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Development of a gradient elution preparative high performance liquid chromatography method for the recovery of the antibiotic ertapenem from crystallization process streams

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Dedicated to Prof. Georges Guiochon.

Abstract

A gradient elution preparative chromatography method was developed for the recovery of the antibiotic ertapenem from crystallization mother-liquor streams. The preparative HPLC method that was developed on the lab-scale employs an analytical size column of conventional dimensions (25 cm × 0.46 cm) packed with Kromasil C8 stationary phase. Gradient elution was used with aqueous acetic acid and acetonitrile as mobile phases. A target of processing approximately 30 mg of ertapenem per half an hour at a flow rate of 1.5 mL/min with high yield and adequate rejection of all major impurities was achieved. This corresponds to a productivity of ∼0.6 kg ertapenem as free acid per kilogram of stationary phase per day (kkd). The scalability of the method was demonstrated by using a 5 cm i.d. column configuration to generate 10 g of purified ertapenem. This work complements a previous study improving on the productivity and throughput of the method by employing gradient elution and the use of crystallization to remove some key impurities that are chromatographically difficult to resolve [A. Vailaya, P. Sajonz, O. Sudah, V. Capodanno, R. Helmy, F.D. Antia, J. Chromatogr. A 1079 (2005) 80].

Keywords: Antibiotics; Carbapenem; Ertapenem; Preparative chromatography; Scale-up; Process streams; Product recovery

1. Introduction

Ertapenem sodium is a novel synthetic 1β-methylcarbapenem antibiotic that is structurally related to β-lactam antibiotics such as penicillins and cephalosporins [2]. It has activity against a wide range of Gram-negative and Gram-positive aerobic and anaerobic bacteria and a long half-life that allows once-daily dosing. Ertapenem shows stability against both β-lactamase and renal dehydropeptidase I, and therefore does not have to be combined with an inhibitor such as cilastatin for protection against enzymatic hydrolysis. The chemical structure of ertapenem is shown in Fig. 1. The ertapenem molecule consists of a carbapenem ring and a thiaproline amide side chain. The bi-cyclic 4:5 fused ring is common to all carbapenems, and it is one of the main reasons for the thermal instability of these compounds [2–8].

Ertapenem bulk drug substance is synthesized as a monosodium salt [9–11]. It is the active pharmaceutical ingredient in the formulated drug product, marketed under the trademark INVANZ® by Merck & Co. Inc., Whitehouse Station, NJ, USA. INVANZ® was approved by the United States Food and Drug Administration in November 2001 for the treatment of adult patients with moderate to severe infections, such as complicated intra-abdominal infections, community acquired pneumonia and complicated urinary tract infections, that are caused by specific strains of susceptible microorganisms. INVANZ® is a parenteral antibiotic that is administered intravenously or intramuscularly [2,3].

Ertapenem sodium synthesis is carried out with a yield of approximately 60% [9,11]. Product losses to process mother-liquor streams during the crystallization of the monosodium salt contribute approximately to 10–20% of the mass balance,

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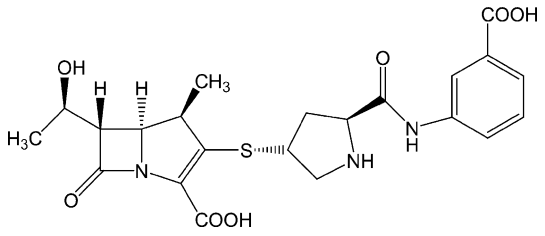

Fig. 1. Chemical structure of ertapenem as free carboxylic acid.

indicating that the incorporation of an effective recovery process for ertapenem could be economically quite valuable. The recovery of product from waste streams is generally a very difficult task. This is because waste streams often have a very complex composition and low product concentrations. Consequently, much developmental work is required to find practical solutions for making product recovery from process streams possible and economically feasible. Because of the complexity of the sample matrix, process development is often conducted empirically. In the case of ertapenem, an additional difficulty is associated with the instability of the drug [1,11]. The presence of degradation products along with process-related impurities, solvents and salts in process streams makes ertapenem recovery even more challenging.

The availability of a wide range of stationary phases with unique selectivities combined with the high efficiencies of packed columns makes reversed phase preparative chromatography a powerful technology for industrial-scale purification of complex process streams. As a result, the use of preparative chromatography in process development is increasingly gaining popularity in the pharmaceutical industry. Several applications of complex preparative HPLC purifications have appeared in the scientific literature recently [12–25]. However, none of these applications are associated with antibiotic purification or product recovery from complex process streams such as crystallization mother-liquors.

In a previous study, the development of an isocratic preparative HPLC method was discussed [1]. An approach based on pH mismatch between the feed and the eluent was used. High-purity ertapenem could be obtained by this method, however, the productivity was somehow limited due to the pH mismatch, and also because of the isocratic elution mode. Such an approach was not considered robust and productive enough to employ routinely for processing large quantities of mother-liquor. In this paper the development of a gradient elution preparative HPLC method on laboratory-scale using an analytical size column is described for the recovery of the antibiotic ertapenem from crystallization streams. The method employs gradient elution instead of isocratic elution to enhance the productivity throughput of the process as well as the concentration of the recovered product. It was found that some key impurities that are chromatographically difficult to resolve are rejected by using crystallization [1]. This fact led to a significantly improved chromatographic purification process that is economically more feasible than the previously reported chromatographic procedure employing isocratic elution and pH mismatch.

The scalability of the method is demonstrated on a 5 cm i.d. column configuration. The development of the preparative HPLC method is one part of the process for the recovery of ertapenem. As discussed in our previous study [1], the complete approach also involves extraction of mother-liquors to generate HPLC feed and, after the purification by reversed phase preparative chromatography, evaporation of recovered ertapenem followed by recycling of the recovered product into the manufacturing process stream.

2. Experimental

2.1. Chemicals

Distilled water was purified using a HYDRO System (Hydro Service & Supplies Inc., Garfield, NJ, USA). Sodium hydroxide (50%), *ortho* phosphoric acid (85%), glacial acetic acid and sulfuric acid were purchased from Fisher Scientific (Fisher Scientific, Fair Lawn, NJ, USA). Acetonitrile, methanol and 2-propanol were obtained from EM Science (Gibbstown, NJ, USA) and 1-ethyl-pyrrolidinone (NEP), 3-methyl-1-butanol (*iso*-amyl alcohol, IAA) and sodium chloride from Aldrich (St. Louis, MO, USA). Ertapenem sodium reference standard was supplied by Merck Sample Repository (Merck Research Laboratories, Rahway, NJ, USA). Process mother-liquors were obtained from Merck Manufacturing Division, Danville, PA, USA.

2.2. Preparation of HPLC feed samples

The feed samples for preparative HPLC were derived from ertapenem crystallization mother-liquor streams containing ca. 3–5 g/L of ertapenem. They were used neat, lyophilized or extracted. The following procedures describe the lyophilization and extraction techniques applied.

2.2.1. Lyophilization

Prior to lyophilization the amount of organic solvents in the sample was reduced under vacuum at subambient temperatures using a rotary evaporator. The mother-liquor samples were then lyophilized at ambient temperature using a Labconco 12 port chamber 75228 freeze dryer (Labconco Corp., Kansas City, MO, USA). This procedure took about 12–24 h. After lyophilization was complete, the samples were stored in glass vials at −70 °C.

2.2.2. Extraction

Extraction using IAA (*iso*-amyl alcohol) was used to concentrate ertapenem in the mother-liquor and to remove most of the organic solvents. Two IAA extractions were performed. For the first extraction, the mother-liquors were concentrated with 6 mg/mL sodium chloride then contacted with 3 volumes of IAA. An equal volume of IAA to aqueous was used in the second extraction. For small-scale experiments the mother-liquor was agitated in a 1000 mL Erlenmeyer flask at 0–5 °C with a magnetic stir bar, for larger scale in a 6 gallon agitated blow-can. The blow-can jacket was maintained at 0 °C resulting in a batch temperature of ∼10 °C. The IAA extraction reduced the