

## GENERAL REFEREE REPORTS

## Committee on Food Nutrition

### Fat-Soluble Vitamins

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### Summary

Potential major changes are being considered by some of the international regulatory bodies which will impact on fat-soluble vitamins (FSV): The Codex Alimentarius (1) wishes to introduce maximum level of vitamins with Upper Safe Limits for infant formula and AOAC Official Methods are recommended for FSV analysis. The UK Food Standards Agency (2) made a comprehensive review and reported risk assessments for FSV: vitamin A, beta-carotene, vitamin D, vitamin E, and vitamin K. Guidance levels were established for vitamin A, vitamin D, and vitamin K and safe upper levels were established for beta-carotene and vitamin E. The European Union (EU) is also proposing major changes in a draft revision of the EU directive on infant formula and follow-up formula which would mean major reformulation changes for FSV and other nutrients.

Another topic of increasing importance is the use of nomenclature rules for expressing vitaminic activity in food; "vitamin E" is an example of a complex situation. The definition of the vitamin affects how the analyst expresses analytical results and may lead to problems in comparing values from different sides of the Atlantic.

The analysis of the fat-soluble vitamins A, E, D, and K<sub>1</sub> is covered by the AOAC Official Methods in chapters 45 (vitamins and other nutrients) and 50 (infant formula, baby foods, and enteral products). Committee E has issued no new methods in 2003 or 2004 for FSV. A summary of the current AOAC methods is given in Table 1. Many of these methods are outdated. Recent methods issued by CEN and ISO are given in Tables 2 and 3, respectively. Some method harmonization work between the various organizations would be useful in the area of FSV.

The main focus should be on carotenoids analysis, since the current AOAC procedures (Methods **941.15** [1947]; **970.64** [1974]; **938.04** [1938]) are rather out-dated and need replacing. Validation of a horizontal multi-method for added

and natural carotenoids in food products should be a priority. At the moment, the supplements area has the priority and a collaborative study protocol has been submitted for review for "Determination of carotene in supplements and raw materials by reversed-phase HPLC," study Ka014, Study Director Jonathan DeVries. Another area which needs some attention is the simultaneous liquid chromatographic (LC) analysis of FSV in vitamin premixes and concentrates which are used for fortification purposes. A large variety of premixes are currently used within the food and pet food industry for which the current methods of analysis may not always be appropriate.

A review of recent literature publications is given below. For analysis of vitamins A, D, and E, the sample preparation step normally involves saponification of the entire sample matrix or an isolated lipid fraction, followed by liquid extraction with solvents like petroleum ether or *n*-hexane. A need exists to simplify these tedious steps. Rapid direct-extraction procedures were reported by Rodas-Mendoza et al. (3) for the determination of retinol acetate, -, -, -tocopherol, and -tocopherol acetate in infant formula. Two extraction procedures were evaluated using either dichloromethane-methanol or ethanol-hexane on one sample of infant formula. While the technique appears to be promising, further validation on a wide range of infant formula and reference materials of known FSV content would be necessary before the procedure can be applied on a routine basis. A direct-extraction was also selected by Hewavitharana et al. (4) for extraction of all 8 vitamin E homologs from chicken meat. In contrast, for cereals Panfili et al. (5) noted that hot saponification and solvent extraction gives considerably higher recoveries of tocopherols and tocotrienols compared to a direct extraction without saponification. Perretti et al. (6) evaluated supercritical fluid extraction (SFE) for FSV determination in cheese and salami. This approach merits further study using broader classes of food products.

An HPLC-fluorescence detection procedure was reported by Iwase (7) for determining retinol palmitate in nutritional supplements containing emulsifying agents. Monosodium glutamate was chosen as the sample-dissolving agent with a cleanup on a C<sub>2</sub> SPE column.

A validated collaboratively tested HPLC method was issued by the Food Standards Agency, UK, for determination

**Table 1. Analytical methods for fat-soluble vitamins and carotenoids published in *Official Methods of Analysis of AOAC INTERNATIONAL***

Method No.	Method title	First Action	Final Action	Analytical technique
960.45	Vitamin A in margarine	1960	Surplus 1980	Spectrophotometric
974.29	Vitamin A in mixed feed, premixes, and human and pet foods, (Codex adopted-AOAC method)	1974	Yes	Colorimetric
992.04	Vitamin A (retinol isomers) in milk and milk-based infant formula (Codex adopted-AOAC method)	1992	1995	Liquid chromatographic
992.06	Vitamin A (retinol) in milk-based infant formula (Codex adopted-AOAC method)	1992	No	Liquid chromatographic
2001.13	Determination of vitamin A in foods	2001	No	Liquid chromatographic
936.14	Vitamin D in milk, vitamin preparations, and feed concentrates	1936	Yes	Rat bioassay
932.16	Vitamin D in poultry feed supplements	1932	Yes	Chick bioassay
975.42	Vitamin D in vitamin preparations	1975	1977	Colorimetric
979.24	Vitamin D in vitamin preparations	1979	1980	Liquid chromatographic
980.26	Vitamin D in multivitamin preparations	1980	1980	Liquid chromatographic
981.17	Vitamin D in fortified milk and milk powder	1981	1982	Liquid chromatographic
982.29	Vitamin D in mixed feeds, premixes, and pet foods	1982	1983	Liquid chromatographic
985.27	Vitamin D in vitamin AD concentrates	1985	None	Liquid chromatographic
992.26	Vitamin D3 (cholecalciferol) in ready-to-feed milk-based infant formula (Codex-AOAC method)	1992	1995	Liquid chromatographic
995.05	Vitamin D in infant formula and enteral products	1995	No	Liquid chromatographic
2002.05	Determination of cholecalciferol (Vitamin D3) in selected foods (Joint AOAC-NMKL method)	2002	No	Liquid chromatographic
948.26	-Tocopheryl acetate (supplemental) in foods and feeds	1948	1980	Colorimetric
971.30	-Tocopherol and -tocopheryl acetate in foods and feeds	1971	1972	Colorimetric
975.43	Identification of RRR- or <i>all</i> - <i>rac</i> -alpha-tocopherol in drugs and food or feed supplements	1975	1980	Polarimetric
988.14	Tocopherol isomers in mixed tocopherols concentrate (FCC, USP, AOAC method)	1988	No	Gas chromatographic
989.09	-Tocopheryl acetate in supplemental vitamin E concentrates	1989	No	Gas chromatographic
992.03	Vitamin E activity ( <i>all</i> - <i>rac</i> -tocopherol) in milk-based infant formula	1992	1996	Liquid chromatographic
974.30	Menadione sodium bisulfite (water-soluble vitamin K <sub>3</sub> ) in feed premixes	1974	1975	Gas chromatographic
992.27	<i>trans</i> Vitamin K1 (phyloquinone) in ready-to-feed, milk-based, infant formula	1992	1995	Liquid chromatographic
999.15	Vitamin K in milk and infant formula	1999	No	Liquid chromatographic
938.04	Carotenoids in macaroni products	1938	Yes	Colorimetric
941.15	Carotene in fresh plant materials and silages	1941	Yes	Spectrophotometric
970.64	Carotenes and xanthophylls in dried plant materials and mixed feeds	1970	1974	Spectrophotometric

of vitamin D<sub>3</sub> in pet foods (8). Apart from milk and egg yolk, little recent data exist for content of vitamin D and its metabolites in food products. Clausen et al. (9) measured the contents of vitamin D<sub>3</sub> and the biologically active metabolite 25-hydroxyvitamin D<sub>3</sub> in raw and cooked pork cuts by 2 step-HPLC. The 25-hydroxylated form has a higher biological activity than D<sub>3</sub> (factor of 1.5 or 5 depending on system of calculation).

The British Standards Institution (10) published an official method for determining vitamin K<sub>1</sub> in infant formula, which is similar to AOAC Official Method 999.15. Weizmann et al. (11) reported a study of vitamin K<sub>1</sub> and the dehydrogenation product dihydrophyloquinone (dK) in fast foods and snack foods. This involved, extraction with hexane-isopropanol followed by solid-phase extraction (SPE) on silica columns and reversed-phase HPLC. Dumont

**Table 2. Methods published by CEN for fat-soluble vitamins**

Reference	Title	Year published	Matrixes validated	Analytical technique
EN 12823-1	Foodstuffs—Determination of vitamin A by HPLC—Part 1: Measurements of <i>all-trans</i> -retinol and 13- <i>cis</i> -retinol	2000	Margarine, milk powder	HPLC
EN 12823-2	Foodstuffs—Determination of vitamin A by HPLC—Part 2: Measurement of $\beta$ -carotene	2000	Margarine, vitaminized drink, pudding powder, mixed vegetables	HPLC
EN 12821	Foodstuffs—Determination of vitamin D by HPLC—measurement of cholecalciferol (D <sub>3</sub> ) and ergocalciferol (D <sub>2</sub> )	2000	Margarine, milk powder, oat flakes	HPLC
EN 12822	Foodstuffs—Determination of vitamin E by HPLC—measurement of $\alpha$ -, $\beta$ -, $\gamma$ -, and $\delta$ -tocopherol	2000	Margarine, milk powder, oats powder	HPLC
EN 14148	Foodstuffs—Determination of vitamin K <sub>1</sub> by HPLC	2003	Milk powder, milk, and infant formula	Colorimetric

et al. (12) reported a similar method for mixed dishes and processed foods.

Carotenoids occupy a large part of the FSV literature at the moment. Cooper (13) and Kiokias and Gordon (14) evaluated the contribution of carotenoids in health and disease. These effects are well known and include provitamin A activity, enhancement of the immune system, and reduction of the risk of degenerative diseases, e.g., cancer, cardiovascular disease, and macular degeneration. Many publications focus on developing methods for specific groups of foods which are then used to provide data for nutritional databases.

Cortés et al. (15) reviewed current HPLC techniques for carotenoids analysis. Extraction is still a difficult problem. In some products solvent extraction is sufficient, but in high-fat products saponification is necessary. Saponification is also used to evaluate the presence of carotenoid esters but may lead to losses of xanthophylls. Saponification may also lead to formation of *cis*-isomers. Various compounds can be used as internal standards, including  $\beta$ -apo-8'-carotenal (*trans*), Sudan 1, canthaxanthin, echinenone, nonapreno- $\beta$ -carotene or, *all-trans*-retinol palmitate.

Reversed-phase chromatography using gradient-elution with either C18 or C30 columns is generally applied. Cortés et al. (15) observed that carotenoids may undergo losses and transformation on the HPLC column, and the addition of ammonium carbonate to the organic phase can improve recovery. Diode array detection (DAD) is very useful for identifying carotenoids in fruits and vegetables, taking advantage of the specific spectral data available for many of these pigments, and circumvents the lack of commercially available standards. However, mass spectrometry (MS) is becoming increasingly the detection method of choice for identification.

A few examples of the many recent publications are outlined. An important study was made by Jewell et al. (16) for determination of lutein and zeaxanthin contents in infant formula and human milk. The method involved saponification and extraction of the FSV into hexane prior to HPLC analysis. Lin and Chen (17) determined the various *cis-trans*-forms of  $\beta$ -carotene, lutein, and lycopene in tomato juice. This involved extraction with ethanol–hexane (4 + 3, v/v) and separation on a C30 HPLC column using a gradient mobile

**Table 3. Methods published by ISO for fat-soluble vitamins**

Reference	Title	Year published	Matrixes validated	Analytical technique
ISO 14565	Animal feeding stuffs—determination of vitamin A content	2000	Animal feeding stuffs and pet food	HPLC
ISO 6867	Animal feeding stuffs—determination of vitamin E content	2000	Animal feeding stuffs and pet food	HPLC
ISO 14892	Dried skimmed milk—determination of vitamin D content	2002	Dried skimmed milk	HPLC
ISO 12080-2	Dried skimmed milk—determination of vitamin A content: Part 2	2000	Dried skimmed milk	HPLC
ISO 12080-1	Dried skimmed milk—determination of vitamin A content—Part 1	2000	Dried skimmed milk	HPLC

phase of acetonitrile–butanol (7 + 3, v/v) and methylene chloride with detection at 476 nm. Humphries and Khachik (18) determined lutein, zeaxanthin, and related geometrical isomers in fruits, vegetables, wheat, and pasta products. Surles et al. (19) compared carotenoid profiles ( $\alpha$ -carotene, lutein, and lycopene) in different colored carrots (purple to white);  $\alpha$ -apo-8-carotenyl deconate was used as an internal standard. Carotenoids were extracted with alternative washes of dichloromethane and acetone with determination by gradient elution HPLC with DAD. Burns et al. (20) used HPLC–DAD to study carotenoids and tocopherols in fruits and vegetables. de Sá and Rodriguez-Amaya (21) carefully evaluated the analysis of carotenoids after stir-frying of several vegetables. Saponification of acetone extracts was necessary to remove chlorophyll degradation products that interfered with the HPLC analysis. Great care was necessary at the saponification/washing step to avoid significant losses of lutein.

Carotenoids are often added to foods as food colors, as well as for their role in health. Multi-methods for the analysis of this large range of compounds are rather scarce. Several methods are listed in the German official collection of methods according to paragraph 35, *LMBG* (2002) as well as by the AOAC *Official Methods of Analysis*. However, a multi-method was reported by Breithaupt (22) for the determination of the following carotenoid food additives: norbixin, bixin, capsanthin,  $\alpha$ -apo-8-carotenal,  $\alpha$ -apo-8-carotenoic acid ethyl ester,  $\alpha$ -carotene, and lycopene in processed foods. Accelerated solvent extraction (ASE) was compared with a manual extraction using a solvent mixture methanol–ethyl acetate–light petroleum (1 + 1 + 1). Echinonene was added as an internal standard. Recoveries for all analytes were 91–97% for ASE, with the exception of norbixin (67%) and between 89–103% for the manual procedure. Separations were obtained using RP  $C_{30}$  column with DAD, and for unequivocal identification, the mass spectra of all analytes were recorded using LC-atmospheric pressure chemical ionization (APCI)-MS. For  $\alpha$ -carotene, the *cis* and *trans* isomers were summed and reported as *all-trans*  $\alpha$ -carotene. Scotter et al. (23) described a procedure for determining *trans*- $\alpha$ -carotene and *trans*- $\alpha$ -apo-8-carotenal in a wide range of food products using HPLC–DAD. The *cis*-forms were only measured semiquantitatively because standards were not available. Solvent extraction was made with ethyl acetate, acetone, and ethanol (2 + 1 + 1). For high-fat products, lipase was added to hydrolyze lipids before extraction.

LC/MS is playing an increasingly important role in carotenoids research. Glaser et al. (24) performed qualitative

and quantitative determinations of carotenoid stereoisomers in a variety of spinach samples by use of matrix solid-phase dispersion (MSPD) before HPLC–UV, HPLC–APCI–MS, and HPLC–NMR on-line coupling. Pott et al. (25) investigated the carotenoids profile in mango using LC/MS with a  $C_{30}$  separation. Kurilich et al. (26) developed an LC–APCI–MS procedure for quantifying some  $^{13}C$ -labeled carotenoids and vitamin  $K_1$  in kale (*Brassica oleracea*).

Favaro et al. (27) investigated the geometrical retinol isomers and carotene in enteral feeding formulas. By using a normal-phase HPLC column and a mobile phase consisting of 1-octanol in *n*-hexane, 6 retinol isomers could be identified. The (*Z*) isomers have lower vitamin A activity.

Bhosdale et al. (28) evaluated Raman resonance spectroscopy for rapid nondestructive estimation of carotenoid levels in intact fruits and vegetables and their juices. A high correlation was found between total carotenoids measured by Raman spectroscopy and HPLC for several types of ripe tomatoes. Individual calibrations need to be established for different types of fruits and vegetables. However, various types of diluted juices (carrot, tomato, orange, grape, and vegetable mix) could be included in a single calibration.

## References

- (1) Codex Alimentarius Commission (2004) ALINORM 03/27/26 <http://www.codexalimentarius.net>
- (2) United Kingdom Food Standards Agency (2003) Expert Group on Vitamins and Minerals, <http://www.foodstandards.gov.uk/multimedia/pdfs/vitmin2003.pdf>
- (3) Rodas-Mendoza, B., Morera-Pons, S., Castellote-Bargallo, A.I., & Lopez-Sabater, M.C. (2003) *J. Chromatogr. A* **1018**, 197–202
- (4) Hewavitharana, A.K., Lanari, M.C., & Becu, A. (2003) *J. Chromatogr. A* **1025**, 313–317
- (5) Panfili, G., Fratianni, A., & Irano, M. (2003) *J. Agric. Food Chem.* **51**, 3940–3944
- (6) Perretti, G., Marconi, O., Montanari, L., & Fantozzi, P. (2004) *Lebensm. Wiss. Technol.* **37**, 87–92
- (7) Iwase, H. (2003) *J. Chromatogr. A* **1008**, 81–87
- (8) Food Standards Agency (2003) Laboratory of the Government Chemist, London, UK
- (9) Clausen, I., Jakobsen, J., Leth, T., & Ovesen, L. (2003) *J. Food Compos. Anal.* **16**, 575–585
- (10) BS EN 14148 (2003) British Standards Institution, London, UK
- (11) Weizmann, N., Petersen, J.W., Haytowitz, D., Pehrsson, P.R., de Jesus, V.P., & Booth S.L. (2004) *J. Food Compos. Anal.* **17**, 379–384
- (12) Dumont, J.F., Peterson, J., Haytowitz, D., & Booth, S.L. (2003) *J. Food Compos. Anal.* **16**, 595–603
- (13) Cooper, D.A. (2004) *J. Nutr.* **134**, 221S–224S
- (14) Kiokias, S., & Gordon, M.H. (2004) *Food Rev. Int.* **20**, 99–121
- (15) Cortés, C., Esteve, M.J., Frigola, A., & Torrefrosa, F. (2004) *Food Chem.* **52**, 2203–2212

- (16) Jewell, V.C., Mayes, C.B.D., Tubman, T.R.J., Northrop-Clewes, C.A., & Thurnham, D.I. (2004) *Eur. J. Clin. Nutr.* **58**, 90–97
- (17) Lin, C.H., & Chen, B.H. (2003) *J. Chromatogr. A* **1012**, 103–109
- (18) Humphries, J.M., & Khachik, F. (2003) *J. Agric. Food Chem.* **51**, 1322–1327
- (19) Surles, R.L., Weng, N., Simon, P.W., & Tanumihardjo, S.A. (2004) *J. Agric. Food Chem.* **52**, 3417–3421
- (20) Burns, J., Fraser, P.D., & Bramley, P.M. (2003) *Phytochemistry* **62**, 939–947
- (21) de Sá, M.C., & Rodriguez-Amaya, D.B. (2004) *J. Food Compos. Anal.* **17**, 37–51
- (22) Breithaupt, D.E. (2004) *Food. Chem.* **86**, 449–456
- (23) Scotter, M.J., Castle, L., Croucher, J.M., & Olivier, L. (2003) *Food Addit. Contam.* **20**, 115–126
- (24) Glaser, T., Lienau, A., Zeeb, D., Krucker, M., Dachtler, M., & Albert, K. (2003) *Chromatographia* **57** (Suppl.), S19–S25
- (25) Pott, I., Marx, M., Neidhart, S., Muhlbauer, W., & Carle, R. (2003) *J. Agric. Food. Chem.* **51**, 4527–4531
- (26) Kurilich, A.C., Britz, S.J., Clevidence, B.A., & Novotny, J.A. (2003) *J. Agric. Food Chem.* **51**, 4877–4883
- (27) Favaro, R.M.D., Iha, M.H., & de-Lourdes-Pires-Bianchi, M. (2003) *J. Chromatogr. A* **1021**, 125–132
- (28) Bhosdale, P., Ermakov, I., Ermakova, M.R., Gellermann, W., & Bernstein, P.S. (2004) *J. Agric. Food Chem.* **52**, 3281–3285

### Selected Study Director Topics

Vitamins A and E in Foods by HPLC **2001.13**: Collaborative study for vitamin E should be re-run when possible using an international collaborative study protocol. Study Director, Jonathan W. DeVries and Karlene Silvera.

### Water-Soluble Vitamins

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### Summary

In earlier General Referee reports, it was often mentioned that progress in establishing new vitamin methods for foods is rather slow. To identify gaps in the water-soluble vitamin area, AOAC Official Methods of Analyses are summarized in Table 1.

From Table 1, it can be concluded that the analytical techniques used for the determination of water-soluble vitamins are mostly outdated. HPLC is used in few of the methods. However, a method for the LC analysis of vitamin B<sub>6</sub> in infant formula was collaboratively tested by AOAC and has just recently achieved First Action status. Results of a collaborative study for the LC determination of vitamin C in fruit juices and related products were not satisfactory for AOAC First Action.

For enforcement reasons, most of the important matrixes, like infant formula, vitamin preparations, and enriched foods, seem to be reasonably covered. However, it can be questioned if, for instance, compositions of vitamin preparations currently available on the market can be correctly analyzed by methods described above. For instance, Kall et al. (2000) described the degradation of folic acid during extraction of multivitamin-mineral preparations possibly caused by the presence of copper ions. It can be imagined that at the time Method **944.12** was finalized the combination of folic acid and copper was neither tested nor available on the market.

Not only for enforcement reasons but also for nutritional studies like dietary intake studies or epidemiological studies which look into associations between intake of vitamins and development of diseases, it is essential to have up-to-date and correct analytical data.

It is therefore essential that available Official Methods for the determination of water-soluble vitamins shall be updated to the currently common analytical techniques and available matrixes on the market.

Until this is accomplished, it might be useful to look into other standardized methods for the determination of water-soluble vitamins, like methods of the European Committee for Standardization (CEN). Table 2 list methods published by CEN in the area of water-soluble vitamins.

The CEN working group on vitamins is currently working on new standards for the determination of niacine and niacinamide, a method for biotin, and a method for the individual vitamin B<sub>6</sub> vitamers (pyridoxine, pyridoxal, and pyridoxamine). All methods are using HPLC as the analytical technique.

Several members of the CEN working group are currently working (independently) on methods for the single-run determination of several water-soluble vitamins in pre-mixes/vitamin preparations (tablets, capsules, etc.). It is to be expected that this work will lead to collaborative tested European Standards.

The currently published CEN methods are collaboratively tested according to ISO 5725.

CEN methods gained a more official status within the European Food Law. According to regulation 882/2004 of the European Parliament and of the Council of April 29, 2004, official controls need to be performed to ensure the verification of compliance with feed and food law, animal health, and animal welfare rules. These rules are applicable from January 1, 2006. Article 11 states that sampling and analysis methods used in the context of official controls shall comply with relevant Community rules, or, if none exist, internationally recognized rules or protocols, for example, those that the CEN has accepted or those agreed in national legislation, or, if none exist, other methods fit for the intended purpose or developed in accordance with scientific protocols.

**Table 1. Analytical methods for water-soluble vitamins published in *Official Methods of Analysis of AOAC INTERNATIONAL*, 17th Edition**

Method No.	Method title	First Action	Final Action	Analytical technique
938.12	Thiamin Hydrochloride (Vitamin B1) in Vitamin Preparations	1938	1938, Surplus 1965	Growth bioassay
942.23	Thiamin (Vitamin B1) in Human and Pet Foods	1942	1942	Fluorometric
953.17	Thiamin (Vitamin B1) in Grain products	1953	1953	Fluorometric
957.17	Thiamin (Vitamin B1) in Bread	1957	1960	Fluorometric
986.27	Thiamin (Vitamin B1) in Milk-Based Infant formula	1986	1988	Fluorometric
940.33	Riboflavin (Vitamin B2) in Vitamin Preparations	1940	1960	Microbiological
970.65	Riboflavin (Vitamin B2) in Foods and Vitamin Preparations	1970	1971	Fluorometric
981.15	Riboflavin in Foods and Vitamin Preparations	1981	1982	Automated
985.31	Riboflavin in Ready-to-Feed Milk-Based Infant Formula	1985	1988	Fluorometric
944.13	Niacin and Niacinamide (Nicotinic Acid and Nicotinamide) in Vitamin Preparations	1944	1960	Microbiological
961.14	Niacin and Niacinamide in Drugs, Foods, and Feeds	1961	1962	Colorimetric
968.32	Niacinimide in Multivitamin Preparations	1968	1969	Spectrophotometric
975.41	Niacin and Niacinamide in Cereal Products	1975	1976	Automated
981.16	Niacin and Niacinamide in Foods, Drugs, and Feeds	1981	1982	
985.34	Niacin and Niacinamide (Nicotinic Acid and Nicotinamide) in Ready-to- Feed Milk-Based Infant Formula	1985	1988	Microbiol-turbidimetric
945.73	Calcium Pantothenate in Vitamin Preparations	1945	1969 Surplus 1974	Spectrophotometric
945.74	Pantothenic acid in Vitamin Preparations	1945	1960	Microbiological
992.07	Pantothenic acid in Milk-Based Infant Formula	1992	1995	Microbiological turbidimetric
961.15	Vitamin B6 (Pyridoxine, Pyridoxal, Pyridoxamone) in Food Extracts	1961	1975	Microbiological
985.32	Vitamin B6 (Pyridoxine, Pyridoxal, Pyridoxamine) in Ready-to-Feed Milk-Based Infant Formula	1985	1988	Microbiological
952.20	Cobalamin (Vitamin B12 activity) in Vitamin Preparations	1952	1960	Microbiological
986.23	Cobalamin (Vitamin B12 activity) in Milk-Based Infant Formula	1986	1988	Turbidimetric
944.12	Folic Acid (Pteroylglutamic Acid) in Vitamin Preparations	1944	1960	Microbiological
992.05	Folic Acid in Infant Formula	1992	1995	Microbiological
967.21	Ascorbic acid in Vitamin Preparations	1967	1968	Titrimetric
967.22	Vitamin C (total) in Vitamin Preparations	1967	1968	Microfluorometric
984.26	Vitamin C (Total) in Food	1984	1968	Semiautomated fluorometric
985.33	Vitamin C (Reduced Ascorbic Acid) in Ready-to-Feed Milk-Based Infant Formula	1985	1988	Titrimetric

**Table 2. Published methods by CEN in the water-soluble vitamin area**

Method No.	Title	Year published	Matrixes validated	Analytical technique used
EN 14122	Determination of Vitamin B1	2003	Whole meal flour, milk powder, mixed vegetables, pig's liver <sup>a</sup>	HPLC
EN 14152	Determination of Vitamin B2	2003	Milk powder, pig's liver <sup>a</sup>	HPLC
EN 14166	Determination of Vitamin B6	2001	Whole meal flour, milk powder, mixed vegetables, pig's liver <sup>a</sup>	Microbiological
EN 14164	Determination of Vitamin B6	2002	Baby food, biscuit, cereal, yeast, tube-feeding solution, chocolate powder, cereal, powdered milk	HPLC
EN 14131	Determination of Folic Acid	2003	Whole meal flour, milk powder, mixed vegetables, pig's liver <sup>a</sup>	Microbiological
EN 14139	Determination of Vitamin C	2003	Orange juice, liquid soup, powdered milk, freeze-dried soup, breakfast cereal, baby food	HPLC

<sup>a</sup> Matrixes are available as Certified Reference Material at IRMM in Geel, Belgium.