BS ISO 16308:2014

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Water quality — Determination of glyphosate and AMPA — Method using high performance liquid chromatography (HPLC) with tandem mass spectrometric detection

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National foreword

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Water quality — Determination of glyphosate and AMPA — Method using high performance liquid chromatography (HPLC) with tandem mass spectrometric detection

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

The committee responsible for this document is ISO/TC 147, *Water quality*, Subcommittee SC 2, *Physical, chemical and biochemical methods*.

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Introduction

Glyphosate [*N*-(phosphonomethyl)glycine] is a non-selective broad-spectrum herbicide. The efficiency of this compound makes it a top selling and one of the most widely used herbicides in the world since it entered the market in 1974. Together with its main degradation product, aminomethylphosphonic acid (AMPA), glyphosate is one of the most detected substances in water bodies in many developed countries. Note also that AMPA can be released during sewage treatment, e.g. due to breakdown of detergent formulations for textiles.

Glyphosate and AMPA belong to the aminophosphonate family and have specific physico-chemical properties that require the development of complex analytical methods for analysis and detection. The difficulty in analysis is mainly linked to the high solubility of glyphosate and AMPA and their chelating nature. To solve these problems, their pre-column derivatization with 9-fluorenylmethylchloroformate (FMOC-Cl) to form less polar derivatives allows a better separation using liquid chromatography.

Gluphosinate, another aminophosphonate, is less commonly subject to regulation and can be determined simultaneously, provided it can be demonstrated that there is no interference with the sample under analysis.

There is currently an International Standard for the determination by liquid chromatography and fluorometric detection; however, the determination by HPLC–ESI–MS/MS can be much more specific (unambiguous identification) and more sensitive (limits of quantification of approximately 30 ng/l for both glyphosate and AMPA). This International Standard is based on this analytical technique and is intended for laboratories involved in the regulatory control of the aquatic environment. Many such laboratories are now equipped with this kind of apparatus.

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WARNING — Persons using this International Standard should be familiar with normal laboratory practice. This International Standard does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

IMPORTANT — It is absolutely essential that tests conducted in accordance with this International Standard be carried out by suitably qualified staff.

1 Scope

This International Standard specifies a method for the determination of dissolved fraction of glyphosate and its major metabolite, aminomethylphosphonic acid (AMPA), in drinking water, ground water, and surface water at concentrations of 0,03 µg/l to 1,5 µg/l. It does not apply to marine or salty water. This method can be applicable to other types of waters, provided the method is validated for each case.

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.

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 5667-3, *Water quality — Sampling — Part 3: Preservation and handling of water samples*

ISO 8466-1, *Water quality — Calibration and evaluation of analytical methods and estimation of performance characteristics — Part 1: Statistical evaluation of the linear calibration function*

3 Principle

Glyphosate and AMPA (dissolved fraction after filtration) are derivatized using 9-fluorenylmethylchloroformate (FMOC-Cl) (5.17) to lower their polarity and increase the retention of compound in a separation on a reverse phase column (e.g. C18) as well as to improve the mass spectrometric detection. If the mass spectrometer has sufficient detection capability, it is possible to omit the solid phase extraction and to analyse the analytes by direct injection (see 8.2.1).

The derivatized sample is purified by liquid/liquid extraction and then concentrated by solid phase extraction (SPE).

The analysis is performed by high performance liquid chromatography coupled with tandem mass spectrometry via an electrospray source (HPLC–ESI–MS/MS), using matrix-matched calibration.

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Table 1 — Substances addressed

Name	Formula	Molecular mass g/mol	CAS-RN[a]
Glyphosate *N*-(phosphonomethyl)glycine	$C_3H_8NO_5P$	169,1	1071–83–6
AMPA Aminomethylphosphonic acid	CH_6NO_3P	111,0	1066–51–9

[a] CAS-RN Chemical Abstracts Service Registry Number

NOTE Gluphosinate, belonging to the aminophosphonate family, can be determined simultaneously, provided it can be demonstrated that there is no interference with the sample (matrix) subject to analysis.

4 Interferences

This method is validated for hard water containing up to 3,2 mmol/l of the sum of calcium and magnesium. For waters with a higher calcium and magnesium content, it may be necessary to increase the concentration of EDTA-Na_2 (5.16) at the derivatization step (see Annex D).

It can prove necessary to include the acidification step described in Annex D even for some water types below 3,2 mmol/l of the sum of calcium and magnesium. The laboratory shall check the necessity of this procedure for its routine samples.

The presence of free chlorine, e.g. in treated waters, can cause losses of glyphosate by oxidation. Consequently sodium thiosulfate shall be used (see Clause 7).

5 Reagents

Unless otherwise stated, all reagents and solvents shall be of sufficient purity, e.g. “for trace analysis”.

5.1 Deionized water.

5.2 Ultra-pure water, complying with grade 1 of ISO 3696.

5.3 Nitrogen, N_2, purity ≥ 99,996 % volume fraction.

5.4 Laboratory detergent, alkaline.

5.5 Sodium thiosulfate, $Na_2S_2O_3$.

5.6 Acetonitrile, C_2H_3N, HPLC grade.

5.7 Methanol, CH_4O, HPLC grade.

5.8 Ethanol, C_2H_6O, 95 %, HPLC grade mass fraction.

5.9 Ethyl acetate, $C_4H_8O_2$, HPLC grade.

5.10 Ammonium acetate, $C_2H_7O_2N$.

5.11 Triethylamine, $C_6H_{15}N$.

5.12 Ammonium hydroxide, NH_4OH, 28 % mass fraction.

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5.13 Formic acid, CH_2O_2.

5.14 Hydrochloric acid, HCl, 300 g/l.

5.15 Glacial acetic acid, $C_2H_4O_2$.

5.16 Ethylenediaminetetraacetic acid (EDTA), disodium salt dihydrate, $C_{10}H_{14}N_2O_8Na_2{\cdot}2H_2O$, with a minimum purity of 99 % mass fraction.

5.17 9-Fluorenylmethyl chloroformate,(FMOC-Cl), $C_{15}H_{11}ClO_2$, with a minimum purity of 97 % mass fraction.

FMOC-Cl is used to prepare the **derivatization reagent**, FMOC-Cl solution, 50 mg/ml, in acetonitrile (5.6). This solution can be stored at –18 °C ± 3 °C for 6 months.

For direct injection (8.2.1), use a FMOC-Cl solution of 0,5 mg/ml in acetonitrile.

5.18 Reference substances, according to Table 1.

5.18.1 Glyphosate, *N*-(phosphonomethyl)glycine, $C_3H_8NO_5P$, purity > 98 % mass fraction.

5.18.2 AMPA, aminomethylphosphonic acid, CH_6NO_3P, purity > 98 % mass fraction.

5.18.3 1,2-$^{13}C_2$,^{15}N-labelled glyphosate, surrogate standard, purity > 98 % mass fraction.

5.18.4 ^{13}C,^{15}N-labelled AMPA, surrogate standard, purity > 98 % mass fraction.

5.19 Calibration solutions.

Individual stock solutions of glyphosate (5.18.1) and AMPA (5.18.2), 100 mg/l, prepared in ultra-pure water (5.2). These solutions can be stored at 4 °C ± 3 °C for 1 month.

Individual stock solutions of 1,2-$^{13}C_2$,^{15}N-labelled glyphosate (5.18.3) and ^{13}C,^{15}N-labelled AMPA (5.18.4) 100 mg/l, prepared in ultra-pure water (5.2). These solutions can be stored at 4 °C ± 3 °C for 1 month.

Multi-substance working solution of surrogates: 1,2-$^{13}C_2$,^{15}N-labelled glyphosate and ^{13}C,^{15}N-labelled AMPA, 20 µg/l, prepared in ultra-pure water (5.2). This solution can be stored at 4 °C ± 3 °C for 1 month.

NOTE Stock and calibration solutions can be stored longer, provided the adequate justifications are given regarding stability.

5.20 Triethylammonium acetate buffer, 0,1 % triethylamine (5.11) solution adjusted to pH 9,5 with glacial acetic acid (5.15) (mobile phase).

5.21 Sodium tetraborate, decahydrate, $Na_2B_4O_7{\cdot}10H_2O$.

5.22 Borate-Na buffer, 0,05 mol/l; pH = 9,2.

Dissolve 19 ± 0,1 g of sodium tetraborate (5.21), decahydrate in 1 l of water (5.1). This solution can be stored for approximately 1 mo at 4 °C ± 3 °C.

5.23 Mineral water, containing less than 3,2 mmol/l divalent cations (Mg^{2+} and total Ca^{2+}), for preparing matrix-matched calibration.

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