

# Guide to Method Format

(Method shown is incomplete to allow space for description.)

**Locator number** identifies method by chapter, subchapter, and sequence within the subchapter for easy cross referencing and access. 4 = chapter 4; .10 = subchapter 10; .03 = the third method found in Chapter 4, subchapter 10. The locator number is not the permanent number and is included only for convenient accessibility.

**Chemical names** of pesticides and drugs are given at end of pertinent chapter.

**Calculation symbols** are identified and show correct units.

**Chemical Abstracts Service Registry Number.** A unique identifier that may be used to search a number of data-retrieval systems.

4.10.03

**AOAC Official Method 996.13**  
**Ethoxyquin in Feeds**  
**Liquid Chromatographic Method**  
**First Action 1996**  
**Final Action 1997**

(Applicable for determination of 0.5–300 g/g ethoxyquin in dry extruded pet food or meat meal.)

See Table 996.13 for the results of the interlaboratory study supporting acceptance of the method.

**A. Principle**

Ethoxyquin is extracted with acetonitrile. Extract is analyzed by isocratic liquid chromatography with fluorescence detection.

**B. Apparatus**

(a) *Liquid chromatograph (LC)*.—Generating 1500–200 psi; with peak area integrator (manual or computer), isocratic LC pump, and column heater. Operating conditions: injection volume, 20 µL; flow rate, 1.3 mL/min; temperature, 35°C; fluorescence detector output, analog to digital conversion; detector settings: excitation, 360 nm; emission, 432 nm.

(b) *LC column*.—250 × 4.6 mm id, C<sub>18</sub> octadecylsilane, 5 µm spherical, 100 Å pore size.

**C. Reagents**

- (a) *Water*.—LC grade.
- (b) *Acetonitrile*.—LC grade.

**D. Preparation of Standard Solutions**

(a) *Ethoxyquin standard stock solution*.—400 µg/mL. Weigh the equivalent of 0.1000 g liquid ethoxyquin into 250 mL amber volumetric flask and dilute to volume with acetonitrile. (Note: Amount of ethoxyquin needed for preparation of stock solution is based on purity of liquid, e.g., for purity of 93.5%, amount of liquid ethoxyquin = 0.100/0.935 = 0.1070 g.)

**H. Calculations**

Calculate concentration of ethoxyquin, g/g or ppm, in test sample from calibration curve (using linear regression with line forced through zero intercept) as follows:

$$\text{Ethoxyquin, g/g or ppm} = \frac{C \cdot 1.5 \cdot F}{W}$$

where *C* = ethoxyquin concentration from LC calibration curve, µg/mL; 1.5 = volume of acetonitrile added to test solution, mL; *F* = dilution factor; *W* = weight of test portion, g.

Reference: *J. AOAC Int.* **80**, 725(1997).

CAS-91-53-2 (ethoxyquin) 6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline

Revised: March 1998

**Permanent number** identifies method by year of adoption or first appearance in *Official Methods of Analysis of AOAC INTERNATIONAL*. 996 = First Action 1996; .13 = sequence of adoption in 1996.

**Title** may include analyte and matrix, type of method, and official status.

**Applicability statement** addresses utility and limitations on use of method or other information.

**Specifications** for necessary laboratory apparatus and reagent preparations. See also *Definition of Terms and Explanatory Notes*.

**Method** may be divided into several descriptive sections.

**References** direct the user to the published collaborative study and any subsequent revisions in the method. Other informative references may be included.