Determination of Cholinergic Compounds in Ophthalmic Solutions Using a Reagent-Free™ Ion Chromatography System

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Carbachol is a choline ester and a positively charged quaternary ammonium compound belonging to a class of drugs referred to as cholinergic. It is used in the treatment of glaucoma and in wash solutions during ophthalmic surgery to lower intraocular pressure (1). Diminished concentrations of carbachol in ophthalmic formulations may prevent effective reduction of intraocular pressure and cause several deleterious effects such as iris prolapse. Analytical methods are used to ensure carbachol concentrations remain at therapeutically active levels in these solutions.

This paper discusses an ion chromatographic method for determination of carbachol and other pharmaceutically important compounds in ophthalmic solutions which offers an improvement upon the current USP monograph (USP 30 NF 25) (2) by taking advantage of the sensitivity of suppressed conductivity detection and eliminating the need to prepare color reagents.

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 eliminates eluent preparation errors, is easy to use, and ensures retention time reproducibility. Suppressed conductivity detection allows carbachol to be determined in ophthalmic solutions with a simple 1:1000 sample dilution.

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

Standards were prepared using carbachol chloride and bethanechol chloride (USP reference standards P/N 1092009 and P/N 1071009). Choline standards were prepared by allowing carbachol to hydrolyze in a 0.1N NaOH solution for 5 days. Because wash solutions for surgical procedures are not commercially available, over-the-counter eyecare solutions (disinfecting lens solution and saline solution) were spiked with carbachol. Both preparations contain large amounts of sodium, therefore sample dilution is necessary to prevent column overload. Injection volume was 25 µL for all standards and samples.

Results and Discussion
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 diluted ophthalmic solution (n= 30 over 5 days), with spiked recoveries >90% for carbachol added to different ophthalmic solutions. A more extensive evaluation of this application is discussed in reference 3.

Figure 1: 1 mg/L carbachol, choline and bethanechol fortified into (a) 1:1000 diluted lens solution and (b) 1:1000 diluted saline solution. Column: IonPac CG17, CS17 4-mm; eluent: 5 mM methanesulfonic acid; eluent source: EGC II MSA cartridge; temperature: 25 °C; flow rate: 1.0 mL/min; injection volume: 25 µL; detection: suppressed conductivity, CSRS ULTRA II. Peaks (a): 1 = sodium, 0.031 mg/L; 2 = choline, 0.988 mg/L; 3 = carbachol, 0.967 mg/L; 4 = bethanechol, 0.985 mg/L. Peaks (b): 1 = sodium, 0.026 mg/L; 2 = potassium, 1.177 mg/L; 3 = choline, 0.961 mg/L; 4 = carbachol, 0.981 mg/L; 5 = bethanechol, 0.937 mg/L.

Conclusion
This RFIC application demonstrates quantification of carbachol, choline, and bethanechol in the presence of sodium in lens and saline solutions. The method improves the existing USP method for quantifying carbachol by delivering reproducible, accurate results without the need for a time consuming colorimetric assay.

References
(3) “Determination of carbachol in ophthalmic solutions using a Reagent Free™ Ion Chromatography system”, Application Note 194, Dionex Corporation, Sunnyvale, CA.