
**Infant formula and adult
nutritionals — Simultaneous
determination of total vitamins B₁, B₂,
B₃ and B₆ — Enzymatic digestion and
LC-MS/MS**

*Formules infantiles et produits nutritionnels pour adultes —
Détermination simultanée de la teneur en vitamines B₁, B₂, B₃ et B₆
— Digestion enzymatique et CL-SM/SM*

ISO 21470:2020

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 34, *Food products*, in collaboration with AOAC INTERNATIONAL. It is being published by ISO and separately by AOAC INTERNATIONAL. The method described in this document is equivalent to the AOAC Official Method 2015.14: *Simultaneous Determination of Total Vitamins B₁, B₂, B₃, and B₆ in Infant Formula and Related Nutritionals by Enzymatic Digestion and LC-MS/MS*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Infant formula and adult nutritionals — Simultaneous determination of total vitamins B₁, B₂, B₃ and B₆ — Enzymatic digestion and LC-MS/MS

1 Scope

This document specifies a method for the simultaneous quantitative determination of four water-soluble vitamins in infant formula and related nutritional products, including relevant forms of vitamins B₁, B₂, B₃ and B₆ by enzymatic digestion and UHPLC-MS/MS. This document is not intended to be used on products where vitamins have not been added.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <http://www.electropedia.org/>

3.1

adult nutritional

nutritionally complete, specially formulated food, consumed in liquid form, which may constitute the sole source of nourishment, made from any combination of milk, soy, rice, whey, hydrolysed protein, starch and amino acids, with and without intact protein

3.2

infant formula

breast-milk substitute specially manufactured to satisfy, by itself, the nutritional requirements of infants during the first months of life up to the introduction of appropriate complementary feeding

[SOURCE: Codex Standard 72-1981]

4 Principle

Samples are prepared by enzymatic digestion with papain and α -amylase to hydrolyse protein and complex carbohydrate and acid phosphatase to free phosphorylated vitamin forms. Stable-isotope labelled internal standards are incorporated into the sample preparation to correct for variability in both the sample preparation and instrument response. A series of six mixed working standard solutions spanning two orders of magnitude in vitamin concentration are used to generate calibration curves based on the peak response ratio of the analyte to its stable-isotope labelled internal standard.

Prepared samples and working standard solutions are injected onto ultra-high pressure liquid chromatograph (UPLC) interfaced to a triple-quadrupole mass spectrometer (MS/MS) for analysis. The MS/MS is configured to monitor precursor-fragment ion pairs for each analyte and internal standard. This reaction forms the basis for method selectivity. Analytes are quantified by least squares regression using the response ratio of the analyte to its internal standard.

5 Reagents and materials

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and distilled or demineralized water or water of equivalent purity.

5.1 Niacinamide (nicotinamide) (MW = 122,12), primary reference standard, e.g. USP Reference Standard, catalogue #1462006¹⁾. Follow the manufacturer's storage and handling directions.

5.2 Niacin (nicotinic acid) (MW = 123,11), primary reference standard, e.g. USP Reference Standard, catalogue # 1461003¹⁾. Follow the manufacturer's storage and handling directions.

5.3 Pyridoxine hydrochloride (MW = 205,64), primary reference standard, e.g. USP Reference Standard, catalogue # 1587001¹⁾. Follow the manufacturer's storage and handling directions.

5.4 Riboflavin (MW = 376,36), primary reference standard, e.g. USP Reference Standard, catalogue # 1603006¹⁾. Follow the manufacturer's storage and handling directions.

5.5 Thiamine hydrochloride (MW = 337,27), primary reference standard, e.g. USP Reference Standard, catalogue #1656002¹⁾. Follow the manufacturer's storage and handling directions. Measure the moisture content of the powder prior to use or use the supplier certificate of analysis (COA) moisture value.

5.6 Pyridoxamine dihydrochloride, Fluka Analytical Standard, catalogue #P9380¹⁾.

5.7 Pyridoxal hydrochloride, Sigma, catalogue #P9130¹⁾.

5.8 ²H₄-Niacinamide, CDN Isotopes, catalogue #D-3457¹⁾.

5.9 ²H₄-Nicotinic acid, CDN Isotopes, catalogue #D-4368¹⁾.

5.10 ¹³C₄-Pyridoxine: pyridoxine:HCl (4,5-bis(hydroxymethyl)-¹³C₄), Cambridge Isotope Laboratory, catalogue #CLM-7563¹⁾.

5.11 ²H₃-Pyridoxal, IsoSciences, catalogue #7098¹⁾.

5.12 ²H₃-Pyridoxamine, IsoSciences, catalogue #7099¹⁾.

5.13 ¹³C₄-Thiamine chloride, IsoSciences, catalogue #9209¹⁾.

5.14 ¹³C₄, ¹⁵N₂-Riboflavin, IsoSciences, catalogue #7072¹⁾.

5.15 Acid phosphatase, type II from potato, 0,5 U/mg to 3,0 U/mg, Sigma, catalogue #P3752¹⁾.

5.16 Papain from *Carica papaya*, ≥ 3 U/mg, Sigma, catalogue #76220¹⁾.

5.17 α-amylase from *aspergillus oryzae*, 150 U/mg, Sigma, catalogue #A9857¹⁾.

5.18 Hydrochloric acid concentrated (substance concentration $c = 12 \text{ mol/l}$), ACS grade, or equivalent.

5.19 Ammonium formate, for mass spectrometry (purity ≥ 99,0 %), Fluka 70221 or equivalent¹⁾.

1) This is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

5.20 Glacial acetic acid, Sigma ACS reagent grade, or equivalent¹⁾.

5.21 Formic acid, Sigma ACS reagent grade, or equivalent¹⁾.

5.22 Laboratory water, 18,0 MΩ, < 10 µg/kg TOC, or equivalent.

5.23 Methanol, Fisher LC-MS/MS Optima grade or EMD Omni-Solve LC-MS grade¹⁾.

5.24 Ethylenediaminetetracetic acid, disodium salt dihydrate (EDTA), ACS grade (99 % to 101 %), or equivalent.

5.25 Potassium phosphate dibasic, ACS grade (purity > 98 %), or equivalent.

5.26 meta-Phosphoric acid, ACS grade (33,5 % to 36,5 %), or equivalent.

5.27 Buffer solutions for pH meter calibration, pH = 4,0, 7,0 and 10,0.

5.28 Phosphoric acid, 85 g/100 g, ACS grade, or equivalent.

5.29 Potassium hydroxide, 40 g/100 g, ACS grade, or equivalent.

6 Standard and solution preparation

6.1 Mobile phases and prepared solutions

6.1.1 Mobile phase A, substance concentration $c = 0,020$ mol/l ammonium formate in water.

Using a graduated cylinder, transfer 500 ml laboratory water to a mobile phase reservoir. Add 0,631 g of ammonium formate (5.19) and mix well. Expiration is three days.

6.1.2 Mobile phase B, methanol.

6.1.3 HCl solution, $c = 0,12$ mol/l.

Add approximately 300 ml of water to a 500 ml graduated cylinder. Add 5,0 ml ± 0,1 ml of concentrated HCl solution (5.18) and swirl to mix. Bring to 500 ml with laboratory water and mix well.

6.1.4 Acetic acid solution, 1,0 ml/100 ml.

Add approximately 30 ml of water to a 500 ml graduated cylinder. Add 5,0 ml ± 0,1 ml of glacial acetic acid (5.20) and swirl to mix. Bring to 500 ml with laboratory water and mix well.

6.1.5 Weak needle wash, 10 ml/100 ml methanol in water, expiration three months. Alternatively, use weak needle wash as recommended by the supplier.

6.1.6 Strong needle wash, methanol or as recommended by the supplier.

6.1.7 Ammonium formate solution, $c = 0,050$ mol/l.

Using a graduated cylinder, transfer 1 400 ml of laboratory water to an appropriate reservoir. Add 4,41 g of ammonium formate (5.19) and mix well. One 400 ml is adequate for 6 working standards and 32 samples. Scale as needed. Expiration is three days.

6.1.8 Mixed enzyme solution.

Using a graduated cylinder, transfer 200 ml of ammonium formate buffer (6.1.7) to an appropriate reservoir. Add 200 mg \pm 10 mg of acid phosphatase (5.15), 80 mg \pm 5 mg of α -amylase (5.17) and 400 mg \pm 10 mg of papain (5.16). Mix for 10 min with a magnetic stir plate and stir bar. Check pH and adjust to $4,25 \pm 0,25$ with formic acid (5.21, approximately 100 μ l). 200 ml is adequate for 6 working standards and 32 samples. Scale as needed. Prepare fresh daily.

6.2 Stable isotope labelled compounds, individual, internal standard stock solutions

6.2.1 Internal standard stock solutions have an expiration of six months. However, the following guidelines can be used to troubleshoot internal standards and, when documented as part of routine system suitability checks, extend the expiration dates indefinitely.

Based on US FDA bioanalytical method validation guidelines, which state that the lowest-level calibration shall be five times the analyte response of the blank,^[9] the channel of the non-labelled analyte of interest shall be monitored to ensure the stable isotope-labelled internal standard does not contribute more than 20 % of the area count of the lowest-level calibration standard. No response should be generated in any other channels being monitored in the method, as this is a sign of contamination, in which case fresh solution should be prepared or fresh lot of material should be ordered.

The area count of the internal standard should be at least three times the area count of the analyte in the lowest-level calibration standard and the lowest level matrix-based QC sample.

6.2.2 $^2\text{H}_4$ -Niacinamide stock solution, mass concentration $\rho \approx 560$ $\mu\text{g/ml}$.

Weigh 14,0 mg \pm 0,1 mg into a tared weighing vessel. Quantitatively transfer to a 25 ml volumetric flask with laboratory water and fill to the mark with laboratory water. Mix well and transfer to a 50 ml amber bottle and store refrigerated (2 $^\circ\text{C}$ to 8 $^\circ\text{C}$). For expiration, see 6.2.1.

6.2.3 $^2\text{H}_4$ -Nicotinic acid stock solution, $\rho \approx 500$ $\mu\text{g/ml}$.

Weigh 12,5 mg \pm 0,1 mg into a tared weighing vessel. Quantitatively transfer to a 25 ml volumetric flask with laboratory water and fill to the mark with laboratory water. Mix well and transfer to a 50 ml amber bottle and store refrigerated (2 $^\circ\text{C}$ to 8 $^\circ\text{C}$). For expiration, see 6.2.1.

6.2.4 $^{13}\text{C}_4$ -Pyridoxine stock solution, $\rho \approx 70$ $\mu\text{g/ml}$.

Weigh 7,0 mg \pm 0,1 mg into a tared weighing vessel. Quantitatively transfer to a 100 ml volumetric flask with laboratory water and fill to the mark with laboratory water. Mix well and transfer to a 100 ml amber bottle and store refrigerated (2 $^\circ\text{C}$ to 8 $^\circ\text{C}$). For expiration, see 6.2.1.

6.2.5 $^2\text{H}_3$ -Pyridoxal stock solution, $\rho \approx 40$ $\mu\text{g/ml}$.

Weigh 4,0 mg \pm 0,1 mg into a tared weighing vessel. Quantitatively transfer to a 100 ml volumetric flask with laboratory water and fill to the mark with laboratory water. Mix well and transfer to a 100 ml amber bottle and store refrigerated (2 $^\circ\text{C}$ to 8 $^\circ\text{C}$). For expiration, see 6.2.1.

6.2.6 $^2\text{H}_3$ -Pyridoxamine stock solution, $\rho \approx 40$ $\mu\text{g/ml}$.

Weigh 4,0 mg \pm 0,1 mg into a tared weighing vessel. Quantitatively transfer to a 100 ml volumetric flask with laboratory water and fill to the mark with laboratory water. Mix well and transfer to a 100 ml amber bottle and store refrigerated (2 $^\circ\text{C}$ to 8 $^\circ\text{C}$). For expiration, see 6.2.1.

6.2.7 $^{13}\text{C}_4$ -Thiamine chloride stock solution, $\rho \approx 100 \mu\text{g/ml}$.

Weigh $5,0 \text{ mg} \pm 0,1 \text{ mg}$ of $^{13}\text{C}_4$ -thiamine into a tared weighing vessel. Quantitatively transfer to a 50 ml volumetric flask with HCl solution (6.1.2) and fill to the mark with HCl solution (6.1.2). Mix well and transfer to a 100 ml amber bottle and store refrigerated (2°C to 8°C). For expiration, see 6.2.1.

6.2.8 $^{13}\text{C}_4$, $^{15}\text{N}_2$ -Riboflavin stock solution, $\rho \approx 73 \mu\text{g/ml}$.

Weigh $7,3 \text{ mg} \pm 0,1 \text{ mg}$ of $^{13}\text{C}_4$, $^{15}\text{N}_2$ -riboflavin into a tared weighing vessel. Quantitatively transfer to a 100 ml volumetric flask with acetic acid solution (6.1.3) and fill to the mark with acetic acid solution (6.1.3). Mix well and transfer to a 100 ml amber bottle and store refrigerated (2°C to 8°C). For expiration, see 6.2.1.

6.2.9 Internal standard stock mixture (ISSM).

Combine 2 500 μl of ammonium formate solution (6.1.7) with 250 μl of $^2\text{H}_4$ -niacinamide stock solution (6.2.2), 250 μl of $^2\text{H}_4$ -nicotinic acid stock solution (6.2.3), 250 μl of $^{13}\text{C}_4$ -pyridoxine stock solution (6.2.4), 200 μl of $^2\text{H}_3$ -pyridoxal stock solution (6.2.5), 50 μl of $^2\text{H}_3$ -pyridoxamine stock solution (6.2.6), 250 μl of $^{13}\text{C}_4$ -thiamine stock solution (6.2.7) and 250 μl of $^{13}\text{C}_4$, $^{15}\text{N}_2$ -riboflavin stock solution (6.2.8). Volume provides sufficient ISSM for 6 working standards and 32 samples. Scale as needed. Prepare fresh daily.

6.2.10 Phosphate buffer solution, $\text{pH} = 5,0$ ($0,010 \text{ mol/l}$ potassium phosphate dibasic, 1 g/100 g EDTA, 2 g/100 g metaphosphoric acid).

Weigh $20,0 \text{ g} \pm 0,2 \text{ g}$ of EDTA into a tared weighing vessel and quantitatively transfer to a 2 000 ml beaker containing approximately 1 800 ml laboratory water and add a magnetic stir bar.

Weigh $34,8 \text{ g} \pm 0,1 \text{ g}$ of potassium phosphate dibasic into a tared weighing vessel and quantitatively transfer to the 2 000 ml beaker already containing approximately 1 800 ml laboratory water and EDTA. Mix by stirring on a magnetic stir plate until both the EDTA and potassium phosphate dibasic is completely dissolved.

Weigh $40,0 \text{ g} \pm 0,2 \text{ g}$ of metaphosphoric acid into a tared weighing vessel and quantitatively transfer to the 2 000 ml beaker containing approximately 1 800 ml laboratory water, EDTA, and potassium phosphate dibasic. Mix by stirring on a magnetic stir plate until the metaphosphoric acid is completely dissolved.

Adjust the pH of the solution to $\text{pH} = 5,00 \pm 0,02$ using 40 g/100 g potassium hydroxide or 85 g/100 g phosphoric acid. Quantitatively transfer the solution to a 2 000 ml volumetric flask and dilute to volume with laboratory water. Expiration: 48 hours.

6.3 Stock standard solutions of native compounds**6.3.1 Vitamin standard stock mixture (VSSM).**

Accurately weigh the indicated amounts for the following standards using separate weighing funnels or other appropriate weighing vessels and quantitatively transfer to a 100 ml volumetric flask using phosphate buffer ($\text{pH} = 5$).

- a) Niacinamide (5.1): $70,5 \text{ mg} \pm 0,5 \text{ mg}$.
- b) Thiamine hydrochloride (5.5): $10,5 \text{ mg} \pm 0,2 \text{ mg}$.

Determine the moisture of the thiamine hydrochloride reference standard (5.5) as directed on the container immediately prior to weighing or use moisture content from the supplier COA. The per cent moisture determined for the reference standard is used to calculate the concentration of thiamine in the VSSM.

- c) Riboflavin (5.4): $7,0 \text{ mg} \pm 0,2 \text{ mg}$.

d) Pyridoxine hydrochloride (5.3): 10,8 mg \pm 0,2 mg.

Fill to volume with phosphate buffer (pH = 5) solution. Heat and slowly stir until the standards have completely dissolved (riboflavin dissolves more slowly) and the solution is clear. Do not heat the solution for more than 40 min and do not exceed 90 °C. Store refrigerated (2 °C to 8 °C). Expiration: three months.

6.3.2 Nicotinic acid stock solution, ρ = 550 mg/ml.

Accurately weigh 13,7 mg \pm 0,1 mg niacin primary reference standard (5.2). Quantitatively transfer the nicotinic acid to a 25 ml volumetric flask. Add laboratory water to a total volume of about 20 ml and swirl until completely dissolved. Bring to volume with laboratory water. Mix well. Expiration: three months.

6.3.3 Pyridoxal stock solution, ρ = 140 mg/ml.

Accurately weigh 17,0 mg \pm 0,5 mg pyridoxal dihydrochloride standard (5.7). Quantitatively transfer to a 100 ml volumetric flask. Add laboratory water to a total volume of about 70 ml and swirl until completely dissolved. Bring to volume with laboratory water. Mix well. Expiration: three months.

6.3.4 Pyridoxamine stock solution, ρ = 160 mg/ml.

Accurately weigh 23,0 mg \pm 0,5 mg pyridoxamine hydrochloride standard (5.6). Quantitatively transfer to a 100 ml volumetric flask. Add laboratory water to a total volume of about 70 ml and swirl until completely dissolved. Bring to volume with laboratory water. Mix well. Expiration: three months.

6.3.5 Mixed working standard (MWS).

Combine 500 μ l VSSM (6.3.1), 25 μ l pyridoxamine stock (6.3.4), 25 μ l pyridoxal stock (6.3.3), and 65 μ l nicotinic acid stock solutions (6.3.2) in a 10 ml volumetric flask containing approximately 5 ml of ammonium formate solution (6.1.7). Bring to volume with ammonium formate solution (6.1.7) and mix well. Prepare fresh daily.

6.4 Working standard solution preparation

6.4.1 Working solution (WS) 1.

Add 20 μ l of MWS (6.3.5) and 980 μ l of ammonium formate (6.1.7) to a 50 ml centrifuge tube. Add 100 μ l of ISSM (6.2.9), and vortex to mix. Prepare fresh daily.

6.4.2 Working solution (WS) 2.

Add 50 μ l of MWS (6.3.5) and 950 μ l of ammonium formate (6.1.7) to a 50 ml centrifuge tube. Add 100 μ l of ISSM (6.2.9), and vortex to mix. Prepare fresh daily.

6.4.3 Working solution (WS) 3.

Add 100 μ l of MWS (6.3.5) and 900 μ l of ammonium formate (6.1.7) to a 50 ml centrifuge tube. Add 100 μ l of ISSM (6.2.9), and vortex to mix. Prepare fresh daily.

6.4.4 Working solution (WS) 4.

Add 200 μ l of MWS (6.3.5) and 800 μ l of ammonium formate (6.1.7) to a 50 ml centrifuge tube. Add 100 μ l of ISSM (6.2.9), and vortex to mix. Prepare fresh daily.

6.4.5 Working solution (WS) 5.

Add 500 µl of MWS (6.3.5) and 500 µl of ammonium formate (6.1.7) to a 50 ml centrifuge tube. Add 100 µl of ISSM (6.2.9), and vortex to mix. Prepare fresh daily.

6.4.6 Working solution (WS) 6.

Add 1 000 µl of MWS (6.3.5). Add 100 µl of ISSM (6.2.9), and vortex to mix. Prepare fresh daily.

6.5 Summary of standard and solution preparation

See Table 1.

Table 1 — Summary of standard and solution preparation

Compound	Mass mg	Purity	Mois- ture cor- rection	Volume stock solution ml	Aliquot stock µl	Volume of MWS ml	Aliquot of MWS (6.3.5) µl	Aliquot of ISSM (6.2.9) µl	Final volume ml
Niacinamide (5.1)	70,5 ± 0,5	0,999 ^a	1,000	100	500	10	see 6.4	100	30
Thiamine HCl (5.5)	10,5 ± 0,2	0,997 ^a	0,961 ^b	100	500	10	see 6.4	100	30
Riboflavin (5.4)	7,0 ± 0,2	0,986 ^a	1,000	100	500	10	see 6.4	100	30
Pyridoxine (5.3)	10,8 ± 0,2	0,999 ^a	1,000	100	500	10	see 6.4	100	30
Pyridoxal (5.7)	17,0 ± 0,5	0,990 ^a	1,000	100	25	10	see 6.4	100	30
Pyridoxamine (5.6)	23,0 ± 0,5	0,980 ^a	1,000	100	25	10	see 6.4	100	30
Niacin (nicotinic acid) (5.2)	13,7 ± 0,1	0,998 ^a	1,000	25	65	10	see 6.4	100	30
^a Purity of the standard as defined by the manufacturer.									
^b Moisture correction (1 – moisture content, from measurement or from the COA provided by the manufacturer).									

7 Apparatus

7.1 Waters® Acquity BEH C18 column²⁾ or equivalent, 2,1 mm x 100 mm, 1,7 µm.

7.2 UHPLC system, Waters Acquity Classic²⁾, or equivalent.

7.3 Tandem quadrupole mass spectrometer with ESI probe, Waters Xevo TQ-S²⁾, or equivalent.

7.4 Analytical balances.

A balance capable of accurately weighing 5,00 mg (for standards), a six-place balance, an analytical five-place balance for samples and a top-loading two-place balance capable of weighing to several hundred grams.

7.5 Water purifier, Millipore Milli-Q Water Purification System²⁾, or equivalent.

2) This is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

- 7.6 Water bath shaker**, capable of maintaining 37 °C, Lab-Line Orbit²⁾, or equivalent.
- 7.7 Bottle-top dispenser**, capable of dispensing volumes of approximately 24 ml.
- 7.8 pH meter**, capable of measuring pH = 4,0 to pH = 5,0.
- 7.9 Vortex mixer**.
- 7.10 Multi-position magnetic stir plate**.
- 7.11 Room light shields**, A.L.P. Protect-A-Lamp²⁾, UV cutoff at 460 nm, or equivalent. Alternatively, the use of amber (brown) glassware and vials can be used.
- 7.12 Graduated cylinders**, various sizes, including 10 ml, 100 ml, 500 ml and 1 000 ml.
- 7.13 Beakers**, various sizes, including 100 ml, 200 ml, 400 ml, 600 ml, 1 000 ml and 2 000 ml.
- 7.14 Volumetric flasks**, various sizes, including 10 ml, 25 ml, 50 ml, 100 ml, 250 ml and 2 000 ml.
- 7.15 Mobile phase bottles**, glass, various sizes, including 250 ml, 500 ml, 1 000 ml and 2 000 ml.
- 7.16 Disposable plastic Pasteur pipettes**.
- 7.17 Amber bottles**, volume capacity of 50 ml and 100 ml (for stock standard storage).
- 7.18 Weighing vessels**, various, including disposable weighing boats and glass weighing funnels.
- 7.19 Positive displacement pipettes**, 10 µl, 100 µl, 250 µl and 1 000 µl, Gilson Microman: Part #F148501, #F148504, #F148505 and #F148506²⁾.
- 7.20 Positive displacement pipette tips**, 10 µl, 100 µl, 250 µl and 1 000 µl, Gilson Capillary Piston: Part #F148312, #F148314, #F148014 and #F148560²⁾.
- 7.21 Plastic syringes**, 3 ml.
- 7.22 Syringe filters**, polytetrafluorethylene (PTFE) 0,45 µm syringe filters, Acrodisc²⁾ 25 mm, or equivalent.
- 7.23 Plastic centrifuge tubes**, 50 ml, self-standing, Superior Scientific, Ltd.²⁾, or equivalent.
- 7.24 Autosampler vials**, Waters autosampler vials; 9 mm amber with screw top 12 mm x 32 mm pre-split PTFE-silicon septa; Waters Part # 186000847C, or equivalent²⁾.
- 7.25 PTFE coated magnetic stir bars**.