Nederlandse norm

NEN-EN 17049

(en)

Diervoeders: Methoden voor analyse en monsterneming - Identificatie van tylosine, spiramycine, virginiamycine, carbadox en olaquindox op subadditieve niveaus in mengvoeders - Bevestigingsanalyse door LC-MS

Animal feeding stuffs: Methods of sampling and analysis - Identification of tylosin, spiramycin, virginiamycin, carbadox and olaquindox at sub-additive levels in compound feed - Confirmatory analysis by LC-MS

Vervangt NEN-EN 17049:2016 Ontw.

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EUROPEAN STANDARD

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English Version

Animal feeding stuffs: Methods of sampling and analysis - Identification of tylosin, spiramycin, virginiamycin, carbadox and olaquindox at sub-additive levels in compound feed - Confirmatory analysis by LC-MS

Aliments des animaux: Méthodes d'échantillonnage et d'analyse - Identification de la tylosine, spiramycine, virginiamycine, du carbadox et de l'olaquindix dans les aliments composés pour animaux à des concentrations inférieures à celles des additifs - Analyse de confirmation par CL-SM

Futtermittel: Probenahme- und Untersuchungsverfahren - Identifizierung von Tylosin, Spiramycin, Virginiamycin, Carbadox und Olaquindox in Konzentrationen unterhalb von Zusatzstoffen in Mischfuttermitteln - Bestätigungsanalyse mittels LC-MS

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Contents

Page

European foreword

This document (EN 17049:2018) has been prepared by Technical Committee CEN/TC 327 “Animal feeding stuffs - Methods of sampling and analysis”, the secretariat of which is held by NEN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by August 2018, and conflicting national standards shall be withdrawn at the latest by August 2018.

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1 Scope

This European Standard specifies a high performance liquid chromatography – mass spectrometry (LC-MS/MS) method for the identification of tylosin, spiramycin, virginiamycin, carbadox and olaquindox in animal feeds.

The method is suitable for the identification of low concentrations of tylosin, spiramycin, virginiamycin, carbadox and olaquindox in compound animal feeds. A limit of identification of 1 mg/kg for tylosin, spiramycin and virginiamycin, 4 mg/kg for carbadox and 3 mg/kg for olaquindox should be obtained by using the described method. The method was fully validated during a collaborative study (see Annex A).

Since tylosin, spiramycin and virginiamycin are fermentation products consisting of a mixture of several closely related compounds, the analysis is based on detection and identification of the most abundant constituents. For tylosin the marker is tylosin A, for spiramycin the marker is spiramycin I and II and for virginiamycin the marker is virginiamycin M1 and S1. The other isomers and forms can be readily detected with the same method but adjustment of the MS parameters according to the molecular mass of precursor and product ions need to be made. Carbadox and olaquindox are analysed as such.

2 Normative references

There are no normative references in this document.

3 Principle

The compounds are extracted from the feed with a mixture of water and methanol. An aliquot of the liquid phase is diluted and applied to a pre-conditioned SPE column. After washing of the SPE column, compounds of interest are eluted with methanol. The obtained extract is evaporated and re-dissolved in dilute formic acid. The resulting extract is analysed by LC-MS/MS. Separation is carried out on a silica-based C18 bonded phase column and detection is performed by mass spectrometry in multiple reaction monitoring mode.

The validation of this method was performed at concentration levels that were calculated on a weight (w/w) basis. Expression of working ranges in terms of w/w concentration is common practice in residue analysis of veterinary drugs, in fact Maximum Residue Limits (MRL) are exclusively expressed on a w/w basis. For feed additives however, tolerances have been expressed traditionally as microbiological activity. To translate the validation experiments concerning the level at which they were performed, to units expressed as microbiological activity, the w/w concentrations should be corrected for the microbiological potency of the preparation used for spiking experiments.

4 Reagents and materials

WARNING — Use all solvents and solutions in a fume hood. Wear safety glasses, protective clothing and avoid skin contact.

4.1 General

All reagents are of 'Analytical reagent' grade or better unless otherwise stated. Throughout this method, “water” means demineralized water with a conductivity of at least 10 MΩ.cm^{-1}. Guaranteed purity is required for each lot of reference standard.

4.2 Reagents and materials

4.2.1 Acetonitrile (LC-MS grade)

4.2.2 Methanol (LC-MS grade)

4.2.3 Formic acid (LC-MS grade)

4.2.4 Tylosin

4.2.5 Spiramycin

4.2.6 Virginiamycin

4.2.7 Carbadox

4.2.8 Olaquindox

4.3 Solutions

4.3.1 HPLC Mobile phase A: Formic acid 5mM

Measure 200 µl formic acid (4.2.3) and transfer to a volumetric flask of 1 000 ml, make up to the mark with water. Filter and degas before use.

4.3.2 HPLC Mobile phase B: Formic acid 50 mM/ acetonitrile (10/90, v/v)

Measure 200 µl formic acid (4.2.3) and transfer to a volumetric flask of 1 000 ml, add 100 ml water and make up to the mark with acetonitrile (4.2.1). Filter and degas before use.

4.4 Standard solutions

4.4.1 Stock solution tylosin (500 µg/ml)

Weigh between 10 and 50 mg of tylosin standard substance (4.2.4) and transfer to a brown glass bottle. Calculate the required amount of methanol (4.2.2) and add that amount (on a weight basis) to obtain a standard solution of 500 µg/ml. Store this stock solution in the dark at 4-8 °C. Under these conditions it is stable for at least one month.

4.4.2 Stock solution spiramycin (500 µg/ml)

Weigh between 10 and 50 mg of spiramycin standard substance (4.2.5) and transfer to a brown glass bottle. Calculate the required amount of methanol (4.2.2) and add that amount (on a weight basis) to obtain a standard solution of 500 µg/ml. Store this stock solution in the dark at 4-8 °C. Under these conditions it is stable for at least one month.

4.4.3 Stock solution virginiamycin (500 µg/ml)

Weigh between 10 and 50 mg of virginiamycin standard substance (4.2.6) and transfer to a brown glass bottle. Calculate the required amount of methanol (4.2.2) and add that amount (on a weight basis) to obtain a standard solution of 500 µg/ml. Store this stock solution in the dark at 4-8 °C. Under these conditions it is stable for at least one month.

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