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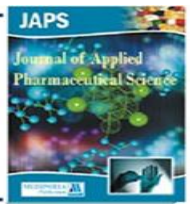

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UPLC method development and validation for Cefditoren Pivoxil in active pharmaceutical ingredient

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ABSTRACT

The objective of the study was to develop UPLC method for the determination of purity of Cefditoren Pivoxil in API and its validation. UPLC is a better technique than HPLC in terms of performance and speed, so it was selected. The method was developed using Acetonitrile and Ammonium Acetate buffer (pH 6.7) and Kromacil column C_{18} (50×2.1mm, 3.5µ) as a stationary phase at a flow rate of 0.25ml/min. Validation was done by linearity, precision, and robustness studies. The precision was found to be within the limits. The linearity studies indicated the drug obeys Beer's law and revealed the specified range of linearity for drug was between 80µg/ml and 120µg/ml. The robustness was observed from the insignificant variation in the analysis by changes in flow rate, mobile phase ratio, wavelength, column oven temperature and pH. Forced Degradation study revealed the drug degraded initially by the influence of acid, alkali, and peroxide. Solution stability study showed the drug was not stable for more than 2 h at 25˚C but stable at 5˚C. It can be concluded that the proposed method was simple, precise, and robust and can be useful for determination of purity of Cefditoren Pivoxil in API by using UPLC.

Key words: UPLC, Cefditoren Pivoxil, active pharmaceutical ingredient (API), method development, validation.

INTRODUCTION

High performance liquid chromatography (HPLC) has proven to be the predominant technology used in laboratories worldwide during the past 30 plus years (Beckett and Stenlake, 2004; Sharma, 2004). Waters Corporation has taken the principles of HPLC and further adapted them to create Ultra Performance Liquid Chromatography (UPLC™), a new separation technique with increased speed, sensitivity and resolution (Swartz *et al.*, 2004; Swartz, 2005). The performance of a column can be measured in terms of the height equivalent to the theoretical plates (HETP) which is calculated from the column length (L) and the column efficiency, or number of theoretical plates (N). N is calculated from an analyte retention time (tR) and the standard deviation of the peak (s). H = L/N.

UPLC instrumentation involves a Binary solvent manager, sample manager, detector. The types of UPLC techniques include Normal phase chromatography (NP-UPLC), Reverse phase chromatography (RP-UPLC), Size exclusion chromatography, Ion exchange chromatography and Bio-affinity chromatography (Swartz, 2004; Chatwal and Anand, 2004). Chromatographic methods are commonly used for the quantitative and qualitative analysis of raw materials, drug substances, drug products and compounds in biological fluids. The objective of a test method is to generate reliable and accurate data regardless of whether it is for acceptance, release, stability or

pharmacokinetics study (Galen, 2002). The various steps to be performed for UPLC method development involve solubility studies to establish the solubility of the API in a number of aqueous and organic solvents, selection of the mobile phase, selection of the detector and detector wavelength, and selection of isocratic or gradient mode of elution. For UPLC method development optimization of some critical parameters is required (Srivastava *et al.*, 2010). They include selection of the buffer, pH of the buffer and the mobile phase, column, column temperature, test concentration and injection volume (Snyder *et al.*, 1997).

Validation of a method is the process by which a method is tested by the developer or user for reliability, accuracy and preciseness of its intended purpose. Methods validation should not be a one-time situation to fulfill agency filing requirements, but the methods should be validated and also designed by the developer or user to ensure ruggedness or robustness (Fajgelj and Ambrus, 2000). There is no single validation approach that must always be employed for a new method. Validation approaches include (Ermer and Miller, 2005) zero-blind method, single-blind method, double-blind method and inter-laboratory collaborative study. The parameters involved for validation of UPLC methods include precision, accuracy, Linit of Detection (LOD), Limit of Quantitation (LOQ), specificity, linearity, ruggedness, robustness, solution stability, and system suitability(capacity factor, resolution, tailing factor, theoretical plate number) (Burgess, 2000; Bliesner, 2006). The acceptance criteria for the different characteristics of validation are mentioned in ICH Q2A guidelines.

The drug used in the present study is Cefditoren Pivoxil which is a cephalosporin category antibiotic (Ebrahim and Balbisi, 2002). It is used to treat uncomplicated skin and skin structure infections, community-acquired pneumonia, acute bacterial exacerbation of chronic bronchitis, pharyngitis, and tonsillitis. Thus the objective of the present study is to develop UPLC method for the determination of purity of Cefditoren Pivoxil in API and validation of the same. There are very few works that has been done on this drug by HPLC but no method has been mentioned by UPLC technique.

MATERIALS AND METHODS

Materials

Cefditoren Pivoxil was obtained from Daiichi Sankyo Life Sciences, India as a gift sample. All the other chemicals and reagents used were of analytical grade.

Method development of Cefditoren Pivoxil by UPLC

Five methods (Method 1 to 5) with varying parameters were tested for best resolution, Peak Shape, and minimum Run Time (Willard *et al.*, 1996). Table 1 gives the UPLC parameters for each method and Table 2 shows the UPLC methodology applied for them. The Method 2 with Kromasil 100 C-18 (50x2.1mm), 3.5µ, flow rate (0.25mL/min) was found optimized based on UPLC analysis, for determination of percentage purity (Ahuja and Rasmussen, 2007; Srinivasa, 2011).

Table 1 The various UPLC parameters for method development of Cefditoren Pivoxil.

PARAMETERS	METHOD 1	METHOD 2	METHOD 3	METHOD 4	METHOD 5
COLUMN	Kromasil 100 C-18, 50×2.1mm, 3.5µ	Kromasil 100 C-18, 50×2.1mm, 3.5µ	Kromasil 100 C-18, 50×2.1mm, 3.5µ	Kromasil 100 C-18, 50×2.1mm, 3.5µ	Kromasil Eternity C-18, UPLC, 2.1×100mm, 2.5µ
MOBILE PHASE	Ammonium Acetate : Acetonitrile (ACN)	Ammonium Acetate : ACN	Formic Acid : ACN	Trifluoroacetic acid : ACN	Ammonium Acetate : ACN
FLOW RATE	0.25 mL/ min.	0.25 mL/ min.	0.25 mL/ min.	0.25 mL/ min.	0.25 mL/ min.
RUN TIME	7 min.	5 min.	5 min.	5 min.	5 min.
DETECTION	232 nm	232 nm	232 nm	232 nm	232 nm
COLUMN TEMP.	40 ºC	40 ºC	40 ºC	40 ºC	40 ºC
SAMPLE TEMP.	5 ºC	5 ºC	5 ºC	5 ºC	5 ºC
INJECTION VOLUME	5 µL	5 µL	5 µL	5 µL	5 µL

Table 2 UPLC gradient methodology for method development of Cefditoren Pivoxil.

Method 1	Run time (min)	0	1	2	3.5	4.5	5.5	7
	Buffer (%)	80	80	50	20	20	80	80
	Acetonitrile (%)	20	20	50	80	80	20	20
Method 2	Run time (min)	0	0.5	1	2.5	3.5	4	5
	Buffer (%)	80	80	50	20	20	80	80
	Acetonitrile (%)	20	20	50	80	80	20	20
Method 3	Run time (min)	0	0.5	1	2.5	3.5	4	5
	Buffer (%)	80	80	50	20	20	80	80
	Acetonitrile (%)	20	20	50	80	80	20	20
Method 4	Run time (min)	0	0.5	1	2.5	3.5	4	5
	Buffer (%)	80	80	50	20	20	80	80
	Acetonitrile (%)	20	20	50	80	80	20	20
Method 5	Run time (min)	0	0.5	1	2.5	3.5	4	5
	Buffer (%)	80	80	50	20	20	80	80
	Acetonitrile (%)	20	20	50	80	80	20	20

Table 3 Percentage purity of Cefditoren Pivoxil in API by UPLC Method 2.

S.NO	RT (min)	AREA	% AREA
1	1.854	5897	0.49
2	2.260	1189486	98.56
3	2.599	5717	0.47

Determination of percentage purity of Cefditoren Pivoxil in API using optimized method

The same UPLC method development parameters and gradient technique as that of Method 2 was employed for determination of percentage purity of Cefditoren Pivoxil (Jerkovich *et al*., 2003; Satinder and Henrika, 2007).

Validation of developed and optimized method

The validation of developed method was done by using the parameters (Maxwell 1994; Riley and Ronsanske, 1996; Ermer and Miller, 2005) which include System suitability (retention time, peak area), Precision (system precision, method precision), Linearity, Forced degradation study (acid, alkali and peroxide degradation), Robustness (flow rate, wavelength, mobile phase ratio, column temperature, pH), and Solution stability at 25ºC and 5ºC.

Statistical analysis

Statistical analysis and significance was carried out using correlation coefficient, standard deviation and relative standard deviation (RSD) with the help of Microsoft Excel, 2007.

RESULTS AND DISCUSSION

Chromatograms depicting the method development of Cefditoren Pivoxil

For Method 1, the chromatogram (Figure 1) obtained was found to have good resolution, less tailing and sharp peak. The retention time (RT) was at 3.18, but to reduce the time of analysis, another set of trials were performed. The chromatogram (Figure 2) obtained for Method 2 was found to have a good resolution with sharp peak. The RT was at 2.26 min with 5 min run time. The chromatogram (Figure 3) obtained for Method 3 was not properly separated; the RT was at 2.20 min having a total run time of 5 min. For Method 4, the chromatogram (Figure 4) obtained was not properly separated; the RT was at 2.04 min having a total run time of 5 min. The chromatogram (Figure 5) for Method 5 was having a poor resolution and peak was not properly separated with the RT at 2.77 min having a total run time of 5 min.

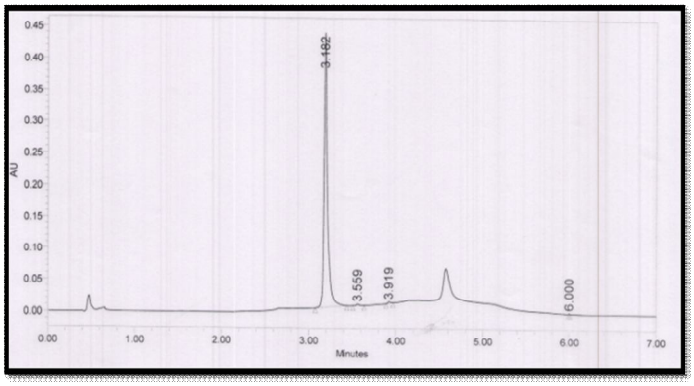

Fig 1 UPLC chromatogram for Method 1.

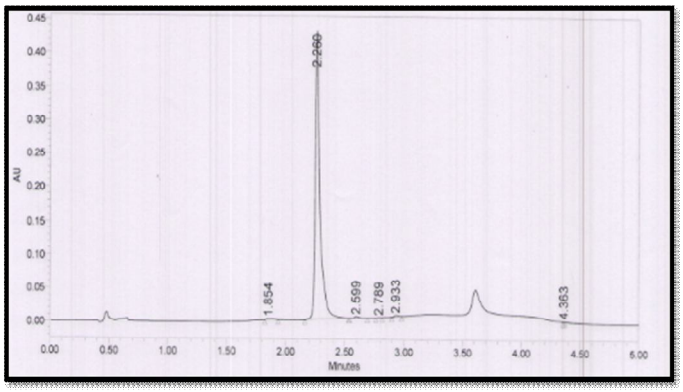

Fig 2 UPLC chromatogram for Method 2.

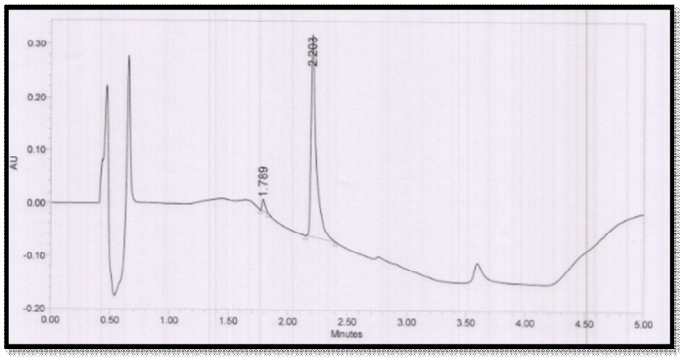

Fig 3 UPLC chromatogram for Method 3.

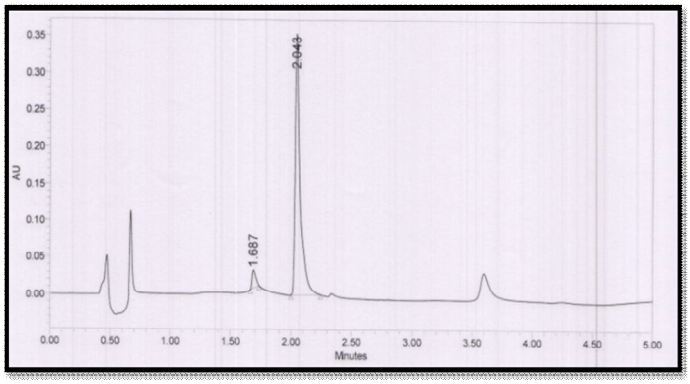

Fig 4 UPLC chromatogram for Method 4.

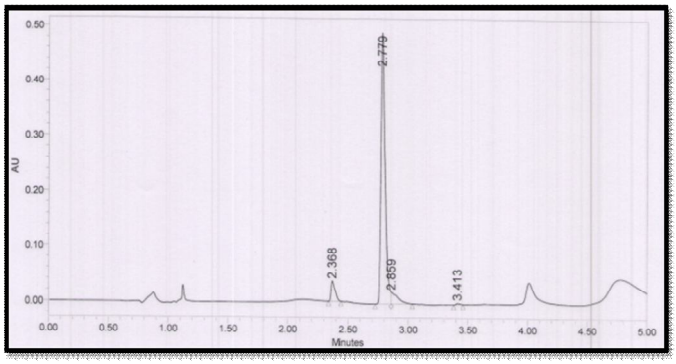

Fig 5 UPLC chromatogram for Method 5.

Determination of percentage purity of Cefditoren Pivoxil using Method 2 in API

The results are given in Table 3 and chromatogram (Figure 2) obtained by optimized UPLC method (Method 2) have a purity of 98.56% and it contained a maximum impurity of 0.49 %.

Validation of developed method for Cefditoren Pivoxil

System suitability

The UPLC chromatogram and its data for system suitability is given in Table 4. The % RSD of the retention time, peak area responses and that of tailing factor for six replicate injections of sample solution were 0.102%, 0.506% and 0.403% respectively which are within the limits specified (% RSD NMT 2.0%). The average number of theoretical plates (N) for the newly developed method was 14355 which are within the limits specified (NLT 2000). The data reveals that the method has a good resolution and fine separation.

Precision

The UPLC chromatogram data for system precision and method precision are shown in Table 5 and Table 6 respectively. Chromatogram data for system precision revealed that % RSD of the retention time and peak area responses for six replicate injections of sample solution was 0.11% and 0.26% which is within the limits specified (% RSD NMT 2.0%). The data for method precision revealed that % RSD of the peak area responses from six injections (each in duplicate) of sample solution was 1.10% which is within the limits specified (% RSD NMT 2.0%).

Table 4 UPLC chromatogram data for system suitability validation of Cefditoren Pivoxil.

INJECTION NO.	RT (min)	AREA	U.S.P. TAILING	U.S.P. PLATE COUNT
1	2.260	2087342	1.294990	14356
2	2.257	2090100	1.283502	14351
3	2.258	2066775	1.288125	14348
4	2.258	2086396	1.287506	14360
5	2.256	2097634	1.279446	14361
6	2.253	2092124	1.286739	14354
Mean	2.257	2086728	1.286718	14355
% R.S.D.	0.104	0.506	0.403	0.035
% R.S.D. (Acceptance Criteria)	NMT 2.0 %	NMT 2.0 %	NMT 2.0 %	NLT 2000

Table 5 UPLC chromatogram data for system precision validation of Cefditoren Pivoxil.

INJECTION NO.	R.T. (Min.)	AREA
1	2.260	2099170
2	2.259	2099101
3	2.258	2087875
4	2.258	2086396
5	2.254	2092339
6	2.253	2092334
Mean	2.570	2092869
% R.S.D.	0.11	0.26
% R.S.D. (Acceptance Criteria)	NMT 2.0 %	NMT 2.0 %

Table 6 UPLC chromatogram for method precision validation of Cefditoren Pivoxil.

	AREA		
	Injection 1	Injection 2	MEAN
1	2112317	2110207	2111262
2	2123866	2123501	2123684
3	2123030	2123622	2123326
4	2124167	2124813	2124490
5	2064354	2064464	2064409
6	2118956	2119895	2119426
MEAN			211109
% RSD			1.10

Table 7 UPLC chromatogram data for determination of linearity validation of Cefditoren Pivoxil.

Sample ID Name	Concentration (ppm)	Area			
		Injection 1	Injection 2	Mean	% RSD
Linearity 80 %	40	1831623	1844756	1838190	0.5
Linearity 90 %	45	2043198	2045756	2044477	0.04
Linearity 100 %	50	2299720	2329616	2314668	0.4
Linearity 110 %	55	2593403	2581209	2587306	0.11
Linearity 120 %	60	2724289	2799568	2761929	0.9

Table 8 UPLC chromatogram data for robustness validation of Cefditoren Pivoxil.

Parameters	Changed Value	Area		Mean	% RSD
		Injection-1	Injection-2		
Flow Rate	0.225 ml	2085688	2060616	2073152	0.85
	0.275 ml	1938502	1924082	1931292	0.52
Wavelength	230 nm	2056728	2078458	2067593	0.74
	235 nm	2031349	2056621	2043985	0.87
Mobile Phase Ratio	75:25	2011050	1998228	2004639	0.45
	85:15	1996063	2003785	1999924	0.27
Column Temperature	38˚C	2075823	2097301	2086562	0.72
	42˚C	2083336	2098343	2090840	0.50
pH	6.5	1994959	1966187	1980573	1.02
	7.0	2097941	2047088	2072515	1.73

Linearity

The UPLC chromatogram data for determination of linearity is mentioned in Table 7. The linearity of the optimized method was determined for 5 concentrations and the correlation coefficient was found to be 0.99 for Cefditoren Pivoxil which is within the limits specified (NLT 0.99). It showed that the developed method followed Beer-Lambert's law within the range of 80–120 µg/ml and is linear for determination of percentage purity of Cefditoren Pivoxil.

Forced degradation study

The forced degradation study using UPLC revealed that the drug was completely unstable under the influence of acid, alkali and hydrogen peroxide solution. The main peak in the chromatogram was completely disappearing when it was run initially, in case of acid and peroxide, showing that the drug degraded 100%, while it was 99% for alkali.

Robustness

The UPLC chromatogram data for determining robustness of the method is given in Table 8. The data revealed that % RSD for decrease and increase in flow rate for Cefditoren Pivoxil were 0.85 and 0.52 respectively which are within the limits specified (% RSD NMT 2.0%). The % RSD for 75:25 and 85:15 mobile phase ratios of the drug were 0.45 and 0.27 respectively which are within the limits specified (% RSD NMT 2.0%). % RSD for decrease and increase in column oven temperature were 0.72 and 0.50 respectively which are within the limits specified (% RSD NMT 2.0%). % RSD for decrease and increase in wavelength were 0.74 and 0.87 respectively which are within the limits specified (% RSD NMT 2.0%). % RSD for decrease and increase in pH were 1.02 and 1.73 respectively which are within the limits specified (% RSD NMT 2.0%). From the above study it can be established that the flow rate, mobile phase ratio, column oven temperature, wavelength and pH are robust in the allowable variations.

Table 9 UPLC chromatogram data for solution stability validation at 25ºC of Cefditoren Pivoxil.

S.No.	Trial	Area	Mean	Cumulative % RSD
1	Initial	1334598		
2	1 hrs.	1325242	1329920	0.5
3	2 hrs.	1294621	1318154	1.5
4	3 hrs.	1208484	1290736	4.4
5	4 hrs.	1185397	1269668	5.3
6	5 hrs.	1155931	1250712	6.1
7	6 hrs.	1110566	1291838	6.8

Table 10 UPLC chromatogram data for solution stability validation at 5ºC of Cefditoren Pivoxil.

S.No.	Trial	Area	Mean	Cumulative % RSD
1	Initial	1825430		
2	1 hrs.	1815265	1820348	0.394
3	2 hrs.	1802468	1814388	0.634
4	3 hrs.	1796191	1809839	0.722
5	4 hrs.	1810855	1810042	0.626
6	6 hrs.	1800355	1808427	0.601
7	12 hrs.	1799204	1812609	0.580
8	24hrs	1798266	1806004	0.566

Solution stability

The UPLC chromatogram data for solution stability at 25ºC and at 5ºC are shown in Table 9 and Table 10 respectively. The solution stability study at 25ºC of Cefditoren Pivoxil revealed that the drug was not stable more than two hours. % RSD for 2 h was 1.5 which is within the limits specified (% RSD NMT 2.0%). The solution stability at 5ºC of the drug showed that the drug is stable for 24 h. The cumulative % RSD was within the limits specified (% RSD NMT 2.0%).

CONCLUSION

The present UPLC method was developed for determination of percentage drug purity of Cefditoren Pivoxil in API using Acetonitrile and Ammonium Acetate buffer (pH 6.7) and Kromacil column C_{18} (50 × 2.1mm, 3.5µ) as a stationary phase at a flow rate of 0.25 ml/min. Five methods were taken for development and Method 2 was found to be optimized for the determination of percentage purity. The method was validated using system suitability, precision, linearity, robustness, forced degradation and solution stability studies. The proposed method was found to have a good resolution, fast speed and less consumption of solvent as per the standard procedures.

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