DIONEX

Application Note

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Choline and Acetylcholine

INTRODUCTION

Choline and acetylcholine (Figure 1) are quaternary ammonium cations which are noted for their activities as neurotransmitters. Concentrations in physiological materials, especially in neural tissues, often are determined when investigating biochemical pathways and determining mechanisms of actions of cholinergic drugs. Choline also helps prevent the accumulation of fats in liver tissues[1] and has been investigated as a therapy for memory loss and other brain disfunctions, although evidence suggests that excess dietary choline has little or no effect on brain function[2]. Because choline is essential to proper metabolism, it is often added to vitamin formulations as the bitartrate salt or supplied as a solution of the chloride salt for oral administration. Acetylcholine has been administered in ophthalmic solutions such as Miochol®.

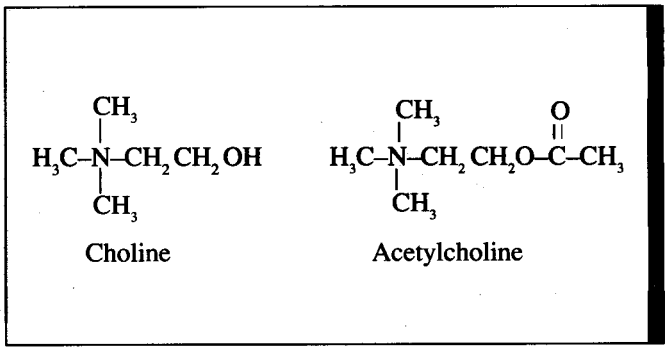

FIGURE 1. STRUCTURES OF CHOLINE AND ACETYLCHOLINE

The most common analytical methods for physiological samples involve either reversed phase/ion pair[3-5] or cation exchange[6, 7] HPLC with postcolumn enzymatic decomposition to hydrogen peroxide followed by DC amperometric detection[8]. These investigators reported detection limits of 0.1 to 10 pmol. Honda[9] reported a similar HPLC method using chemiluminescence detection with a detection limit of 1 pmol. HPLC methods involving precolumn derivatization and either cation exchange[10] or reversed phase/ion pairing[11] followed by ultraviolet (UV) absorption (MDLs of 10 to 100 pmol) have been used successfully for plant extracts[10] and blood plasma[11]. An indirect UV method has also been reported[12], but detection limits for standard solutions were about 10 nmol. The United States Pharmacopeial method[13] for ophthalmic solutions describes a reversed phase/ion pair separation with refractive index (RI) detection.

Efficiencies of the ion pair separations cited above range from 3200 plates/m[9] to 6000 plates/m[11] for choline and 4800 plates/m[9] to 21,000 plates/m[5] for acetylcholine. Cation exchange efficiencies were 760 plates/m[6] to 3700 plates/m[10] for choline and 3100 plates/m[6] to 4800 plates/m[7] for acetylcholine.

The cation exchange method described here exhibits higher efficiencies than existing methods (choline: 30,000 plates/m; acetylcholine: 29,000 plates/m). Detection limits for this method using suppressed conductivity (5 to 7 pmol) are lower than those for the UV methods which require precolumn derivatization and comparable to the less sensitive amperometric methods which use enzymes or enzyme

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reactor columns. Suppressed conductivity detection is more selective than either UV or RI detection methods, allowing detection of only ionic species; however, it is less selective than enzymatic/amperometric detection methods.

EQUIPMENT

Dionex chromatography system consisting of:

- Gradient Pump
- Liquid Chromatography Module
- Conductivity Detector Module or Pulsed Electrochemical Detector
- AutoRegen Accessory
- Eluent Degas Module
- Data acquisition capability

REAGENTS & STANDARDS

Hydrochloric Acid, Reagent Grade (Concentrated, 12M)

Methanol, HPLC Grade

Tetrabutylammonium Hydroxide, 100 mM TBAOH (Dionex Cation Regenerant Solution, p/n 039602)

Choline Bitartrate

Acetylcholine Chloride

CONDITIONS

Column:	OmniPac® PCX-100
Mobile Phase:	75 mM HCl, 1% Methanol
Sample Loop Volume:	10 µL
Flow Rate:	1.0 mL/min.
Suppressor:	CMMS-II
Regenerant:	100 mM TBAOH
Regenerant Flow Rate:	5 mL/min.

PREPARATION OF SOLUTIONS AND REAGENTS

Mobile Phase: 75 mM HCl, 1% Methanol

Add 6.2 mL (7.4 g) of concentrated HCl and 10 mL (8.1 g) of methanol to about 800 mL of 18 MΩ deionized water. Dilute this solution with additional deionized water to a final volume of 1.0 L.

Calibration Standards:

Prepare a stock solution of 10 mM HCl by diluting 0.8 mL (1 g) of concentrated HCl to 1.0 L with deionized water. Dissolve 243 mg of choline bitartrate and 124 mg of acetylcholine chloride in sufficient 10 mM HCl to prepare 100 mL of a solution having a concentration of 1.00 mg/mL (1000 ppm) for each analyte. Dilute this solution to the appropriate concentrations with 10 mM HCl to prepare individual standards. For example, to prepare a 10.0 µg/mL standard dilute 1.00 mL of the 1.00 mg/mL solution to 100 mL.

SAMPLE PREPARATION

Nutrilin Tablets:

Sonicate one crushed tablet in 80 mL of 18 MΩ deionized water. Filter the solution through a 0.45 µm filter and dilute the filtrate to 100 mL. Dilute a 1.00 mL aliquot of this solution to 100 mL with the 10 mM HCl stock solution described under Calibration Standards.

Dietary Choline Chloride Solution:

Dilute 4.00 mL of the dietary choline chloride solution to 1.00 L with 18 MΩ deionized water. Filter the resulting solution through a 0.45 µm filter. Dilute a 1.00 mL aliquot of this solution to 100 mL with the 10 mM HCl stock solution described under Calibration Standards.

DISCUSSION

Choline and acetylcholine are chromatographed by cation exchange. A number of different cation exchange resins may be used but the OmniPac PCX-100 produces peaks of the highest efficiency, 30,000 and 29,000 plates/m for choline and acetylcholine, respectively (Figure 2). The same mobile phase with the IonPac® CS10 produces efficiencies of 17,000 and 14,000 for choline and acetylcholine, respectively. The higher efficiency of the PCX-100 may be a result of the lower hydrophobicity of its cation exchange sites and the consequent reduction in any secondary hydrophobic interaction.

Suppressed conductivity detection is linear ($r^2 = 0.9994$) for both analytes over the range of 1 to 300 ng, and reproducible (RSD=1.9% and 2.8% for choline and acetylcholine, respectively, for ten 100-ng injections). The minimum detection limit (3 x noise) is 500 pg (5 pmol) for choline and 1 ng (7 pmol) for acetylcholine. Suppressed conductivity detection may be used for the simultaneous determination of other ions, such as sodium and potassium, which are present in many formulations.

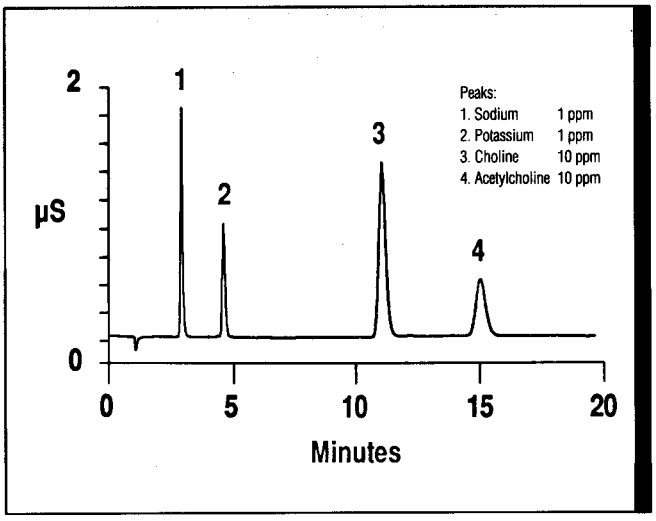

FIGURE 2. CHOLINE AND ACETYLCHOLINE STANDARDS

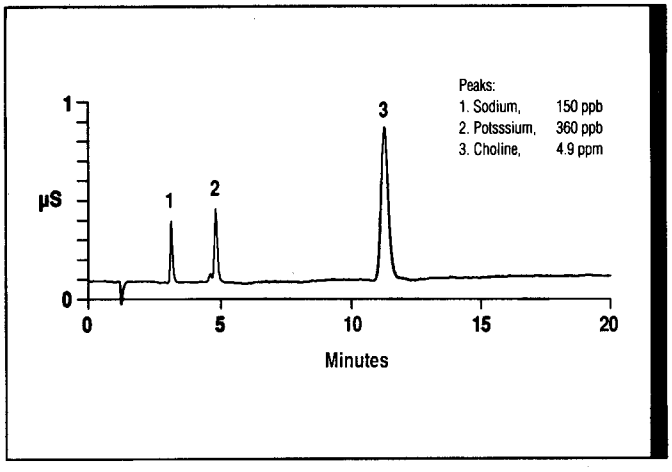

FIGURE 3. NUTRILIN

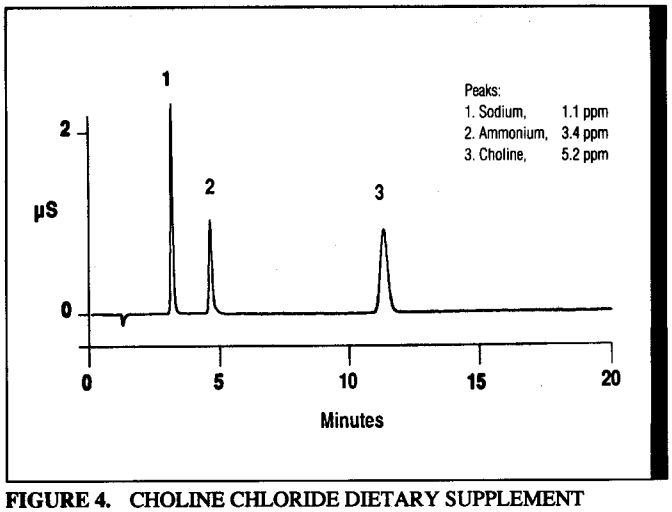

FIGURE 4. CHOLINE CHLORIDE DIETARY SUPPLEMENT

Nutrilin (Figure 3) is a vitamin and mineral formulation manufactured by Puritan-Quartz, containing more than 25 components. The use of conductivity detection simplifies choline determination, compared to refractive index detection or UV absorption, because only ionic components are detected. The pantothenic acid in the Choline Chloride Solution (Figure 4), obtained from Vitamin Research Products, is not detected because it is removed along with most anionic components during eluent suppression.

PRECAUTIONS

The choline bitartrate and acetylcholine chloride salts are hygroscopic. Choline bitartrate should be stored desiccated at room temperature. Acetylcholine chloride should be stored desiccated below 0°C. Although choline chloride is commercially available, it is not recommended as a standard because it is extremely hygroscopic, making accurate weighing difficult.

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