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Exploiting pH mismatch in preparative high-performance liquid chromatographic recovery of ertapenem from mother liquor streams

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Dedicated to the memory of Csaba Horváth, professor, mentor and friend.

Abstract

Preparative chromatography was successfully employed to recover ertapenem from mother liquor streams. The recovery process involved concentration of mother liquor stream by evaporation, purification by reversed-phase preparative high-performance liquid chromatography (HPLC), and removal of chromatographic solvents in the recovered fractions by evaporation. HPLC feed was prepared by stripping off the organic solvents from the mother liquor using a wiped-film evaporator. Purification was first carried out on a 25 cm × 0.46 cm analytical column packed with 10-μm Kromasil C8 particles and then scaled up to a 25 cm × 5 cm preparative column. Gram-level recovery of ertapenem with high purity was achieved by exploiting a novel approach based on pH mismatch between the feed and the eluent. Purified ertapenem streams from preparative HPLC runs were combined, evaporated and recycled into the crystallizer for ertapenem isolation.

Keywords: Reversed-phase; Preparative chromatography; Scale-up; Antibiotics

1. Introduction

The discovery and development of new antibiotics has drawn significant attention in recent years due to the rising prevalence of multidrug resistant bacteria [1–3]. The most popular antibiotics of today are penicillins, cephalosporins, penems and carbapenems, which include a common 4:5 fused lactam ring [4]. Among these antibiotics, the carbapenems have the broadest spectrum of activity against a wide range of Gram-negative and Gram-positive aerobic and anaerobic pathogens [4,5]. They are resistant to most β-lactamases because of the unusual trans-conformation of the hydroxyethyl side chain [6,7]. Most marketed carbapenems, however, have a relatively short elimination half-life of about 1 h, which necessitates frequent parenteral administration. Ertapenem is a long acting synthetic 1β-methylcarbapenem antibiotic that is marketed by Merck & Co., Whitehouse Station, NJ, USA as a disodium salt formulation under the trade name INVANZ for the treatment of a broad spectrum of bacterial infections [8]. It demonstrates enhanced stability towards hydrolysis by human renal dehydropeptidase I enzyme thus offering significant medical and economic advantages [9]. Administered as a once-daily intravenous or intra-muscular dosing, it has been shown to be effective for treating serious upper and lower respiratory tract infections, intra-abdominal infections, complicated skin and skin-structure infections, community-acquired pneumonia, acute pelvic infections and urinary tract infections [10–17].

Ertapenem active pharmaceutical ingredient is synthesized as a monosodium salt [18,19]. During manufacturing a significant amount of ertapenem is lost in the crystallization mother liquor stream. It is estimated that the overall process yield can be increased by as much as 7–10% even if only 50% of ertapenem in the mother liquor is recovered. Developing an efficient and cost-effective process for the recovery and purification of ertapenem from mother liquor streams is therefore important from the viewpoint of large-

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scale manufacturing. Typically, recovery of products from waste streams is challenging because of low product concentrations and the complex nature of waste streams. Long lead-times for development work and high cost of finding economical solutions often impede such recovery efforts. The composition of ertapenem mother liquor crystallization streams is also fairly complex. Owing to the highly strained structure of its β-lactam ring, ertapanem is prone to facile degradation through hydrolysis, methanolysis, and ethanolysis in water at high and low pH [20]. Additionally, at higher ertapenem concentrations, dimers and dehydrated dimers are formed [21]. Several of these degradation products are already present in the mother liquor stream. The major challenges associated with ertapenem recovery and purification from the mother liquor stream include the low concentration of ertapenem (typically from 3 to 5 mg/mL), high impurity levels (60–70 area % by chromatographic analysis) and presence of various salts, acids and organic solvents along with a large number of structurally similar impurities and degradation products. Reversed-phase preparative high-performance liquid chromatography (HPLC) is becoming an increasingly popular industrial purification process for product recovery because it offers a highly efficient system with a wide range of selectivity to achieve the desired level of purification [22]. This was the rationale to investigate a chromatographic recovery process for ertapenem.

The focus of many articles on carbapenems thus far has been the discovery and development of a new carbapenem, its structural characterization and aqueous stability [5–6,20,23–28]. Some studies [29–32] describing analytical methods for the determination of antibiotics in aqueous solutions and biological fluids have appeared in the scientific literature recently but there have been no reports on efficient methods for the preparative purification of carbapenems. In this paper, we report a preparative chromatographic process to recover ertapenem from mother liquor streams. The recovery process involves pretreatment of mother liquor to generate the column feed, purification by reversed-phase preparative HPLC, and treatment of purified fractions for recycling. A novel approach based on pH mismatch between column feed and mobile phase is employed during the purification step to separate all process-related impurities and degradation products from ertapenem and recover material with high purity. Process feasibility is first demonstrated in the lab using an analytical-scale column. Then the scale-up is demonstrated on a 5 cm i.d. column.

2. Experimental

2.1. Materials

Ertapenem sodium is described as [4*R*-[3(3*S**,5*S**), 4α,5β,6β(*R**)]]-3-[[5-[[(3-carboxyphenyl)amino]carbonyl]-3-pyrrolidinyl]thio]-6-(1-hydroxyethyl)-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid monosodium salt. Its chemical structure is shown in Fig. 1. Reference standards of ertapenem sodium and isolated dimers (I + II, III and dimer-H_2O) were supplied by the sample repository at Merck Research Labs., Rahway, NJ, USA and stored at −70 °C until further use. The purity of reference standard was determined to be 88.6%. Process mother liquors containing ertapenem were obtained from Technical Operations at Merck Manufacturing Division, Danville, PA, USA and stored at −70 °C to prevent degradation. Throughout the paper, dimers and dehydrated dimers will be referred to as "dimers".

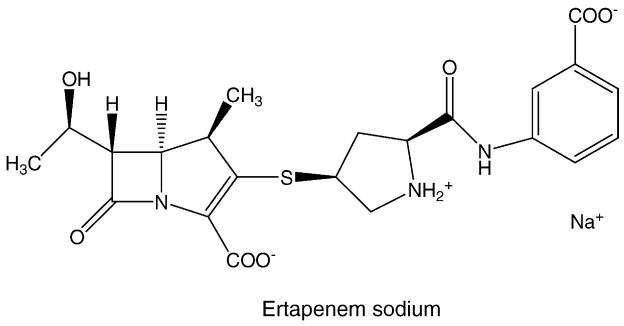

Fig. 1. Chemical structure of ertapenem sodium.

Distilled water deionized with a HYDRO System (Garfield, NJ, USA) was used in experiments. Glacial acetic acid, 50 wt.% sodium hydroxide and 85 wt.% orthophosphoric acid were obtained from Fisher Scientific, Fair Lawn, PA, USA. HPLC grade acetonitrile was obtained from EM Science, USA. Sodium bicarbonate was purchased from Fisher Scientific, Fair Lawn, PA, USA.

2.2. Apparatus

Pope wiped-film evaporators (Pope Scientific, Saukville, WI, USA) with minimal contact time were used to provide one-pass or two-pass concentration of ertapenem mother liquors and preparative HPLC rich cuts. A 2 in. diameter evaporator was used for initial small-scale studies whereas a 4 in. i.d. model was used for prep-scale processing. Both models had jacketed glass bodies, agitated stainless steel rotors supplied with tachometers, spring-loaded polymer wipers, and were heated with a Haake or FTS recirculating bath containing a 50/50 antifreeze/water solution. A separate Neslab recirculator provided this solution to a series of external condensers. The target distillate cut was controlled for each pass by setting the jacket service temperature and adjusting the feed rate on a digitally controlled Cole-Parmer peristaltic pump. The vacuum level provided by a vacuum pump was set at 35 mbar. The vacuum traps were cooled with dry ice/acetone. The feed and bottoms stream volumes were measured after each run and the actual distillate cut was calculated from the relationship: Feed volume − Bottoms volume/Feed volume. The concentration factor was calculated as the ratio of the Feed Volume/Bottoms Volume.