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RESEARCH ARTICLE

RP HPLC Method Development and Validation of Imipenem and Cilastatin in Bulk and Pharmaceutical Dosage Forms

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ABSTRACT

A new method was established for simultaneous estimation of Imipenem and Cilastatin by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Imipenem and Cilastatin by using Zodiac silRP C18 4.6×250mm 3.0µm, flow rate was 1.0ml/min, mobile phase ratio was ACN: pH 3 buffer (70: 30 % v/v) ($KH_2PO_4$and K_2HPO_4) pH 3 (pH was adjusted with orthophosphoric acid),detection wavelength was 240nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The % purity of Imipenem and Cilastatin was found to be 100.27% and 99.87% respectively. The system suitability parameters for Imipenem and Cilastatin such as theoretical plates and tailing factor were found to be 2885, 1.25 and 2235 and 1.33, the resolution was found to be3.48. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study Imipenem and Cilastatin was found in concentration range of 50µg-250µg and 5µg-50µg and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 99.56% and 99.48%, %RSD for repeatability was 1.2 and 2, % RSD for intermediate precision was 1.1 and 1.1 respectively. The precision study was precise, robust, and repeatable.LOD value was 0.03 and 2.17, and LOQ value was 0.11 and 6.6 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Imipenem and Cilastatinin API and Pharmaceutical dosage form.

Keywords: Zodiac silRP C18 column, Imipenem and Cilastatin, HPLC method

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CONTENTS

1. Introduction

Imipenem (Primaxin among others) is an intravenous β-lactam antibiotic discovered by Merck scientists Burton Christensen, William Leanza, and Kenneth Wildonger in the mid-1970s. Carbapenems are highly resistant to the β-lactamase enzymes produced by many multiple drug-resistant Gram-negative bacteria, thus play a key role in the treatment of infections not readily treated with other antibiotics.

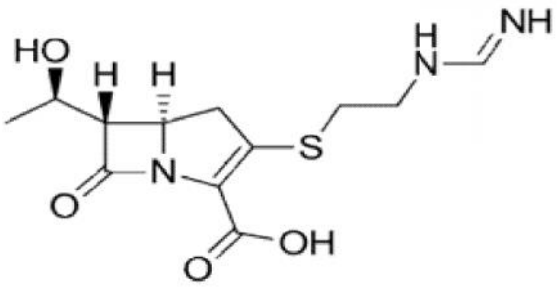

Fig 1: Structure of Imipenem

Imipenem alone is an effective antibiotic and can be given without cilastatin. Cilastatin itself does not have antibiotic activity, although it has been proved to be active against a zinc-dependent beta-lactamase that usually confers antibiotic resistance to certain bacteria, more precisely, the carbapenem family of antibiotics.

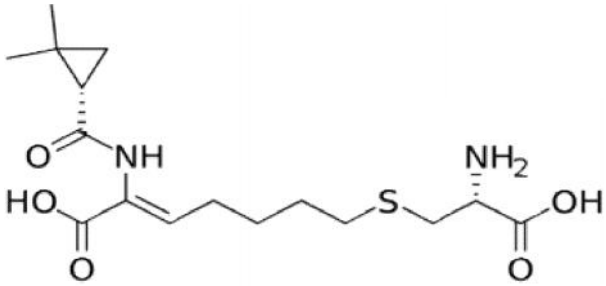

Fig 2: Structure of Cilastatin

2. Materials and Methods

Instrumentation:

System Alliance Waters 2690 separation module, Pump Analytical HPLC isocratic pump, Detector Photo diode array detector, Software Empower 2 software, Column Agilent (250×4.6mm, 5µ) C-18 RP-column, Sonicator Analytical Technologies Limited- Ultrasonic cleaner. U.V double beam spectrophotometer LABINDIA, UV 3000^{+}pH meter, Weighing machine.

Chemicals:

Imipenem and Cilastatin, KH_2PO_4, Water and Methanol for HPLC, Acetonitrile for HPLC, Ortho phosphoric Acid, K_2HPO_4.

Optimized chromatographic conditions

Column : Zodiac silRP C18 4.6×250mm 3.0µm
Mobile phase ratio : ACN: pH 3 buffer (70: 30 % v/v)
Detection wavelength: 240 nm
Flow rate : 1.0ml/min
Injection volume : 20µl
Run time : 10min

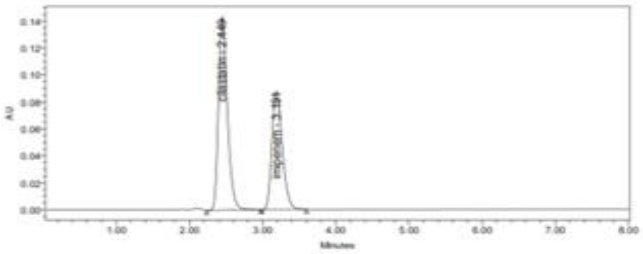

Fig 3: Chromatogram from optimized conditions

Observation: The separation was good, peak shape was good, so we conclude that there is no required for reduce the retention times of peaks, so it is taken as final method.

Sample solution preparation:

10 mg of Imipenem and 1 mg Cilastatin tablet powder were accurately weighed and transferred into a 10 ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and making volume up to the mark with the same solvent(Stock solution). Further pipette 10ml of the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

Standard solution preparation:

10 mg Imipenem and 1mg Cilastatin working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

Method Validation

- ✓ System Suitability
- ✓ Linearity
- ✓ Specificity
- ✓ Precision (Repeatability & Intermediate precision)
- ✓ Accuracy
- ✓ Limit of Detection and Limit of Quantification
- ✓ Robustness

3. Results and Discussion

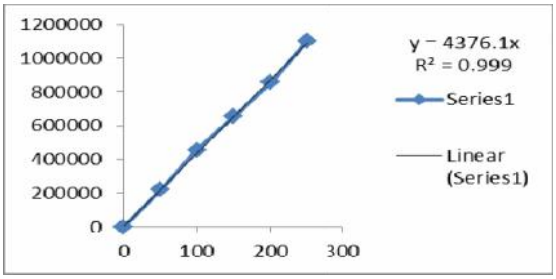

Fig 4: Showing calibration graph for Imipenem

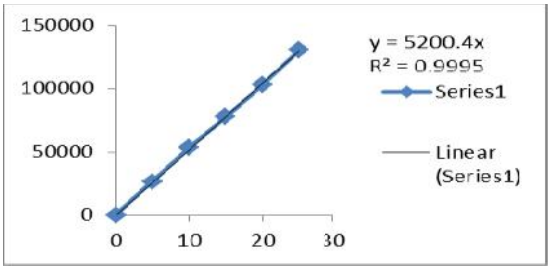

Fig 5: Showing calibration graph for Cilastatin

Table 1: Assay results

S.NO	Peak Name	RT	Area	Height	USP Plate Count	USP Tailing
1	Cilastatin	2.449	1214356	143778	1938.96	1.32
2	Cilastatin	2.467	1217143	139906	1847.94	1.36
3	Cilastatin	2.511	1230473	134589	1741.58	1.43
4	Imipenem	3.191	861161	18187	2438.26	1.26
5	Imipenem	3.268	855169	83700	2358.16	1.30
6	Imipenem	3.336	868604	81477	2243.15	1.36

Table 2: Linearity Results for Imipenem

S.No	Linearity Level	Concentration	Area
1	I	50 ppm	107359
2	II	100 ppm	221497
3	III	150 ppm	329389
4	IV	200 ppm	448105
5	V	250 ppm	570352
Correlation Coefficient			0.999

Table 3: Linearity Results for Cilastatin

S.No	Linearity Level	Concentration	Area
1	I	5ppm	26472
2	II	10 ppm	53841
3	III	15ppm	77655
4	IV	20ppm	102541
5	V	25ppm	130567
Correlation Coefficient			0.999

Table 4: Repeatability results for Cilastatin

S.No	Peak Name	RT	Area	Height	USP Plate Count	USP Tailing
1	Cilastatin	3.174	1592260	146364	2973.61	1.49
2	Cilastatin	3.174	1600516	147364	2973.61	1.49
3	Cilastatin	3.323	1620416	144812	2873.05	1.52
4	Cilastatin	3.360	1605813	145422	2912.90	1.52
5	Cilastatin	3.740	1670034	136518	2654.63	1.50
6	Cilastatin	3.842	1571138	141267	2898.11	1.43
Mean			1610030			
Std.Dev.			33600.6			
%RSD			2.0			

Table 5: Repeatability results for Imipenem

S.No	Peak Name	RT	Area	Height	USP Plate Count	USP Tailing
1	Imipenem	4.002	1197254	141033	2852.74	1.37
2	Imipenem	4.673	1171214	84462	2672.17	1.38
3	Imipenem	4.863	1207502	84306	2557.54	1.40
4	Imipenem	4.920	1212400	84282	2627.38	1.38
5	Imipenem	5.450	1203215	82319	2444.22	1.38
6	Imipenem	5.637	1184663	85374	2506.06	1.32
Mean			1196041.2			
Std.Dev.			15477.3			
%RSD			1.2			

Table 6: Intermediate precision results for Cilastatin

S.No	Peak Name	RT	Area	Height	USP Plate Count	USP Tailing
1	Cilastatin	3.054	1747364	146364	2973.61	1.49

2	Cilastatin	3.261	1720516	147364	2973.61	1.49
3	Cilastatin	3.532	1690416	144812	2873.05	1.52
4	Cilastatin	3.954	1705813	145422	2912.90	1.52
5	Cilastatin	4.125	1710034	136518	2654.63	1.50
6	Cilastatin	3.824	1721138	141267	2898.11	1.43
Mean			1715880			
Std.Dev.			19101.4			
%RSD			1.1			

Table 7: Intermediate precision results for Imipenem

S.NO	Peak Name	RT	Area	Height	USP Plate Count	USP Tailing
1	Imipenem	4.754	1637254	141033	2852.74	1.37
2	Imipenem	4.847	1621214	84462	2672.17	1.38
3	Imipenem	4.961	1657502	84306	2557.54	1.40
4	Imipenem	4.647	1602400	84282	2627.38	1.38
5	Imipenem	5.964	1643215	82319	2444.22	1.38
6	Imipenem	5.789	1634663	85374	2506.06	1.32
Mean			1632708			
Std.Dev.			18975.3			
%RSD			1.1			

Table 7: Showing results for Limit of Detection

Drug name	Standard deviation(σ)	Slope(s)	LOD(µg)
Cilastatin	371827.90	563365963	2.17
Imipenem	5401.60	479884400	0.0372

Table 8: Showing results for Limit of Quantification

Drug name	Standard deviation(σ)	Slope(s)	LOQ(µg)
Cilastatin	371827.90	563365963	6.60
Imipenem	5401.60	479884400	0.112

Table 9: Showing system suitability results for Cilastatin

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	2590	1.39
2	**1**	**2294**	**1.27**
3	1.2	2146	1.26

Table 10: Showing system suitability results for Imipenem

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	5435	1.04
2	**1**	**4891**	**1.03**
3	1.2	4781	1.04

Table 11: Showing system suitability results for Cilastatin

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	2347	1.44
2	*Actual	2294	1.27
3	5 % more	2239	1.13

Table 12: Showing system suitability results for Imipenem

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	5437	0.99

2	*Actual	4891	1.03
3	5 % more	4817	1.05

4. Conclusion

In the present research, a fast, simple, accurate, precise, and linear RP-HPLC method has been developed and validated for Imipenem and Cilastatin and hence it can be employed for routine quality control analysis.

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