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Determination of biapenem in a medicinal product by micellar electrokinetic chromatography with sweeping in an enhanced electric field☆

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ABSTRACT

The elaborated method of micellar electrokinetic chromatography (MEKC) used to separate biapenem from its related substances was successfully implemented using sweeping under an enhanced electric field, followed by UV absorption detection at 200 nm. The best results were obtained with formic buffer (22.5 mM) pH 4.3 and sodium dodecyl sulfate (150 mM) added to the electrolyte as the sweeping agent. Neutral capillary (60/50 cm; 50 μm ID) with reverse polarity and voltage values of 22 kV, were used throughout the investigation.

The optimized method of biapenem determination, validated in terms of linearity, accuracy and precision, provides a detection limit of 0.5 μg/mL at S/N = 3 for biapenem. The repeatability of the CE system, expressed by relative standard deviations (RSD) in the migration times, for biapenem and its degradation products varied from 0.14 to 1.48%, whereas for the corrected peak areas RSD were about 0.68–8.43%. Satisfactory separation was achieved within 20 min of electrophoresis; moreover all carbapenems (imipenem, meropenem, ertapenem, doripenem and biapenem) were separated from each other during analysis. The evaluated MEKC method was applied to the analysis of a medicinal product containing biapenem – Omegacin® 0.3 g for intravenous drip infusion.

1. Introduction

Biapenem (Fig. 1) (marketing authorization holder – Meiji Seika Pharma Co., Ltd.) is a new parenteral carbapenem antibiotic, approved for use in 2002 in Japan, with a broad spectrum of activity encompassing many Gram-positive and Gram-negative aerobic and anaerobic bacteria, including species producing β-lactamases. Clinical trials have demonstrated its effectiveness in the treatment of many moderate to severe infections of the intra-abdominal area, lower respiratory tract, including bacterial pneumonia and complicated urinary tract [1]. Biapenem has a β-methyl substitution at C-1 on the carbapenem moiety to reduce the ability of dihydropeptidase-I (DHP-I) to attack the β-lactam unit.

A few methods have been developed to measure plasma levels of biapenem using a microbiological assay [2] and high-performance liquid chromatography (HPLC) with UV detection [3,4]. Xia and co-workers, while studying the stability of biapenem in aqueous solution by a reverse-phase HPLC method, identified the degradation products using a liquid chromatography–tandem mass spectrometric (LC–MS/MS) method as well as determined the structure of dimer B by nuclear magnetic resonance (NMR) spectroscopy [5]. During this study, several related substances were disclosed: three hydrolysis degradation products, impurities I and II, a pair of inter-converting tautomeric isomers, both with open rings, impurity III, a synthetic process impurity as well as two dimers: (A) an open ring dimer, and (B) dimer of biapenem with a symmetrical structure (Fig. 2). Additionally, Cielecka-Piontek et al. have investigated extensively the stability and kinetics of biapenem in the presence of degradation products in aqueous solution, using derivative spectrophotometry for this purpose [6].

The advantages of capillary electrophoresis (CE) in comparison with HPLC are well known. Among the many benefits of CE that may be mentioned, its extraordinarily high efficiency of separation, environmentally friendly nature associated with minimum sample and reagent consumption, and fast separation speed, all contribute to CE being regarded as a cost-effective analytical tool. Unfortunately, it also has several disadvantages. One of the main drawbacks of CE with UV detection is its poor concentration sensitivity, associated with a short optical path length equal to the capillary diameter. Hence several strategies have been proposed to improve detection limits in CE systems with UV detection, including sweeping as a superior and general approach to an on-line

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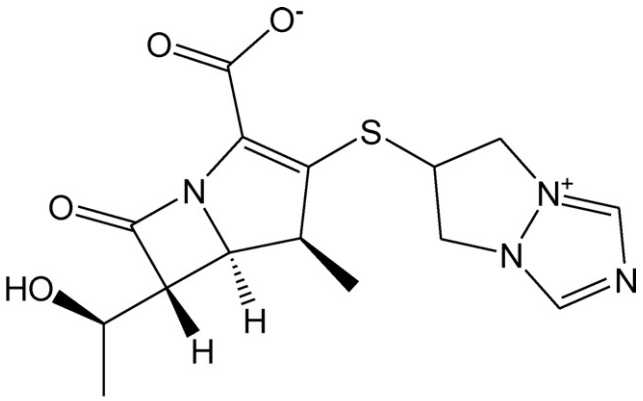


Biapenem

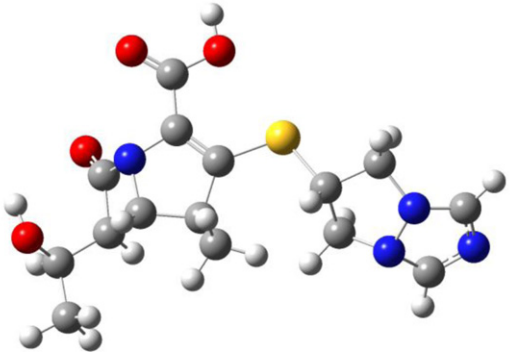


Fig. 1. Chemical structure and equilibrium geometries of biapenem [31].

sample preconcentration technique. In the past few years, many publications as well as excellent review articles on sweeping have been presented [7–13].

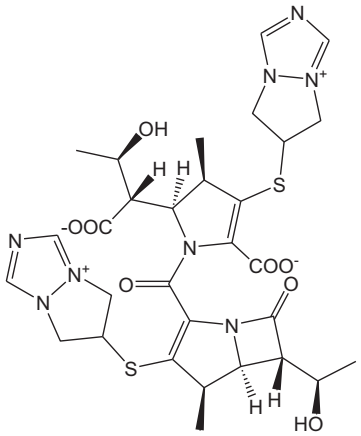
The aim of this study was to develop a fast, accurate and sensitive CE method for the quantitative determination of biapenem in the presence of related substances in a medicinal product. To the best of our knowledge, this is the first study using a sweeping-micellar electrokinetic chromatography (MEKC) technique to analyse biapenem in pharmaceutical dosage form. However, over the past few years, several CE methodologies have been reported for the analysis of carbapenem antibiotics, mainly applying capillary zone electrophoresis (CZE) and MEKC separation modes [14–17].

2. Materials and methods

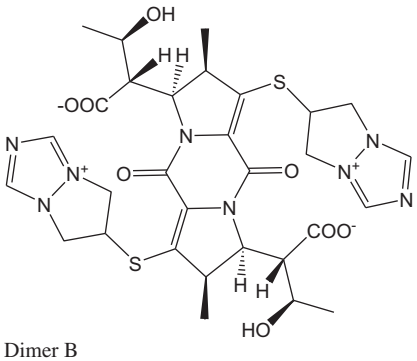
2.1. Materials

Biapenem for infusion (Omegacin® 0.3 g for intravenous drip infusion) was supplied by Meiji Seika Pharma Co., Ltd. (Tokyo, Japan). Biapenem hydroxide, an inner salt reference standard was purchased from Molekula Limited (Dorset, United Kingdom). Doripenem monohydrate reference standard was kindly supplied by Shionogi (Osaka, Japan). The ertapenem sodium reference standard was obtained from Merck Research Laboratories (Rahway, NJ, USA). Certified reference standards of imipenem and meropenem were obtained from the European Directorate for the Quality of Medicines and HealthCare (Strasbourg, France).

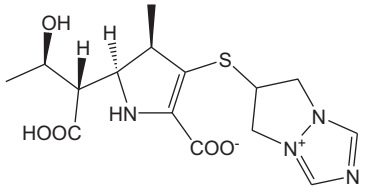
Formic acid was purchased from Merck KGaA (Darmstadt, Germany). Citrate/MES buffer, pH 6.0 was provided by Beckman Coulter, Inc. (Fullerton, CA, USA). Other reagents such as phosphoric acid (Merck KGaA, Darmstadt, Germany), acetic acid



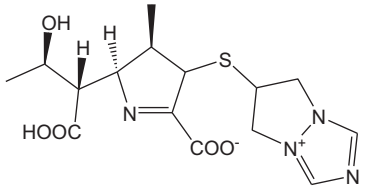
Dimer A



Dimer B



Impurity I



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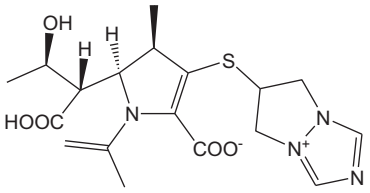

Impurity III

Fig. 2. Proposed molecular structures of biapenem impurities [5].

(AppliChem GmbH, Darmstadt, Germany), sodium acetate (POCH, Gliwice, Poland), citric acid (POCH, Gliwice, Poland), sodium citrate (AppliChem GmbH, Darmstadt, Germany), malic acid (Sigma–Aldrich, Steinheim, Germany), tartaric acid (POCH, Gliwice,