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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".

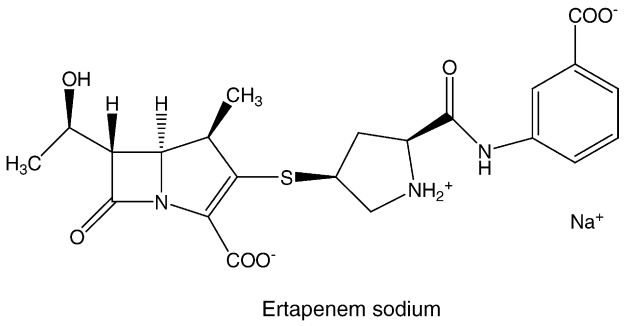

Fig. 1. Chemical structure of ertapenem sodium.

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.

Three HPLC instruments were employed in the present study. An Agilent 1100 series analytical HPLC system (Agilent Technologies, Palo Alto CA, USA) equipped with a quaternary pump (Model G1311A) and a diode array detector (Model G1315A) was modified for conducting lab-scale preparative chromatography experiments. The Agilent 1100 autosampler was bypassed and a Rheodyne 77251 manual injector with a 2-mL loop was placed between the mixer and the column to allow large-volume injections on an analytical-scale column. Short 1/16th-in. diameter tubings were used to minimize dead volume and sample loss. A 1000 μL Hamilton syringe was used to fill the injection loop with the sample. Kromasil C8 columns (Eka Chemicals, GA, USA) having a dimension of 25 cm × 0.46 cm and packed with 10 μm particles were used for lab-scale preparative chromatography experiments. The system was operated by an HP (Palo Alto, USA) personal computer using Agilent's Chemstation software, Revision A 0901 (1206). A Varian HPLC system (Palo Alto, CA, USA) equipped with two Dynamax Model SD-1 gradient pumps, one Dynamax SD-200 pump for sample introduction and a Model UV-1 absorbance detector was used for preparative HPLC scale-up runs. The system was operated using the Varian Star Chromatography Workstation software version 5.5.2. Bulk C8 stationary phase having particle size of 10 μm and a pore size of 100 Å was purchased from Kromasil (Eka Chemicals) for preparative chromatography. A Prochrom Series LC50VE self-packing dynamic axial compression HPLC column skid of 5 cm i.d. was employed for column packing. The column was packed with Kromasil C8 stationary phase using a slurry technique with isopropanol/acetone as solvents. An amount of 325 g of packing material was used to give an effective column length of approximately 25 cm. Another Agilent 1100 Series HPLC system (Agilent Technologies) equipped with a quaternary pump (Model G1311A), an autosampler (Model G1329A) and a diode array detector (Model G1315A) was used for ertapenem analysis (purity and concentration) of mother liquor samples and fractions collected from preparative HPLC experiments. The HPLC system was controlled by a DEC (Nashua, NH, USA) personal computer using Agilent's Chemstation software, Revision A 0901 (1206). A Rheodyne Model 7125I injector (Rheodyne, CA, USA) was used to inject ertapenem samples.

2.3. Procedures

2.3.1. Evaporation

HPLC feed for prep-scale studies was prepared from ertapenem mother liquors as follows. The samples were defrosted from a fully frozen state at −70 °C, diluted by adding one part of deionized water to four parts of mother liquor (25% or 1.25 times dilution), and treated with 2.00–2.67 equivalents of solid sodium bicarbonate (1.13–1.50 g added per liter of mother liquors). One liter of the treated liquor at a time was cooled in ice baths (−10 to +2 °C) and slowly fed to the evaporator while the remaining treated liquor was held in a refrigerator (+2 °C) until needed. A water–ice bath was used to keep the aqueous bottoms cold during collection and the final bottoms product streams were frozen at −70 °C after the pH of the collected sample was adjusted to 7.2 by adding sodium hydroxide.

Preparative HPLC rich cuts, held at −70 °C freezer, were defrosted for 10–15 min using a 40 °C water bath. The pH was adjusted from 3.8 to 6.8 using 5.1 g of sodium bicarbonate per liter of rich cuts and the pH-adjusted feed was held at 0–5 °C throughout processing. The treated rich cuts were fed at 15–20 mL/min to the 4 in. Pope evaporator operating at approximately 250 rpm and 30–60 mbar pressure with 40–55 °C re-circulation in the service jacket. Concentration was completed in one-pass. A small (5 ml) deionized water follow-flush was used to rinse the feed lines and evaporator occasionally when it was necessary to stop the feed as well as at the end of the charge. A water–ice bath was used to keep the aqueous bottoms cold during collection and the final bottoms product stream was frozen at −70 °C after processing.

2.3.2. Preparative chromatography

The protocol for lab-scale preparative HPLC experiments was established as follows: mobile phase A was made up of deionized water and glacial acetic acid (0.1% by volume), while mobile phase B was HPLC grade acetonitrile. The pH of mobile phase A was established across the range from 3.3 to 5.8 by adding sodium hydroxide. The column temperature was set at 25 °C. Since the Agilent 1100 HPLC instrument has a UV detector cell designed for analytical separation, it was limited in its ability to monitor the ertapenem peak due to saturation under overloaded conditions. Detection at higher wavelength, i.e. 300, 340 or 360 nm, was found to be ideal for monitoring the separation and determination of cut points for fraction collection. A 25 cm × 0.46 cm Kromasil column packed with 10 μm C8 stationary phase was conditioned once as follows. It was washed for an hour with 90% B at a flow rate of 1.5 mL/min; then the manual injector was turned to the load position and equilibrated along with the column with 5% B for 1 h at the same flow rate. The gradient profile employed for the experiments consisted of an equilibration cycle followed by an elution cycle and finally a gradient wash cycle. The details of this method are as follows: equilibrate for 3 min at either 1 or 5% B, elute with 13% B for 14 min, then ramp up to 90% B in 8 min, and finally equilibrate at initial condition for 5 min. The overall run time of the method at a column flow rate of 1.5 mL/min was 30 min including 5 min of column equilibration at the initial mobile phase condition. The evaporated mother liquor sample was diluted appropriately with deionized water and then adjusted with dilute acetic acid to a desired pH prior to injection onto the column. After filling the loop of the manual injector with the sample using a 1 mL syringe, the modified analytical HPLC was started without using the Agilent 1100 auto injection system. Fractions were collected at desired intervals during the run and either analyzed real-time or immediately frozen at −70 °C for future analysis. After completion of the HPLC run the in-

jection port and the syringe were rinsed with deionized water and the rinse solutions were analyzed to determine the exact amount of ertapenem injected onto the column.

As with lab-scale experiments, two mobile phases, aqueous stream (A) made up of deionized water and glacial acetic acid (0.1%, v/v) and HPLC grade acetonitrile (B), were employed in the scale-up of preparative HPLC on a 5 cm i.d. column. The pumps for each stream were then primed by pumping their streams to waste for 3 min at a flow rate of 200 mL/min. The column was equilibrated with 5% acetonitrile at a total flow rate of 118 mL/min until the base line at the UV wavelength of 360 nm leveled off. The evaporated mother liquor feed at pH 7.2 containing approximately 30 g/L of ertapenem was diluted 1.8-fold with deionized water to a pH of 6.5 and ertapenem concentration of approximately 15 g/L. The adjusted feed stream was then loaded onto the column at a flow rate of 50 mL/min using the feed pump after eluent flow to the column was turned off. Once the feed stream was injected, the gradient program was activated as follows: equilibration cycle for 9.5 min at 5% B, then a step gradient to 13% B in 30 s to initiate the isocratic elution cycle for 12 min followed by a wash cycle, i.e., a steep gradient to 90% B in 9 min. Fractions were collected by capturing the column eluent stream into polyethylene bottles. Each collected fraction was sampled for characterization and frozen at −70 °C.

2.3.3. Analysis

Ertapenem analysis for impurity profile and concentration was performed using an HPLC analytical method employing a 25 cm × 0.46 cm YMC basic column (Waters, Milford, MA, USA) packed with 5 μm C8 stationary phase and 0.05% phosphoric acid at pH of 2.2 (A) and acetonitrile (B) mobile phase system. The temperature was set at 25 °C with column flow rate of 1.5 mL/min. Sample injection volume was set at 10 μL. UV detection of ertapenem and its impurities was monitored at 220 nm. At this wavelength, the response of the detector was linear in the concentration range analyzed for all components investigated. The following gradient was employed for elution: hold at 10% B for 5 min, then ramp up linearly to 30% B in 15 min followed by another linear ramp to 90% B in 5 min. At the end of gradient run the column was equilibrated for 5 min at the initial mobile phase condition. The analytical method was able to separate ertapenem and all impurities and degradates with high resolution, thus providing accurate impurity profile and concentration data. All impurity profile results were reported as area % (a%) of the main peak and of individual impurities, whereas concentration results were reported in mg/mL or g/L.

A 0.2 mg/mL ertapenem standard solution for concentration measurement was prepared in the following manner. Ertapenem reference standard was defrosted for 15 min inside a dry box with relative humidity <10% and quickly weighed since it is unstable at ambient conditions. Water content of the defrosted reference standard was also determined inside the dry box to ensure the sample had not picked up moisture. Approximately 20 mg of the standard was accurately weighed into a suitable sized volumetric flask and diluted with 100 mL of deionized water to prepare the 0.2 mg/mL ertapenem standard solution. The prepared standard solutions were injected onto the chromatographic column immediately or stored in LC vials in the freezer for later use.

Mother liquor samples were defrosted for 10 min and diluted 25 times with deionized water prior to injecting onto the chromatograph. Evaporated mother liquor samples for preparative HPLC purification were defrosted for 10 min and diluted 100 times prior to HPLC analysis. Syringe and waste line rinses were diluted 25 times with deionized water prior to analysis. Preparative HPLC fractions containing ertapenem were diluted at least 20–25 times with deionized water prior to HPLC analysis.

3. Results and discussion

3.1. Chromatographic recovery process

A typical ertapenem mother liquor sample contains several process-related impurities and degradation products as shown in the analytical HPLC impurity profile in Fig. 2. The structures of these impurities were identified by LC/MS and NMR and are shown in Figs. 3 and 4. The major impurities in the mother liquor are ring-opened hydrolysis product at 10–30 a% and dimers I + II (inter-converting tautomeric isomers), dimer III and dimer-H_2Oa and dimer-H_2Ob at 10–20 a% combined. Acetic acid adduct and methanolysis

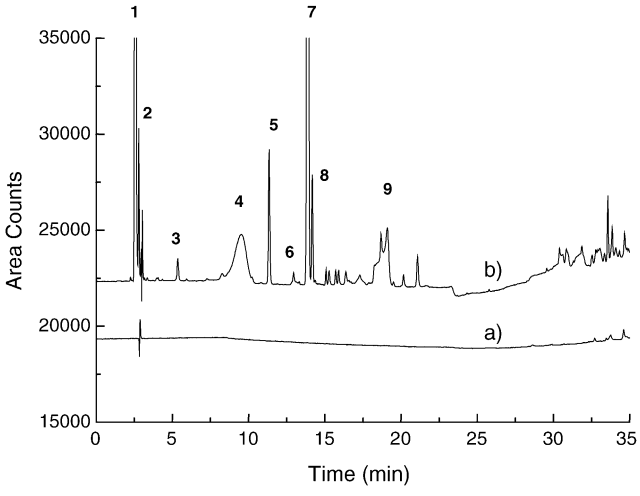

Fig. 2. Analytical HPLC chromatograms illustrating the impurity profile of (a) acetonitrile and (b) a typical mother liquor stream. (1) tetramethyl guanidine, (2) 1-ethyl-2-pyrrolidinone, (3) proline *m*-aminobenzoic acid, (4) ring-opened hydrolysis product, (5) oxazinone, (6) methanolysis and acetic acid adduct, (7) ertapenem, (8) *p*-aminobenzene ertapenem and, (9) dimers (dimers I + II, III, dimer-H_2Oa and dimer-H_2Ob). Instrument: Agilent HP1100 HPLC; detection: UV at 220 nm; mobile phase: acetonitrile (B)/0.05% (v/v) phosphoric acid (A), pH at 2.2; temperature: 25 °C; column: YMC basic C8 (25 cm × 4.6 mm × 5 μm); flow rate: 1.5 mL/min; gradient profile: hold at 10% B for 5 min, then ramp up linearly to 30% B in 15 min followed by linear ramp to 90% B in 5 min; sample injection volume: 10 μL.

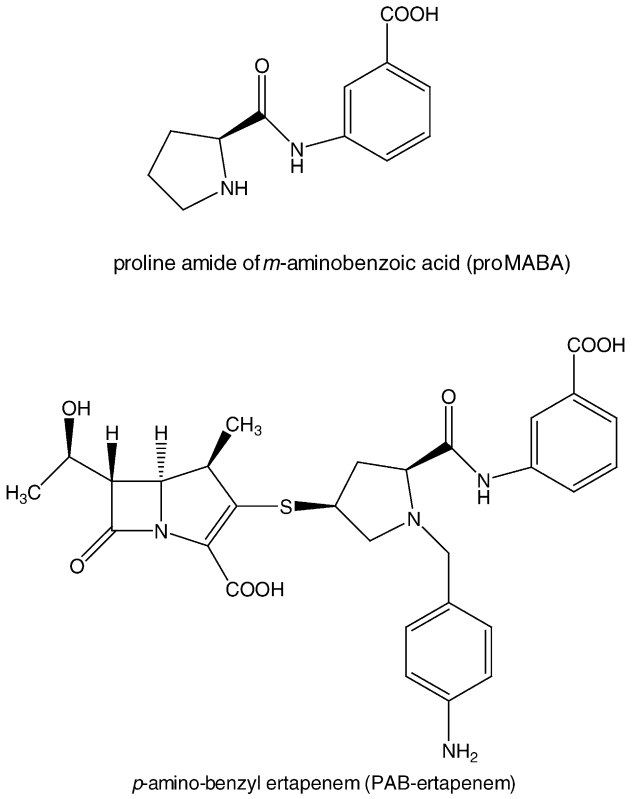

Fig. 3. Chemical structures of process-related impurities in ertapenem mother liquor stream.

impurities elute very close to each other in the impurity profile assay. Their combined levels vary significantly in mother liquors, from as low as 1 a% to as high as 10 a% relative to ertapenem, depending on storage conditions. Other impurities are present at much lower levels, such as oxazinone at 5 a%, *p*-aminobenzyl ertapenem (PAB-ertapenem) at 0.5–1 a% and proline amide of *m*-aminobenzoic acid (proMABA) at 3 a%, but they are not well rejected during crystallization and, therefore, are considered critical from the viewpoint of recovery. In addition, organic solvents, such as methanol and *n*-propanol, constitute 65% (v/v) of the mother liquor stream, along with 0.5% of isoamyl alcohol and trace amounts of 1-ethyl-2-pyrrolidinone. Also present in mother liquor sample are salts and process reagents, such as sodium chloride, sodium acetate, tetramethyl guanidine and diphenyl phosphate. Ertapenem concentration of mother liquor as determined by HPLC assay using an ertapenem reference standard is typically 3–5 g/L.

The mother liquor matrix is not ideal for reversed-phase chromatography due to the presence of a significant amount of organic solvents. Initial experiments with a YMC basic C8 analytical-scale column (25 cm × 0.46 cm), in the linear chromatography mode, yielded adequate separation and good peak shapes when up to 10 μL of a mother liquor sample containing 3–5 mg/mL of ertapenem was injected onto the chromatograph. However, chromatographic separation started deteriorating as injection volume was increased further and considerable breakthrough of ertapenem was observed at overloaded (non-linear chromatography) conditions. Not surprisingly, at higher injection volumes of mother liquor the sample matrix becomes increasingly important and the presence of large amounts of organic solvents does not provide adequate conditions for ertapenem binding to the chromatographic media. It was clear that the mother liquor stream had to be pre-treated to remove organic solvents for reversed-phase preparative HPLC to be effective in ertapenem recovery. Treatment of mother liquor was also necessary to increase the concentration of ertapenem so that a larger amount could be injected onto the preparative chromatograph while keeping the injection volume reasonably small for convenience.

An economical recovery process for ertapenem would involve recycling of recovered ertapenem into the synthetic process. Such a strategy places stringent constraints on the quality of the recycled stream. For instance, the recycled stream would need to have an impurity profile similar to that of the crystallization batch and not contain any new solvents or impurities. In order to meet these requirements, chromatography-related solvents needed to be stripped away from the HPLC ertapenem stream. Fig. 5 illustrates the schematic of a three-step recovery process that was employed in the current study. First, the mother liquor stream post ertapenem crystallization was evaporated to prepare the feed for chromatographic purification. Second, reversed-phase preparative HPLC was employed to purify and recover ertapenem from the treated mother liquor stream. Finally, the HPLC rich cut stream containing the recovered product was evaporated to remove HPLC solvents and recycled into the crystallizer.

3.2. Preparation of HPLC feed

Wiped-film evaporation of the mother liquor stream was investigated to generate the feed stream suitable for preparative HPLC purification. The goal of the evaporation step was to concentrate the mother liquor stream to approximately 15–25 g/L of ertapenem and reduce the combined level of organic solvents, namely methanol and *n*-propanol, from 67% (v/v) to <1% (v/v). Experiments utilizing either one-pass or two-pass operations with 2 in. Pope lab-scale evaporator were conducted to evaluate evaporation performance. Table 1 lists the experimental conditions employed and the results obtained in terms of ertapenem concentration factor and solvent reduction. As seen, initial experiments (1 and 2) with fresh mother liquor streams utilizing one-pass evaporation failed to achieve the desired ertapenem concentration and solvent reduction even with distillate cuts as high as 79%. The evaporated mother liquor still contained approximately 6% (v/v) methanol and 24% (v/v) *n*-propanol. Addition of deionized water to the mother liquor stream prior to one-pass operation was determined to enhance the removal of the organic solvents but a high distillate cut was required to ensure the combined level of organic solvents below 1% (v/v). Consequently, analysis of data from experiments at 41% and 79%

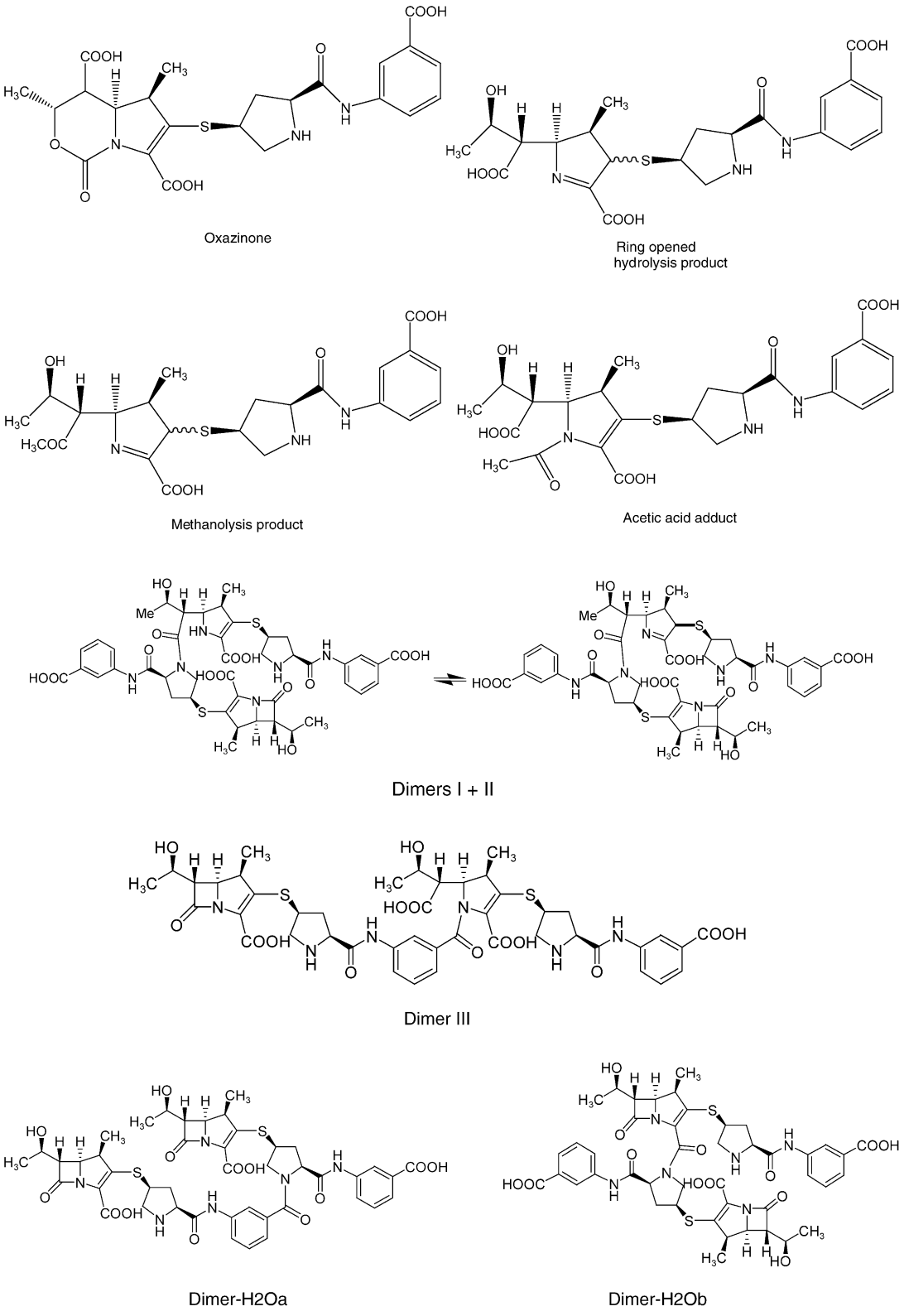

Fig. 4. Chemical structures of degradation products in ertapenem mother liquor stream.

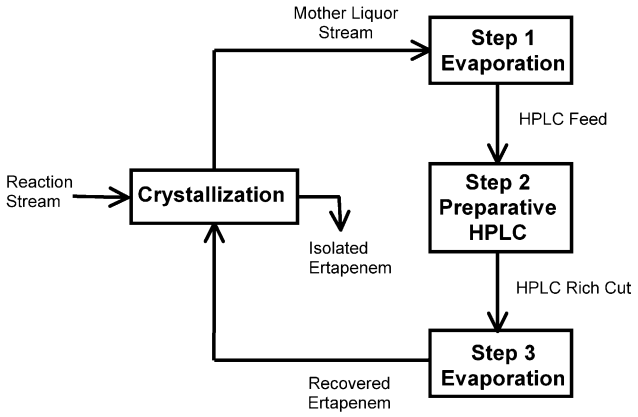

Fig. 5. Schematic of a chromatographic recovery process.

distillate cuts with no dilution and at 84% distillate cut with 30% dilution showed that moving the feed point via dilution was the only practical way to enhance solvent removal, particularly *n*-propanol. Approximately fourfold concentration of the mother liquor stream was achieved in using the conditions of experiment 3 with one-pass operation.

In contrast, two-pass operations of the 2 in. Pope laboratory-scale evaporator system provided more flexibility in terms of achieving solvent reduction and concentration with regard to dilution and distillation cut quantities. Experiments 4 through 6 were two-pass operations conducted with varying conditions of water dilution and distillate cut volumes as shown in Table 1. In all cases significant solvent reduction and concentration were achieved.

Stability tests showed that ertapenem in the evaporated mother liquor degraded at the rate of 0.26 a% per hour even when stored at 5 °C. This is probably owing to the concentration of acetic acid in the mother liquor stream. By treating the mother liquor with sodium bicarbonate prior to evaporation and adjusting the evaporated stream to a pH of 7.2 with sodium hydroxide, the degradation rate was decreased by a factor of two. Data presented in Table 1 for experiment 7 indicates that the performance of two-pass evaporation did not change with this modification in the treatment of mother liquor. Using the conditions of experiment 7, approximately 8 L of mother liquor were processed in two passes using 4 in. Pope evaporator system to generate 22 g of ertapenem with an average yield of 95.1%. The evaporated mother liquors were analyzed by HPLC and found to contain approximately 30 g/L of ertapenem.

3.3. Preparative HPLC purification

3.3.1. pH effects in linear chromatography

Ertapenem, process-related impurities and degradation products have titratable acidic and/or basic groups so that retention behavior in reversed-phase chromatography is expected to be sensitive to changes in the mobile phase pH. For instance, ertapenem has two carboxylic acids groups and one cyclic amine group with pK_a values of 2.7, 4.0 and 7.1, respectively. On the other hand, both the ring opened and oxazinone impurities contain an additional carboxylic acid group, whereas dimers have two additional carboxylic acid groups with one or two additional cyclic amine groups. Likewise, methanolysis and acetic acid adduct degradation products contain an additional cyclic amine and carboxylic acid group, respectively. A YMC Basic C8 analytical-scale column (25 cm × 0.46 cm) was employed to investigate the effect of mobile phase pH on chromatographic retention behavior of some of these key impurities as well as ertapenem and thus to determine ideal conditions for adequate purification through enhanced selectivity. The mobile phase system consisted of acetonitrile (B) and several solutions containing 0.1% (v/v) acetic acid (A) adjusted with sodium hydroxide to different pH values ranging from 3.3 to 5.8. Solutions of ertapenem reference standard, isolated dimers, and preparative HPLC fractions containing acetic acid adduct and methanolysis impurities were used in the experiments. Fig. 6 illustrates the dependence of chromatographic retention behavior of ertapenem, dimers, acetic acid adduct and methanolysis product on the mobile phase pH under gradient elution conditions. When eluent pH is increased from 3.3 to 5.8, retention times of all compounds decrease dramatically. This is because carboxylic acid groups in the compounds are deprotonated at higher pH resulting in higher polarity and, therefore, decreased retention. It is also observed that the retention time for dimers at any given pH is longer than that for ertapenem due to a larger non-polar moiety of the dimers that is available

Table 1
Experimental conditions and performance of evaporation of mother liquor stream

Experiment no.	No. of passes	Dilution[a] per pass (%)	Distillation cut per pass (%)	Ertapenem concentration factor	Methanol concentration (%v/v)	*n*-Propanol concentration (%v/v)
1	1	0	41	1.7	20.4	35.9
2	1	0	79	4.8	6.0	23.9
3[b]	1	30	84	4.7	0.04	0.04
4	2	80100	3838	0.7	0.70	0.50
5	2	2520	5966	4.7	0.17	0.25
6	2	250	7738	5.6	0.10	0.10
7[b]	2	2525	5264	3.7	0.30	0.60

Jacket temperature, 40–55 °C; flow rate, 10–20 mL/min; pressure, 30–50 mbar.
[a] Deionized water added as a percentage of mother liquor stream per pass.
[b] With sodium bicarbonate.

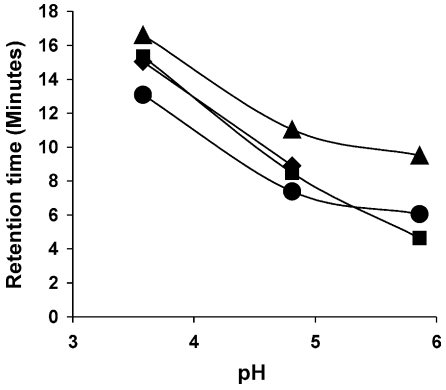

Fig. 6. Effect of eluent pH on retention behavior of ertapenem (●), acetic acid adduct (■), methanolysis product (▲) and dimers (♦). Experimental conditions: column: YMC Basic C8, 25 cm × 4.6 mm × 5 μm; temperature: 25 °C; detection: UV at 220 nm; mobile phase: acetonitrile (B)/0.1% (v/v) acetic acid (A), pH adjusted with NaOH; flow rate: 1.5 mL/min; gradient profile: 5–45% B in 60 min; sample injection volume: 10 μL.

for hydrophobic interactions with the chromatographic surface. Additionally, both methanolysis and acetic acid adduct impurities elute later than ertapenem at lower pH but a reversal of elution order for ertapenem and acetic acid adduct impurity is observed at eluent pH > 5.25. Another interesting behavior to note is that the selectivity between methanolysis product and acetic acid adduct impurity increases as eluent pH is increased in the range investigated. Results of the experiments clearly indicate that pH plays a significant role in governing retention behavior of these compounds.

3.3.2. Feed and mobile phase pH mismatch in non-linear chromatography

In linear chromatography, the amount of sample injected onto the chromatograph is very small and, therefore, sample matrix does not play a significant role in affecting chromatographic retention behavior. However, when a large amount of sample is injected onto the chromatograph, the effect of sample environment on chromatographic retention cannot be neglected. In fact, it plays a significant role in determining initial binding of sample constituents to the stationary phase. Given the observed effects of eluent pH on chromatographic retention of ertapenem and its impurities, it was deemed important to qualitatively understand the role of feed pH in governing retention behavior under overloaded conditions.

Several preparative HPLC experiments were conducted on Kromasil C8 analytical-scale column (25 cm × 0.46 cm) packed with 10 μm particles primarily by varying the pH of sample feed and eluent to determine their interplay on chromatographic separation and to identify a set of conditions that result in adequate removal of all major impurities. Table 2 lists the experimental conditions and the parameters that were varied. The eluent consisted of acetonitrile (B) and 0.1% (v/v) acetic acid (A) adjusted to the desired pH with sodium hydroxide. Evaporated mother liquor samples were utilized in these experiments in order to ensure adequate binding and to mitigate the effect of organic content of the sample on chromatographic retention. The pH of the evaporated mother liquor was adjusted lower by adding dilute acetic acid but could not be adjusted below 4 as the sample precipitates. Approximately 16–20 mg of ertapenem was injected onto the chromatograph in each experiment while maintaining injection volume to approximately 1 mL. Column temperature was set to 25 °C with UV detection at 300 nm.

In each experiment, evaporated mother liquor feed was analyzed for ertapenem purity and concentration. The amount of ertapenem injected onto the chromatograph was also determined to permit evaluation of chromatographic performance. Following sample injection, several fractions were collected and analyzed for ertapenem purity and concentration. The rich cuts were identified, combined and re-analyzed for determining overall chromatographic yield, i.e., the percentage of injected ertapenem that is recovered as purified ertapenem. Table 2 also lists the chromatographic performance of each of these experiments in terms of rich cut impurity profile and chromatographic yield.

Data presented in Table 2 for experiment 1 clearly indicates that adequate purification is not achieved when both eluent and feed pH are ≥5.8. A significant amount of ertapenem breakthrough is observed with rich cut containing only 34.5 a% of ertapenem and very high levels of dimers. This breakthrough at high eluent pH is consistent with the finding that ertapenem binding is severely impaired at such conditions, resulting in inadequate separation. At the other extreme, complete rejection of dimers, oxazinone, ring opened and PAB-ertapenem is obtained when lower pH (≤5.8) in both eluent and feed is employed in experiments 5 through 7 but separation is poor as far as removal of acetic acid adduct and methanolysis impurities are concerned. This is because retention behavior and selectivity of ertapenem and its impurities are initially dominated by pH of the sample feed rather than that of the eluent. At feed pH of 4.8 or 5.8, selectivity between ertapenem and acetic acid adduct is considerably reduced based on data presented in Fig. 6. Once the sample environment is displaced by that of the eluent, retention behavior of ertapenem and its impurities is governed by the eluent pH. However, the effect of eluent pH on chromatographic retention does not appear to be sufficient to offset the selectivity disadvantage imposed by feed pH during initial stages of chromatographic separation. As a result, the acetic acid adduct impurity co-elutes with ertapenem in experiment 6. On the other hand, ideal separation conditions are achieved in experiments 3 and 4 by exploiting a significantly larger mismatch between eluent and sample feed pH of 3.3 and 6.4, respectively. This can also be explained on the basis of the finding in Fig. 6 that a high pH environment results in the reversal of elution order for ertapenem and acetic acid adduct. In the initial stage of chromatographic separation, retention behavior is dominated by high pH of the feed until eluent displaces the sample environment. This is the key driver for enhanced selectivity between methanolysis product and acetic acid adduct as well as the reversal of elution order between ertapenem and acetic acid adduct. Thus, the

Table 2
Experimental conditions and performance of analytical-scale preparative HPLC experiments to investigate the interplay of feed and eluent pH in influencing the separation of ertapenem and its impurities

Experiment no.	Sample feed pH	Eluent pH	ACN[a] equil. conc. (%v/v)	Rich cut impurity profile (area %)							Yield (%)
				Ertapenem	PAB ertapenem	Ring opened	Oxazinone	Dimers	Acetic acid adduct	Methanolysis product	
1	6.4	5.8	1	34.5	0	5.6	0.05	16.6	<1	2	93
2	6.4	4.3	1	96.1	0	0	0	0	0	1	83
3	6.4	3.3	5	97.4	0	0	0	0.25	0	0.6	95
4	6.4	3.3	1	97.5	0	0	0	0	0.14	2	87
5	5.8	4.3	1	91.5	0	0	0	0	5.1	1.1	92
6	5.8	3.3	5	93.9	0	0	0	0	5	0	94
7	4.8	3.3	5	85.3	0	0.1	0	0	7.7	3.3	–

[a] Acetonitrile.

acetic acid adduct goes ahead of ertapenem and methanolysis product stays back under the influence of the high pH environment of sample feed. This selectivity advantage and preferred elution order is not compromised even when an eluent with a much lower pH of 3.3 is employed. As a result, adequate purification of ertapenem is achieved under the greatest pH mismatch condition. HPLC analysis of fractions collected before and after the rich cut also confirms this hypothesis. As seen in Table 2, ertapenem purity of >97 a% is achieved with significant removal of all major impurities, including methanolysis and acetic acid adduct impurities, for experiments 3 and 4. The chromatographic yield is ≥87% for these experiments suggesting that preparative HPLC at such loading and pH conditions is an efficient approach to ertapenem recovery. Similar results were also obtained in experiment 2 by slightly increasing the pH of the mobile phase to 4.3 and changing the equilibration concentration of acetonitrile to 1%. It is noted, however, that the primary contaminant in the rich cut is the methanolysis impurity. Although data in Fig. 6 under linear elution chromatographic conditions may seem to suggest adequate separation between the methanolysis impurity and the product, this is not completely achieved in experiments 3 and 4. The presence of a small amount of the methanolysis impurity in the rich cut is likely due to the tag along effect of sample overloading in non-linear chromatography.

3.3.3. Preparative HPLC on 0.46 cm i.d. column

A preparative HPLC run was carried out on a Kromasil C8 analytical-scale (25 cm × 0.46 cm) column packed with 10 μm particles to investigate the feasibility of reversed-phase preparative HPLC in the purification and recovery of ertapenem from mother liquor streams. An evaporated mother liquor sample containing approximately 30 g/L of ertapenem was employed in the preparative HPLC run. The sample was diluted by addition of 0.5 mL of deionized water to 0.6 mL of sample and the resulting solution pH was 6.7. HPLC analysis of the sample indicated 11.2 a% of acetic acid adduct and methanolysis impurities combined and ertapenem concentration of 14.8 g/L. Approximately 1.1 mL of this sample was injected onto the chromatograph. Based upon experimental findings described in Table 2, preparative HPLC conditions exploiting the pH mismatch were employed in this run. Fig. 7 illustrates the preparative HPLC chromatogram obtained for the run. Several fractions were collected during the run and analyzed by HPLC to determine overall performance of the separation. Based on this analysis, fractions with high concentration and desired purity of ertapenem were combined to make up the rich cut. In this experiment the rich cut was determined to comprise of fractions collected between 8.1 to 14.1 min during the course of the run. The rich cut was also analyzed by HPLC and its impurity profile is illustrated in Fig. 8. The analysis shows ertapenem purity of 95.1 a% with only 0.7 a% acetic acid adduct and methanolysis combined and ertapenem concentration of approximately 1.2 g/L. Other major impurities were also removed to <1 a% in the rich cut. Oxazinone and ring opened hydrolysis impurities were found in large quantities in the early fractions whereas dimers were rejected in the later fractions. A majority of acetic acid adduct was found in the fraction collected immediately before the rich cut while methanolysis product was rejected in the fraction collected after the rich cut. The chromatographic yield of the run was determined as 73.3%.

3.3.4. Preparative HPLC on 5 cm i.d. column

The scale-up of the preparative HPLC recovery process for ertapenem was demonstrated on a 5 cm i.d. Kromasil C8 column packed with 10 μm particles. The stationary phase

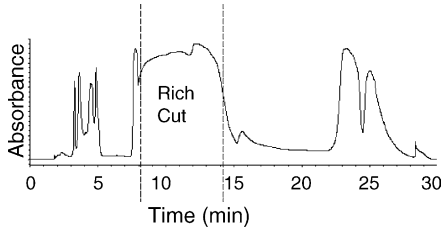

Fig. 7. Chromatogram of an analytical scale preparative HPLC run. Experimental conditions: instrument: Agilent HP1100 HPLC modified with 2-mL manual injection loop; detection: UV at 300 nm; mobile phase: acetonitrile (B)/0.1% (v/v) acetic acid (A), pH at 3.3; temperature: 25 °C; column: Kromasil C8 (25 cm × 4.6 mm × 10 μm); flow rate: 1.5 mL/min; gradient profile: hold at 5% B for 3 min, then a step gradient to 13% B and hold for 15 min followed by a steep gradient to 90% B in 7 min; sample: 1.1 mL of diluted evaporated mother liquor at pH 6.6.

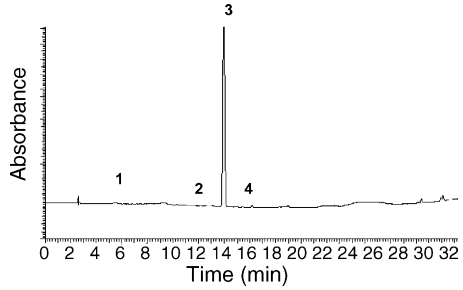

Fig. 8. Analytical HPLC chromatogram illustrating impurity profile of the sample of combined rich cuts from analytical scale preparative HPLC run. (1) Ring-opened hydrolysis product, (2) methanolysis and acetic acid adduct, (3) ertapenem and (4) unknown impurity. Instrument: Agilent HP1100 HPLC; detection: UV at 220 nm; mobile phase: acetonitrile (B)/0.05% (v/v) phosphoric acid (A), pH at 2.2; temperature: 25 °C; column: YMC basic C8 (25 cm × 4.6 mm × 5 μm); flow rate: 1.5 mL/min; gradient profile, hold at 10% B for 5 min, then ramp up linearly to 30% B in 15 min followed by linear ramp to 90% B in 5 min; sample injection volume: 10 μL.

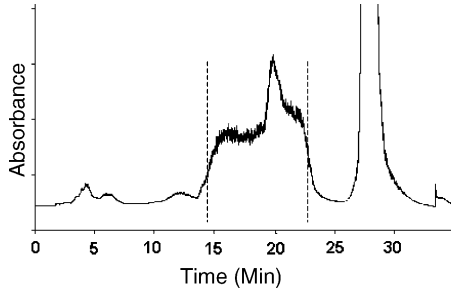

Fig. 9. Chromatogram of a preparative HPLC scale-up run. Instrument: Varian HPLC system; detection: UV at 360 nm; mobile phase: acetonitrile (B)/0.1% (v/v) acetic acid (A), pH at 3.3; temperature: 25 °C; column skid: 5.0 cm i.d. Prochrom Series LC50VE self-packing dynamic axial compression; packing: 10 μm Kromasil C8; column length: 25 cm; flow rate: 118 mL/min; gradient profile: hold at 5% B for 9.5 min, then a step gradient to 13% B in 30 s and hold for 12 min followed by a steep gradient to 90% B in 9 min; sample: 118 mL of diluted evaporated mother liquor at pH 6.5.

bed was packed to a height of 25 cm and column efficiency was determined to be adequate using an ertapenem probe. To mimic the experimental conditions of the analytical-scale preparative HPLC run a scale-up factor of 118 was used by taking the square of the ratio of column diameters in the scale-up run to that in the analytical-scale run. Using this factor the amount of sample needed for the 5 cm preparative HPLC run was determined to be approximately 1.5–1.9 g of ertapenem contained in 118 mL of evaporated mother liquor feed sample. Likewise, a flow rate of 1.5 mL/min in the 0.46 cm preparative HPLC run scaled up to a flow rate of 177 mL/min for a 5 cm i.d. run. However, owing to pressure limitations on the column flow rate due to the column skid/housing, the scale-up run was capped at 118 mL/min, equivalent to a flow rate of 1.0 mL/min for the analytical-scale run. Because of this change in column flow rate, the gradient profile was adjusted so that the chromatographic residence time remained unchanged during each of the equilibration, elution and gradient wash cycles.

Approximately 60 mL of the evaporated mother liquor sample containing 30 g/L of ertapenem was diluted with 50 mL of deionized water and the pH of the diluted sample was measured at 6.6. HPLC analysis of the sample indicated 11.2 a% of acetic acid adduct and methanolysis impurities combined and 14.8 g/L of ertapenem. Approximately 110 mL of this sample was injected onto the chromatograph. Based on experimental findings described in Table 2, preparative HPLC conditions exploiting pH mismatch were employed in this run. Fig. 9 illustrates the preparative HPLC chromatogram obtained for the scale-up run. Fractions were collected at specific time intervals and analyzed by HPLC to determine product purity. The fractions beginning from 14.8 to 22.5 min were combined to make up the rich cut stream. The region corresponding to these fractions on the preparative chromatogram is shown in Fig. 9. The sample of combined rich cuts was analyzed by HPLC and the impurity profile is displayed in Fig. 10. Analysis shows ertapenem purity of 97.6 a% with 0.5 a% of ring-opened hydrolysis, 0.5 a% of methanolysis and 0.2 a% of acetic acid adduct impurities. All other major impurities were less than 0.1 a%. Several runs were conducted at this scale to generate 10 g of purified ertapenem. The average chromatographic yield for these runs was calculated to be 82%. The throughput of the chromatographic process was calculated as 5 g/L/h of ertapenem processed per liter of bed per hour. The total eluent and acetonitrile requirements for ertapenem purification are 2800 and 560 L, respectively, per kilogram of ertapenem processed.

3.4. Evaporation of preparative HPLC rich cut for recycle

Evaporation was used again for the post-treatment of rich cuts from preparative HPLC runs. Experiments with simulated HPLC rich cut streams at high/low distillate cut showed that acetonitrile is readily removed to 0.02–0.20% (v/v) in one pass and the stream is concentrated by a factor of 1.5–2.2. Us-

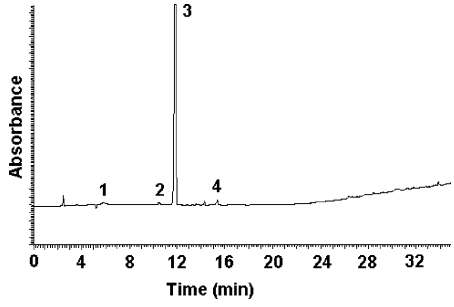

Fig. 10. Analytical HPLC chromatogram illustrating impurity profile of the sample of combined rich cuts from preparative HPLC scale-up run. (1) Ring-opened hydrolysis product, (2) methanolysis and acetic acid adduct, (3) ertapenem and (4) unknown impurity. Instrument, Agilent HP1100 HPLC; detection, UV at 220 nm; mobile phase, acetonitrile (B)/0.05% (v/v) phosphoric acid (A), pH at 2.2; temperature, 25 °C; column: YMC basic C8 (25 cm × 4.6 mm × 5 μm); flow rate, 1.5 mL/min; gradient profile, hold at 10% B for 5 min, then ramp up linearly to 30% B in 15 min followed by linear ramp to 90% B in 5 min; sample injection volume, 10 μL.

ing these conditions, a 4 in. Pope evaporator was employed to treat the pooled rich cuts for recycle. Evaporation led to a slight increase in the level of ring-opened hydrolysis impurity (1–2 a%) owing to the degradation of ertapenem. Therefore, the yield of this step was at 90%, somewhat lower than that for the evaporation of mother liquors.

Recycle stream specifications were established for a recycle stream volume to crystallization batch volume ratio of 1:10. Characterization of the isolated product indicated that the quality of ertapenem was not compromised by recycling the recovered ertapenem as long as the following recycled product specifications were met: ring-opened hydrolysis product <3 a%, dimers <4 a%, solvents <2% (v/v) and all other impurities <1 a%. The evaporated rich cut stream met all the stringent specifications for recycling. The overall yield of the chromatographic recovery process for ertapenem was measured as 70%.

4. Conclusion

The present study describes the development and scale-up of a recovery process for ertapenem active pharmaceutical ingredient from crystallization mother liquor streams. The process involves evaporation of mother liquor streams containing ertapenem, purification by reversed-phase preparative HPLC and recycle of evaporated HPLC rich cuts into the crystallization process. Purification is achieved by employing a novel approach that exploits a significant mismatch between the pH of HPLC feed and eluent to selectively remove all the major impurities. The process is demonstrated with a 25 cm × 5 cm preparative-scale HPLC column to generate several grams of recovered ertapenem.

As shown in this study, feed size is important to properly exploit the features of pH mismatch. Therefore, the feed size was optimized for a given column length and particle size. However, some more scale-up studies are warranted prior to commercialization to further optimize the chromatographic separation process in terms of column length and particle size, thus resulting in enhanced productivity and reduced overall cost of operation. Although the isocratic mode of elution, which is employed in the current process for HPLC purification, offers the advantage of ease of operation in terms of selecting cut points for fraction collection, it has a low throughput, consumes a considerable amount of acetonitrile and results in rich cuts that are very dilute in product concentration. Given the constraint on the size of the vessels that can be used to handle solvents during manufacturing, the rich cuts from isocratic elution have to be concentrated considerably before proceeding further. This requirement places a stringent demand on the ability and performance of the unit operation that is selected for solvent concentration. One approach to address this issue is to employ the gradient mode of elution. The increasing content of the organic modifier in the mobile phase during gradient runs automatically concentrates the product as it traverses through the HPLC column. As a result, the rich cut from a gradient run typically has a three to fivefold higher concentration of the product than that observed in an isocratic run. This can significantly soften the demand on the evaporator for the treatment of rich cuts as well as improve the throughput of the process. However, HPLC methods based on gradient elution have to be carefully assessed in order to insure that the resolution of the chromatographic separation, and hence the quality of the recovered product, is not compromised. This could be particularly challenging in the case of ertapenem recovery where pH mismatch between the HPLC feed and the eluent is exploited for achieving the desired purification. This will be the subject of investigation in a subsequent paper.

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