Challenges in the Analytical Method Development and Validation for an Unstable Active Pharmaceutical Ingredient

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Abstract

A sensitive high-performance liquid chromatography (HPLC) impurity profile method for the antibiotic ertapenem is developed and subsequently validated. The method utilizes an Inertsil phenyl column at ambient temperature, gradient elution with aqueous sodium phosphate buffer at pH 8, and acetonitrile as the mobile phase. The linearity, method precision, method ruggedness, limit of quantitation, and limit of detection of the impurity profile HPLC method are found to be satisfactory. The method is determined to be specific, as judged by resolving ertapenem from in-process impurities in crude samples and degradation products that arise from solid state thermal and light stress, acid, base, and oxidative stressed solutions. In addition, evidence is obtained by photodiode array detection studies that no degradate or impurity having a different UV spectrum coeluted with the major component in stressed or unstressed samples. The challenges during the development and validation of the method are discussed. The difficulties of analyzing an unstable active pharmaceutical ingredient (API) are addressed. Several major impurities/degradates of the API have very different UV response factors from the API. These impurities/degradates are synthesized or prepared by controlled degradation and the relative response factors are determined.

Introduction

Impurity profile methods are standard tools in the pharmaceutical industry for characterizing active pharmaceutical ingredients and dosage forms. The expectations for validation of these methods have been clearly expressed by United States and international regulatory agencies (1–4) and are largely consistent worldwide (5). At the most basic level, the validation of an impurity profile method seeks to demonstrate that it is suitable for its intended use. For the pharmaceutical industry, the ability to consistently identify and quantitate impurities is critical to establishing the properties of the material studied for safety and efficacy. Validation is crucial to ensuring that the data set used to assess the suitability of the drug is consistent, and to establish the basis against which all future commercial supplies will be judged for quality (4). Ideally, validation will include spiking of authentic samples of impurities to establish the method's ability to separate and quantitate the most significant impurities. When individual isolated impurities are not available for spiking, crude samples or bulk drug that has been purposely degraded can be used (4).

In some cases the UV response factors of a drug substance and the relevant impurity are very different. Then a correction factor needs to be applied or the impurities are, in fact, being overestimated (3,6). In cases of potential underestimation, it is important to investigate any new impurities to ensure levels remain below the qualification threshold. β-Lactam compounds can present a challenge in this regard because several known impurities in these drug substances have lower relative response factors than the active pharmaceutical ingredient (API) (e.g., ertapenem's major impurity, the ring-opened hydrolysis product, has a relative response of 0.87 to the API).

Ertapenem is a broad-spectrum 1β-methylcarbapenem antibiotic. The bulk drug substance, ertapenem, is a monosodium salt. Ertapenem is the active pharmaceutical ingredient used in the formulated drug product Invanz (Merck & Co., Whitehouse Station, NJ). The clinical applications of Invanz currently include the treatment of adult patients with moderate to severe infections, such as complicated intra-abdominal infections, community acquired pneumonia, and complicated urinary tract infections caused by specific strains of susceptible microorganisms (7–11). The molecular structure of ertapenem is shown in Figure 1. It consists of a carbapenem ring and a side chain. Hydrolysis of the highly strained ring system is one very important characteristic of the molecule that accounts for the instability of carbapenem antibiotics in water and leads to the ring-opened hydrolysis degradate (Figure 2A). In the presence of alcohols such as ethanol, a corresponding ethanolysis product can be formed (Figure 2B).

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Ethanol is used in the synthetic process and can appear as a residual solvent in the final API. In addition to the hydrolysis and ethanolysis products, other degradates can form in aqueous solution, especially at higher ertapenem concentrations. These impurities are dimers and dehydrated dimers of ertapenem (12). The two most important dimers appear only as equilibrium mixture in solutions [i.e., dimers I+II (Figure 2C)]. The instability of ertapenem is a very important issue that needs to be addressed appropriately during the development of a suitable impurity profile method. Degradation has to be prevented or at least minimized during all stages of the method, starting from the sample preparation procedure to the final analysis.

The goal of this work is to present the development and validation of a sensitive and robust high-performance liquid chromatography (HPLC) impurity profile method for ertapenem bulk drug substance. Another goal is to show how the major impurities or degradates were prepared and characterized and how their UV response factors relative to ertapenem were measured.

Experimental

Chemicals

Ertapenem samples were obtained as monosodium salt. They were supplied by Merck Sample Repository (Merck Research Laboratories, Rahway, NJ). The water used was distilled and purified by a HYDRO system (Hydro Services & Supplies, Garfield, NJ). Sodium hydroxide (50%) and ortho phosphoric acid (85%) were purchased from Fisher Scientific (Fisher Scientific, Fair Lawn, PA). Acetonitrile was obtained from EM Science (Gibbstown, NJ). 3-*N*-Morpholino (MOPS) (propane sulfonic acid, SigmaUltra grade, pK_a = 7.2, purity > 99.5%) was obtained from Sigma (St. Louis, MO); 4-morpholineethane sulfonic acid monohydrate (MES) (pK_a = 6.1, purity 98%); and 4-2-hydroxyethyl-1-piperazine-propanesulfonic acid (EPPS) (pK_a = 8.0, purity 99%) were purchased from Aldrich (St. Louis, MO). Hydrogen peroxide (American Chemical Society reagent) was obtained as a solution of 30% (w/w) in water (Aldrich).

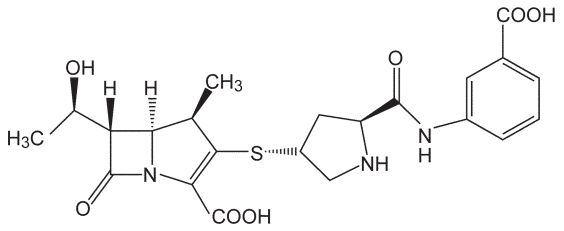

Figure 1. Molecular structure of ertapenem sodium API as free carboxylic acid.

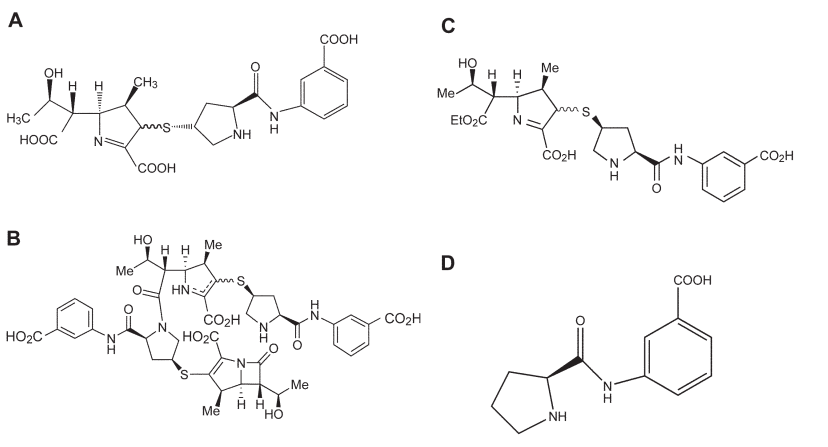

Figure 2. Molecular structures of major impurities/degradates of ertapenem. Ring-opened hydrolysis product (A), dimer I+II degradates (B), ethanolysis products I and II (C), and proMABA (D).

Equipment

An Agilent 1100 Series HPLC system equipped with an autoinjector, sample tray cooler, quaternary pump, column oven, and diode array detector (Agilent Technologies, Palo Alto, CA) was used for analytical chromatography. A model ES 2000 benchtop light chamber (Enviromental Specialties, Raleigh, NC) was used for photolytic stress studies.

Preparation of materials

The buffer solutions were prepared by dissolving the desired amount of MOPS, MES, or EPPS in water. The solutions were titrated to pH 7.0, 5.5, or 9 with aqueous sodium hydroxide solution. Ertapenem standard solutions were prepared using ertapenem monosodium reference standard and water as diluent.

Solution stability studies

Solution stability was evaluated under the following conditions: (*i*) in water diluent at 5°C; (*ii*) in water diluent at ambient temperature; (*iii*) in aqueous 10mM MOPS solution (pH = 7.0); (*iv*) in aqueous 0.1M MES (pH = 3.7); and (*v*) in aqueous 0.1M EPPS (pH = 9.3). Ertapenem was prepared in the diluent of interest, and several injections were made at intervals of 1–2 h while maintaining the autosampler temperature at the desired value.

Degradation studies

The degradation studies involved (*i*) solid state thermal stress, (*ii*) solid state photolytic stress, (*iii*) solution acid stress, (*iv*) solution base stress, and (*v*) oxidative solution stress.

For the solid state thermal stress study, an aliquot of ertapenem was stressed by storage at 125°C for 5 min. The photolytic stress of ertapenem was achieved by exposing a sample of ertapenem to UV and white fluorescent light sources, providing an overall illumination of 60,000 lux h and an integrated near UV energy of 96 W h/m^2. The cool white fluorescent

light had an output similar to that specified in International Standards Organization 10977 (13). The near UV light had a spectral distribution from 320 to 400 nm with a maximum energy emission between 350 and 370 nm and a significant proportion of UV in both the 320–360 and 360–400 nm bands. A protected sample of ertapenem, wrapped in aluminum foil, was used as a dark control to evaluate the contribution of thermally induced change to the total observed change. The authentic sample and the dark control were placed in separate glass Petri dishes and spread across the dish to give a thickness of no more than 3 mm, in accordance with ICH guidelines (14). Both samples were placed in the light chamber (maintained at 25°C throughout the study) and exposed to the light sources for 6 h.

The solution stress studies were conducted by dissolving ertapenem (0.5 g/L) in the desired diluents [e.g., acetic buffer (0.1M MES, pH = 3.7) or basic buffer (0.1M EPPS, pH = 9.0)] for 17 and 7.5 h, respectively. For the oxidative solution stress study, a sample solution of ertapenem having a concentration of ~ 0.2 mg/mL was prepared using 0.03% (w/w) hydrogen peroxide in water as diluent. The solution was stored in the autosampler tray at ~ 5°C and injected thereafter at approximately 2-h intervals. Concurrently, also at 2-h intervals, a control sample (i.e., ertapenem dissolved in water only) was injected for comparison.

For all sample injections, diode array spectra were collected from 190 to 350 nm at 1.0-nm steps.

Preparation and isolation of impurities

Dimers I+II

The preparation and isolation of pure dimer I+II degradates was completed in four steps: (*i*) controlled degradation of a concentrated ertapenem solution; (*ii*) isolation of dimer I+II by preparative HPLC; (*iii*) removal of excess acetonitrile using a Rotavap; and (*iv*) lyophilization. The development of the procedures for the preparation and isolation of dimers I+II are outlined in detail in the literature (12).

Ring-opened

The ring-opened hydrolysis product was prepared by adding ertapenem to a diluted sodium hydroxide solution (15,16). The resulting solution was then lyophilized to yield pure ring-opened degradate as sodium salt.

Proline meta-amino benzoic acid

The proline meta-amino benzoic acid (proMABA) is an in-process impurity from the side chain of ertapenem. The proMABA impurity was synthetically prepared as an HCl salt (15).

Results and Discussion

Development of the HPLC method

The initial development of the impurity profile method for ertapenem sodium focused on the separation of the major impurities and degradates that are present in the bulk drug substance. These impurities are the ring-opened hydrolysis product, side-chain impurity proMABA, ethanolysis product, and several dimers (14). The structures of these impurities are shown in Figure 2. After screening several columns, methods could be developed on a Phenomenex Customsil column, Prodigy column (Phemomenex, Torrance, CA), and Metachem Inertsil Phenyl column (Metachem, Torrance, CA). These methods were all able to separate the major impurities; however, it was found that the Inertsil phenyl column gives better peak shapes while maintaining a similar or better resolution of all impurities than the other two columns. For this reason it was chosen for further development of the method. The initial method that was developed at a low pH was able to separate all impurities from the main component ertapenem; however, all dimers coeluted as a cluster of peaks (see Figure 3A). For this reason, it was necessary to further improve the method. The ertapenem molecule, as well as the dimeric degradates, possesses several carboxylic and amine groups. Therefore a study of the influence of pH on the separation was performed (see Figure 3). The study revealed that the dimers are well resolved at pH 8. At this pH, dimers I+II elute in front of ertapenem and the other dimers (dimer III, dimer-H_2Oa, and dimer-H_2Ob) elute after it. The final optimized method that was developed on the Inertsil phenyl column was designed to allow for simple operation and easy implementation in the factory. Gradient elution and an injection volume of 10 µL were used to enhance sensitivity. The choice of the monitoring UV wavelength of 230 nm was made because this wavelength corresponds to the location of the adsorption maximum of the carbapenem ring system, thus further optimizing the selectivity and sensitivity of the method for carbapenem antibiotics. The HPLC method was established with the following conditions:

HPLC system: Agilent 1100 column; Inertsil phenyl (25 × 0.46 cm) (Metachem); flow rate, 1.0 mL/min; injection volume,

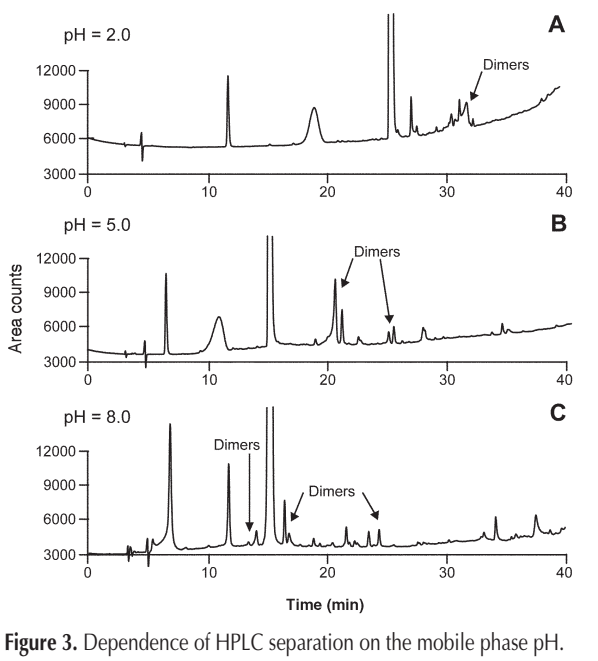

Figure 3. Dependence of HPLC separation on the mobile phase pH.

10 µL; sample tray temperature, 5°C; column temperature, ambient; and mobile phase, (A) 0.1% sodium phosphate buffer in water (pH = 8.0) and (B) acetonitrile. Gradient profile (including equilibration): 98% A–2% B (v/v) to 95% A–5% B in 3 min; 95% A–5% B to 85% A–15% B in 22 min; 85% A–15% B to 75% A–25% B in 10 min; and hold for 10 min. Detection: UV at 230 nm.

Typical chromatograms of a water blank and an ertapenem solution are shown in Figure 4. Ertapenem elutes at approxi-

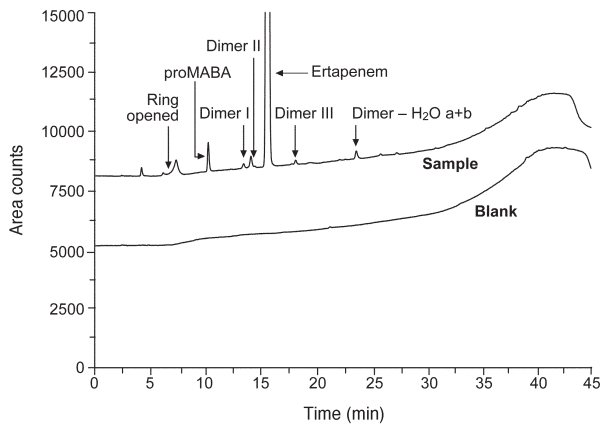

Figure 4. Chromatogram of a typical ertapenem sodium API sample using the optimized HPLC method.

Table I. Linearity of the HPLC Method*

Percent of target concentration	Concentration (mg/mL)	Ertapenem peak area counts
10%	0.0237	525776 524918
50%	0.1068	2362832 2364247
75%	0.1526	3353627 3350332
100%	0.2048	4571801 4470646
125%	0.2510	5532810 5589096
150%	0.3188	7047786 7032724

* R^2 = 0.99985.

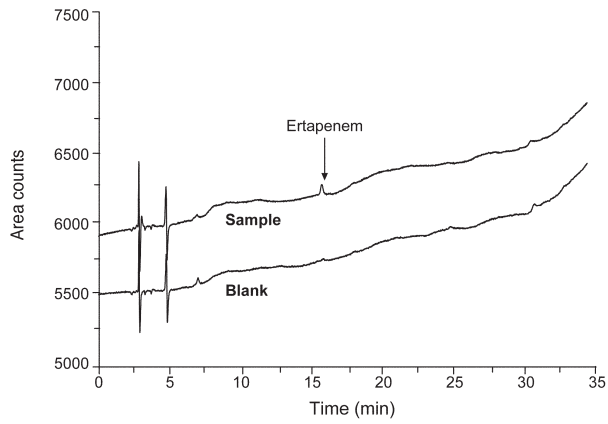

Figure 5. Chromatogram of ertapenem sodium API at the LOD.

Table II. LOD and LOQ of the HPLC Method

Ertapenem concentration (µg/mL)	Ertapenem peak area counts	%RSD
1.804	40520 40409 40111	0.5
0.902	20362 20246 20021	0.9
0.451	9856 9680 9697	1.0
0.2255	4863 4930 4798	1.4
0.1128	2420 2411 2480	1.5
0.0564	1333 1239 1309	3.8
0.0282	873 948 860	5.3
0.0141	358 326 373	6.8

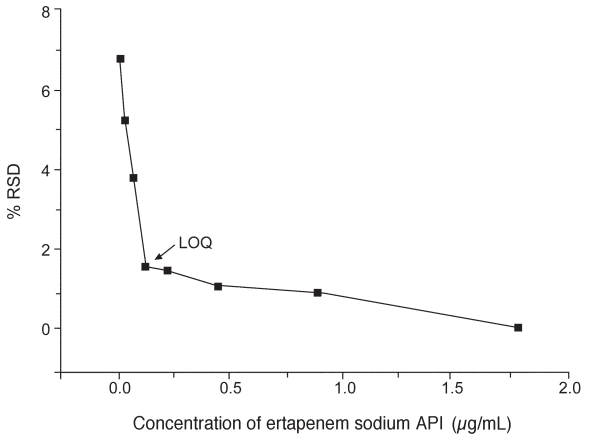

Figure 6. Determination of the LOQ of the ertapenem sodium API HPLC method.

mately 15 min and is well separated from proMABA, the ring-opened degradate, and dimers. All dimer degradates are well resolved from each other.

Validation of the HPLC method

The HPLC method was validated by determining the linearity, limits of detection (LOD) and quantitation (LOQ), method precision, selectivity, ruggedness, and solution stability. Suitability criteria for the method were also established. The validation was performed consistent with expectations for use of impurity profile methods in a pharmaceutical manufacturing environment (3,4).

Linearity

Linearity of the detector response for ertapenem was evaluated over the concentration range of 10–150% of the target concentration (0.2 mg/mL) (i.e., 0.02–0.3 mg/mL). Solutions of ertapenem standard were prepared using serial dilutions. Two injections were made at each concentration. The detector response of ertapenem was found to be linear over the entire range (see Table I) with a regression coefficient R^2 = 0.99985. This is satisfactory considering the wide concentration range investigated.

LOD and LOQ

The LOD and LOQ were determined by making triplicate injections of low level standards prepared by serial dilutions. A solution of ertapenem was prepared at the target concentration (0.18 mg/mL) and then diluted 6400 times to 0.000028 mg/mL (representative of a 0.016% level with respect to the target concentration). The peak arising from the diluted solution was detected under the recommended chromatographic conditions with a signal-to-noise (s/n) ratio > 3 (see Figure 5). Therefore, the LOD of ertapenem, and for impurities with a similar UV response factor, is at least 0.016%. To determine the LOQ, solutions with concentrations of ertapenem ranging from 0.000014 to 0.0018 mg/mL were prepared, three injections of each sample were made, and the percent relative standard deviation (%RSD) of the area counts of ertapenem from each sample was calculated (see Table II) and plotted versus the concentration of the sample (see Figure 6). The LOQ was taken as the concentration at the inflection point on the plot. Therefore, the LOQ for the method was 0.0001 mg/mL, which represents the 0.05% level with respect to the target concentration.

Method precision/ruggedness

The precision of the method was evaluated by making six replicate injections on each of 2 days. Every injection was made from a freshly prepared solution of ertapenem standard. The average area percent of the total impurities obtained for 2 days (12 injections) was 2.7% with a %RSD of 2.2% (see Table III). This demonstrated satisfactory injection precision. The ruggedness of the method was evaluated by making six replicate injections on columns from two different lots on each of 2 days. Every injection was made from a freshly prepared solution of ertapenem. The average area percent of the total impurities obtained for 2 days (12 injections) was 2.7% with %RSD of 2.8% (see Table III).

Solution stability

An important issue to consider is the stability of ertapenem in solution. It is well known that carbapenem antibiotics are unstable because of the highly strained fused ring system. Nucleophiles can attack the carbapenem ring and lead to its opening, primarily forming the ring-opened hydrolysis product when in aqueous solution. Solution stability was evaluated in

Table III. Method Precision/Ruggedness of the HPLC Method

	Sample No.						Average	Standard deviation
	1	2	3	4	5	6		
HPLC column 1, day 1								
Average total impurities = 2.76 area-%								
%RSD = 2.0%								
Total impurities (area-%)	2.80	2.82	2.80	2.67	2.76	2.73	2.76	0.056
HPLC column 1, day 2								
Average total impurities = 2.69 area-%								
%RSD = 1.5%								
Total impurities (area-%)	2.65	2.75	2.73	2.68	2.70	2.65	2.69	0.041
HPLC column 2								
Average total impurities = 2.66 area-%								
%RSD = 2.1%								
Total impurities (area-%)	2.60	2.68	2.66	2.76	2.64	2.63	2.66	0.056

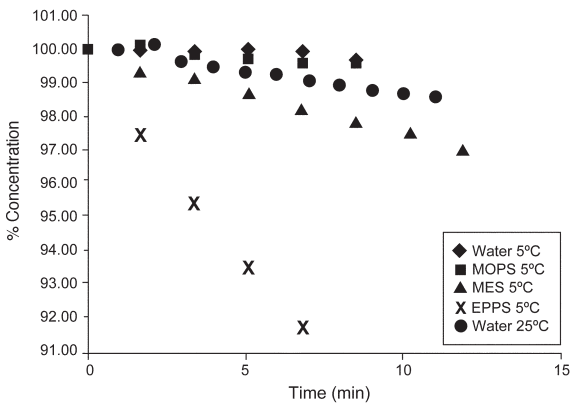

Figure 7. Stability of ertapenem sodium API at 5°C in various diluents at a diluted concentration of 0.2 g/L. Change of relative area counts versus time for water and three buffers used: MOPS (pH 7), MES (pH 5.5), and EPPS (pH 9.5).

water diluent (pH = 5.6) at 5°C and at ambient temperature and with non-nucleophilic buffers [i.e., aqueous 10mM MOPS solution (pH = 7.0), aqueous 0.1M MES solution (pH = 3.7), and aqueous 0.1M EPPS solution (pH = 9.3)]. Figure 7 shows the results of the stability studies. The solutions were found to be most stable at pH 5.6 and 7.0 at 5°C and least stable at pH 9.3. Even at the most stable condition, however, the dimer I impurity gradually converted to dimer II in less than 2 h (see Table IV). Therefore, solutions need to be freshly prepared prior to each injection to obtain consistent impurity profile results.

Selectivity

The selectivity of the method was examined by injecting crude samples and samples that were stressed thermally and photolytically, and by the use of acid, base, and hydrogen peroxide.

The chromatogram of a crude ertapenem sample is shown in Figure 8A. This chromatogram demonstrates the separation of ertapenem from all the impurities. Similar chromatograms are shown in Figures 8B–F for ertapenem that was thermally or photolytically stressed or stressed by acid, base, or peroxide. Significant levels of numerous degradation products were observed in the thermally stressed ertapenem sample, but all were separated from the main ertapenem peak (see Figure 8B). The area-% of total impurities increases from 3.4% to 15.4%, with the increase of the ring-opened compound from 0.4% to 3.6%, total ethanolysis products from 0.4% to 2.0%, and total dimers from 1.4% to 6.8%. The impurity profile of the photolytic stressed sample is shown in Figure 8C. Ertapenem was stressed by storage under UV/white light at 25°C for 6 h. The level of total impurities in the photolytically stressed sample was 2.7% versus 2.4% for the dark control sample. The level of the ring-opened degradate was 1.0% for both the photolytically stressed sample and dark control sample. The level of total dimers was 1.0% for the photolytic stressed sample and 0.8% for the dark control sample. Only one unknown degradate [relative retention time (RRT) 0.71] at < 0.05 area-% was formed in the photolytically stressed sample, which was not detected in the dark control sample; the impurity is well separated from the ertapenem peak. The differences in total impurities and total dimers observed in the photolytically stressed sample versus the dark control sample are not significant because of the elevated levels of degradates from the thermal degradation observed in both samples caused by the ambient temperature conditions of the experiment. In the acid-stressed sample, several degradation products were observed, but all were separated from the ertapenem peak (see Figure 8D). The area-% of total impurities increased from 3.4% to 5.6%, with the increase of the ring opened compound from 0.4% to 2.1%, and total ethanolysis products from 0.4% to 0.5%. The level of total dimers remained the same. In the base stressed ertapenem sample, significant levels of several degradation products were observed, but all were separated from the ertapenem peak (see Figure 8E). The area-% of total impurities increased from 3.4% to 11.7%, with the increase of the ring-opened compound from 0.4% to 7.1%, and an unknown impurity (RRT 1.06) from not detected to 1.5%. Levels of total ethanolysis products and total dimers remained the same. In the oxidative stress study, significant levels of several degradation products not detected in an unstressed sample were formed. These degradation products ranged in area-% from < 0.05% to 4.1%. However, as can be seen in Figure 8F, all peaks were separated from the ertapenem peak. The area-% of the total impurities increased from 2.0% to 14.3%, the ring-opened hydrolysis product increased from 0.8% to 3.7%, and the total dimers decreased from 0.6% to 0.3%.

In all selectivity experiments, the UV spectra taken at the upslope, apex, and downslope of the main peak were superimposable, indicating that no impurities with dissimilar UV characteristics were coeluting.

Method robustness

Robustness was evaluated by deliberately varying the method parameters. At each varied condition, solutions of two ertapenem sodium samples were injected to ensure that the HPLC system was well equilibrated and the retention time of the ertapenem main peak was reproducible. Method robustness has been shown to be satisfactory with respect to variations in flow rate (0.95 to 1.05 mL/min), column temperature (20–30°C), mobile phase pH (7.9 to 8.1), mobile phase composition (± 0.5% acetonitrile), injection volume (8 to 12 µL), and re-equilibration time (10–35 min). The effect of the change of the method parameters on the total impurity levels of two different ertapenem sodium lots is shown in Table V (i.e., the %RSD values stay within a reasonably low range for all method parameter variations). The relatively high RSD value of 6.3% that was obtained for the mobile phase pH change experiments

Table IV. Solution Stability of Ertapenem API in Water at 5°C

	Time (h)					
	0	**1.7**	**3.4**	**5.1**	**6.8**	**8.5**
Ring opened (area-%)	2.95	3.06	3.18	3.21	3.28	3.49
Side chain impurity (area-%)	0.84	0.84	0.84	0.84	0.83	0.84
Dimer I (area-%)	0.37	0.22	0.19	0.17	0.17	0.17
Dimer II (area-%)	1.08	1.21	1.21	1.23	1.22	1.23
Dimer III (area-%)	0.52	0.52	0.51	0.50	0.51	0.50
Ethanolysis product I (area-%)	0.10	0.08	0.07	0.06	0.06	0.04
Ethanolysis product II (area-%)	0.16	0.18	0.17	0.15	0.15	0.17
Dimer H_2O (area-%)	0.23	0.23	0.23	0.24	0.24	0.24
Total impurities (area-%)	6.97	6.94	7.03	6.96	7.05	7.30

is still sufficient in the current case because ertapenem is an unstable drug substance. The value appears high because the total amount of impurities is still low, and the RSD relates to the total impurities. In contrast, the absolute value of the standard deviation that is also listed in Table V is quite low (i.e., 0.13 area-%).

System suitability

As a final part of the impurity profile method validation, it is necessary to establish system suitability criteria. The system suitability tests are based on the concept that the equipment, electronics, analytical operations, and samples to be analyzed constitute an integral system that can be evaluated as such (4).

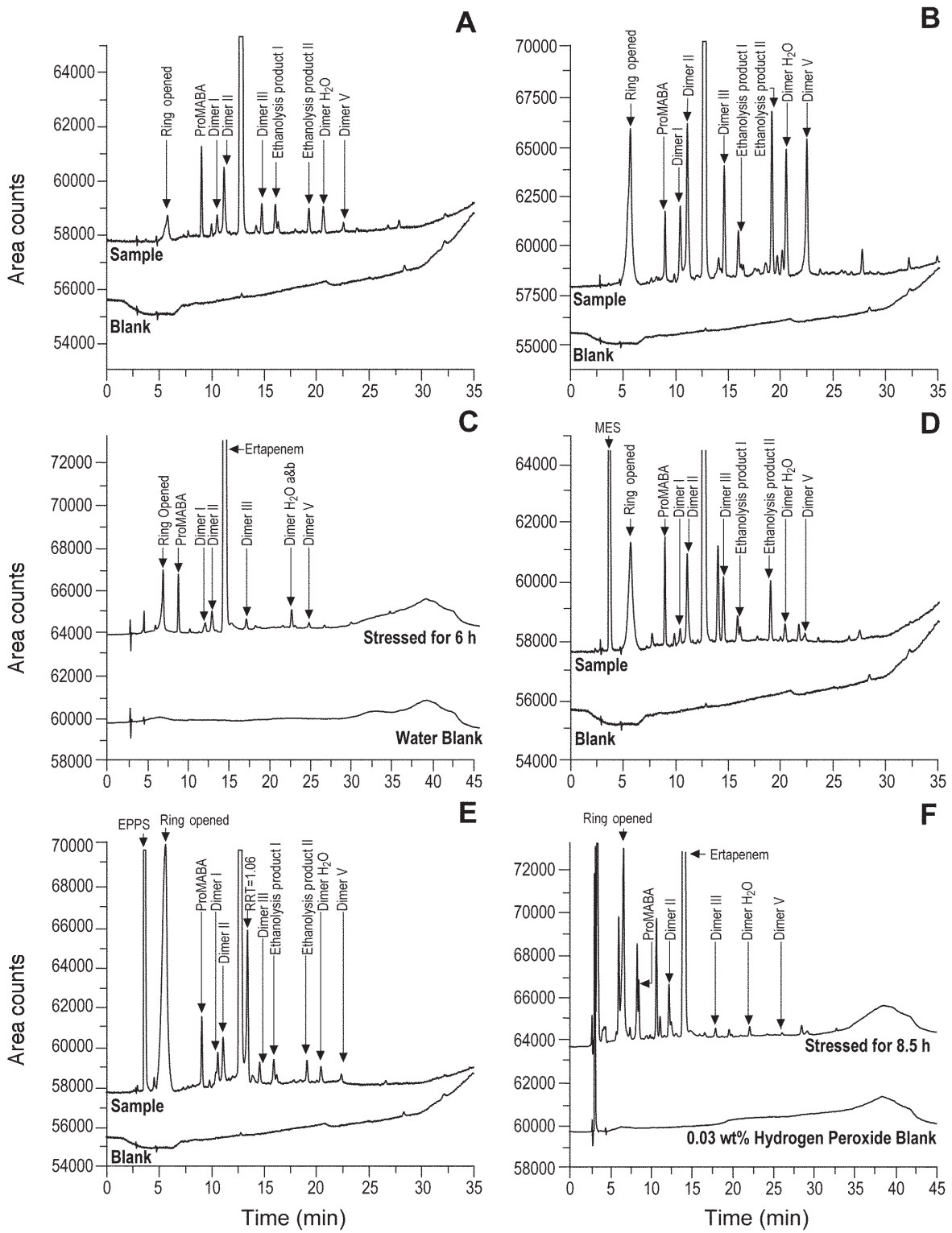

Figure 8. HPLC chromatograms of crude and stressed ertapenem sodium samples. crude sample (A), solid state thermal stress sample (B), solid state photolytic stress sample (C), acid solution stress sample (D), base solution stress sample (E), and oxidative solution stress sample (F).

These evaluations ensure that data generated from multiple systems are comparable. System suitability requirements for the impurity profile method were established by examining the existing database of previously analyzed samples. The following system suitability specifications were set: (*i*) precision: The precision of consecutive injections of three fresh preparations of the sample should have < 1 % RSD on area-% of the main peak. (*ii*) Peak tailing factor at 5% of maximum peak height: the tailing factor of ertapenem should be < 2. (*iii*) Resolution: the resolution between ertapenem and dimer II should be > 2. These suitability criteria were found to be sufficient to ensure that an accurate impurity profile can be obtained with the developed HPLC method.

Table V. Method Robustness: Effect of the Change of Various Method Parameters on the Total Impurity Levels in Two Typical Bulk Drug Samples

Method parameter	Ertapenem sodium sample #1 *Total impurities*	Ertapenem sodium sample #2 *Total impurities*
Flow rate	3.54 ± 0.11 area-% RSD = 3.1%	2.17 ± 0.10 area-% RSD = 4.6%
Temperature	3.74 ± 0.03 area-% RSD = 0.9%	2.50 ± 0.02 area-% RSD = 0.9%
Mobile phase pH	3.19 ± 0.09 area-% RSD = 2.7%	2.01 ± 0.13 area-% RSD = 6.3%
Mobile phase composition	3.44 ± 0.11 area-% RSD = 3.2%	2.10 ± 0.07 area-% RSD = 3.3%
Injection amount	3.55 ± 0.04 area-% RSD = 1.2%	2.35 ± 0.02 area-% RSD = 1.0%
Re-equilibration time	3.36 ± 0.04 area-% RSD = 1.3%	1.71 ± 0.03 area-% RSD = 1.6%

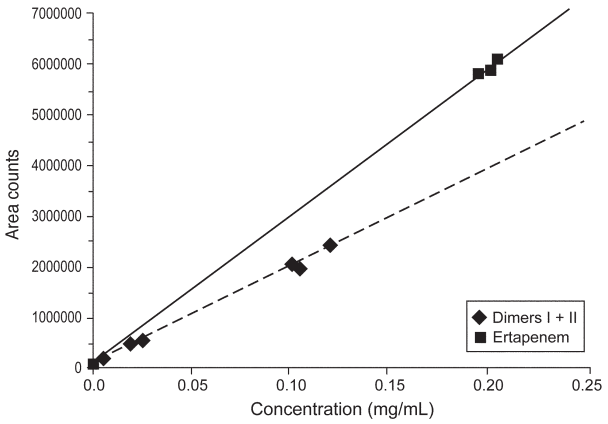

Figure 9. Determination of the relative response factor of dimer I+II degradates versus ertapenem. Area counts versus concentration.

Table VI. Impact of Relative UV Response Factors of the Major Impurities in Ertapenem Sodium API

Impurity	Area-% un-corrected	Area-% corrected for RRFs
Ring opened	0.6	0.7
proMABA	0.4	0.2
Dimers I+II	0.3	0.5
Other impurities	0.2	0.2
Total impurities	1.5	1.6

Determination of the relative UV response factors

Several impurities that are typically present in the API at levels exceeding 0.1% needed to be prepared, isolated, and structurally characterized. The relative UV response of these impurities versus ertapenem also needed to be determined. The following section discusses the determination of the relative response factors (RRF) of dimers I+II, the ring-opened hydrolysis product, and proMABA. The other impurities given in the chromatograms were not isolated because the levels did not exceed 0.1%.

Dimer I+II degradates

Dimer I+II degradates are the major dimers present in the API. An assigned purity of 54.2% (expressed as free acid) was found for dimer I+II based on determination of the purity by HPLC, moisture by Karl Fischer titration, residual solvents by headspace GC, salt levels by ion exchange chromatography using indirect photometric detection or conductivity detection and flame emission (or both) (17,18). The relative response factor was obtained by determining the ratio of the slope of a linearity plot for dimers I+II and ertapenem. Solutions of ertapenem reference standard were prepared at 100% of the target concentration (0.2 mg/mL), solutions of dimers I+II were prepared at 4%, 20%, and 100%. The detector response was found to be linear over the entire dimer concentration range. The regression coefficient R^2 was 0.9988 (slope of dimer I+II). An RRF of 0.66 was calculated as the ratio of slope of sample (dimers) over slope of standard (see Figure 9).

Ring-opened hydrolysis product

The ring-opened compound is a hydrolysis product of ertapenem. The purity of the isolated ring-opened hydrolysis product was 75.7% as carboxylic acid. The RRF of the ring-opened compound, determined similarly to the case of dimers I+II, was found to be 0.87.

Sidechain impurity (proMABA)

The proMABA is an in-process impurity from the side chain of ertapenem. The relative response factor (RRF) of the proMABA impurity was determined versus ertapenem. Solutions of a proMABA HCl salt sample were analyzed. The purity of the synthesized proMABA impurity was 95.7% as HCl salt. The RRF of proMABA was determined to be 1.68.

Impact of the RRF on the impurity profile of typical ertapenem lots

It is necessary to have relative response factors determined for high level impurities present in API or drug product. The use of accurately measured RRFs provides the evidence that a specific lot is still within specifications based on individual or total impurity levels. The ring-opened hydrolysis product, dimer I+II degradates, and proMABA were the major impurities that were found at levels greater than 0.1% in the ertapenem sodium API. Based on the calculated RRF values, the ring-opened was underestimated by 12%, dimer I+II degradates are underestimated by 34%, and the proMABA impurity overestimated by 68%. The impurity levels of a selected batch of ertapenem sodium API based on uncorrected and RRF corrected area-% data are given in Table VI. The impurity levels in the corrected data have been adjusted by dividing the impurity area-% by the calculated RRF. RRF values of 1.0 were used for impurities, which were present at levels < 0.1%. It is seen from Table VI that the impact of the relative response factors is minimal for a typical ertapenem sodium API sample, with an increase of only 0.1% in the given ertapenem sodium API lot.

Conclusion

A robust and sensitive HPLC impurity profile method for the unstable antibiotic ertapenem sodium API and its primary degradates and process impurity was developed and validated. The method employs gradient elution HPLC with UV detection. The injection precision, linearity, LOQ, LOD, selectivity, accuracy, ruggedness, and stability were evaluated and found to be satisfactory. The method can be used routinely to ensure the quality of manufactured ertapenem sodium API. The major impurities were isolated and characterized and the relative response factors versus ertapenem reference standard were obtained.

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