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Research Article

HPLC-UV Method for the determination of Biapenem in Pharmaceutical Dosage Forms.

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Abstract

A simple, selective, rapid, precise and economical reverse phase high pressure liquid chromatographic method has been developed for the estimation of Biapenem in pharmaceutical Tablet dosage form. The mobile phase consisted of 50:50 % (v/v) of Methanol & 10mM Potassium dihydrogen phosphate operated on isocratic mode. The flow rate is 1.0 ml/min. Chromatographic determination of Biapenem was performed on Agilent Zorbax SB C_{18} column (150 X 4.6 mm id, ODS 2, 5µm). The wavelength of detection is 300 nm. The injection volume is 20µL. The retention time of Biapenem is 2.72 ± 0.10 minutes. The developed method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation and solution stability. The influence of Acid, Alkaline, Oxidative Stress, Photolytic stress conditions on Biapenem was studied. Results indicated complete degradation in Alkaline medium. The proposed method has been successfully used for the estimation in dosage forms.

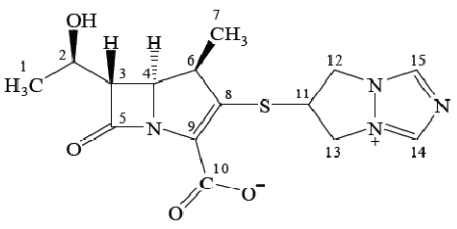
Keywords: Biapenem; Antibiotics; RP- HPLC;

1. Introduction

Biapenem (L-627) [Lederle (Japan), Ltd] (Fig. 1) is a parenteral carbapenem that possesses antibacterial activities against a wide range of Gram-positive and -negative bacteria [1]. It is stable to human renal dehydropeptidase-I (DHP-I) and therefore does not require the co-administration of a DHP-I enzyme inhibitor [2]. Single and repeated iv doses of biapenem have been reported to be well tolerated by healthy young volunteers, showing linear pharmacokinetics within the dosage range of 20–600 mg [3]. This tolerability has been also demonstrated in the elderly [4]. In normal subjects, biapenem is cleared mainly by urinary excretion. However, the predominant concern in terms of adverse reactions to imipenem/cilastatin is the increased tendency to cause seizures compared with other β-lactams, and the risk of producing a seizure is highly associated with inadequate dose adjustment in relation to renal function. [5]. Koeppe *et al* [6] reported the pharmacokinetics of biapenem in patients with various degrees of reduced renal function

Biapenem is usually unstable. Xia et al. [7] had identified the degradation products in biapenem aqueous solution by LCMS. However, the LCMS method is not appropriate for the routine quality control. Identification and content determination of drug related impurities in a drug product is one key area of quality control. Traditionally, HPLC with UV detection (LCUV) is the most widely used analytical technique for quality control of small molecule drugs. In the present study we report a validated stability indicating method for the determination of Biapenem in pharmaceutical dosage forms as per ICH guidelines [8].

Fig-1: Structure of Biapenem



2. EXPERIMENTAL

2.1. Reagents and chemicals

Potassium dihydrogen phosphate (AR Grade, Merck ltd), Methanol (HPLC grade, Merck ltd), Milli-Q water, Biapenem (Gift sample from Sigma Aldrich,). All other chemicals are of the highest grade commercially available unless otherwise specified.

2.2. Apparatus and chromatographic conditions

The Chromatographic system consisted of a Shimadzu Class VP Binary pump LC-10ATvp, SIL-10ADvp Auto sampler, CTO-10Avp Column Temperature Oven, SPD-10Avp UV-Visible Detector. All the components of the system are controlled using SCL-10Avp System Controller. Data acquisition was done using LC Solutions software.

The mobile phase consisted of 50:50% (v/v) of Methanol & 10mM Potassium dihydrogen phosphate operated on isocratic mode. The flow rate is 0.7 ml/min. Chromatographic determination of Biapenem was performed on Agilent Zorbax SB C_{18} column (150 X 4.6 mm id, ODS 2, 5µm). The wavelength of detection is 300 nm. The injection volume is 20µL.

2.3. Preparation of standard solutions, Calibration Standards & Quality Control Samples

Stock solutions of Biapenem (5mg/mL) was prepared separately in a volumetric flask and labeled accordingly. Suitable dilutions of Biapenem were prepared using 50:50 %v/v Methanol & Milli-Q water as diluent Solution. A Linear Calibration curve containing 7 non-zero standards were prepared using diluent solution in the concentration range of 5.35 – 101.15 µg/mL. The linear standard calibration standard sample is then transferred into the auto sampler for analysis. Samples for Specificity (Sample with Drug; Blank Sample were also prepared accordingly).

For the preparation of quality control samples, a separate stock containing approximately the same concentration of the drug substance is prepared and labeled as quality control stock. From this stock, quality control samples were prepared at three concentration levels namely LQC (25.29 µg/mL), MQC (50.57 µg/mL), HQC (75.86 µg/mL) so as to obtain low, median and high concentration quality control samples. The performance of the linear calibration curve is then evaluated using quality control samples.

2.4. Assay

The assay of tablets containing Biapenem is done using the procedure given in Indian Pharmacopoeia for tablets. Briefly, twenty tablets, each containing 80 mg of Biapenem as labeled claim were weighed and finely powdered; a quantity of powder equivalent to 80.0 mg of Biapenem was weighed and transferred to a 20 mL volumetric flask. To this 10 mL of methanol was initially added and vortexed thoroughly. The final volume is made up to volume with methanol. The final solution was mixed well. This mixture is then carefully filtered using 0.45 µm membrane filter. The filtrate is then taken and suitably diluted and injected for analysis. The assay content was evaluated using the regression equation of linear calibration curve.

2.6 Method Validation

2.6.1 System Suitability

The system suitability was assessed by six replicate analysis of the drug at a concentration of 50.57 µg/ml. The acceptance criterion is ± 2 % for the percent coefficient of the variation for the retention time for the drug.

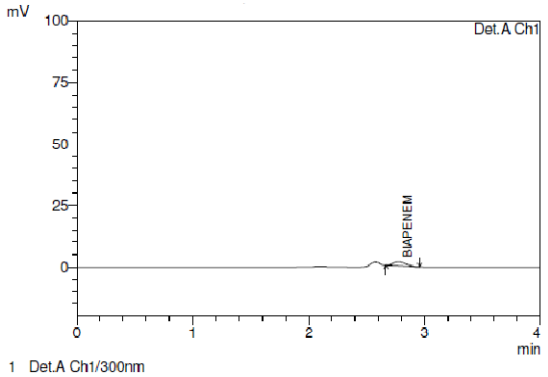
2.6.2 Detection and Quantitation Limits (Sensitivity)

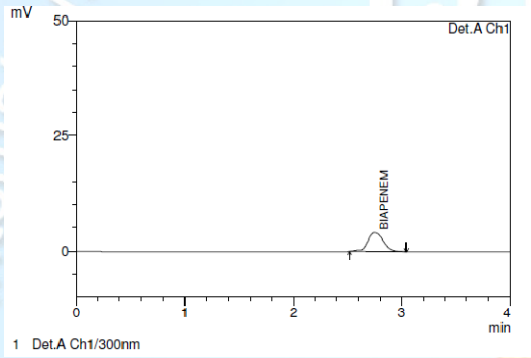
Limits of detection (LOD) and quantification (LOQ) (**Fig-2**) were estimated from both linearity calibration curve method and signal to noise ratio method. The detection limit was defined as the lowest concentration level resulting in a peak area of three times the baseline noise. The quantification limit was defined as the lowest concentration level that provided a peak area with signal to noise ratio higher than 5, with precision (%CV) and accuracy with (±) 20%.

2.6.3 Linearity (Calibration Curve)

The calibration curve was constructed with eight concentrations ranging from 5.35 to 101.15 µg/mL. The linearity was evaluated by linear regression analysis, which was calculated by least square method. It is depicted in (**Fig- 3**).

Fig-2: Chromatograms shown below indicate limit of Detection (LOD) above and Limit of Quantitation (LOQ) below.





2.6.4 Accuracy and Precision

Accuracy of assay method was determined for both intra-day and inter-day variations using triplicate analysis of the QC samples. Precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day). Repeatability refers to the use of the analytical procedure within the laboratory over the shorter period of the time that was evaluated by assaying the QC samples during the same day. Intermediate precision was assessed by comparing the assays on different days (3 days).

2.6.5 Specificity

Specificity of the method was determined by comparing the Blank sample with that of the sample containing Biapenem. (**Fig-4**). A less than 20% interference of the peak area at the retention time of the drug in the blank sample is taken as acceptance criteria for the analyte. Sample Specificity is also observed in the degradation study of the drug. None of the degraded products must interfere with the quantification of the drug.

2.6.6 Stability

The stability of the drug is determined by placing the MQC samples for the short term stability by keeping at room temperature up to 12 hours and then comparing the obtained peak area with that of the similarly prepared fresh sample.

2.6.7 Stress Degradation Studies

For Stress Degradation Analysis, 1 mL aliquots (in duplicate) of samples containing MQC level concentration are treated separately with 100 µL of 0.1N HCl (Acid stress), 0.1N NaOH (Alkaline stress), 5% v/v Hydrogen Peroxide (Oxidative Stress), for 24 Hrs. Samples for Photolytic stress are placed in a transparent glass vial & placed in a UV chamber for 24 Hrs. Samples are then injected for analysis. The results of analysis are then compared with similarly prepared fresh samples.

3.0 RESULTS AND DISCUSSION

3.1 Method Development and Validation

The HPLC procedure was optimized with a view to develop a stability indicating assay method. Functional group analysis revealed the presence of acidic character to the molecule. Therefore we evaluated the chromatographic behavior at different pH values ranging from pH 3.0 to pH 6.5 using various columns like Hypersil-BDS-C18, Symmetry C18, Ymc-pack C18, Ymc-pack pro, Spherisorb C18, Phenomenex C18 have been tried with different buffer salts such ammonium Formate, ortho phosphoric acid, di-potassium hydrogen ortho-phosphate, in combination with acetonitrile, methanol and tetrahydrofuran. However less tailing and high theoretical plates are obtained with Agilent Polaris ODS column C18 150 X 4.6 cm 5µm column. Mobile phase composition consisted of (80:20 v/v) of Methanol and 10mM Sodium acetate (pH adjusted to 3.0 ± 0.1 with glacial acetic acid) on isocratic mode. The flow rate of the method is 1.0 ml/min. Calibration standards were prepared in diluents solution containing 50:50 % v/v of methanol and Milli-Q water. The wavelength of detection is 210nm. The column temperature is maintained at 25 ^{o}C. At the reported flow rate, peak shape was excellent, however increasing or decreasing the flow rate resulted in unacceptable tailing factor and poor peak shape. Hence 1.0 ml/min was optimized flow rate decreasing the consumption of the

mobile phase, which in turn proves to be cost effective for long term routine quality control analysis.

3.2 Method Validation

3.2.1 System Suitability

The % RSD of the peak area and the retention time for both drug and internal standard are within the acceptable range (**Table-1**). The efficiency of the column was expressed as the number of theoretical plates for the six replicate injections was around 9600 ± 25 and the USP tailing factor was 1.29 ± 0.1.

3.2.2 Determination and Quantification Limits (Sensitivity)

Fig-2 represents the chromatogram of limit of detection and limit of quantification. The method is found to be sensitive which can be determined from the data obtained from the (**Table-2)**.

3.3.3 Linearity

The linearity was demonstrated in triplicate. The results of the best fit line (y = mx + c) for the triplicate analysis is given in **Table 3**. The accuracy of the calibration standards was evaluated from the back calculated concentrations **(Table 4)**. All the standards were found to be within the range of 95 – 105 %.

3.3.4 Accuracy and Precision

Accuracy and precision calculated for the QC samples during the intra- and inter –day run are given the (**Table-5)**. The intra-day (day-1) and inter-day accuracy ranged from 98.00 to 102.00 %. The results obtained from intermediate precision (inter-day) also indicated a good method precision .All the data were within the acceptance criteria.

3.3.5 Specificity

Specificity was determined by comparison of the Blank chromatogram with that of the Standard chromatogram (**Fig-4**)

3.3.6 Room Temperature Stability

Stability studies were done for short term stability up to 12 hrs on the bench top for the MQC levels conditions. Stability is calculated as the ratio of the mean peak area of the stability sample to the mean peak area of the fresh sample and expressed as the percentage (n=6). The room temperature stability was found to be 105.61 %. The results are tabulated in **Table-6**.

Fig-3: Linear calibration curve of Biapenem.

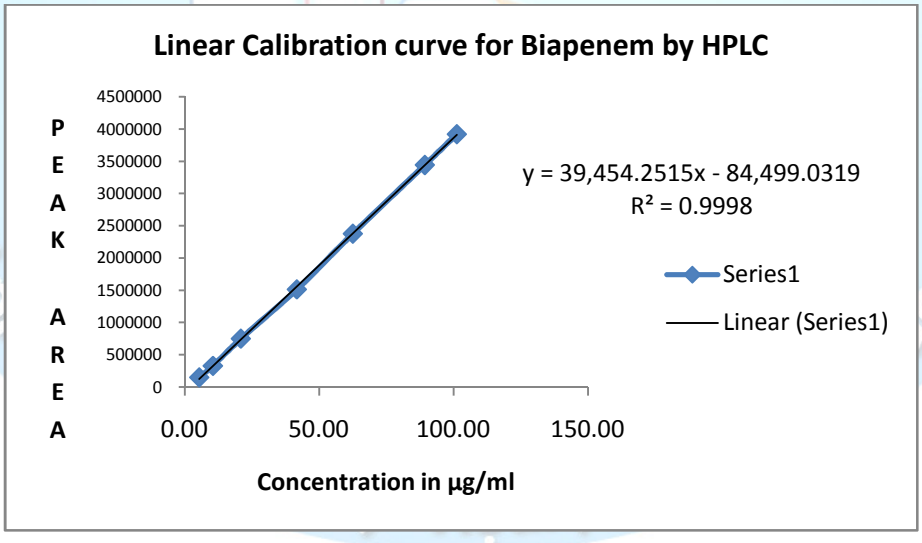
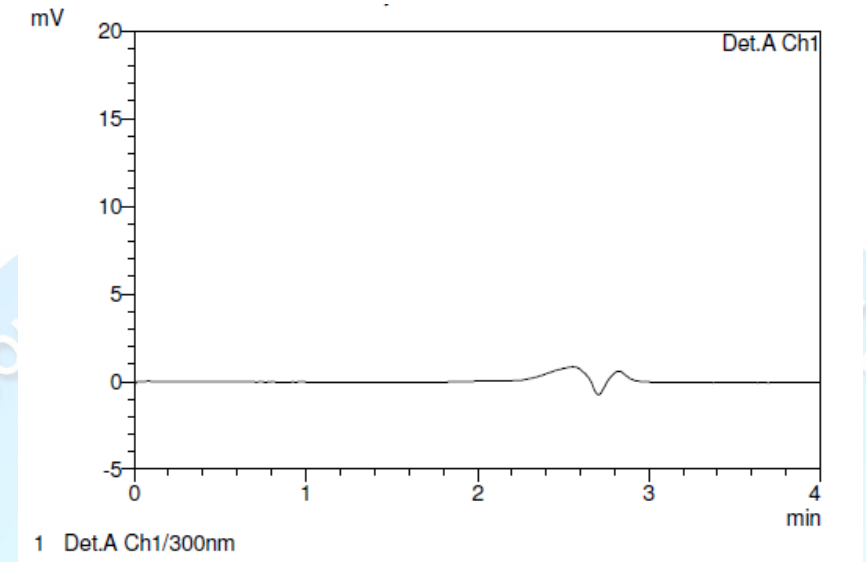


Fig-4: Comparison of Blank Chromatogram (below) to that of sample containing Biapenem (above) standard



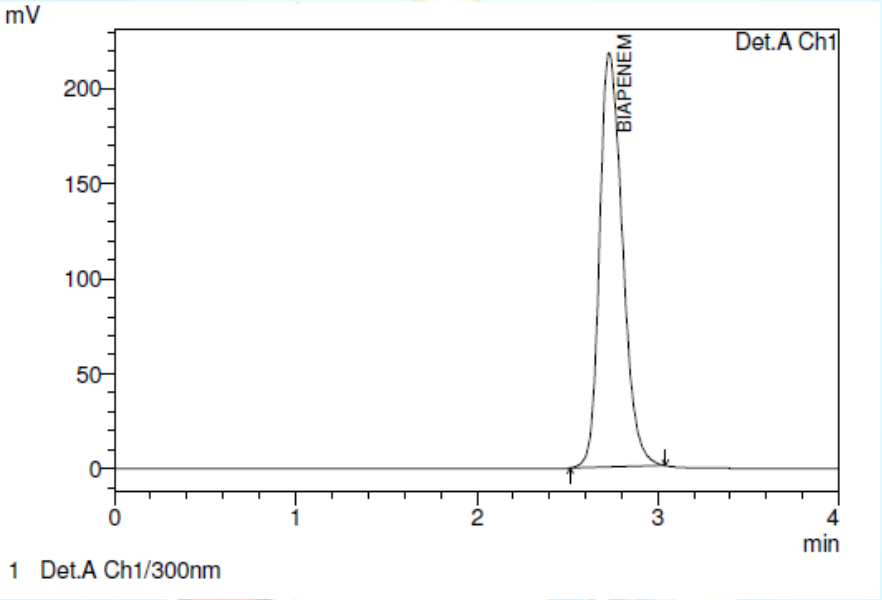


Fig-5: Overlay Chromatogram showing the influence of various stress conditions on Biapenem; Data 1 – Freshly prepared Sample; Data 2 – Oxidative Stress; Data 3 – Photolytic Stress; Data 4 – Acid Stress; Data 5 – Alkaline Stress. Data 5 clearly indicates the spectral degradation of Biapenem due to alkaline instability.

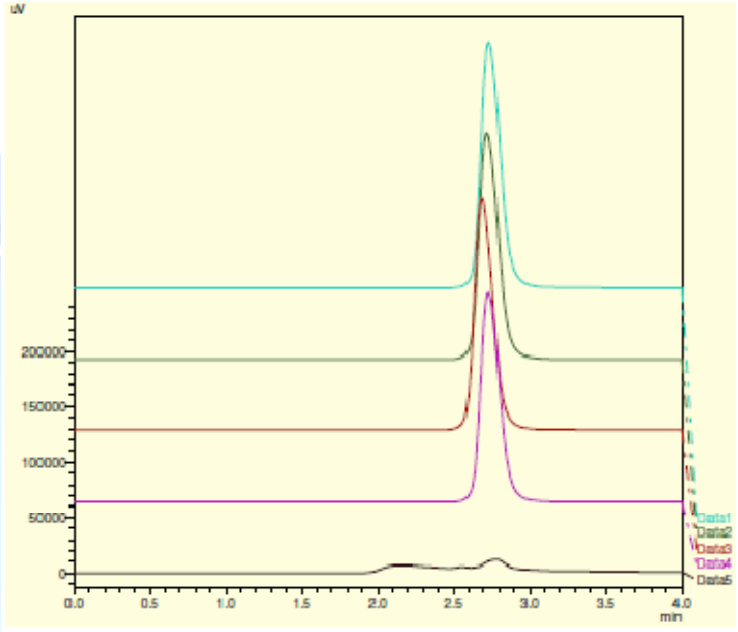


Table 1. System Suitability test for Biapenem

BIAPENEM				
Sr. No	**Retention Time**	**Peak Area**	**Tailing Factor**	**Theoretical Plates**
1	2.70	1911585	1.30	5860
2	2.72	1933717	1.31	5712
3	2.73	1919247	1.31	5694
4	2.72	1954745	1.32	5627
5	2.73	1961497	1.32	5684
6	2.71	1997027	1.31	5568
MEAN	**2.718**	**1946303.000**	**1.312**	**5690.833**
ST DEV	**0.0117**	**31530.38**	**0.01**	**98.27**
% CV	**0.43**	**1.62**	**0.57**	**1.73**

Table 2. Sensitivity of Biapenem by HPLC

LOQ

SR NO	DRUG	
	Retention Time	**Peak Area**
1	2.75	38396
2	2.76	39230
3	2.73	37283
MEAN	**2.7**	**38303.0**
ST DEV	**0.02**	**976.83**
% CV	**0.56**	**2.55**

LOD

SR NO	DRUG	
	Retention Time	**Peak Area**
1	2.77	12451
2	2.77	12682
3	2.77	12409
MEAN	**2.8**	**12514.0**
ST DEV	**0.00**	**147.00**
% CV	**0.00**	**1.17**

Table 3. Results of best-fit line for triplicate analysis

Curve	Slope	Intercept	r^2
1	39454.25	-84499.03	0.9998
2	39697.02	-118079.52	0.9988
3	40124.53	-113599.56	0.9988
Mean	**39758.6**	**-105392.7**	**0.9991**

Table 4. Linearity and Range for Biapenem demonstrating Mean accuracy, carryover effect and specificity of the method (n = 3).

Sr. No	SAMPLE ID	CONC'N (µg/mL)	DRUG		Calculated Conc'n (µg/mL)	Accuracy (%)
			RETENTION TIME	PEAK AREA		
1	BLANK	0.000	NO PEAK	NO PEAK	Nap	NA
2	CC 01	5.35	2.72	115652	5.07	94.73
3	CC 02	10.41	2.73	329183	10.49	100.70
4	CC 03	20.82	2.72	755357	21.29	102.22
5	CC 04	41.65	2.73	1469208	39.38	94.55
6	CC 05	62.47	2.72	2355957	61.86	99.01
7	CC 06	89.25	2.73	3479281	90.33	101.21
8	CC 07	101.15	2.72	3922333	101.56	100.40
9	CO BLANK	0.000	NO PEAK	NO PEAK	NA	NA

- **NA – Not applicable**

Table 5. Results of inter and intra-day accuracy & precision for Biapenem by HPLC

	Nominal Concentration (µg/mL)		
	25.29	50.57	75.86
DAY 1			
MEAN	25.46	49.62	73.82
SD	0.07	1.28	3.14
% CV	0.29	2.58	4.25
DAY 2			
MEAN	25.21	50.28	75.92
SD	0.06	0.95	2.11
% CV	0.27	1.89	2.78
DAY 3			
MEAN	25.18	50.87	75.94
SD	0.11	0.95	1.38
% CV	0.44	1.87	1.82

Table 6. Room Temperature Stability of Biapenem (n = 6).

BIAPENEM

FRESH SAMPLE

SR NO	SAMPLE ID	DRUG	
		RETENTION TIME	PEAK AREA
1	FRESH	2.73	1923652
2	FRESH	2.68	1840851
3	FRESH	2.73	1958151
4	FRESH	2.74	1918823
5	FRESH	2.73	1905948
6	FRESH	2.73	1953856
MEAN			**1926209.00**
STDEV			**24793.32**
% CV			**1.29**

STABILITY SAMPLE

SR NO	SAMPLE ID	DRUG	
		RETENTION TIME	PEAK AREA
1	STABILITY	2.73	1929936
2	STABILITY	2.73	1908284
3	STABILITY	2.73	1894461
4	STABILITY	2.73	1886420
5	STABILITY	2.64	1911349
6	STABILITY	2.73	1897609
MEAN			**1898459.33**
STDEV			**12486.23**
% CV			**0.66**

3.3.7 Stress Degradation

The stress studies involving acid, light (UV) and oxidation revealed that Biapenem was not fully degraded (**Fig 5**). However in alkaline conditions (0.1N NaOH), the drug was instable and the degradation peak eluted earlier accompanied with a drastic peak distortion and increased tailing. Except for alkaline conditions, the drug content was within 95 –105 % for all stress conditions indicating the stability and specificity of the analytical method to differentiate the degradation peaks.

3.3.8 Robustness study

Robustness is the measure of method capacity to remain unaffected by deliberate small changes in the chromatographic conditions. The experimental conditions were deliberately altered to evaluate the robustness of the method. The impact of flow-rate (1.0 ± 0.1 ml/min), and effect of mobile-phase composition (± 5%) on chromatographic parameters such as retention time, theoretical plates, and tailing factor, were studied**.** There was no significant variation due to the variation of mobile phase composition or flow rate variation.

3.4 Application of the method to dosage forms

The HPLC method developed is sensitive and specific for the quantitative determination of Biapenem. Also the method is validated for different parameters, hence has been applied for the estimation of drug in pharmaceutical dosage forms. Biapenem tablets of 80 mg strength from two different manufacturers were evaluated. The amount of Biapenem in tablet 1 is 99.05 ± 0.16 and tablet 2 is 99.35 ± 0.11. None of the tablets ingredients interfered with the analyte peak. The spectrum of Biapenem is extracted from the tablets was matching with that of standard Biapenem showing the purity of peak of Biapenem in the tablets.

Conclusions

The method gave accurate and precise results in the concentration range of 2.53 to 50.57 µg/mL. The mobile phase composition consists of (80:20 v/v) of Methanol and 10 Mm Sodium acetate (pH adjusted to 3.0 with glacial acetic acid), at the flow rate of 1.0 ml/min. The retention times of the drug are 4.40 minutes. The column is Agilent Polaris ODS 2 150 X 4.6mm, C18 column with the particle size of 5µm. A rapid sensitive and specific method for the determination of Biapenem in the pharmaceutical formulations has been developed.

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4. CONCLUSION

The proposed RP-HPLC method for the estimation of Biapenem in dosage form is accurate, precise, linear, rugged, robust, simple and rapid. Hence the present RP-HPLC method is suitable for the quality control of the raw materials, formulations.