

METHOD STATEMENT



Determinand:

Determination of Glyphosate and Aminomethylphosphonic Acid (AMPA).

Matrix:

Sample Type: Treated, Ground and Raw Waters.

Principle of Method:

The method is a large volume direct aqueous injection on-line solid phase extraction procedure. Samples are derivatised with 9-fluorenylmethyl chloroformate (FMOC-Cl) prior to analysis. The FMOC derivatised sample is analysed by high performance liquid chromatography using a triple quadrupole mass spectrometer as a detector. The analytes are separated and then identified and quantified with mass spectrometric detection (MSD) in multiple reaction monitoring (MRM) mode. Quantitation is based on an internal standardisation procedure. In order to decrease the load of the LC column and mass spectrometer with derivatisation by-products e.g. 9-fluorenylmethanol FMOC-OH, a liquid/liquid extraction clean up step is performed with dichloromethane. Acidification of the sample and addition of EDTA-Na₂ is used to minimise complexation of the target compounds with metal ions.

Sampling and Sample Preparation:

Sampling, samples are taken in PE/HD bottles, with screw tops containing sample preservative - sodium thiosulfate 1.8% w/v.

Storage - samples should be analysed as soon as possible after collection. When this is not possible they are stored under refrigeration at 1-5°C until analysis can begin. Maximum permissible storage time prior to analysis is given in table below.

APPENDIX 7 - SAMPLE STABILITY INFORMATION.

Interferences

HPLC-MS/MS is an extremely selective technique and interferences should only be encountered very rarely. Any interfering compounds would have to display the identical MRM transition at the same retention time, this is extremely unlikely in potable water samples. However, any compound, which passes through the extraction procedure, and has a similar liquid chromatographic retention time and mass spectrometric properties to the compound of interest, will cause interference. Samples containing high humic or fulvic loading have been demonstrated to not cause significant ion suppression for the compounds.

Performance of Method:

Range of Application:

Determinand	Operational Calibration Range
AMPA	LOQ - 0.250 µg/L
GLYPHOSATE	LOQ - 0.250 µg/L

Limit of Quantification, Recoveries of Compounds and Uncertainty of measurement:

DETERMINAND	LOQ ng/L	UoM	DIRECT STANDARDS				ELVINGTON TREATED (Hard)	
			LOW STANDARD		HIGH STANDARD		PCV SPIKE	
			RECOVERY	RSD	RECOVERY	RSD	RECOVERY	RSD
GLYPHOSATE	5	6.594%	98.90%	2.27%	99.46%	1.55%	99.61%	3.22%

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AMPA	6	7.279%	99.39%	3.51%	99.24%	2.43%	100.09%	2.51%
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References:

Agilent 1200 Series, Reference Manuals.

Agilent 6400 QQQ LC/MS Techniques and Operation, Agilent Technologies Course Number R1893A, Student Manuals Volumes 1 and 2.

Agilent 6470 Triple Quad LC/MS System, Quick Start Guide

Agilent 6400 Triple Quad LC/MS, Maintenance and Familiarization Guides.

Agilent 6400 Triple Quad LC/MS System, Concept Guide.

Ultratrace-level determination of glyphosate, aminomethylphosphonic acid and glufosinate in natural waters by solid-phase extraction followed by liquid chromatography-tandem mass spectrometry: performance tuning of derivatization, enrichment and detection. Irene Hanke, Heinz Singer, Juliane Hollender, *Analytical and Bioanalytical Chemistry*, 2008, Volume 1175, Issue 2, page 2265-2276.

Determination of glyphosate in groundwater samples using an ultrasensitive immunoassay and confirmation by on-line solid phase extraction followed by liquid chromatography coupled to tandem mass spectrometry. Josep Sanchis, Lina Kantiani, Marta Llorca, Fernando Rubio, Antoni Ginebreda, Josep Fraile, Teresa Garrido, Marinella Farre, *Analytical and Bioanalytical Chemistry*, 2011.

Water quality - Determination of glyphosate and AMPA - Method using high performance liquid chromatography (HPLC) with tandem mass spectrometric detection, Bsi, ISO/TC 147/SC 2 N 1260, ISO/CD 16308 - Revision 2 - clear version, 2012.