DIONEX

Application Note 148

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Determination of Bethanechol by Ion Chromatography

INTRODUCTION

Amines are widely used in various industries, such as the chemical, manufacturing, power, and pharmaceutical industries. In pharmaceuticals, amines may be used in the production of emulsifying agents and medications. Bethanechol chloride, 2-[(aminocarbonyl)oxy]-*N*,*N*,*N*-trimethylpropanaminium chloride, is a quaternary ammonium compound that is structurally and pharmacologically related to acetylcholine. It is administered either as an injection or tablet for the treatment of urinary retention. A method in the *U.S. Pharmacopeia* (USP) 24 NF 19 (page 230) recently proposed that the gravimetric assay for bethanechol chloride be replaced with a more specific and rugged ion chromatography assay that also measures stability.[1] The proposed method specifies the use of a Dionex IonPac® CS14 separator column using a manually prepared methanesulfonic acid (MSA) eluent and suppressed conductivity detection. In this application note, we applied electrolytic on-line generation of MSA, using the EG40 eluent generator to optimize reproducibility, convenience, and method transfer between laboratories. We describe the linearity, method detection limits (MDLs), and potential interferences during the determination of bethanechol and its degradation product, 2-hydroxypropyltrimethylammonium chloride.

EQUIPMENT

Dionex DX-600 ion chromatography system consisting of:

- GP50 gradient pump with vacuum degas option
- ED50A Electrochemical Detector
- EG40 Eluent Generator
- EluGen® EGC-MSA cartridge (Dionex P/N 053922)
- AS50 Autosampler
- AS50 Thermal Compartment with conductivity cell
- Chromeleon® 6.4 Chromatography Workstation

REAGENTS AND STANDARDS

Deionized water, Type I reagent-grade, 17.8 MΩ-cm resistivity or better

Combined Six Cation Standard-II (Dionex P/N 046070)

Bethanechol chloride (U.S. Pharmacopeia)

CONDITIONS

Columns:	IonPac CS14 Analytical, 4 × 250 mm (Dionex P/N 044123) IonPac CG14 Guard, 4 × 50 mm (Dionex P/N 044124)
Eluent:	20 mM MSA
Eluent Source:	EG40
Flow Rate:	1.0 mL/min
Temperature:	30 °C
Injection:	25 µL
Detection:	Suppressed conductivity, CAES™ (Dionex P/N 056118) Power setting 67 mA
Expected Background:	~0.2 µS
Expected Backpressure:	~2100 psi
Run Time:	15 min

PREPARATION OF SOLUTIONS AND REAGENTS

Reagent Water

Use Type I reagent-grade distilled or deionized water with a specific resistance of 17.8 MΩ-cm or greater, filtered through a 0.2-µm filter immediately before use.

Eluent Solution

Generate 20 mM MSA eluent on-line by pumping deionized water through the EG40 with an EGC-MSA cartridge. Chromeleon software tracks the amount of MSA used and calculates the remaining lifetime. Replace the EGC-MSA cartridge when the remaining lifetime drops below 10%.

Alternatively, prepare 20 mM MSA by diluting 50 mL of 0.4 N methanesulfonic acid eluent concentrate (Dionex P/N 057562) to 1.0 L with deionized water. Degas the eluent by sonicating under vacuum for 10 min or by sparging with helium. Store the eluent in plastic labware.

As another alternative, prepare a 1.0 N methanesulfonic acid stock solution. Carefully add 96.10 g of methanesulfonic acid (MSA, >99%, Dionex P/N 033478) to a 1-L volumetric flask containing about 500 mL of deionized water. Dilute to the mark and mix thoroughly. Prepare 20 mM MSA by diluting 20 mL of the 1.0 N MSA stock solution to 1.0 L with deionized water. Degas the eluent and store in plastic labware.

Stock Standard Solutions

1000 mg/L Bethanechol Standard Solution

To prepare the stock standard, weigh 0.050 g bethanechol chloride into a 125 mL plastic bottle, add 50.0 g deionized water, sonicate to dissolve, and mix.

1000 mg/L 2-Hydroxypropyltrimethylammonium (2-HPTA) Standard Solution

To prepare the stock standard solution, weigh 0.050 g bethanechol chloride into a 125 mL plastic bottle, add 50 mL of 0.1 N NaOH, sonicate to dissolve, and mix. Allow five days for bethanechol to completely hydrolyze to 2-HPTA chloride.

Working Standard Solutions

Prepare composite working standards at lower concentrations by diluting appropriate volumes of the stock standards with deionized water. For the calibration shown here, the following standards for bethanechol and 2-HPTA were prepared: 0.02, 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10, 25, 50, 100, 200, 500, and 1000 mg/L. The only exception was that the maximum 2-HPTA was 500 mg/L.

SYSTEM PREPARATION AND SETUP

Prepare the CAES for use by hydrating the eluent chamber. Use a disposable plastic syringe to slowly push approximately 3 mL of deionized water through both the “Eluent-In” port and “Regen-In” port of the suppressor. Allow the suppressor to sit for approximately 20 min to fully hydrate the suppressor. For more information on CAES operation, consult the *Installation and Troubleshooting Instructions for the CAES* (Document No. 031770-02).

Install the EG40, connect it to the network, and configure it with the Chromeleon chromatography data system. Condition the EluGen MSA cartridge, as directed in the EG40 manual, by running a gradient from 1 to 60 mN MSA in 20 min, then 60 mN for 40 min at 1.0 mL/min. For instructions on EG40 installation and use, see the *Operator’s Manual* for the EG40 eluent generator system (Document No. 031373).

Remove the 0.005 in. PEEK backpressure tubing temporarily installed during conditioning of the EluGen cartridge. Install a 4 × 50 mm IonPac CG14 guard column and a 4 × 250 mm IonPac CS14 column. Make sure the system pressure displayed by the pump is at least 2000 psi when 20 mM MSA is delivered at 1.0 mL/min, because the EG40 high-pressure degas tubing assembly requires at least 2000 psi (14 MPa) backpressure to efficiently remove hydrolysis gas from the eluent. If necessary, install backpressure coils supplied with the EG40 ship kit to bring the system pressure between 2000 and 2800 psi. Because the system pressure can rise over time, occasional trimming of the backpressure coil may be necessary to maintain system pressure under 3000 psi. Do not exceed 3000 psi.

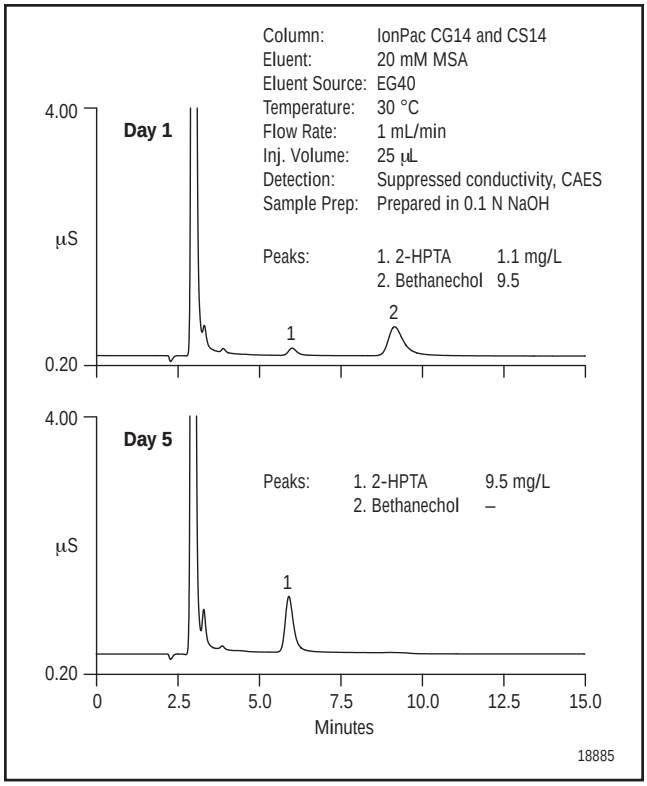

Figure 1. *Conversion of Bethanechol to 2-HPTA in the presence of NaOH.*

Allow the CS14 to properly equilibrate by pumping 20 mM MSA at 1.0 mL/min for approximately 60 min. Prior to sample analysis, analyze a system blank of deionized water. Prepare a 500× dilution of the Six Cation Standard (Dionex P/N 046070) and a make a 25-µL full-loop injection. Subsequently, prepare a 5 mg/L combined standard of bethanechol and 2-HPTA, and make a 25-µL full-loop injection. No peaks should be eluting at the same retention times as the analytes of interest. When duplicate injections of the bethanechol and 2-HPTA standard produce identical retention times, the system is equilibrated.

Peak area precision and accuracy depend on autosampler performance. Replace the water in the flush reservoir daily with freshly filtered and degassed deionized water. Inspect the AS50 daily for bubbles in the sample syringe or its tubing. Purge to remove any bubbles by following the instructions in the AS50 manual.

The precision and accuracy of the AS50 will vary depending on the mode of injection. The most accurate and precise injections can be made with a calibrated sample loop in the full-loop injection mode. To conserve sample, use a partial-loop injection mode. Refer to the AS50 reference manual for a complete discussion of the different injection modes.

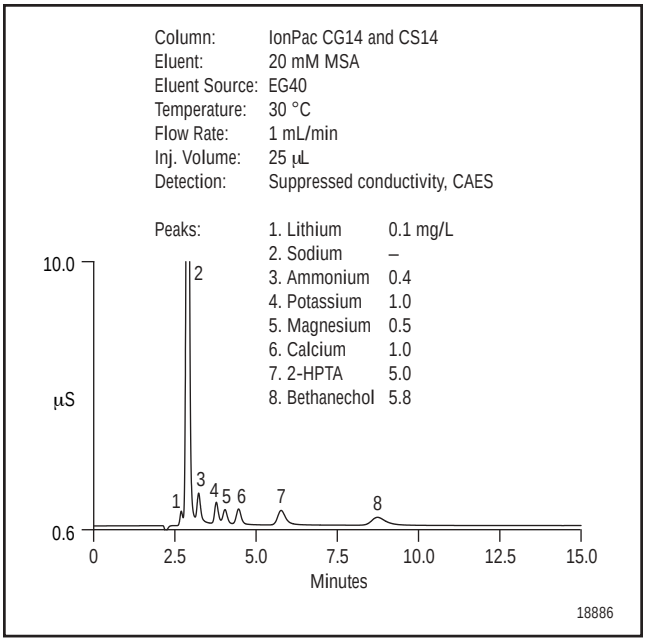

Figure 2. *Separation of Bethanechol and 2-HPTA from common inorganic cations using the IonPac CS14.*

Make sure the correct sample "Loop Size" and "Sample Syringe Volume" are entered in the AS50 Plumbing Configuration Screen.

RESULTS AND DISCUSSION

In the presence of an alkaline solution, bethanechol undergoes hydrolysis to 2-HPTA. In this application note, NaOH was used to prepare the hydrolysis product by combining 0.1 N NaOH with bethanechol and allowing the solution to stand for five days. Figure 1 illustrates the conversion of 9.5 mg/L bethanechol to 2-HPTA from day one to day five.

To determine the system suitability for the analysis of bethanechol and its degradation product, 2-HPTA, the analytes were analyzed in the presence of six common cations (Figure 2). The relative retention values reported by the U.S. Pharmacopeia[1] for Na^+, Mg^{2+}, Ca^{2+}, 2-HPTA, and bethanechol were 1.0, 1.4, 1.6, 2.0, and 2.8, respectively. We found the relative retention values were 1.0, 1.3, 1.5, 2.0, and 2.9, respectively. According to reference 1, the resolution between calcium and 2-HPTA should be greater than 2, with peak efficiency for bethanechol greater than 350 theoretical plates, and a peak tailing factor less than 4.5. The corresponding values from the separation in Figure 2 were 4.66, 2189, and 1.29, respectively.

Table 1. Linear Range and MDLs for Bethanechol and 2-HPTA

Cation	Range (mg/L)	Linearity (r^2)	Calculated MDL* (mg/L)	MDL Standard (mg/L)
Bethanechol	0.02–1000	0.9999	0.01	0.05
2-HPTA	0.02–500	1.0000	0.006	0.02

* The MDLs were calculated as MDL = *(t)* x (S) Where *t* = Student's *t* value for a 99% confidence level and a standard deviation estimate with n – 1 degrees of freedom (*t* = 3.14 for seven replicates of the MDL Standard), and S = standard deviation of the replicate analysis.

Table 1 summarizes the calibration data and MDLs for bethanechol and 2-HPTA. Calibration was linear over four orders of magnitude with correlation coefficients for 2-HPTA and bethanechol of 1.0000 and 0.9999, respectively (see "Appendix"). The intraday precision based on the retention time RSD of 7 replicate injections was 0.31%, and 88 injections of varying analyte amounts during 4 days of a 14-day period yielded a retention time RSD of 1.6%. The high retention time reproducibility is a result of a continuous generation of an exact high-purity MSA concentration by the EG40. The EG40 provides an increased level of automation, decrease in operator error, and greater precision in comparison to a manually prepared eluent.

SUMMARY

This application note discussed the separation and detection of bethanechol and 2-HPTA using the IonPac CS14 column with 20 mM MSA and suppressed conductivity detection. These pharmaceutically important amines were shown to be well resolved from the common inorganic cations that may be present as inactive ingredients. The good day-to-day reproducibility of the retention times of these analytes was possible with the continuous on-line generation of MSA using the EG40.

APPENDIX

The proposed USP method specifies a 50-µL sample injection with a concentration range up to 1000 mg/L bethanechol. It is our experience that injecting concentrations at this level with a 50-µL sample volume will overload the column, resulting in a nonlinear calibration curve. However, we believe a change in the sample loop injection volume is considered a minor modification of the USP method according to the system suitability specifications.[2] Therefore, in this application note, we described the determination of bethanechol using a 25-µL injection. The decrease in injection volume allowed bethanechol to be measured up to 1000 mg/L without overloading the column, resulting in good linearity (r^2 = 0.9999). However, any degradation of bethanechol at this concentration will compromise the linearity.

REFERENCE

1. *Pharmacopeial Forum* **2001** *27(1)*, 155–157.
2. U.S. Pharmacopeia. 24 NF19, **2000,** 24(1), 1923.

SUPPLIER

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