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Research Article

Development and Validation of RP-HPLC Methods for the Estimation of API's in Newanti Injection and Restozeal Tablets

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Abstract: Newanti injection comprises of 0.3 mg Biapenem in powder for injection, used as in vitro agent against anaerobes. Resveratrol is an antioxidant in Restozeal tablets. Since HPLC methods were more accurate, precise and sensitive, the present work describes two simple, precise and accurate HPLC methods for the estimation of pharmaceutical agents in Newanti injection (Method A) and Restozeal tablet dosage form (Method B). The separation was carried out using phenomenex C18 (250 x 4.6 mm, 5 µm particle size) column for both the methods. Mobile phase consisting of acetonitrile and phosphate buffer pH (6) in the ratio of 85:15%, v/v, at the flow rate of 0.8 mL/min and detection wavelength of 294 nm was chosen for method A. Acetonitrile: phosphate buffer pH (5) in the ratio of 80:20%, v/v as the mobile phase at the rate of 0.9 ml/min with the detection wavelength of 315 nm were chosen as operating parameters for Method B. The linearity range was found to be 150-350 µg/ml at the retention time of 3.33 min for biapenem; 50-100 µg/ml at 2.88 min for resveratrol. The liquid chromatography methods were extensively validated for linearity, accuracy, precision, and robustness. All these analytical validation parameters were observed to be in limits, which indicate the usefulness of the methods for determination of biapenem/resveratrol in their marketed formulations.
Keywords: Biapenem, Resveratrol, Validation, RP-HPLC, Acetonitrile, Phosphate buffer (pH 6, pH 5).

INTRODUCTION

Biapenem is chemically (4*R*,5*S*,6*S*)-3-(6,7-dihydro-5*H*- pyrazolo[1,2-a][1,2,4]triazol-8- ium-6-ylsulfanyl)-6-(1-hydroxyethyl)- 4-methyl-7-oxo-1-azabicyclo [3.2.0] hept-2- ene-2-carboxylate, used as invitro agent anerobes. Resveratrol is an anti oxidant, which is chemically 5-[(E)-2-(4-hydroxy phenyl) ethyl] benzene-1,3-diol. Literature survey revealed that there are very few LC methods available for estimation of Biapenem [1-3] and Resveratrol [4-14], but no simplified method has been far reported for estimation of pharmaceutical agents in marketed formulations by HPLC. The purpose of this work was to develop a method in economic point of view and for the purpose of routine analysis.

It was resolved to develop simple, rapid, economic, precise, efficient RP-HPLC methods for quantitative analysis of Biapenem and Resveratrol in marketed formulations with less retention time, runtime, simple mobile phase preparation and to validate the method according with ICH guidelines.

MATERIALS AND METHODS

Equipment

SHIMADZU LC-20AD system with SPD-20A UV-Vis detector equipped with Sphinchrom software was used for method development, double-beam Perkin Elmer (LAMBDA 25) UV-Vis spectrophotometer was used for spectral measurements and ELICO pH meter was used for pH measurements.

Reagents and standards

Biapenem and resveratrol were obtained as a gift sample from Aurobindo pharma Ltd, Hyd.,Triple distilled water of HPLC grade, Acetonitrile of HPLC grade, 0.2 M potassium dihydrogen phosphate (KH_2PO_4), 0.2 M sodium hydroxide (NaOH) and 10 M Potassium hydroxide (KOH) which are of AR grade were used for the experimental work.

Method Development

Chromatographic conditions

The separation was achieved on a phenomenex C18 (250 mm x 4.6 mm, 5 µm) column, with a mobile phase of Acetonitrile : buffer (pH 6) in the ratio of 15:85%, v/v and at a flow rate of 0.8 mL/min and detection was monitored at 294 nm for method A and mobile phase of Acetonitrile : buffer (pH 5) in the ratio of 80:20%, v/v and at a flow rate of 0.9 mL/min for method B. The detection was studied at 315 nm. Both the methods were developed at ambient temperature.

Preparation of mobile phase
Method A
Preparation of Phosphate buffer, pH 6

125.0 ml of 0.2 M potassium dihydrogen phosphate was placed in a 250-ml volumetric flask, 14 mL of 0.2 M sodium hydroxide was added and then volume was made with water.

Method B
Preparation of Phosphate buffer, pH 5

2.72 g of KH_2PO_4 was taken in a 1000-ml volumetric flask and 800 ml of water was added, pH was adjusted with 1M KOH volume was adjusted with water. Acetonitrile and phosphate buffers were filtered through 0.45 µm membrane filter and sonicated before use.

Preparation of stock solutions of Biapenem (Method A)

About 50 mg of biapenem was weighed and dissolved in pH 6 buffer, diluted to 50 ml and sonicated for 10 min.

Preparation of stock solution of Resveratrol (Method B)

50 mg of Resveratrol into a 50 ml was weighed in a clean dry volumetric flask, 30 ml of diluent was added and made to the final volume with diluent.

Sample preparation
Method A

Commercial formulations, twenty samples of biapenem powder for injection were taken, weighed. Average weight was calculated and transferred 0.3 mg into 25 mL volumetric flask, dissolved in phosphate buffer (pH 6), diluted to mark and further diluted to get appropriate concentration. The diluted solutions were filtered through 0.45 µm membrane filter paper and degassed. Six replicates were prepared and 20 µL of each dilution was injected in to the column and chromatographed under above mentioned conditions.

Method B

Twenty capsules were weighed accurately. An amount of drug equivalent to 50 mg of Resveratrol was transferred to a 50 ml volumetric flask, add 30 ml of diluent and made the final volume with diluents, sonicated for 20 min, filtered through 0.45 µm whatmann filter paper, diluted to get approximate concentration each of six replicates and 20 µL was injected in to the column and chromatographed as per above mentioned conditions.

Calibration standards
Method A

Different volumes of stock solution were accurately transferred to 10 ml volumetric flasks to reach 100-350 µg/ml concentration of biapenem. Six replicate solutions of the above range each of 20 µL were injected into the HPLC system.

Method B

Different volumes of stock solutions were accurately transferred to 10 ml volumetric flasks to reach 50-100 µg/ml concentration range for resveratrol. Six replicate solutions of the above range each of 20 µL were injected into the HPLC system.

RESULTS AND DISCUSSIONS
Method optimization

Acetonitrile and phosphate buffer (pH 6) in the ratio of 15:85%, v/v for method A and acetonitrile and phosphate buffer (pH 5) 80:20%, v/v for method B were selected as the mobile phase, as it was found ideal to resolve the peaks with retention time 3.33 ± 0.022 min for method A and 2.880±0.03 min for methods B, respectively and shown in the fig-3 and fig-4, respectively. Detection wavelength was chosen by scanning the analytes over a range of wave length from 190-400 nm in a spectrophotometer and the suitable wavelength was found to be 294 nm for method A and 315 nm for method B, respectively. The overlay UV spectra of both the components and their binary mixture were shown in fig. 1 and fig. 2 and the optimization parameters were produced in table 1.

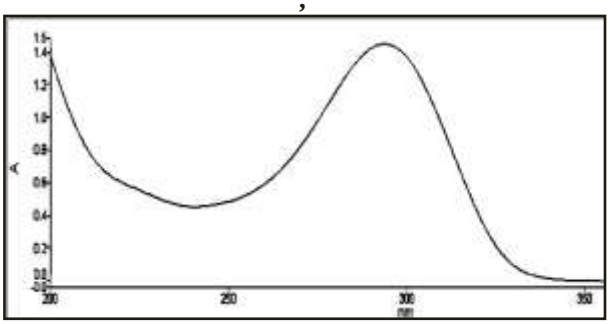

Fig. 1 Absorption sepctrum of Biapenem

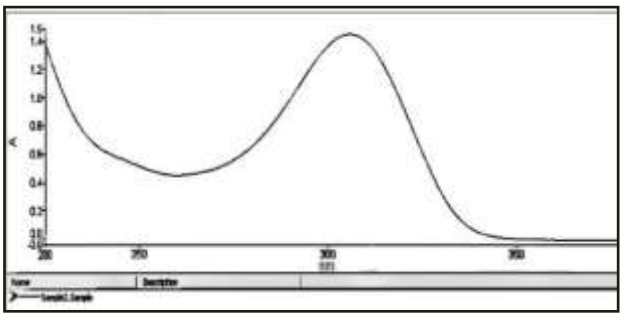

Fig. 2 Absorption spectrum of Resveratrol

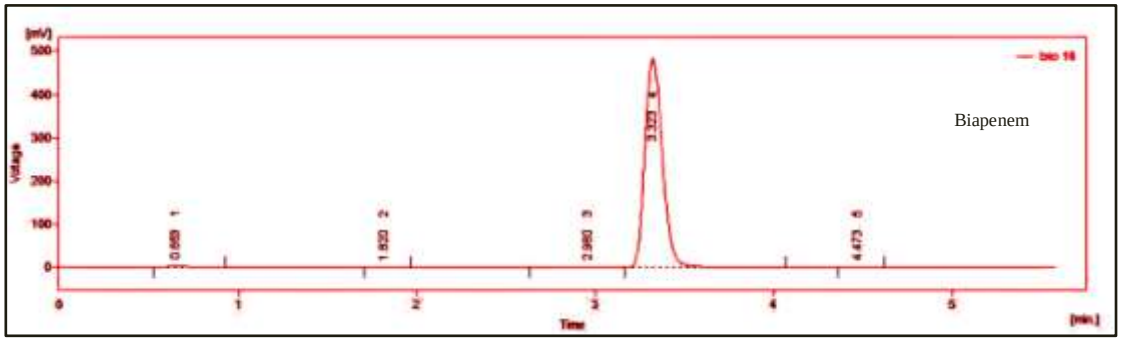

Fig. 3 Chromatogram of Biapenem in Newanti injection

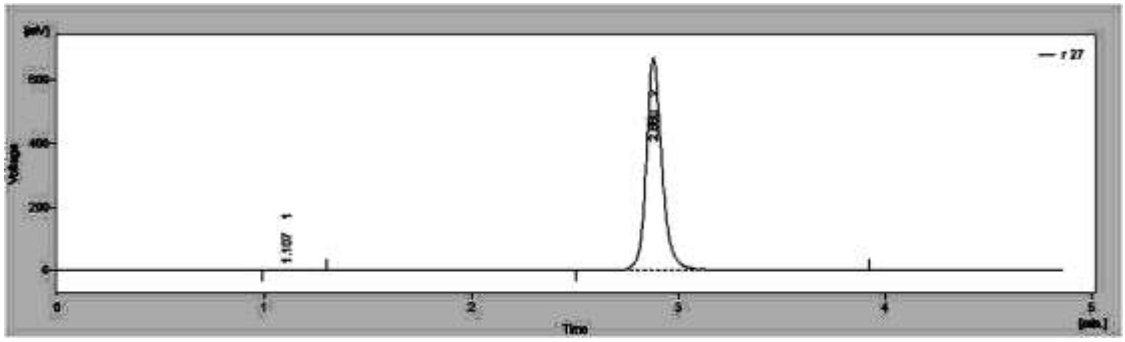

Fig. 4 Chromatogram of Resveratrol

Method validation

After development of HPLC methods, method A and method B were carried out with respect to several parameters like precision, accuracy, linearity, robustness, ruggedness to ensure that the developed method copes with all the requirements for the intended purpose.

System suitability studies

Standard solutions of biapemem (Method A) and resveratrol (Method B) were prepared. Standards set as per test method was injected five times in to HPLC system. The chromatograms were recorded and system suitability parameters were given in table 1.

Table 1: Optimization parameters of Method A and Method B

Parameters	Method A	Method B
	Biapenem	Resveratrol
Column	C_{18} column (250 x 4.6 mm, 5 µ)	
Run time	10 min	
Diluent	Mobile phase	Acetonitrile
Retention time	3.33	2.880
Linearity (µg/mL)	150-350	50-100
LOD (µg/mL)	26	4
LOQ (µg/mL)	80	12
Mean Area	4219	6402
SD	5.913	2.773
%RSD	0.139	0.13
Theoretical plates	17742	20736
Tailing factor	0.85	1.01
Symmetry factor	1.00	1.00

Linearity

Chromatograms were recorded by preparing series of solutions and calibration graph was plotted with area in the Y-axis and concentration of standard solution on the X-axis as follows and results were presented in table 2 and pictured in fig. 5 and fig. 6.

Table 2: Results showing linearity values of Method A and Method B

Sl. No.	Method A		Method B	
	Biapenem		Biapenem	
	Conc. (µg/ml)	Conc. (µg/ml)	Conc. (µg/ml)	Area, mv
1.	150	150	50	4438
2.	200	200	60	5195
3.	250	250	70	5997
4.	300	300	80	6787
5.	350	350	90	7623
6.	-	-	100	8373

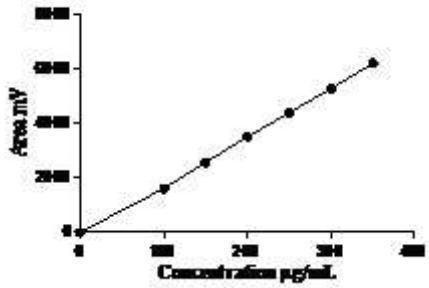

Fig. 5: Linearity plot of Biapenem

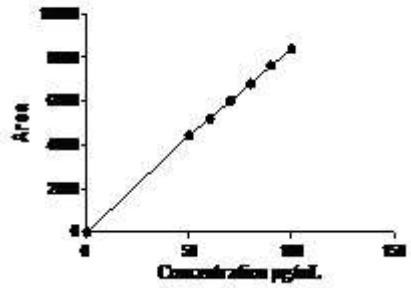

Fig. 6: Linearity plot of Resveratrol

Precision

Method precision and system precision were preformed preparing six replicates of each standard and sample solutions, respectively and the results were presented in table 3.

Table 3: Results showing method precision and system precision

Parameter	Method Precision		System Precision	
	Method A	Method B	Method A	Method B
Sample 1	99.52	99.52	388	4900
Sample 2	101.05	101.05	394	4867
Sample 3	100.07	100.07	386	4967
Sample 4	99.86	99.86	391	5124
Sample 5	99.99	99.99	398	5023
Sample 6	101.61	101.61	390	4978
Mean	100.05	100.05	391	4977
SD	0.8018	0.8018	4.665	91.49
% RSD	0.8018	0.8018	1.19	1.838

SD, Standard Deviation; RSD, Relative Standard Deviation

Accuracy

To evaluate the accuracy of the proposed methods recovery studies were carried out by standard addition method, where a known amount of each drug is added to a pre analyzed tablet powder at 3 levels (50%, 100%, 150%) of the label claim of biapenem and resveratrol. At each level recovery studies were carried out in triplicate and expressed as percent recoveries. The results were presented in table 4 for both Methods.

Table 4 Results of Accuracy studies of Method A and Method B

Sl. No.	Method A		Method B	
	% Recovery	% RSD	% Recovery	% RSD
50%	100.1	0.67	100.4	0.09
100%	100.2	0.23	99.7	0.2
150%	100.08	0.25	100.2	0.18

Robustness

Robustness is measured by reproducibility of test results under normal, expected operational conditions from laboratory to laboratory and from analyst to analyst. Results were found to be acceptable.

Ruggedness

Ruggedness is measured by studying system to system/Analyst to Analyst/column to Column variability and the results were found acceptable.

Limit of detection and Limit of quantification

The LOD and LOQ of the developed methods were determined by analyzing progressively lower concentrations of the standard solutions using optimized chromatographic conditions. The results were shown in table 1.

CONCLUSION

From the present research it was established that the proposed methods were simple, sensitive, reliable, reproducible, rapid and economical and can be employed for routine analysis. The methods were proved to be superior to most of the reported methods and were successfully applied in pharmaceutical formulations.

REFERENCES

1. Ikeda K, Ikawa K, Ikeda A, Nishikawa Y and Morikawa N; A simple and rapid determination of biapenem in plasma by high-performance liquid chromatography. Journal ofChromatography Bio Analytical Technology, Biomed Life Sciences, 2006; 844(1): 148-152.
2. Michalska K, Cielecka PJ, Pajchel G and Tyski S; Determination of biapenem in a medicinal product by micellar electrokinetic chromatography with sweeping in an enhanced electric field. Journal of Chromatography Analysis, 2013; 1282(10): 153-160.
3. Kameda K, Ikawa K, Ikeda K, Morikawa N, Nakashima A, Ohge H, Sueda T. HPLC method for measuring meropenem and biapenem concentrations in human peritoneal fluid and bile: application to comparative pharmacokinetic investigations. Journal of Chromatographic Sciences, 2010; 48(5): 406-411,
4. Gurinder Singh, Roopa S. Pai, Vinay Pandit, Development and validation of a HPLC method for the determination of *trans*-resveratrol in spiked human plasma. Journal of Advanced Pharmaceutical Technology & Research, 2012; 3(2): 130-136.
5. Chen X, Stereospecific determination of cis & trans resveratrol in rat plasma by HPLC; application of pharmacokinetic study. Biomed chromatography, 2007; 21(3): 257-65.
6. Z.Pineiro, M. Palma,C.G. Barroso, Determination of trans-resveratrol in grapes by pressurised liquid extraction and fast high-performance liquid chromatography. Journal of Chromatography A, 2006; 1110: 61–65.
7. Richerd B.van Breemen, Yan Wand, An LCMS method for analyzing total resveratrol in grape juice and wine. Journal of agricultural and food chemistry, 2002; 50(3): 431-435.
8. Ludovic Le Corre, Anne Leger-Enreille, Nassera Chalabi, Laetiti Delort, Yves-Jean Bignon, and Dominique J. Bernard-Gallon, determined HPLC Analysis of Trans-Resveratrol in Human Plasma After Red Wine Consumption. The Open Chemical and Biomedical Methods Journal, 2008; 1: 7-10.
9. Laszlo Mark, Martin S. Pour Nikfardjam, Peter Avar, and Robert Ohmacht, Validated HPLC Method for the Quantitative Analysis of Trans-Resveratrol and Trans-Piceid in Hungarian Wines. Journal of Chromatographic Science, 2005; 43: 445-449.
10. Nuno Ratola, Joaquim Luis Faria and Arminda Alves, Analysis and Quantification of trans-Resveratrol in Wines from Alentejo Region (Portugal) Food Technol. Biotechnol, 2004; 42 (2): 125–130
11. Romero-Perez, Rosa M. Lamuela-Ravento, Cristina Andres-L, and Carmen de la Torre-Boronat M, Quantitative Extraction of Resveratrol and Piceid Isomers in Grape Berry Skins. Effect of Powdery Mildew on the stilbene Content. Journal of Agriculture and Food Chemistry, 2001; 49: 210-215.
12. S.S. Lee , S.M. Lee , M. Kim , J. Chun , Y.K. Cheong d, J. Lee, Analysis of trans-resveratrol in

peanuts and peanut butters consumed in Korea. Food Research International, 2004; 37: 247–251

13. AndreH A. Souto, Manuel C. Carneiro, Marcus Seferin, Marcos J. H. Senna, Andressa Conz, Kristiane Gobbi, Determination of trans-Resveratrol Concentrations in Brazilian Red Wines by hplc. Journal of food composition and analysis, 2001; 14: 441-445.
14. Careri M , Direct HPLC analysis of t-resevetrol and quercetin in red wine, grape and wine making by products. Journal of agriculture and food chemistry, 2003; 51(18):5226-31.