

STN	Krmivá pre zvieratá Metódy odberu vzoriek a analýz Stanovenie pyrolizidínových alkaloidov v krmivách pre zvieratá metódou LC-MS/MS	STN EN 17683 46 7092
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Animal feeding stuffs - Methods of sampling and analysis - Determination of pyrrolizidine alkaloids in animal feeding stuff by LC-MS/MS

Táto norma obsahuje anglickú verziu európskej normy.
This standard includes the English version of the European Standard.

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English Version

Animal feeding stuffs - Methods of sampling and analysis - Determination of pyrrolizidine alkaloids in animal feeding stuff by LC-MS/MS

Alimentation animale - Méthodes d'échantillonnage et
d'analyse - Dosage des alcaloïdes pyrrolizidiniques
dans l'alimentation animale par CL-SM/SM

Futtermittel - Probenahme- und
Untersuchungsverfahren - Bestimmung von
Pyrrolizidinalkaloiden in Futtermitteln mittels LC-
MS/MS

This European Standard was approved by CEN on 17 January 2023.

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EN 17683:2023 (E)**European foreword**

This document (EN 17683:2023) 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of an identical text or by endorsement, at the latest by September 2023, and conflicting national standards shall be withdrawn at the latest by September 2023.

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Introduction

Pyrrolizidine alkaloids (PA) are secondary metabolites of flowering plants. Ingestion of high doses results in acute liver damage. In animal studies some PA have proven to be genotoxic carcinogens. Therefore, PA are undesired substances in food and feed [1], [2]. Poisoning in animals has been reported with known outbreaks attributed to *Heliotropium*, *Trichodesma*, *Senecio*, and *Crotalaria species*. In general, grazing animals will avoid PA-bearing plants. However, if weedy crops are used for the production of hay, silage or other plant derived feed materials the animals can no longer exercise discrimination when feeding because the toxins survive storage processes and are completely intermixed with the feed. Therefore, analytical methods for the control of PA levels in animal feed are needed [1], [2]. This document describes methods of sampling and analysis for the determination of pyrrolizidine alkaloids in animal feeding stuff by LC-MS/MS.

The method has been successfully validated in a collaborative trial for the matrices complete feed for horses, supplementary feed for horses, supplementary feed for rodents, hay, alfalfa and grass silage. Validation was carried out for the PA and concentrations ranges as described in Clause 1. It was demonstrated that the PA isomeric pairs senecivernine and senecionine as well as senecivernine-N-oxide and senecionine-N-oxide cannot be determined individually due to insufficient chromatographic separation. However, the sums of the individual PA of the isomeric pairs were quantified with sufficient reproducibility. Co-elution of other PA-isomers not included in the scope of the method need to be taken into account. A list of potentially co-eluting isomers is presented in Commission Regulation (EU) 2020/2040 [3].

Although the calibration range of the method protocol is specified from 10 µg/kg to 300 µg/kg, the results of the collaborative study showed, that the dilution of sample extracts with blank sample extracts enables for the quantitation of concentrations exceeding the calibration range. Satisfactory reproducibility was achieved when quantifying up to 1 428 µg/kg for individual PA and up to 887 µg/kg for the sum of isomeric pairs.

WARNING — The use of this protocol involves hazardous materials, operations and equipment. This protocol does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this protocol to establish appropriate health and safety practices and determine the compatibility with regulatory limitations prior to use.

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1 Scope

This document specifies a method for the quantitative determination of pyrrolizidine alkaloids (PA) in the concentration ranges shown in Table 1 in complete and supplementary feed and in forages by liquid chromatography tandem mass spectrometry (LC-MS/MS) after solid phase extraction (SPE) clean-up.

Table 1 — Summary of concentration ranges per PA tested in the collaborative trial

Tested pyrrolizidine alkaloid (PA)	Abbreviation	Tested concentration range ^a (µg/kg)	
		From	To
Echimidine	Em	20	435
Echimidine-N-oxide	EmN	5	30
Erucifoline	Er	20	245
Erucifoline-N-oxide	ErN	20	370
Europine	Eu	15	330
Europine-N-oxide	EuN	25	285
Heliotrine	Hn	25	280
Heliotrine-N-oxide	HnN	25	245
Jacobine	Jb	20	230
Jacobine-N-oxide	JbN	20	215
Lasiocarpine	Lc	20	350
Lasiocarpine-N-oxide	LcN	5	250
Intermedine	Im	25	560
Intermedine-N-oxide	ImN	5	395
Lycopsamine	La	25	500
Lycopsamine-N-oxide	LaN	20	280
Monocrotaline	Mc	20	360
Monocrotaline-N-oxide	McN	20	365
Retrorsine	Re	250	375
Retrorsine-N-oxide	ReN	5	285
Senecionine^b	Sc	25	205
Senecionine-N-oxide^b	ScN	5	300
Senecivernine^b	Sv	20	205
Senecivernine-N-oxide^b	SvN	5	165
Senkirkine	Sk	20	275
Seneciphylline	Sp	25	225
Seneciphylline-N-oxide	SpN	5	225
Trichodesmine	Td	5	250
Intermedine + Lycopsamine	Im+La	50	890
Intermedine-N-oxide + Lycopsamine-N-oxide	ImN+LaN	5	645
Senecivernine + Senecionine	Sv+Sc	30	280
Senecivernine-N-oxide + Senecionine-N-oxide	SvN+ScN	10	380

^a Rounded figures

^b Individual PA of the isomeric pairs Sv+Sc and SvN+ScN were not evaluated statistically due to insufficient chromatographic separation.

NOTE 1 A second method was part of the method validation collaborative main trial. For this method PA-N-Oxides are reduced by adding zinc powder to the extract of the feed material. The following steps correspond to the first and main method. Quantitative results for each PA except the otonecine type PA senkirine represent the sum of the free PA base and its corresponding N-oxide.

NOTE 2 Due to insufficient numbers of data for some analyte-matrix combinations statistical evaluation was not valid for standardization. Received data indicated the methods applicability in experienced laboratories with appropriate quality assurance measures. Therefore, the method description is included as an informative annex (Annex D).

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN ISO 3696, *Water for analytical laboratory use - Specification and test methods (ISO 3696)*

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