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Ion chromatography

Choosing the appropriate cation-exchange column for quaternary amine determinations

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Keywords

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Goal

To evaluate and identify cation-exchange columns suitable for applications involving quaternary ammonium compound determinations

Introduction

Quaternary ammonium compounds (QACs) are widely used in industrial, agriculture, and pharmaceutical products. QACs comprise a nitrogen atom surrounded by four covalently bound organic moieties, whereby one permanent positive charge arises at the nitrogen atom. The QACs include linear naturally occurring alkylammonium compounds such as choline and acetylcholine.[1] Some of these, such as bethanechol, are therapeutically important. Bethanechol is an amine similar to acetylcholine and is administered for the treatment of urinary retention.[2] Methacholine chloride, a synthetic analogue of the neurotransmitter acetylcholine, is used as a parasympathomimetic broncho-constrictor agent to assess bronchial asthma of subjects in respiratory function labs and epidemiological field studies.[3] QACs also include pyridinium compounds, such as diquat and paraquat, which are used as herbicides.[1] Diquat and paraquat are applied on the leaves as a defoliant, desiccant, and herbicide. They pose a challenge to food safety and are considered a health risk.[4] Parkinson's disease has been linked to exposures of diquat and paraquat.[5-8] Diquat is used more often in lakes and rivers to minimize aquatic weeds, whereas paraquat is applied in no-till farming as a contact herbicide for grass and weeds. Paraquat blocks ferredoxin, thereby inhibiting photosynthesis and generating reactive oxygen species. Due to its toxicity, paraquat is restricted to commercial applications. Other QACs, such as mepiquat and chlormequat, are used as plant growth regulators. The World Health Organization classifies them as slightly hazardous.[9]

Studies on the fates and toxicity of QACs are challenging due to the compounds' chemical properties and associated analytical difficulties. Traditionally, they are determined colorimetrically after complexation with anionic dyes. This method is unsuitable for complex environmental matrices where anionic surfactants are also present.[10] Scientific instrumentation and methods based on cation-exchange chromatography and thin-layer chromatography, GC/MS methods based on thermal decomposition and detection of Hofmann degradation products, potentiometric determination with selective electrodes, and infrared spectroscopic methods have been increasingly replaced by high pressure liquid chromatography (HPLC).[11] More recently, QACs are commonly determined by GC-MS or HPLC-MS methods,[11] but these methods are not ideal because of the ionic and non-volatile nature of QACs. Ion chromatography (IC) is a major technique for determining QACs, and several IC methods have been published for the determination of QACs.[2,4,12-15] The IC separation can precede a conductivity or a mass spectrometric detector to yield a sensitive assay for the determination of QACs.[4,15]

The goal of this work is to evaluate and identify cation-exchange columns suitable for applications involving QAC determinations. Four Thermo Scientific™ Dionex™ IonPac™ columns (CS17, CS19-4µm, CS20, and CS21-4µm) were evaluated. A Thermo Scientific™ Dionex™ Reagent-Free Ion Chromatography (RFIC™) system that produced an electrolytically generated methane sulfonic acid (MSA) eluent was used for this work. QACs were separated with a high-performance cation-exchange column and detected by suppressed conductivity using a Thermo Scientific™ Dionex™ CDRS™ 600 suppressor. The results for retention time, peak area, peak asymmetry, and efficiency are included here, along with recommendations for choosing a column for QAC determinations.

Experimental

Equipment

- A Dionex ICS-5000+ Reagent-Free Ion Chromatography (RFIC) system* including
  - DP Dual Pump with degas option
  - DC detector compartment
- Thermo Scientific™ Dionex™ AS-AP autosampler (P/N 074926) with cooling tray option (recommended)
- Thermo Scientific™ Dionex™ Eluent Generator Cartridge EGC 500 MSA (P/N 075779)
- Thermo Scientific™ Dionex™ CR-CTC 500 Continuously Regenerated Cation Trap Column (P/N 075551)
- Thermo Scientific™ Dionex™ CDRS 600, 2 mm suppressor (P/N 088670)
- 2.5 µL and 5 µL sample loops
- 10 mL polypropylene autosampler vials with caps (P/N 074228)

* [Note- Equivalent or improved results can be achieved using the Thermo Scientific™ Dionex™ ICS-6000 HPIC system.]

Columns

- Columns: Thermo Scientific™ Dionex™ IonPac™ CS17 2 × 250 mm (P/N 060561), Thermo Scientific™ Dionex™ IonPac™ CG17 Guard 2 × 50 mm (P/N 060563)
- Columns: Thermo Scientific™ Dionex™ IonPac™ CS19-4µm 2 × 250 mm (P/N 078836), Thermo Scientific™ Dionex™ IonPac™ CG19 Guard 2 × 50 mm (P/N 078839)
- Columns: Thermo Scientific™ Dionex™ IonPac™ CS20 2 × 250 mm (P/N 302606), Thermo Scientific™ Dionex™ IonPac™ CG20 Guard 2 × 50 mm (P/N 302607)
- Columns: Thermo Scientific™ Dionex™ IonPac™ CS21-4µm 2 × 150 mm (P/N 303348), Thermo Scientific™ Dionex™ IonPac™ CG21 Guard 2 × 30 mm (P/N 303349)

Reagents and chemicals

- Diquat dibromide monohydrate (Sigma P/N S-1752)
- Paraquat dichloride hydrate (Sigma P/N 36541)
- Chlormequat chloride (Sigma P/N 11-102-0552)
- Mepiquat chloride (Sigma P/N 11-101-3665)
- Succinylcholine chloride (Sigma P/N 460111000)
- Choline chloride (Sigma P/N C7527)
- Acetylcholine (Sigma P/N A6625)
- Methacholine chloride (Sigma P/N PHR1943)
- Carbachol (Sigma P/N PHR1511)
- Bethanechol chloride (Sigma P/N PHR2357)
- Thermo Scientific™ Dionex™ Cation-II standard (P/N 046070)

Preparation of reagents

1,000 mg/L amine stock solutions:

Dissolve appropriate amounts of amine salts (e.g., 13.41 mg of choline chloride) in DI water in 125 mL polypropylene bottles. Adjust the volume to 100 g with DI water. Store the bottles at 4 °C until use.

10 and 100 mg/L amine standard solutions:

Prepare 100 mg/L amine standard solutions by diluting the 1,000 mg/L stock solutions 10-fold as follows. In a 125 mL polypropylene bottle, add 10 mL of 1,000 mg/L stock solution. Adjust the weight to 100 g with DI water.

Dilute the 100 mg/L standard solution 10-fold to prepare a 10 mg/L solution.

Store all solutions at 4 °C until use.

Conditions

Individual chromatographic conditions used for each of the four columns are listed in Table 1.

Table 1. Conditions for quaternary amine separations on four cation exchange columns

	Dionex IonPac column			
	CS17	CS19-4µm	CS20	CS21-4µm
Flow rate (mL/min)	0.3	0.3	0.3	0.3
Injection volume (µL)	5	5	2.5	2.5
Eluent conc. (mM)	10	35, 40	40, 50	2.5, 3, 5, 5.25, 6.5, 7.5
Column temp. (°C)	40	40	40	40
Elution mode	Isocratic	Isocratic	Isocratic	Isocratic
Run time (min)	10 or 30	10 or 30	8 or 12	10 or 12 min
Suppressor current (mA)	9	26, 30	36, 44	3, 3, 5, 5, 6, 7

Results and discussion

Separation

This work aims to compare chromatographic parameters, such as peak area, peak asymmetry, and efficiency, to identify the best column for separating the quaternary amines studied here. For each of the four columns, an isocratic elution was used to have compound retention be similar for a better comparison of peak efficiency and asymmetry. Two different amine concentration levels of 10 and 100 mg/L were used. The 100 mg/L level was used for amines with a low peak response at 10 mg/L. Chromatographic conditions used for each column are listed in Table 1.

Figure 1 shows all 10 amines separated on a Dionex IonPac CS17 column using 10 mM MSA at a column temperature of 40 °C. Using an isocratic separation at 10 mM MSA, peak asymmetry and efficiency data were collected for the 10 amines.

Next, similar experiments were performed on a Dionex IonPac CS19-4µm column. As discussed above, retention times on the Dionex IonPac CS19-4 µm column were optimized to approximate those obtained on the Dionex IonPac CS17 column. Figure 2 shows the chromatography obtained on the Dionex IonPac CS19-4 µm column. Elution of late-eluting succinyl choline, diquat, and paraquat requires eluent concentrations of 35 mM MSA or higher.

Similar retention time matching was performed for the Dionex IonPac CS20 and Dionex IonPac CS21-4µm columns. Figures 3 and 4 show the separation of amines obtained for these two columns.

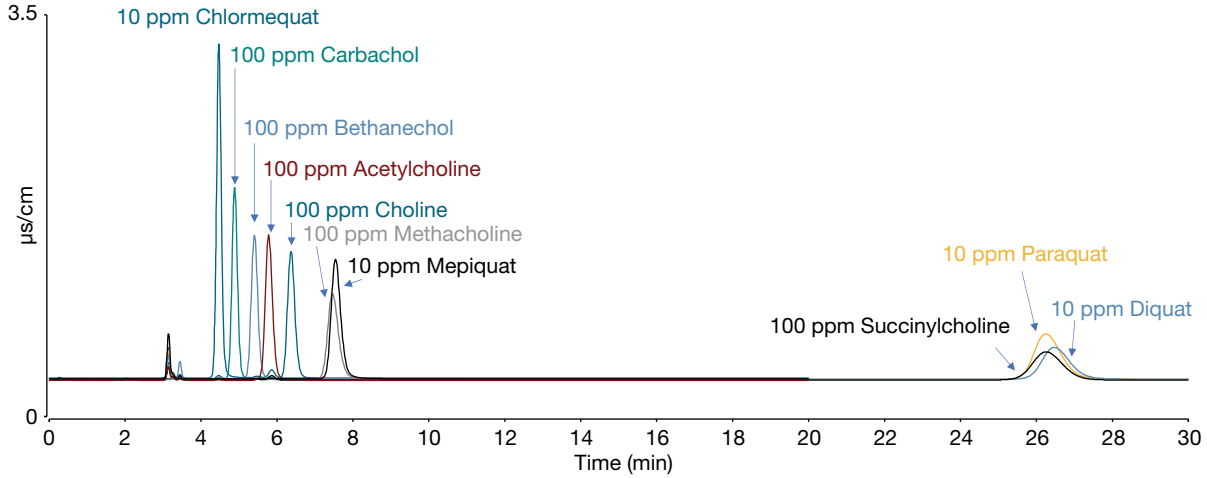

Figure 1. Separation of 10 amines using 10 mM MSA on a Dionex IonPac CS17 column

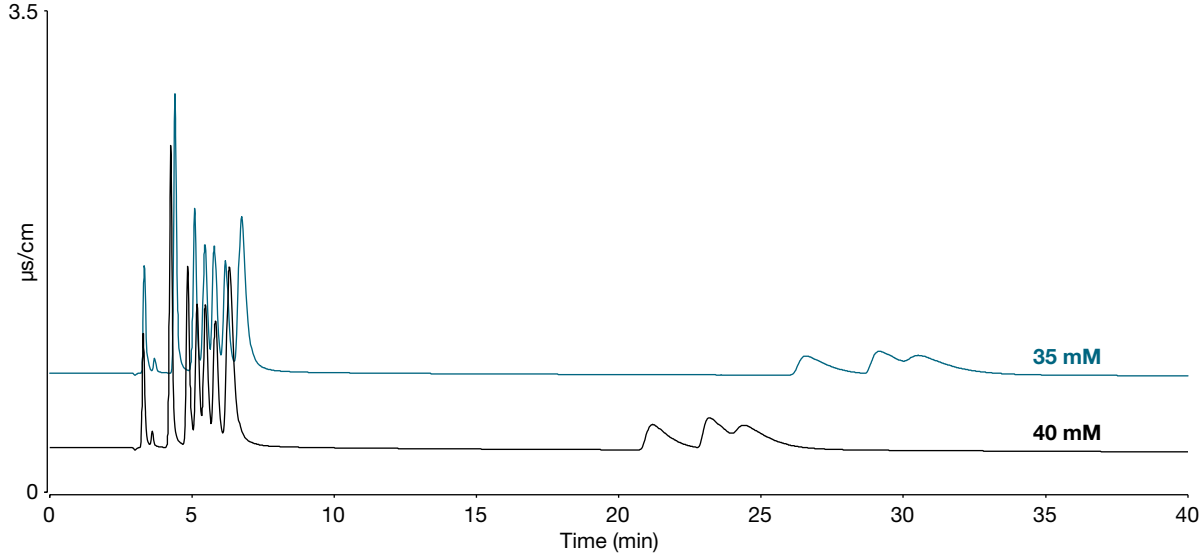

Figure 2. Separation of 10 amines on a Dionex IonPac CS19-4µm column using different MSA concentrations

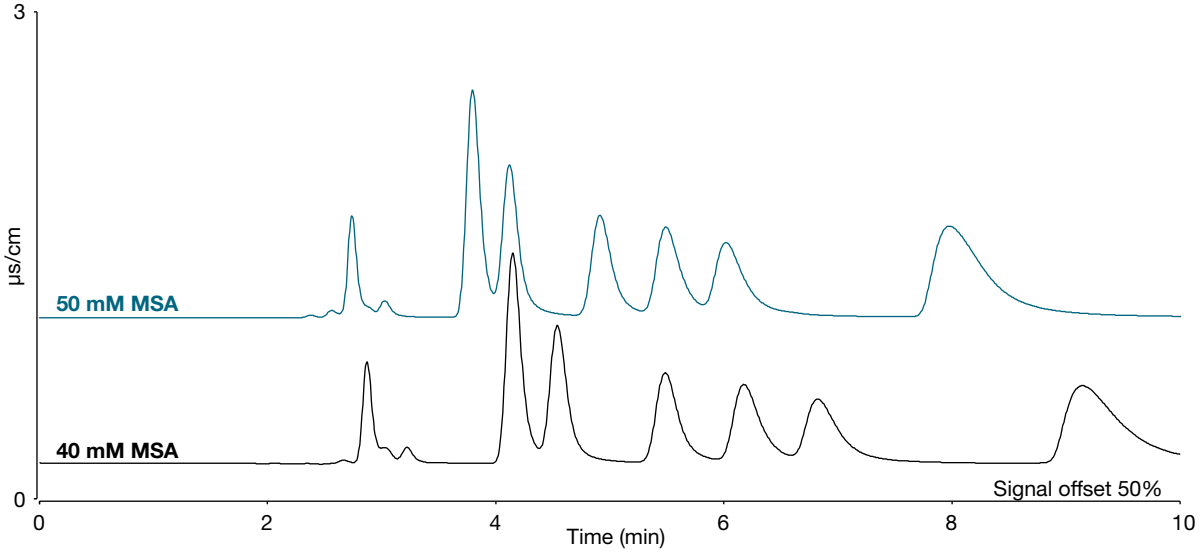

Figure 3. Separation of 10 amines using 40 and 50 mM MSA on a Dionex IonPac CS20 column

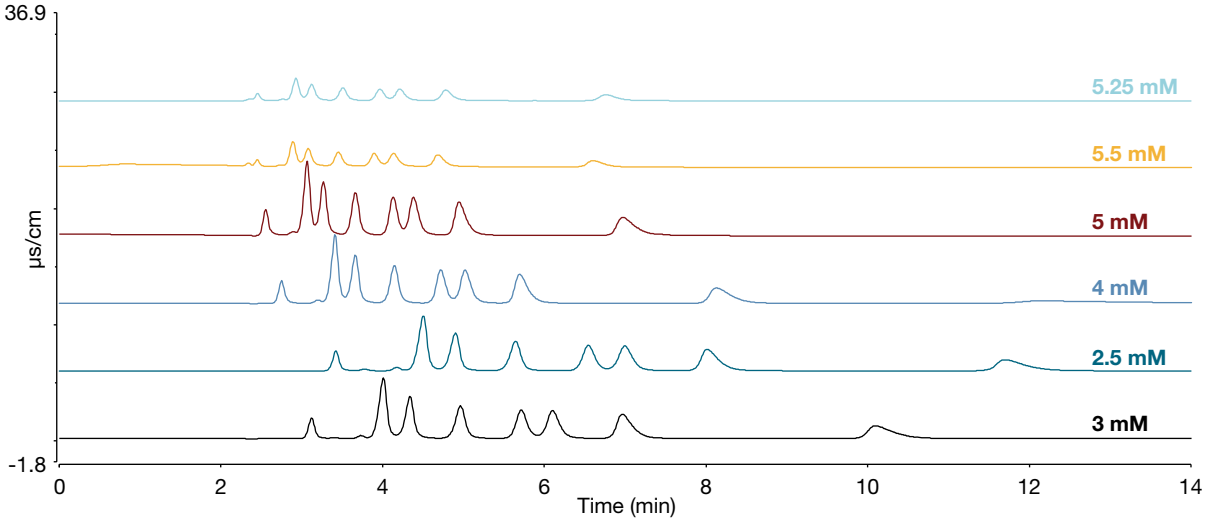

Figure 4. Separation of 10 amines using various MSA concentrations on a Dionex IonPac CS21-4µm column

The retention times on the four columns are within ±0.5 min for most compounds (Table 2). For amines eluting after 25 min, the difference in retention time ranges from 0.1 to 1.6 min.

Peak asymmetry and efficiency calculations

The calculated average peak asymmetry and efficiency values are summarized in Table 3. The Dionex IonPac CS17 and Dionex IonPac CS21-4µm columns show better peak asymmetry and efficiency values than the Dionex IonPac CS19-4µm and Dionex IonPac CS20 columns. The asymmetry values of the Dionex IonPac CS17 and Dionex IonPac CS21-4µm columns are comparable for amine peaks 1 to 4 (choline to acetylcholine). However, the Dionex IonPac CS17 column shows better (lower) peak asymmetry values for later eluting amines. At the same time, the Dionex IonPac CS21-4µm column shows higher peak efficiency values for most of the peaks. Only for diquat and paraquat are the peak efficiency values better on the Dionex IonPac CS17 column, but they are not resolved on this column. The higher peak efficiencies observed on the Dionex IonPac CS21-4µm column are probably best explained by its smaller particle packing compared to the Dionex IonPac CS17 column.

Table 2. Amine retention time data on Dionex IonPac columns

Analyte	Retention time (min)			
	Dionex IonPac CS17 column	Dionex IonPac CS19-4µm column	Dionex IonPac CS20 column	Dionex IonPac CS21-4µm column
Choline	4.5	4.4 (35 mM)*	4.2 (40 mM)	4.5 (2.5 mM)
Carbachol	4.8	5.1 (35 mM)	4.5 (40 mM)	4.9 (2.5 mM)
Bethanechol	5.4	5.5 (35 mM)	5.4 (40 mM)	5.6 (2.5 mM)
Acetylcholine	5.8	5.8 (35 mM)	5.4 (50 mM)	6.1 (3 mM)
Chlormequat	6.4	6.2 (35 mM)	6 (50 mM)	6.5 (2.5 mM)
Methacholine	7.5	6.8 (35 mM)	8 (50 mM)	7 (5 mM)
Mepiquat	7.5	6.8 (35 mM)	8 (50 mM)	8 (2.5 mM)
Paraquat	26.2	23.3 (40 mM)		24.9 (6.5 mM)
Diquat	26.5	21.3 (40 mM)		24.9 (7.5 mM)
Succ. choline	26.0	25.6 (35 mM)		25.9 (5.25 mM)

* Note- Values included in parentheses indicate the MSA concentration used for corresponding retention time

Table 3. Amine asymmetry and efficiency data on Dionex IonPac columns

Peak number	Peak	Avg. Asymmetry (n=3)				Avg. Efficiency (n=3)			
		Dionex IonPac CS17 column	Dionex IonPac CS19-4µm column	Dionex IonPac CS20 column	Dionex IonPac CS21-4µm column	Dionex IonPac CS17 column	Dionex IonPac CS19-4µm column	Dionex IonPac CS20 column	Dionex IonPac CS21-4µm column
1	10 ppm Choline	1.09	1.28	1.41	1.08	6098	9417	4835	9259
	100 ppm Choline	1.09	1.38	1.46	1.06	5749	9030	4593	8275
2	10 ppm Carbachol	1.07	1.34	1.46	1.08	5590	8308	4620	9277
	100 ppm Carbachol	1.08	1.36	1.47	1.06	5154	8016	4501	8422
3	10 ppm Bethanechol	1.09	1.45	1.51	1.08	4707	6463	3784	8931
	100 ppm Bethanechol	1.09	1.47	1.61	1.09	4412	6340	3655	8153
4	10 ppm Acetylcholine	1	1.56	2.07	1.2	4490	6388	2918	8347
	100 ppm Acetylcholine	1.12	1.66	1.59	1.1	4282	6303	3336	8467
5	10 ppm Chlormequat	1.12	1.68	1.78	1.23	4822	6471	3138	7636
6	10 ppm Mepiquat	1.21	2.0	2.45	1.46	4151	4534	2024	7120
7	10 ppm Methacholine	1.19	1.8	1.76	1.38	3241	3607	2036	6205
	100 ppm Methacholine	1.26	2.08	2.24	1.59	3079	3731	1779	5560
8	10 ppm Paraquat	1.22	3.47	-	3.1	6074	3206	-	5447
9	10 ppm Diquat	1.19	3.03	-	3.1	5902	3077	-	5087
10	10 ppm Succinyl choline	1.06	1.89	-	1.2	4685	2882	-	7357
	100 ppm Succinyl choline	1.12	3	--	1.87	4767	2533	-	6135

Table 4. QAC separation applications and suitable column suggestions

Analytes	Sample	Reference	Comments	Dionex IonPac column suggestion
Chlormequat, mepiquat	Fresh fruit, vegetable, and juice	9	LC/MS method	CS21-4µm
Diquat, paraquat	Food	16	LC/MS method	CS21-4µm
Methacholine, β-methylcholine*, acetylcholine	Bronchoconstrictor formulation	12	Method uses Dionex IonPac CS17 column	CS21-4µm
Bethanechol, carbochol, choline	Ophthalmologic solution	14	Method uses Dionex IonPac CS14 column	CS21-4µm
Choline, succinyl choline	DI water	13	Method uses Dionex IonPac CS19 column	CS21-4µm
	Blood plasma	3	Method uses indirect detection of choline after enzymatic conversion	CS21-4µm
Chlormequat, mepiquat, diquat, paraquat	Food and beverage	17	IC-MS/MS method uses Dionex IonPac CS21-4µm column	CS17**
Chlormequat, mepiquat, diquat, paraquat	Food	15	IC-MS/MS method uses Dionex IonPac CS21-4µm column	CS17**

*Analyte not tested in this work

** Replacement or alternative (diquat, paraquat little to no resolution)

Overall, the peak asymmetry and efficiency values for the amines tested here are superior on the Dionex IonPac CS17 and Dionex IonPac CS21-4µm columns than the other two columns tested. These results can be used to guide column selection for different applications. Table 4 shows various suitable columns for applications involving QACs. The table also discusses currently used methods and a suitable column alternative based on the results reported here. For most applications, the Dionex IonPac CS21-4µm column can be used. In cases where diquat and paraquat resolution is not required, the Dionex IonPac CS17 column can be used as an alternative.

Conclusion

Here we have compared quaternary amine separation on four different cation-exchange columns. The column selection is based on the best efficiency. Chromatographic peak efficiency is important because it results in increased sensitivity, better resolution, and ease of quantitation. Comparing columns side by side allows choice of a suitable column for a specific application. The data for the four columns may also suggest the appropriate or alternate column based on the required analytes.

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