

Stofnaam	Olaquinox
Type methode	HPLC
Te onderzoeken in	Diervoeders
Minimum bepaalbaarheids grens	1,5 mg/kg
Herhaalbaarheid	RSD _r (relatieve standaarddeviatie) voor diervoeders op de volgende niveaus: 2.5 mg/kg: 6.6% 10 mg/kg 3.6%
Reproduceerbaarheid	RSD _R voor diervoeders op de volgende niveaus: 2.5 mg/kg: 19% 10 mg/kg: 13%
Categorie	A
Titel	Determination of low level contents of olaquinox in feedingstuffs by High Performance Liquid Chromatography CANFAS / SMT4-CT98-2216 / final method olaquinox / 2003-01-31.

BEPALING VAN OLAQUINDOX

1. Purpose and scope

The method is for the determination of olaquinox in feedingstuffs. The limit of determination (=quantification) is 1.5 mg/kg. The limit of detection is 0.3 mg/kg

2. Principle

The sample is extracted by a water-methanol mixture. The content of olaquinox is determined by reversed-phase high-performance liquid chromatography (HPLC) with UV-detection at 380 nm.

3. Reagents

3.1. Methanol

3.2. Methanol, HPLC grade

3.3. Water, HPLC grade

3.4. Mobile phase for HPLC

Water (3.3)-methanol (3.2) mixture, 900+100 (V + V)

3.5. Standard substance: pure olaquinox 2-[N-2'-(hydroxyethyl)carbamoyl]-3-methylquinoxaline-N¹,N⁴-dioxide, E 851

3.5.1. Olaquinox stock standard solution, 25 µg/ml

Weigh to the nearest 0.1 mg, 5 mg of olaquinox (3.5) in a 200 ml graduated flask and add ca. 190 ml water. Then place the flask for 10 min in a ultrasonic bath (4.1). After ultrasonic treatment, bring the solution to room temperature, make up to the mark with water and mix. Wrap the flask with aluminium foil and store in a refrigerator. At this temperature of $\leq 4^{\circ}\text{C}$ the solution is stable for 1 month.

3.5.2. Calibration solutions

Into a series of 50 ml graduated flasks transfer 0.5, 1.0, 2.5, 5.0 and 10.0 ml of the stock standard solution (3.5.1). Make up to the mark with water (3.3) and mix. These solutions correspond to concentrations of olaquinox of 0.25, 0.5, 1.25, 2.5 and 5.0 µg/ml respectively.

These solutions must be prepared fresh each day.

4. Apparatus

4.1. Ultrasonic bath

4.2. Mechanical shaker

4.3. Membrane filter, 0.45 µm

4.4. HPLC equipment with variable wavelength ultraviolet detector

4.4.1. Liquid chromatographic column, 250 mm x 4mm, C 18, 5 µm packing, or equivalent. See remark 7.2.

5. Procedure

Note: *Olaquinox is light sensitive. Carry out all procedures under subdued light or use amber glass ware.*

5.1. General

5.1.1. Blank feed

For the performance of the recovery test (5.1.2) a blank feed should be analysed to check that neither olaquinox nor interfering substances are present. The blank feed should be similar in type to that of the sample and on analysis olaquinox or interfering substances should not be detected.

5.1.2. Recovery test

A recovery test should be carried out by analysing the blank feed which has been fortified by addition of a quantity of olaquinox, similar to that present in the sample. To fortify at a level of 2.5 mg/kg, transfer 1 ml of the stock standard solution (3.5.1) to a 250 ml conical flask, add 10 g of the blank feed, mix thoroughly and leave for 10 min mixing again several times before proceeding with the extraction step (5.2). In stead of 40 ml water, 39 ml water should be added in the extraction step. Alternatively, if a blank feed similar in type to that of the sample is not available (see 5.1.1), a recovery test can be performed by means of the standard addition method. In this case, prepare two independent laboratory sample aliquots (A and B) of the feed to be examined. Spike one of them (A), before extraction with a quantity of olaquinox, similar to that already present in the sample. Both samples are analysed. Calculate the analyte content in sample A and B and calculate the recovery by subtraction.

5.2. Extraction

Weigh to the nearest 0.01 g, approximately 10 g of the sample. Transfer to a 250 ml conical flask, add 10 ml of methanol (3.1) and place the flask for 5 min in the ultrasonic bath (4.1). Add 40 ml water and leave in the ultrasonic bath for further 15 min. Remove the flask from the ultrasonic bath, shake it for 30 min on the shaker (4.2) and filter through a folded filter or a glass fibre filter (GFA, Whatman) (see remark 7.1). It is highly recommended to filter the clear samples by using a membrane filter (4.3) additionally. Proceed to the HPLC determination (5.3).

5.3. HPLC determination

5.3.1. Parameters:

The following conditions are offered for guidance, other conditions may be used provided that they give equivalent results; gradient elution may not be used.

Analytical column (4.4.1): See remark 7.2.

Mobile Phase (3.4): water (3.3) - methanol (3.2) mixture, 900 + 100 (V+ V)

Flow rate: 1.5 - 2 ml/min

Detection wavelength: 380 nm

Injection volume: 50 µl -100 µl

Check the stability of the chromatographic system, injecting several times the calibration solution (3.5.2) containing 1.25 µg/ml, until constant peak heights and retention times are achieved.

5.3.2. Calibration graph

Inject each calibration solution (3.5.2) several times and determine the mean peak heights (areas) for each concentration. Plot a calibration graph using the mean peak heights (areas) of the calibration solutions as the ordinates and the corresponding concentrations in µg/ml as the abscissae.

5.3.3. Sample solution

Inject the sample extract (5.2) and determine the peak height (area) of the olaquinox peaks.

6. Calculation of the results

From the height (area) of the olaquinox peaks of the sample solution determine the concentration of the sample solution in µg/ml by reference to the calibration graph (5.3.2).

The olaquinox content w (mg/kg) of the sample is given by the following formula:

$$w = \frac{c * 50}{m}$$

in which:

c = olaquinox concentration of the sample extract (5.2) in µg/ml

m = mass of the test portion in g

7. Remarks

7.1 Instead of filtration through a folded filter a centrifugation step could be carried out. If plastic vials are used for centrifugation, a recovery study should be carried out to validate this application.

7.2 The following columns could be recommended: Hypersil ODS 5 µm 200 x 4.6 mm, Spherisorb ODS 2 5 µm 250 x 4.6 mm, LUNA C18(2) 5 µm 250 x 4.6 mm.