



Analysis of Taurine in Infant Formulas and Adult Nutritionals by Hydrophilic Interaction Liquid Chromatography-Mass Spectrometry: First Action 2022.03

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Abstract

Background: Taurine is recognized as an essential growth factor and as being critical in the maintenance of functional tissue regulation.

Objective: To evaluate the analytical performance of a hydrophilic interaction liquid chromatography–tandem mass spectrometry (HILIC-MS/MS) method for compliance with AOAC Standard Method Performance Requirements (SMPR) for taurine analysis described in SMPR 2014.013.

Method: Following protein precipitation with Carrez solutions, taurine is extracted and separated by HILIC with detection by triple quadrupole MS using multiple reaction monitoring (MRM). Stable isotope labeled (SIL) 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 meet the requirements specified in the SMPR with a linear range of 0.27–2700 mg/hg RTF (ready-to-feed), a limit of detection of 0.14 mg/hg RTF, acceptable recovery of 97.2–100.1%, and acceptable repeatability of 1.6–6.4% relative standard deviation. Additionally, the method was found to have no statistically significant bias compared with reference values for National Institute of Standards and Technology (NIST) 1849a certified reference material (CRM) (P-value = 0.95) and 1869 CRM (P-value = 0.31), and with results from AOAC 997.05 (P-value = 0.10).

Conclusions: A recent review of the method and validation data by the Stakeholder Program on Infant Formula and Adult Nutritionals (SPIFAN) Expert Review Panel (ERP) found that this method met all the criteria for analysis of taurine specified in SMPR 2014.013 and voted to adopt this method as First Action AOAC Official Method 2022.03.

Highlights: A method for the analysis of taurine in infant formulas and adult nutritionals by HILIC-MS/MS is described. A single laboratory validation (SLV) study demonstrated the applicability of the method to meet requirements of SMPR 2014.013. In December 2022, the SPIFAN ERP voted to adopt this method as First Action AOAC Official Method 2022.03.

Introduction

Taurine, 2-aminoethanesulfonic acid, is an essential growth factor and is critical for the maintenance of functional tissue regulation(1). Taurine is a b-amino acid and is not incorporated into any protein but instead is found only in the free form. Taurine is a major intracellular free amino acid in humans and is a conditionally essential micronutrient, as a deficiency of taurine may have serious consequences, such as cardiomyopathy, renal dysfunction, and developmental abnormalities, for individuals with low serum levels (2). Taurine is found in significantly lower concentrations in bovine milk compared with human milk and is absent in soy protein; hence, fortification of taurine in infant formulas is necessary and allows formula-fed infants to maintain serum levels equivalent to those of their counterparts who are fed on their mother's milk (3). Precise, accurate, rapid, high throughput analytical methods for taurine are needed for routine testing to ensure that infant formulas and adult nutritional products are manufactured to meet product specifications. Additionally, reference methods using contemporary techniques are needed to guarantee product compliance with global regulations. Currently, there is an Official Method for taurine, AOAC 997.05, in which samples are deproteinized with Carrez solutions, taurine is derivatized pre-column with dansyl chloride, and chromatographic separation is achieved by reversed-phase HPLC with either UV or fluorescence detection (4).

An alternative method based on hydrophilic interaction liquid chromatography-tandem mass spectrometry (HILIC-MS/MS) for the routine compliance testing of taurine in infant formulas and adult/pediatric nutritional formulas was recently reported (5). As part of the SPIFAN program to update reference methods for micronutrients in infant formula and adult nutritionals, taurine was identified as a priority for development of a new reference method. A full single-laboratory validation (SLV) study was undertaken to determine the applicability of this method to the analysis of taurine in infant formulas and adult nutritionals, and to demonstrate compliance with the Standard Method Performance Requirements (SMPR) specified in SMPR 2014.013 (6, 7).

In December 2022, this method and associated SLV data were assessed by the Stakeholder Program on Infant Formula and Adult Nutritionals (SPIFAN) Expert Review Panel (ERP), and the method was approved for First Action Official Method status.

Validation Design

The method was validated as prescribed in the recommended guidelines for SPIFAN SLV (7). More than six levels were used, and the linear range interval extended beyond the bounds of the upper and lower concentrations of the analyte in samples. The limit of detection was estimated from replicate analyses ($n = 10$) of an adult nutritional formula with a low concentration of taurine. Samples selected for precision studies were analyzed in duplicate on each of 6 days using multiple analysts and instruments as practical for the different days. Spike recovery was determined from SPIFAN matrixes with each sample spiked at three concentration levels. In addition to the validation requirements, method bias was evaluated against two National Institute of Standards and Technology (NIST) certified reference materials (CRM) and against another method, AOAC 997.05, a HPLC-UV method for taurine (4).

AOAC Official Method 2022.03

Taurine in Infant Formulas and Adult Nutritionals

HILIC-MS/MS

First Action 2022

[Applicable to the determination of taurine in infant formulas and adult/pediatric nutritional formulas.]

Caution: Refer to Material Safety Data Sheet for all chemicals prior to use. Use all appropriate personal protective equipment and follow good laboratory practices.

A. Principle

After protein precipitation with Carrez solution, taurine in the sample extract is separated by hydrophilic interaction liquid chromatography (HILIC) with detection by tandem mass spectrometry (MS/MS) using multiple reaction monitoring (MRM). Stable isotope labeled (SIL) taurine internal standard is used for quantitation to correct for losses in extraction and any variation in ionization.

B. Apparatus

- (a) UHPLC system.—Nexera, Shimadzu, Kyoto, Japan, consisting of a dual-pump system, a sample injector unit, a degasser unit, and a column oven (or an equivalent instrument).
- (b) Triple quadrupole mass spectrometer.—Triple Quad 6500, Sciex, Framingham, MA (or an equivalent instrument).
- (c) Column.—Kinetex HILIC, 2.6 μm , 4.6 $\times$ 100 mm, Phenomenex, Torrance, CA (or equivalent column).
- (d) Balance.—Digital readout to four decimal places.
- (e) Schott bottles.—500 mL, 1 L.

- (f) Measuring cylinders.—Glass, 100 mL, 1 L.
- (g) Volumetric flasks.—25, 100 mL.
- (h) Disposable centrifuge tubes.—Polypropylene: 15, 50 mL.
- (i) Autopipets.—200 µL, 1 mL, 5 mL.
- (j) Pipet tips.—Polypropylene: 200 µL, 1 mL, 5 mL.
- (k) Disposable syringe.—3 mL.
- (l) Disposable syringe filters.—Nylon, 0.22 µm, 13 mm.
- (m) Centrifuge.—Suitable for 15 mL and 50 mL centrifuge tubes.
- (n) Microcentrifuge vials.—2 mL.
- (o) Cryogenic vials.—2 mL.
- (p) HPLC vials, septa, and caps.

C. Reagents

- (a) Taurine.—Purity $\geq$ 99.5%; Sigma (or equivalent standard material). InChI Key: XOAAWQZATWQOTB-UHFFFAOYSA-N.
- (b) $^{13}\text{C}_2^{15}\text{N}$ taurine.—Purity $\geq$ 98%; Cambridge Isotope Laboratories (or equivalent standard material). InChI Key: XOAAWQZATWQOTB-VMIGTVKRSA-N.
- (c) Potassium hexacyanoferrate trihydrate.—Reagent grade. InChI Key: NLYKFUFUIUWPPIZ-UHFFFAOYSA-N.
- (d) Zinc acetate dihydrate.—Reagent grade. InChI Key: BEAZKUGSCHFXIQ-UHFFFAOYSA-L.
- (e) Acetonitrile.—LC-MS grade. InChI Key: WEVYAHXRMPXWCK-UHFFFAOYSA-N.
- (f) Formic acid.—LC-MS grade. InChI Key: BDAGIHXXWSANSRUHFFFAOYSA-N.
- (g) Water.—Reagent grade ($\geq$ 18 M Ω). InChI Key: XLYOFNOQVPJJNP-UHFFFAOYSA-N.

D. Preparation

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

E. Standard Preparation

- (a) Stable isotope labeled stock standard (SILSS) ~0.1 mg/mL.—Store in freezer for up to 6 months.
- (1) Dispense the contents of a 10 mg vial of $^{13}\text{C}_2^{15}\text{N}$ taurine into a 100 mL volumetric flask.
 - (2) Dissolve in ~9 mL water; vortex-mix thoroughly until dissolved.
 - (3) Dilute to volume with water; shake to mix.
 - (4) Immediately dispense aliquots (~1.3 mL) into cryogenic vials and freeze at $< -15^\circ\text{C}$.
- (b) Non-labeled stock standard (NLSS) ~1 mg/L.—Store in refrigerator for up to 1 week.
- (1) Weigh accurately approximately 100 mg taurine into a 100 mL volumetric flask.
 - (2) Dissolve in ~90 mL water; vortex-mix thoroughly until dissolved.
 - (3) Dilute to volume with water; shake to mix.
 - (4) Store in refrigerator under nitrogen at $4-7^\circ\text{C}$.
- (c) Non-labeled working standard (NLWS) 0.1 mg/mL.—Make fresh daily.
- (1) Pipet 2.5 mL NLSS into a 25 mL volumetric flask. Dilute to volume with water.
- (d) Calibration standards.—Make fresh daily (Table 2022.03A).
- (1) Calibration Standard 1 (CS1).—Pipet 0.3 mL SILSS, 0.03 mL NLWS, and 5.97 mL water into a 15 mL centrifuge tube.
 - (2) Calibration Standard 2 (CS2).—Pipet 0.3 mL SILSS, 0.15 mL NLWS, and 5.85 mL water into a 15 mL centrifuge tube.
 - (3) Calibration Standard 3 (CS3).—Pipet 0.3 mL SILSS, 0.3 mL NLWS, and 5.7 mL water into a 15 mL centrifuge tube.
 - (4) Calibration Standard 4 (CS4).—Pipet 0.3 mL SILSS, 1.5 mL NLWS, and 4.5 mL water into a 15 mL centrifuge tube.
 - (5) Calibration Standard 5 (CS5).—Pipet 0.3 mL SILSS, 3 mL NLWS, and 3 mL water into a 15 mL centrifuge tube.
 - (6) Standards (CS1–CS5) in 15 mL centrifuge tube are put through the analytical method in the same manner as samples. See F(c)(1).

F. Sample Preparation

- (a) Slurry preparation.—Make fresh prior to analysis.
- (1) Accurately weigh ~5 g powder into a 50 mL disposable centrifuge tube. Record weight.
 - (2) Accurately weigh ~40 g water into the powder. Record weight.
 - (3) Cap firmly and vortex mix until dissolved.
 - (4) Shake each slurry immediately prior to weighing to ensure homogeneity.
 - (5) Accurately weigh ~1.0 mL slurry into a 15 mL centrifuge tube. Record weight.
 - (6) Add 0.3 mL SILSS to each sample centrifuge tube.
 - (7) Add 5 mL water. Cap and shake to mix.
- (b) Ready-to-drink liquid preparation.—Make fresh prior to analysis.
- (1) Accurately weigh ~1 mL liquid into a 50 mL disposable centrifuge tube. Record weight.
 - (2) Add 0.3 mL SILSS to each sample centrifuge tube.
 - (3) Add 5 mL water. Cap and shake to mix.
- (c) Extraction.
- (1) Add 0.1 mL Carrez I solution and 0.1 mL Carrez II solution. Shake to mix.
 - (2) Stand for 20 min. Shake to mix every 5 min.
 - (3) Centrifuge at $2000 \times g$ for 10 min.
 - (4) Syringe filter ~1–2 mL of supernatant into a microcentrifuge vial.
 - (5) Transfer 0.1 mL extract in the microcentrifuge vial into an HPLC vial; add 0.9 mL acetonitrile. Cap and vortex mix.

G. Chromatography

- (a) Set up the UHPLC system with settings given in Table 2022.03B.

H. Mass Spectrometry

- (a) Set up the mass spectrometer with settings in Table 2022.03C.
- (b) The specific compound parameters to be used are given in Table 2022.03D.

I. System Suitability

- (a) At the beginning of every analytical run:
- (1) Run: 3 × taurine calibration standard (CS3).
 - (2) Repeatability as RSD_r of ratio of peak areas of taurine and $^{13}C_2^{15}N$ taurine should be less than 2%.
 - (3) Repeatability as RSD_r of retention time in seconds should be less than 5%.

J. Calculations

- (a) Concentration of $^{13}C_2^{15}N$ taurine in stable isotope labelled stock standard (SILSS):

$$SILTSS_{Tconc} = \frac{10}{100} \times 1000$$

where $SILSSTconc$ = concentration of $^{13}C_2^{15}N$ taurine in SILSS ($\mu g/mL$); 10 = mass of $^{13}C_2^{15}N$ taurine in SILSS (mg); 100 = final of volume of SILSS (mL); 1000 = concentration conversion from mg to μg .

- (b) Concentration of taurine in non-labeled stock standard (NLSS):

$$NLSS_{Tconc} = \frac{100}{100} \times 1000$$

where $NLSSTconc$ = concentration of taurine in NLSS ($\mu g/mL$); 100 = mass of taurine in NLSS (mg); 100 = final of volume of NLSS (mL); 1000 = concentration conversion from mg to μg .

- (c) Concentration of taurine in non-labeled working standard (NLWS):

$$NLWS_{Tconc} = NLSS_{Tconc} \times \frac{2.5}{25}$$

where $NLWS_{Tconc}$ = concentration of taurine in NLWS ($\mu g/mL$); $NLSS_{Tconc}$ = concentration of taurine in NLSS ($\mu g/mL$); 2.5 = volume of NLSS in NLWS (mL); 25 = final of volume of NLWS (mL).

- (d) Concentration of taurine in calibration standards (CS1–CS5):

$$CS1_{Tconc} = NLWS_{Tconc} \times \frac{0.03}{6.3}$$

$$CS2_{Tconc} = NLWS_{Tconc} \times \frac{0.15}{6.3}$$

$$CS3_{Tconc} = NLWS_{Tconc} \times \frac{0.3}{6.3}$$

$$CS4_{Tconc} = NLWS_{Tconc} \times \frac{1.5}{6.3}$$

$$CS5_{Tconc} = NLWS_{Tconc} \times \frac{3.0}{6.3}$$

where $CS1$ – $CS5_{Tconc}$ = concentration of non-labeled taurine in $CS1$ – $CS5$ ($\mu\text{g/mL}$); $NLWS_{Tconc}$ = concentration of non-labeled taurine in $NLWS$ ($\mu\text{g/mL}$); 0.03, 0.15, 0.3, 1.5, 3.0 = volume of $NLWS$ in $CS1$ – $CS5$ (mL); 6.3 = final volume of $CS1$ – $CS5$ (mL).

- (e) Concentration of $^{13}\text{C}_2^{15}\text{N}$ taurine in calibration standards

$$CS1$$
– $CS5_{Tconc} = SILSS_{Tconc} \times \frac{0.3}{6.3}$

where $CS1$ – $CS5_{Tconc}$ = concentration of $^{13}\text{C}_2^{15}\text{N}$ taurine in $CS1$ – $CS5$ ($\mu\text{g/mL}$); $SILSS_{Tconc}$ = concentration of $^{13}\text{C}_2^{15}\text{N}$ taurine in $SILSS$ ($\mu\text{g/mL}$); 0.3 = volume of $SILSS$ in $CS1$ – $CS5$ (mL); 6.3 = final volume of $CS1$ – $CS5$ (mL).

- (f) Determine the linear regression curve $y = mx + b$ (using the “least squares” method) for the ratio of peak areas (non-labeled taurine/ SIL taurine) versus the ratio of concentrations (non-labeled taurine/ SIL taurine) for the five calibration standards with the y-intercept forced through zero.
- (g) The concentration of taurine in the sample is calculated as:

$$\text{Result}_{\text{Taurine}} = \frac{PA_{\text{NLT}}}{PA_{\text{SILT}}} \times \frac{SILSS_{Tconc}}{L} \times \frac{SILSS_{alqt}}{S_{\text{mass}}} \times \frac{100}{1000}$$

where $\text{Result}_{\text{Taurine}}$ = taurine concentration in sample (mg/hg); PA_{NLT} = peak area of taurine in sample (no units); PA_{SILT} = peak area of SIL taurine in sample (no units); $SILSS_{Tconc}$ = concentration of $^{13}\text{C}_2^{15}\text{N}$ taurine in $SILSS$ ($\mu\text{g/mL}$); L = slope of calibration curve (no units); $SILSS_{alqt}$ = volume of $SILSS$ aliquot spiked to sample (mL) (0.3 mL); 1000 = concentration conversion factor (mg/g to mg/g); 100 = concentration conversion factor (mg/g to mg/hg); S_{mass} = mass of sample (g).

- (h) Mass of powder in slurried sample:

$$S_{\text{mass}} = \frac{D_{\text{mass}}}{(D_{\text{mass}} + W_{\text{mass}})} \times A_{\text{mass}}$$

where S_{mass} = the mass of sample (g); D_{mass} = the mass of powder used to make the slurry (g) (see F(a)(1)); W_{mass} = the mass of water used to make the slurry (g) (see F(a)(2)); A_{mass} = the mass of the aliquot of slurried sample used in the analysis (g) (see F(a)(5)).

- (i) Report the result as mg/hg to 1 decimal place.

Results and Discussion

Method Performance

HILIC was identified to be the most appropriate separation technique for the analysis of polar molecules such as taurine. In the development of this method, several considerations were applied to provide a method that was simple to use, rapid, and high throughput. This method also meets the performance characteristics necessary for it to be considered a fit-for-purpose reference method.

There are many possible approaches to protein removal from the sample matrix; this method uses Carrez solutions because they afford significant advantages in health and safety compared with other precipitants that are commonly used, such as trichloroacetic acid.

The chromatographic separation is isocratic, and, as no late eluting compounds were found to carry over between samples, the need to use a gradient mobile phase system and to consequently allow time for column equilibration was eliminated, thereby allowing for a rapid analysis time and a high sample throughput.

The method uses a stable carbon and nitrogen isotope labelled internal standard, avoiding the pitfalls that can be associated with deuterated internal standards, such as label exchange, and possible chromatographic separation of deuterated compound from non-deuterated compound.

Method Validation

The linear range interval should extend beyond the bounds of the upper and lower concentrations of the analyte in samples (7). The range of taurine concentrations in linearity standards was 0.06–600 µg/mL, equivalent to a sample powder concentration of 0.27–2700 mg/hg RTF (ready-to-feed), which extends beyond both the lower limit and the upper limit of the range specified in SMPR 2014.013 (0.5–2500 mg/hg RTF). Linearity was evaluated by testing nine calibration standards through the analytical procedure on three different days. Regression and residual plots of the ratios of the peak areas of analyte taurine to internal standard against the ratios of the concentrations of analyte taurine to internal standard demonstrated acceptable correlation coefficients (≥ 0.9983), with the residuals plot showing no discernible pattern (Figure 1).

Precision as repeatability and intermediate precision was assessed by testing duplicate pairs of samples ($n = 12$) of different products on six separate days. The estimated repeatability for the method was 1.6–6.4% RSD_r with corresponding HorRat_r values within or better than the expected range of 0.3–1.3 (8).

Repeatability at the concentration ranges specified in SMPR 2014.013 was evaluated by spiking an adult nutritional formula at three concentration levels in the ranges: low (~2 mg/hg RTF); medium (~20 mg/hg RTF); high (~200 mg/hg RTF). The estimated values for repeatability at 2, 20, and 200 mg/hg RTF level were 4.5, 1.8, and 1.8% RSD_r, respectively, each of which complies with the limits specified in the SMPR (Table 1).

Intermediate precision values were estimated to be within 3.4–7.3%, showing that the method would likely provide acceptable precision in a multi-laboratory study. Intermediate precision is not a requirement of the SMPR.

The limit of detection determined as the method detection limit must be based on the analysis of samples that have been taken through the whole measurement procedure using results calculated with the same equation as for the test samples (9).

The limit of detection was estimated from replicate analyses ($n = 10$) of an adult nutritional formula with a low concentration of taurine and was calculated to be 0.04 mg/hg RTF with the corresponding limit of quantification of 0.14 mg/hg RTF, which complies with the SMPR.

Accuracy for the method was assessed in several ways: as bias against a CRM, as bias against the current AOAC Official Method, and as spike recovery. Two CRMs were evaluated (NIST 1849a, 36.6 ± 1.8 mg/hg; NIST 1869, 37.2 ± 3.2 mg/hg). Differences between the measured value and the reference value are determined with the mean and standard deviation of the differences, and the test-statistic is calculated. The probability of the observation ($H_0 = 0$, $\alpha = 0.05$) under the null hypothesis was assessed (10). The calculated P-values for both materials exceeded 0.05 [NIST 1849a ($P = 0.95$) and NIST 1869 ($P = 0.31$)], indicating that there was no bias between the reference values and the measured values. Bias against a CRM is not a method performance requirement given in the SMPR (Table 2).

The method was also evaluated for bias against AOAC 997.05, a HPLC UV method for taurine (4), by testing a set of different products and comparing the results using a paired t-test. The calculated P-value of 0.10 indicates that there was no statistically significant difference between this method and AOAC 997.05. Bias against a reference method is not a method performance requirement given in SMPR 2014.013 (Table 3).

Recovery was evaluated using an adult nutritional formula with a low concentration of endogenous taurine. Replicate samples were spiked at three concentration levels: low (~0.7 mg/hg); medium (~7 mg/hg); and high (~70 mg/hg). Spiked and unspiked samples were analyzed in triplicate on three separate days. Average recoveries were estimated to be 97.2–100.1% (95% confidence interval: 95.6–101.0%), within the limits specified by the SMPR and consistent with the recoveries expected (80–115%) at ~10 mg/hg concentration (11).

Additionally, recovery at the concentration ranges specified in the SMPR were evaluated by spiking an adult nutritional formula made up as RTF samples (25 g powder/200 g water) at three concentration levels in the ranges: low (~2 mg/hg RTF); medium (~20 mg/hg RTF); and high (~200 mg/hg RTF). Spiked and unspiked samples were analyzed in triplicate on three separate days. The average method recoveries were estimated to be 99.2–101.4% (95% confidence interval: 96.1–105.3%), within the limits set in the SMPR (Table 4).

Robustness is normally expressed as the lack of influence on the test results of operational variables in the analytical method. A robustness trial is used to evaluate the effect on test results of the variation in conditions normally expected from a test method. The robustness of the method was assessed by conducting a Plackett–Burman trial (12, 13) with graphical and statistical interpretation of the results (14, 15). The factors assessed were volume of water added to the sample (5.2, 4.8 mL); volume of Carrez I and Carrez II solutions added (0.12, 0.08 mL); wait time (25, 15 min); centrifuge speed (2200, 1800 rcf); extract volume (0.11, 0.09 mL); acetonitrile volume (0.91, 0.89 mL); and a dummy factor (left-hand, right-hand). The seven factors were assessed in the study, and whether statistically significant changes occurred in the measured results because of the method changes for each factor was evaluated. The candidate method was found to be robust for the method parameters evaluated, and the results obtained were normally distributed, with variances conforming to those expected by chance.

As with similar methods exploiting SIL internal standard quantitation, critical method parameters include accurate measurement of sample weights, as well as the amount of internal standard added to the samples and to the calibration standards. These parameters in the method are tightly controlled by using appropriately calibrated pipets and balances.

Conclusions

A SLV study of a HILIC-MS/MS method for the analysis of free taurine 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. The method was shown to be accurate, precise, and fit for purpose.

CRedit Author Statement

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

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Conflict of Interest

All authors declare no conflict of interest.

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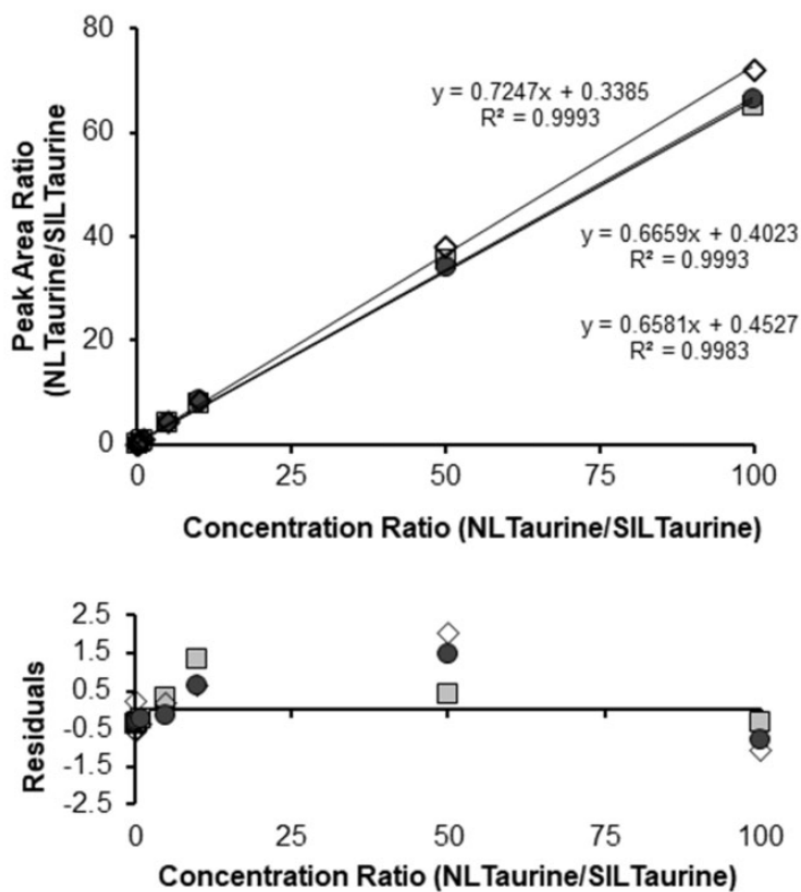


Figure 1. Linearity plot of ratio of peak area to concentration ratio for standard containing non-labeled taurine (NL) and stable isotope labelled (SIL) taurine

Table 1. Precision of taurine in powder samples by LC-MS method

Sample	Repeatability		Intermediate precision (% RSD _{IR})
	(% RSD _r)	(HorRat _r)	
NIST 1869 certified reference material powder ^a	6.4%	0.6	7.3
Partially hydrolyzed milk-based infant formula powder	4.6%	0.4	4.9
Bovine milk-based infant formula powder	2.8%	0.2	3.4
Soy-based infant formula powder	4.5%	0.4	5.0
FOS/GOS infant formula powder ^{b,c}	3.9%	0.4	5.2
Child elemental powder	3.5%	0.3	3.7
NIST 1849a certified reference material powder	1.6%	0.1	4.0

^a NIST = National Institute of Standards and Technology^b FOS = Fructooligosaccharides^c GOS = Galactooligosaccharides

Table 2. Results for bias experiment against NIST 1849a and 1969 CRM^{a,b}

Parameter	NIST 1849a	NIST 1869
Reference value (mg hg ⁻¹)	6.4%	37.2
Uncertainty (mg hg ⁻¹)	4.6%	3.2
Coverage factor (k)	2.0	2.0
Degrees of freedom (df _{CRV}) ^c	60	60
Mean ($\bar{x}$, mg hg ⁻¹)	36.7	35.4
Standard deviation (s, mg hg ⁻¹)	1.47	2.59
Number of replicates (n)	24	24
95% confidence interval (mg hg ⁻¹)	36.0–37.3	34.3–36.5
t _{stat}	0.067	1.025
Degrees of freedom (df)	72.36	72.24
P-value	0.95	0.31

^a NIST = National Institute of Standards and Technology

^b CRM = certified reference materials

^c Calculated for k, using a 2-sided t-distribution at $\alpha = 0.05$. An empirical model for doing this calculation was obtained by fitting $1/DF$ versus $\log t_{0.05}$ to a 4th-order polynomial for integral values of DF from 2 to 100 DF. The maximum relative error over the fitted range was 0.06%

Table 3. Results for bias experiment against AOAC 997.05

Parameter	Reference method	LC-MS/MS method
Mean ($\bar{x}$, mg hg ⁻¹)	36.9	37.8
Standard deviation (s , mg hg ⁻¹)	2.70	3.14
Number of replicates (n)		16
Mean difference ($\bar{d}$, %)		-0.9%
Standard deviation difference (s_m , mg hg ⁻¹)		1.8
t_{stat}		-1.78
Degrees of freedom ($df = n - 2$)		14
P-value		0.06

Table 4. Taurine recovery of spiked adult nutritional formula sample

Spiked adult nutritional formula	SMPR limit ^b	Recovery, %		
		Mean	Standard deviation	95% confidence interval
Low concentration	100 ± 12%	99.2	4.6	96.1–102.2%
Medium concentration	100 ± 10%	102.3	4.3	99.5–105.2%
High concentration	100 ± 7%	101.4	5.9	97.6–105.3%

^a RTF = ready-to-feed.^b SMPR = Standard Method Performance Requirements.