

#### 4.1.12 - Animal Feed / Animal Feed--General

## 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  $\mu$ L loop.

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

(c) *HPLC column*.—4 or 5  $\mu$ 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  $\mu$ 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  $\text{Na}_2\text{CO}_3$  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%  $\text{H}_3\text{PO}_4$  to 100 mL with water.

(h) *Phosphate buffer solution*.—0.5M. pH 6.2. Dissolve 30.6 g  $\text{KH}_2\text{PO}_4$  and 4.36 g  $\text{K}_2\text{HPO}_4$  in about 400 mL water and adjust pH to 6.2 with  $\text{H}_3\text{PO}_4$ . 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  $\mu\text{g/mL}$ . Solutions are stable 1 week at 4°C.

(j) *Mobile phase*.—*Solvent A*.—0.02M phosphate buffer, pH 3.0. Dissolve 2.72 g  $\text{KH}_2\text{PO}_4$  in about 800 mL water and adjust pH to 3.0 with  $\text{H}_3\text{PO}_4$ . 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  $\mu\text{m}$  disposable syringe filter. Pipet 250  $\mu\text{L}$  filtered hydrolysate into 2 mL reaction vial and evaporate to dryness under gentle stream of N. Keep temperature  $<70^{\circ}\text{C}$  to avoid loss of taurine.

**(b) Derivatization.**—Dissolve the residue obtained from **D(a)** in 100  $\mu\text{L}$  water and Vortex mix. Add 0.5 mL  $\text{Na}_2\text{CO}_3$  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  $65^{\circ}\text{C}$  for 30 min. Cool to room temperature and add 100  $\mu\text{L}$  2%  $\text{H}_3\text{PO}_4$  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  $\mu\text{m}$  disposable syringe filter into HPLC autosampler vial (solution may be stored for up to 2 days at  $-20^{\circ}\text{C}$  until ready for analysis).

Treat 100  $\mu\text{L}$  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  $\mu\text{L}$  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  $\mu\text{L}$  aliquots of calibrant and test derivatives and establish peak response (area or height) using an electronic integrator or computer package.

Construct a multiple level calibration with forced origin at 0. Reject the analysis if the 0.00  $\mu\text{g/mL}$  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:

$$\text{Taurine, mg/kg} =$$

where  $R_{\text{test}}$  = peak response (area or height) of taurine in test extract;  $S$  = slope of calibration curve; 100 = volume of reconstituted extract ( $\mu\text{L}$ ); 250 = volume of extract

taken for derivatization ( $\mu\text{L}$ ); 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).

*Revised: March 2002*

**[Table 999.12A: Interlaboratory study results for total taurine in cat and dog food](#)**

**[Table 999.12B: Gradient program](#)**

© 2002 AOAC INTERNATIONAL