

Extension of AOAC Official Method 999.14 (Choline in Infant Formula and Milk) to the Determination of Choline in Dietary Supplements

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AOAC Official Method 999.14 is applicable for the determination of choline in milk and infant formulas. To date, its use has not been extended beyond these matrices. We modified Official Method 999.14 and applied it to the determination of choline in a range of choline-containing dietary supplements. Dietary supplement tablets, capsules, wafers, softgels, liquid products, and drink powders were included. We found that the standard curve could be extended to cover a wider range of choline concentrations and defined a procedure for the use of Norit for samples in which the vitamin C content was high enough to interfere with the analysis. Recoveries of choline added to infant formula powders and to representative dietary supplement tablets, capsules, powdered drink mix, and wafer products were 85–114%. The use of Norit during the procedure did not affect the recovery of choline added to infant formula powders or to dietary supplements. An alkaline digestion was included for use with a product containing lecithin as the sole source of choline. Ten of 11 dietary supplement products analyzed by the modified method contained amounts of choline at or above declarations found on the product labels. The remaining product contained about 40% of the label-declared amount of choline.

Choline, a dietary constituent, is an important component of membrane phospholipids. It provides a source of labile methyl groups, participates in transmembrane signaling, exerts a lipotropic action on the liver, is important for lipid and cholesterol transport and metabolism, and serves as a precursor for the neurotransmitter acetylcholine (1, 2). Choline is widely distributed in foods, with most of it in the form of phosphatidylcholine in membranes. Milk, liver, eggs, and peanuts are rich in choline.

Choline is also available as a dietary supplement of choline chloride or choline bitartrate and as lecithin, which usually contains about 25% phosphatidylcholine or 3–4% choline by weight. Foods and dietary supplements may now carry nutrient content claims, including claims about choline content, as authorized under the U.S. Food and Drug Administration Modernization Act (3).

Relatively few methods for the analysis of choline appear in the literature. Recently published methods report the use of proton nuclear magnetic resonance spectroscopy (4), liquid chromatography/electrospray ionization-isotope dilution mass spectrometry (5), and choline biosensors (6) for the quantitation of choline and its metabolites in several matrices. Instrumentation requirements of some of these methods may put them out of the reach of laboratories needing rapid and routine analyses for choline in foods and dietary supplements.

We studied the recently adopted AOAC Official Method 999.14 (Choline in Infant Formula and Milk—Enzymatic Colorimetric Method; 7, 8) to determine its applicability to the analysis of choline in matrices such as dietary supplements that may bear choline content claims. We used standard reference material (SRM) 1846 (Infant Formula), which has a noncertified reference value for choline, as an in-house quality assurance/quality control (QA/QC) material. We analyzed 5 infant formula powders and 11 dietary supplements of different types.

Experimental

Principle

The test sample is digested in acid (or, if needed, alkali) at 70°C, thereby releasing most of the choline. Following pH adjustment, treatment of hydrolysates with Norit (Fisher Scientific Co., Fairland, NJ) is included if needed to reduce interference from vitamin C. Residual choline phospholipids are then cleaved with phospholipase D, and the free choline moiety is subjected to choline oxidase with subsequent liberation of hydrogen peroxide. In the presence of peroxidase, phenol is oxidized and a quinoneimine chromophore is formed with 4-aminoantipyrine. Both enzymatic and color-forming reactions are formatted to occur concurrently. Absorbance is measured at 505 nm and choline

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