

GENERAL REFEREE REPORTS

Committee on Food Nutrition

Fat-Soluble Vitamins

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Summary

One of the major upcoming issues is the increasingly tough legislation being enacted, or being discussed, for regulatory compliance in fortified food products. The European Union is moving toward harmonized rules for addition of vitamins and minerals in food products. Some countries, like Belgium, China, and Brazil, have placed strict tolerances (\pm) around the declared values for nutritional parameters. Mexican authorities are currently discussing legislation for tolerances of $\pm 10\%$ relative for declared fortified nutrients. The levels of fat-soluble vitamins (FSV) will surely be closely scrutinized because of potential health risks associated with overdosing, in particular vitamin D.

The introduction of stricter regulatory compliance places even more importance on the need for well-validated internationally accepted analytical methods of high precision. The analysis of the FSV A, E, D, and K₁ are covered by the AOAC Official Methods in Chapters 45 (Vitamins and Other Nutrients) and 50 (Infant Formula, Baby Foods, and Enteral Products). Some of the older procedures for FSV are not well validated and are not really suitable for checking the new stricter regulatory compliance regulations around the world. The set of methods available for FSV needs a thorough review, and where several procedures exist for the same analysis, it is suggested that only the best-in-class methods should be retained, and poorly validated procedures should be withdrawn. As a first step it is recommended that 2 methods be withdrawn:

(1) *Vitamin D (981.17)*.—Four methods are available for the determination of vitamin D, based on liquid chromatography (LC) with UV detection (Table 1). Method **981.17** has largely been superseded by the 3 other procedures. The former method has no validation data available to show that it is fit for purpose. The other 3 procedures adequately cover the determination of vitamin D₃, and so it is recommended to withdraw Method **981.17**. (At the Annual Meeting, the Food Nutrition Committee voted to withdraw this method.)

It should be noted in the 3 remaining procedures to determine vitamin D₂; then vitamin D₃ can be used as an internal standard as described in EN 12181 method Foodstuffs—Determination of vitamin D by

HPLC—measurement of cholecalciferol (D₃) and ergocalciferol (D₂).

(2) *Vitamin K (992.27)*.—Two methods, based on LC, are available for determination of vitamin K₁ (Table 2) with similar fields of application. Method **992.27** has largely been superseded by Method **999.15**. The main advantages of **999.15** are greater specificity, since it uses a post-column reaction with fluorescence detection rather than UV detection at 254 nm; better precision; and harmonization with the European EN 14148 procedure.

It is thus recommended that Method **992.27** be withdrawn. (At the Annual Meeting, the Food Nutrition Committee voted to defer withdrawal of this method until a method modification can be issued for Method **999.15**, allowing for the use of a C₃₀ column to separate *cis* and *trans* isomers.)

The Food Nutrition Committee has issued no new methods in 2005 for FSV. However a high-performance liquid chromatography (HPLC) method for determining β -carotene in dietary supplements and raw materials was collaboratively studied (1). The reversed-phase LC procedure requires visible light absorbance for detection and quantitation. If high levels of alpha-carotenes are present, a second LC system is used for additional separation and quantitation of the carotene species. The recommendation from this study is that this method should be adopted as an AOAC Official Method.

Some recent publications have focused on checking nutritional labels for compliance. Rodríguez-Comesana et al. (2) described a validated HPLC-UV method for control of nutritional labels for beverages with added vitamins, screening β -carotene and ascorbic acid contents. The results obtained with 13 beverages showed that in virtually all drinks, the vitamin contents were much higher than those declared on the nutritional label. In Spain, Herrero-Barbudo et al. (3) reviewed retinol, α - and γ -tocopherols, and carotenoids in natural and vitamins A- and E-fortified dairy products. Compared with label claims, over-fortification predominated, although both under- and over-fortification of vitamins A and E were found in fluid milks.

The well-known reference book by Ball (4), *Vitamins in Foods: Analysis, Bioavailability, and Stability*, was recently updated. An excellent chapter reviews the determination of FSV by HPLC. Perales et al. (5) critically reviewed HPLC techniques for determination of vitamin D in milk and milk infant formulas.

A review of recent literature publications is given below for analysis of FSV. One of the major bottlenecks in analysis of FSV is due to the time-consuming solvent extraction step after saponification. One way to speed up these extractions and reduce the considerable consumption of solvents is to use solid-phase extraction (SPE) in place of liquid-liquid extractions. Fedder and Ploger (6) reported the use of Oasis®

Table 1. AOAC methods for vitamin D by liquid chromatography

Method No.	Method title	First Action	Final Action	Matrixes validated	Vitamin D forms measured	Comments
981.17	Vitamin D in fortified milk and milk powder	1981	1982	Fluid milk, milk powder	D ₂ or D ₃	No internal standard; factor of 1.25 used to correct for saponification losses
992.26	Vitamin D ₃ (cholecalciferol) in ready-to-feed (RTF) milk-based infant formula (Codex-AOAC method)	1992	1995	RTF milk-based infant formula	D ₃	No internal standard; factor of 1/0.86 used to correct for saponification losses
995.05	Vitamin D in infant formula and enteral products (LC method)	1995	No	Infant formula, enteral products	D ₃ and previtamin D ₃	Vitamin D ₂ used as internal standard
2002.05	Determination of cholecalciferol (vitamin D ₃) in selected foods (joint AOAC-NMKL method)	2002	No	Fortified milk, infant formula, gruel, margarine, cooking oil, fish oil	D ₃	General procedure; vitamin D ₂ used as internal standard

columns in place of liquid-liquid extractions for determining vitamins A and E in animal feeds. Intermediate reproducibility for the method was 21 and 11% of the average results for vitamins A and E, respectively. Recovery of vitamin A was 80% and 110% for vitamin E, as calculated using a National Institute of Standards and Technology (NIST) infant formula reference material. Ostermeyer and Schmidt (7) took a similar approach by using a disposable Kieselguhr cartridge to extract vitamin D₃ and provitamin D₃ from saponified fish before HPLC with electrochemical determination. Heudi et al. (8) used Chromabond® XTR for extraction of vitamins A, D, and E from fortified food products. Chase et al. (9) described an interlaboratory verified method for the determination of vitamins A and E in milk and soya-based infant formula using matrix solid-phase dispersion. It is recommended to evaluate SPE as an alternative to solvent extraction for analysis of vitamins A, D, and E. This could be made initially as a single-laboratory validation (SLV).

Another approach is to avoid saponification and to extract the FSV directly. Chavaz-Servin et al. (10) described a

simultaneous analysis of vitamins A and E in infant milk-based formula by normal-phase HPLC with diode array detection (DAD). The analysis included retinol acetate and palmitate, and α -tocopherol acetate. This procedure involved protein precipitation and vitamin extraction with ethanol, followed by re-extraction into hexane. A short narrow-bore column (50 × 2.1 mm, 3 mm particle size) was used for LC separation. For cereals, Seon et al. (11) observed that higher recoveries were obtained with a direct solvent extraction than with a saponification/solvent extraction. Samples were dispersed in hot water, followed by addition of isopropanol and MgSO₄, with Polytron homogenization. Normal-phase HPLC with UV detection was applied. A simple colorimetric method was reported to determine the yield of microencapsulation of α -tocopherol (12).

The contents of vitamin K₁, 2,3'-dihydrophyloquinone (dK) and menaquinone (MK-4) were determined in grains, cereals, fast-food breakfasts, and baked goods using an HPLC procedure (13). Vitamin K₁ was determined (14) in olive oil, chard, and human plasma by reversed-phase HPLC-UV with

Table 2. AOAC methods for vitamin K by liquid chromatography

Method No.	Method title	First Action	Final Action	Matrixes validated	Vitamin K forms measured	Comments
992.27	<i>trans</i> Vitamin K ₁ (phyloquinone) in ready-to-feed (RTF), milk-based infant formula	1992	1995	RTF milk-based infant formula	<i>trans</i> Vitamin K ₁	Determined by LC with UV detection at 254 nm, nonspecific
999.15	Vitamin K ₁ in milk and infant formula (LC method)	1999	No	Infant formula and milk (fluid, RTF, and powdered)	Total vitamin K ₁ (phyloquinone)	Procedure harmonized with EN 14148; post-column reaction with fluorescence detection gives better specificity

acetonitrile–dichloromethane–methanol (60 + 20 + 20, v/v/v) as eluent on a C18 I-Bondapak column, and in green leafy and flower vegetables using HPLC-fluorescence detection (15).

Jasinghe and Perera (16, 17) reported the use of UV radiation to generate vitamin D₂ from ergosterol in edible mushrooms. The highest vitamin D₂ content (184 ± 5.71 mg/g dry matter (DM)) was observed in oyster mushrooms irradiated with UV-B at 35°C and around 80% moisture. Mushrooms thus treated could be used as concentrates for vitamin D fortification purposes.

Estes et al. (18) described how temperature programming can be used to optimize LC separations for vitamins A, E, and carotenoids. Castanheira et al. (19) investigated the errors caused by the use of volumetric glassware in vitamins analysis.

A validated method for the quantification of vitamin A in human milk was reported by Lopez et al. (20). Samples (500 µL) were saponified with potassium hydroxide–ethanol, extracted with hexane, evaporated to dryness and reconstituted with methanol. A reversed-phase (RP)-C₁₈ column, a methanol–water (91 + 9, v/v) mobile phase, and a fluorescence detector (excitation 330 nm; emission 470 nm) were used for the separation and quantification of vitamin A. The method characteristics were linearity ($r^2 = 0.9995$), detection limit (0.010 µg/mL), quantification limit (0.025 µg/mL), precision [repeatability relative standard deviation (RSD_r) = 9.0% within 1 day; reproducibility relative standard deviation (RSD_R) = 8.9% several days], and accuracy (recovery = 83.8%).

A rapid and direct HPLC-UV method for determination of γ - and α -tocopherols in human milk was reported (21). Samples were diluted in hexane with addition of an internal standard (α -tocopherol acetate). The chromatographic system consisted of a short column (50 × 2.1 mm id, 3 µm particle size) that allowed the separation of γ - and α -tocopherols in <6 min. The direct method was compared with standard saponification/solvent extraction procedures using either (1) UV detector or (2) light scattering detector. The direct method gave about 20% higher recoveries than (1) and similar results to (2).

The analysis of carotenoids continues to occupy a large part of the FSV literature. Some key references are given below. A general review of recent literature for HPLC analysis of carotenoids was reported by Lu and Liang (22). Schieber and Carle (23) reviewed the effects of processing on *trans-cis* isomerization with particular attention to β -carotene, lycopene, lutein, and zeaxanthine. Rodriguez-Bernado de Quiros and Costa (24) reviewed the analysis of carotenoids in vegetables and plasma samples.

An LC-mass spectrometry (MS) procedure was reported (25) for determination of lycopene and β -carotene in fruits and vegetables. Extraction was carried out by tetrahydrofuran–methanol (1 + 1, v/v). Separation was carried out with 2 coupled columns, first a C₁₈ and then a dC₁₈, using a mobile phase of methanol–tetrahydrofuran–acetonitrile (60 + 30 + 10, v/v/v) in isocratic mode. The molecular ion was selected for quantification in selective ion monitoring

mode. Chemical ionization atmospheric pressure in positive mode was used for quantification of the carotenoids.

The use of high-speed counter-current chromatography was reported (26) for extracting lutein, violaxanthin, and β -carotene from marigold flowers, squashes, and potatoes, and for isolation of (all-E)-lutein and (all-E)-zeaxanthin from plant materials and dietary supplements (27).

A new approach to extraction was adopted by Larsen and Christensen (28), who extracted carotenoids from green vegetables with acetone, followed by the selective removal of the chlorophylls and esterified fatty acids from the organic phase using a strongly basic resin (Ambersep 900 OH). The mean recoveries (%) for (all-E)-violaxanthin, (all-E)-lutein epoxide, (all-E)-lutein, neolutein A, and (all-E)- β -carotene after saponification of the vegetable extracts with Ambersep 900 OH were 99–104%, while the mean recovery (%) for (9 and prime; Z)-neoxanthin was 119% and that of (all-E)-neoxanthin and neolutein B was 90 and 72%, respectively.

A major study was reported by Chun et al. (29), who surveyed the tocopherol and tocotrienol contents of raw and processed fruits and vegetables in the U.S. diet. The methodology involved saponification, solvent extraction, and normal-phase LC.

Several quality control (QC) methods have been reported for determining tocopherols in oils. Near infrared (NIR) spectroscopy was used (30) to determine natural α -tocopherol levels in vegetable oils. This method was calibrated using partial least-squares (PLS) regression, and validated. Results showed that the NIR method compared favorably with HPLC in both precision and recovery accuracy. A rapid Fourier transform infrared (FT-IR) procedure was reported (31) to determine tocopherols, tocotrienols, and plastochromanol 8 in various oils. It was calibrated against reference values obtained by HPLC, and an excellent correlation was obtained using PLS analysis. α -Tocopherol was determined in refined bleached and deodorized palm olein by FT-IR (32). This method was calibrated with an HPLC procedure ($r = 0.9922$). Method accuracy was similar to that of HPLC. Synchronous fluorescent spectroscopy appears to have some potential as a rapid QC procedure for determining total tocopherols content in vegetable oils. This alternative method was calibrated against an HPLC procedure using PLS regression (33).

A need exists within the food industry for simple and more rapid QC procedures for FSV as an alternative to the time-consuming and expensive HPLC methods. A step in this direction has been made by SciMed Labs (Canada), who have developed immunoassay kits for vitamins A and D in milk and dairy products. First trials show that these procedures require some further optimization to render them sufficiently robust for routine analyses. Immunoassay kits are also planned for vitamins E and K (34).

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Water-Soluble Vitamins

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Summary

In the reporting period (June 2005–June 2006), the reference book, *Vitamins in Foods: Analysis, Bioavailability, and Stability*, by G.F.M. Ball, CRC Press, was updated. The book presents the latest information on vitamin analysis, bioavailability, and stability in foods. The following articles related to Water-Soluble Vitamins analysis in foods were published:

Simultaneous Determination of Vitamins

Marszall et al. (1) published a method for the simultaneous determination of thiamin hydrochloride, pyridoxine hydrochloride, and cyanocobalamin in pharmaceutical formulations and dietary supplements using coulometric electrochemical and UV detection. Zafra-Gomez et al. (2) published a method for the isolation and simultaneous determination of the vitamins thiamin, riboflavin, niacin, pantothenic acid, pyridoxine, folic acid, cyanocobalamin, and ascorbic acid in food samples. The most relevant advantages of the proposed method are the simultaneous determination of the 8 more common vitamins in enriched foods and a reduction of the time required for quantitative extraction, because the method consists merely of the addition of a precipitation solution and centrifugation of the sample. Furthermore, this method saves a substantial amount of reagents as compared with official methods, and minimal sample manipulation is required. The chromatographic separation is carried out using reversed-phase high-performance liquid chromatography (RP-HPLC) on a C18 column, and the vitamins are detected at different wavelengths by either fluorescence or UV-visible detection. The proposed method was applied to the determination of water-soluble vitamins in 2 enriched ultra-high temperature (UHT) milk beverages marketed for children, an enriched milk infant formula, UHT milk, and a milk powder certified reference material (CRM 421, BCR) with recoveries ranging from 90 to 100%.

These methods are not compared to single-vitamin methods. Also, the extractions used are not suitable to determine endogenous (bound) vitamins in food samples and are generally applied for enriched foods and supplements. The

methods described are not suitable if total vitamin amounts in food samples need to be determined.

Vitamin B

Lebiedzińska and Szefer (3) published values of vitamins B (thiamine, riboflavin, pyridoxine, and niacin) in grain and cereal-grain food, soy products, and seeds. The concentrations of vitamins were determined by microbiological analytical methods.

Vitamin B₅

Haughy et al. (4) published a method for the determination of pantothenic acid in foods by optical surface plasmon resonance (SPR) biosensor immunoassay. The sensitivity of the SPR assay technology coupled with the specificity of the ligand-binding protein interaction allows a rapid assay with a minimum of sample preparation required. A range of (fortified) foods, including National Institute of Standards and Technology (NIST) reference samples, were tested in 3 independent laboratories and the results were tested by microbiological assay as well as liquid chromatography/mass spectrometry (LC/MS) methods. Statistically, there was significant equivalence between the independent analytical techniques over the range of samples, despite the radically different analytical principles.

Vitamin B₆

Gatti and Gioia (5) published an HPLC method for the determination of vitamin B₆ vitamers and riboflavin (vitamin B₂) in milk and pharmaceuticals. The method involves a (gradient) HPLC method with fluorescence detection at different programmed wavelengths and has been developed for the simultaneous analysis of B₆ vitamers, including pyridoxal 5'-phosphate, 4-pyridoxic acid, pyridoxal, pyridoxine, and pyridoxamine and vitamin B₂ in commercial vitaminized milk and in human milk. Chromatographic separations were performed on a reversed-phase column. The fluorescence intensity of pyridoxal 5'-phosphate was enhanced by post-column photochemical conversion. In addition, an isocratic method without photochemical conversion was proposed for the analysis of vitamins B₆ and B₁₂ in pharmaceuticals.

Vitamin B₁₂

Heudi et al. (6) published a method for the determination of vitamin B₁₂ in food products and in premixes by RP-HPLC and immunoaffinity extraction. Vitamin B₁₂ was extracted from food products with 50 mM sodium acetate buffer at pH 4.0, followed by a purification step on an immunoaffinity column before the LC analysis. An enzymatic hydrolysis step with pepsin before the purification step efficiently released the bound vitamin B₁₂, and, thus, allowed obtaining total vitamin B₁₂ content in food products. Vitamin B₁₂ was monitored by UV at 361 nm after its separation on a reversed-phase narrow-bore column with a gradient of mobile phase made of water-acetonitrile and 0.025% trifluoroacetic acid. The method was successfully applied to several food products.

Different data obtained after the determination of vitamin B₁₂ in different food products by the developed HPLC method and microbiological assay demonstrated that the values of the 2 sets were comparable and that the bias was not significantly different from zero at 95% confidence.

Pakin et al. (7) described a method for the determination of free vitamin B₁₂ in various foods by RP-HPLC and fluorimetric detection. The method includes purification by immunoaffinity column and a precolumn conversion of vitamin B₁₂ into the fluorescent α -ribazole. An enzymatic hydrolysis with pepsine before the purification made it possible to release vitamin B₁₂ bound to proteins and, thus, to obtain the total vitamin B₁₂ content of these foods. The method has a low quantification limit (3 ng/g), which allows quantification in most foods.

A method that was developed for the determination of vitamin B₁₂ by HPLC electrospray ionization-mass spectrometry (HPLC-ESI-MS) has been applied successfully to fortified foods and multivitamin-multimineral tablets (8).

Vitamin C

Fontannaz et al. (9) published an HPLC method for the determination of total ascorbic acid and isoascorbic acid in fortified food products and premixes. The method is based on the acidic extraction of ascorbic acid in the presence of reducing agent Tris [2-carboxyethyl] phosphine (TCEP) and decylamine as ion pairing agent. According to the authors, ascorbic acid and isoascorbic acid often co-elutes when extracted in extraction buffers as metaphosphoric acid. Reducing agents such as cystein or dithiothreitol are frequently used to convert dehydroascorbic acid into its reduced form. Recently it was demonstrated that TCEP offers more efficient reduction of dehydroascorbic acid at low pH compared to that of dithiothreitol.

Hernández et al. (10) described a comparative evaluation of methods for the determination of vitamin C in tropical fruits. Two analytical methods used ion-pair HPLC for the detection of ascorbic acid, but differed in the preparation of the sample. Samples were extracted with 3% metaphosphoric acid, 8% acetic acid, or 0.1% oxalic acid. The latter extraction solvent proved to be unacceptable in some cases.

Güçlü et al. (11) described the use of copper(II)-neocuproine reagent to oxidize ascorbic acid to dehydroascorbic acid. The copper-reagent is milder and therefore a more selective oxidant than conventional Fe(III)-1,10-phenanthroline reagent used for the same assay. It seems that this feature makes the proposed method superior for real samples such as fruit juices containing weak reductants such as citrate, oxalate, and tartarate. Two papers from Chinese authors (12, 13) described, respectively, a specific use of an electrochemical determination of ascorbic acid in fruits on a vanadium oxide polypropylene carbonate modified electrode, and a direct fluorimetric determination of ascorbic acid by the supramolecular system of ascorbic acid with β -cyclodextrin derivative.

Folic Acid

A method for the determination of folic acid by capillary electrophoresis (CE) with chemiluminescence (CL) detection has been successfully applied in commercial pharmaceutical tablets and apple juices (14).

Biotin

Höller et al. (15) published a method for the quantification of biotin in feed, food, tablets, and premixes using HPLC-MS/MS. Key steps in sample preparation were an alkaline extraction or a hydrolysis with sulfuric acid followed by enzymatic digest with papain. In particular, samples with low biotin content needed the latter combination of extraction steps for an optimal release of biotin from the sample matrix. Results of the method were in good agreement with results obtained by microbiological assay; for comparison of the new HPLC-MS/MS method with the microbiological assay, 15 samples have been analyzed in duplicate according to the 2 procedures. Additionally, only 2 reference materials from NIST have been analyzed by HPLC-MS/MS. Of the 17 samples investigated, results of 9 samples differed by less than $\pm 15\%$, and in 4 cases each the HPLC-MS/MS results were higher or lower by more than 15% than those from the microbiological assay. Other differences were attributed to the principles of the microbiological assay.

Reference Materials

A new category of Standard Reference Materials (SRMs) based on dietary supplements is under development by the NIST, with certified values for organic constituents and selected trace elements (16). These materials are provided primarily for use in method development and as control materials. The SRMs will assist manufacturers of dietary supplements in characterizing raw materials for potency, authenticity, and contamination or adulteration. In addition, the SRMs will assist in self-assessment of consistency and quality in finished products. The goal of this ongoing effort is to provide tools to the dietary supplement industry and measurement communities that will lead to improved quality of dietary supplements, and ultimately reduce public health risks that could potentially be associated with these products.

Update on European Committee Standardization (Working Group: Vitamins and Carotenoids)

The working group had its last meeting in April 2006 in Budapest, Hungary.

Topics discussed included status of work, new items on the work program, periodical review of existing CEN standards, and possible future work.

Status of Work

CEN has published a European standard (EN 14663) for the determination of vitamin B₆ in foods by HPLC. Vitamin B₆ vitamers pyridoxine, pyridoxal, and pyridoxamine are extracted from food by acid hydrolysis and dephosphorylated and deglycosylated enzymatically using

acid phosphatase and β -glucosidase. The individual vitamers (pyridoxine, pyridoxal, pyridoxamine) are separated by HPLC and quantified by fluorescence detection. This method was successfully validated for infant food, potato puree, vegetables with ham, and multivitamin drink at levels from 0.034 to 1.21 mg/100 g.

European standards published since the last meeting in 2005 are 2 corrigenda (additional corrections/improvements to the existing Standards) related to Standards for the determination of vitamin B₁ (EN 14122) and vitamin B₂ (EN 14152) in foods. It appeared from research in Denmark that Takadiastase, described as a possible source for the dephosphorylation of vitamins B₁ and B₂ phosphates in these standards, did not work properly anymore. The CEN working group on vitamins and carotenoids concluded that EN 14122 (vitamin B₁) and EN 14152 (vitamin B₂) contained errors in the wording pertaining to the dephosphorylating enzyme. A suitable text was proposed to alert the user of the standards for the potential variability in enzyme activity. The text of the standards was amended in corrigenda to the existing methods.

New Items on the Work Program

The working group discussed drafts of a method for the determination of biotin in foods and a draft of a method for the determination of niacin (vitamin B₃) in foods. Both are HPLC methods. The working group agreed that these methods are suitable to work out as European standards. Drafts are included in the work program for further elaboration.

Periodical Review of Existing CEN Standards

Five years after publication, European standards are due for review. The working group discussed the need for having 3 standards for the determination of vitamin B₆ in foods. It was concluded to retain all standards because every method has its own application, which is described in the scope. All standards are used in one or more European countries.

The working group decided to revise the standard for the determination of vitamin D (EN 12821) and the standard for the determination of vitamin A (retinol; EN 12823-1). The standard for vitamin D will be updated with additional validation data of other matrixes, and an improved text for the purity determination of vitamin A will be included in revised versions of the standards. Standards for the determination of vitamin A (β -carotene; EN 12823-2) and vitamin E (EN 12822) were approved for another 5 years.

Possible Future Work

Vitamins and supplements.—The Food Standards Agency (UK) tested a multivitamin procedure for supplements (B₁, B₂, B₃, B₅, B₆, and folic acid). A report is expected in June 2006. The working group will discuss the possibility to elaborate this method as a European Standard.

Astaxanthin/cantaxanthin.—The working group identified the need for possible future standards for the determination of astaxanthin and canthaxanthin in, e.g., salmon. A proposed method seemed to be suitable. However, this method cannot be published as a European standard because of the absence of

interlaboratory validation data. The secretariat will check the possibility of publishing the proposed method as a technical report.

Vitamin C with new available premixes.—From work in the enforcement by the Food and Consumer Product Safety Authority in The Netherlands, it appeared that vitamin C from several samples, in particular meal replacement products, could not be extracted (completely) by the usual metaphosphoric acid extraction. This means that the European standard in its current form will not quantify the amount of vitamin C in products containing certain newly available premixes. Other experts confirmed these experiences. Sometimes, vitamins in beadlets coated with cellulose cannot be released. It seemed that oxalic acid as extraction solvent might be a good alternative for metaphosphoric acid. However, fat-coated vitamin C premixes also can cause problems. Experts were invited to collect information on any problems with the release of vitamins due to resistant coating for discussion at the next meeting.

Evaluation of Biacore Test Kits for the Determination of Riboflavin (Vitamin B₂), Pantothenic Acid (Vitamin B₅), Cyanocobalamin (Vitamin B₁₂), Folic Acid, and Biotin in Foods and Supplements by Optical Biosensor Immunoassay

Biacore submitted applications to obtain Performance-Tested MethodsSM status of 4 test kits for the determination of vitamin B₂, vitamin B₅, vitamin B₁₂, and biotin, respectively. All kits are used together with a Biacore Q, Qflex[®].

The following kits were granted Performance-Tested status in 2006; all matrixes are food-based and fortified, such as infant formula, breakfast cereals, fortified drinks, milk powder, etc., as well as dietary supplements: Qflex[®] Kit Vitamin B₂ PI; Qflex[®] Kit Vitamin B₁₂ PI; Qflex[®] Kit Biotin; Qflex[®] Kit Pantothenic Acid PI.

Along with the Folic Acid kit, these methods will now proceed to AOAC's Official MethodsSM Program. Vitamins mentioned will be collaboratively tested in different matrixes, such as infant formula, breakfast cereals, fortified drinks, and dietary supplements.

Vitamin Assays with Microbiological Vitamin Detection in Microtiter Plate Format

R-Biopharm brought several test kits on the market for the analysis of water-soluble vitamins in food products, animal feed, and pharmaceutical products. The microtiter plates for these microbiological assays are coated with specific microorganisms, which grow according to the presence or absence of vitamins in the sample. After sample, standard, and assay medium are placed into the wells, the plates are incubated and subsequently evaluated using a microtiter plate reader. Test kits for several water-soluble vitamins are available.

International Workshop on Vitamin Analysis

David Woollard and Erik Konings led a workshop on vitamin analysis, which was organized in collaboration with governmental organizations Instituto Nacional de Tecnologia Industrial (INTI) from Argentina and Laboratoria Tecnológico del Uruguay (LATU) in Uruguay. It was a 5-day workshop, which took place at the laboratories in Argentina (3 days) and Uruguay (2 days). The workshop stimulated the cooperation between technicians of the several laboratories in Argentina and Uruguay in the field of vitamins. Furthermore, the workshop improved their knowledge of AOAC and European (CEN) methods of analysis in this field, which encouraged future collaboration between AOAC, CEN, and South American countries.

Selected Study Director Topics

Biacore methods for the determination of riboflavin (vitamin B₂), pantothenic acid (vitamin B₅), cyanocobalamin (vitamin B₁₂), folic acid, and biotin in foods and supplements by optical biosensor immunoassay will be Study Director Topics in 2006/2007. Pathik Vyas of AgriQuality in New Zealand is assigned as Study Director for these topics.

Recommendations

Determination of biotin, folic acid, vitamins B₂, B₅, and B₁₂ in fortified foods and dietary supplements by surface plasmon resonance.—Study Director Pathik Vyas, Agriquality Laboratory Services Auckland, PO Box 41, Auckland, New Zealand, Tel: 64 9 626 8216, Fax: 64 9 626 8282, E-mail: vyasp@agriquality.com. Extend the scope of the AOAC Official Method for LC analysis of vitamin B₆ in infant formula (**2004.07**) to also include validation data from the determination of vitamin B₆ in baby food, biscuit, cereals, yeast, tube-feeding solution, chocolate powder, and powdered milk as described in CEN ENV 14164 (the determination of vitamin B₆ in foodstuffs by HPLC).

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