



BSI Standards Publication

Animal feeding stuffs — Determination of inorganic arsenic by hydride generation atomic absorption spectrometry (HG-AAS) after microwave extraction and separation by solid phase extraction (SPE)

National foreword

This British Standard is the UK implementation of EN 16278:2012.

The UK participation in its preparation was entrusted to Technical Committee AW/10, Animal feeding stuffs.

A list of organizations represented on this committee can be obtained on request to its secretary.

This publication does not purport to include all the necessary provisions of a contract. Users are responsible for its correct application.

© The British Standards Institution 2012. Published by BSI Standards Limited 2012

ISBN 978 0 580 66999 6

ICS 65.120

Compliance with a British Standard cannot confer immunity from legal obligations.

This British Standard was published under the authority of the Standards Policy and Strategy Committee on 31 July 2012.

Amendments issued since publication

Date	Text affected
------	---------------

EUROPEAN STANDARD
NORME EUROPÉENNE
EUROPÄISCHE NORM

EN 16278

July 2012

ICS 65.120

English Version

**Animal feeding stuffs - Determination of inorganic arsenic by
hydride generation atomic absorption spectrometry (HG-AAS)
after microwave extraction and separation by solid phase
extraction (SPE)**

Aliments des animaux - Dosage de l'arsenic inorganique
par spectrométrie d'absorption atomique par génération
d'hydrides après extraction par micro-ondes

Futtermittel - Bestimmung von anorganischem Arsen mit
Atomabsorptionsspektrometrie-Hydridtechnik (HD-AAS)
nach Mikrowellen-Extraktion und Trennung durch
Festphasenextraktion (SPE)

This European Standard was approved by CEN on 17 May 2012.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the CEN-CENELEC Management Centre or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the CEN-CENELEC Management Centre has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and United Kingdom.



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

Management Centre: Avenue Marnix 17, B-1000 Brussels

Contents

Page

Foreword.....	3
1 Scope	4
2 Normative references	4
3 Principle	4
4 Reagents	4
5 Apparatus and equipment	6
6 Procedure	7
7 Calculation	10
8 Precision	10
9 Test report	11
Annex A (informative) Results of the interlaboratory tests.....	12
Annex B (informative) Flowchart Animal feeding stuffs — Determination of inorganic arsenic by hydride generation atomic absorption spectrometry (HG-AAS) after microwave extraction and separation by solid phase extraction (SPE)	13
Bibliography	18

Foreword

This document (EN 16278:2012) has been prepared by Technical Committee CEN/TC 327 “Animal feeding stuffs”, 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 January 2013, and conflicting national standards shall be withdrawn at the latest by January 2013.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] 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.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

1 Scope

This European Standard describes a procedure for the determination of inorganic arsenic in animal feeding stuffs of marine origin by Solid Phase Extraction (SPE) and Hydride Generation Atomic Absorption Spectrometry (HG-AAS). The method has been successfully tested in a collaborative trial with a working range from 0,19 mg/kg to 2,7 mg/kg (HORRAT values < 2 ; HORRAT value is Horwitz –Ratio value). The LOQ of the method is usually approximately 0,1 mg/kg or lower.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. 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)*

EN ISO 6497, *Animal feeding stuffs — Sampling (ISO 6497)*

EN ISO 6498, *Animal feeding stuffs — Guidelines for sample preparation (ISO 6498)*

3 Principle

Inorganic arsenic consists of arsenite, As(III) and arsenate, As(V). This standard describes a method for the determination of inorganic arsenic (i.e. the sum of As(III) and As(V)). A representative test portion of the sample is treated with a diluted hydrochloric acid and hydrogen peroxide solution using microwave assisted heating. Hereby inorganic arsenic species are extracted and As(III) is oxidised to As(V). The inorganic arsenic is selectively separated from other arsenic compounds using solid phase extraction (SPE) and the concentration of inorganic arsenic is determined by HG-AAS in the SPE eluate. The gaseous hydride is transferred into a heated measuring cell (cuvette) by means of a carrier gas stream and decomposed. Since arsenic (III) and arsenic (V) show a different sensitivity to the hydride technique, it is necessary to reduce arsenic (V) to arsenic (III) in order to avoid incorrect measurements.

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

4 Reagents

The concentration of arsenic in the reagents and water used shall be low enough not to affect the results of the determination. All reagents shall be of analytical grade or similar unless otherwise specified.

Use water conforming to grade 2 of EN ISO 3696.

4.1 Hydrochloric acid (HCl), mass fraction of $\geq 30\%$ of approximately $\rho(\text{HCl}) \geq 1,15 \text{ g/ml}$.

NOTE Only use hydrochloric acid available with high purity or which was cleaned by a sub-boiling distillation.

4.2 Diluted hydrochloric acid.

4.2.1 0,4 mol/l as eluent for the SPE.

Dilute e.g. 20 ml of hydrochloric acid (4.1) to 0,5 l with water.

4.2.2 0,055 mol/l for the extractant solution.

Dilute e.g. 5,8 ml of hydrochloric acid (4.1) to 1 l with water.

4.2.3 2,6 mol/l for sample pre-reduction.

Dilute e.g. 270 ml of hydrochloric acid (4.1) to 1 l with water.

4.2.4 4,7 mol/l for HG-AAS measurements.

Dilute e.g. 500 ml of hydrochloric acid (4.1) to 1 l with water.

4.3 Hydrogen peroxide (H₂O₂), mass fraction not less than 30 %.

4.4 Extractant solution, volume fraction of 3 % H₂O₂ in 0,055 mol/l HCl.

Dilute e.g. 20 ml hydrogen peroxide (4.3) to 200 ml with 0,055 mol/l hydrochloric acid (4.2.2).

4.5 Acetic acid, ρ (CH₃COOH) = 1,05 g/ml.

4.6 Diluted acetic acid, 0,5 mol/l for washing of SPE columns.

Dilute e.g. 15 ml of acetic acid (4.5) to 0,5 l with water.

4.7 Sodium hydroxide pellets, mass fraction of ≥ 98 %.

4.8 Sodium hydroxide, 0,1 mol/l for adjustment of pH.

Dilute 0.4 gram sodium hydroxide pellets (4.7) to 100 ml with water.

4.9 Ammonium carbonate, mass fraction of $\geq 99,999$ %.

4.10 Ammonium carbonate buffer solution, 40 mmol/l for sample buffering.

Dissolve e.g. 0,384 g ammonium carbonate (4.9) in water and dilute to 100 ml with water.

4.11 Methanol (CH₃OH), HPLC grade, for conditioning of SPE sorbent.

4.12 Sodium borohydride, mass fraction of ≥ 96 %.

4.13 Sodium borohydride solution for hydride generation.

Dissolve e.g. 2,5 g of sodium hydroxide pellets (4.7) in a small amount of water; add 2,5 g of sodium borohydride (4.12) and dilute to 500 ml with water. Prepare a fresh solution daily prior to the hydride generation step and, when necessary, filter before use.

Commercially available sodium borohydride solutions may also be used.

NOTE The required concentrations may vary slightly with the system and the instructions of the relevant manufacturer shall therefore be observed.

WARNING — It is essential to observe the safety instructions for working with sodium borohydride. Sodium borohydride forms hydrogen with acids and this can result in an explosive air/hydrogen mixture. A permanent extraction system shall be provided at the point where the measurements are carried out.

4.14 L-Ascorbic acid, mass fraction of $\geq 99,7$ %.

4.15 Potassium iodide, mass fraction of $\geq 99,5$ %.