

Overview of Testing Methods for Formula Products (Part I)

Government Laboratory, Hong Kong



Topics covered

- Fatty acids
- Oil-soluble vitamins
- Water-soluble vitamins
- Other nutrients: choline, myo-inositol, L-carnitine, taurine



References

- CODEX STAN 234-1999 (amendment 2013)
- Method guidance notes on nutritional composition and nutrition labelling of infant formula, follow-up formula and prepackaged food for infants and young children, Centre for Food Safety, Food and Environmental Hygiene Department (Draft)



Test Methods – fatty acids

- Codex suggested test methods
 - ✓ AOAC 996.06 (GC-FID)
 - ✓ AOCS Ce 1h-05 (09) (GC-FID)
- Other standard test methods
 - ✓ AOAC 2012.13 (GC-FID)



Test Methods – fatty acids

- ✓ AOAC 996.06 (GC-FID)
- Homogenized sample digested with HCl and/or NH_4OH depending on food matrices.
- Digested solution extracted with diethyl ether and petroleum ether.
- Ether extract evaporated, derivatized with BF_3 reagent.
- Sample further extracted with H_2O /hexane mixture. Hexane layer for GC-FID analysis.



CAP132W Part IV Other Requirements

- linoleic acid : α -linolenic acid $\geq 5:1$ and $\leq 15:1$
- lauric acid + myristic acid $\leq 20\%$ of total fatty acids
- trans fatty acids $\leq 3\%$ of total fatty acids
- erucic acid $\leq 1\%$ of total fatty acids
- If docosahexaenic acid (DHA) added
 - arachidonic acid \geq DHA
 - eicosapentaenoic acid \leq DHA



CAP132W Part IV Other Requirements

- Minimum α -tocopherol equivalent (α -TE)/g polyunsaturated fatty acids (PUFA) required based on number of fatty acid double bonds:
 - ✓ 0.5 mg α -TE/g linoleic acid (18:2)
 - ✓ 0.75 mg α -TE/g α -linolenic acid (18:3)
 - ✓ 1.0 mg α -TE/g arachidonic acid (20:4)
 - ✓ 1.25 mg α -TE/g eicosapentaenoic acid (EPA) (20:5)
 - ✓ 1.5 mg α -TE/g docosahexaenoic acid (DHA) (22:6)
- Vitamin E (α -tocopherol) prevents peroxidation of PUFA. More double bonds require larger amount of vitamin E.



CAP132W Part IV Other Requirements

- Considering the five polyunsaturated fatty acids (PUFA) alone:

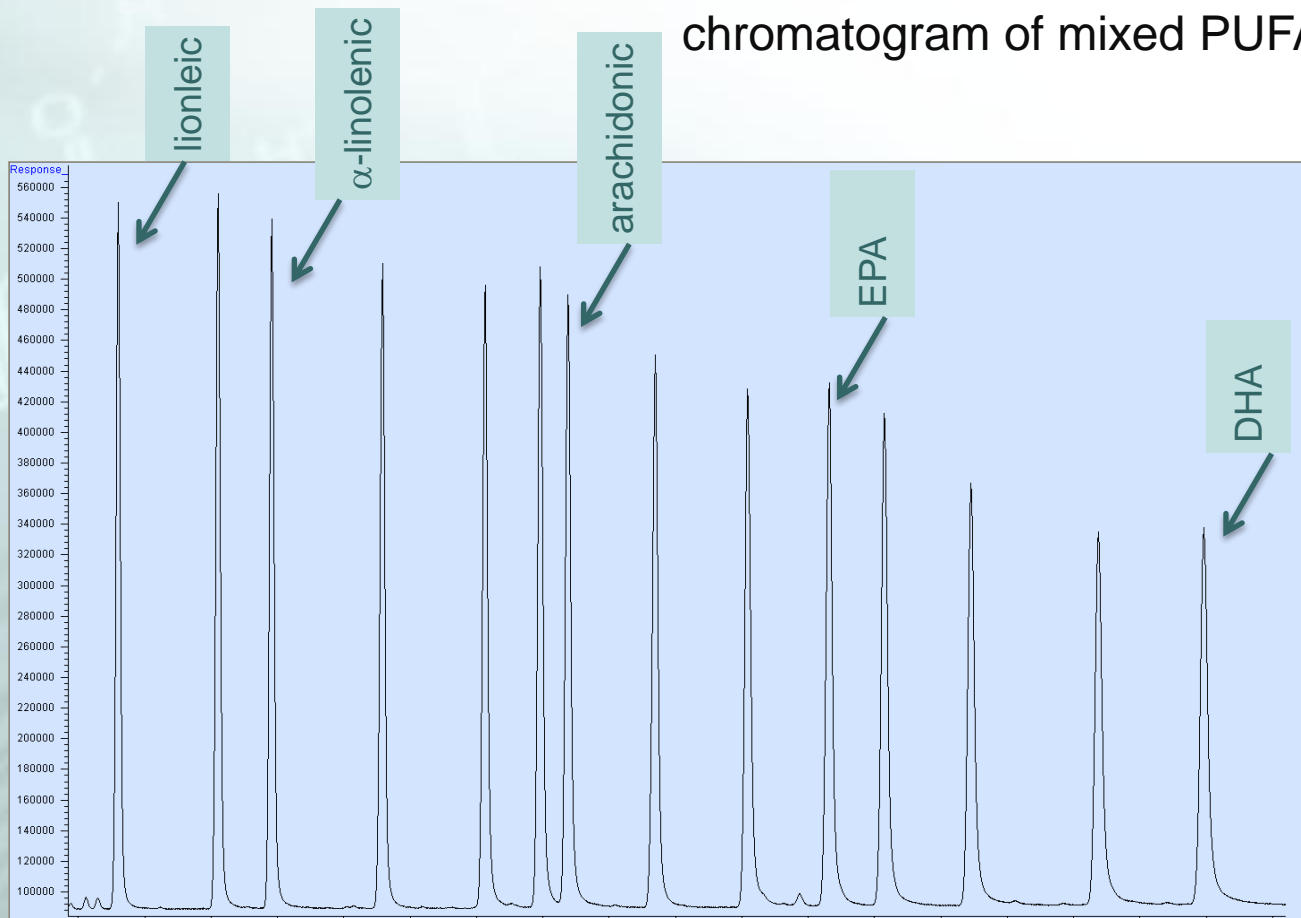
Minimum α -TE/g PUFA (mg/g PUFA) required:

$$\frac{0.5 \times [\textit{linoleic}] + 0.75 \times [\alpha - \textit{linolenic}] + 1.0 \times [\textit{arachidonic}] + 1.25 \times [\textit{EPA}] + 1.5 \times [\textit{DHA}]}{[\textit{linoleic}] + [\alpha - \textit{linolenic}] + [\textit{arachidonic}] + [\textit{EPA}] + [\textit{DHA}]}$$



CAP132W Part IV Other Requirements

chromatogram of mixed PUFA standards

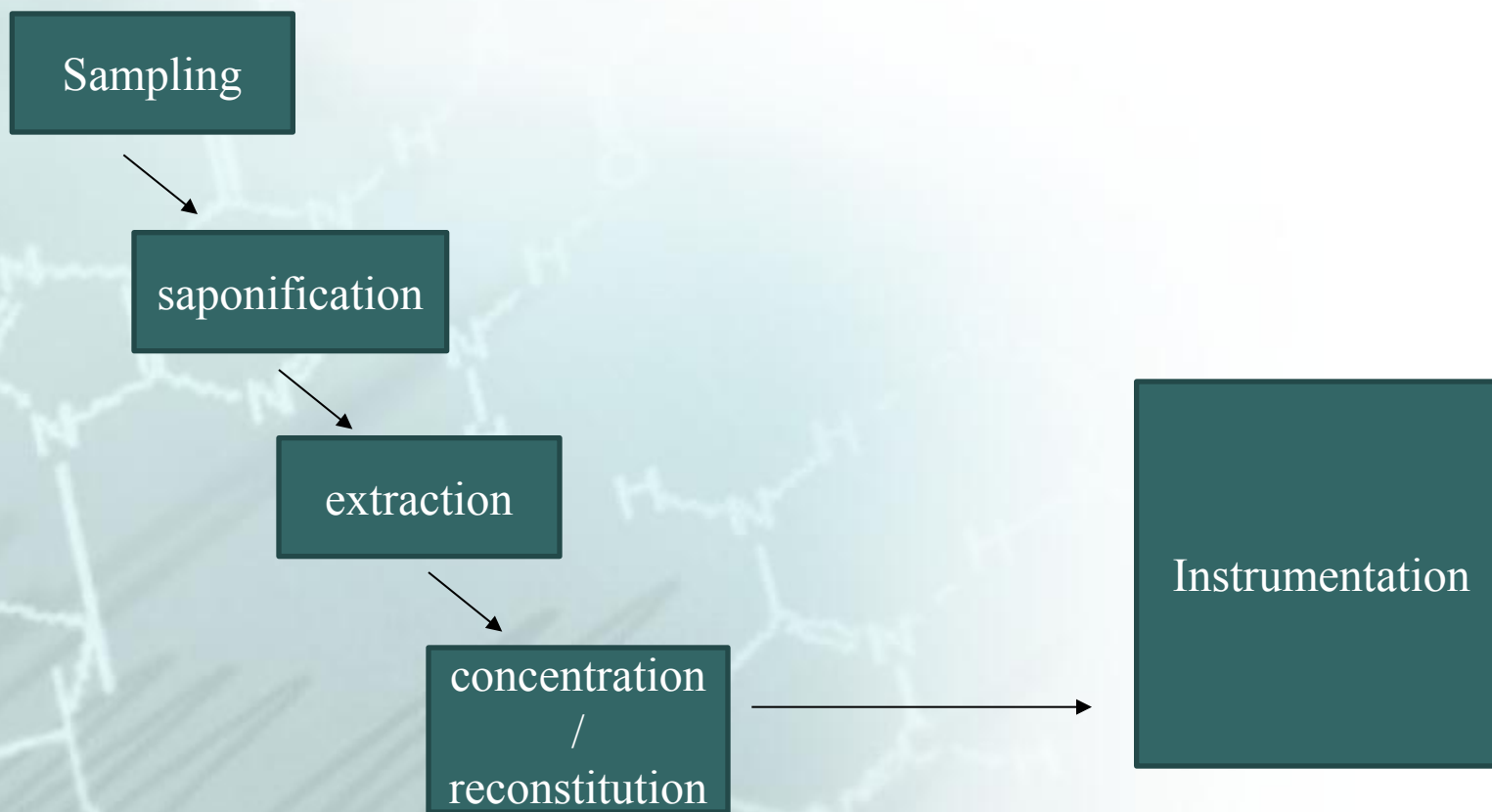


Oil-soluble Vitamins

- Vitamin A (retinol)
- Vitamin D (cholecalciferol and ergocalciferol)
- Vitamin E (α -tocopherol)
- Vitamin K (phylloquinone)



Oil-soluble Vitamins – General methodology



Oil-soluble vitamins – General methodology

- Saponification (alkaline hydrolysis)
 - ✓ Oil-soluble vitamins are naturally embedded in food lipids → separation from fat required
 - ✓ Saponification affects ester linkages, releasing fatty acids from glycerides and phospholipids, and also vitamins from any combined forms.
 - ✓ Charged fatty acids and glycerides become soluble in polar media



Oil-soluble vitamins – General methodology

■ Extraction

- ✓ Removes fatty acids and triglycerides.
- ✓ Unsaponifiable fatty components, including fat-soluble vitamins remain in organic layer.

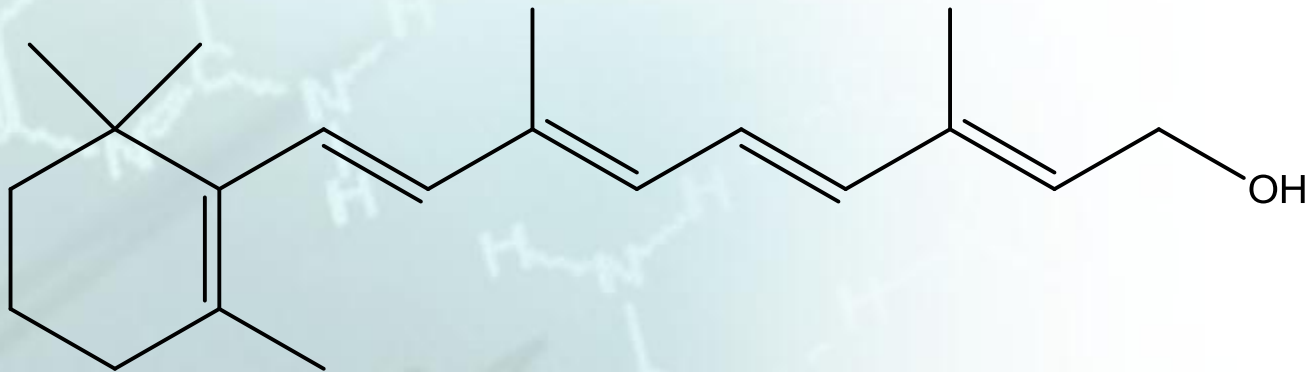
■ Instrumentation

- ✓ HPLC-UV(FLD)
- ✓ UPLC-MS/MS



Vitamin A (Retinol)

- Vitamin A (Retinol)



Test Methods – Vitamin A (Retinol)

- Codex suggested test methods

- ✓ AOAC 992.04 (HPLC-UV)
- ✓ AOAC 992.06 (HPLC-UV)
- ✓ EN 12823-1:2014 (HPLC-UV/FLD)

- Other standard test methods

- ✓ AOAC 2011.07 (UPLC-MS/MS)
- ✓ AOAC 2011.15 (HPLC-UV)
- ✓ AOAC 2012.09 (HPLC-UV)
- ✓ AOAC 2012.10 (HPLC-UV)



Test Methods – Vitamin A (Retinol)

- ✓ AOAC 992.04 (HPLC-UV)
- Sample dissolved in water, added with pyrogallol (antioxidant) and ethanolic KOH. Solution stirred for 18h in dark.
- Digested solution extracted with hexane:diethyl ether (85:15), centrifuged. Organic layer evaporated and reconstituted with heptane for HPLC-UV analysis.



Test Methods – Vitamin A (Retinol)

- ✓ AOAC 992.06
- Sample dissolved in water, added with pyrogallol (antioxidant) and KOH. Solution digested in 70 °C shaking water bath for 25 min.
- Digested solution extracted with hexane-methylene chloride (3:1). Residues evaporated and reconstituted with mobile phase (Hexane: isopropyl alcohol = 100: 0.25 v/v) for HPLC analysis.



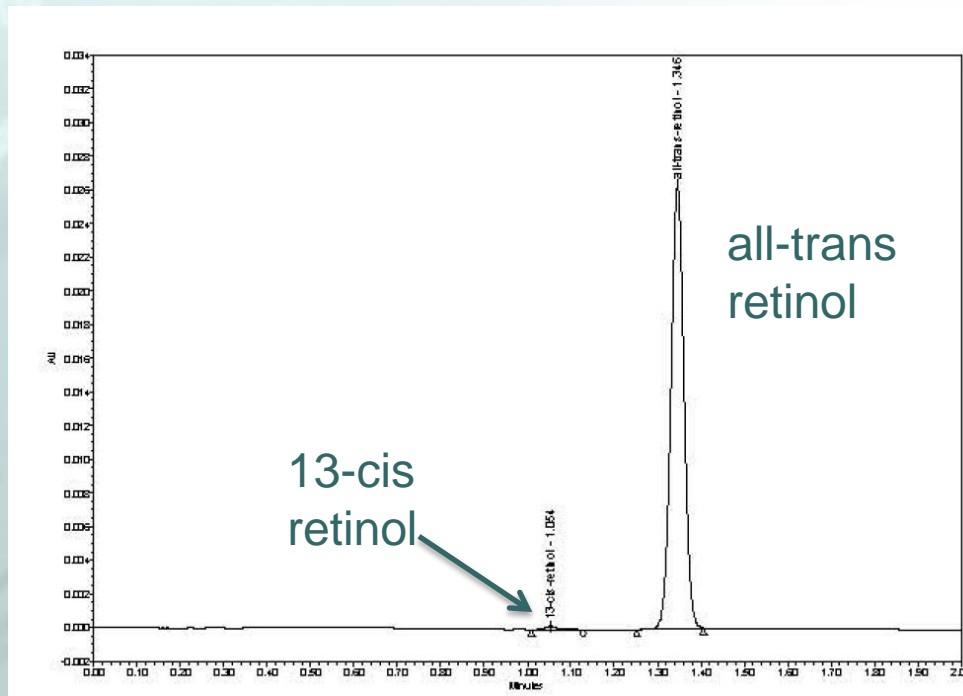
Definition – Vitamin A

Infant and follow-up formula	Prepackaged food for Infant and young children
All-trans retinol	Sum of retinol and beta-carotene



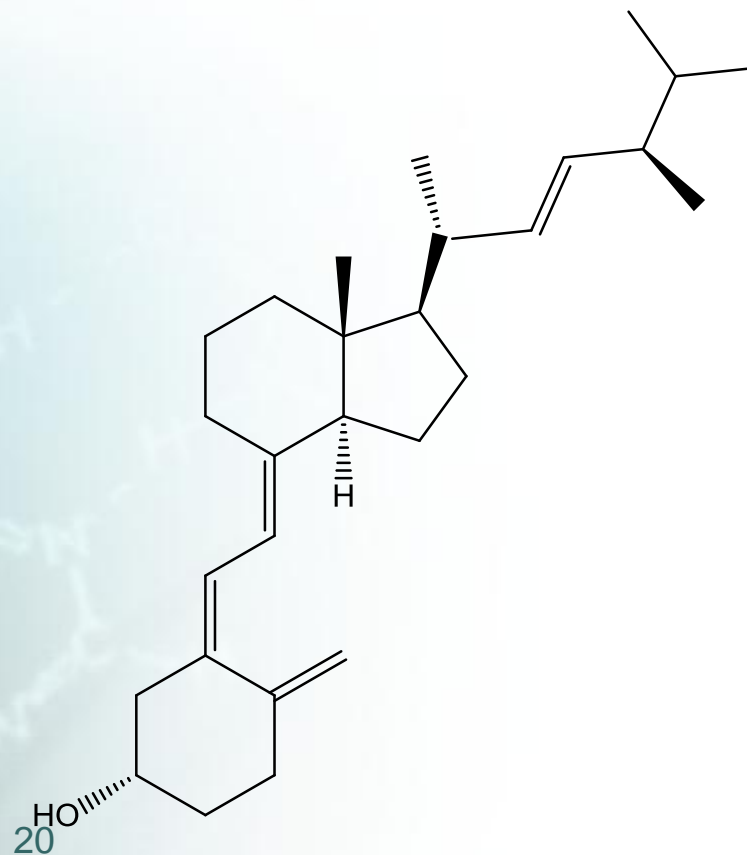
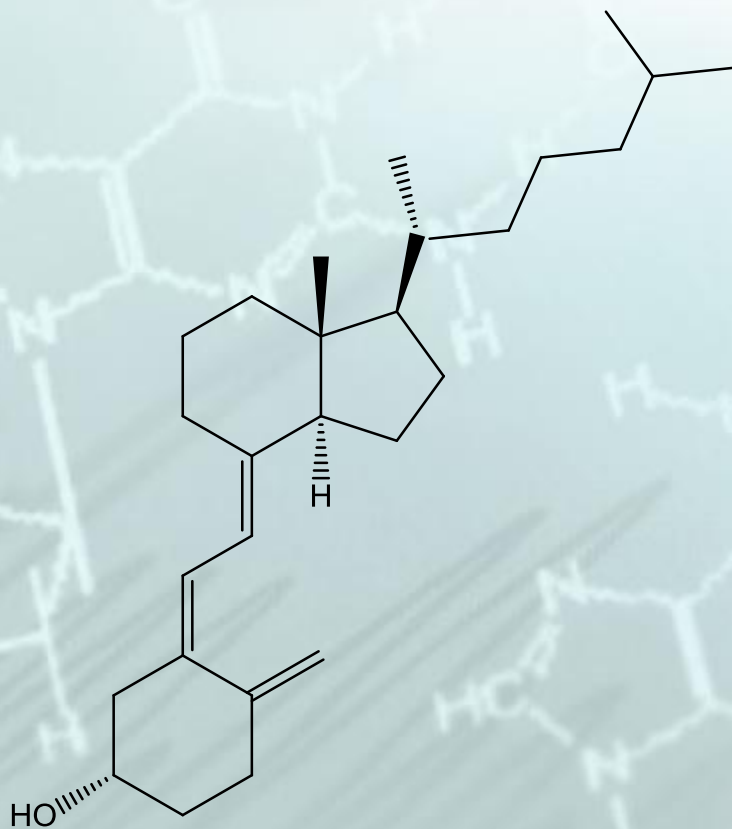
Definition – Vitamin A

- Definition for IF and FF includes *all-trans retinol* BUT excludes *cis retinol*



Vitamin D

- Vitamin D₃ (Cholecalciferol) & Vitamin D₂ (Ergocalciferol)



Test Methods – Vitamin D

- Codex suggested test methods
 - ✓ EN 12821:2009 (HPLC-UV)
 - ✓ NMKL 167:2000 (HPLC-UV)
 - ✓ AOAC 992.26 (HPLC-UV)
 - ✓ AOAC 995.05 (HPLC-UV)
- Other standard test methods
 - ✓ AOAC 2011.11 (UPLC-MS/MS)
 - ✓ AOAC 2011.12 (UPLC-MS/MS)
 - ✓ AOAC 2011.13 (UPLC-MS/MS)
 - ✓ AOAC 2012.11 (UPLC-MS/MS)



Test Methods – Vitamin D

- ✓ AOAC 995.05 (HPLC-UV)
- Test portion added with ethanolic KOH and shaken for 30 min at 60 °C
- Digested solution extracted with hexane which was drained through Na_2SO_4 . Hexane residues evaporated and redissolved in dichloromethane-IPA solution.
- Residues passed through SPE column, evaporated and reconstituted with acetonitrile for HPLC-UV analysis.



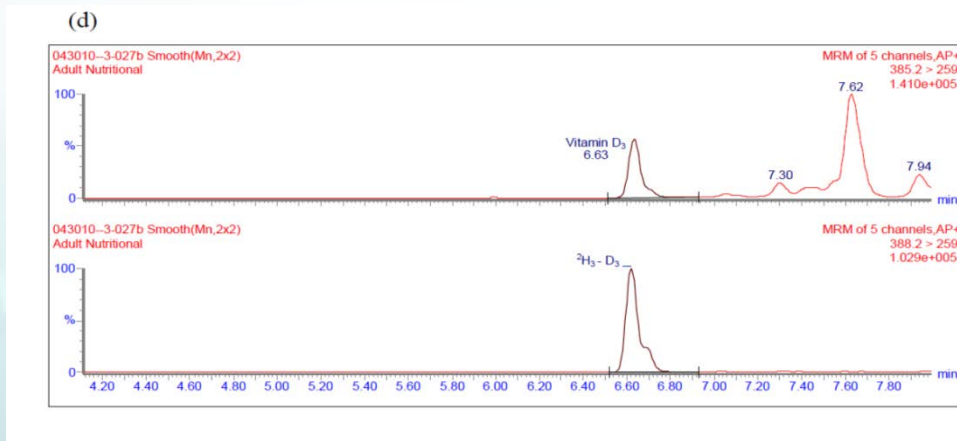
Test Methods – Vitamin D

- ✓ AOAC 2011.13 (UPLC-MS/MS)
- Test portion added with vitamin D₂ and D₃ isotope-labelled internal standards, sodium ascorbate, methanol, KOH and shaken for 30 min at 75±2 °C.
- Digested solution added with acetonitrile, ether-pentane (20:80), centrifuged, ether-pentane layer separated, evaporated, and reconstituted with methanol.
- UPLC-MS/MS analysis.

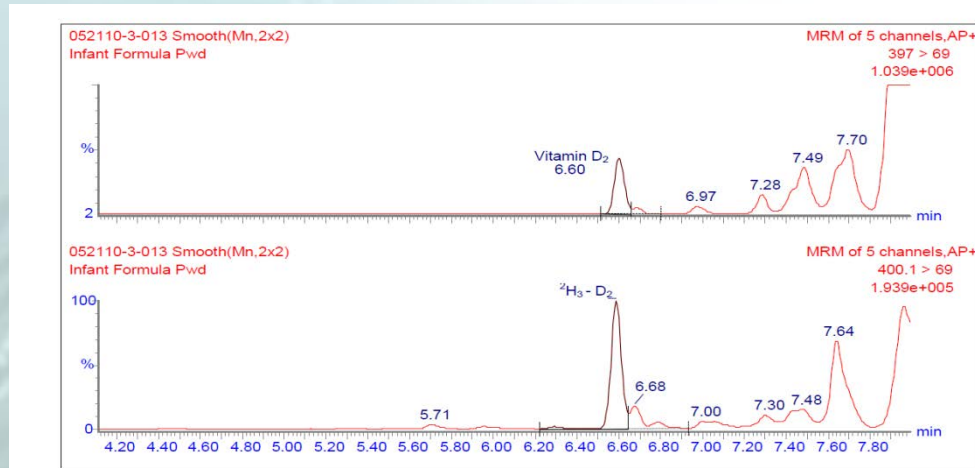


Test Methods – Typical Vitamin D chromatograms

Vitamin D₃

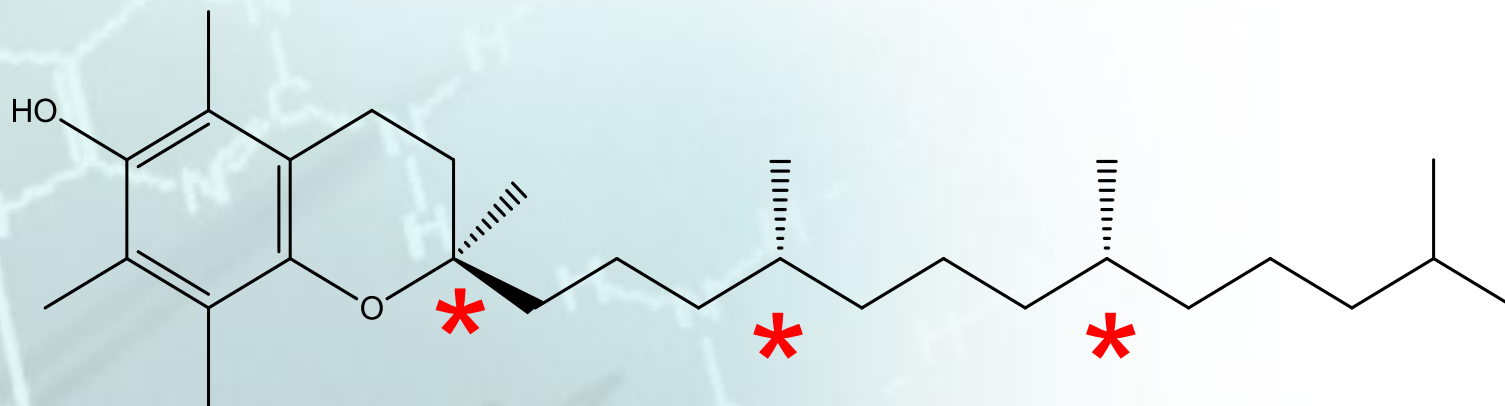


Vitamin D₂



Vitamin E (α -Tocopherol)

- Vitamin E (α -Tocopherol)



* Chiral Centre



Test Methods – α -Tocopherol

- Codex suggested test methods
 - ✓ EN 12822: 2014 (HPLC-UV/FLD)
 - ✓ AOAC 992.03 (HPLC-UV)
- Other standard test methods
 - ✓ AOAC 2012.09 (HPLC-UV)
 - ✓ AOAC 2012.10 (HPLC-UV)



Test Methods – α -Tocopherol

- ✓ AOAC 992.03 (HPLC-UV)
- Sample dissolved in water, added with pyrogallol (antioxidant) and KOH. Solution digested in 70 °C shaking water bath for 25 min.
- Digested solution extracted with hexane-methylene chloride (3:1). Residues evaporated and reconstituted with mobile phase (Hexane: isopropyl alcohol = 99.92: 0.08 v/v) for HPLC analysis.



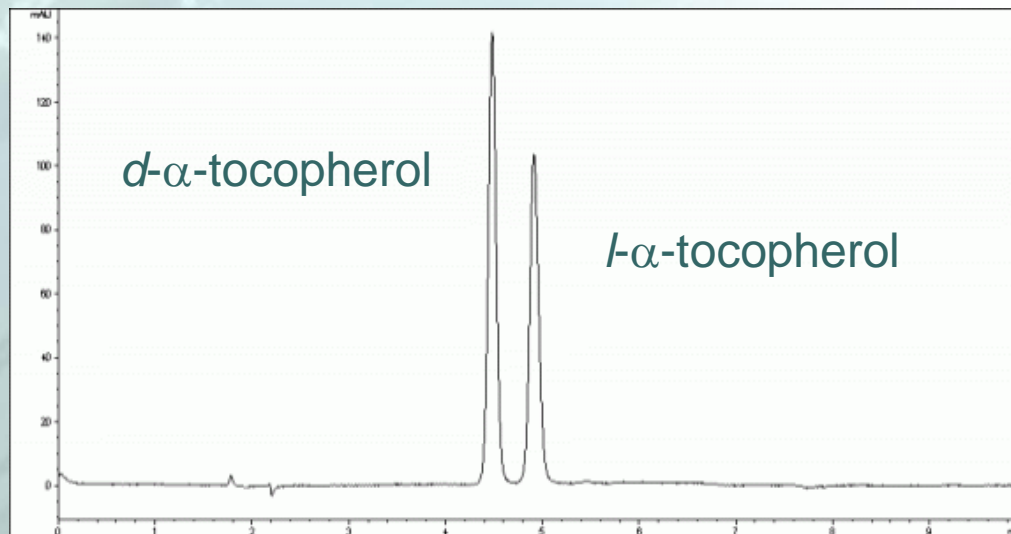
Test Methods – α -Tocopherol

- ✓ EN 12822: 2014 (HPLC-UV/FLD)
- Sample saponified by refluxing with ethanol/methanol, water, antioxidant (e.g. pyrogallol) and KOH for 15-40 min at 80-100 °C.
- Digested solution extracted with suitable solvent (e.g. n-hexane) 4-5 times. Residues evaporated and reconstituted with mobile phase (3% 1,4-dioxane in n-hexane)
- HPLC-FLD (ex: 295nm, em: 330 nm)



α -Tocopherol – chirality

- All standard methods do **NOT** consider separation of enantiomers of α -Tocopherol (i.e. *d* & *l* forms)
- Definition in CAP132W **REQUIRES** enantiomeric separation.
- Use of chiral column: e.g. Chiralcel OD-H column



Definition - Vitamin E

Infant Formula	Follow-up Formula
<p><i>d</i>-alpha-tocopherol, calculated in terms of alpha-Tocopherol Equivalent (α-TE) or International Unit (IU) with 1IU as being equivalent to 0.67 mg</p>	<p>alpha-tocopherol compounds, calculated in α-TE or IU, (i) (for alpha-tocopherol compounds from any natural source) with 1 IU as being equivalent 0.67 mg α-TE; or (ii) (for alpha-tocopherol compounds from synthetic source) with 1 IU as being equivalent to 0.45 mg α-TE</p>

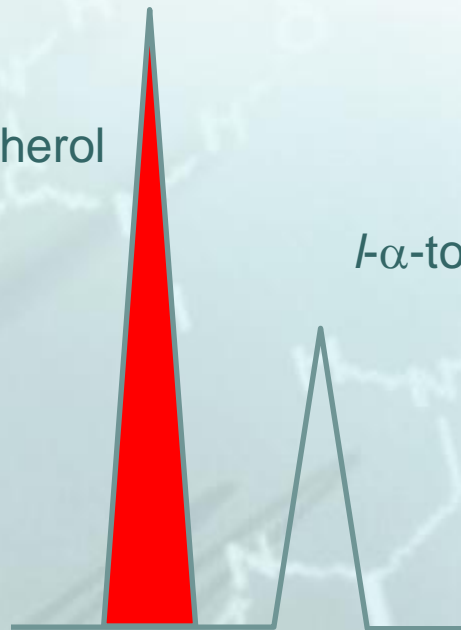



α -Tocopherol – chirality and determination in Infant Formula

- *d*- α -tocopherol in IF

d- α -tocopherol

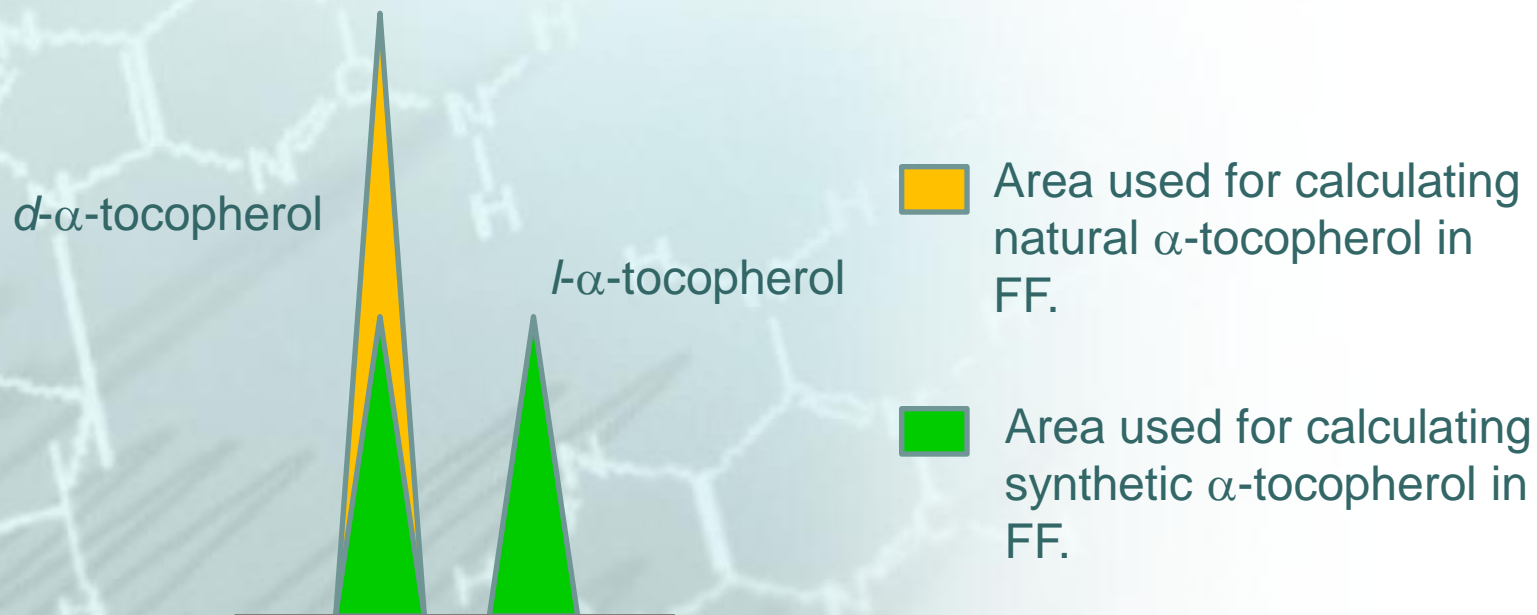
l- α -tocopherol



 Area used for calculating *d*- α -tocopherol in Infant Formula.

α -Tocopherol – chirality and determination in Follow-up Formula

- Synthetic source: assume racemic mixture of *d* and *l* forms (i.e. 1:1); area of *l*- α -tocopherol $\times 2$
- Natural source: area of *d*- α -tocopherol minus *l*- α -tocopherol



Test Methods – Vitamin K₁ (Phylloquinone)

- Codex suggested test methods
 - ✓ AOAC 999.15 (HPLC-FLD)
 - ✓ EN 14148:2003 (HPLC-FLD)
- Other standard test methods
 - ✓ AOAC 992.27 (HPLC-UV)



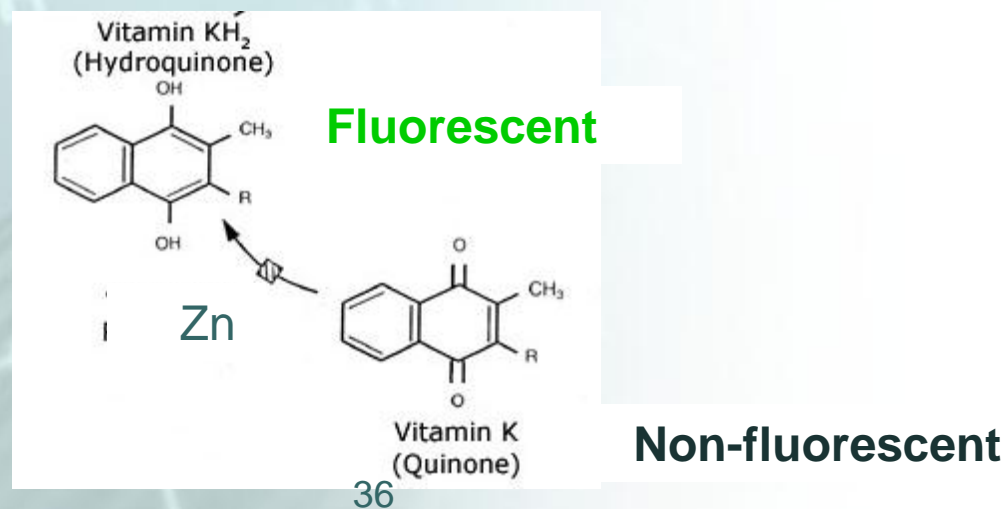
Test Methods – Vitamin K₁ (Phylloquinone)

- ✓ AOAC 999.15 and EN 14148:2003 (HPLC-FLD)
- Sample dissolved in warm water, added with phosphate buffer, lipase powder, mixed, and incubated at 37 ± 2 °C for 2h.
- Digested solution extracted with hexane. Residues evaporated and reconstituted with methanol.
- HPLC determination: post-column Zn reduction; fluorescence detection (ex: 243nm; em: 430nm)



Test Methods – Vitamin K₁ (Phylloquinone)

- Phylloquinone is unstable in alkaline solution → alkaline saponification cannot be employed
- Post-column Zn powder reduction: reduction of quinone into fluorescent hydroquinone by solid-phase reductive column packed with metallic zinc particles.



Test Methods – Other considerations

- Unstable to light and oxidizing agent
- Vitamin K is unstable to alkali
- Protect from light:
 - ✓ Use of non-UV lamps
 - ✓ Brown glassware
- Immediately cool down after digestion
- Verification of standard concentration for each batch of analysis (e.g. E1%)



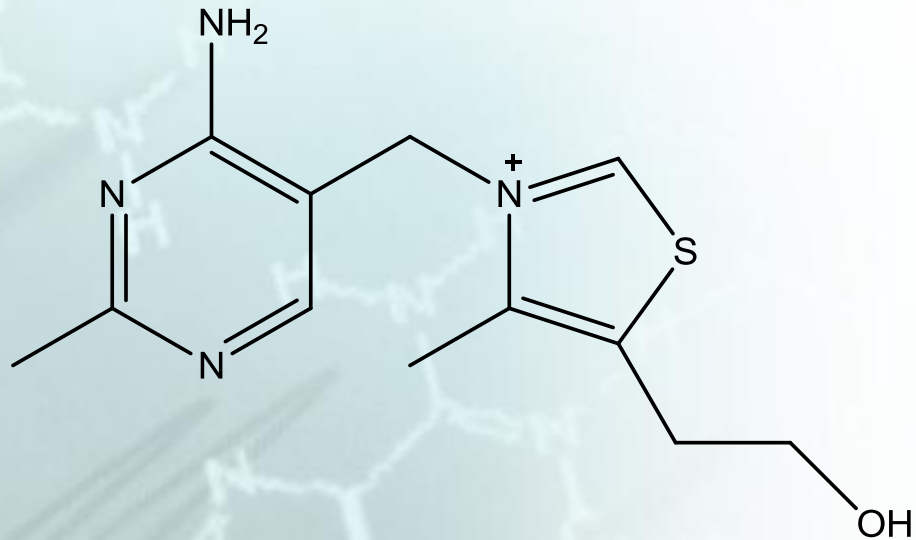
Water-soluble Vitamins

- Vitamin B₁ (Thiamine)
- Vitamin B₂ (Riboflavin)
- Vitamin B₃ (Niacin)
- Vitamin B₅
(Pantothenic acid)
- Vitamin B₆
(Pyridoxine, Pyridoxal,
Pyridoxamine)
- Vitamin B₉ (folic acid)
- Vitamin B₁₂
- Biotin
- Vitamin C



Vitamin B₁ (Thiamine)

- Vitamin B₁ (Thiamine)



Test Method – Thiamine

- Codex suggested test methods
 - ✓ EN 14122:2003 (HPLC-FLD)
 - ✓ AOAC 986.27 (Fluorimetry)



Test method - Thiamine

- ✓ EN 14122:2003 (HPLC-FLD)
- Homogenized test sample digested in hydrochloric acid or sulfuric acid solution ($\text{pH} < 2.0$) for 30 min at 121 °C or 60 min at 100 °C.
- Extract adjusted to optimal pH with sodium acetate solution, added with enzyme (e.g. taka-diaxase) and underwent incubation. Sample diluted with distilled water.



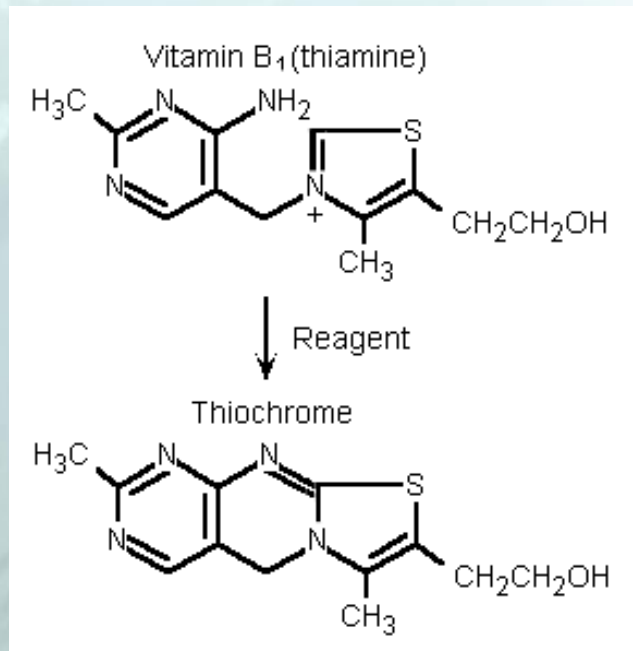
Test method - Thiamine

- Sample filtered for HPLC-FLD with pre- or post-column oxidation (ex: 366 nm; em: 435 nm)
- Taka-diaastase: liberation of bound thiamine into free form



Test method - Thiamine

- ✓ Thiamine is oxidised to thiochrome using post-column reaction with alkaline potassium hexacyanoferrate III solution.



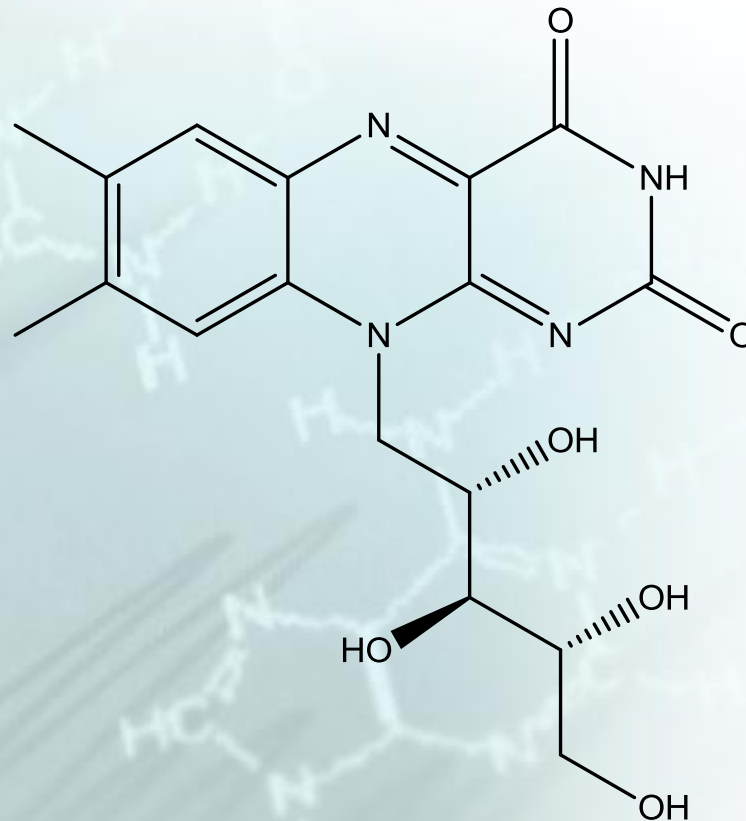
Non-fluorescent

Fluorescent



Vitamin B₂ (Riboflavin)

- Vitamin B₂ (Riboflavin)



Test Method – Riboflavin

- Codex suggested test methods
 - ✓ EN 14152:2014 (HPLC-FLD)
 - ✓ AOAC 985.31 (Fluorimetry) (points to AOAC 970.65)



Test method - Riboflavin

- ✓ EN 14152:2014 (HPLC-FLD)
- Homogenized test sample digested in hydrochloric acid or sulfuric acid solution ($\text{pH} < 2.0$) for 30 min at 121 °C or 60 min at 100 °C.
- Extract adjusted to optimal pH with sodium acetate solution, added with dephosphorylating enzyme (e.g. taka-diaxase) and underwent incubation. Sample diluted with dilute acetic acid.



Test method - Riboflavin

- Sample filtered. Aliquot diluted with compatible solvent for HPLC-FLD analysis (ex: 468 nm; em: 520 nm)
- Riboflavin is **fluorescent** itself, ∴ post-column reaction is not required.
- Taka-diastase: liberation of bound riboflavin into free form



Thank you !



Overview of Testing Methods for Formula Products (Part II)

Government Laboratory, Hong Kong



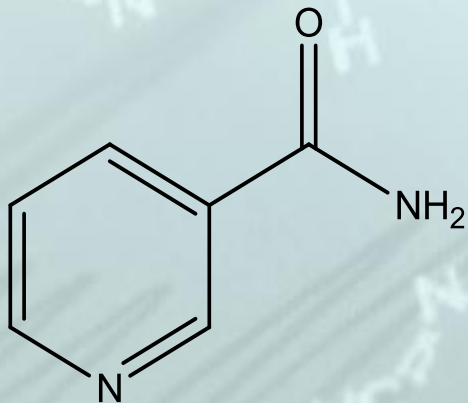
Topics covered

- Fatty acids
- Oil-soluble vitamins
- **Water-soluble vitamins**
- **Other nutrients: choline, myo-inositol, L-carnitine, taurine**

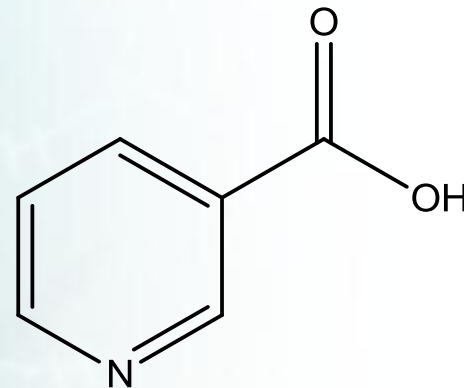


Niacin

- Vitamin B₃
- Infant formula : Nicotinamide and Nicotinic acid
- Follow-up formula: Nicotinamide



Nicotinamide



Nicotinic acid



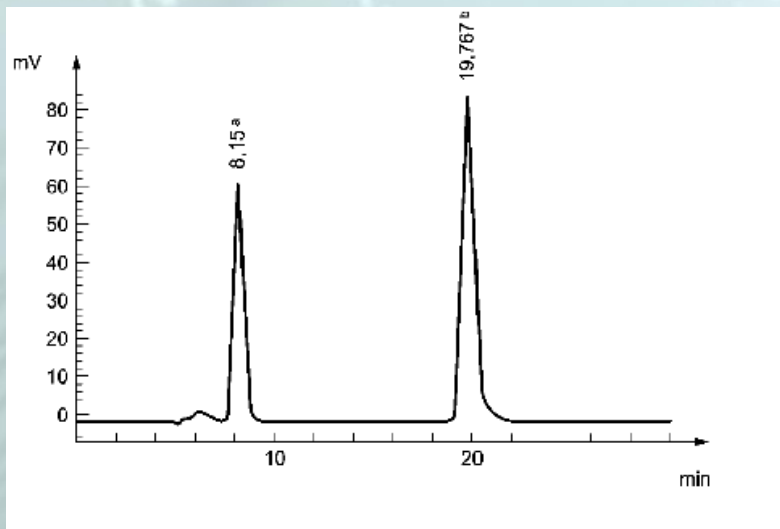
Test Method – Niacin

- Codex suggested test methods
 - ✓ EN 15652 (HPLC)
 - ✓ AOAC 985.34 (Microbioassay)
- Other standard test methods
 - ✓ AOAC 960.46 (Microbiological method)



Test Method – Niacin

- ✓ EN 15652 (HPLC)
- Niacin vitamers are extracted from food by an acid, an enzymatic or an acid/alkaline treatment.
- Quantified by HPLC with a fluorimetric detection after a post-column derivatization with UV irradiation.



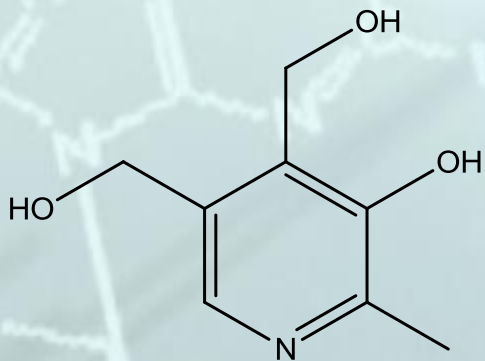
Example of a HPLC separation of nicotinic acid and nicotinamide standards using post-column derivatization

a nicotinic acid
b nicotinamide

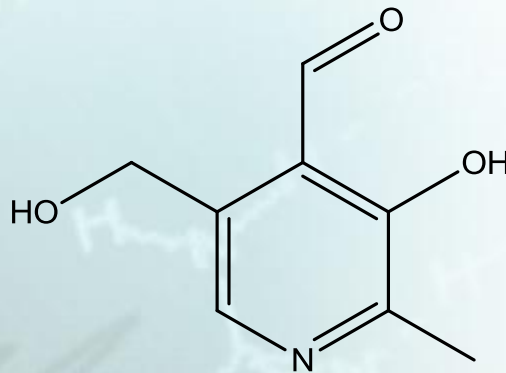


Vitamins B₆

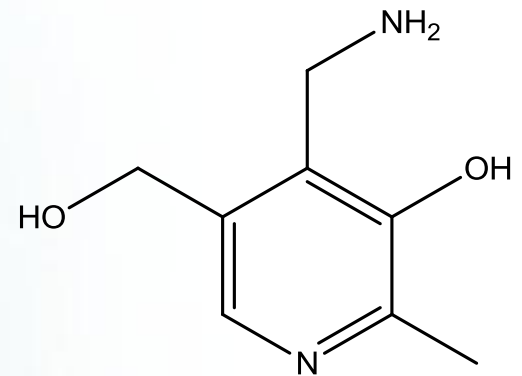
- Vitamin B₆ (Pyridoxine, Pyridoxal, Pyridoxamine)



Pyridoxine



Pyridoxal



Pyridoxamine



Test Method – Vitamin B₆

- Codex suggested test methods
 - ✓ EN 14164 (HPLC)
 - ✓ AOAC 2004.07 (HPLC)
 - ✓ AOAC 985.32 (Microbioassay)
 - ✓ EN 14166 (Microbioassay)
 - ✓ EN 14663 (HPLC)



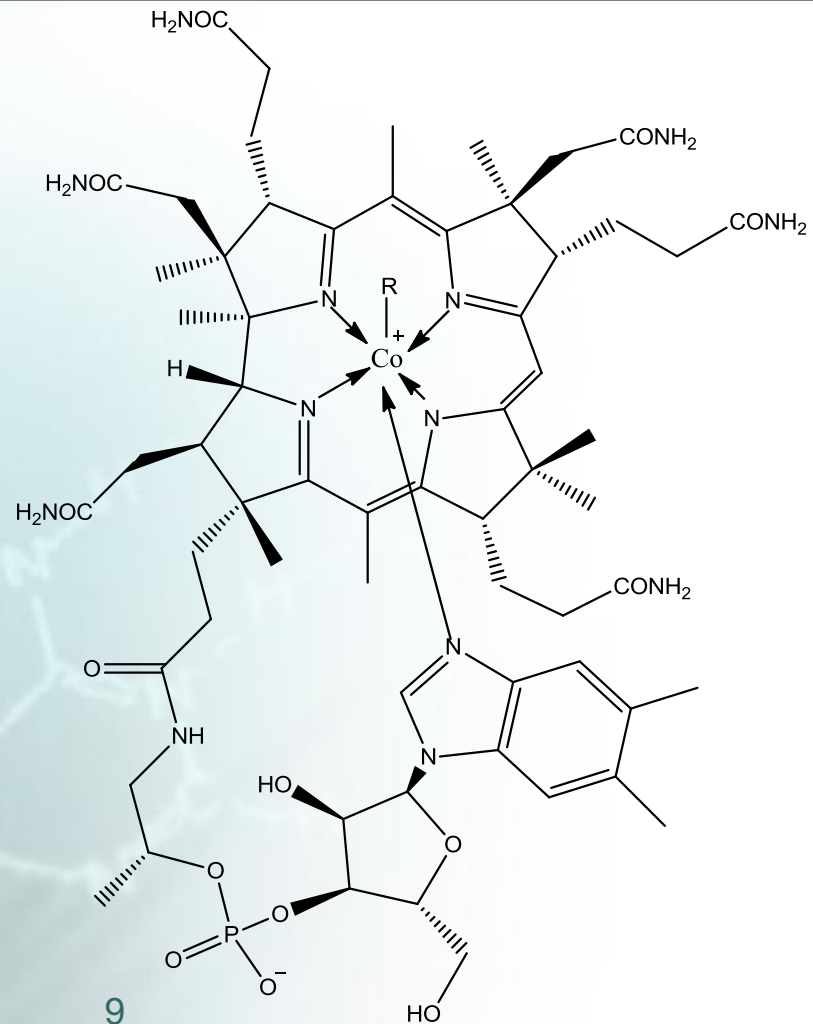
Test Method – Vitamin B₆

- ✓ EN 14164 (HPLC)
- Pyridoxal, pyridoxamine and pyridoxine are extracted from food by acid hydrolysis and dephosphorylated enzymatically using acid phosphatase.
- By reaction with glyoxylic acid in the presence of Fe²⁺ as a catalyst, pyridoxamine is transformed into pyridoxal, which is then reduced to pyridoxine by the action of sodium borohydride in alkaline medium.
- Pyridoxine is then quantified by HPLC with a fluorometric detection.



Vitamins B₁₂

- Vitamin B₁₂



Test Method – Vitamin B₁₂

- Codex suggested test methods
 - ✓ AOAC 986.23 (Turbidimetric Method)
- Other standard test methods
 - ✓ AOAC 2011.01, AOAC 2011.16 (Surface plasmon resonance)
 - ✓ AOAC 2011.08 (HPLC)
 - ✓ AOAC 2011.09 (HPLC)
 - ✓ AOAC 2011.10 (HPLC)
 - ✓ AOAC 2014.02 (UPLC)



Test Method – Vitamin B₁₂

- ✓ AOAC 986.23 (Turbidimetric Method)
- Protect solutions from undue exposure to light.
- Sample is suspended in phosphate buffer. After removing protein by precipitation, extract is diluted with basal medium containing all required growth nutrients except Vitamin B₁₂.
- The growth response of *Lactobacillus leichmannii* to extracted Vitamin B₁₂ is compared to the growth response to calibrant solutions with known concentration.



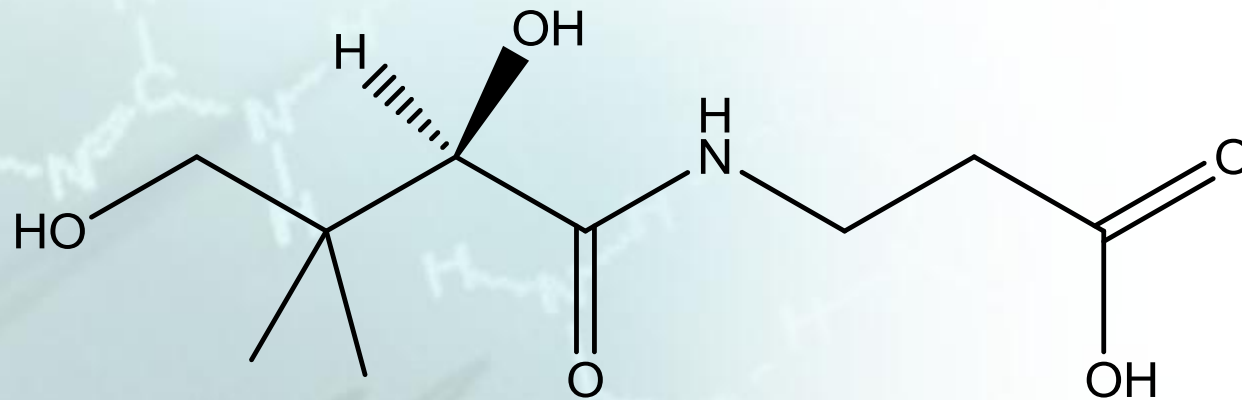
Test Method – Vitamin B₁₂

- ✓ AOAC 2014.02 (UPLC)
- Vitamin B₁₂ is extracted from the sample using sodium acetate buffer in the presence of sodium cyanide at 100°C for 30 min.
- Extract is purified and concentrated with an immunoaffinity column.
- Vitamin B₁₂ is determined by ultra-high-performance liquid chromatography with UV detection at 361 nm.



Pantothenic acid

- Vitamin B₅



Test Method – Pantothenic acid

- Codex suggested test methods
 - ✓ AOAC 992.07 (Microbioassay)
- Other standard test methods
 - ✓ AOAC 2012.16 (UPLC-MS/MS)



Test Method – Pantothenic acid

- ✓ AOAC 992.07 (Microbioassay)
 - Infant formula is treated with alkaline intestinal phosphatase, which removes triphosphoadenine from coenzyme A, and avian liver peptidase, which breaks peptide linkage.
 - Bound pantothenic acids are released as the free form, and assayed by turbidimetric microbial growth response.



Test Method – Pantothenic acid

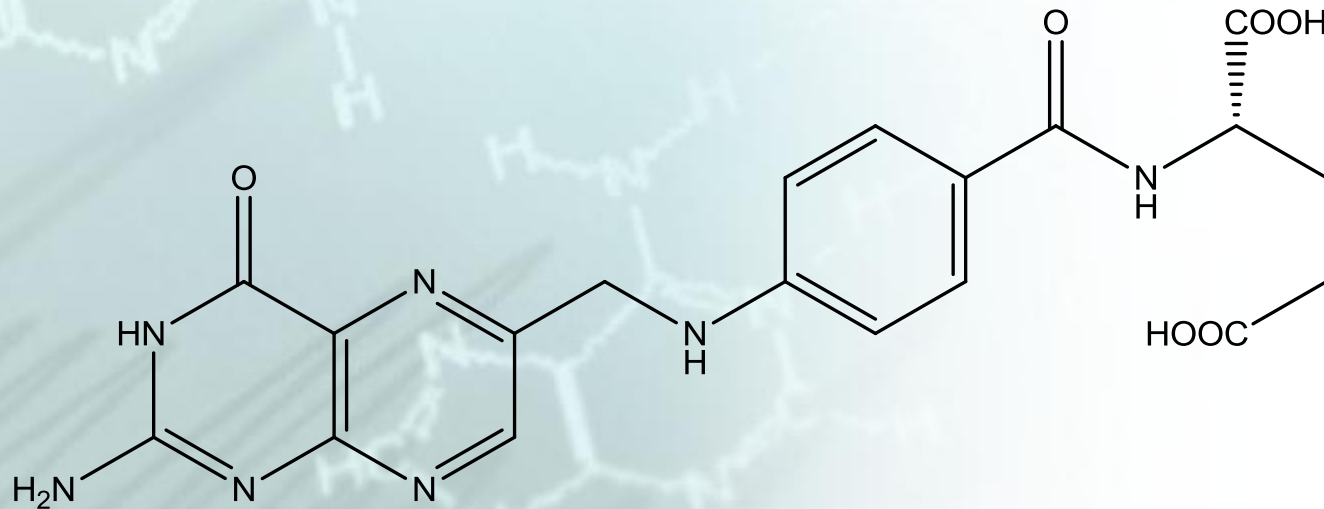
- ✓ AOAC 2012.16 (UPLC-MS/MS)
 - Pantothenic acid is extracted by using a 0.4 M ammonium acetate buffer solution (pH 3.8).
 - After filtration, the final solution is subjected to UPLC-MS/MS.



Folic acid

- Vitamin B₉
- For infant and follow-up formula:

N-pteroyl-L-glutamic acid



Test Method – Folic acid

- Codex suggested test methods
 - ✓ AOAC 992.05 (Microbioassay)
 - ✓ EN 14131 (Microbioassay)

- Other standard test methods
 - ✓ AOAC 2011.05 (Optical biosensor assay)
 - ✓ AOAC 2011.06 (UPLC-MS/MS)
 - ✓ AOAC 960.46 (Microbiological method)



Test Method – Folic acid

- ✓ EN 14131 (Microbioassay)
- Samples suspended in phosphate buffer are heated to enable extraction of folates. Naturally occurring polyglutamates are hydrolysed with γ -glutamyl hydrolase to polymono- or polydi-glutamates.
- Extracted folates are diluted with basal medium containing all required growth nutrients except folates. The growth response of *Lactobacillus casei*, subsp. *rhamnosus* to extracted folates is followed turbidimetrically and is compared to the growth response to calibrant solutions with known concentration.



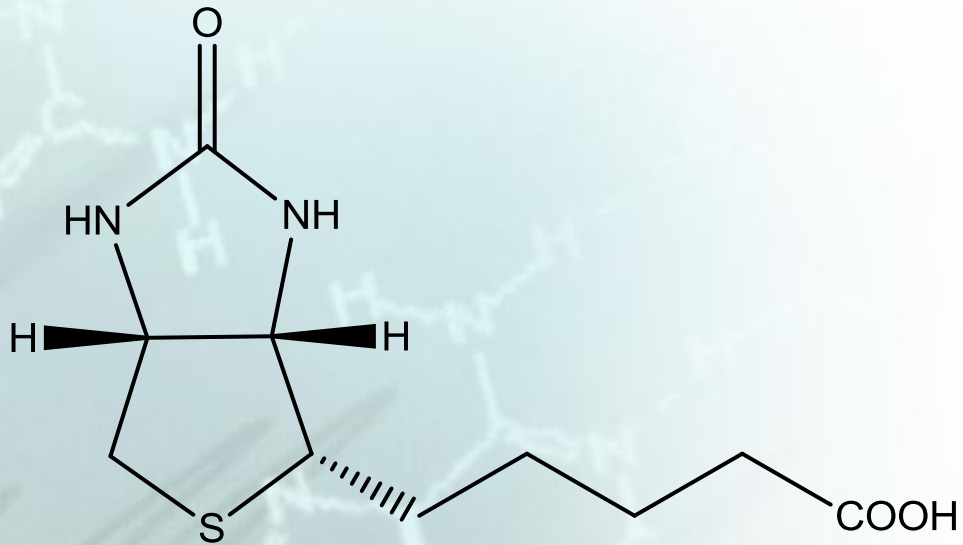
Test Method – Folic acid

- ✓ AOAC 2011.06 (UPLC-MS/MS)
- Folic acid is extracted by phosphate buffer solution (pH 6).
- Extract is cleaned by weak anion exchange (WAX) SPE.
- The final solution is analyzed by UPLC-MS/MS.



Biotin

- Vitamin B₇ (Biotin)



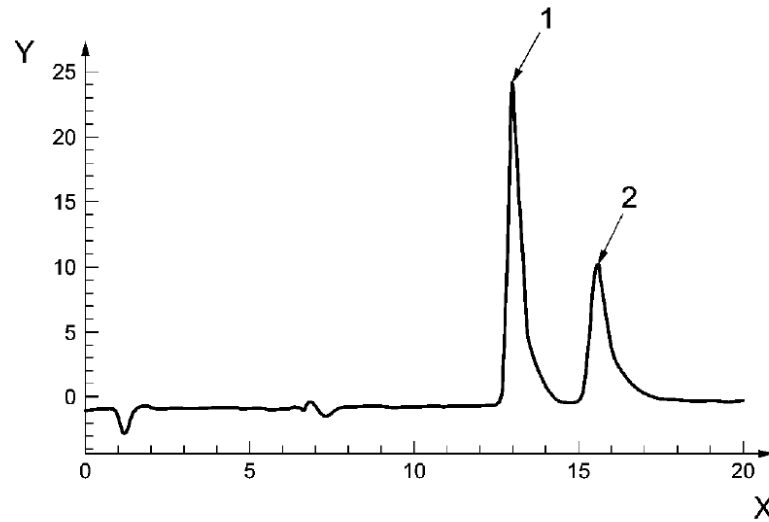
Test Method – Biotin

- Codex suggested test methods
 - ✓ EN 15607 (HPLC)
 - D-biotin is extracted after an enzymatic treatment and quantified by HPLC with post-column binding reaction.
 - Standard solution is stable for 1 day or 2 months at -18 °C.



Test Method – Biotin

✓ EN 15607 (HPLC)



Key

X Time in min
Y Fluorescence intensity
1 d-biotin
2 d-biocytyl

Figure A.1 — Example of a HPLC separation of d-biotin and d-biocytyl standards using post-column derivatization



General Procedures of Vitamins B Test Methods (1)

- Microbiological method

Sampling

Enzyme
digestion

Microbial
cultures

Turbidimetric
analysis



General Procedures of Vitamins B Test Methods (2)

- HPLC

Sampling

Enzyme digestion
or Hydrolysis

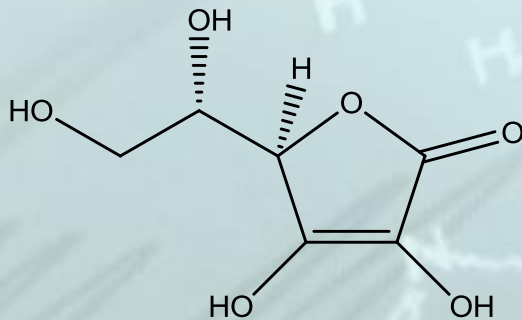
Extraction

HPLC
or
HPLC-MS

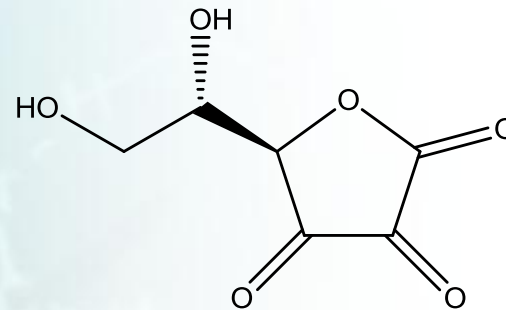


Water-soluble Vitamin – Vitamin C

- Infant formula: ascorbic acid & dehydroascorbic acid
- Follow-up formula: ascorbic acid
- Sensitive to light & oxygen



Ascorbic acid



Dehydroascorbic acid



Test Method – Vitamin C

- Codex suggested test methods
 - × EN 14130:2003 (HPLC) (**withdrawn**)
- Other standard test methods
 - ✓ AOAC 985.33 (Titration)
 - ✓ AOAC 2012.21 (HPLC)
 - ✓ AOAC 2012.22 (UPLC)



Test Method – Vitamin C

- ✓ AOAC 985.33 (Titration)
 - Ascorbic acid is estimated by titration with coloured oxidation-reduction indicator, 2,6-dichloroindophenol.
 - EDTA is added as chelating agent to remove Fe and Cu interferences.



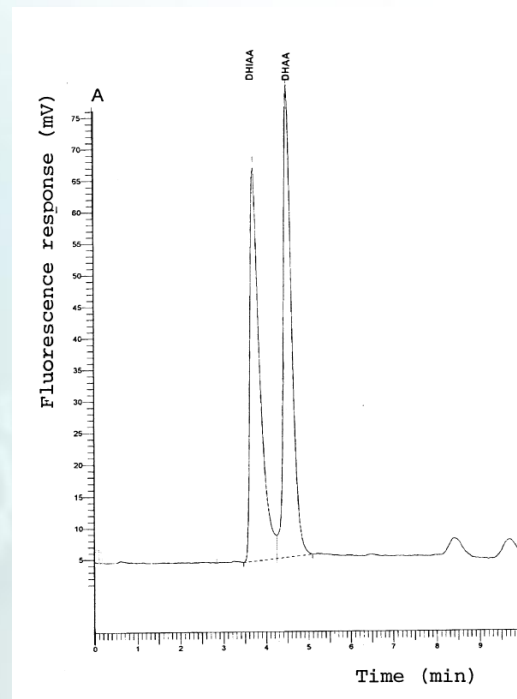
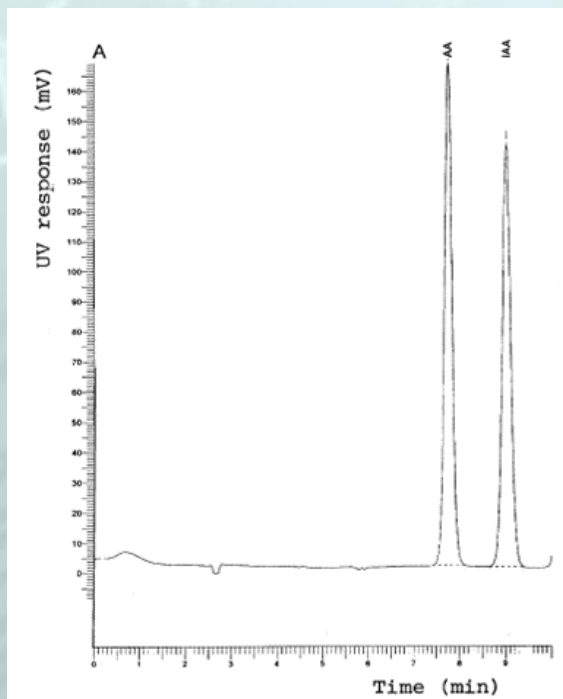
Test Method – Vitamin C

- ✓ AOAC 2012.21 (HPLC), AOAC 2012.22 (UPLC)
- Vitamin C is extracted with “ethylenediamine tetraacetic acid (EDTA), tris (2-carboxyethyl) phosphine (TCEP) hydrochloride, and metaphosphoric acid” or “trichloroacetic acid (TCA) and TCEP”
- These reagents precipitate proteins and stabilize vitamin C.
- Analyzed by HPLC-UV.



Test Method – Vitamin C

- ✓ Other literatures
- Kall M.A, Andersen C. (1999) “Improved method for simultaneous determination of ascorbic acid and dehydroascorbic acid, isoascorbic acid and dehydroisoascorbic acid in food and biological samples” *Journal of Chromatography B* 730:101-111.



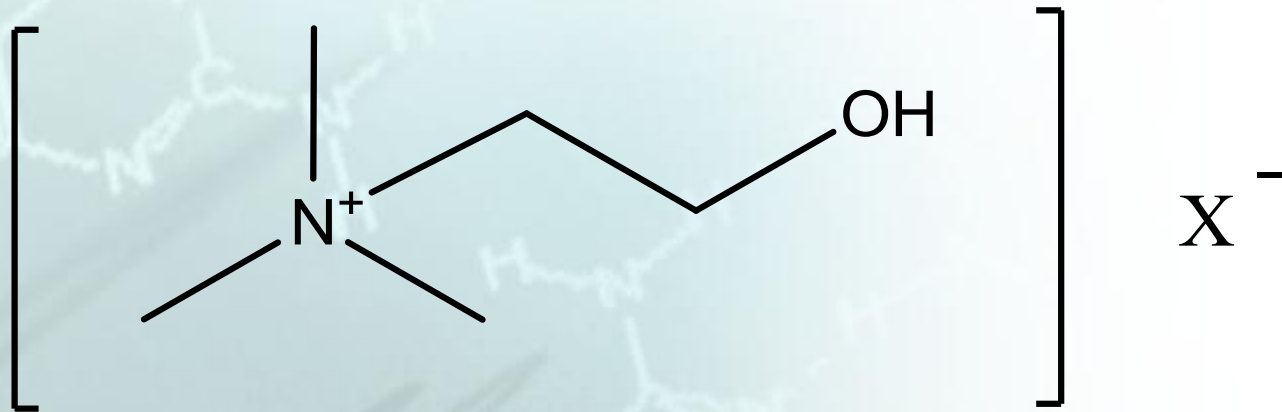
Other Nutrients

Chemical structures & Standard test methods



Other Nutrients (1)

- Choline



Test Method – Choline

- Codex suggested test methods
 - ✓ AOAC 999.14 (Enzymatic colorimetric method)
- Other standard test methods
 - ✓ AOAC 2012.18 (UPLC-MS/MS)
 - ✓ AOAC 2012.20 (IC)



Test Method – Choline

- ✓ AOAC 999.14 (Enzymatic colorimetric method)
- Sample is acid digested at 70°C to release most of the bound choline.
- Residual choline phospholipids are cleaved with phospholipase D and free choline is subjected to choline oxidase with liberation of H₂O₂. In the presence of peroxidase, phenol is oxidized and a quinoneimine chromophore is formed with 4-aminoantipyrine.
- Absorbance is measured at 505 nm and choline content is calculated from a calibration curve.



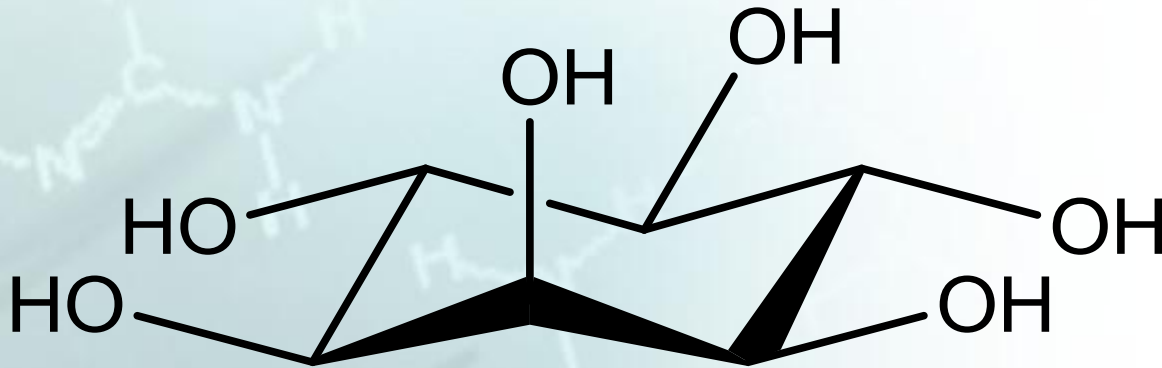
Test Method – Choline

- ✓ AOAC 2012.20 (IC)
- Microwave-assisted acid hydrolysis is used to digest and release bound choline from infant formula and adult nutritional samples.
- Following hydrolysis, separation of choline from common cations is achieved on a Dionex IonPac CS19 column, and choline is detected by suppressed conductivity.



Other Nutrients (2)

- Myo-inositol



Test Method – Myo-inositol

- Codex suggested test methods

- ✓ NA

- Other standard test methods

- ✓ AOAC 2011.18 (HPLC)

- ✓ AOAC 2012.12 (IC)



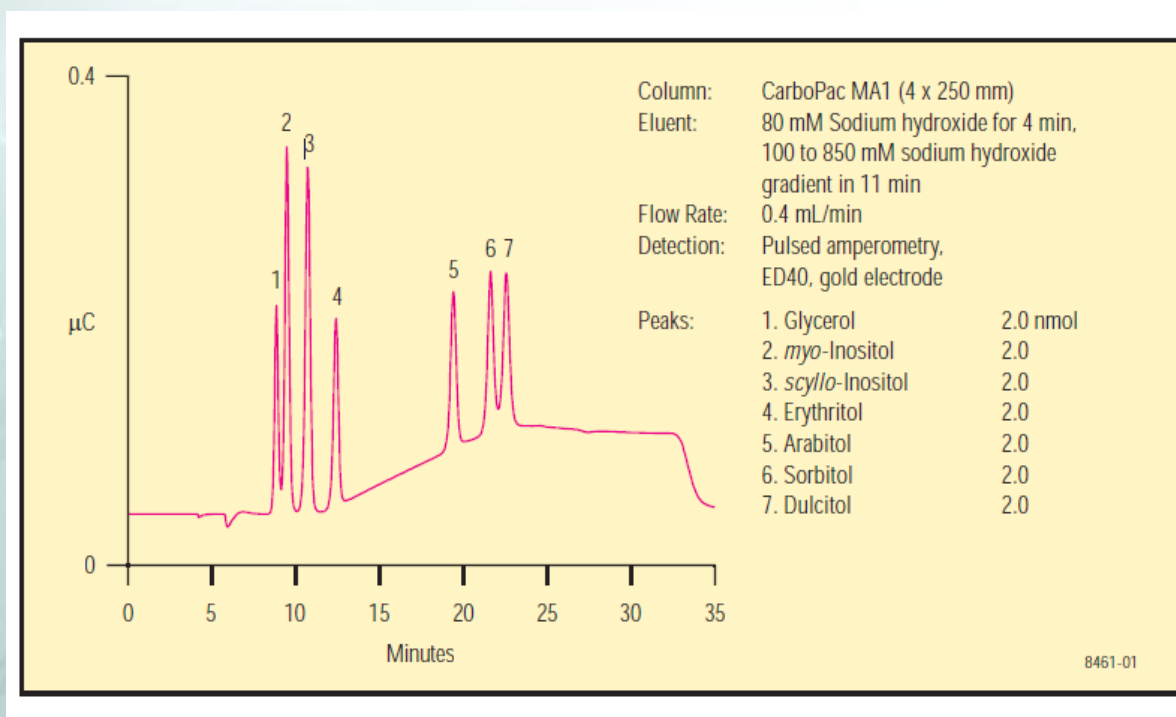
Test Method – Myo-inositol

- ✓ AOAC 2012.12 (IC)
- Total myo-inositol is extracted after acid hydrolysis and enzymatic treatment.
- Separation of myo-inositol from interferences is achieved on a CarboPac MA1 column (or equivalent), and myo-inositol is detected by electrochemical (pulsed amperometry) detector.



Test Method – Myo-inositol

➤ CarboPac MA1 column (or equivalent)

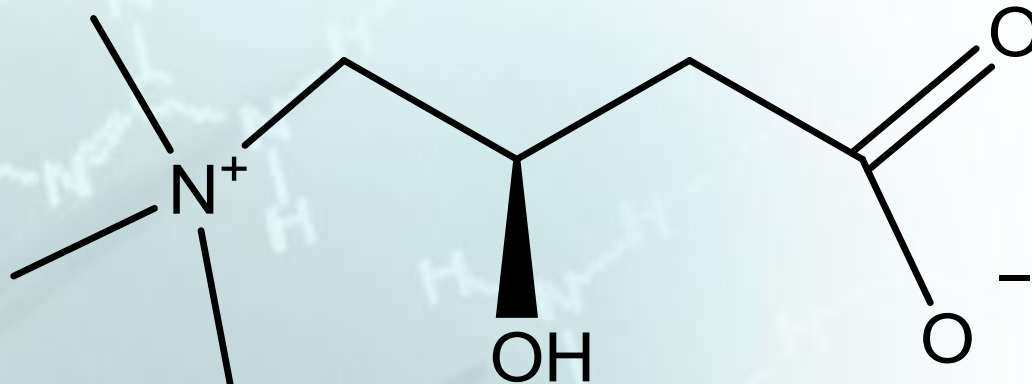


Source : CarboPac™ MA1 Column for Carbohydrate Alditol and Aldose Analysis, DIONEX



Other Nutrients (3)

- L-carnitine



Test Method – L-Carnitine

- Codex suggested test methods
 - ✓ NA
- Other standard test methods
 - ✓ AOAC 2012.17 (LC-MS/MS)



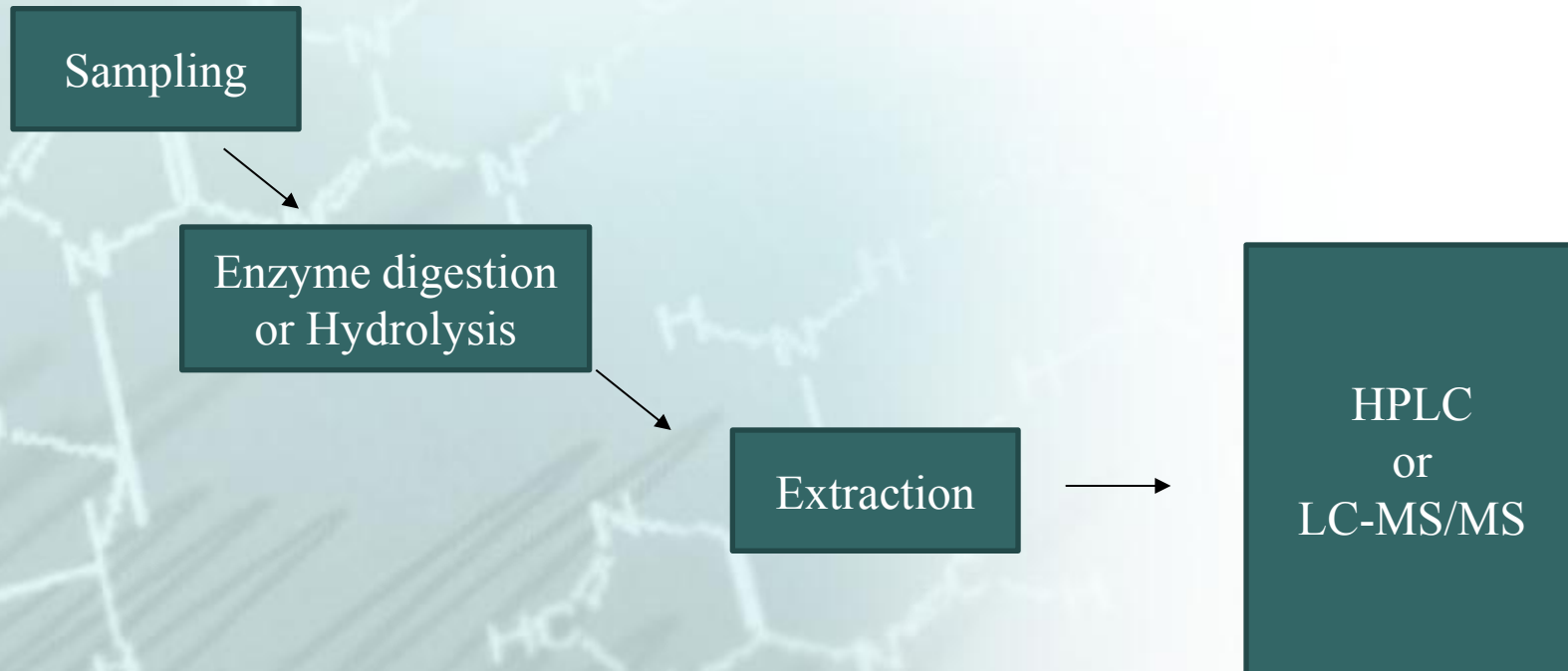
Test Method – L-Carnitine

- ✓ AOAC 2012.17 (LC-MS/MS)
- Total L-Carnitine is extracted after saponification.
- After dilution and filtration, the extract is analyzed by LC-MS/MS.



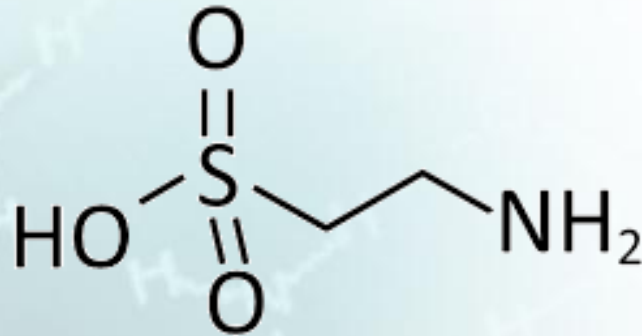
General Procedures of Other Nutrients Test Methods

- HPLC



Other Nutrients (4)

- Taurine



Test Method – Taurine

- Codex suggested test methods
 - ✓ NA
- Other standard test methods
 - ✓ AOAC 997.05 (HPLC)



Test Method – Taurine

- ✓ AOAC 997.05 (HPLC)
- Sample is reconstituted with water and protein and fat are removed by precipitation.
- Aliquot is treated with dansyl chloride to form taurine derivative, which is separated and measured by liquid chromatography with UV or fluorescence detection.



Summary

- Infant & follow-up formula contain more than 30 nutrients according to new regulation
 - Protein, fat, carbohydrates
 - Oil-soluble vitamins
 - Water-soluble vitamins and other nutrients
 - Minerals and trace elements
- Definition of some nutrients are different for IF and FF → Different analytical methods being applied
- International standards cover the analysis of most of the nutrients



Thank you !

