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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF CEFPODOXIME PROXETIL AND LEVOFLOXACIN HEMIHYDRATE IN COMBINED DOSAGE FORM

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Keywords:

RP-HPLC, Simultaneous estimation, System suitability, Cefpodoxime Proxetil, Levofloxacin hemihydrate

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ABSTRACT: A simple, accurate, precise, rapid and economical RP-HPLC method was developed for the estimation of Cefpodoxime Proxetil (CPD) and Levofloxacin hemihydrate (LVX) in tablet dosage form. In Agilent – 1100/1200 Series, using C18 (250 x 4.6 mm x 5µ) column and with mobile phase composition of Phosphate buffer: Acetonitrile (pH 6.0) (50:50 v/v) at a flow rate of 1 ml/min was used. Detection was carried out at 240 nm. Retention time of CPD and LVX was found to be 5.64 & 5.97 min and 2.79 min, respectively. The method has been validated for linearity, accuracy and precision, LOD, LOQ and System suitability according to ICH Q2R1 Guideline.

INTRODUCTION: Cefpodoxime is an oral third generation cephalosporin antibiotic. It is active against most Gram positive and Gram negative bacteria. It is commonly used to treat acute otitis media, pharyngitis, and sinusitis. Chemically it is 1-(isopropoxycarbonyloxy) ethyl (6R, 7R)-7-[2-(2-amino-4 thiazolyl) - (Z) – 2 - (methoxyimino) acetamido] – 3 - methoxymethyl – 3 – cephem - 4-carboxylate. Levofloxacin is an antibiotic, anti bacterial agent and anti infective agent. Chemically it is (2S)-7-fluoro-2-methyl-6-(4-methylpiperazin-1-yl)-10-oxo-4-oxa-1-azatricyclo [7.3.1.0^ {5,13}]trideca-5(13),6,8,11-tetraene-11-carboxylic acid. The tablet Glevopod contains the combination of Cefpodoxime Proxetil (200mg) and Levofloxacin Hemihydrate (250mg). Glenmark pharmaceuticals Ltd. had manufactured the tablet.

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A literature survey revealed that only a few methods based on HPLC [3, 4, 5], Spectrometry and HPTLC [6, 7, 8, 9] were reported for the determination of Cefpodoxime and Levofloxacin individually but no single method is reported for the simultaneous estimation Cefpodoxime and Levofloxacin in pharmaceutical dosage form. Hence in the present study a physical mixture of Cefpodoxime and Levofloxacin was being taken for simultaneous estimation by HPLC method. This present investigation describes a rapid, accurate and precise UV method of Cefpodoxime and Levofloxacin in combination using Phosphate buffer:Acetonitrile (pH 6.0) (50:50 v/v) as a mobile phase.

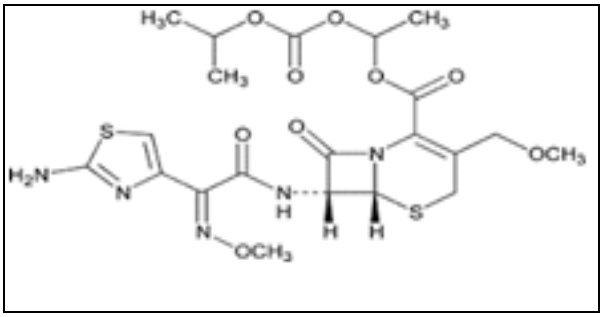


FIG.1: STRUCTRE OF CEFPODOXIME PROXETILE

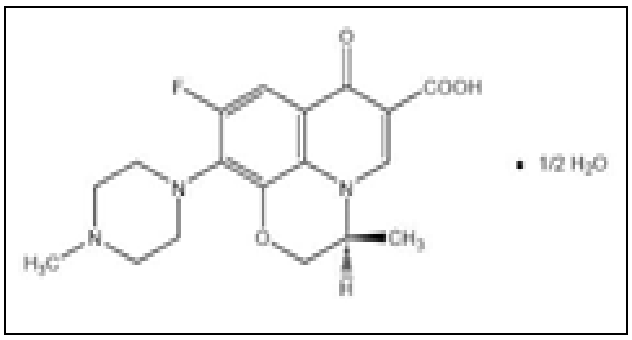


FIG. 2: STRUCTURE OF LEVOFLOXACIN HEMIHYDRATE

MATERIALS AND METHODS:

Apparatus and Instruments:

- Model: Agilent – 1100/1200 Series.
- Column: C18 (250 x 4.6 mm, 5 µm)
- Injector: An auto injector
- Detector: PDA Detector
- Software: Chem - 32 Software
- Analytical balance: Electronic analytical balance (Mettler Toledo)
- Volumetric flasks and pipettes (Borosil)

Reagents and Chemicals:

- Cefpodoxime Proxetil (Gift sample, Sunrise Remedies, Ahemdabad)
- Levofloxacin Hemihydrate (Gift sample, Sunrise Remedies, Ahemdabad)
- Distilled water (HPLC grade-SD Fine chemical Pvt. Ltd)
- Acetonitrile (HPLC grade-SD Fine chemical pvt. Ltd)
- Methanol (HPLC grade-SD Fine chemical pvt. Ltd)
- Potassium dihydrogen ortho phosphate (SD Fine chemical pvt. Ltd)
- Ortho Phosphoric acid (SD Fine chemical pvt. Ltd)
- Potassium hydroxide (SD Fine chemical pvt. Ltd)

Preparation of Standard Solution: All chemicals and reagents used were of AR/HPLC grade. Pure standards of CPD and LVX were obtained from Sunrise remedies, Ahmedabad.

Preparation of buffer: A 6.8 gm of potassium dihydrogen orthophosphate was dissolved in 900 ml of Distilled water. Then the pH was adjusted to 6.0 with KOH. Then the volume was made up to 1000 ml and was filtered through 0.45µm membrane filter and degassed.

Preparation of mobile phase: A degassed mixture of Phosphate buffer and Acetonitrile in the ratio of 50:50 (v/v) was prepared and the mixture was filtered through 0.45 µ membrane filters and it was degassed.

Preparation of standard stock solution Cefpodoxime Proxetil (CPD) (1000 µg/ml): Accurately weighed CPD 100 mg was transferred into 100 ml volumetric flask and dissolved in acetonitrile and diluted up to the mark with acetonitrile to give a stock solution having strength 1000 µg/ml.

Preparation of standard stock solution Levofloxacin Hemihydrate (LVX) (1250 µg/ml): Accurately weighed LVX 125 mg was transferred into 100 ml volumetric flask and dissolved in acetonitrile and diluted up to the mark with acetonitrile to give a stock solution having strength 1250 µg/ml.

Preparation of standard solution of binary mixtures of CPD and LVX: Accurately weighed 4 mg of CPD and 5 mg of LVX were transferred to 50 ml volumetric flask and diluted up to mark with diluent to give concentration of 80 µg/ml of CPD and 100 µg/ml of LVX.

Calibration Curve of Cefpodoxime Proxetil and Levofloxacin Hemihydrate: Calibration curves were prepared by taking appropriate aliquots 0.2, 0.4, 0.6, 0.8, 1 and 1.2 ml of stock solution of CPD and 0.2, 0.4, 0.6, 0.8, 1 and 1.2 ml stock solution of LVX in 10 ml vol. flask and dilute up to the mark with acetonitrile to give 20-120µg/ ml of CPD and 25-150 µg/ ml of LVX.

The standard solution was run for 10 minutes using mobile phase at a flow rate of 1 ml/min. The graph of peak area vs concentration was plotted, regression equation and correlation co- efficient for both drugs were obtained.

Chromatographic separation: Standard solutions of 20-120 µg/ml of CPD and 25-150 µg/ml LVX were injected in column with 20 µl micro-syringe. The chromatogram was run for appropriate minutes with mobile phase Phosphate buffer: Acetonitrile

(50:50v/v). The detection was carried out at wavelength 240 nm. The chromatogram was stopped after separation achieved completely. Data related to peak like area, height, retention time, resolution etc.

Validation of RP-HPLC Method:

(A) Linearity: Aliquots of standard solutions of CPD and LVX in range 20-120 µg/ml and 25-150 µg/ml respectively, was prepared from working standard solution and injected to system with stated chromatographic conditions and analyzed. The graph of peak area obtained versus respective concentration was plotted. The mean area with its standard deviation and % relative standard deviation of peak were calculated.

(B) Precision:

I. Repeatability: Three different standard solutions of CPD (40, 80 and 120 µg/ml) were prepared from working standard solution and injected three times to system with stated chromatographic conditions and analyzed. Three different standard solutions of LVX (50, 100 and 150 µg/ml) were prepared from working standard solution and injected three times to system with stated chromatographic conditions and analyzed.

II. Intraday precision: Standard solutions CPD (40, 80, and 120 µg/ml) and LVX (50, 100, and 150 µg/ml) were prepared from working standard solution and injected in to system with stated chromatographic conditions and analyzed, three times in a day.

III. Interday precision:

Standard solutions CPD (40, 80, and 120 µg/ml) and LVX (50, 100, and 150 µg/ml) were prepared from working standard solution and injected in to system with stated chromatographic conditions and analyzed, three days.

(C) Accuracy:

Procedure:

Preparation of Sample Solution:

Tablet Solution X: CPD (1000 µg/ml) + LVX (1250 µg/ml) (final dilution contain CPD 1000 µg/ml and LVX 1250 µg/ml)

Standard solution Y: CPD (1000 µg/ml) &

Standard solution Z: LVX (1250 µg/ml)

The amount of CPD and LVX was calculated at each level (80%, 100% & 120%) and % recoveries were computed.

(D) Robustness: It should show the reliability of an analysis with respect to deliberate variations in method parameters.

In case of liquid chromatography, examples of typical variations are

- Influence of variations of pH(±0.2) in a mobile phase
- Influence of variations in mobile phase(±2) composition,
- Flow rate. (±0.2)

(E) Specificity: Specificity is a procedure to detect quantitatively the analyte in the presence of components that may be expected to be present in the sample matrix. While selectivity is the procedure to detect qualitatively the analyte in presence of components that may expected to be present in the sample matrix. Specificity of developed method was established by spiking of CPD and LVX in hypothetical placebo (i.e. might be expected to be present) and expressing that analytes peak were not interfered from excipients.

(F) LOD and LOQ: The LOD (Limit of Detection) was estimated from the set of 6 calibration curves used to determine method linearity. The LOD may be calculated as

$$\mathbf{LOD = 3.3 \times (S.D./Slope)}$$

Where,
SD = Standard deviation of the response
Slope = Mean slope of calibration curves

The LOQ (Limit of Quantitation) was estimated from the set of 6 calibration curves used to determine method linearity. The LOQ may be calculated as

$$\mathbf{LOQ = 10 \times (S.D./Slope)}$$

Where,
SD = Standard deviation of the response
Slope = Mean slope of calibration curves

Analysis of Tablet Formulation: This method was applied to determine CPD and LVX in tablet dosage form. Total 20 tablets were accurately weighted and triturated with glass mortar and

pestle. The powder equivalent to 8 mg of CPD and 10 mg of LVX was taken in 100 ml volumetric flask; acetonitrile was added and the flask was kept in an ultrasonic bath for 10 min. The volume was made up to mark and the solution was filtered through 0.45 micro membrane filter. The diluted solution was analyzed under optimized chromatographic conditions. The areas of resulting peak were measured at 240 nm.

RESULT AND DISCUSSION:

Selection of wavelength: Both Cefpodoxime Proxetil and Levofloxacin Hemihydrate show reasonably good response at 230 nm. Mention in **Fig.**

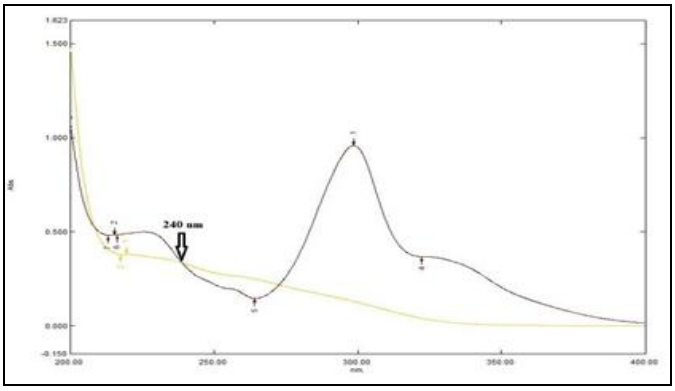


FIG.3: OVERLAY UV SPECTRUM OF CEFPODOXIME PROXETIL AND LEVOFLOXACIN HEMIHYDRATE SHOWING SELECTION OF WAVELENGTH DETECTION

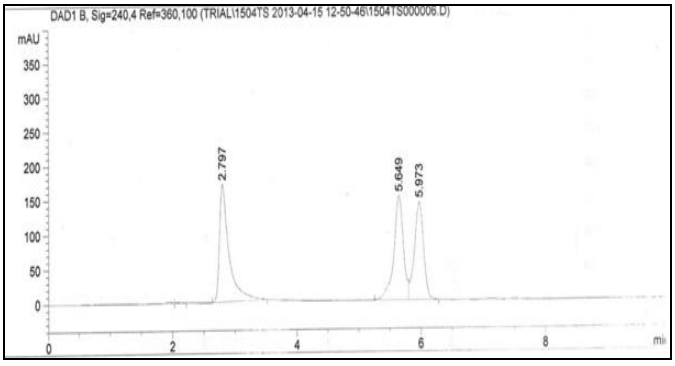


FIG. 4: CHROMATOGRAM OF CEFPODOXIME PROXETIL AND LEVOFLOXACIN

Hemihydrate in Phosphate buffer:Acetonitrile (50:50v/v) (Flow rate-1 ml/min):

TABLE 1: OBSERVED VALUES FOR SYSTEM SUITABILITY TEST

Parameters	Data obtained	
	CPD	LVX
Theoretical plates per column	8674	2422
Symmetry factor/Tailing factor	1.16	0.42
Resolution	12.02	

Method Validation:

(A)Linearity and Range:

The linearity range for CPD and LVX was found to be in the range of 20-120 µg/ml and 25-150 µg/ml respectively. Correlation co-efficient for calibration curve of CPD and LVX was found to be 0.999 and 0.998 respectively.

The regression line equation for CPD and LVX are as following:

For CPD: $y = (29.98) \times (x) + 34.92$

For LVX: $y = (15.87) \times (x) – 119.7$

Where,

y = Corresponding peak area

x = Concentration of drug in µg/ml

TABLE: 2 LINEARITY DATA FOR CPD

Sr.no	Concentration (µg/ml)	Area Mean±S.D. (n=3)	%RSD
1.	20	611.36 ± 5.1903	0.8489
2.	40	1239.09 ± 3.8617	0.3116
3.	60	1871.69 ± 3.5996	0.1923
4.	80	2420.63 ± 3.9861	0.1646
5.	100	3044.29 ± 4.6287	0.1521
6.	120	3616.42 ± 4.2351	0.1171

TABLE: 3 LINEARITY DATA FOR LVX

Sr.no	Concentration (µg/ml)	Area Mean±S.D. (n=3)	%RSD
1.	25	288.8 ± 4.2571	1.4738
2.	50	690.91 ± 3.8136	0.5519
3.	75	1045.53 ± 1.7303	0.1654
4.	100	1461.75 ± 3.4045	0.2329
5.	125	1825.44 ± 3.0759	0.1685
6.	150	2302.82 ± 3.6074	0.1566

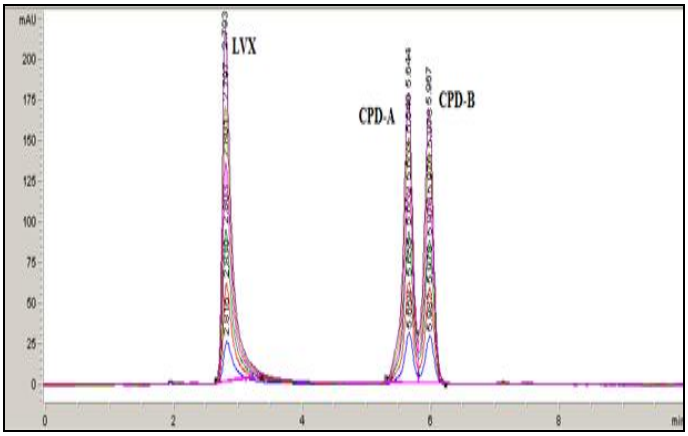
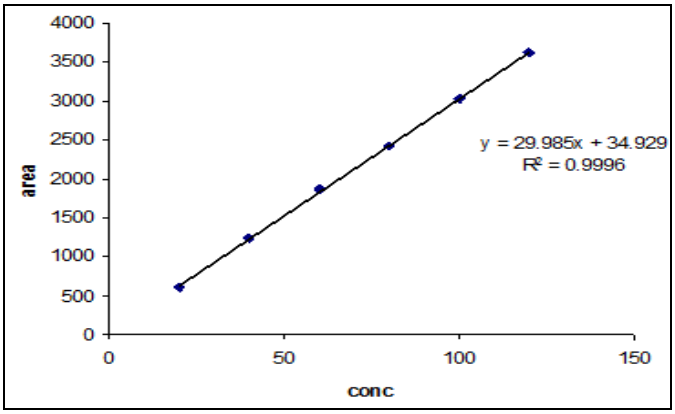
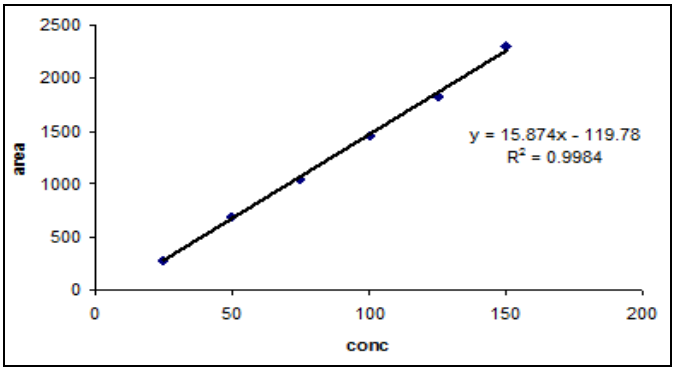


FIG. 5: OVERLAY CHROMATOGRAM OF DIFFERENT CONCENTRATIONS OF BINARY MIXTURE OF CPD AND LVX



CALIBRATION CURVE FOR CPD



CALIBRATION CURVE FOR LVX

(B) Precision:

I. Repeatability: The data for repeatability of peak area measurement for CPD and LVX, based on three measurements of three different solution of CPD and LVX, are depicted in **Table 4** and **5** respectively.

TABLE: 4 REPEATABILITY DATA FOR CPD

Sr. no.	Concentration (µg/ml)	Area Mean (Mean ± S.D)	% RSD (n=6)
1	40	1240.68 ± 11.54	0.9304
2	80	2425.97 ± 24.52	1.0111
3	120	3613.65 ± 11.78	0.3261

TABLE: 5 REPEATABILITY DATA FOR LVX

Sr. no.	Concentration (µg/ml)	Area Mean (Mean ± S.D)	% RSD (n=6)
1	50	693.24 ± 7.65	1.1037
2	100	1455.68 ± 18.22	1.2521
3	150	2300.09 ± 13.11	0.5696

II. Intraday precision:

The data for intraday precision for CPD and LVX is shown in **Table 5.**

TABLE 5: INTRADAY PRECISION DATA FOR ESTIMATION OF CPD AND LVX

CPD			LVX		
Conc. (µg/ml)	Area Mean±S.D. (n=3)	% RSD	Conc. (µg/ml)	Area Mean±S.D. (n=3)	% RSD
40	1242.25 ± 9.1386	0.7356	50	693.24 ± 5.2542	0.7579
80	2420.37 ± 11.2545	0.4649	100	1461.52 ± 12.6153	0.8631
120	3615.93 ± 12.505	0.3458	150	2302.63 ± 15.9831	0.6941

III. Interday Precision: The data for Interday precision for CPD and LVX is shown in **Table 6.**

TABLE 6: INTERDAY PRECISION DATA FOR ESTIMATION OF CPD AND LVX

CPD			LVX		
Conc. (µg/ml)	Area Mean±S.D. (n=3)	% RSD	Conc. (µg/ml)	Area Mean±S.D. (n=3)	% RSD
40	1236.47 ± 15.9559	1.2904	50	691.67 ± 10.1900	1.4732
80	2419.20 ± 16.6104	0.6866	100	1461.64 ± 13.8049	0.9444
120	3616.37 ± 15.1707	0.4195	150	2301.26 ± 17.3607	0.7544

(C) Accuracy: The data for Accuracy for CPD and LVX is shown in **Table 7** and **8**.

TABLE 7: RECOVERY DATA FOR CPD

Conc. level	Amount Of Sample Drug Taken (µg/ml)	Amount Of Standard Drug Added (µg/ml)	Area	Amount recovered (µg/ml)	% Recovery	%Mean Recovery ± SD	%RSD
80	80	64	465973.9	64.59	100.93	100.29±0.7497	0.7475
	80	64	466810.3	64.30	100.48		
	80	64	464961.5	63.66	99.46		
100	80	80	513190.1	80.15	100.19	99.82±0.3361	0.3367
	80	80	513290.1	79.63	99.53		

	80	80	511964.1	79.78	99.73		
120	80	96	561471.4	96.42	100.44	100.09±0.3413	0.3409
	80	96	558792.3	95.77	99.76		
	80	96	557263.9	96.08	100.08		

TABLE 8: RECOVERY DATA FOR LVX

Conc. level	Amount Of Sample Drug Taken (µg/ml)	Amount of Standard Drug Added (µg/ml)	Area	Amount recovered (µg/ml)	% Recovery	%Mean Recovery ± SD	%RSD
80	100	80	465973.9	79.45	99.09	99.89±1.5500	1.5516
	100	80	466810.3	81.32	99.54		
	100	80	464961.5	78.97	98.55		
100	100	100	513190.1	99.59	99.49	99.91±1.4868	1.4881
	100	100	513290.1	101.53	99.53		
	100	100	511964.1	98.61	98.97		
120	100	120	561471.4	120.04	100.13	99.92±0.8421	0.8428
	100	120	558792.3	120.84	99.17		
	100	120	557263.9	118.83	98.63		

D) Robustness: The data for Robustness for CPD and LVX is shown in **Table 9** and **10**.

TABLE 9: ROBUSTNESS DATA FOR CPD

Sr. no.	Flow rate +0.2	Flow rate -0.2	M.P + 2	M.P - 2	pH + 0.2	pH - 0.2
1	2330.11	2501.71	2408.46	2443.82	2412.43	2453.11
2	2313.19	2584.99	2396.31	2397.09	2378.38	2389.61
3	2390.06	2585.11	2448.08	2464.72	2436.17	2426.86
%RSD	1.7228	1.8815	1.1197	1.4219	1.2058	1.3167

TABLE 10: ROBUSTNESS DATA FOR LVX

Sr. no.	Flow rate +0.2	Flow rate -0.2	M.P + 2	M.P - 2	pH + 0.2	pH - 0.2
1	1298.06	1550.22	1469.11	1414.05	1411.55	1429.41
2	1286.52	1538.79	1483.43	1383.86	1396.35	1391.46
3	1315.91	1507.54	1451.94	1432.14	1444.37	1413.16
%RSD	1.1388	1.4419	1.0738	1.7298	1.7315	1.3491

(E) Limit of Detection (LOD) & Limit of Quantification (LOQ):

TABLE 11: LOD AND LOQ DATA FOR CPD AND LVX

Parameters	CPD	LVX
LOD	1.2305	1.9874
LOQ	3.7289	6.0226

(F) Applicability of the method:

Analysis of marketed formulation: Applicability of the proposed method was tested by analyzing the commercially available tablet formulation GLEVO-POD. The results are shown in **Table.**

TABLE 12: ANALYSIS OF MARKETED FORMULATION

Tablet	mg/tablet		Assay (% of label claim*) Mean ± S. D.	
	CPD	LVX	%CPD	%LVX
GLEVO-POD	200	250	99.85 ± 0.4571	100.03 ± 1.2871

*Average of six estimations

The assay results were comparable to labeled value of each drug in tablet dosage form. These results indicate that the developed method is accurate, precise, simple and rapid. It can be used in the routine quality control of dosage form in industries.

CONCLUSION:

- The proposed method is simple, sensitive and reproducible and hence can be used in routine for determination of Cefpodoxime proxetil and Levofloxacin Hemihydrate in pharmaceutical preparations.

- The values of percentage recovery and standard deviation show that the proposed methods were reproducible, accurate and precise.

- The developed methods can be used for routine quantitative estimation of Cefpodoxime proxetil and Levofloxacin Hemihydrate in their combined pharmaceutical preparation.

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