

Update on AOAC Analytical Methods for Nutrients

William Horwitz
Food and Drug Administrator
Washington, District of Columbia

During the past decade, the development of "new" methods of analysis for nutrients, or even the application of what are now standard methods for other substances, has been limited. Most of the activity has been in applying previously approved methods for nutrients in foods to infant formulas. From the point of view of database production, perhaps such stability is a desirable attribute. The purpose of a database is to solidify, coordinate, and preserve knowledge; one of the worst things that can happen to a compiler is a slow, unrecognized drift of what was thought to be a stable reference point.

Over the last half century, chemical, microbiological, and bioassay procedures have been developed for nutrients, and many of them have been validated by the application of method-performance protocols of the Association of Official Analytical Chemists (AOAC). The AOAC has been testing and adopting methods of analysis required to enforce laws and regulations for over a century. Its methods are accepted professionally and legally on an international basis. AOAC relies upon its sponsoring agencies, primarily to US Food and Drug Administration, the US Department of Agriculture, and the corresponding organizations in Canada, other countries, and the states for the actual performance of the laboratory studies required to gain approval of methods as "official."

Many of the currently approved methods for nutrients were validated 20 to 40 years ago and have not been updated to take advantage of modern separation and measurement technology. For example, validated automated methods for nutrients exist only for niacin, riboflavin, and vitamin C. Although innumerable methods exist in the literature proposing "new and improved" high-performance liquid chromatographic methods for the fat-soluble vitamins in foods -- vitamin A, vitamin D, and vitamin E -- such a method has been adopted only for vitamin D in milk, milk powder, and mixed feeds. The structural components of food, primarily protein and fiber-related components, ap-

pear to interfere with the release of the minor and trace nutrients to the measurement operations.

Although a vast literature exists on methodology for nutrients, very few methods have gone to an interlaboratory methods-performance trial. Many methods that behave admirably in the hands of the proud developers fail miserably when subjected to the abuses inflicted by other laboratories. Part of the problem of development of new methods for nutrients lies with the lack of stable reference standards to ensure a common reference point for all laboratories. Furthermore, knowledge of the variability of the nutrient concentrations across laboratories is important to indicate the relative standing of analytical variability compared to the variability introduced from other sources such as physical sampling of raw commodities (processing tends to smooth out commodity variability), uncontrolled serving sizes, and total food intake of individuals.

Table 1 provides the official AOAC method number for nutrients in the latest edition (1990) of *Official Methods of Analysis of the AOAC*, a reference to the last interlaboratory method-performance study published in the *Journal of the Association of Official Analytical Chemists*, and a brief personal comment on the quality of the results. The high variability of published results for nutrients that exist in the literature strongly suggests that little attention has been paid in the past to quality control of the analytical operations. It is possible that a considerable fraction of the variability ascribed to varieties, geography, and seasonal factors of raw commodities is merely a manifestation of lack of quality control of the nutrient analyses. In the single commodity area of infant formulas, which had the benefit of legislation mandating adherence to nutrient standards, a decade of investigational work is expected to result, at the AOAC meeting of August 1991, in a complete set of methods for measuring all of the nutrients required to be stated on the label of this commodity.

Table 1. Reference to Analytical Methods in the Vitamins and Other Nutrients Chapter in the 15th Edition (1990) of Official Methods of Analysis of the Association of Official Analytical Chemists

Analyte Matrix	Method Type	Method Number*	Latest JAOAC Reference
Chemical Methods			
Vitamin A			
Foods	Colorimetric	974.29	63,0468 (1980)
The official method is still the antimony trichloride spectrophotometric procedure. Carotenes can be determined simultaneously in high fat products. Note there is no general HPLC method for Vitamin A.			
Carotenes	Spectrophotom	941.15	53,0186 (1970)
in Fresh Plants			
Although monohydroxy and dihydroxy pigments are reported, there is no provision for these analytes in the referenced method, 941.15.			
Thiamine			
Foods	Fluorometric	942.23	64,1336 (1981)
Grains	Fluorometric	953.17	38,0722 (1955)
Bread	Fluorometric	957.17	43,0047 (1960)
All three methods are based upon the classical thiochrome fluorometric measurement. A number of simplifications are available for enriched foods, in which the thiamine is present in free form and does not have to be released from its compounds.			
Riboflavin			
Foods	Fluorometric	970.65	53,0542 (1970)
Latest version was also applied to concentrates and feed supplements.			
	Automated Fluorometric	981.15	62,1041 (1979)
Extensive study but results from one laboratory are somewhat out of line.			
Niacin and Niacinamide			
Foods	Colorimetric	961.14	45,0449 (1962)
Excellent parameters but only for bran, bread, cereal, and flour.			
Cereals	Automated Colorimetric	975.41	58,0799 (1975)
Automated method much superior to manual method; microbiological method highly variable as applied to cereals and baked pet food.			
Foods	Automated Colorimetric	981.16	62,1027 (1979)
Manual method erratic; automated method much superior. Even among-laboratories precision better for automated method than for manual or microbiological method for a variety of foods.			
Vitamin C (Ascorbic Acid)			
Juices	Titrimetric	967.21	50,0798 (1967)
Foods	Automated Fluorometric	984.26	66,1371 (1983)
All assays, except those by the unofficial diphenylhydrazine method, are excellent.			
Vitamin D			
Milk and Powder	HPLC	981.17	65,1228 (1982)
Variability high but acceptable.			
Mixed Feeds	HPLC	982.29	66,0751 (1983)
Variability high but acceptable. This assay requires conscientious quality control. On the practice test sample, the range of 18 laboratories was a factor of 4; on real test samples, the range was 5-10. One laboratory reported 146 for a 3 ppm concentration.			
* The Method Number is constructed from the last three digits of the year of adoption and the consecutive item number separated by a decimal point.			

Analyte Matrix	Method Type	Method Number*	Latest JAOAC Reference
Vitamin E			
Alpha-tocopherol (AT)	Colorimetric Obsolete TLC method; apparently problems exist in nomenclature and units.	971.30	54,00001 (1971)
Calcium Pantothenate			
	Spectrophotometric The only chemical method is for pharmaceutical preparations.	*945.73*	52,0449 (1969)
Sodium			
Foods for SDU	Ion Sel. Electrometric Ion selective electrode method is very simple, but lower limit is about 10 ppm Na. Precautions necessary to avoid ordinary laboratory contamination.	976.25	59,1131 (1976)
Fat			
Foods	CHCl ₃ -MeOH Extn Fat values are method-specific; individual commodities have classical methods, but composite products use MeOH-CHCl ₃ and must be thoroughly homogenized. Relative standard deviations as high as 20% can be expected at 1-5% fat levels.	983.23	66,0927 (1983)
Cholesterol			
Multicomponent Foods	GLC Precision parameters relatively poor apparently from both homogenization and fat extraction.	976.26	59,0046 (1976)
Noodles	Fluorometric Very good parameters but limited to noodles.	969.14	51,1220 (1968)
Dietary Fiber			
Foods	Enzymatic-Grav. Problems with this analyte are very well known.. Values are method-specific, and reproducibility at 1-5% levels and below is very poor.	985.29	69,0259 (1986)
Protein, carbohydrates, sugars, etc.			
Numerous methods available for individual commodities. See overall evaluation in 73,0661 (1990) and for dairy products in 72,0784 (1989).			
<i>Microbiological Methods</i>		<i>Titrimetric</i>	<i>960.46E</i>
		<i>Tubidimetric</i>	<i>960.46F</i>
Cobalamin			
B ₁₂ Activity	Microbiological Studies performed on relatively high activity products with excellent results.	952.20	45,0529 (1959)
Folic Acid	Microbiological Studies performed on relatively high activity products with excellent results.	944.12	42,0529 (1959)
Niacin and Niacinamide	Microbiological Ten commodities as part of the automated method study provided acceptable results.	944.13	62,1027 (1959)
Pantothenic Acid	Microbiological Excellent results but only for three products and by five laboratories.	945.74	42,0853 (1957)
Riboflavin	Microbiological Acceptable results but only a few test samples.	940.33	32,0461 (1949)
Amino Acids	Microbiological Excellent results but the studies were conducted with solutions.	960.47	43,0034 (1960)
Vitamin B6			
Pyridoxine, -al, -amine	Microbiological Results are acceptable for most foods, but liver is exception.	961.15	53,0546 (1970)

Analyte Matrix	Method Type	Method Number	Latest JAOAC Reference
Bioassy Methods			
Thiamine	Growth Rat growth method has same precision as chemical methods.	*938.12*	25,0451 (1942)
Vitamin D	Rat Bioassy is in terms of healing scores, and is not amenable to statistical analysis. Very few laboratories can perform this assay.	936.14	46,0160 (1963)
Infant Formula			
Proximates Milk-based	Grav./Titrimetric	986.25	69,0777 (1986)
Elements CA, Cu, Fe, Mg, Mn P, K, Na, Zn	ICP Emission Atom Absorp.	984.27 985.35	67,0985 (1984) 68,0514 (1985)
Phosphorus Milk-based	Spectrophotometric	986.24	69,0777 (1986)
Chloride Milk-based	Potentiometric	986.24	69,0777 (1986)
Thiamine Milk-based	Fluorometric	986.27	69,0777 (1986)
Riboflavin Milk-based	Fluorometric	985.31	68,0514 (1985)
Vitamins A, D, and E Milk-based	Not recommended for approval		
Vitamin B6 Milk-based	Microbiological/Turb.	985.32	68,0514 (1985)
Vitamin C Milk-based	Titrimetric	985.33	68,0514 (1985)
Niacin and Niacinamide Milk-based	Microbiological/Turb.	985.34	68,0514 (1985)
Cobalamin (vitamin B12 activity) Milk-based	Microbiological/Turb.	986.23	69,0777 (1986)
Pantothenic Acid Milk-based	Microbiological/Turb.	43.025 [84]	69,0777 (1986)
<p>Variability is high but acceptable (RSDR 20%; HORRAT 1.5); note adopted in expectation of obtaining "improved" method. Improved quality control required since some laboratories not aware they were producing outliers.</p> <p>The results from the method-performance studies for the proximates, inorganic nutrients, and water-soluble vitamins are acceptable, those for vitamins A and D are borderline, but those for vitamin E are unacceptable.</p>			
<p>* = Surplus (No longer used) SDU = Special Dietary Use Grav. = Gravimetric Titr. = Titrimetric Turb. = Turbidimetric</p>			