Simultaneous Estimation of Levofloxacin and Cefpodoxime Proxetil in Tablet Formulation by Ratio Spectra Derivative Spectroscopy

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Abstract: *A simple, sensitive, rapid, accurate and precise method for simultaneous determination of Levofloxacin and Cefpodoxime Proxetil in combined tablet dosage form has been developed. The method is based on ratio spectra derivative spectrophotometry. The amplitudes 250 nm and 267 nm in first derivative of the ratio spectra were selected to determine Levofloxacin and Cefpodoxime Proxetil respectively in combined formulation. The developed method was showing linearity in concentration range of 4-24 µg/ml for Levofloxacin and Cefpodoxime Proxetil with the correlation coefficient (R^2) 0.999 and 0.998, respectively. Results of analysis were validated statistically and by recovery studies. The percent relative standard deviation (% RSD) of inter-day and intra-day precision studies were found to be within limits of not more than 2 % indicating that the present method is precise as per ICH guidelines Q2 (R1). The developed method can be used for routine estimation of Levofloxacin and Cefpodoxime Proxetil in bulk and pharmaceutical dosage form.*

Key Words: *Levofloxacin, Cefpodoxime Proxetil, Ratio Derivative Spectroscopy*

1. INTRODUCTION:

1.1. Levofloxacin (LEVO) is having its IUPAC name as (3S)-9-fluoro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic acid. LEVO is a broad spectrum antibiotic of fluoroquinolone class of drug having greater activity towards gram positive bacteria and lesser activity toward gram-negative bacteria. It is also knows as respiratory quinolone. It inhibits bacterial type II topoisomerases, DNA gyrase and topoisomerase IV. [1]

1.2. Cefpodoxime proxetil (CP) is an oral third generation cephalosporin antibiotic used to treat a variety of bacterial infections. Its IUPAC name is 1-propan-2-yloxycarbonyloxyethyl (6R, 7R)-7-[[(2z)-2-(2-amino-1,3-thiazol-4-yl)-2-methoxyiminoacetyl]amino]-3-(methoxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-caboxyate.
Cefpodoxime proxetil is indicated for the treatment of mild to moderate infections caused by susceptible strains of the designated microorganisms. [2-4]

Literature survey reveals few UV spectrometric methods reported like absorption ratio method [5-7], simultaneous equation method [5] and absorption correction method [8]. An RP-HPLC method for determination of LEVO and CP with other drugs is also reported in literature.[9, 10] To the best of our knowledge no Ratio Spectra Derivative Spectroscopy method has been reported for estimation of Levofloxacin and Cefpodoxime Proxetil in combination. The present work describes a Ratio spectra derivative spectroscopy method in bulk and pharmaceutical dosage form (MACPOD LX) according to the International conference on harmonization (ICH) guidelines Q2 R (1).[11]. The structure of LEVO (a) and CP (b) is shown in figure 1 and its overlapped in spectra is shown in figure 2.

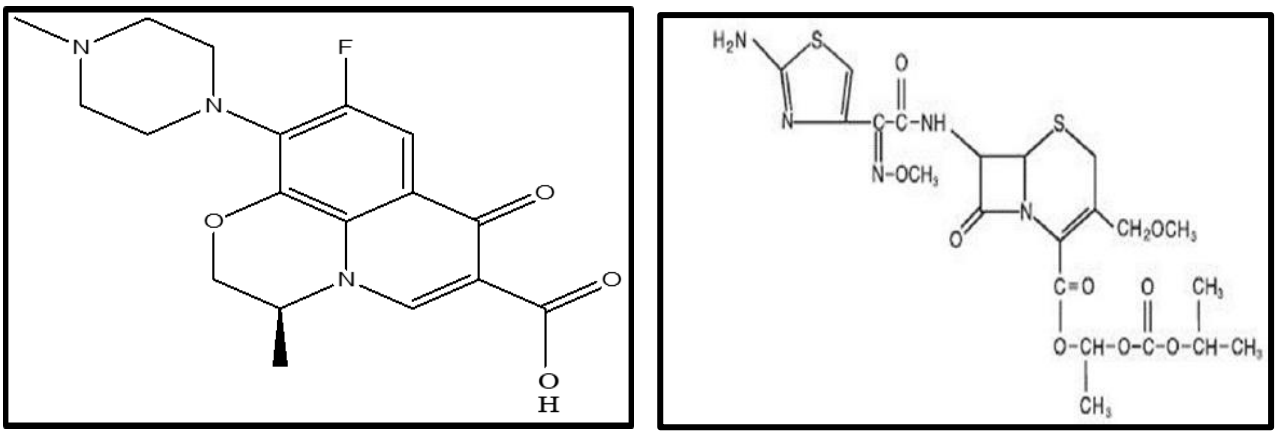


Figure 1. Structure of (a) Levofloxacin and (b) Cefpodoxime Proxetil

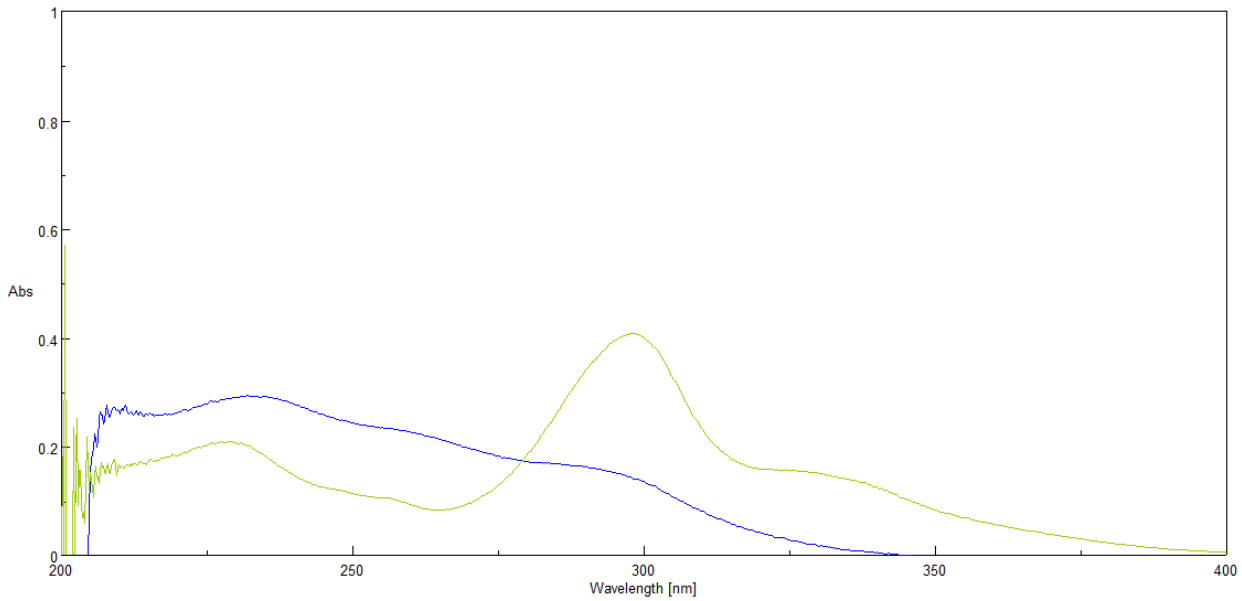


Figure 2.Overlayed spectra of LEVO and CP

2. MATERIALS AND METHODS:

2.1. EQUIPMENT:

The instrument used in the present study was JASCO double beam UV/Visible spectrophotometer (Model UV-730) with fixed slit width of 2 nm connected to a computer with spectra manager software. All weighing were done on electronic balance (Shimadzu AY 120).

2.2. CHEMICALS AND REAGENTS:

Pure drug sample of LEVO and CP were used without further purification. All chemicals used in spectrophotometric analysis were of analytical grade.

2.3. PHARMACEUTICAL FORMULATION:

Commercial tablets of MACPOD LX having its label claim as each film coated tablet contains Cefpodoxime Proxetil IP equivalent to anhydrous Cefpodoxime 200 mg and Levofloxacin hemihydrate IP equivalent to Levofloxacin 250mg were procured from the local market.

3. PROCEDURE:

3.1. PREPARATION OF STANDARD STOCK SOLUTION

Standard stock solution of LEVO was prepared by dissolving 100 mg of drug in 100 ml of methanol to get concentration of 1000 µg/ml. 2.5 ml of stock solution was further diluted to 25 ml with methanol to get a working standard solution of concentration 100 µg/ml. In similar manner standard stock solution of CP was prepared to get a working solution of concentration 100 µg/ml.

3.2. Preparation of working stock solution

Working stock solutions were prepared from standard stock solution of 100 µg/ml by appropriate dilution with methanol to obtain final concentration of 4, 8, 12, 16, 20 and 24µg/ml for LEVO and CP.

4. EXPERIMENTAL WORK:

The method involves dividing the spectrum of mixture into the standardized spectra for each of the analyte and deriving the ratio to obtain spectra that is independent of analyte concentration used as a divisor. Using appropriate dilutions of standard stock solution the two solutions were scanned separately. The ratio spectrums of different LEVO standards at increasing concentrations are obtained by dividing each with the stored spectrum of the standard solution of CP (12 µg/ml, Scaling factor 4) by computer aid are shown in figure 3 (I) and the first derivative of these spectra traced with the interval of $\Delta\lambda$=21nm (the influence of the first derivative of the ratio spectra was tested to obtain the optimum wavelength interval, $\Delta\lambda$ =21 nm was considered to be suitable) are illustrated in figure 3 (II). Wavelength 250 nm was selected for the quantification of LEVO in LEVO+CP mixture. The ratio and ratio derivative spectra of the solutions of CP at different concentrations traced with the interval of $\Delta\lambda$ =21nm by using the standard spectrum of LEVO (8 µg/ml, Scaling factor 4) as divisor by computer aid are demonstrated in figure 4 (I) and (II), respectively.

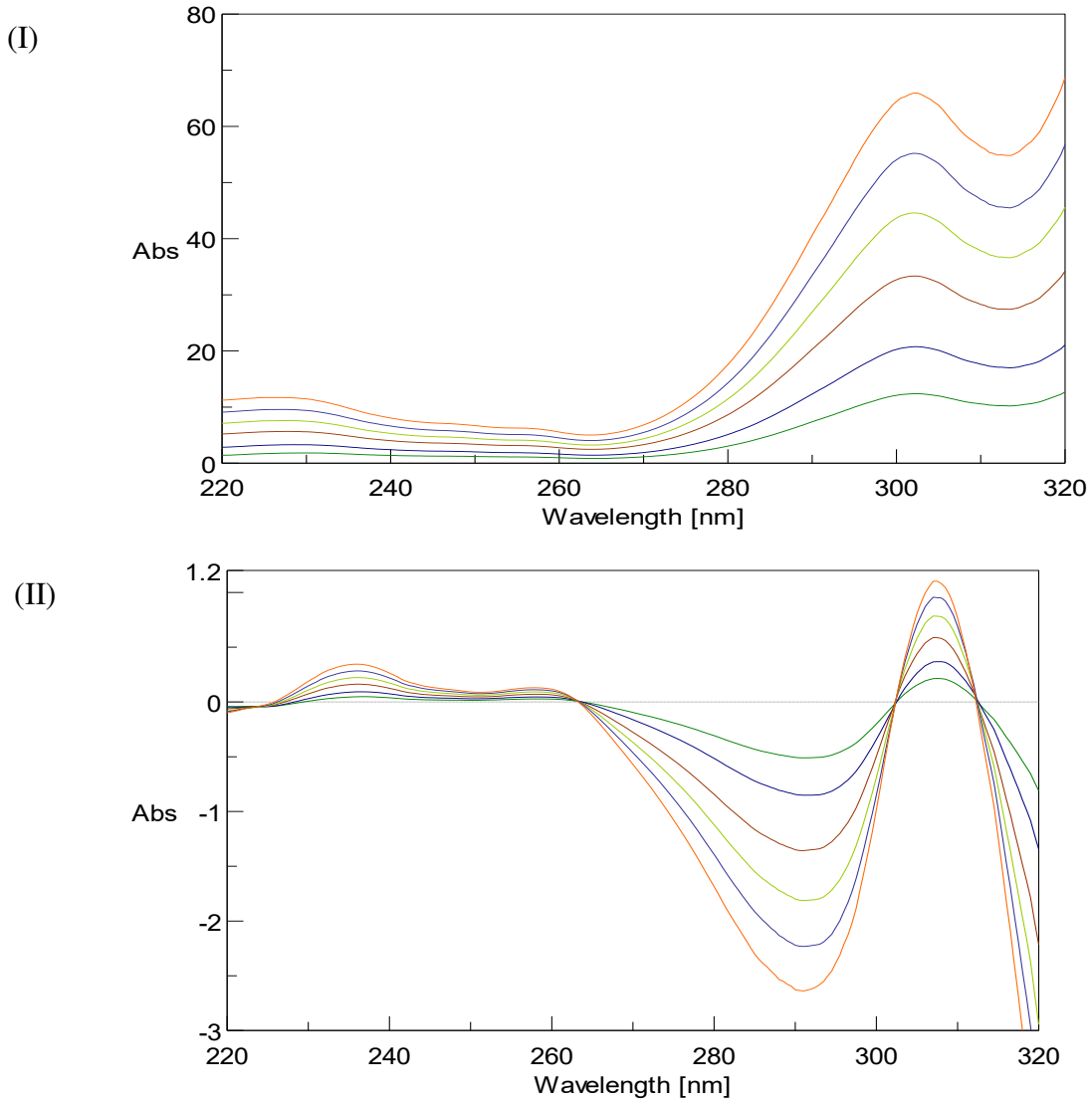
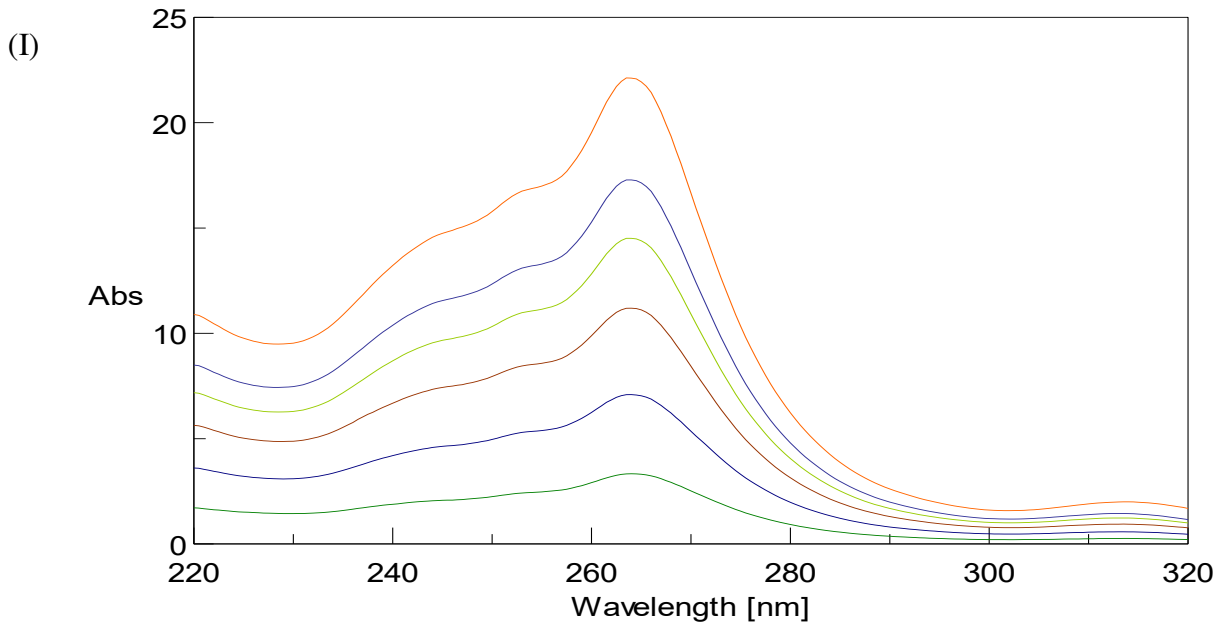


Figure 3.Ratio spectra (I) and first derivative of the ratio spectra (II) of (a) 4 µg/ml, (b) 8 µg/ml,(c) 12 µg/ml, (d) 16 µg/ml, (e) 20 µg/ml, (f) 24 µg/ml solution of LEVO when 12 µg/ml solution of CP is used as divisor (Δλ=21 nm)



(II)

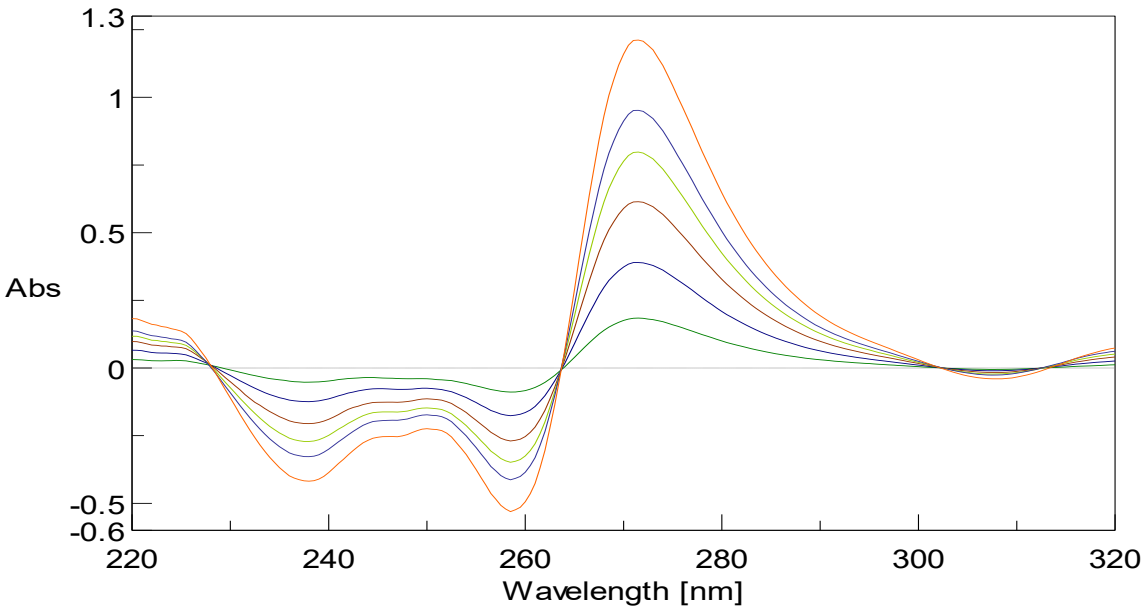


Figure 4. Ratio spectra (I) and first derivative of the ratio spectra (II) of (a) 4 µg/ml, (b) 8 µg/ml,(c) 12 µg/ml, (d) 16 µg/ml, (e) 20 µg/ml, (f) 24 µg/ml solution of CP when 8 µg/ml solution of LEVO is used as divisor (Δλ=21 nm)

Wavelength 267 nm was selected for the quantification of CP in LEVO+ CP mixture. Measured analytical signals at these wavelengths are proportional to the concentrations of the drugs. The coincident first derivative ratio spectra of pure and sample solution for estimation of LEVO and CP are shown in the figure 5.

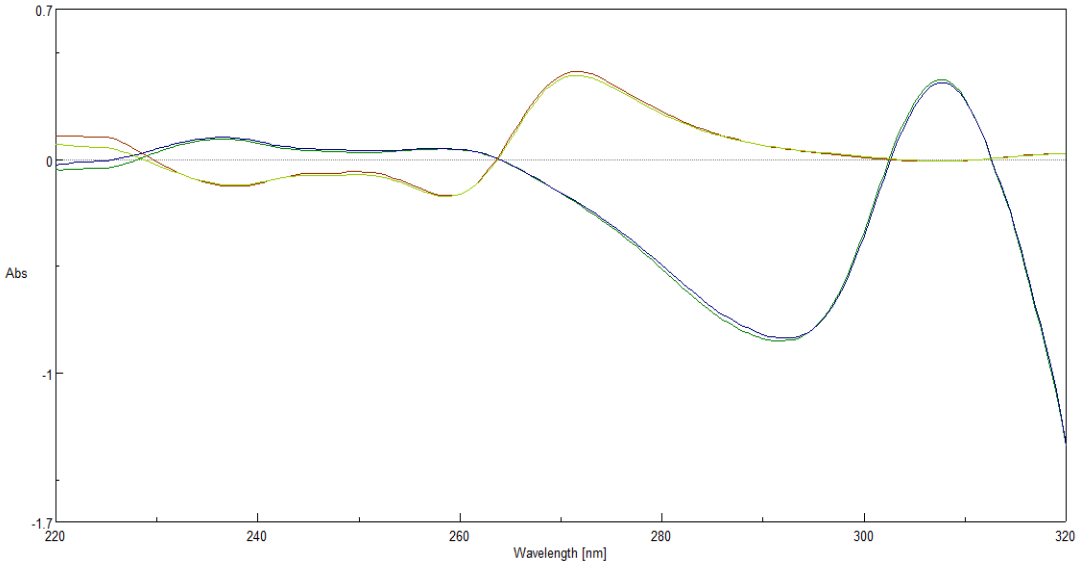


Figure 5. Coincident first derivative ratio spectra of (a_1) 8 µg/ml of pure LEVO and (a_2) sample solution (12 µg/ml of CP as divisor); (b_1) 8 µg/ml of pure CP and (b_2) sample solution (8 µg/ml of LEVO as divisor)

5. RESULTS AND DISCUSSION

Under experimental conditions described, calibration curve, assay of tablets, recovery studies and precision were performed. A critical evaluation of proposed method was performed by statistical analysis of data where slopes, intercept, correlation coefficient are shown in Table 1. The linearity range selected for LEVO and CP was 4-24 µg/ml with the correlation coefficient (R^2) as 0.999 and 0.998 respectively.

Table 1. Optical characteristics of the proposed method

Parameters	LEVO	CP
λ_{max}	250nm	267 nm
Beer's law limit (µg/ml)	4-24 µg/ml	4-24 µg/ml
Molar Absorbtivity	3.83×10^{-3}	2.94×10^{-2}
Regression Equation (y = mx + c)	y = 0.004x - 0.0015	y = 0.0303x - 0.0078
Slope (m)	0.004	0.0303
Intercept (c)	-0.0015	-0.0078
Correlation Coefficient	0.9991	0.9985

5.1. ASSAY AND ACCURACY:

Results of analysis of commercial formulation are reported in Table 2. Low standard deviation values of determination indicate reproducibility of the method. Recovery studies were carried out by the addition of standard drug solution to preanalyzed tablet sample solution at three different concentration levels within the range of linearity for both the drugs. Results of recovery studies are shown in Table 3.

Table 2. Results of Analysis of Commercial Formulation

Drug	Label Claim (mg / tablet)	% of Label claim estimated	Standard Deviation	% RSD
LEVO	250	100.55	1.565	1.557
CP	200	100.34	1.649	1.643

Table 3. Recovery studies of LEVO and CP

Level of % Recovery	% Mean Recovery		Standard Deviation		% RSD	
	LEVO	CP	LEVO	CP	LEVO	CP
50	99.04	99.78	0.816	1.286	0.824	1.289
100	99.32	100.88	0.778	0.451	0.783	0.447
150	98.50	101.50	0.709	0.493	0.719	0.486

5.2. PRECISION:

The precision of the method was demonstrated by intraday (within a day) and interday (within days) variation studies. In the Intraday studies, 3 replicates of 3 different concentrations (12, 16, 20) were analyzed in a day and percentage RSD was calculated. For the interday variation studies, 3 different concentrations (12, 16, 20) were analyzed on 3 consecutive days and percentage RSD was calculated. The results obtained for intraday and interday variations are shown in Table 4.

Table 4. Precision of LEVO and CP

UV Method	Conc	Intraday Precision				Interday Precision			
		LEVO		CP		LEVO		CP	
		% Recovery	% RSD	% Recovery	% RSD	% Recovery	% RSD	% Recovery	% RSD
Ratio Derivative spectrometry	12	100.79	0.919	99.23	0.951	100.65	0.801	100.51	1.28
	16	101.12	1.043	100.87	0.893	101.07	0.992	99.85	1.18
	20	100.38	1.013	98.30	0.268	100.47	1.086	98.35	0.18

6. CONCLUSION:

The developed method was found to be simple, sensitive, accurate, precise and repeatable and can be used for routine analysis of LEVO and CP in bulk and pharmaceutical dosage form without any interference from the excipients. The method was validated as per ICH guidelines. Statistical analysis proved that the method is repeatable for analysis of LEVO and CP.

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