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Determination of Choline in Dry Milk and Infant Formula

INTRODUCTION

Recent research indicates that choline plays an important role in cardiovascular and liver health and in reproduction and development. Choline may even help improve memory and physical performance. Milk, eggs, organ meats, and other meats are good sources of choline, whereas grains, fruits, and vegetables are poor sources. Choline is essential to proper metabolism, and is therefore often added to vitamin formulations, animal feeds, infant formulas, and sports drinks. It is usually added to these products as the bitartrate or chloride salt and supplied as a solution for oral administration.¹

This Application Note describes methods for extraction of free and bound choline from dry milk and infant formula and its determination in the mg/L range by ion chromatography. The method also allows mineral ions (Na^+ , K^+ , Mg^{2+} , Ca^{2+}) to be determined simultaneously with choline.

EQUIPMENT

Dionex DX-500 Ion Chromatography System consisting of:

- GP40 Gradient Pump
- CD20 Conductivity Detector
- AS40 Automated Sampler
- LC20 Chromatography Enclosure with rear-loading valve.
- PeakNet Chromatography Workstation

REAGENTS AND STANDARDS

Deionized water (DI H_2O), Type I reagent grade, 18 M Ω -cm resistance or better

Concentrated Sulfuric acid (18.0 M), ACS reagent grade (95–98%) (J.T. Baker or other)

Hydrochloric acid, ultrapure reagent, ULTREX® II, 36.9% (J.T. Baker or equivalent)

Choline bitartrate, 99% reagent grade or better (Aldrich or other)

BRL (Bethesda Research Laboratories) TRIS (Tris(hydroxymethyl)aminomethane)

Filter Paper, quantitative grade 494 (VWR)

CONDITIONS

Columns: IonPac® CS12A Analytical, 4 x 250 mm (P/N 46073)
IonPac CG12A Guard, 4 x 50 mm (P/N 46074)

Eluent: 18 mN Sulfuric acid

Flow Rate: 1.0 mL/min

Sample Volume: 10 μL

Detection: Suppressed Conductivity, CSRS®-II AutoSuppression® recycle water mode

System

Backpressure: 7.90–11.37 MPa (1200–1650 psi)

Background

Conductance: 0.3–3 μS

PREPARATION OF SOLUTIONS AND REAGENTS

Standard Solutions

Stock choline hydroxide solution (1000 mg/L)

Dry approximately 7–10 g of choline bitartrate (MW 253.25) at 102 °C to a constant weight. Dissolve 2.092 g of dry choline bitartrate in 1000 mL of deionized water to prepare 1000 mg/L standard. The standard is stable for one week when stored at 4 °C.

Stock choline hydroxide solution for the recovery experiment (10000 mg/L)

Dissolve 2.092 g of dry choline bitartrate in 100 mL of deionized water.

Working Standards Solution

Dilute 1000 mg/L standard solution as required with deionized water to prepare the appropriate working standards.

Stock Eluent Solution

1.0 N Sulfuric acid

Weigh 972.80 g of deionized water into an eluent bottle. Degas water for approximately 5 minutes. Carefully add 27.20 mL of concentrated sulfuric acid directly to the bottle.

Working Eluent

18 mN Sulfuric acid

Weigh 982 g of deionized water into an eluent bottle. Degas water for approximately 5 minutes. Carefully add 18 mL of 1.0 N sulfuric acid solution directly to the bottle. Mix and then quickly transfer the eluent bottle to the instrument and pressurize the bottle with helium at 0.055 MPa (8 psi).

Extracting Solution

1 M Hydrochloric acid

Weigh 909.70 g of deionized water into an eluent bottle. Degas for approximately 5 minutes. Tare the bottle and carefully add 90.3 mL of ultrapure reagent-grade hydrochloric acid directly to the bottle.

Preparation of 50 mM TRIS buffer

Weigh 6.057 tris(hydroxymethyl)aminomethane into a 1-L flask containing approximately 500 mL of water. Dissolve the TRIS and adjust the pH to 8.0 by the addition of 1.0 M hydrochloric acid. Add water to a final volume of 1.00 L.

SAMPLE PREPARATION AND EXTRACTION

- Accurately weigh 5 g of dry milk sample into a 50-mL conical plastic tube with a cap (VWR).
- Add 30 mL of 1 M hydrochloric acid, cap, and mix by shaking until well dispersed.
- Place the flasks in a water bath at 70 °C for 3 hours, shaking every hour. Occasionally loosen or temporarily remove stoppers during the early heating stage to avoid excessive pressure build-up.
- After 3 hours, cool to room temperature.
- Filter hydrolysate through a filter paper (VWR, Quantitative Filter Paper) into a 100-mL volumetric flask. Rinse the filter with water.
- Adjust the total volume of the filtrate in a volumetric flask ($\pm .08$) to 100-mL with water.* This filtrate may be stored at 4 °C for three days.
- Prior to analysis, a final dilution with water should be made so that the amount of choline is in the calibrated linear range (10–200 mg/L). A 1 to 5 dilution is often appropriate.

* Note: As a precaution, phospholipase D may be added to the filtrate to release choline that may still be present as phosphatidylcholine. Choline recoveries were found to be the same with or without phospholipase D treatment for the samples analyzed in this Application Note.

Preparation of Phospholipase D Solution

1. Dissolve 150 U of phospholipase D in 200 mL of 50 mM TRIS.
2. Add 1.0 mL of enzyme solution to 1.0 mL of sample extract. Incubate at 37 °C for 15 minutes and cool to room temperature prior to analysis.

Determination of Choline Recovery from the Milk Samples

Add 400 μL of 10000 mg/L choline standard to 5 g of each dry milk or infant formula sample, and follow the extraction procedure described above. The final spike concentration is 10 mg/L if the final sample filtrate volume (after dilution) is 400 mL.

REPRODUCIBILITY OF CHOLINE RECOVERY FROM MILK SAMPLES

Choline was extracted from duplicates of eight milk samples and analyzed by IC.

RESULTS AND DISCUSSION

Acid digestion releases bound choline from milk samples. In this Application Note we used hydrochloric acid to extract choline from infant formula samples prior to IC analysis.

An equilibrated IC system will demonstrate a background conductance between 0.3 and 3.0 μS . Peak-to-peak noise is typically 2 nS and system backpressure is between 7.90 and 11.37 MPa (1200 and 1650 psi). A system blank is determined by using deionized water as the sample. This blank establishes the baseline and confirms the lack of contamination in the system.

Figure 1A shows that a typical chromatogram of a 100 ppm choline standard and Figure 1B an infant formula sample (Similac). The choline peak is well resolved from other peaks in the preparation. The concentrations of inorganic cations, ammonium and choline can be determined in the same run.

Figure 2 shows that the detection of choline is linear in the mg/L (ppm) range. The choice of the calibration range is based on the amount of choline usually found in milk samples.

To evaluate recovery, a known amount of choline standard was added to each of the dry milk samples. The total amount of choline was then determined following the same hydrolysis, filtration, and separation processes. Table 1 shows the recovery results. Over 95% recoveries of choline from the nonfat dry milk and low-iron infant formula were obtained.

Table 2 shows the results of an experiment in which eight infant formula and milk powder samples and their blind duplicates were analyzed. These data demonstrate good agreement for the duplicate samples that were run as part of a collaborative study². Sample pairs were revealed upon completion of the study.

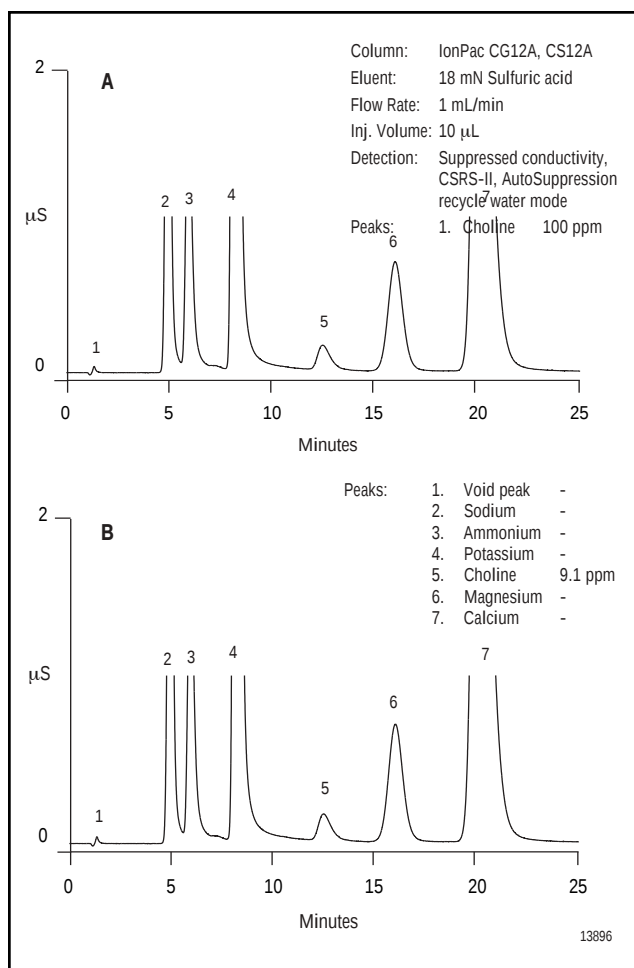


Figure 1A 100 ppm choline standard

Figure 1B Infant formula sample

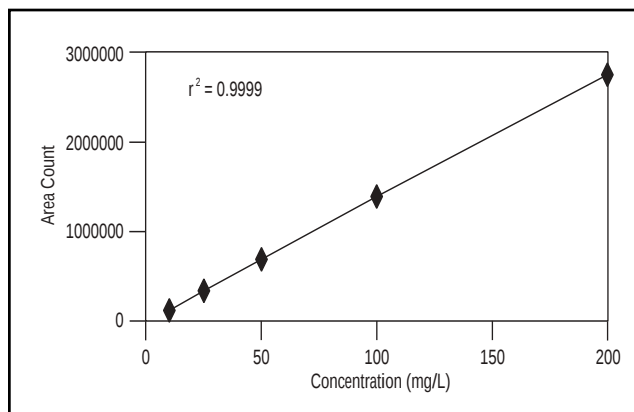


Figure 2 Choline calibration

Table 1 Choline recoveries in milk samples

Sample Name	Amount Present (mg/L)	Amount Added (mg/L)	Total Recovered (mg/L)	Recovery (%)
Similac	9.1	10.0	18.5	94
Dry milk	14.4	10.0	23.9	95

Table 2 Precision of the choline analysis in blind duplicate infant formula and milk powder samples

Sample	Sample Concentration (mg/100 g)		% Difference (A-B)/Larger Value
	Sample A	Sample B	
1	75.0	75.3	0.40
2	60.5	62.1	2.58
3	152.7	152.0	0.46
4	39.3	39.4	0.25
5	84.1	78.6	6.54
6	111.1	103.6	6.75
7	92.2	89.2	3.25
8	109.6	110.2	0.54

SUMMARY

The method outlined in this Application Note accurately quantifies mg/L (ppm) amounts of choline in milk samples.

REFERENCES

1. Canty, D., Lecithin and Choline Redeemed. *Nutrition Science News*. October 1997, 1-6.
2. Wollard, D.C. and Indyk, H.E., *J. AOAC Int.* (2000) 83, 131-138.

LIST OF SUPPLIERS

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