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Simultaneous Determination of Cefepime, Cefotaxime and Ceftriaxone in Pharmaceutical formulation by Ultra-Fast Liquid Chromatography with PDA Detection

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Abstract: *A simple, rapid and accurate Ultra-fast liquid chromatography (UFLC) method was developed for simultaneous determination for cefepime, cefotaxime and ceftriaxone in pharmaceutical formulation. The chromatographic separation was conducted on UFLC Shimadzu connected with PDA detector; using column Phenomenex, Prodigy, ODS3, 5 µm, (250 x 4.6 mm) with a mobile phase was isocratic consisted of Acetonitrile and 0.025M KH2PO4 pH 3.0 in the ratio of 15:85 (by volume) and was delivered to the system at a flow rate of 1.5 ml/min. An injection volume of 20 µl was used for cefepime, cefotaxime and Ceftriaxone. The detection wavelength (λ_{max}) was 260 nm for cefepime, cefotaxime and Ceftriaxone. The calibration curve of cefepime in mobile phase was linear with correlation coefficient (r2) = 0.9998; over a concentration range of 25.0 – 100.0 mg/L for; with a retention time of 2.37 minutes, the calibration curve of cefotaxime in mobile phase was linear with correlation coefficient (r2) = 0.9993; over a concentration range of 50.0 – 200.0 mg/L for; with a retention time of 5.90 minutes. While the calibration curve of Ceftriaxone in mobile phase was linear with correlation coefficient (r2) = 0.9974; over a concentration range of 100.0 – 400.0 mg/L for; with a retention time of 4.15 minutes. The relative standard deviation (RSD) was found to be < 2. The validated HPLC method was successfully applied to the analysis for cefepime, cefotaxime and Ceftriaxone in pharmaceutical dosage form.*

Keywords: *UFLC, Cefepime, Cefotaxime, Ceftriaxone, PDA detector; Method Validation.*

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I. Introduction

Cefepime is fourth-generation, semisynthetic, broad spectrum, cephalosporin antibiotic for parenteral administration. Chemically, it is 7-[α-(2-aminothiazol-4-yl)-α-(z)methoxyimino acetamido]-3-(1-methylpyrrolidino)-methyl-3-cephem-4 carboxylate, it is characterized by the presence of a positively charged quaternerized N-methyl-pyrrolidine substitution at the 3 position of the cephem moiety, making cefepime a zwitter ion as shown in Fig. 1. It is official in The United States Pharmacopeia and the British Pharmacopeia. It is used clinically for the treatment of lower respiratory tract, intra-abdominal, urinary tract, skin and soft tissue infections and also used for prophylaxis in biliary tract and prostate surgery. Cefepime/TAZ is one of the dosage form combination already licensed and used in Indian hospitals [1, 2].

A literature survey revealed that several liquid chromatography methods had been reported for the determination of cefepime alone [3–6].

Chemically, cefotaxime (Fig. 2) is (6R,7R)-3[(acetyloxy) methyl]-7-[[(2Z)-(2-amino-4-thiazolyl)(methoxyamino)-acetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2- carboxylic acid. Chemically, ceftriaxone (Fig. 3) is (6R,7R)- 7-[[(2Z)-(2-amino-4-thiazolyl)(methoxyamino)-acetyl]amino]- 8-oxo-3-[[1,2,5,6-tetrahydro-2-methyl-5,6-dioxo-1,2,4-triazin-3-yl)-thio]methyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2- carboxylic acid. Cefotaxime and ceftriaxone are third generation broad spectrum cephalosporins for parenteral administration and are bactericidal and mainly used in the treatment of various bacterial infections caused by gram positive and Gram-negative micro-organisms [7,8].

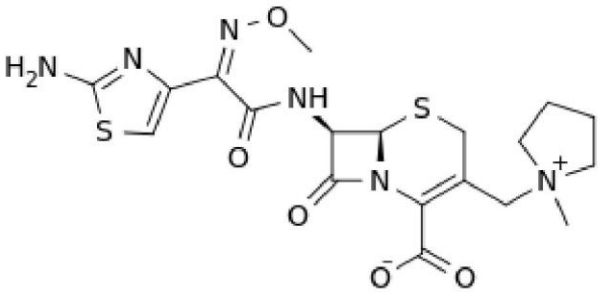

Fig. 1: The Chemical structure of cefepime.

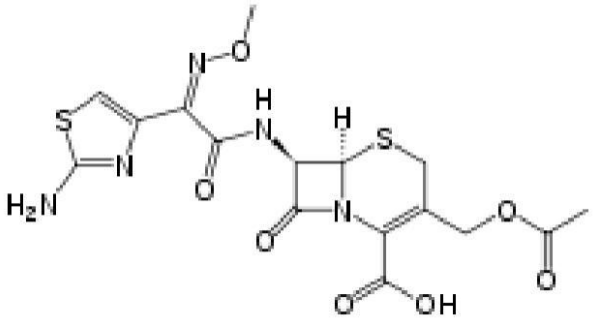

Fig. 2: The Chemical structure of Cefotaxime.

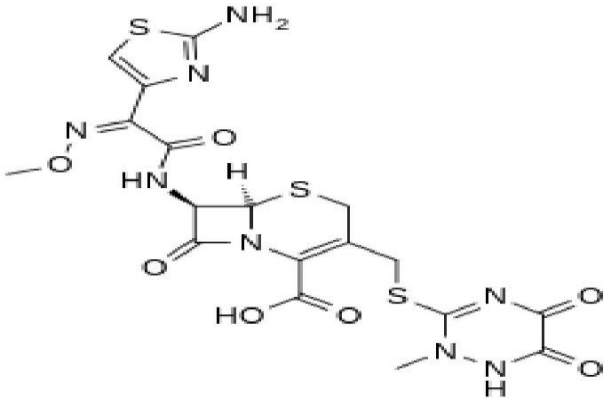

Fig. 3: The Chemical structure of Ceftriaxone.

The analytical methods focused on separation and determination of a mixture of these compounds by methods used capillary electrophoresis [9], TLC [10,11], HPLC with ammeter [12], voltmeter [13] and UV detector [14,15].

The aim of this study was to find a valid, rapid and sensitive analytical procedure using HPLC for the separation and as well as the assay of a mixture of Cefepime, Cefotaxime and Ceftriaxone.

II. Materials and Methods

Materials

All chemicals and reagents used were HPLC grade. Pure standards of Cefepime, Cefotaxime and Ceftriaxone were obtained from Chinese. Acetonitrile was HPLC grade from Romil. Water for chromatography from Merck. Potassium dihydrogen orthophosphate was from EL Nasr Pharmaceuticals Chemicals.

Methods

Analytical procedure for simultaneous determination of Cefepime, Cefotaxime and Ceftriaxone in Pharmaceuticals dosage form:-

Chromatographic conditions: The UFLC experimental conditions were optimized on the Prodigy, ODS3 column (250 mm × 4.6 mm internal diameter, 5 µm particle size) that was purchased from Phenomenex, USA. The optimum mobile phase was prepared by mixing Acetonitrile and 0.025M KH2PO4 pH 3.0 in the ratio of 15:85 (by volume). The mobile phase was filtered by using a 0.45 µm nylon membrane filter. A wavelength of

260 nm was chosen since it was found to be the most appropriate for the determination of the three active ingredients because both the drugs have sufficient absorption at this wavelength. The flow rate used was 1.5 ml/minute. The injection volume was 20 µl. The total run time of the system was about 8.0 minutes.

Preparation of stock and working standard solution

A 50 mg of Cefepime, 50 mg Cefotaxime and A 50 mg of Ceftriaxone working standard were weighed and transferred into a 100 ml volumetric flask. 85 ml of the distilled water was added and shake on vortex for 2 min; then was sonicated for 5.0 minutes. Working standard solutions were prepared and further diluted in distilled water to contain a mixture of Cefepime, Cefotaxime and Ceftriaxone in over the linearity range from 25.0 – 100.0 mg/L, 50.0 – 200.0 mg/L and 100.0 – 400.0 mg/L respectively.

Analytical method validation

a. ***Selectivity:*** It provides an indication of the selectivity and specificity of the procedure. The method is to be selective, if the main peak is well resoluted from any other peak by resolution of minimum 2 This could be done injecting placebo and compare it with that of standard and dissolution samples, then peak purity was ascertained by use of PDA

b. ***Linearity:*** is defined by the correlation coefficient, which should be found NLT 0.99, using peak area responses, Linearity for single point standardization should extend to at least 20% beyond the specification range and include the target Conc. This was performed by preparing 6 different concentrations, and then making 3 replicates of each concentration The linear working range were determined from the constructed standard calibration curve

c. ***Intraday Precision:*** This study was conducted by performing multiple analyses on a suitable number of portions of a homogeneous sample. This was performed by assaying multiple aliquots with the same concentration starting from the first step to the final step of analysis. The analytical precision of the method was determined by the relative standard deviation.

d. ***Inter-day Reproducibility (Method Ruggedness***): The degree of reproducibility determined by analysis of samples from homogeneous lot of materials, under different but typical test conditions The method is to be rugged, at any item if the pooled %RSD of the total number of replicates that have been made in this item is within the acceptance criteria, 3 replicates of a single sample of powder material are used for each determination. First day: 3 replicates, on a second day: 3 replicates, then on third day: 3 replicates of freshly prepared test from the same sample are analyzed, under the same conditions.

e. ***Accuracy and Recovery:*** Accuracy was evaluated by spiking standard solution. The measurements are made at a concentration of standard mix, which is found to be the target concentration, and at suitable intervals around this point. The test samples was spiked with known quantities of standard Cefepime, Cefotaxime and Ceftriaxone using three determinations over three concentrations level covering the specified range. Relative recoveries of standard Cefepime, Cefotaxime and Ceftriaxone used in the standards were evaluated by comparing their peak area with those obtained from the calibration curve equation.

III. Results and Discussion

The proposed HPLC method required fewer reagents and materials, and it is simple and less time consuming. This method could be used in quality control test in pharmaceutical industries. The chromatogram of Cefepime, Cefotaxime and Ceftriaxone were shown in Fig. 4. There was clear resolution between Cefepime, Cefotaxime and Ceftriaxone with retention time of 2.37, 5.90 and 4.15 minutes; respectively.

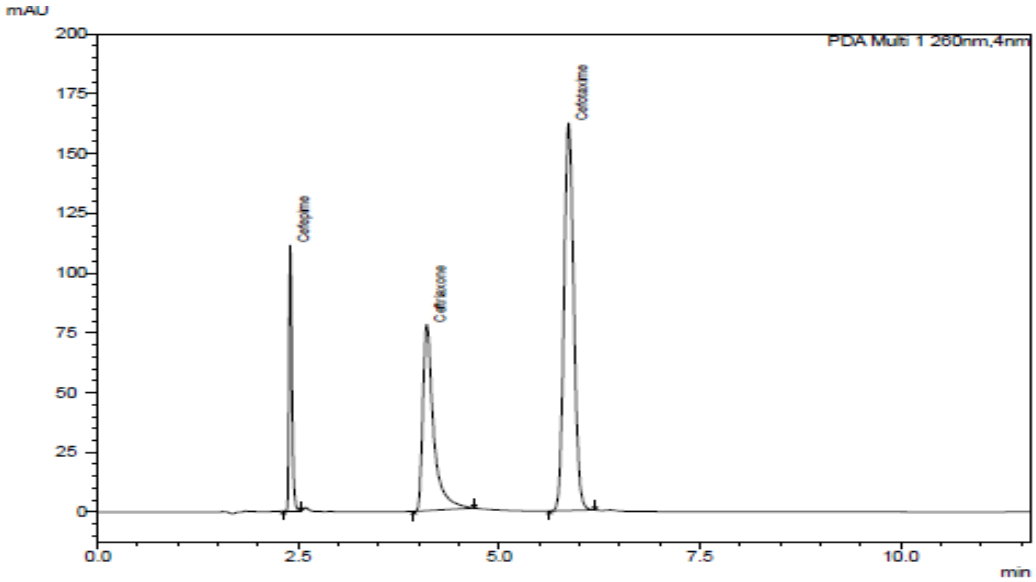

Fig. 4: UFLC Chromatogram for cefepime, cefotaxime and ceftriaxone.

Specificity

Generally, the specificity of a method is its suitability for the analysis of a compound in the presence of potential impurities. Placebo, standards, and sample test solutions were all injected at the same wavelength of 260 nm to demonstrate the specificity of the optimized method. A comparison of the retention times of Cefepime, Cefotaxime and Ceftriaxone in sample solutions and in the standard solutions were exactly the same. Fig. 5 showed that there were no interferences at the retention times for Cefepime, Cefotaxime and Ceftriaxone due to the placebo. Therefore, the proposed method is suitable for the quantification of the active ingredients in dosage form.

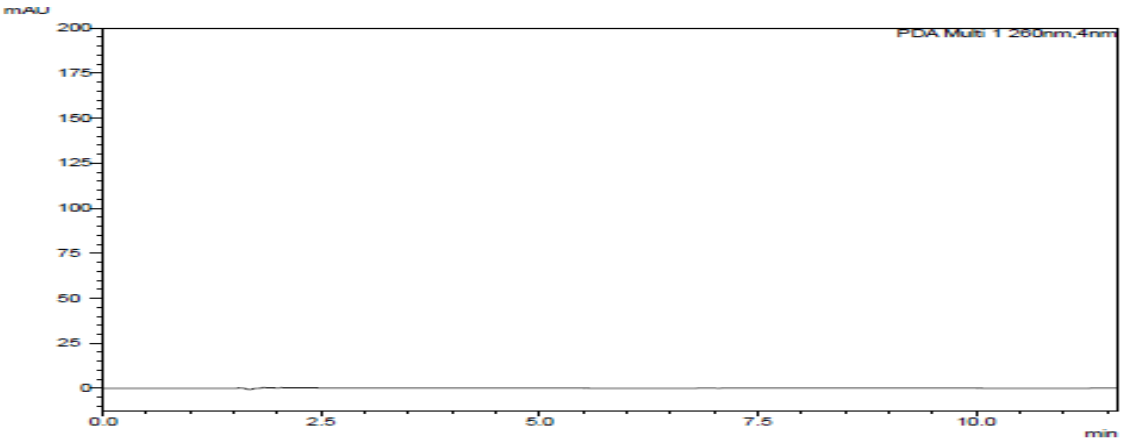

Fig. 5: UFLC chromatogram of Placebo

Linearity

Calibration curves were plotted over concentration of 25.0-100.0 mg/L for Cefepime, 50.0-200.0 mg/L for Cefotaxime and 100.0-400.0 mg/L for Ceftriaxone. Accurately measured working standard solutions of Cefepime (25.0, 40.0, 50.0, 80.0 & 100.0 ml) as shown in Fig. 6 and data are shown in Table 1, Cefotaxime (50.0, 80.0, 100.0, 160.0 & 200.0 ml) as shown in Fig. 7 and data are shown in Table 2, Ceftriaxone (100.0, 160.0, 200.0, 320.0 & 400.0 ml) as shown in Fig. 8 and data are shown in Table 3. Each of the concentrations was injected in triplicate to get reproducible response. Calibration curves were constructed by plotting peak area versus concentration. Each reading was average of three determinations. They were represented by the linear regression equation.

$Y_{Cefepime} = 7378.8423x - 59714.0287$, $r^2 = 0.9998$

$Y_{Cefotaxime} = 18748.3587x - 356275.1976$, $r^2 = 0.9993$

$Y_{Ceftriaxone} = 5318.60293x - 358002.29256$, $r^2 = 0.9974$

Slopes and intercepts were obtained by using regression equation (Y = mx + c) and least square treatment of the results used to confirm linearity of the method developed.

Table 1: UFLC analytical parameters for linearity of cefepime

% of Working Concentration	**cefepime**	
	Concentration (µg/ml)	**Observed peak area (mean)**
50%	25	128051
80%	40	235308
100%	50	305599
160%	80	527709
200%	100	681522
Slope:	**7378.8423x**	
Intercept:	**-59714.0287**	
r^2	**0.9998**	

Table 2: UFLC analytical parameters for linearity of cefotaxime

% of Working Concentration	**Cefotaxime**	
	Concentration (µg/ml)	**Observed peak area (mean)**
50%	50	614303
80%	80	1106280
100%	100	1523577
160%	160	2618511
200%	200	3417484
Slope:	**18748.3587x**	
Intercept:	**-356275.1976**	
r^2	**0.9993**	

Table 3: UFLC analytical parameters for linearity of ceftriaxone

% of Working Concentration	**Ceftriaxone**	
	Concentration (µg/ml)	**Observed peak area (mean)**
50%	100	212428
80%	160	474438
100%	200	660863
160%	320	1367061
200%	400	1771150
Slope:	**5318.60293x**	
Intercept:	**-358002.29256**	
r^2	**0.9974**	

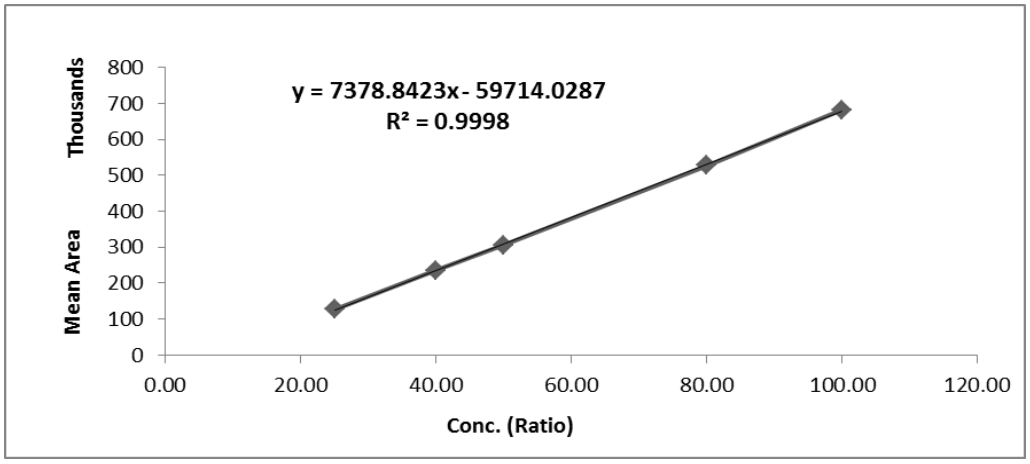

Fig. 6: Calibration curve for cefepime.

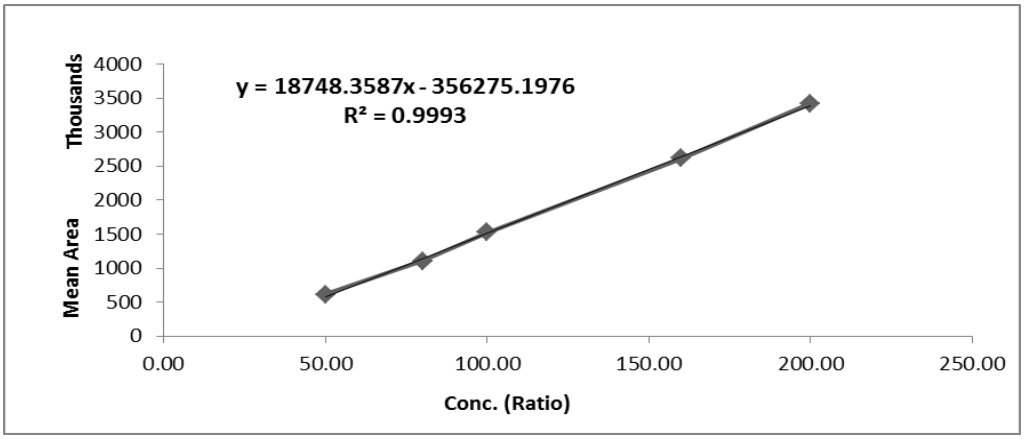

Fig. 7: Calibration curve for cefotaxime.

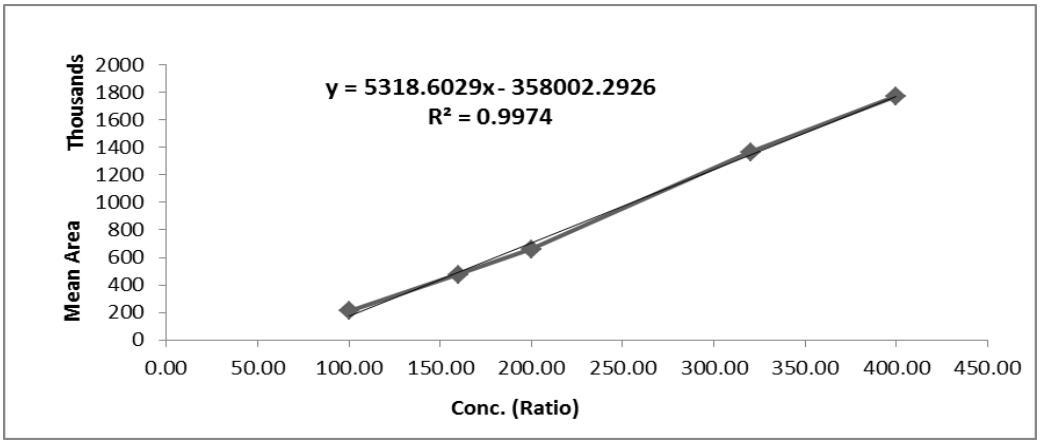

Fig. 8: Calibration curve for Ceftriaxone.

Quantification limit

The limit of detection (LOD) and limit of quantification (LOQ) of the developed method was determined by injecting progressively low concentrations of the standard solutions using the developed methods. The LOD is the lowest concentration of the analyte that can be detected with signal to noise ratio (3:1) and LOQ is the lowest concentration that can be quantified with acceptable precision and accuracy with signal to noise ratio (10:1). The LOD of Cefepime, Cefotaxime and Ceftriaxone found to be 6.67 mg/L. The LOQ of Cefepime, Cefotaxime and Ceftriaxone found to be 20.0 mg/L.

Accuracy

Accuracy was calculated by addition of standard drugs to preanalyzed sample at 3 different concentration levels and computing percentage recoveries. Standard limit of % recovery study is 98 - 102 % as per ICH guideline. From the studies it was concluded that % recovery study of Cefepime, Cefotaxime and Ceftriaxone complies with standard limit of ICH guideline. Results of accuracy were proven by the Table 4, 5 and 6.

Table 4: Accuracy and recovery results of cefepime in pharmaceutical market as Pimfast vial.

Working Concentration, (µg/ml)	Peak area (Mean*)	Found concentration (µg/ml)	% Recovery
40 µg/ml	237510	40.28	**100.70**
50 µg/ml	307885	49.82	**99.64**
80 µg/ml	537931	80.99	**101.24**

Table 5: Accuracy and recovery results of cefotaxime in pharmaceutical market as Rametax vial.

Working Concentration, (µg/ml)	Peak area (Mean*)	Found concentration (µg/ml)	% Recovery
80 µg/ml	1122461	78.87	**98.59**
100 µg/ml	1533224	100.78	**100.78**
160 µg/ml	2610955	158.27	**98.92**

Table 6: Accuracy and recovery results of ceftriaxone in pharmaceutical market as Rameceftrax vial.

Working Concentration, (µg/ml)	Peak area (Mean*)	Found concentration (µg/ml)	% Recovery
160 µg/ml	486829	158.84	**99.28**
200 µg/ml	688157	196.70	**98.35**
320 µg/ml	1336733	318.64	**99.58**

Solution Stability

In this study, the mobile phases, the standard solutions, and the sample solution were subjected to long term (3 days) stability studies. The stability of these solutions was studied by performing the experiment and looking for changes in separation, retention, and asymmetry of the peaks which were then compared with the pattern of the chromatogram of freshly prepared solutions

System suitability

The system suitability was determined by injecting six replicates of the standard solutions and analyzing each active ingredient for its peak area, peak tailing factor, resolution, number of theoretical plates, and capacity factor. The values obtained demonstrated the suitability of the system for the analysis of the above drug combinations System suitability parameters might be fall within ± 3% standard deviation range during routine performance of the methods.

IV. Conclusion

The validated HPLC method developed for the quantitative quality control determination of Cefepime, Cefotaxime and Ceftriaxone in combination was evaluated for system suitability, specificity, linearity, range, accuracy (recovery), precision (repeatability and intermediate precision), and robustness. This method enables simultaneous determination of Cefepime, Cefotaxime and Ceftriaxone because of good separation and resolution of the chromatographic peaks. As a result, the proposed HPLC method could be adopted for the quantitative quality control and routine analysis of dosage form.

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