

# Use of Ion Chromatography to Determine Cholinergic Compounds in Ophthalmic Solutions

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## ABSTRACT

Carbachol and bethanechol are positively charged quaternary ammonium compounds and choline-esters, belonging to a class of drugs referred to as cholinergic. They are used in the treatment of glaucoma and in wash solutions during ophthalmic surgery to lower intraocular pressure.<sup>1</sup> Diminished concentrations of carbachol in ophthalmic formulations may prevent effective reduction of intraocular pressure and cause several deleterious effects including iris prolapse, therefore an analytical method is needed to ensure that the concentrations of these drugs remain at a therapeutically active level. The current USP monograph (USP 29-NF 24) describes a colorimetric method for the determination of carbachol in ophthalmic solutions.<sup>2</sup> However colorimetric methods can have significant measurement errors and are both time-consuming and labor-intensive.

Here we examine an ion chromatographic method to determine carbachol and bethanechol and other pharmaceutically important compounds in over-the-counter eye care products. Carbachol, choline, and bethanechol were separated using an IonPac® CS17 column with electrolytically generated methanesulfonic acid eluent, and detected by suppressed conductivity. The reagent-free ion chromatography (RFIC™) system eliminated eluent preparation errors, was easy to use, and ensured retention time reproducibility. The sensitivity of suppressed conductivity detection allows determination of carbachol and bethanechol in ophthalmic solutions with a simple dilution of sample.

## INTRODUCTION

Carbachol and bethanechol chloride are positively charged quaternary ammonium compounds with a choline-ester used primarily for ophthalmic applications, such as glaucoma treatment or ophthalmic surgery. Carbachol is a potent cholinergic agent which constricts the iris, reducing intraocular pressure in patients with glaucoma, while bethanechol chloride is structurally and pharmacologically related to carbachol.<sup>1,3</sup> Analytical methods are needed to ensure that drug concentrations in ophthalmic formulations remain at therapeutically active levels during manufacturing. The current USP monograph (USP 29-NF 24) describes a colorimetric method for determination of carbachol in ophthalmic solutions, but these methods can be both time- and labor-intensive, and yield significant measurement errors.<sup>2</sup> Dionex Application Note 148 reports an RFIC method for determination of bethanechol chloride.<sup>3</sup> In the method described here,

optimized conditions for carbachol and bethanechol were used to determine linearity, method detection limits (MDL), and to demonstrate the separation of these drugs from other ingredients typically found in ophthalmic solutions. We performed an accelerated stability study of both drugs under extreme alkaline conditions for 5 days and determined that the method parameters can also be used for the analysis of the breakdown products of carbachol and bethanechol; specifically: choline and 2-hydroxypropyltrimethylammonium (2-HPTA). This RFIC method can be used to determine carbachol, bethanechol, choline, and 2-HPTA in 25 min. Use of eluent generation eliminated eluent preparation errors and ensured high retention time reproducibility.

## EXPERIMENTAL

### Conditions

Columns:	IonPac® CS17 4 mm Analytical 4 × 250 mm, IonPac CG17 4 mm Guard 4 × 50 mm
Eluent:	5 mM Methanesulfonic Acid
Eluent Source:	EluGen® II MSA
Flow Rate:	1.0 mL/min
Temperature:	30 °C
Injection Vol.:	25 µL
Detection:	Suppressed conductivity, CSRS® Ultra 4 mm P/N 053948, recycle mode, 20 mA

## EQUIPMENT

The study was performed using a Dionex ICS-2000 RFIC system. This is an integrated ion chromatography system consisting of an eluent generator, pump with in-line vacuum degas, column heater, AS Autosampler, and Chromeleon® chromatography management software.

Standards were prepared using carbachol chloride and bethanechol chloride (USP reference standards P/N 1092009 and P/N 1071009, respectively). Choline and 2-HPTA standards were prepared by hydrolyzing carbachol and bethanechol in 0.1N NaOH for 5 days. Because wash solutions for surgical procedures were not commercially available, we spiked over-the-counter eyecare solutions with carbachol. For more extensive details of the method, refer to the application in reference (4).

## RESULTS AND DISCUSSION

Figure 1 shows a chromatogram of a 1 mg/L carbachol standard along with several commonly occurring cations that may potentially interfere with the analysis. All the compounds were well separated from carbachol, and did not interfere with its determination.

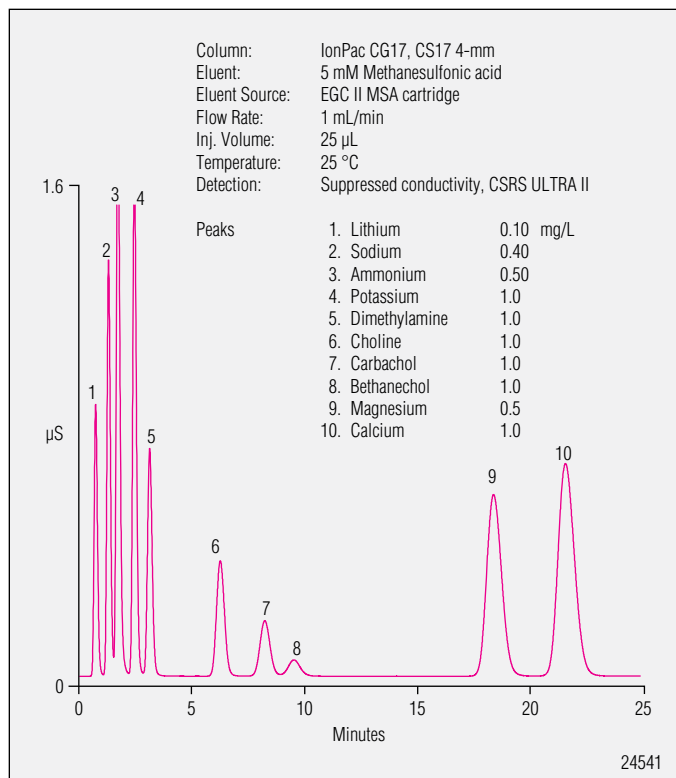


Figure 1. 1 mg/L carbachol, choline, and bethanechol with mixed cation standard.

Figure 2 shows the decomposition of carbachol to choline in the presence of NaOH, on Day 1 and Day 5 of exposure to 0.1N NaOH.

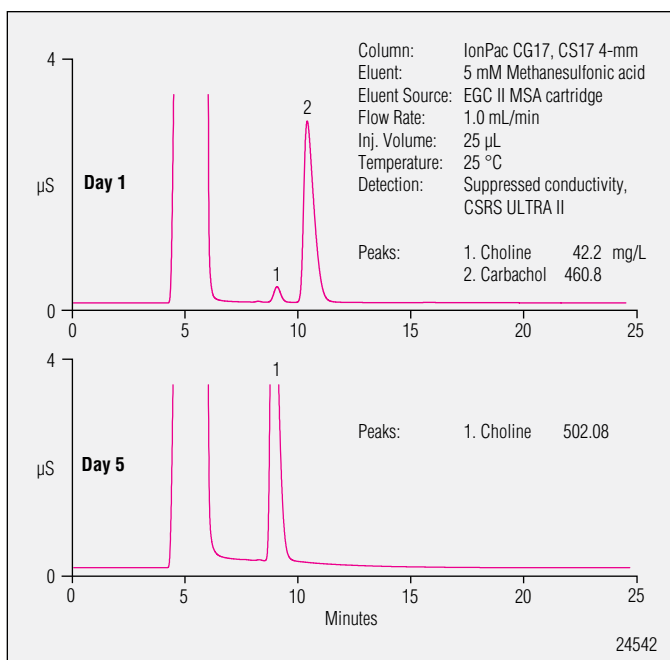


Figure 2. Conversion of carbachol to choline in NaOH.

Figure 3 shows the decomposition of bethanechol to 2-HPTA in an alkaline solution.

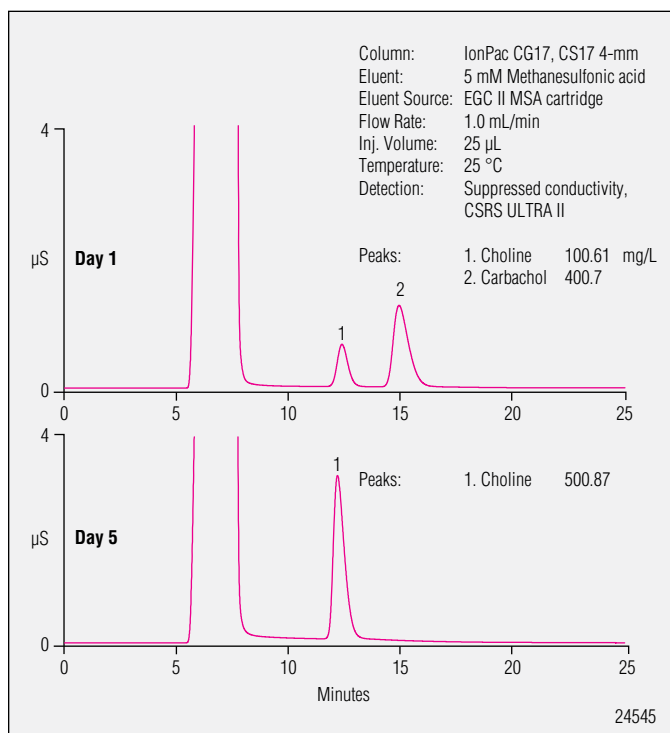


Figure 3. Conversion of bethanechol to 2-HPTA in an alkaline solution.

Figure 4 shows separation of 1 mg/L of carbachol, choline, and bethanechol in diluted disinfecting lens solution and saline solution.

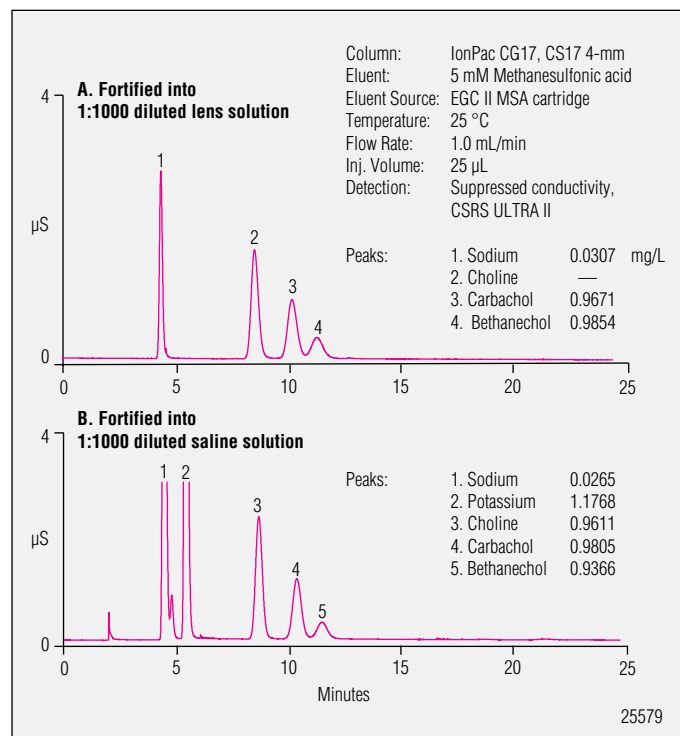


Figure 4. 1 mg/L carbachol, choline, and bethanechol.

Table 1 summarizes the calibration data for a typical calibration curve obtained by injecting calibration standards of carbachol, choline, bethanechol, and 2-HPTA.

Table 1. Linear Ranges for Carbachol, Choline, Bethanechol, and 2-HPTA				
Cation	Range (mg/L)	r <sup>2</sup>	Offset	Slope
Carbachol	0.02–1000	0.99998	–0.036	0.085
Choline	0.02–500	0.99999	–0.011	0.036
Bethanechol	0.02–1000	0.99928	–0.007	0.036
2-HPTA	0.02–500	0.99997	–0.011	0.028

Table 2 summarizes the MDL determinations of carbachol, choline, bethanechol, and 2-HPTA.

Table 2. Determination of MDLs for Carbachol, Choline, Bethanechol, and 2-HPTA					
Cation	Range (mg/L)	MDL Standard (mg/L)	RSD	S/N	*Calculated MDL (µg/L)
Carbachol	0.02–1000	0.02	0.12	5.61	5
Choline	0.02–500	0.01	0.04	2.96	1
Bethanechol	0.02–1000	0.05	0.05	2.8	2
2-HPTA	0.02–500	0.05	0.12	3.3	5

\* MDLs were calculated as MDL = (t) × (SD) 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 SD = standard deviation of the replicate analysis.<sup>5</sup>

Carbachol was spiked into two over-the-counter ophthalmic solutions, and the precision, recovery, linearity, and MDL were evaluated. The results from the linearity and MDL studies for both matrices are summarized in Table 3, and the precision and recovery data are shown in Table 4.

Table 3. Linear Range and Detection Limits of Carbachol in Two Ophthalmic Solutions					
Cation	Range (mg/L)	MDL Standard (mg/L)	RSD	S/N	*Calculated MDL (µg/L)
Lens solution	0.01–500	0.99999	0.02	4.12	4
Saline solution	0.01–500	0.99995	0.02	13.5	3

\* MDLs were calculated as MDL = (t) × (SD) 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 SD = standard deviation of the replicate analysis.<sup>5</sup>

Table 4. Recovery of Carbachol from Two Eye Care Products			
Matrix	Amount Added (mg/L)	Recovery	Precision (RSD)
Lens solution	0.5	96	0.77
Saline solution	0.5	98	0.67

Table 5 summarizes a short-term experiment in which between-day reproducibility was measured by injecting five replicates each day for 6 days of a 5 mg/L standard. The precision based on the retention time RSD was 0.043% with saline and 0.101% with lens solution.

Table 5. Reproducibility				
Matrix	Concentration (mg/L)	RSD		
		Retention Time	Height	Area
Lens solution	5	0.10	0.78	0.88
Saline solution	5	0.043	0.74	0.89

## CONCLUSION

- Carbachol and bethanechol were linear over four orders of magnitude (0.02 to 1000 mg/L) with an estimated lower limit of detection of 5 and 2 µg/L, respectively.
- The separation was reproducible with retention time and peak area precisions of <0.1% and <1% RSD, respectively for 0.5 mg/L carbachol spiked into the diluted ophthalmic solution (n= 30 injections over 5 days)
- The method was accurate with spiked recoveries of >90% for carbachol added to different ophthalmic solutions.
- Analysis time for all four compounds; carbachol, bethanechol, choline, and 2-HPTA was 25 minutes.

## REFERENCES

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3. Dionex Corporation "Determination of Bethanechol by Ion Chromatography", Application Note 148, Sunnyvale, CA, LPN 1510, March, 2003.
4. Dionex Corporation "Determination of Carbachol in Ophthalmic Solutions Using a Regent Free Ion Chromatography System", Application Note 194, Sunnyvale, CA, LPN 1967, April, 2008.

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LPN 2125-01 09/08  
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