OXFORD

Journal of AOAC INTERNATIONAL, 106(2), 2023, 384–388

https://doi.org/10.1093/jaoacint/qsac132
Advance Access Publication Date: 27 October 2022
Short Communication

HUMAN NUTRIENT METHODS

Rapid Analysis of Taurine in Infant Formula and Adult Nutritionals by Hydrophilic Interaction Liquid Chromatography–Mass Spectrometry

Brendon D. Gill * and Jackie E. Wood

Fonterra Co-operative Group Ltd, Laboratory Operations, PO Box 7, Waitoa 3341, New Zealand

*Corresponding author's e-mail: brendon.gill@fonterra.com

Abstract

Background: Taurine is recognized as an essential growth factor and as being critical in the maintenance of functional tissue regulation.
Objective: A rapid compliance method for the analysis of taurine that is applicable to infant formula and milk-based nutritional products is described.
Method: Following protein precipitation with Carrez solutions, taurine in the sample extract is separated by hydrophilic interaction liquid chromatography (HILIC) with detection by triple quadrupole mass spectrometry using multiple reaction monitoring (MRM). Stable isotope-labeled taurine internal standard is used for quantification to correct for losses in extraction and variations in ionization in the ion source.
Results: The method was shown to be accurate, with acceptable recovery of 99.6% (range = 91.1–106.5%). Results for National Institute of Standards and Technology (NIST)-certified reference materials showed no statistical bias for NIST 1849a ($P = 0.96$) and NIST 1869 ($P = 0.88$) when compared with reference values. No bias was found when results were compared with those of an international reference method, AOAC *Official Method*^{SM} **997.05** ($P = 0.18$). Repeatability was estimated to be 3.1% RSD_r (range: 2.4–4.0%, HorRat: 0.3), and intermediate precision was estimated to be 4.9% RSD_{iR} (range: 2.2–7.7%).
Conclusions: Successful single-laboratory validation demonstrates that this rapid method is suitable for use in high-throughput laboratories as part of routine product compliance release testing of taurine in nutritional products.
Highlights: A method for the analysis of taurine in infant formula and adult nutritionals by hydrophilic interaction liquid chromatography–mass spectrometry (LC–MS) is described. The method is suitable for use in high-throughput laboratories for routine product compliance testing of taurine. A single-laboratory validation study demonstrated the method to be accurate, precise, and fit for purpose.

Taurine, 2-aminoethanesulfonic acid, is recognized as an essential growth factor and as being critical in the maintenance of functional tissue regulation (1). As taurine is a β-amino acid, it is not incorporated into any protein and is found only in the free form. Taurine is a major intracellular free amino acid in humans and is considered to be a conditionally essential micronutrient because a deficiency of taurine can have serious consequences for individuals with low serum levels (2, 3). When breast milk substitutes are used, dietary supplementation is now regarded as being necessary because of the absence of taurine in soy protein and the significantly lower taurine content in bovine milk compared with human milk. Taurine-fortified infant formulas enable infants to maintain serum levels equivalent to those of their counterparts fed on their mother's milk (4, 5).

Taurine is a small molecule without a strong chromophore or fluorophore; hence, detection of the extracted taurine generally

Received: 15 August 2022; **Revised:** 9 October 2022; **Accepted:** 12 October 2022

requires derivatization or electrochemistry, similar to many other amino acids or carboxylic acids (6).

As part of amino acid profiles, taurine analysis was commonly performed using ion-exchange columns to separate underivatized amino acids, followed by post-column ninhydrin reaction in discrete amino acid analyzers (7). However, these have been replaced largely by HPLC techniques that are less expensive and more versatile (6). A number of pre-column derivatization chemistries, including dansyl chloride (8), *o*-phthalaldehyde (9), 4-fluoro-7-nitrobenzofurazan (10), and 2,4-dinitrofluorobenzene (11), have been used for taurine analysis in milk and beverages.

In AOAC *Official Method*SM **997.05**, following deproteinization of the liquid sample with Carrez solutions, taurine is derivatized pre-column with dansyl chloride, with chromatographic separation achieved by reversed-phase HPLC with either UV or fluorescence detection (12). This technique has been widely used for taurine analysis in the dairy industry for the last two decades (13–15).

In recent years, LC–MS methods for taurine have been developed using standard addition or a nonlabeled internal standard for calibration (16–18). The purpose of this study was to develop a simple, rapid method with minimal sample preparation for free taurine analysis with the reliability, accuracy, and specificity achievable using stable isotope label internal standardization.

Experimental

Apparatus

(a) *HPLC system*.—Nexera X2 UHPLC system consisting of two LC-30AD pumps, an SIL-30AC autosampler, a CTO-20AC column oven, a CBM-20A control module, and a DGU-20A5R degasser unit (Shimadzu, Kyoto, Japan).
(b) *Mass spectrometer*.—6500 QTrap triple quadrupole detector with Analyst software version 1.6 (Sciex, Foster City, CA).
(c) *UHPLC column*.—Kinetex HILIC column, 2.6 μm, 4.6 mm × 100 mm (Phenomenex, Torrance, CA).
(d) *Centrifuge*.—Heraeus Multifuge X3 centrifuge (ThermoFisher, Torrance, CA).
(e) *Analytical balance*.—Mettler-Toledo (Columbus, OH) AE 260 analytical delta range (±0.1 mg) or equivalent, calibrated with National Institute of Standards and Technology (NIST; Gaithersburg, MD) traceable calibration weights.
(f) *Vortex mixer*.—Genius 3 (IKA, Wilmington, NC).
(g) *Micropipettes*.—Eppendorf Research Plus, 20–200 μL and 0.1–1 mL (Hauppauge, NY).
(h) *Centrifuge tubes*.—Polypropylene, 15 and 50 mL (ThermoFisher, Waltham, MA)
(i) *Syringes*.—3 mL Luer-lock (Hapool, Shandong, China).
(j) *Syringe filters*.—Nylon, 0.2 μm pore size × 13 mm i.d. (Merck Millipore, Carrigtwohill, Cork, Ireland).
(k) *Graduated cylinders*.—100 and 1000 mL.
(l) *Volumetric flasks*.—10, 25, 50, and 100 mL.
(m) *Reagent bottles*.—500 and 1000 mL.
(n) *Microcentrifuge vials*.—2 mL.
(o) *HPLC vials*.—2 mL with Teflon-coated caps.

Reagents

(a) *Formic acid (CHOOH)*.—LC-MS grade (Merck, Darmstadt, Germany).
(b) *Potassium hexacyanoferrate trihydrate ($K_4[Fe(CN)_6]\cdot 3H_2O$)*.—Reagent grade (Merck).
(c) *Zinc acetate dihydrate ($Zn(CH_3COO)_2\cdot 2H_2O$)*.—Reagent grade (Merck).
(d) *Acetonitrile (CH_3CN)*.—LC-MS grade (Merck).
(e) *Taurine ($NH_2C_2H_4SO_3H$)*.—Pharmaceutical secondary standard, certified reference material (Merck).
(f) *$^{13}C_2^{15}N$ taurine ($^{15}NH_2^{13}C_2H_4SO_3H$)*.—Stable isotope-labeled standard (Cambridge Isotope Laboratories, Tewksbury, MA).
(g) *Water (H_2O)*.—Purified to ≥18 MΩ resistivity (Barnstead, Dubuque, IA).

Solutions

(h) *Carrez I solution*.—Dissolve 15 g of potassium hexacyanoferrate trihydrate in 100 mL of water (stored at room temperature, expiry 1 month).
(i) *Carrez II solution*.—Dissolve 30 g of zinc acetate dihydrate in 100 mL of water (stored at room temperature, expiry 1 month).
(j) *Mobile phase*.—Mix 900 mL of acetonitrile, 100 mL of water, and 1 mL of formic acid (prepared fresh each run).

Standards

A stable isotope-labeled taurine standard (~0.1 mg/mL) was made by dispensing the contents of a 10 mg vial of $^{13}C_2^{15}N$ taurine into a 100 mL volumetric flask and dissolving in ~90 mL of water. The volumetric flask was shaken to mix the standard solution until completely dissolved and was then made to volume with water. Aliquots (~1.3 mL) were transferred into cryogenic vials and stored frozen at below −15°C for up to 6 months.

A nonlabeled taurine stock standard (1 mg/mL) was made by weighing accurately 100 mg of taurine into a 100 mL volumetric flask and dissolving in ~90 mL of water. The volumetric flask was shaken to mix the standard solution until completely dissolved and was then made to volume with water. Aliquots (~1.3 mL) were transferred into cryogenic vials and stored frozen at below −15°C for up to 6 months. A 2.5 mL aliquot of this stock standard was transferred to a 25 mL volumetric flask, which was made to volume with water. This nonlabeled taurine working standard (0.1 mg/mL) was kept refrigerated at 4–7°C for up to 1 week.

Calibration standards were made fresh by adding 0.03, 0.15, 0.3, 1.5, or 3.0 mL of nonlabeled taurine working standard and adding 5.97, 5.85, 5.7, 4.5, or 3 mL of water, respectively, to five separate 15 mL polypropylene disposable centrifuge tubes, each containing 0.3 mL of stable isotope-labeled taurine standard. Calibration standards were put through the sample extraction procedure in the same manner as nutritional formula samples.

Samples

Method performance was evaluated using a range of nutritional formula powders, including a partially hydrolyzed milk-based infant formula, two bovine milk-based infant formulas, two soy-based infant formulas, an infant formula fortified with fructooligosaccharides and galactooligosaccharides, a child elemental product, a low-fat adult nutritional product, an ovine milk-based infant formula, an infant formula based on bovine A2 β-casein, and two NIST-certified reference materials, NIST 1849a and NIST 1869.

Sample Preparation

Powder samples were prepared by accurately weighing ~5 g of powder into a 50 mL polypropylene disposable centrifuge tube, taring the balance, and accurately weighing 40 mL of added water. The resulting liquid slurry was firmly capped, shaken, and vortex-mixed to ensure that the powder was completely dissolved. A 1 mL aliquot of the liquid slurry was accurately weighed into a 15 mL polypropylene disposable centrifuge tube, and then 0.3 mL of stable isotope-labeled stock standard and 5 mL of water were added, after which the tubes were capped and vortex-mixed for 20 s each.

Sample Extraction

To centrifuge tubes containing either the calibration standard or sample, 0.1 mL aliquots of Carrez I solution and Carrez II solution were added, after which the tubes were capped and vortex-mixed for 20 s each. The centrifuge tubes were left to stand for 20 min with vortex mixing once every 5 min. The tubes were then centrifuged at 2000 × *g* for 10 min. The supernatant was syringe filtered into a microcentrifuge vial, and 0.1 mL of this extract was transferred to an HPLC vial containing 0.9 mL of acetonitrile. The HPLC vial was capped and vortex-mixed ready for LC–MS analysis the same day.

LC–MS Analysis

The chromatography consisted of a Kinetex HILIC analytical column using a mobile phase comprising a mixture of acetonitrile, water, and formic acid (900:100:0.1, v/v/v). Isocratic elution, with a flow rate of 0.8 mL/min, a column oven temperature of 30°C, a vial chiller temperature of 10°C, and an injection volume of 5 µL, was used.

The electrospray ion source temperature was set to 300°C with an ion-spray voltage of 5500 V. Curtain gas, nebulizer gas (GS1), and drying gas (GS2) were set at 30, 40, and 40 psi, respectively. The collision gas was nitrogen, set at medium flow rate. Detection was performed by electrospray ionization in positive ion mode followed by multiple reaction monitoring (MRM) of target ions (Table 1).

Calculations

A linear calibration plot was constructed, plotting the ratio of peak areas against the ratio of concentrations. The concentration (mg/hg) of taurine in powder samples is given by the following equations:

Table 1. Mass spectrometry parameters

Multiple reaction monitoring transitions, m/z	DP, V[a]	EP, V[b]	CE, V[c]	CXP, V[d]	Dwell time, ms
126.0→108.0[e]	1	10	15	16	150
126.0→65.1[f]			49	16	150
129.0→111.0[g]			15	16	150

[a] DP = Declustering potential.
[b] EP = Entrance potential.
[c] CE = Collision energy.
[d] CXP = Collision cell exit potential.
[e] Taurine quantifier ion.
[f] Taurine qualifier ion.
[g] $^{13}C_2^{15}N$ taurine internal standard.

$$\text{Taurine (mg/hg)} = \frac{PA_{NLT}}{PA_{SILT}} \times \frac{SILSS_{conc}}{L} \times \frac{SILSS_{alqt}}{S_{mass}} \times \frac{100}{1000}$$

where PA_{NLT} = peak area of taurine in sample, PA_{SILT} = peak area of stable isotope-labeled taurine in sample, $SILSS_{conc}$ = concentration of $^{13}C_2^{15}N$ taurine in stable isotope-labeled taurine standard (µg/mL), L = slope of calibration curve, $SILSS_{alqt}$ = volume of stable isotope-labeled taurine standard (mL) aliquot spiked to sample, S_{mass} = mass of sample (g), 100 = mass conversion (g/hg), and 1000 = concentration conversion (µg/mg);

$$S_{mass} = \frac{P_{mass}}{P_{mass} + W_{mass}} \times A_{mass}$$

where S_{mass} = mass of sample (g), P_{mass} = mass of powder (g), W_{mass} = mass of water (g), and A_{mass} = mass of slurry aliquot (g).

Results and Discussion

Method Optimization

A simplified extraction technique based on that used in AOAC Method **997.05** was used for sample extraction. Elimination of the heating and cooling steps of sample extraction prior to addition of the Carrez solutions was found to be beneficial in saving analysis time and did not negatively impact the measured results or the analyte recovery.

Hydrophilic interaction liquid chromatography (HILIC) was identified to be the most appropriate separation technique for the analysis of a polar molecule such as taurine. The chromatographic procedure was modified from that used for the analysis of taurine, glucuronolactone, and glucuronic acid in energy drinks (18). Water is the strong solvent in HILIC; therefore, to avoid problems with peak tailing because of a high water content in the vial, sample extracts were diluted 1:10 with acetonitrile in the HPLC vials to match the elution strength of the mobile phase.

Optimization of the ion source parameters was performed by direct infusion of taurine and isotope-labeled taurine standards. Product ion scans were used to identify major fragment ions, and the most intense MRM transitions were selected for quantitation, with the second most intense ion being selected as the qualifier ion. Unambiguous identification of taurine was assured by selecting a specific elution time window for monitoring characteristic quantifier and qualifier ions, with performance criteria for the ion ratios set at ±25%. The chromatography was optimized for the elution of taurine with a run time of 8 min (Figures 1 and 2).

Method Validation

Detector linearity was performed by the analysis of taurine standard solutions at nine concentration levels (0.06–600 µg/mL ≡ 0.27–2700 mg/hg). Linearity of the method was demonstrated by plotting the instrument response as peak area ratios against the concentration ratios of nonlabeled taurine to stable isotope-labeled taurine. The range exceeds both the lower end and the upper end of the method calibration range by one order of magnitude. The value of the calibration correlation coefficient was calculated as 0.9994, with a residuals plot showing no discernible pattern.

Accuracy of the method was demonstrated in three ways: (i) as bias against reference values in certified reference materials; (ii) as bias against AOAC *Official Method* **997.05** (12); (iii) as spike recovery.

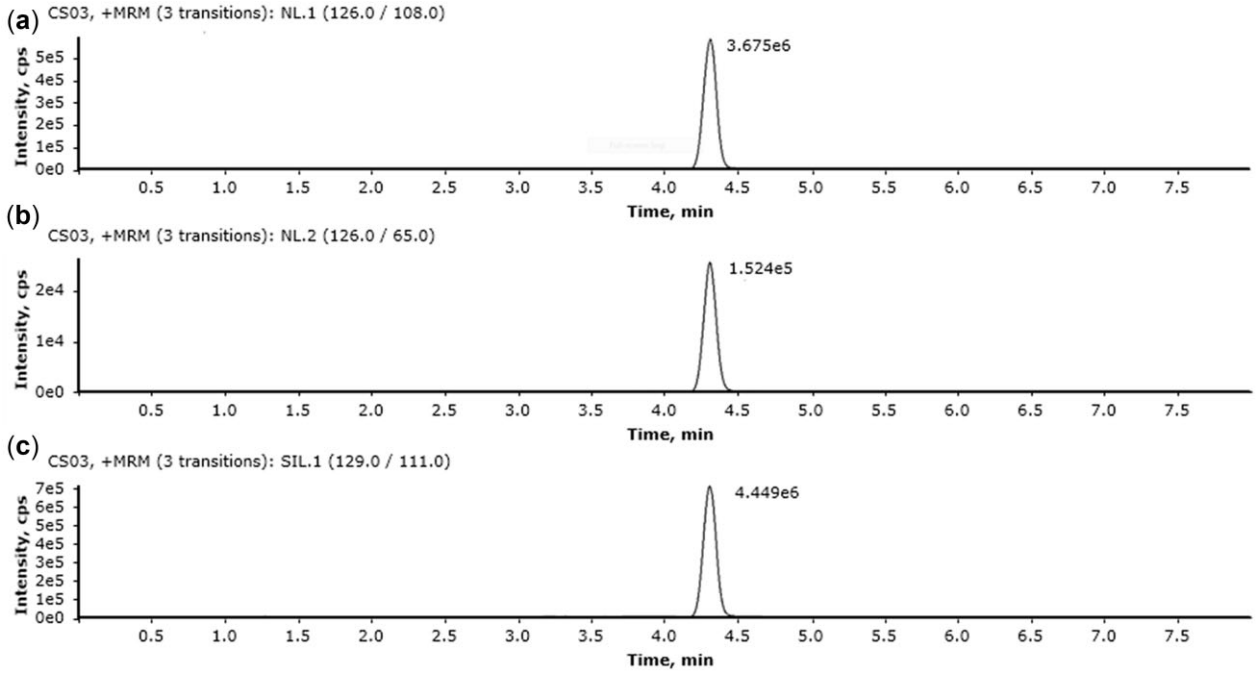

Figure 1. Chromatograms of taurine in a standard mixture: (a) quantifier ion: 126→108; (b) qualifier ion: 126→65; (c) internal standard: 129→110 *m/z*.

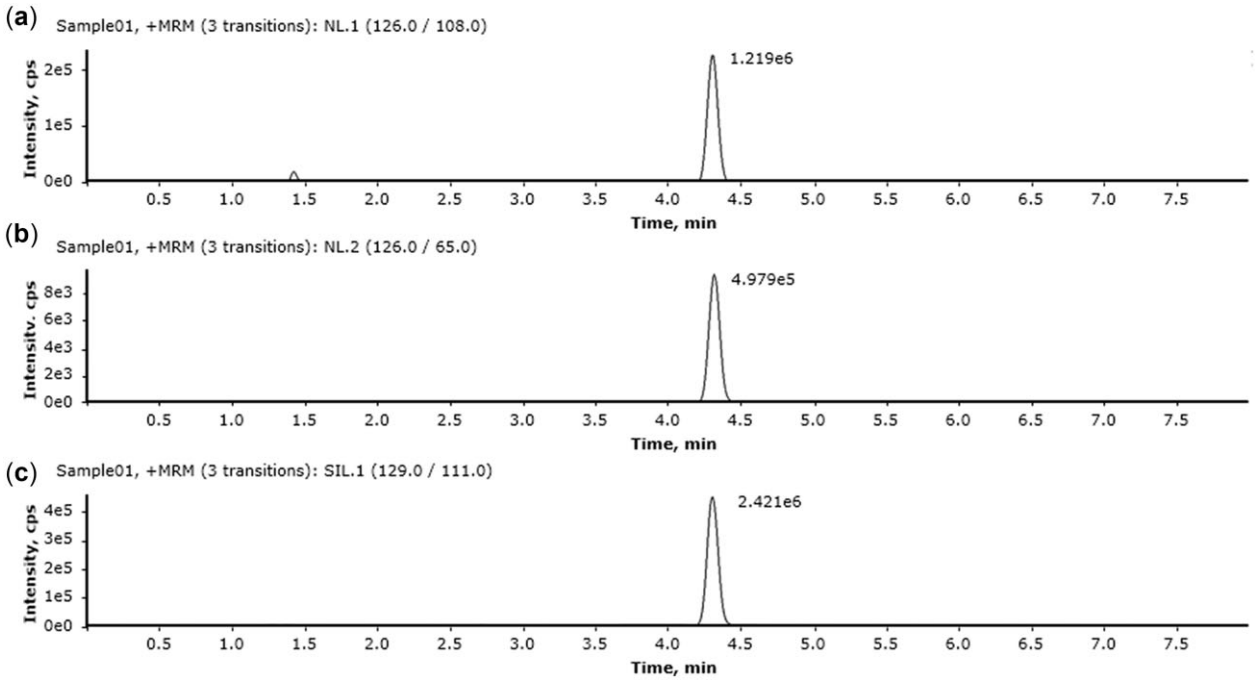

Figure 2. Chromatograms of taurine in an infant formula sample: (a) quantifier ion: 126→108; (b) qualifier ion: 126→65; (c) internal standard: 129→110 *m/z*.

Bias against certified reference materials was evaluated by duplicate testing of NIST 1849a and NIST 1869 certified reference materials over three different days. A paired *t*-test found no bias between results obtained by the HILIC-MS/MS method and the reference value for taurine for either NIST 1849a ($\alpha = 0.05$, $n = 6$, $P = 0.96$) or NIST 1869 ($\alpha = 0.05$, $n = 6$, $P = 0.88$). Bias against AOAC Method **997.05** reference method was evaluated by the testing of 12 infant formula samples by the reference method and the HILIC-MS/MS method. No evidence of bias between results was found in a paired *t*-test of the reference method and the HILIC-MS/MS method results ($\alpha = 0.05$, $P = 0.18$, $n = 12$). Recovery was evaluated by spiking an infant formula in triplicate with a taurine standard to concentrations equivalent to 0.7, 7.0, and 70 mg/hg of taurine in infant formula. The mean recovery value was estimated to be 99.6% (range = 91.1–106.5%). The recovery values were within the expected 85–100% range for samples at the 0.01% (10 mg/hg) concentration level specified in the AOAC *Official Methods of Analysis* (19).

Precision was estimated by the analysis of pairs of duplicates of each infant formula sample ($n = 12$) over three different days. Repeatability was estimated to be 3.1% RSD_r (range: 2.4–4.0%, HorRat: 0.3), and intermediate precision was estimated to be 4.9% RSD_{iR} (range: 2.2–7.7%,). The HorRat value demonstrated that the precision was within the expected range specified in the AOAC *Official Methods of Analysis* (19).

The limit of detection and the limit of quantitation were estimated from the precision of the complete method applied to 10 replicates of a sample containing low levels of analyte (20). The values for the limit of detection and the limit of quantitation were calculated to be 0.39 and 1.30 mg/hg, respectively. The described method is suitable for application to analyze taurine in infant formulas, as these typically contain taurine at concentrations that are at least an order of magnitude higher than the estimated limit of quantitation.

A seven-factor Plackett–Burman robustness trial was performed in a manner described previously (21). Method robustness was evaluated by measuring concentrations of taurine in a milk-based infant formula with the method modified by use of binary levels on either side of the optimized values selected for each of the following factors: volume of water added (5.2 mL, 4.8 mL), volume of Carrez solution added (0.12 mL, 0.08 mL), standing time (25 min, 15 min), centrifuge speed (2200 × *g*, 1800 × *g*), extract volume (0.11 mL, 0.09 mL), acetonitrile volume (0.91 mL, 0.89 mL), and a dummy factor. The impact of each of the factors on the measured results was calculated to be less than the margin of error, indicating that the method was robust and that any variation was due to random variation alone. As with similar methods exploiting stable isotope-labeled internal standard quantitation, critical method parameters include accurate recording of sample weight, the concentration and volume of internal standard added to samples, and the ratio of taurine to internal standard in the calibration standards. These parameters were tightly controlled by using appropriately calibrated pipets and balances traceable to international measurement standards.

The method described can report a single result in less than 2 h, with significant sample throughput of more than 70 samples per day by a single analyst.

Conclusions

A rapid HILICMS/MS method for analysis of free taurine in infant formula is described. The method is suitable for use in high-throughput laboratories for routine product compliance testing of taurine in infant formulas and adult nutritional products. A single-laboratory validation study of the method was performed, showing it to be accurate, precise, and fit for purpose.

CRediT Author Statement

Brendon Gill: Conceptualization, Formal analysis, Visualization, Writing—original draft. Jackie Wood: Writing—review & editing.

Acknowledgments

Rob Crawford, Bharathi Sadipiralla, and Cherilyn Bunning (Fonterra) are gratefully acknowledged for their support in providing time and resources for the completion of this validation study.

Conflict of Interest

All authors declare no conflict of interest.

References

1. Huxtable, R.J. (1992) *Physiol. Rev.* **72**, 101–163. doi:10.1152/physrev.1992.72.1.101
2. Sturman, J.A., & Chesney, R.W. (1995) *Pediatr. Clin. North Am.* **42**, 879–897. doi:10.1016/S0031-3955(16)39022-8
3. Hayes, K.C., & Sturman, J.A. (1981) *Annu. Rev. Nutr.* **1**, 401–425. doi:10.1146/annurev.nu.01.070181.002153
4. Chesney, R.W., Helms, R.A., Christensen, M., Budreau, A.M., Han, X., & Sturman, J.A. (1998) *Adv. Exp. Med. Biol.* **442**, 463–476. doi:10.1007/978-1-4899-0117-0_56
5. Cataldi, T.R.I., Telesca, G., & Bianco, G. (2004) *Anal. Bioanal. Chem.* **378**, 804–810. doi:10.1007/s00216-003-2388-x
6. Gill, B.D., Indyk, H.E., & Woollard, D.C. (2016) *J. AOAC Int.* **99**, 30–41. doi:10.5740/jaoacint.15-0247
7. Nicolas, E.C., Pfender, K.A., Aoun, M.A., & Hemmer, J.E. (1990) *J. AOAC Int.* **73**, 627–631. doi:10.1093/jaoac/73.4.627
8. Woollard, D.C., & Indyk, H.E. (1993) *Food Chem.* **46**, 429–437. doi:10.1016/0308-8146(93)90017-A
9. Omer, M.M.A., Omar, M.M.A., Abdelaziz, M.A., Thiel, A., & Elbashir, A.A. (2019) *J. Food Chem. Nanotechnol.* **5**, 1–7. doi:10.17756/jfcn.2019-065
10. Sawabe, Y., Tagami, T., & Yamasaki, K. (2008) *J Health Sci.* **54**, 661–664. doi:10.1248/jhs.54.661
11. Yan, H.Y., Qiao, F.X., Tian, M., & Row, K.H. (2012) *J. Liq. Chromatogr. Relat. Technol.* **36**, 35–43. doi:10.1080/10826076.2011.644050
12. *Official Methods of Analysis* (2019) 21st Ed., AOAC INTERNATIONAL, Rockville, MD, Method **997.05**, www.eoma.aoac.org (accessed July 25, 2022)
13. Christiansen, S., Guo, M., & Kjelden, D. (2010) *Int. Dairy J.* **20**, 630–636. doi:10.1016/j.idairyj.2009.12.005
14. Walsh, H. (2014) in *Human Milk Biochemistry and Infant Formula Manufacturing Technology*, M. Guo (Ed), Woodhead Publishing, Cambridge, UK, pp 311–344
15. Woollard, D.C., & Indyk, H.E. (1997) *J. AOAC Int.* **80**, 860–865. doi:10.1093/jaoac/80.4.860
16. Chaimbault, P., Alberic, P., Elfakir, C., & Lafosse, M. (2004) *Anal. Biochem.* **332**, 215–225. doi:10.1016/j.ab.2004.06.014
17. de Person, M., Hazotte, A., Elfakir, C., & Lafosse, M. (2005) *J. Chromatogr. A* **1081**, 174–181. doi:10.1016/j.chroma.2005.05.052
18. Ricciutelli, M., Caprioli, G., Cortese, M., Lombardozzi, A., Strano, M., Vittori, S., & Sagratini, G. (2014) *J. Chromatogr. A* **1364**, 303–307. doi:10.1016/j.chroma.2014.08.083
19. *Official Methods of Analysis* (2019) 21st Ed., AOAC INTERNATIONAL, Rockville, MD, Appendix K, www.eoma.aoac.org (accessed July 25, 2022)
20. Magnusson, B., & Örnemark, U. (Eds) (2014) *Eurachem Guide: The Fitness for Purpose of Analytical Methods—A Laboratory Guide to Method Validation and Related Topics*, 2nd Ed., Eurachem, www.eurachem.org (accessed July 25, 2022)
21. Gill, B.D., Indyk, H.E., Kumar, M.C., Sievwright, N.K., & Manley-Harris, M. (2010) *J. AOAC Int.* **93**, 966–973. doi:10.1093/jaoac/93.3.966