SLOVENSKI STANDARD
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Kakovost vode - Določevanje glifosata in AMPA - Metoda s tekočinsko kromatografijo visoke ločljivosti (HPLC) s fluorometrijsko detekcijo

Water quality - Determination of glyphosate and AMPA - Method using high performance liquid chromatography (HPLC) and fluorometric detection

Qualité de l'eau - Dosage du glyphosate et de l'AMPA - Méthode par chromatographie liquide à haute performance (CLHP) et détection fluorimétrique

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Water quality — Determination of glyphosate and AMPA — Method using high performance liquid chromatography (HPLC) and fluorometric detection

Qualité de l'eau — Dosage du glyphosate et de l'AMPA — Méthode par chromatographie liquide à haute performance (CLHP) et détection fluorimétrique

Reference number
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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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.

ISO 21458 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 2, *Physical, chemical and biochemical methods*.

Water quality — Determination of glyphosate and AMPA — Method using high performance liquid chromatography (HPLC) and fluorometric detection

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. Some of the solvents used in the procedure are toxic and dangerous. Exercise caution when handling them.

IMPORTANT — It is absolutely essential that tests conducted according to this International Standard be carried out by suitably trained staff.

When this method is applied to its analysis, surface water shall be tested for additional and for multiplicative interferences.

1 Scope

This International Standard specifies a method for the determination of glyphosate and its major metabolite, aminomethylphosphonic acid (AMPA), in drinking water, ground water and surface water. The lowest limit of determination is about 0,05 µg/l. This method may be applicable to other types of waters provided the method is validated for each case.

2 Normative references

The following referenced documents are indispensable for the application 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.

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

ISO 6058, *Water quality — Determination of calcium content — EDTA titrimetric method*

ISO 6059, *Water quality — Determination of the sum of calcium and magnesium — EDTA titrimetric method*

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

This method is based on derivatization of glyphosate and AMPA (see Table 1) by using 9-fluorenylmethyl chloroformate (FMOCCl) in basic conditions followed by analysis using liquid chromatography on a polar phase linked to a fluorescence detector.

The quantification of the compounds is performed by using external calibration or by internal standard calibration or by applying the standard addition method. The compounds are identified by comparing the retention times or by using the standard addition method.

Table 1 — Compounds determined by this method

Name	Molecular formula	Molar mass g/mol	CAS No.
Glyphosate *N*-(phosphonomethyl)glycine	$C_3H_8NO_5P$	169,1	1071-83-6
AMPA Aminomethylphosphonic acid	CH_6NO_3P	111,0	1066-51-9

4 Interferences

Substances that produce a response at the selected wavelengths and for which the retention times are identical to those of the compounds to be analysed may cause overlapping peaks during the determination. It is necessary to take this into account especially when testing water samples other than ground water or drinking water samples.

The presence of divalent cations, such as calcium, copper, iron, and zinc, may lead in some cases to an underestimation of glyphosate and AMPA due to complex formation (see Reference [9]), in which case pretreatment is required (see 8.1).

The presence of free chlorine used in treated waters may cause losses of glyphosate by oxidation, in which case sodium thiosulfate is used to mitigate its effects (see Clause 7).

5 Reagents

Use only reagents of recognized analytical or HPLC grade, if available, unless otherwise specified. Reagents shall not contain any substance that interferes with the compounds to be analysed.

5.1 **Water**, complying with grade 1 as defined in ISO 3696, or better.

5.2 **Sodium thiosulfate**, $Na_2S_2O_3$, CAS No.: 7772-98-7.

5.3 **Acetonitrile**, CH_3CN, HPLC grade, CAS No.: 75-05-8.

5.4 **Diethyl ether**, $C_4H_{10}O$, CAS No.: 60-29-7.

5.5 **Reference substances** (see Table 1).

5.5.1 **Glyphosate**, purity > 97 % mass fraction, CAS No.: 1071-83-6.

5.5.2 **AMPA**, purity > 97 % mass fraction, CAS No.: 1066-51-9.

5.5.3 **Internal standard**, purity > 97 % mass fraction (see Annex A for typical compounds).

Commercially available solutions may be used, e.g. 10 µg/ml in water.

5.5.4 **Stock solution of glyphosate and AMPA**, 1,0 g/l.

Using glyphosate (5.5.1) and AMPA (5.5.2), prepare a stock solution containing 1,0 g/l of each compound in water (5.1), e.g. dissolve 20 mg of each compound in water (5.1) in a 20 ml one-mark volumetric flask (6.8).

This solution may be stored at 4 °C ± 3 °C for about 1 year.

5.5.5 Intermediate concentration solution of glyphosate and AMPA, 30 µg/l.

Proceed with subsequent dilutions of stock solution of glyphosate and AMPA (5.5.4) in order to obtain a mass concentration of 30 µg/l.

NOTE The solution can be obtained by adding 200 µl of stock solution (5.5.4) to 20 ml of water (5.1) in a one-mark volumetric flask (6.8); then adding 60 µl of this solution to 20 ml of water (5.1) in another one-mark volumetric flask (6.8).

This solution may be stored at 4 °C ± 3 °C for about 1 week.

5.5.6 Stock solution of internal standard, 30 µg/l.

Dissolve an internal standard (5.5.3) in water (5.1) and dilute to obtain a mass concentration of 30 µg/l in a one-mark volumetric flask (6.8).

This solution may be stored at 4 °C ± 3 °C for about 1 week.

5.6 Potassium hydroxide solution, c(KOH) = 3 mol/l, CAS No.: 1310-58-3,.

Dissolve 16,8 g of potassium hydroxide in 70 ml to 80 ml of water (5.1) in a 100 ml one-mark volumetric flask (6.8) and make up to the mark with water.

This solution may be stored at 4 °C ± 3 °C for about 3 months.

5.7 Hydrochloric acid, c(HCl) = 4 mol/l, CAS No.: 7647-01-0.

To 340 ml of water (5.1), slowly add 160 ml of concentrated HCl (37 % mass fraction) under magnetic stirrer agitation in a 500 ml one-mark volumetric flask (6.8).This solution may be stored at 4 °C ± 3 °C for about 3 months.

5.8 Potassium dihydrogenphosphate solution, $c(KH_2PO_4)$ = 0,05 mol/l, pH 5,4, CAS No.: 7778-77-0.

Dissolve 6,8 g ± 0,05 g of potassium dihydrogenphosphate in 1 000 ml of water. Adjust to pH = 5,4 ± 0,05 with potassium hydroxide solution (5.6).

This solution may be stored for about 1 week.

5.9 Oxalic acid dihydrate solution, $\rho(C_2H_2O_4 \cdot 2\ H_2O)$ = 100 g/l, CAS No.: 6153-56-6.

Dissolve 50 g ± 0,05 g of oxalic acid dihydrate in 500 ml of water.

This solution may be stored at 4 °C ± 3 °C for about 2 months.

5.10 Mobile phase.

Mix 300 ml acetonitrile (5.3) and 700 ml of potassium dihydrogenphosphate buffer (5.8).

This solution can be stored for about 1 week.

5.11 Sodium tetraborate decahydrate solution, $c(Na_2B_4O_7 \cdot 10\ H_2O)$ = 0,05 mol/l, CAS No.: 1303-96-4.

Dissolve 19 g ± 0,1 g of sodium tetraborate decahydrate in 1 000 ml of water.

This solution may be stored at 4 °C ± 3 °C for about 1 month.

5.12 9-Fluorenylmethyl chloroformate solution, $\rho(C_{15}H_{11}ClO_2)$ = 1 mg/ml, CAS No.: 28920-43-6.

Dissolve 50 mg of 9-fluorenylmethyl chloroformate (FMOCCl; 97 % mass fraction) in 50 ml of acetonitrile (5.3).

This solution may be stored at 4 °C ± 3 °C for about 2 weeks.

5.13 Phosphoric acid, 85 % (acidimetric), CAS No.: 7664-38-2.

6 Apparatus

Equipment or components that may come into contact with the sample shall be free from any residue that could cause unacceptable interference in the blanks.

The use of glassware may cause losses of glyphosate by adsorption. Consequently, the use of disposable materials is recommended wherever possible (see 6.1 and 6.2). For the same reason, check that any filters used for samples prior to HPLC analysis do not lead to losses of substances.

6.1 Disposable plastic test tubes with screw caps, of capacity about 20 ml, for the derivatization.

6.2 Polyolefin bottles, of capacity about 50 ml, for sampling.

6.3 Syringe, for the standard solution of glyphosate and AMPA at the mass concentration 30 µg/l (5.5.5) and internal standard at a mass concentration of 30 µg/l (5.5.6).

6.4 Analytical column: polar phase column: e.g. silica-bonded NH_2 type chromatographic column, particle size 3 µm to 5 µm, inner diameter 2 mm to 4,6 mm, length 250 mm. A suitable guard-column is recommended.

Other types of columns may be used, e.g. reverse phase C_{18} column, material with particle size 3 µm to 5 µm inner diameter 2 mm to 4,6 mm, length 250 mm. See elution conditions in Figure C.2 when using C_{18} column.

6.5 Vials, standard 2 ml vials with polytetrafluoroethylene caps.

CAUTION — Do not clean vials with any detergent or corrosive agents before use.

6.6 High performance liquid chromatograph (HPLC), including:

6.6.1 System, allowing the elution of mobile phase (5.10) with either manual or automatic injection designed for analytical work.

6.6.2 Degassing system.

6.6.3 HPLC column oven with temperature control unit, 35 °C.

6.6.4 Fluorescence detector, λ_{exc} = 260 nm; λ_{em} = 310 nm.

6.7 pH meter.

6.8 One-mark volumetric flasks, ISO 1042 [10] class A, of capacities 20 ml, 100 ml, 500 ml.

CAUTION — Do not clean one-mark volumetric flasks with any detergent or corrosive agents before use.

7 Sampling

Fill the polyolefin bottles (6.2) to the top with the water to be analysed.

For samples containing free chlorine, add about 2 mg of sodium thiosulfate (5.2) or any other chlorine reducing agent per 100 ml of sample.

The water samples to be analysed may be stored in the dark at 4 °C ± 2 °C for 1 week or frozen in polyolefin bottles (6.2) for 1 month. The freezer temperature should be at least −20 °C.

8 Procedure

8.1 Pretreatment

Determine the water hardness as specified in ISO 6058 and/or ISO 6059. If the measure of hardness, $c(CaCO_3)$, exceeds 3 mmol/l, a sample pretreatment is recommended, in order to release any test substance complexed with divalent cations (Reference [9]). Two preferred pretreatment procedures are proposed (8.1.1 and 8.1.2), the choice of which is left to the user. A third option for pretreatment can be suitable in some cases (8.1.3).

8.1.1 First option for pretreatment

Adjust 3 ml of sample to pH 1 with about 200 µl of hydrochloric acid (5.7). Shake the mixture vigorously and wait for 1 min. Adjust the sample to between pH 6 and pH 7 with potassium hydroxide solution (5.6). Then proceed as specified in 8.2.

8.1.2 Second option for pretreatment

Add 300 µl of oxalic acid dihydrate solution (5.9) to 30 ml of sample. Check that the pH is between 2 to 3. Shake the mixture vigorously and wait for 1 h. Neutralize the sample with potassium hydroxide solution (5.6). Then proceed as specified in 8.2.

8.1.3 Third option for pretreatment

Add 0,625 ml of sodium tetraborate decahydrate buffer (5.11) and 50 µl of EDTA (1 g/l, pH = 9 adjusted by the addition of potassium hydroxide) to 4 ml sample. Shake the mixture. Store it for 24 h at 4 °C ± 3 °C. Without shaking, take 3 ml of the clear sample. Then proceed as described in 8.2.

8.2 Derivatization

After thawing the sample, if frozen (see Clause 7), mix the solution thoroughly and withdraw an aliquot of 3 ml. Transfer into a disposable plastic test tube with screw cap (6.1). Add 50 µl of the internal standard (5.5.6) to achieve a mass concentration of about 0,5 µg/l.

Add 0,5 ml of sodium tetraborate decahydrate buffer (5.11) and let it stand for about 15 min.

Add about 3 ml of diethyl ether (5.4), mix vigorously for 2 min and allow to settle for 15 min.

Take 1,5 ml from the aqueous phase and add 0,25 ml of acetonitrile (5.3), then add 0,25 ml of FMOCCl (5.12).

Allow the mixture to react at ambient temperature for 60 min. Stop the derivatization by adding 40 µl phosphoric acid (5.13) and mix.

NOTE 1 A reaction time of 30 min is sufficient to reach a yield of 100 %, however, the last 30 min allow for a better elimination of excess reagent. An excess of reagent can lower the life of the analytical column and also causes interferences.

NOTE 2 For some samples, using a slow derivatization results in fewer interfering peaks in the chromatogram. Leave out the extra acetonitrile as an accelerant. Allow the mixture to react at ambient temperature for 18 h. Stop the derivatization by adding 40 µl phosphoric acid (5.13) and mix.

Add 2 ml of diethyl ether (5.4) and mix for 2 min, allow the mixture to stand for 1 h and transfer an aliquot of the aqueous phase to a vial (6.5) for analysis. Analyse the aqueous extract or store it at 4 °C ± 3 °C for up to 2 weeks (4 weeks in a freezer).

If the standard addition approach is used to quantify compounds, prepare three different plastic test tubes with screw caps as described in Table 2. Allow the solutions to stand for 15 min then proceed with derivatization.