

# CEN updates affecting chemical measurements September – December 2019

## **EN 17270:2019 Animal feeding stuffs: Methods of sampling and analysis – Determination of theobromine in feed materials and compound feed, including cocoa derived ingredients, by liquid chromatography**

Theobromine (3,7-dihydro-3,7-dimethyl-H-purine-2,6-Dione) is naturally present in cocoa products and by-products. Theobromine can have an adverse impact on livestock animals and because of this susceptibility to theobromine toxic effects feed manufacturers do not include by-products of cocoa manufacture or confectionery by-products in feeds especially for dogs and horses.

EN 17270 describes a method for the determination of theobromine in compound feed using high performance liquid chromatography coupled to an ultraviolet detector (LC-UV) having a detection range of 27 to 307 mg/kg and validated using a compound feed intended for adult dogs and complementary compound feedstuff for horses.

The standard also describes the method using liquid chromatography coupled to a tandem mass spectrometer detector (LC-MS/MS) with a detection range of 49 to 307 mg/kg.

Both detection systems demonstrated applicability in the determination of theobromine in baking chocolate.

The method validation study in support of EN 17270 is published; Kirstin M Gray and D Thorburn Burns, 2019, Validation of a Method for the Determination of Theobromine in Feed Materials and Compound Feeds by Liquid Chromatography with UV Detection or with Tandem Mass Spectrometry, J. Assoc. Public Analysts, 47, 1-35

## **EN 17256:2019 Animal feeding stuffs: Methods of sampling and analysis – Determination of ergot alkaloids and tropane alkaloids in feed materials and compound feeds by LC-MS/MS**

Ergot alkaloids and tropane alkaloids are toxic compounds that may be present in cereal crops.

Ergot alkaloids are generated by a fungus infection of the cereal producing a number of alkaloids including ergocornine, ergocorninine ergocristine,  $\alpha$ -ergocryptine etc. Tropane alkaloids such as atropine and scopolamine compounds are naturally present in Brassicaceae or Solanaceae plant families that includes mandrake, henbane and deadly nightshade. These tropane alkaloids may contaminate cereals through contamination with seeds from henbane, deadly nightshade etc.

EN 17256 describes a validated method for the determination of specific ergot alkaloids and tropane alkaloids in unprocessed cereals and cereal-based compound feeds using high performance liquid coupled to a tandem mass spectrometer detector (LC-MS/MS).

Validation of the method involved a collaborative trial for the cereals rye, barley, wheat and feed for bovine, porcine and poultry. The range of the method is stated as approximately 10 to 250 µg/kg for individual alkaloids.

### **EN 17194:2019 Animal feeding stuffs: Methods of sampling and analysis – Determination of Deoxynivalenol, Aflatoxin B1, Fumonisin B1 & B2, T-2 & HT-2 toxins, Zearalenone and Ochratoxin A in feed materials and compound feed by LC-MS/MS**

Aflatoxin B1, trichothecenes including deoxynivalenol, fumonisin B1 & B2, ochratoxin A, T-2 toxin, HT-2 toxin and zearalenone are mycotoxins produced by fungi growing on cereals and cereal products, peanuts and dried fruits. Some mycotoxins can cause a variety of adverse health effects in humans including cancer (genotoxic).

Commission Recommendation 2013/165/EU provides indicative levels concerning the presence of T-2 and HT-2 toxin in feed and food along with Regulation (EC) No 1881/2006 setting limits for aflatoxin B1, deoxynivalenol, fumonisin B1 & B2, ochratoxin A, T-2 toxin, HT-2 toxin and zearalenone.

EN 17194 describes a method for determining trichothecenes and zearalenone mycotoxins in feed and compound feed using solid phase extraction (SPE) and determination by liquid chromatography coupled with a tandem mass spectrometer detector (LC-MS/MS).

Solid phase extraction (SPE) involves adding the homogenised feed sample to the acetonitrile/formic acid solution in order to extract the mycotoxins from the feed material followed by centrifuging to separate the feed material from solution. An aliquot of the supernatant solution is removed and combined with a known quantity of stable isotope analogues and evaporated forming a mycotoxin residue that is re-dissolved in the injection solvent for determination by LC-MS/MS.

EN 17194:2019 indicates laboratories are required to meet specified limits of quantitation (LOQ) in order to be able to apply the method over the validation range with the following LOQ limits specified: deoxynivalenol ≤ 100 µg/kg, aflatoxin B1 and B2 ≤ 500 µg/kg, T-2 & HT-2 toxins ≤ 10 µg/kg, zearalenone ≤ 20 µg/kg and ochratoxin A ≤ 10 µg/kg.

EN 17194:2019 supports Regulation (EC) 2017/625 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules (Applicable 14 December 2019).

### **CEN/TR 17421:2019 Animal feeding stuffs: Methods of sampling and**

## **analysis – Recommendations for the organization and evaluation of collaborative studies for multi-analyte methods of analysis**

CEN/TR 17421 provides guidance to laboratories in the design, operation and evaluation of collaborative studies using multi-analyte methods developed by CEN/TC 327 Animal Feeding-Stuffs Working Group.

The guidance helps laboratories to consider the impact of deviations to multi-analyte methods when changes, which might be contrary to prescribed validation protocols, are necessary in conducting collaborative trials to ensure the method is still fit for purpose.

A technical specification (TS) is an approved normative document setting specifications in experimental and/or evolving technologies.

Regular updates to changes in food and feed legislation published by the Government Chemist:

[Food and feed law: Compendium of UK food and feed legislation with associated context and changes during July to September 2019 – Government Chemist Programme Report](#)