# 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

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## NATIONAL FOREWORD



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| See Eesti standard EVS-EN 17049:2018 sisaldab Euroopa standardi EN 17049:2018 ingliskeelset teksti. | This Estonian standard EVS-EN 17049:2018 consists of the English text of the European standard EN 17049:2018. |
| Standard on jõustunud sellekohase teate avaldamisega EVS Teatajas | This standard has been endorsed with a notification published in the official bulletin of the Estonian Centre for Standardisation. |
| Euroopa standardimisorganisatsioonid on teinud Euroopa standardi rahvuslikele liikmetele kättesaadavaks 14.02.2018. | Date of Availability of the European standard is 14.02.2018. |
| Standard on kättesaadav Eesti Standardikeskusest. | The standard is available from the Estonian Centre for Standardisation. |

Tagasisidet standardi sisu kohta on võimalik edastada, kasutades EVS-i veebilehel asuvat tagasiside vormi või saates e-kirja meiliaadressile standardiosakond@evs.ee.

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# 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

This European Standard was approved by CEN on 8 January 2018.



EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

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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.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

This document has been prepared under a mandate given to CEN by the European Commission and the European Free Trade Association.

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 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 $M\Omega.cm^{-1}$. Guaranteed purity is required for each lot of reference standard.