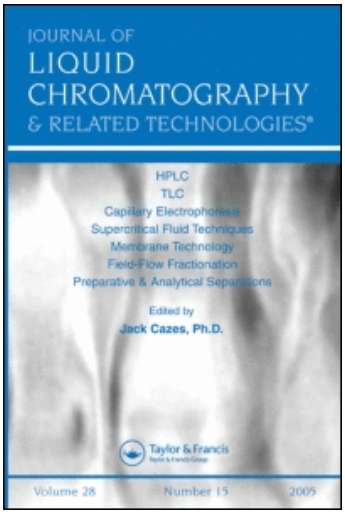

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ACETATE AND CHLORIDE DETERMINATION BY ION EXCHANGE HPLC WITH INDIRECT PHOTOMETRIC DETECTION AND ITS APPLICATION TO A β-METHYLCARBAPENEM ANTIBIOTIC

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ABSTRACT

A high performance liquid chromatographic (HPLC) method, using an anion exchange column with indirect photometric detection, was developed for the determination of acetate and chloride anions in the antibiotic ertapenem sodium. HPLC with indirect photometric detection was used due to the UV-transparent properties of acetate and chloride and the advantage that the method employs simple rugged laboratory chromatographic equipment. The challenges encountered with method optimization are discussed.

The method gives baseline separation of the anions with limit of quantitation values of 0.005 wt % and 0.006 wt % for acetate and chloride, respectively. The method was also validated for precision, linearity, recovery, ruggedness, and solution stability.

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INTRODUCTION

Ertapenem sodium,† [4R-[3(3S*,5S*), 4α,5β,6β,(R*)]]-3-[[5-[[(3-carboxyl-phenyl) 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, structure shown in Figure 1, is a synthetic broad spectrum carbapenem antibiotic with activity against a wide range of Gram-negative and Gram-positive aerobic and anaerobic bacteria.(1,2) It was developed for treatment of a variety of moderate to severe bacterial infections and has been effective and well-tolerated over the course of therapy in clinical trials. Acetate and chloride are used in the process to prepare ertapenem sodium bulk drug; therefore, it was necessary to develop a method to quantitatively determine residual levels of acetate and chloride in the bulk drug substance.

The concentrations of inorganic ions can be determined by ion selective potentiometry,(3) capillary electrophoresis,(4,5) and ion chromatography. The disadvantages of ion-selective potentiometry are that the methods are only capable of analyzing a single ion at a time, are subject to interference problems, and do not provide adequate sensitivity. Capillary electrophoresis with indirect UV detection has been used for anion determinations; however, the technique is not as rugged as HPLC. Conventional ion chromatography,(6) introduced in 1975, requires specialized dual-column instrumentation with conductivity detection. The conductivity detector requires careful maintenance and the response has a limited linear range. Indirect photometric ion exchange chromatography with UV detection permits ion chromatography on a simple HPLC system. Indirect

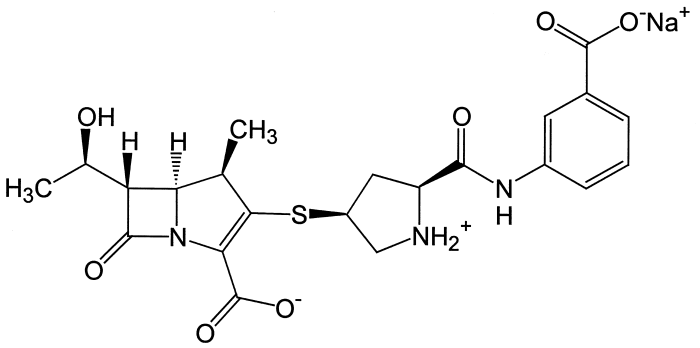

Figure 1. Structure of ertapenem sodium.

† The bulk drug substance ertapenem sodium is isolated as a monosodium salt. Ertapenem is formulated as a disodium salt and is known as INVANZ,™ which is trademark of Merck & Co., Inc., Whitehouse Station, NJ, USA.

photometric chromatography has been widely used for detection of ions lacking chromophores or other suitable properties for simple, routine detection with commonly used detectors.(7-14)

Indirect photometric chromatography with UV detection requires the use of a highly absorbing modifier in the mobile phase. The monitoring wavelength is chosen where the mobile phase additive highly absorbs and the analytes have little or no absorbing properties. A constant concentration of UV absorbing mobile phase additive is used and distributed to the stationary phase. The mobile phase eluting from the column gives a steady detector response. In ion exchange applications, the UV-absorbing counterion used in the mobile phase competes with the UV-transparent analyte ions for the ion exchange sites in the column stationary phase. As the UV-transparent analyte ions elute from the column, the analytes replace the UV-absorbing counterion in the effluent.(15) This leads to a decrease in the absorbance at the detector and produces a negative peak. Alternatively, the analytes can be observed as positive peaks when the polarity of the UV detector is reversed. There is one peak for each separated analyte and additional peaks that are characteristic of the chromatographic system can be observed.

There have been relatively few indirect UV photometric chromatography applications reported for pharmaceuticals.(16-19) This paper will discuss the application of ion exchange HPLC using indirect photometry with UV detection for determination of residual acetate and chloride in ertapenem sodium bulk drug, and the challenges encountered in method optimization. The HPLC indirect photometry method development and subsequent method validation for precision, linearity, sensitivity, recovery, ruggedness, and solution stability are discussed.

EXPERIMENTAL

Apparatus

The high performance liquid chromatograph used in the study consisted of a Thermo Separation Products Model AS3000 autosampler (Thermo Separation Products, Piscataway, New Jersey, USA), Thermo Separation products Model P4000 pump (Thermo Separation Products, Piscataway, New Jersey, USA), ABI Model 785A variable wavelength detector (ABI Analytical, Foster City, California, USA), Perkin-Elmer Access Chrom version 1.7 software (PE Nelson, Cupertino, California, USA).

The column used was a Hamilton PRP-X100, 250 x 4.1 mm (Hamilton Co., Reno, Nevada, USA) with 10 μm spherical particle size and poly(styrene-divinylbenzene) trimethylammonium base ion exchanger. The column is a low capacity 0.2 meq/g anion exchanger.

Materials and Chemicals

Ertapenem sodium bulk drug was prepared by Merck Research Laboratories (Rahway, New Jersey, USA). HPLC grade water was obtained from a HYDRO water purification unit (Garfield, New Jersey, USA). Sodium acetate trihydrate, sodium hydroxide, trace metal grade nitric acid were purchased from Fisher Scientific (Fair Lawn, New Jersey, USA). Sodium chloride and Omnisolv HPLC grade methanol were purchased from EM Science (Gibbstown, New Jersey, USA) and *p*-hydroxybenzoic acid was obtained from Sigma (St. Louis, Missouri, USA). All chemicals and solvents were reagent grade.

Chromatographic Conditions

The mobile phase was prepared by dissolving 270-280 mg *p*-hydroxybenzoic acid in 1.98 L of HPLC-grade water. The pH was adjusted to 9.5 with aqueous sodium hydroxide and 20 mL of methanol added. The mobile phase was well-mixed prior to use. The anion exchange column was equilibrated with mobile phase at 1.5 mL/min for 3 hours at 50°C (unless otherwise noted) prior to use. The monitoring wavelength for the UV detector was 305 nm. The injection volume was 20 µL. The column wash solution was prepared by pipetting 2 mL of concentrated nitric acid into 2 L of methanol and mixing well. The column was washed with the column wash solution for 3 hours at 2.0 mL/min or overnight at 0.5 mL/min.

Standard and Sample Preparation

The acetate and chloride anion standard solution was prepared by accurately weighing appropriate amounts of sodium acetate trihydrate and sodium chloride in a volumetric flask to prepare a 0.3 mg/mL solution. The solution was dissolved and diluted to volume with HPLC-grade water.

Ertapenem sodium samples were prepared at concentrations of 50 mg/mL in HPLC-grade water diluent.

RESULTS AND DISCUSSION

Method Development and Optimization

The objective was to develop a precise, sensitive, and specific method for the quantitation of acetate and chloride anions in ertapenem sodium bulk drug

substance. Method ruggedness was critical to enable method transfer to the factory and subsequent use in manufacturing quality control laboratories.

Dependence of Separation on the Selection of Mobile Phase Additive, Additive Concentration, pH of Mobile Phase, and Wavelength of Detection

The choice of mobile phase additive was determined by choosing an additive, which provided optimum selectivity for the separation of the anion analytes and one, which had high absorbance at longer UV wavelengths where the acetate and chloride analytes are UV transparent. Aromatic acids with different dissociation constants and high molar absorption coefficients were used in order to obtain high detection sensitivity. Trimesic acid, phthalic acid, and *p*-hydroxybenzoic acid were evaluated. Trimesic acid and phthalic acid (polyvalent ions) were found to be stronger displacing ions than *p*-hydroxybenzoic acid. The optimum separation of acetate, chloride, and the ertapenem system peak (formation to be discussed later in this paper) was obtained with the weaker displacing ion *p*-hydroxybenzoic acid.

The concentrations between 1.0 mM and 4.0 mM *p*-hydroxybenzoic acid mobile phase modifier were evaluated. The concentration of mobile phase modifier was found to effect method sensitivity and retention. Sensitivity for detection of acetate and chloride was improved by decreasing the concentration of *p*-hydroxybenzoic acid mobile phase additive from 4.0 mM to 1.0 mM. Therefore, the additive concentration of 1.0 mM was chosen to obtain the desired method sensitivity and separation of acetate and chloride anions in ertapenem sodium.

The monitoring wavelength was chosen where the mobile phase additive highly absorbs and the anion analytes have little or no absorbing properties. The monitoring wavelength of 305 nm was chosen since it is in the wavelength region of maximum absorbance for *p*-hydroxybenzoic acid and the acetate and chloride anions are UV-transparent at this wavelength.

The pH of the mobile phase will affect the charge of the anion analyte and its hydrophobicity. The mobile phase pH must be sufficiently high to ensure that the analytes are ionized, for the analyte to be retained by anion exchange interactions. A pH of 9.5 was chosen for the mobile phase to insure that acetate and ertapenem will be completely ionized, the pKa values are given in Table 1.

Dependence of Separation on Methanol Concentration

The concentration of methanol used in the mobile phase changes the relative polarity of the mobile and stationary phases, and was found to effect the

Table 1. pK_a Values of Acetate (20), Bicarbonate, (20) and Ertapenem Sodium (21)

Component	pK_{a1}	pK_{a2}	pK_{a3}
Acetic Acid	4.76	n/a*	n/a
Carbonic Acid	6.35	10.33	n/a
Ertapenem Sodium	2.72	3.96	7.06

*n/a = not applicable.

retention of acetate and the system peak. The concentrations of 0-1.5 v/v% methanol in the 1.0 mM *p*-hydroxybenzoic acid pH 9.5 aqueous mobile phase were evaluated. It was necessary to have some organic modifier in the mobile phase to optimize the separation between acetate, chloride, and the system peak. The optimum concentration of methanol in the mobile phase was determined as 1 v/v%.

Dependence of Separation on Column Temperature

Column temperatures at ambient conditions (ca. 25°C), 45, 50, and 55°C were evaluated for the method optimization. An increase of the column temperatures from ambient (ca. 25°C) to 45°C led to a decrease in the retention times of acetate and chloride anions by 0.4–1.5 minutes. Increasing the column temperature from 50°C to 55°C did not significantly change the retention times of each analyte. The temperature of 50°C was chosen because it provided short analysis times and was effective in sharpening the acetate and chloride peak shapes. The column stability is excellent at 50°C for extended time periods.

Diluent and Sample Concentration Determination

Solubility and potential absorbance interferences were considered for the diluent selection. Water was chosen as the diluent because ertapenem sodium is very soluble and water does not interfere in the analysis. The sample concentration of 50 mg/mL was selected since it provides the desired method sensitivity for acetate and chloride in ertapenem sodium drug substance.

Dependence of Separation on Flow Rate

Flow rates of 1.0–2.0 mL/min were evaluated for the separation. A flow rate of 1.0 mL/min increased the retention times of acetate and chloride by 2.3-

2.7 minutes and the flow rate of 2.0 mL/min decreased the retention times of each analyte by 1.1-1.3 minutes. A flow rate of 1.5 mL/min provided optimum separation of the analytes with 15 minute standard run times and 60 minute sample run times, with the ertapenem peak eluting at approximately 55 minutes.

Method Challenges

This section will discuss the challenges encountered after the optimized method was applied to large numbers of samples. A description of the observation and measures taken to solve the issue are given.

Ertapenem System Peak Formation

Evaluation of ertapenem sodium samples was found to generate a system peak at approximately 10 minutes. An anion standard solution containing acetate, chloride, and bicarbonate was spiked into an ertapenem sodium sample solution and the bicarbonate peak was not observed in the sample. The system peak is formed at approximately the same retention time as bicarbonate; see water blank, anion standard, sample, and anion standard spiked sample in Figure 2. The observation that low level bicarbonate addition to ertapenem samples does not give rise to a bicarbonate peak as observed in standard solutions, is consistent with formation of a carbon dioxide adduct resulting from interaction of ertapenem sodium with bicarbonate. Carbon dioxide adduct formation has been previously reported for meropenem,(22) also a 1- β-methylcarbapenem antibiotic. Meropenem contains a pyrrolidine moiety as does ertapenem sodium. It is believed that formation of the carbon dioxide adduct creates a chromatographic disturbance (system peak), which interferes with detection of bicarbonate in ertapenem sodium. The ertapenem system peak is well resolved from acetate and chloride anions and does not interfere with their quantification.

Effect of Repeated Injections of Ertapenem Sodium

It was found that the ion exchange column performance decreased when numerous injections of ertapenem sodium were made. Figure 3 gives chromatograms of the sample injected onto the column initially, and after, 1, 3, 6 and 9 injections. Injections of sample solutions at a concentration of 50 mg/mL are required to achieve the desired method sensitivity, so sample loading cannot be decreased. Extended equilibration with the mobile phase did not improve the column performance. The column is restored after regeneration with the

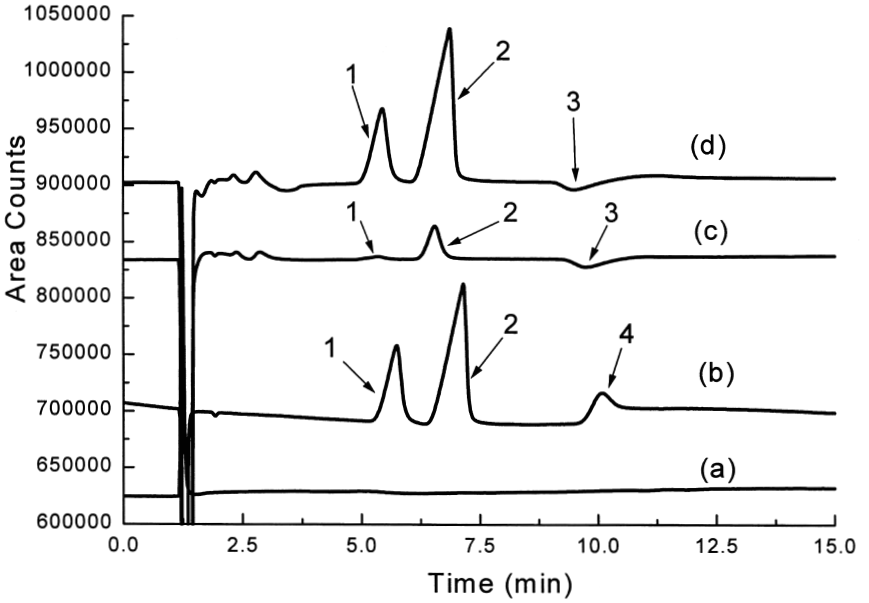

Figure 2. Chromatograms showing formation of the ertapenem system peak. 1 - acetate; 2 - chloride; 3 - ertapenem system peak; 4 - bicarbonate. (a): Water blank. (b): Acetate, chloride, bicarbonate anion standard solution diluted in water. (c): Ertapenem sodium diluted in water. (d): Ertapenem sodium diluted in acetate, chloride, bicarbonate standard solution.

methanolic nitric acid column wash solution. The wash effectively removes adsorbed ertapenem degradation products from the stationary phase. The chromatograms of the sample, before and after the column wash procedure, are given in Figure 4. Incorporation of a column washing step was included in the procedure for the analysis of ertapenem sodium. The column wash step is performed after several sample injections to maintain optimum precision of acetate and chloride results.

Method Validation

Precision

The injection precision was determined by repeated analysis of the acetate and chloride anion standard solution. Six consecutive injections were made. The

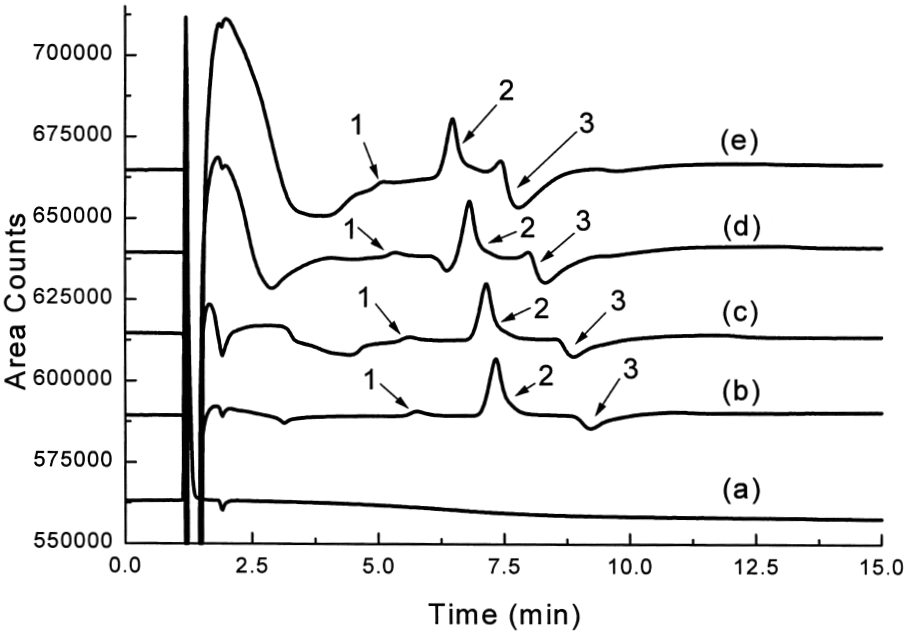

Figure 3. Effect of repeated injections of ertapenem sodium. 1 - acetate; 2 - chloride; 3 - ertapenem system peak. (a): Water blank. (b): Ertapenem sodium diluted in water, injection #1. (c): Ertapenem sodium diluted in water, injection #3. (d): Ertapenem sodium diluted in water, injection #6. (e): Ertapenem sodium diluted in water, injection #9.

results were satisfactory with %RSD values of 1.8% and 1.2% for acetate and chloride anions, respectively.

The method precision was evaluated by analyzing the same sample multiple times on different days. Nine individual determinations of freshly prepared samples and standard solutions were completed by two analytical chemists. The daily average, standard deviation, and % relative standard deviation of the weight % values were satisfactory with %RSD <30% for acetate and %RSD <8% for chloride. The reproducibility of the method was found to be satisfactory for both analytical chemists with comparable %RSD's for each of the analytes on different days. The overall average %RSD's of the weight % of each analyte, between the two analytical chemists, for the 27 individual determinations were compared. The average result of three injections, for each of nine determinations of acetate and chloride anions in the sample is given in Table 2. The %RSD of the average weight %'s determined for acetate and chloride by two analytical chemists, is satisfactory, see results in Table 3. The %RSD's were 10.8% and 0.7% for acetate and chloride anions, respectively. The higher %RSD value for acetate is acceptable due to the low level (<0.01 wt %) present in the sample.

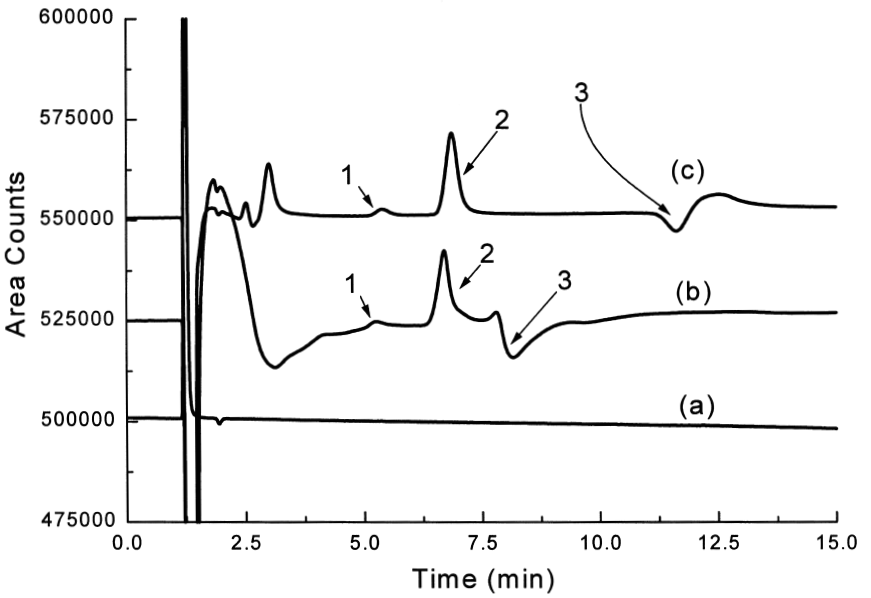

Figure 4. Chromatograms of ertapenem sodium before and after column washing procedure. 1 - acetate; 2 - chloride; 3 - ertapenem system peak; (a): Water blank. (b): Ertapenem sodium diluted in water before column wash procedure. (c): Ertapenem sodium diluted in water after column wash procedure.

Linearity

Linearity was evaluated from triplicate injections of the acetate and chloride standard solutions in the concentration range of 0.0023–0.4663 mg/mL for acetate and 0.0032–0.6306 mg/mL for chloride, which corresponds to 0.005–0.933 wt % acetate and 0.006–1.26 wt % chloride in the sample, with target concentration of 50 mg/mL. The plots for acetate and chloride were linear with correlation coefficient R^2 = 0.9999 and 0.9998 for acetate and chloride, respectively. Therefore, the method is linear for the concentration ranges of 0.005–0.933 wt % acetate and 0.006–1.26 wt % chloride.

Sensitivity-Determination of Limit of Quantitation

Serial dilutions of the anion standard solution were made to establish the limit of quantitation (LOQ) for acetate and chloride. The criteria used to define

Table 2. Precision Results

Weighing* Number	Analytical Chemist #1 Acetate (wt %)	Analytical Chemist #1 Chloride (wt %)	Analytical Chemist #2 Acetate (wt %)	Analytical Chemist #2 Chloride (wt %)
1	0.0074	0.0640	0.0094	0.0636
2	0.0083	0.0596	0.0080	0.0618
3	0.0087	0.0529	0.0062	0.0572
4	0.0110	0.0555	0.0072	0.0583
5	0.0095	0.0565	0.0120	0.0592
6	0.0097	0.0567	0.0055	0.0518
7	0.0085	0.0536	0.0073	0.0607
8	0.0096	0.0564	0.0081	0.0570
9	0.0097	0.0656	0.0076	0.0565
Avg.	0.0092	0.0579	0.0079	0.0585
% RSD	12.4	7.7	28.6	6.4

*The acetate and chloride value for each weighing are the average result of three injections.

LOQ is the lowest concentration at which the injection precision yields ≤15% relative standard deviation (RSD) for three injections; the signal to noise ratio is ≥10:1 and ≤20% RSD is observed in the response factor compared to a five-fold more concentrated standard solution.

The results of the experiments revealed that at a standard concentration of 0.00233 mg/mL acetate, the injection precision was 3.6% RSD, the S/N ratio was 14:1, and 1.3% deviation of the response factor was observed between the 0.00233 mg/mL and the 0.01166 mg/mL standard solution. The LOQ of acetate was determined as 0.002 mg/mL, which is equivalent to 0.005 wt % in the sample at the target concentration of 50 mg/mL.

The results of the studies showed, that at a standard concentration of 0.00315 mg/mL chloride, the injection precision was 2.2% RSD, the S/N ratio is

Table 3. Overall Precision

Anion	Analytical Chemist #1	Analytical Chemist #2	Avg.	Std. Dev.	%RSD
Avg. Acetate (wt %)	0.0092	0.0079	0.0086	0.0009	10.8
Avg. Chloride (wt %)	0.0579	0.0585	0.0582	0.0004	0.7

Table 4. Acetate Recovery Studies

Approx. Acetate Spike Conc. in Sample (wt-%)	Concentration of Acetate in Sample (wt-%)	Difference Between Spiked Sample and Unspiked Sample (wt-%)	Theoretical Concentration Acetate (wt-%)	% Recovery
0.00	0.0102	n/a*	n/a	n/a
0.05	0.0599	0.0497	0.0502	99.0
0.10	0.1099	0.0997	0.1002	99.5
0.20	0.2133	0.2031	0.2006	101.2

*n/a = not applicable.

31:1, and 5.7% deviation of the response factor was observed between the 0.00315 mg/mL and the 0.01577 mg/mL standard solution. The LOQ of chloride was determined as 0.003 mg/mL, which is equivalent to 0.006 wt % in the sample, at the target concentration of 50 mg/mL.

Recovery

An anion standard solution containing 0.58 mg/mL sodium acetate (0.25 mg/mL acetate) and 0.41 mg/mL sodium chloride (0.25 mg/mL chloride) was spiked at three different concentration levels representing 0.05, 0.10, and 0.20 wt % of each analyte into different sample preparations. The unspiked sample was also analyzed in the same HPLC run. The recoveries were satisfactory for both acetate and chloride at the three spiking levels. The recovery results ranged from 99-101% for acetate and 106-108% for chloride; see Tables 4 and 5. The chromatograms are shown in Figure 5.

Table 5. Chloride Recovery Studies

Approx. Chloride Spike Conc. in Sample (wt-%)	Concentration of Chloride in Sample (wt-%)	Difference Between Spiked Sample and Unspiked Sample (wt-%)	Theoretical Concentration Chloride (wt-%)	% Recovery
0.00	0.0713	n/a*	n/a	n/a
0.05	0.1238	0.0525	0.0497	105.7
0.10	0.1765	0.1052	0.0991	106.2
0.20	0.2852	0.2139	0.1984	107.8

*n/a = not applicable.

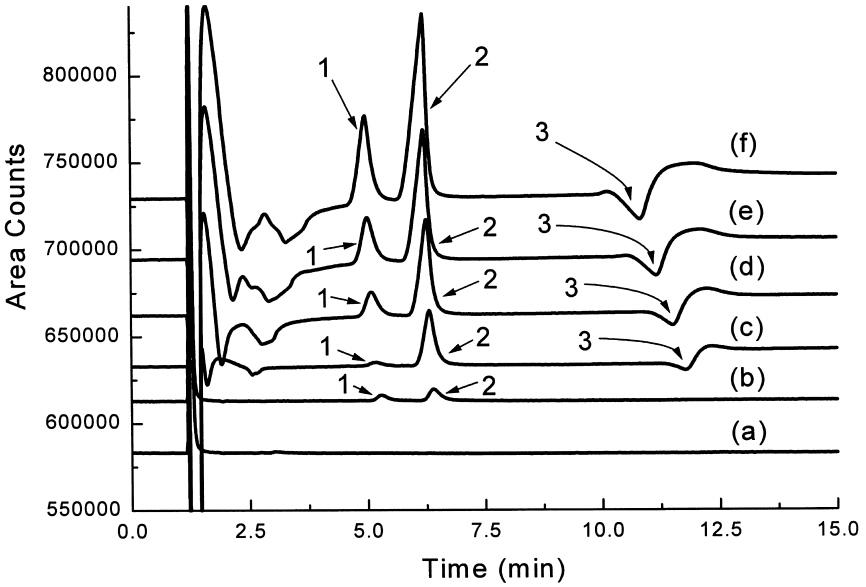

Figure 5. Recovery of acetate and chloride from ertapenem sodium samples. 1 - acetate; 2 - chloride; 3 - ertapenem system peak; (a): Water blank. (b): Acetate and chloride anion solution diluted 25-fold in water. (c): Ertapenem sodium diluted in water. (d): 0.05 wt % acetate and chloride spiked ertapenem sodium. (e): 0.10 wt % acetate and chloride spiked ertapenem sodium. (f): 0.20 wt % acetate and chloride spiked ertapenem sodium.

Ruggedness

Several different lots of the anion exchange HPLC columns were used for the analyses of the same sample. The change in column did not significantly affect the separation of acetate and chloride anions or the level of each anion present in the sample. The results of the analysis using two different columns are shown in Table 6. The %RSD of the results for each column was satisfactory with values of 22.8% and 6.6% for acetate and chloride, respectively. The higher %RSD value for acetate is acceptable due to the low level (<0.01 wt-%) present in the sample.

Solution Stability

The solution stability of the sample and standard solutions was investigated at both ambient and refrigeration storage conditions. The sample solution was

Table 6. Ruggedness: Variation of Analytical Column

	Column Number				
Anion	#1	#2	Avg.	Std. Dev.	%RSD
Avg. Acetate (wt %)	0.0086	0.0119	0.0103	0.0023	22.8
Avg. Chloride (wt %)	0.0582	0.0639	0.0611	0.0040	6.6

held at ambient conditions for 1 day and for 1, 2, and 21 days (3 weeks) at refrigeration in polypropylene vials. When the sample solutions were stored for >1 day at ambient conditions, the solution became discolored and yellow solids formed. The comparison of results between the fresh samples and the stored samples was acceptable for the samples stored at ambient conditions for 1 day, and stored at

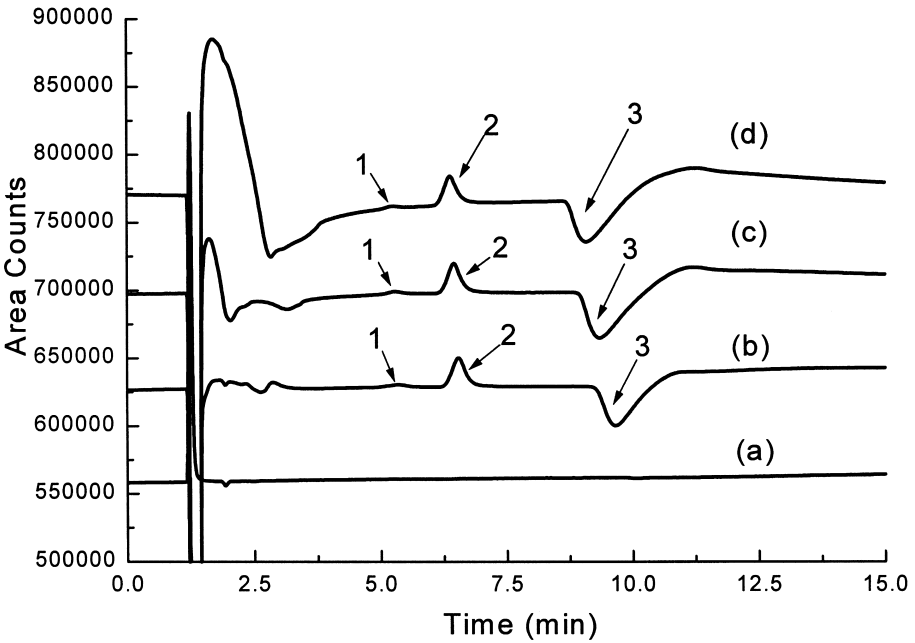

Figure 6. Solution stability of ertapenem sodium samples. 1 - acetate; 2 - chloride; 3 - ertapenem system peak; (a): Water blank. (b): Ertapenem sodium diluted in water, fresh solution. (c): Ertapenem sodium diluted in water and stored at 5°C for 1 day. (d): Ertapenem sodium diluted in water and stored at ca. 25°C for 1 day.

refrigeration for 1 and 2 days (see chromatograms in Figure 6). The chromatography of the sample stored for 3 weeks at refrigeration storage conditions was not optimal, but the results could be quantified. The sample acetate and chloride results are acceptable using standard solutions stored at ambient conditions for 1 and 4 days, with %RSD's <1% for both acetate and chloride. The acetate and chloride standard solution was stable for up to 4 days, at ambient conditions in glass volumetric flasks. It is concluded from these experiments, that the sample solutions should be analyzed within 1 day at ambient conditions and within 2 days at refrigeration storage conditions.

CONCLUSION

HPLC with indirect photometric detection was developed for the quantification of acetate and chloride in ertapenem sodium drug substance and determined to be precise, sensitive, and rugged for routine analysis. Specialized equipment was not required for the analysis. Analysis of ertapenem sodium presented some interesting analytical challenges, which were investigated and solved. The optimized method was further validated for precision, linearity, sensitivity, recovery, ruggedness, and solution stability.

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