

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 µg/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, C₁₈ 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, µg/g or ppm, in test sample from calibration curve (using linear regression with line forced through zero intercept) as follows:

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

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.