectra of CEF standards of increasing concentrations in its binary mixture with OFX were divided by the spectrum of 16 µg/ml OFX. From the ratio spectra obtained [Figure 8], their first derivatives were calculated [Figure 9]. These spectra showed one maxima (259.8nm) and two minimas (271.8 nm and 317 nm). Further, the results also indicated that the measured signals at these wavelengths are proportional to the concentrations of the drug. In the synthetic mixtures, mean recovery and standard deviation were found to be 98.96±1.99, 101.51±1.75 and 99.56±0.93 with peaks 259.8 nm, 271.8 nm and 317 nm respectively. From the above observations, the wavelength of 317 nm was selected for determination of CEF in synthetic mixture and commercial tablets due to its low standard deviation value and suitable mean recovery.

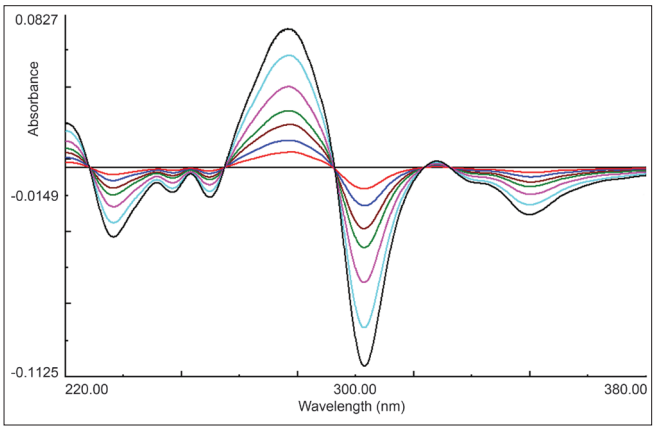

Figure 4: First derivative UV absorption spectra of ofloxacin (2-20 µg/ml)

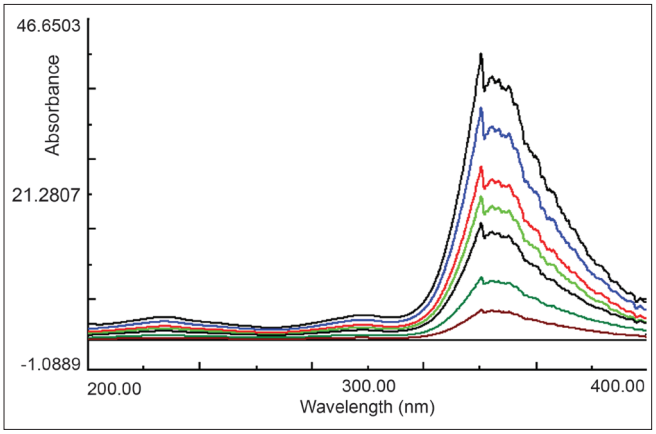

Figure 6: Ratio spectra of OFX (2-20 µg/ml) using 16 µg µg/ml solution of CEF as devisor

Moreover, the data observed in the current investigation also signify that for both drugs the divisor concentration of 16 µg/ml could be the optimum value. Similarly, the Δλ was found to be optimum for the first derivative of the ratio spectra is 4 nm. Calibration graphs were established for standards containing 2-20 µg/ml for OFX at 337.2 nm and for standards containing 2-20 µg/ml CEF at 317 nm. A critical evaluation of the proposed method was performed by the statistical analysis of the experimental data; regression curves were optioned by least square method. The obtained slopes, intercepts and correlation coefficients are summarized in Table 1. The proposed methods were validated as per ICH guideline and the LOD and LOQ for method-I and method II for both the drugs were calculated [Table 1] and tested practically.

Precision

Precision was determined by studying system and intermediate precision. System precision (% RSD) ranged from 0.71 to 1.28 for both the drugs in both methods

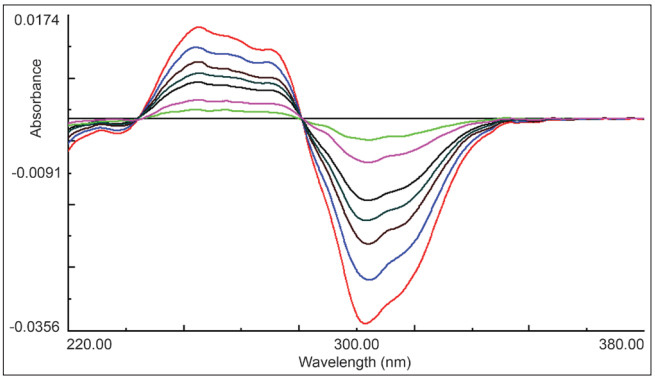

Figure 5: First derivative UV absorption spectra of cefixime (2 to 20 µg/ml)

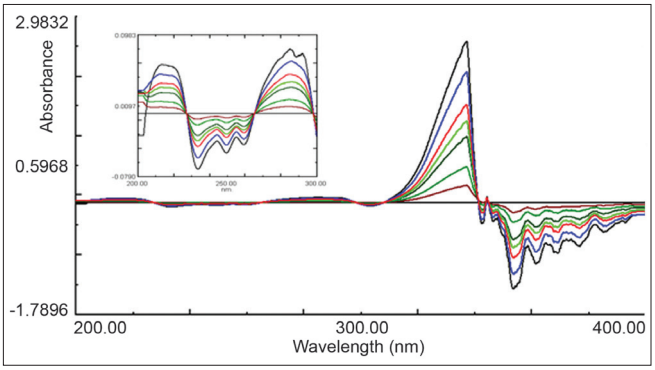

Figure 7: First derivative ratio spectra of OFX (2-20 µg/ml), inside enlarged portion of spectra of 200-300 nm

[Table 1]. The % of RSD for inter and intraday variations for both drugs in both methods is less than 1.76%. RSD values found were well with the acceptable range indicating that these methods have excellent repeatability and intermediate precision in the current experimental condition.

Accuracy

The data observed with the current methods showed excellent mean % recovery values, close to 100% and their low standard deviation (RSD 1.38) which represents high accuracy of the proposed analytical methods. The validity and reliability of the proposed methods was further assessed by determining the mean percentage recovery at 80, 100 and 120% level. The average % recovery ranges from 99.38 to 101.103 for OFX and CEF form both the methods and are presented in Table 2.

Estimation in formulation

The content of the OFX and CEF in the tablet was analyzed. Following the application of the methods % amount of OFX and CEF was found to be between 99.788 and 100.952 with standard deviation not more than 1.5% [Table 3]. The observed assay values were equivalent to the quantity claimed in the label indicating that the interference of excipient matrix is insignificant in the estimation of OFX and CEF by proposed analytical methods.

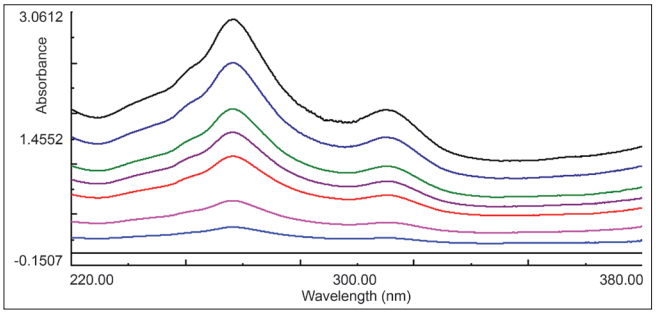

Figure 8: Ratio spectra of CEF (2 to 20 μg/ml) using 16 μg/ml solution of OFX as devisor

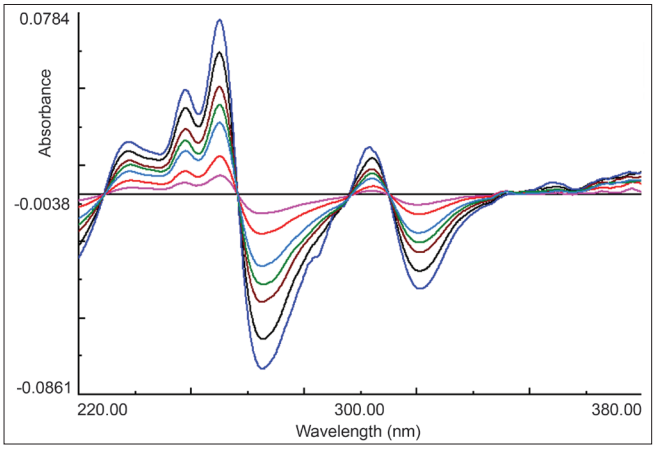

Figure 9: First derivative of the ratio spectra of CEF (2 to 20 μg/ml)

Conclusions

The objective of the current study was to develop a simple, economical, rapid, accurate, and precise and nature friendly method for the simultaneous estimation of OFX and CEF in dosage forms, bulk drugs and dissolution studies. Two UV spectroscopic indirect methods were assessed to estimate

Table 1: Validation parameters for first and ratio first derivative spectroscopic methods

Parameters assessed	Method I*		Method II**	
	OFX	CEF	OFX	CEF
Beer's law range (μg/ml)	2-20	2-20	2-20	2-20
Wavelength (nm)	282.8	318.6	337.2	317
Correlation coefficent(r2)	0.9997	0.9999	0.9996	0.9997
Slope	0.00034	0.00114	0.128	0.0024
Intercept	6.52x10-5	-4.07x10-5	0.023	2.68x10-4
LOD	0.25	0.38	0.21	0.11
LOQ	0.74	1.16	0.64	0.31
%RSD				
System precision	1.28	0.61	0.94	1.12
Intraday precision	0.34	0.65	0.69	0.48
Interday precision	1.76	1.58	1.43	1.28

* First derivative Spectrophotometric method; **First derivative of the ratio spectra; OFX – Ofloxacin; CEF – Cefixime trihydrate; %RSD – %Relative standard deviation

Table 2: Recovery studies

Amount of pure drug added (μg/ml)		% Recovery			
		Method I		Method II	
OFX	CEF	OFX	CEF	OFX	CEF
4	4	101.69	99.11	98.18	99.23
5	5	99.84	101.31	100. 81	101.86
6	6	101.78	101.48	99.15	101.29
Average		101.103	100.633	99.38	100.793
± RSD (%)		1.095	1.321	1.33	1.383

OFX – Ofloxacin; CEF – Cefixime trihydrate; %RSD – %Relative standard deviation

Table 3: Results of assay of formulation by first and ratio first derivative spectroscopic methods

Weight taken (mg each)	% Label claim			
	Method I*		Method II**	
	OFX	CEF	OFX	CEF
10.1	99.03	100.84	99.23	101.15
10.0	101.36	101.93	101.66	99.23
10.2	99.36	101.58	100.67	99.15
10.1	100.74	98.81	99.91	101.61
10.2	101.12	101.60	101.31	98.16
Avg.	100.43	100.952	100.55	99.788
± RSD (%)	1.208	1.262	0.996	1.506

* First derivative Spectrophotometric method; **First derivative of the ratio spectra; OFX – Ofloxacin; CEF – Cefixime trihydrate; %RSD – %Relative standard deviation

the drug content from the formulations and bulk drugs. The observed data indicate the feasibility of first and ratio first derivative spectrophotometry methods in assessing OFX and CEF simultaneously from binary mixture. The recovery of both the drugs from the formulations was in good agreement with the label claim, which suggested noninterference of formulations excipients in the estimation. Moreover, the present methods were rapid as compared to sophisticated chromatographical techniques. Hence it can be said the objective of the current study was achieved,

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