

4.1.12

AOAC Official Method 999.12
Taurine in Pet Food
First Action 1999

(Applicable to the determination of 150–2000 mg total taurine/kg wet or dry cat or dog foods.)

See Table 999.12A for the results of the interlaboratory study supporting acceptance of method.

A. Principle

The test portion is hydrolyzed with HCl and the extracted taurine reacted with dansyl chloride to form a fluorescent derivative determined by reverse-phase HPLC.

B. Apparatus

(a) *Liquid chromatograph*.—Gradient elution system, automatic sampler with 20 μ L loop.

(b) *Fluorescence detector*.—Excitation wavelength, 298 nm; emission wavelength, 550 nm.

(c) *HPLC column*.—4 or 5 μ m silica C8 or C18 reverse-phase column. (Note: A variety of specific column types may be accommodated with appropriate manipulation of the gradient elution profile.)

(d) *Filtration system*.—PVDF disposable syringe filters, 0.2 μ m (Gelman Acrodisc, or equivalent).

(e) *Vortex mixer*.

C. Reagents

(a) *Dilute hydrochloric acid*.—6M. Add slowly 500 mL concentrated HCl to 500 mL water.

(b) *Sodium carbonate solution*.—0.2M. pH 9.7. Dissolve 21.2 g Na_2CO_3 in about 800 mL water. Adjust pH to 9.7 with 6M HCl, (a), using pH meter and dilute to 1 L. Solution is stable at least 6 months at room temperature.

(c) *Acetonitrile*.—HPLC grade.

(d) *Acetonitrile–water solution*.—70 + 30 (v/v).

(e) *Acetone*.—Minimum 99.5%.

(f) *Dansyl chloride solution*.—(1) *Stock solution* (100 mg/mL).—Dissolve 1.000 g dansyl chloride (Sigma Chemical Co., 95%) in acetone, (e), in 10 mL volumetric flask and dilute to volume. Solution is stable 1 month when stored in the dark. (2) *Working solution*.—Prepare immediately before use. Dilute 1 mL stock solution, (1), to 10 mL with acetonitrile–water solution, (d).

(g) *Orthophosphoric acid solution*.—2% (v/v). Dilute 2.35 mL 85% H_3PO_4 to 100 mL with water.

(h) *Phosphate buffer solution*.—0.5M. pH 6.2. Dissolve 30.6 g KH_2PO_4 and 4.36 g K_2HPO_4 in about 400 mL water and adjust pH to 6.2 with H_3PO_4 . Dilute to 500 mL with water.

(i) *Taurine standard solutions*.—(1) *Stock solution* (1 mg/mL).—Dissolve 100.0 mg taurine (Sigma Chemical Co., 99%) in water and dilute to 100 mL. Solution is stable 1 week at 4°C. (2) *Working standard solutions*.—Dilute aliquots of stock solution with water to prepare solutions containing 0, 10, 20, 50, 80, and 100 μ g/mL. Solutions are stable 1 week at 4°C.

(j) *Mobile phase*.—*Solvent A*.—0.02M phosphate buffer, pH 3.0. Dissolve 2.72 g KH_2PO_4 in about 800 mL water and adjust pH to 3.0 with H_3PO_4 . Dilute to 1 L with water. Filter through membrane, B(d). *Solvent B*.—Mix 600 mL acetonitrile with 400 mL mobile phase A.

D. Isolation and Derivatization

Grind representative quantity (dry pet food) to a fine powder. Homogenize the content (wet pet food) of one unit with a homogenizer.

(a) *Hydrolysis*.—Weigh 400 mg ground dry food or 800 mg homogenized wet food into a 25 mL screw cap reagent bottle. Add 10 mL 6M HCl, C(a). Cap bottle and hydrolyze mixture in oven at 110°C for 16 h. Cool to room temperature, transfer hydrolysate quantitatively to a 25 mL volumetric flask, and dilute to mark with water. Filter approximately 2 mL diluted hydrolysate through 0.2 μ m disposable syringe filter. Pipet 250 μ L filtered hydrolysate into 2 mL reaction vial and evaporate to dryness under gentle stream of N. Keep temperature <70°C to avoid loss of taurine.

(b) *Derivatization*.—Dissolve the residue obtained from D(a) in 100 μ L water and Vortex mix. Add 0.5 mL Na_2CO_3 solution, C(b), and Vortex mix for 10 s. Add 0.5 mL dansyl chloride working solution, C(f)(2), and Vortex mix for 10 s. Cap the vial and place in oven or heating block at 65°C for 30 min. Cool to room temperature and add 100 μ L 2% H_3PO_4 solution. Vortex mix for 10 s. Add 0.5 mL 0.5M phosphate buffer and 0.3 mL water and Vortex mix for 10 s. Transfer reaction mixture to a 2 mL syringe and pass through a 0.2 μ m disposable syringe filter into HPLC autosampler vial (solution may be stored for up to 2 days at –20°C until ready for analysis).

Treat 100 μ L of each working standard solution, C(i)(2), the same as the reconstituted residue above.

E. HPLC Determination

Use flow rate, 1.5 mL/min and gradient program, percent solvents A and B, C(j), and Table 999.12B. Optimize separation conditions to achieve satisfactory separation of the taurine peak.

Establish stable HPLC performance by repeatedly injecting a 20 μ L aliquot of a calibrant derivative using the gradient elution profile, Table 999.12B, which may be varied to suit individual analytical column characteristics.

Inject 20 μ L aliquots of calibrant and test derivatives and establish peak response (area or height) using an electronic integrator or computer package.

Table 999.12A Interlaboratory study results for total taurine in cat and dog food

Product	No. of labs	No. outliers excluded	Avg., mg/kg	RSD _r , %	r, mg/kg	RSD _R , %	R, mg/kg
Wet cat food 1	8	1	526	4.1	61	6.1	90
Wet cat food 2	7	2	514	3.9	56	6.6	94
Wet dog food 3	9	0	167	10.0	47	10.2	48
Wet dog food 4	9	0	333	7.4	69	16.1	150
Dry cat food 5	7	2	936	3.2	84	8.9	233
Dry cat food 6	8	0	2256	5.7	361	13.3	839

Table 999.12B Gradient program

Time (min)	Percent	
	A	B
0	80	20
21	71	29
25	20	80
40	20	80
42	80	20
47	80	20

Construct a multiple level calibration with forced origin at 0. Reject the analysis if the 0.00 µg/mL calibrant fails to give a zero response and determine the cause of the interference. Calibration line should be linear.

F. Calculation

Calculate total taurine (mg/kg) as follows:

$$\text{Taurine, mg/kg} = \frac{R}{S} \frac{100}{250} \frac{25}{W}$$

where R_{test} = peak response (area or height) of taurine in test extract; S = slope of calibration curve; 100 = volume of reconstituted extract (µL); 250 = volume of extract taken for derivatization (µL); 25 = final volume of test extract (mL); W = weight of test portion (g).

To convert a result from mg/kg into g/100 g, divide the figure obtained by 10 000.

Reference: *J. AOAC Int.* **83**, 784(2000).

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