

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

EESTI STANDARDI EESSÕNA

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English Version

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Identification of tylosin, spiramycin, virginiamycin,
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Aliments des animaux: Méthodes d'échantillonnage et
d'analyse - Identification de la tylosine, spiramycine,
virginiamycine, du carbadox et de l'olaquinox 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 Olaquinox
in Konzentrationen unterhalb von Zusatzstoffen in
Mischfuttermitteln - Bestätigungsanalyse mittels LC-
MS

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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of an identical text or by endorsement, at the latest by August 2018, and conflicting national standards shall be withdrawn at the latest by August 2018.

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WARNING — The method described in this standard implies the use of reagents that pose a hazard to health. The standard does not claim to address all associated safety problems. It is the responsibility of the user of this standard to take appropriate measures for the health and safety protection of the personnel prior to use of the standard and to ensure that regulatory and legal requirements are complied with.

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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 olaquinox in animal feeds.

The method is suitable for the identification of low concentrations of tylosin, spiramycin, virginiamycin, carbadox and olaquinox 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 olaquinox 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 olaquinox 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.