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Determination of etimicin and related impurities in etimicin sulfate

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Keywords

Dionex IonPac AmG-3µm C18 column, aminoglycoside, Dionex ICS-5000+ HPIC system, PAD, electrochemical detection, drug substance, antibiotic, ion-pair reversed-phase HPLC, China Pharmacopoeia, Dionex ICS-6000 HPIC system

Goal

To validate the etimicin sulfate China Pharmacopoeia (ChP) monograph method for etimicin content and impurities using a Thermo Scientific™ Dionex™ IonPac™ AmG-3µm C18 column

Introduction

Etimicin is a broad-spectrum, water-soluble antibiotic belonging to the group of aminoglycoside antibiotics. It is valuable in the treatment of serious infections caused by gram-negative bacteria and some gram-positive bacteria such as those that infect the digestive system or cause perioperative infection. Etimicin is a semi-synthetic aminoglycoside antibiotic prepared from gentamicin C1a by introduction of an ethyl group at the 1-N-position.[1] Although etimicin is the main product, some byproducts such as 3-N-ethyletimicin can also be formed in small amounts during synthesis. Differences in antimicrobial potency and toxicity necessitate the limitation and control of the amount of impurities in commercial samples.

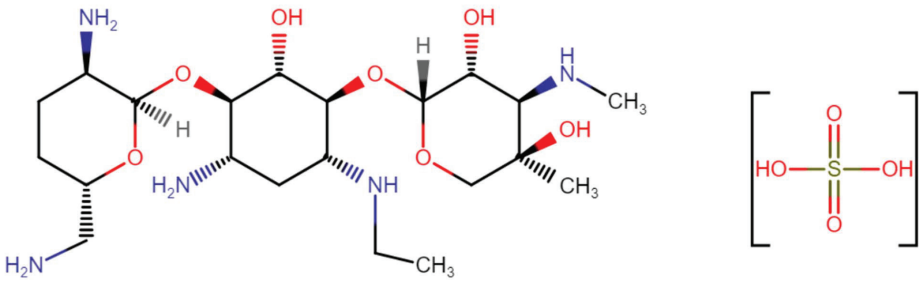

Figure 1. Structure of etimicin sulfate

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The number of impurities makes the chromatographic analysis challenging. Detection of etimicin and impurities is problematic because they lack good UV-absorbing chromophores. Ion-pairing reversed-phase liquid chromatography is widely used to separate aminoglycosides by using volatile perfluorinated carboxylic acids, such as trifluoroacetic acid (TFA) and pentafluoropropionic acid (PFPA), and this separation method has been paired with electrochemical detection. Pulsed amperometric detection (PAD), a powerful detection technique with a broad linear range and very low detection limits, is ideally suited for detecting aminoglycoside antibiotics and their impurities. Electrochemical detection has advantages relative to other techniques in that an oxidation potential can be selected for specific analytes while other compounds remain undetected. Derivatization is not required for detection, which simplifies the analysis. The analysis of etimicin sulfate in pharmaceutical formulations based on ion-pairing HPLC-PAD is described in the China Pharmacopoeia.[2]

The Dionex IonPac AmG-3µm C18 columns are specifically designed for ion-pairing reversed-phase analysis of various aminoglycoside antibiotics. The stationary phase is prepared through the covalent bonding of C18 ligands onto a polymer-encapsulated silica media, which ensures ultra-stability when exposed to varied mobile phase conditions such as low pH, high temperature, different organic solvents, and highly aqueous solutions.[3] The Dionex IonPac AmG-3µm column is packed in a PEEK column body rather than stainless steel. A stainless steel column, as well as steel used in conventional HPLC systems, can release significant levels of metal contamination, particularly when corrosive eluents are used. Metal ions can interfere with electrochemical detection.

In this application note, the etimicin sulfate analysis method in the ChP monograph was evaluated with a Dionex IonPac AmG-3µm C18 column. Key performance parameters were evaluated including system suitability separation, linearity, limits of detection, and precision. One sample was analyzed and the percentage of etimicin in that sample was determined. Impurity was also determined and compared with ChP Etimicin Sulfate acceptance criteria.

Experimental conditions

Equipment

- Thermo Scientific™ Dionex™ ICS-5000⁺ HPIC™ system including*:
  - Dionex ICS-5000⁺ DP Pump module
  - Dionex ICS-5000⁺ DC Detector/Chromatography module with ED Electrochemical Detector
  - Thermo Scientific™ Dionex™ AS-AP Autosampler with sample tray cooling, 250 µL sample syringe (P/N 074306),1200 µL buffer line (P/N 074989), and 1.5 mL vial trays (P/N 074936)
- Dionex ICS-5000⁺ ED Electrochemical Detector Cell (P/N 072044)
- ED conventional working electrode, gold, 3 mm (P/N 063723) with 5 mil gasket (P/N 063550)
- Reference electrode pH, Ag/AgCl (P/N 061879)
- Knitted reaction coil, 375 µL, unpotted (P/N 043700)
- Three-way manifold (P/N 048227)
- Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) Software, version 7.2.5

*This method can be run on a single Dionex ICS-5000⁺ or Dionex ICS-6000 system using a Thermo Scientific™ Dionex™ AXP pump to add the post-column reagent.

The procedure for system preparation and setup can be found in Thermo Scientific Application Note 72647[4] with support from specific product manuals.[5–8]

Consumables

- Glass autosampler vials 1.5 mL with slit septum (P/N 055427)
- Thermo Scientific™ Nalgene™ Rapid-Flow™ Sterile Disposable Filter Units with Nylon Membrane (1000 mL, 0.2 µm pore size, Fisher Scientific P/N 09-740-46)
- Helium ultrahigh purity

Reagents and standards

- Deionized (DI) water, Type I reagent grade, 18 MΩ·cm resistivity or better
- Trifluoroacetic acid (Fisher Scientific P/N PI28901)
- Pentafluoropropanoic acid (Sigma-Aldrich P/N 245917-50G)
- Sodium sulfate (Sigma-Aldrich P/N 71959-250G)
- Sodium hydroxide 50% (w/w) (Fisher Scientific P/N SS254-500)
- Acetonitrile (Fisher Scientific P/N A955-4)
- ChP Etimicin sulfate reference standard*
- ChP Netilmicin sulfate reference standard*

*Kindly provided by a Chinese pharmaceutical company

Sample

An etimicin sample was obtained from a pharmaceutical company in China.

Chromatographic conditions

Columns:	Dionex IonPac AmG-3µm C18 Guard, 4 × 30 mm (P/N 302694) Dionex IonPac AmG-3µm C18 Analytical, 4 × 150 mm (P/N 302693)
Eluent:	0.2 M trifluoroacetic acid, 0.05% pentafluoropropanoic acid, 1.5 g/L sodium sulfate, adjust to pH 3.5 with NaOH, 4% acetonitrile
Flow rate:	0.8 mL/min*
Column temperature:	35 °C
Injection volume:	20 µL (Full loop)
Autosampler temperature:	5 °C
Reference electrode:	Ag/AgCl
Working electrode:	Conventional electrode gold, 3 mm diameter with a 5-mil gasket
Post-column reagent	0.76 M NaOH
Post-column reagent flow rate:	0.3 mL/min with delivered by pump 2
Detection:	Pulsed amperometric detection (Electrochemical detector)
Detection compartment temperature:	35 °C
Detection waveform:	Gold, Carbohydrates, 4-Potential (Table 1)
System backpressure:	~2700 psi
Run time:	50 min

*The ChP monograph describes the column as follows: Type C18 size 250 mm, ID 4.6 mm; 5-µm packing. The diameter of the Dionex IonPac AmG-3µm C18 column is 4 mm. Therefore, the flow rate was adjusted from 1 mL/min (ChP monograph condition) to 0.8 mL/min.

Table 1. Carbohydrates, 4-potential waveform

Time (s)	Voltage (V)	Integration
0	0.1	Off
0.2	0.1	On
0.4	0.1	Off
0.41	-2.0	Off
0.42	-2.0	Off
0.43	0.6	Off
0.44	-0.1	Off
0.5	-0.1	Off

Preparation of solutions and reagents

10-fold concentrated eluent

To prepare 2 L of 10x concentrated eluent (except acetonitrile):

1. Add 30 g of sodium sulfate to a 2 L glass eluent bottle that contains ~200 mL DI water and sonicate for 15 min to dissolve the sodium sulfate.
2. Add 456 g of trifluoroacetic acid into the glass eluent bottle.
3. Add 15.6 g of pentafluoropropanoic acid into the glass eluent bottle.
4. Add 320 g of 50% (w/w) NaOH into the solution, add degassed DI water to approximately 1800 mL. The pH of the solution should be around 3.5; if not, adjust it to be 3.5 with 50 % (w/w) NaOH.
5. Add extra degassed DI water to the eluent bottle until the total weight is 2350 g.
6. Sonicate the 2 L eluent bottle for 30 min with the cap on but untighten it to allow carbon dioxide to escape.

Note: Weigh trifluoroacetic acid and pentafluoropropanoic acid using a balance in a fume hood.

Eluent

To prepare 2 L of eluent:

1. Weigh 235 g of 10-fold concentrated eluent to a glass 2 L volumetric flask.
2. Add 63.2 g of acetonitrile to the glass 2 L volumetric flask.
3. Bring the volume to 2 L with degassed DI water.
4. Immediately transfer this solution to a glass eluent bottle.
5. Degas the eluent by sparging with helium gas for at least 10 min and blanket it with helium at 6 to 8 psi.

Post-column reagent (0.76 M NaOH)

To prepare 1 L of post-column reagent, degas 954 g of DI water by sparging helium gas for at least 10 min in a plastic eluent bottle, add 40 mL of 50% (w/w) NaOH into the eluent bottle. Immediately blanket it with helium at 6 to 8 psi. Gently swirl the bottle to complete mixing. Always maintain the eluents under 6 to 8 psi of helium to reduce diffusion of atmospheric carbon dioxide. Prepare new NaOH eluent if left un-blanketed for more than 30 min.

Note: It is very important to degas the eluent and post-column reagent by sparging with helium gas rather than ultrasonic agitation to avoid a slow decrease in peak area response over time.

Stock standard solutions

Etimicin stock, 1 mg/mL

Dissolve 43 mg of ChP grade etimicin sulfate in 25 mL of eluent.

Note: The etimicin sulfate reference standard claims to contain 58% etimicin.

Netilmicin stock, 1 mg/mL

Dissolve 43 mg of ChP grade netilmicin sulfate in 25 mL of eluent.

Working standard solutions

Etimicin standard, 0.25 mg/mL

Dilute 5 mL of etimicin stock to 20 mL with eluent.

Etimicin standard, 25 µg/mL

Dilute 2 mL of etimicin (0.25 mg/mL) to 20 mL with eluent.

Netilmicin standard, 0.25 mg/mL

Dilute 5 mL of netilmicin stock to 20 mL with eluent.

System suitability solution (25 µg/mL of ChP etimicin RS and 25 µg/mL of ChP netilmicin RS in eluent)

Mix 1 mL of etimicin standard (0.25 mg/mL) and 1 mL of netilmicin standard (0.25 mg/mL) and then dilute to 10 mL with eluent.

Sample preparation

Sample stock solution, 1 mg/mL

Dissolve 43 mg of etimicin sulfate sample in 25 mL of eluent.

Sample solution (a), 0.25 mg/mL

Dilute 5 mL of etimicin sample stock to 20 mL with eluent.

Sample solution (b), 25 µg/mL

Dilute 2 mL of etimicin sample solution (a) to 20 mL with eluent.

Sample solution (c), 2.5 µg/mL

Dilute 2 mL of etimicin sample solution (b) to 20 mL with eluent.

Use sample solutions (a) and (c) for impurity analysis. Use sample solution (b) for the etimicin assay.

Note: Store all standard and samples in a refrigerator after preparation.

Results and discussion

System suitability

The system suitability was evaluated using chromatograms of a system suitability standard and a 2.5 µg/mL etimicin standard. In the ChP monograph for etimicin sulfate, the system suitability requirement specifies resolution between netilmicin and etimicin as > 4. The monograph also specifies signal-to-noise (S/N) ratio > 10 for 2.5 µg/mL etimicin. Figure 2 shows the first chromatogram using a Dionex IonPac AmG-3µm C18 column set. Netilmicin and etimicin were well separated. Peak resolution between netilmicin and etimicin is 5.43, exceeding the ChP requirement of 4. Figure 3 shows the chromatogram of 2.5 µg/mL etimicin. Etimicin is sensitively detected. The S/N of 2.5 µg/mL etimicin is 77.1, easily exceeding the ChP requirement of 10. The system suitability requirements are met for all parameters (Table 2).

Table 2. System suitability

Test	ChP Criterion	Measured
Resolution between Netilmicin and Etimicin	> 4	5.43
S/N (Etimicin 2.5 µg/mL)	> 10	77.1

Column:	Dionex IonPac AmG-3µm C18 Guard, 4 × 30 mm (P/N 302694) Dionex IonPac AmG-3µm C18 Analytical, 4 × 150 mm (P/N 302693)
Eluent:	0.2 M Trifluoroacetic acid, 0.05% Pentafluoropropanoic acid, 1.5 g/L Sodium sulfate adjust to pH 3.5 with NaOH, 4% Acetonitrile
Inj. volume:	20 µL
Column temperature:	35 °C
Flow rate:	0.8 mL/min
Post-column reagent:	0.76 M NaOH (0.3 mL/min)
Detection:	Pulsed Amperometric Detector (Waveform: Carbohydrates, 4-Potential)

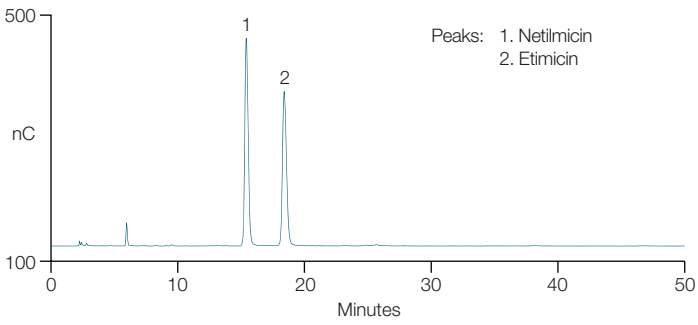

Figure 2. Separation of a system suitability standard (etimicin 25 µg/mL + netilmicin 25 µg/mL) using a Dionex IonPac AmG-3µm C18 column

Column:	Dionex IonPac AmG-3µm C18 Guard, 4 × 30 mm (P/N 302694) Dionex IonPac AmG-3µm C18 Analytical, 4 × 150 mm (P/N 302693)
Eluent:	0.2 M Trifluoroacetic acid, 0.05% Pentafluoropropanoic acid, 1.5 g/L Sodium sulfate adjust to pH 3.5 with NaOH, 4% Acetonitrile
Inj. volume:	20 µL
Column temperature:	35 °C
Flow rate:	0.8 mL/min
Post-column reagent:	0.76 M NaOH (0.3 mL/min)
Detection:	Pulsed Amperometric Detector (Waveform: Carbohydrates, 4-Potential)

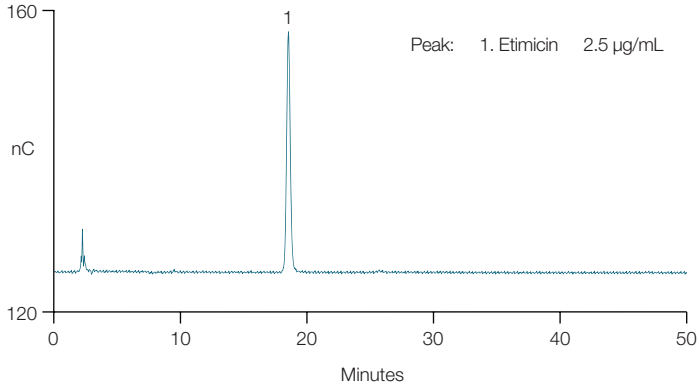

Figure 3. Etimicin standard (2.5 µg/mL)

Linearity

The linearity of etimicin electrochemical response to concentration was investigated in the concentration range of 0.25 to 25 µg/mL (0.25, 1, 2.5, 5, 10, 25 µg/mL). Figure 4 shows the calibration curve; the coefficient of determination is 0.9993. This reveals that a sample concentration of 25 µg/mL is within the response linear range and can be used for sample analysis.

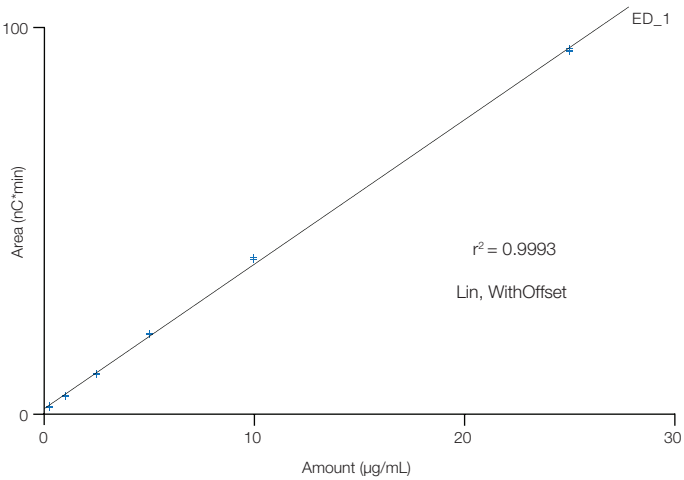

Figure 4. Calibration of etimicin

Method limits of detection and quantification

The United States Pharmacopeia chapter on method validation specifies a S/N of 3 for the determination of the limit of detection (LOD) and a S/N of 10 for the determination of the limit of quantitation (LOQ).[9] ChP has the same definitions for the LOD and LOQ.

To determine the LOD and LOQ, the baseline noise was first determined by measuring the peak-to-peak noise in a representative 1-min segment of the baseline where no peaks elute but close to the etimicin peak. The LOD and LOQ were then calculated from the average peak height of three injections of etimicin (0.25 µg/mL). Table 3 summarizes the LOD and LOQ of etimicin in sample solution and in etimicin sulfate powder.

Method precision

Method precision performance was evaluated with six replicate injections of an etimicin standard (25 µg/mL) over 27 h (injected every five hours). Figure 5 shows an overlay of the chromatograms from the precision analysis.

As shown in Table 4, the relative standard deviation (RSD) of peak area for six injections of etimicin standard is 0.373%.

Table 3. LOD and LOQ

Analyte	LOD (µg/mL) in Sample Solution	LOQ (µg/mL) in Sample Solution	LOD in Etimicin Sulfate Powder (µg/g)	LOQ in Etimicin Sulfate Powder (µg/g)
Etimicin	0.0571	0.190	228	761

Table 4. Peak area precision of six injections of etimicin standard, 25 µg/mL

Injection	Etimicin Peak Area (nC*min)
1	95.7
2	96.1
3	96.4
4	95.9
5	95.7
6	95.4
RSD	0.373%

Column:	Dionex IonPac AmG-3µm C18 Guard, 4 × 30 mm (P/N 302694) Dionex IonPac AmG-3µm C18 Analytical, 4 × 150 mm (P/N 302693)
Eluent:	0.2 M Trifluoroacetic acid, 0.05% Pentafluoropropanoic acid, 1.5 g/L Sodium sulfate adjust to pH 3.5 with NaOH, 4% Acetonitrile
Inj. volume:	20 µL
Column temperature:	35 °C
Flow rate:	0.8 mL/min
Post-column reagent:	0.76 M NaOH (0.3 mL/min)
Detection:	Pulsed Amperometric Detector (Waveform: Carbohydrates, 4-Potential)

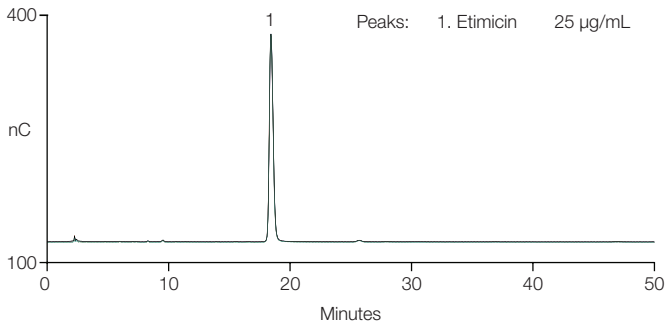

Figure 5. Overlay of 6 injections of etimicin standard (25 µg/mL)

Sample analysis

Etimicin assay

Etimicin standard (25 µg/mL) and sample solution (b) were used for etimicin assay. Figure 6 shows the separation of an etimicin ChP standard. Figure 7 shows the separation of etimicin sample (25 µg/mL). A few impurities were detected and separated from etimicin.

The percentage of etimicin in etimicin sample was calculated using the peak areas obtained from the chromatograms shown in Figures 6 and 7. The calculation method is shown below:

% Etimicin in sample = *(Rs/Rd)* ×100

Rs = Peak area of a sample solution

Rd = Peak area of a standard solution

The sample had 89% etimicin.

Column:	Dionex IonPac AmG-3µm C18 Guard, 4 × 30 mm (P/N 302694) Dionex IonPac AmG-3µm C18 Analytical, 4 × 150 mm (P/N 302693)
Eluent:	0.2 M Trifluoroacetic acid, 0.05% Pentafluoropropanoic acid, 1.5 g/L Sodium sulfate adjust to pH 3.5 with NaOH, 4% Acetonitrile
Inj. volume:	20 µL
Column temperature:	35 °C
Flow rate:	0.8 mL/min
Post-column reagent:	0.76 M NaOH (0.3 mL/min)
Detection:	Pulsed Amperometric Detector (Waveform: Carbohydrates, 4-Potential)

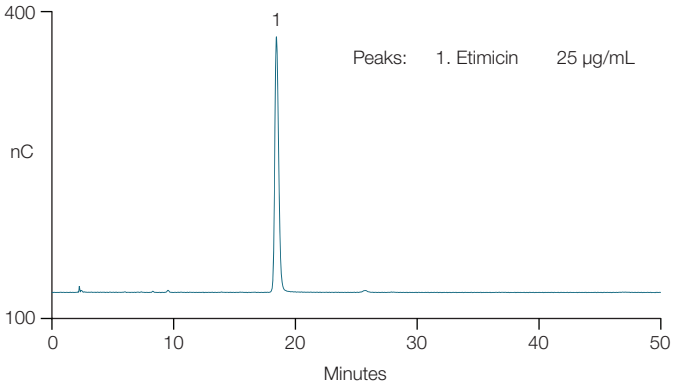

Figure 6. Separation of an etimicin ChP reference standard (25 µg/mL) using a Dionex IonPac AmG-3µm C18 column

Column:	Dionex IonPac AmG-3µm C18 Guard, 4 × 30 mm (P/N 302694) Dionex IonPac AmG-3µm C18 Analytical, 4 × 150 mm (P/N 302693)
Eluent:	0.2 M Trifluoroacetic acid, 0.05% Pentafluoropropanoic acid, 1.5 g/L Sodium sulfate adjust to pH 3.5 with NaOH, 4% Acetonitrile
Inj. volume:	20 µL
Column temperature:	35 °C
Flow rate:	0.8 mL/min
Post-column reagent:	0.76 M NaOH (0.3 mL/min)
Detection:	Pulsed Amperometric Detector (Waveform: Carbohydrates, 4-Potential)

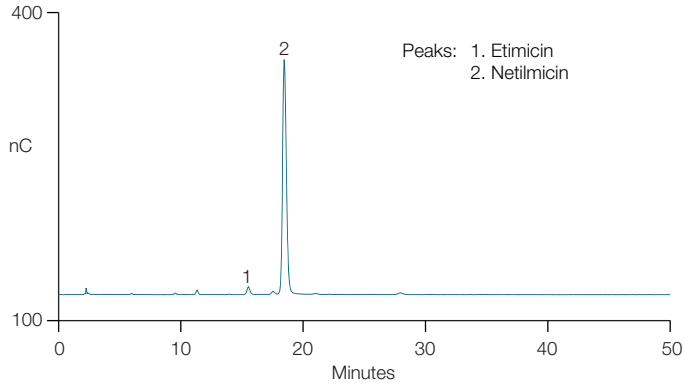

Figure 7. Separation of etimicin sample (25 µg/mL) using a Dionex IonPac AmG-3µm C18 column

Percentage of impurities in etimicin sulfate samples

Sample solutions (a) and (c) were used for impurities analysis. Figure 8 shows the chromatogram of etimicin standard solution (0.25 mg/mL).

Column:	Dionex IonPac AmG-3µm C18 Guard, 4 × 30 mm (P/N 302694) Dionex IonPac AmG-3µm C18 Analytical, 4 × 150 mm (P/N 302693)
Eluent:	0.2 M Trifluoroacetic acid, 0.05% Pentafluoropropanoic acid, 1.5 g/L Sodium sulfate adjust to pH 3.5 with NaOH, 4% Acetonitrile
Inj. volume:	20 µL
Column temperature:	35 °C
Flow rate:	0.8 mL/min
Post-column reagent:	0.76 M NaOH (0.3 mL/min)
Detection:	Pulsed Amperometric Detector (Waveform: Carbohydrates, 4-Potential)

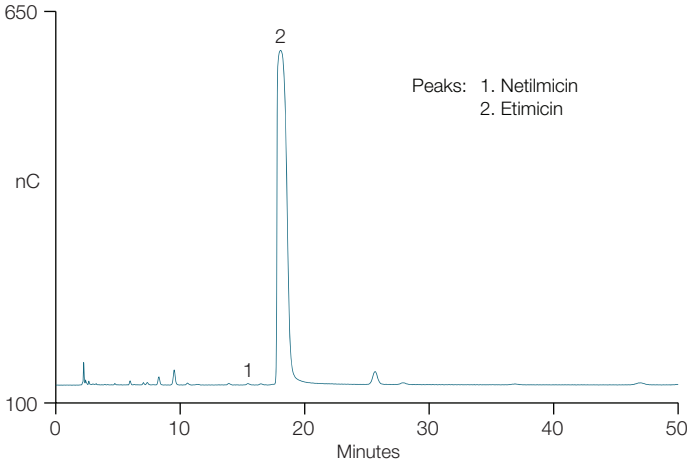

Figure 8. Separation of etimicin standard (0.25 mg/mL) using a Dionex IonPac AmG-3µm C18

Figures 9 and 10 show the chromatograms of sample solutions (a) and (c), respectively. The ten times greater concentration of these samples compared to the samples used for the assay allow the impurity peaks to be more easily observed.

ChP monographs describe acceptance criteria for impurity levels in commercial samples. For that purpose, all impurities were calculated using the peak areas of impurities obtained from the chromatogram of the etimicin sample solution (a) (Figure 9) and compared to the peak area of the etimicin obtained from the chromatogram of etimicin sample solution (c) (Figure 10).

% of impurity in sample =(*r1*/*r2*)

r1 = peak response of each individually impurity from the 0.25 mg/mL sample solution (a)

r1 = peak response of etimicin from the 2.5 µg/mL sample solution (c)

Table 5 shows the percentage of netilmicin and total impurities of standard and sample and compared with the ChP acceptance criteria. The etimicin standard passed the ChP netilmicin impurity and the other impurity criteria. The sample did not pass the ChP total impurities criterion.

Column:	Dionex IonPac AmG-3µm C18 Guard, 4 × 30 mm (P/N 302694) Dionex IonPac AmG-3µm C18 Analytical, 4 × 150 mm (P/N 302693)
Eluent:	0.2 M Trifluoroacetic acid, 0.05% Pentafluoropropanoic acid, 1.5 g/L Sodium sulfate adjust to pH 3.5 with NaOH, 4% Acetonitrile
Inj. volume:	20 µL
Column temperature:	35 °C
Flow rate:	0.8 mL/min
Post-column reagent:	0.76 M NaOH (0.3 mL/min)
Detection:	Pulsed Amperometric Detector (Waveform: Carbohydrates, 4-Potential)

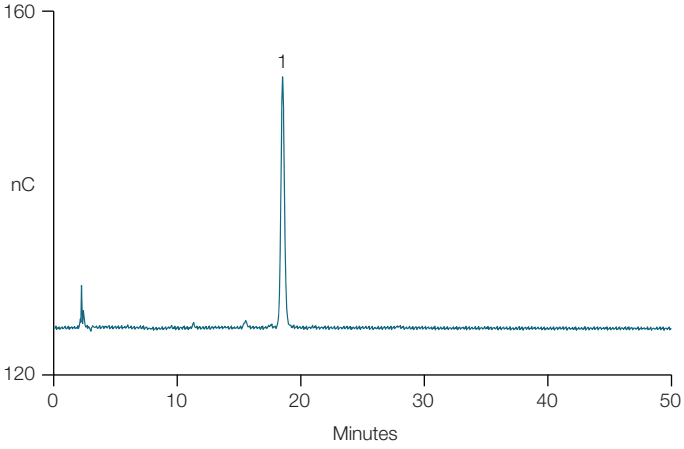

Figure 9. Separation of etimicin sample (2.5 µg/mL) using a Dionex IonPac AmG-3µm C18

Column:	Dionex IonPac AmG-3µm C18 Guard, 4 × 30 mm (P/N 302694) Dionex IonPac AmG-3µm C18 Analytical, 4 × 150 mm (P/N 302693)
Eluent:	0.2 M Trifluoroacetic acid, 0.05% Pentafluoropropanoic acid, 1.5 g/L Sodium sulfate adjust to pH 3.5 with NaOH, 4% Acetonitrile
Inj. volume:	20 µL
Column temperature:	35 °C
Flow rate:	0.8 mL/min
Post-column reagent:	0.76 M NaOH (0.3 mL/min)
Detection:	Pulsed Amperometric Detector (Waveform: Carbohydrates, 4-Potential)

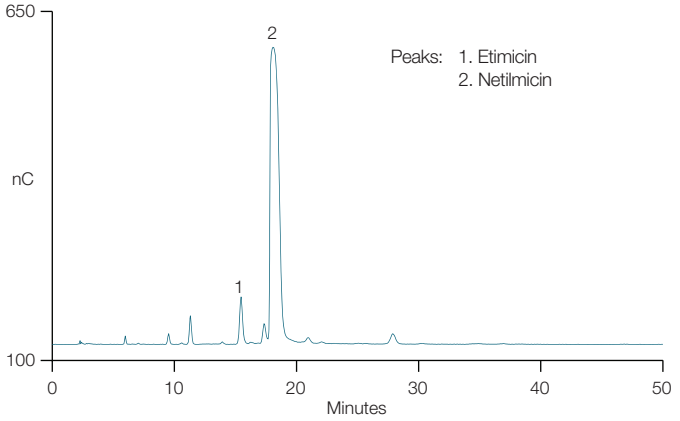

Figure 10. Separation of etimicin sample (0.25 mg/mL) using a Dionex IonPac AmG-3µm C18

Table 5. Percentage of impurity in etimicin sulfate

	Netilmicin	Any Other Individual Impurity	Total Impurities
Standard	0.0677	<0.806	2.02
Sample	2.33	<2.33	6.65
ChP Acceptance Criterion	2.5	2.5	5

Robustness

To achieve good retention time and peak area reproducibility, it is very important to prepare the eluent and post column reagent consistently. The 10-fold concentrated eluent is prepared to minimize both the number of eluent preparation steps and the variation between eluent batches. It is also very important to prepare eluents using chemicals that are free of impurities like the chemicals listed in this application note. Proper column performance may not be achieved when an alternate supplier of chemicals or lower purity water is used.

The eluent and post column reagent should be degassed with helium sparging and blanketed with helium to protect from carbon dioxide contamination.[11] Nitrogen does not work as well as helium. Background signal increasing over time usually indicates that the post-column reagent is not protected from carbon dioxide well enough. This background increase causes a concomitant decrease in peak area.

Rinse the cell body, working electrode, and gasket thoroughly with DI water and dry with a lab wipe but do not touch the working electrode gold surface with any paper products as this can contaminate the working electrode. Polishing of the working electrode becomes necessary when contaminants block the analytes´ access to the gold surface, leading to a decrease of detector response. A color change of the gold electrodes surface is a good indicator for an electrode fouling, as well. During the three month project period, the working electrode was only polished at the start of the project and remained untouched throughout the method evaluation. Always check a possible impact of the post-column sodium hydroxide solution on the peak area stability before polishing the electrode. The procedure for polishing the working electrode can be found in the product manual.[8] The reference electrode should be replaced after about six months of use. When the method is operating as expected the pH reading should be 12.4–12.6. If that reading is not achieved and the post-column reagent is being delivered at the expected flow rate, then replace the reference electrode. It is good practice to have a spare reference electrode available to maximize instrument uptime.

Retention time robustness was evaluated by comparing two column sets from two different lots. The retention time change was < 1%. Retention time robustness was also evaluated for 600 sample and standard injections using the same column (the ratio of sample to standard injections was 3:4). The retention time decreased < 2% during those 600 injections.

Peak area robustness was evaluated with replicate injections of an etimicin standard (25 µg/mL) over three days (injected every 5–6 h). The peak area RSD was < 1% with no obvious trending (Figure 11).

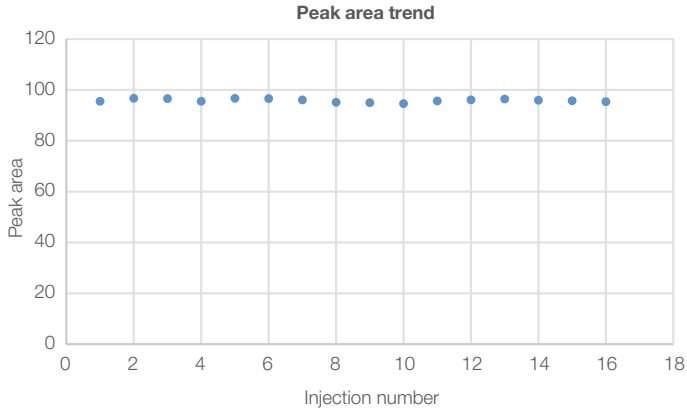

Figure 11. Peak area trend of etimicin standard (25 µg/mL) over three days

Conclusion

This application note demonstrates that the ChP Etimicin Sulfate monograph etimicin assay method and the method for organic impurities can be successfully executed with a Dionex IonPac AmG-3µm C18 column on an ICS-5000⁺ system. The separation, linearity, reproducibility, and sensitivity meet or exceed the current ChP Etimicin Sulfate monograph performance requirements. This method is reliable and can be used for the routine monitoring of etimicin.

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