Stability-indicating derivative spectrophotometry method for the determination of biapenem in the presence of its degradation products

Judyta Cielecka-Piontek1*, Aran Lunzer2, Anna Jelińska1

1Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Poznan University of Medical Sciences, 60-780 Poznan, Poland
2Tanaka Laboratory in Hokkaido University, W 7, Kita-ku, Sapporo 060-8638, Japan

Received 5 July 2010; Accepted 7 October 2010

Abstract: A first-derivative UV spectrophotometric method, with or without the subtraction technique, was developed for the determination of biapenem in pharmaceutical dosage form in the presence of its degradation products. The method was based on the measurement of first-derivative amplitudes at zero crossing point (λ = 312 nm) and the peak-to-zero technique and validated with regard to linearity, limits of detection and quantitation, selectivity and precision. The observed rate constants for the degradation of biapenem were comparable to those obtained in the stability-indicating HPLC method.

Keywords: Biapenem • Degradation • First-derivative UV

1. Introduction

Biapenem (Fig. 1) is a new parenteral carbapenem antibacterial agent, approved for use in Japan. It has a broad-spectrum activity against Gram-positive and Gram-negative aerobic and anaerobic bacteria [1–3]. Similarly to meropenem, ertapenem and doripenem; biapenem has a 1-β-methyl side chain that provides resistance to the renal enzyme I-dehydropeptidase [4].

The literature reports only the use of high-performance liquid chromatography for the identification of degradation products of biapenem in aqueous solutions. It has been proven that below pH 3.50, impurities I, II and dimer B of biapenem reached the top (Fig. 2) and in solutions of pH above 3.50, open-ring hydrolysis products and an open-ring dimer of biapenem were formed (Fig. 3). An isocratic HPLC method was developed for determining biapenem in plasma [5] and peritoneal fluid [6]. For the identification of the impurities of biapenem preparative a gradient RP-HPLC, LC-M/MS and NMR method were used [7].

Derivative spectrophotometry (first derivative, first derivative of ratio spectra and bivariate analysis) was used for the stability-indicating determination of ertapenem [8,9], meropenem [10] and doripenem [11].

In this work spectrophotometric studies of the degradation of biapenem were conducted in order to develop a simple and fast analytical method, for the quantification of biapenem in the presence of its degradation products (dimers A–B and impurities I–III).

2. Experimental Procedure

2.1. Chemicals

Biapenem for injection, OMEGACIN® (Meiji Seika Kaisha, Ltd. Tokyo Japan), is a white sterile powder. Injection vials contain 300 mg of biapenem. Purity of powdered biapenem was 98%.

All other chemicals and solvents were obtained from Merck KGaA (Germany) and were of analytical grade. High-quality pure water was prepared using the Millipore purification system (Millipore, Molsheim, France, model Exil SA 67120).
2.2. Apparatus and experimental conditions
A UV-VIS Lambda 20 (Perkin Elmed) spectrophotometer equipped with 1.0 cm-in-width quartz cells and controlled by the UV WinLab software was utilized. The first derivatives of the zero-order spectra of test solutions were recorded over the ranges 200.0 – 320.0 nm (Δλ = 4 nm) and at a scaling factor of 10. The amplitudes of the first derivative peaks of biapenem were measured at 278 nm and at 312 nm.

2.3. Spectrophotometric procedure
Standard solutions for spectrophotometric determination were prepared as follows: 25.0 mg of biapenem was accurately weighed, transferred into a 25 mL volume flask, and dissolved with distilled water (solution A). Working solutions ((1.60–9.60) × 10⁻² mg mL⁻¹) were prepared from solution A.

2.4. Validation of the UV method
The UV method was validated according to the International Conference on Harmonisation Guidelines [12].

2.4.1. Linearity
Calibration plots \(D^1 = f(c)\) were obtained in the range \((1.60–9.60) \times 10^{-2}\) mg mL⁻¹ by plotting the measured peak amplitude at the first derivative curve, at \(\lambda = 278\) nm and \(\lambda = 312\) nm.

2.4.2. Precision
Precision of the assay was determined in relation to repeatability (intra-day) and intermediate precision (inter-day). In order to evaluate the repeatability of the method six samples were determined during the same day for three concentrations \((3.20 \times 10^{-2}, 6.40 \times 10^{-2}, 9.60 \times 10^{-2}\) mg mL⁻¹). The intermediate precision (at the biapenem concentration \(6.40 \times 10^{-2}\) mg mL⁻¹) was studied by comparing the assays performed on two different days.

2.4.3. Limit of detection (LOD) and limit of quantitation (LOQ)
The LOD and LOQ parameters were determined from the regression equation:

\[
\text{LOD} = \frac{3.3 \times S_y}{a}, \quad \text{LOQ} = \frac{10 \times S_y}{a};
\]

where \(S_y\) is a standard error and \(a\) is the slope of the corresponding calibration curve.

2.5. Kinetic studies of biapenem in aqueous solutions
The determination of biapenem in the presence of its degradation products was studied at 313 K in hydrochloric acid (pH 0.82, 2.05), phosphate buffers (pH 2.14, 3.25 and 6.48, 7.31), acetate buffer (pH 3.88, 4.99), borate

---

Figure 1. Chemical structure of biapenem

Figure 2. Proposed molecular structure of impurity I (a), impurity II (b) and dimer B (c) [7].
buffer (pH 8.23, 10.11) and carbonate buffer (pH 10.57). The pH values of the reaction solutions, and those of the buffer standards used to calibrate the pH-meter were measured at the temperature of reactions. The pH values of the reaction solutions in HCl were calculated from the equation pH = -\log f_{HCl} [HCl] [13]. The ionic strength of all solutions was adjusted to 0.5 mol L\(^{-1}\) with a solution of sodium chloride (4 mol L\(^{-1}\)).

Degradation was initiated by dissolving an accurately weighed 5.0 mg of OMEGACIN\(\textregistered\) in 25.0 mL of reaction solution equilibrated to 313 K in stoppered flasks. At selected times, samples of reaction solutions (1.0 mL) were collected and instantly cooled with a mixture of ice and water. Samples from the pH range 0.85–2.05 and 8.23–10.57 were neutralized with 0.8 mL of NaOH solution or HCl of suitable concentration. In case of neutralization of borate and carbonate buffers, HCl concentration were calculated for neutralization of base components of these buffers ([NaBO\(_2\)] or [Na\(_2\)CO\(_3\)]). For samples from this range, 1.0 mL of distilled water was added to each of them. For other samples 1.8 mL of distilled water was added.

### 3. Results and Discussion

#### 3.1. Method development and validation

The zero-order absorption spectra of biapenem and its degradation products showed spectral interferences. In the first-derivative spectra the interferences were considerably reduced. At zero-crossing wavelengths of 278 and 312 nm the first-derivative spectra were linear.

The UV method was validated; validation parameters are listed in Table 1. The calibration curves for both methods were linear and described by the equation y = (125.2 ± 9.6)x at λ = 278 nm and y = (210.8 ± 5.6)x at λ = 312 nm (n = 13). The parameters of regression were calculated for f = n–2 degrees of freedom and α = 0.05. The values b, calculated from the equation y = ax + b, were not significant. The method had good intra-day repeatability (RSD from 0.41% to 2.16%) and inter-day repeatability (RSD 0.64% and 0.96%) (Table 1). Only in case of precision determined for 120% of concentration of biapenem used in kinetic studies was slightly bigger than 2%. The comparison of precision of
stability-indicating derivative spectroscopy and HPLC method [14] showed that the calculated values \( F \) were found to be lower than the corresponding theoretical ones, what indicates the good precision also of the UV method (Table 1).

3.2. Spectrophotometric studies of the degradation of biapenem

The UV absorption spectra of biapenem and its degradation products overlapped, while their first-derivative spectra showed significant differences in values of peak amplitudes, which (\( \lambda = 312 \) nm) permitted the determination of biapenem in the presence of degradation products. During the degradation of biapenem depending on the pH of a solution, different degradation products were formed [7]. Below pH 3.50, impurities I and II and dimer B (dimer of symmetrically-structured biapenem) (Fig. 2), with the absorption maximum similar to that of biapenem, reached the top. Due to the application of first-derivative (\( D_1 \)) UV spectrophotometry (\( \lambda = 312 \) nm) for determination of biapenem in the pH range 0.82–3.25 was necessary to use the subtraction technique because the value (\( D_1 \))$_{\text{max}}$ decreased to the value (\( D_1 \))$_{\infty}$ > 0 over a time period from \( t_0 \) to \( t_\infty \) (Fig. 4a). In solutions of pH above 3.50, open-ring hydrolysis products and an open-ring dimer of biapenem were formed (Fig. 3), which did not disturb in the application of first-derivative (\( D_1 \)) UV spectrophotometry (\( \lambda = 312 \) nm) for the determination of biapenem in the presence of its products degradation in the acetate (pH = 3.88–4.71), phosphate (pH = 6.48–7.51), borate (pH = 8.23–10.11) and carbonate (pH = 10.57) buffers (Figs. 4b–c).

Similarly to the degradation of ertapenem [9], meropenem [10] and doripenem [11], shifts of the amplitude maximum of the first derivatives of biapenem, resulting from the formation of the degradants, were observed. The products of biapenem degradation caused a bathochromic effect in the phosphate (pH=6.48–7.51), borate (pH=8.23–10.11) and carbonate (pH = 10.57) buffers (Fig. 4c) and a hypochromatic effect in the acetate buffer (pH = 3.88–4.71) (Fig. 4b).

3.3. Kinetic studies of biapenem in aqueous solutions

During the degradation of biapenem in hydrochloric acid (pH = 0.83–2.05) and phosphate buffer (pH = 2.40–3.25) the value (\( D_1 \))$_{\text{max}}$ decreased to value (\( D_1 \))$_{\infty}$ > 0 over a period of time from \( t_0 \) to \( t_\infty \) and the reaction reached a steady state. The pseudo-first order reaction was described by the equation:

\[
\ln (D_1 - D_1\infty) = \ln(D_1 - D_1\infty)_0 - k_{obs} \times t
\]

The plots \( \ln (D_1 - D_1\infty) = f(t) \) were linear and the slopes of the plots (with the negative sign) corresponded the observed rate constants (Fig. 5).

During the degradation of biapenem in phosphate buffer (pH = 6.48–7.31), acetate buffer (pH = 3.88–4.71), borate buffer (pH = 8.23–10.11) and carbonate buffer (pH = 10.57) the pseudo-first order reaction was described by the equation:

\[
\ln D_1 = \ln D_1^0 - k_{obs} \times t
\]

The observed rate constants were equal to the slopes of the plots \( \ln (D_1) = f(t) \) with the negative sign (\( -k_{obs} \)).

Figure 5. Semilogarithmic plots \( \ln D_1 = f(t) \) (I) and \( \ln (D_1 - D_1\infty) = f(t) \) (II) for the degradation of biapenem in 0.05 mol L$^{-1}$ HCl at 313 K.

Table 1. The statistical analysis of the results of the determination of biapenem with the UV method

| Parameters                                      | UV (first derivative) |
|------------------------------------------------|-----------------------|
| \( \lambda \) (nm)                             | \( \lambda = 278 \) nm | \( \lambda = 312 \) nm |
| Concentration range (mg mL$^{-1}$)             | \( (1.60-9.60) \times 10^2 \) | \( 3.20 \) |
| Slope                                          | 210.81                | 160.19                |
| Correlation coefficient – \( r \)              | 0.9933                | 0.9941                |
| LOD (mg mL$^{-1}$)                             | \( 1.08 \times 10^2 \) | \( 3.27 \times 10^2 \) |
| LOQ (mg mL$^{-1}$)                             | \( 3.7 \times 10^2 \) | \( 1.13 \times 10^2 \) |
| Concentration of biapenem 10$^{-2}$ mg mL$^{-1}$ | 0.52                  | 0.45                  |
| Intra-day repeatability [%]                    | 0.41                  | 0.58                  |
| Values \( F_{\text{cal}} \) 5.05/Values \( F_{\text{cal}} \) | 2.16                  | 1.33                  |
| Inter-day repeatability [%] for 6.40 \( \times 10^2 \) mg mL$^{-1}$ | 6.40                  | 1.78                  |
Table 2. Observed rate constants and coefficients a for the degradation of biapenem in the pH range 0.85–10.57, at 313 K

| pH   | HPLC method | UV method | t₀ |
|------|-------------|-----------|----|
|      | 10² × a min⁻¹ | kobs s⁻¹ | 10² × a min⁻¹ | kobs s⁻¹ |    |
|      | hydrochloric acid |          |           |          |    |
| 2.05 | -2.97 ± 0.28  | (4.95 ± 0.47) 10⁻³ | -3.02 ± 0.2 | (5.04 ± 2.03) 10⁻² | 0.4840 |
| 2.14 | -3.38 ± 0.13  | (5.63 ± 0.10) 10⁻⁴ | -3.52 ± 0.21 | (5.87 ± 0.17) 10⁻⁵ | 1.7771 |
| 2.45 | -0.86 ± 0.02  | (1.43 ± 0.03) 10⁻⁴ | -0.83 ± 0.02 | (1.39 ± 3.55) 10⁻⁵ | 0.0679 |
| 3.88 | -16.30 ± 0.13  | (2.82 ± 0.21) 10⁻⁵ | -14.00 ± 0.06 | (2.33 ± 0.11) 10⁻⁵ | 0.0443 |
| 4.71 | -2.30 ± 0.18  | (3.83 ± 3.00) 10⁻⁴ | -2.09 ± 0.29 | (3.49 ± 0.49) 10⁻⁵ | 0.7249 |
| 6.26 | -8.54 ± 0.82  | (2.37 ± 1.36) 10⁻⁵ | -8.09 ± 0.23 | (2.25 ± 3.86) 10⁻⁵ | 1.5584 |
| 7.31 | -9.86 ± 0.33  | (2.74 ± 5.62) 10⁻⁵ | -9.43 ± 0.05 | (2.62 ± 0.82) 10⁻⁵ | 2.0712 |
| 8.23 | -42.0 ± 3.48  | (7.01 ± 0.58) 10⁻³ | -38.10 ± 2.12 | (6.36 ± 3.54) 10⁻² | 2.0311 |
| 10.11 | -120.0 ± 19.30  | (2.00 ± 0.32) 10⁻² | -101 ± 16.80 | (1.69 ± 2.80) 10⁻² | 1.7177 |
| 10.57 | -11.70 ± 1.29  | (1.95 ± 0.21) 10⁻³ | -11.50 ± 0.04 | (1.92 ± 0.08) 10⁻³ | 0.1069 |

*observed rate constants calculated from first-derivative spectra by using subtraction technique
**observed rate constants calculated from first-derivative spectra, calculated by parallelism test

The observed rate constants determined by using the UV and HPLC methods [14]. The parallelism test was used to verify that the values of kobs determined by either of the methods were statistically insignificant (Table 2).

4. Conclusions

The proposed first-derivative UV method, with or without the subtraction technique, allows simple and selective quantitative analysis for the determination of biapenem in pharmaceutical preparation and in the presence of its degradation products. It is also faster and more cost-effective than the HPLC method.

Acknowledgements

The authors are grateful to Aran Lunzer for providing the substance for research. This study was supported by a grant from the State Committee for Scientific Research, Poland (no. N N405 683040).

References

[1] A. Watanabe, H. Kikuchi et al., J. Infect. Chemother. 5, 171 (1999)
[2] M. Hikida, M. Mori, T. Kitta, Chemother. 42, 101 (1994)
[3] K. Aldridge, N. Morice, D. Schiro, Antimicrob. Agents Chemother. 38, 889 (1994)
[4] M. Hikida, K. Kawashima, K. Nishiki et al., Antimicrob. Agents Chemother. 36, 481 (1992)
[5] I. Kayo, I. Kazuro., I. Aki, N.N. Yoshimi, M. Norifumi, J. Chrom. B 844, 148 (2006)
[6] I. Kayo, I. Kazuro, M. Norifumi, K. Keiko, U. Nami, O. Hiroki et al., J. Chrom. B 867, 20, (2008)
[7] M. Xia, T.J. Hang, F. Zhang, L. Xiao-Min, X.Y. Xia, J. Biomed. Pharm. Anal. 49, 937 (2009)
[8] Y.H. Nagiba, M. Ezzat, A. Nariman, R. Mamdouh, Spectrochimica Acta Part. A. 72, 915 (2009)
[9] M. Zajac, J. Cielecka-Piontek, A. Jelińska, Anal. Chem. 51, 761 (2006)
[10] A. Nariman, M. Ezzat, Y.H. Nagiba, R. Mamdouh, R. Talanta, 77, 28 (2008)
[11] J. Cielecka-Piontek, A. Jelińska, Spectrochim. Acta A Mol. Biomol. Spectrosc. 77, 554 (2010)
[12] Validation of analytical procedures, Proceeding of the International Conference of Harmonisation (ICH), Commission of the European Communities (2009)
Stability-indicating derivative spectrophotometry method for the determination of biapenem in the presence of its degradation products

[13] E. Pawełczyk, T. Hermann, The Fundamentals of stability drugs (PZWL, Warsaw, 2006) (in Polish)

[14] J. Cielecka-Piontek, A. Lunzer, A. Krause, P. Zalewski, A. Jelińska, Acta Chromatographica 2010 (in press)