

ENVIRONMENTAL ANALYSIS

A TURN-KEY GUARANTEED ANALYSER FOR THE ROUTINE MEASUREMENT OF GLYPHOSATE AND AMPA IN DRINKING WATER (EUROPEAN DRINKING WATER DIRECTIVE 98/83/EC) AND OTHER ENVIRONMENTAL WATER SAMPLES

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Solution Note

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Abstract

Agilent is now offering a comprehensive analyser package providing a robust automated system for the routine analysis of Glyphosate and AMPA in treated drinking water samples. The system is all manufactured and supported by Agilent and includes instrument hardware, proven validated methods conforming to the required Quality standards and operational consumables. Agilent's installation, commissioning and training program (with clearly defined timelines) means the receiving laboratory can rapidly plan for on-site validation and subsequent routine operation within a matter of weeks. It should be noted that the same methodology can be applied to other water types and the same instrument hardware can be applied to the analysis of an extensive range of polar organics with details available on request from Agilent.



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Introduction

Glyphosate is a broad-spectrum systemic herbicide widely used in agriculture, forestry and urban landscape management. Microbial biodegradation of glyphosate occurs in soil, aquatic sediment and water to produce its major metabolite AMPA. Glyphosate is chemically stable in water and is not subject to photochemical degradation. Its low mobility in soil minimises the potential for groundwater contamination. However, it can enter surface waters after direct use near aquatic environments or through run-off or leaching from terrestrial applications. Glyphosate is generally considered to be of low toxicity.

However, due to its high usage rate (it is currently the most commonly used agricultural pesticide) and the fact that there are increasing health and environmental concerns over its toxicity, it is important to have a robust, routine method available for the accurate determination of both Glyphosate and AMPA.



The European Drinking Water Directive 98/83/EC prescribes maximum admissible concentrations of 0.1 µg/L for individual pesticides and 0.5µg/L for total pesticides, although these limits are not based on toxicological considerations. A method capable of achieving LODs (limits of detection) of 0.010 µg/L (10 ng/L) in drinking waters would therefore be desirable.

Glyphosate and AMPA are both highly polar compounds, soluble in water and almost insoluble in organic solvents. They are also non-volatile compounds of relatively low molecular weight. These properties make them difficult to extract and analyse at residue levels by simple, straightforward techniques such as GC or HPLC. Derivatisation of these compounds is required to achieve chromatographic separation and improve their detectability.

Analytical Technique

This document outlines a robust, routine method for the determination of Glyphosate and AMPA in potable, borehole and river waters using an Agilent 1200/6460 LC/QQQ (high performance liquid chromatography with mass spectrometer triple quadrupole detection) system. This system incorporates fully automated online solid phase enrichment of an aliquot of water after offline derivitisation with FMOC (fluorenylmethyl-oxycarbonyl chloride) reagent. The range of application for this method is up to 1.0 µg/L, with LODs of less than 10 ng/L being achievable.

Sample Preparation

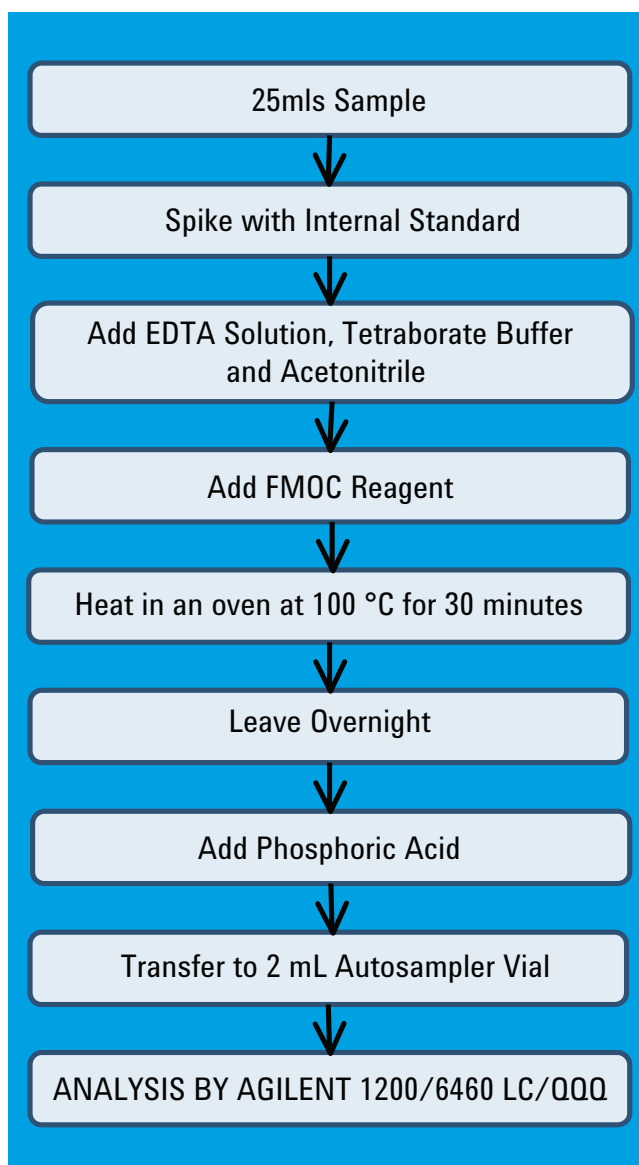
- Offline Derivatisation with FMOC Reagent
- Reagents Required – EDTA Solution (0.1M), Tetraborate Buffer (30g/L), Acetonitrile, FMOC Reagent (20mM in Acetonitrile) and Phosphoric Acid

Instrumentation

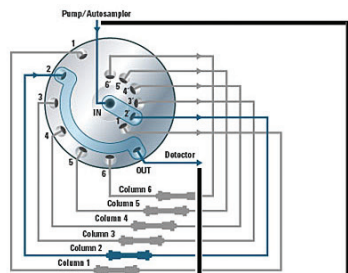
Agilent 1200 Series HPLC System consisting of the following:

- Quaternary Pump G1311B
- Autosampler G1329C with Extended Injection Volume Option and Multi-Draw Kit
- 2 Micro Vacuum Degassers G1379B
- External Valve Drive G1170A
- 6 Position / 14 Port Valve G4234A
- 2 Position / 6 Port Valve G4231A
- Binary Pump G1312B
- Column Compartment G1316A

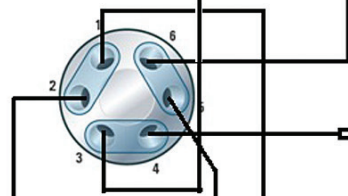
Agilent 6460 QQQ System with Jet Stream Interface



Selection of trapping column



Load position



to waste



Figure 1: Schematic diagram of Agilent 1200/6460 LC/QQQ System.

Online Enrichment Setup

900 μ L of the derivatised sample is loaded onto the SPE cartridge from a standard 2 mL vial in the enrichment autosampler using the quaternary loading pump. The SPE cartridge is then desorbed in 'backflush' mode using the gradient from the high pressure gradient pump and trapped analytes are separated on the analytical column.

Instrumentation

| LC Conditions | | | |
|------------------------|--|---------------|-----|
| Analytical Column | ZORBAX Eclipse Plus C18 2.1x150mm, 3.5 µm | | |
| Column Temperature | 60 °C | | |
| Injection Volume | 900 µL | | |
| Mobile Phase | A = 2.2 mM Ammonium Acetate in Water B = Acetonitrile | | |
| Run Time | 14 minutes | | |
| Flow Rate | 0.4 mL/min | | |
| Gradient Program | Time (minutes) | Gradient: % A | % B |
| | 0 | 90 | 10 |
| | 1 | 90 | 10 |
| | 7 | 30 | 70 |
| | 7.1 | 90 | 10 |
| MS (QQQ) Conditions | | | |
| Acquisition Parameters | MRM Mode, ESI Negative | | |
| Gas Temperature | 325 °C | | |
| Drying Gas Flow | 8 L/min Nitrogen | | |
| Nebuliser Pressure | 45 psi | | |
| VCap Voltage | 4500 V | | |
| Sheath Gas Temperature | 400 °C | | |
| Sheath Gas Flow | 11 L/min Nitrogen | | |
| Nozzle Voltage | 1000 V | | |

Mass Spectrometer in MRM Mode

| Compound | Ion Type | Precursor Ion (m/z) | Product Ion (m/z) | Fragmentor Voltage (V) | Collision Energy (V) | Polarity |
|------------------------------|--------------|---------------------|-------------------|------------------------|----------------------|----------|
| Glyphosate | Quantitation | 390.2 | 167.8 | 100 | 6 | Negative |
| Glyphosate Internal Standard | Quantitation | 393.2 | 170.8 | 100 | 6 | Negative |
| AMPA | Quantitation | 332.0 | 109.8 | 100 | 6 | Negative |
| AMPA Internal Standard | Quantitation | 334.0 | 111.8 | 100 | 6 | Negative |

Chromatograms and Calibrations

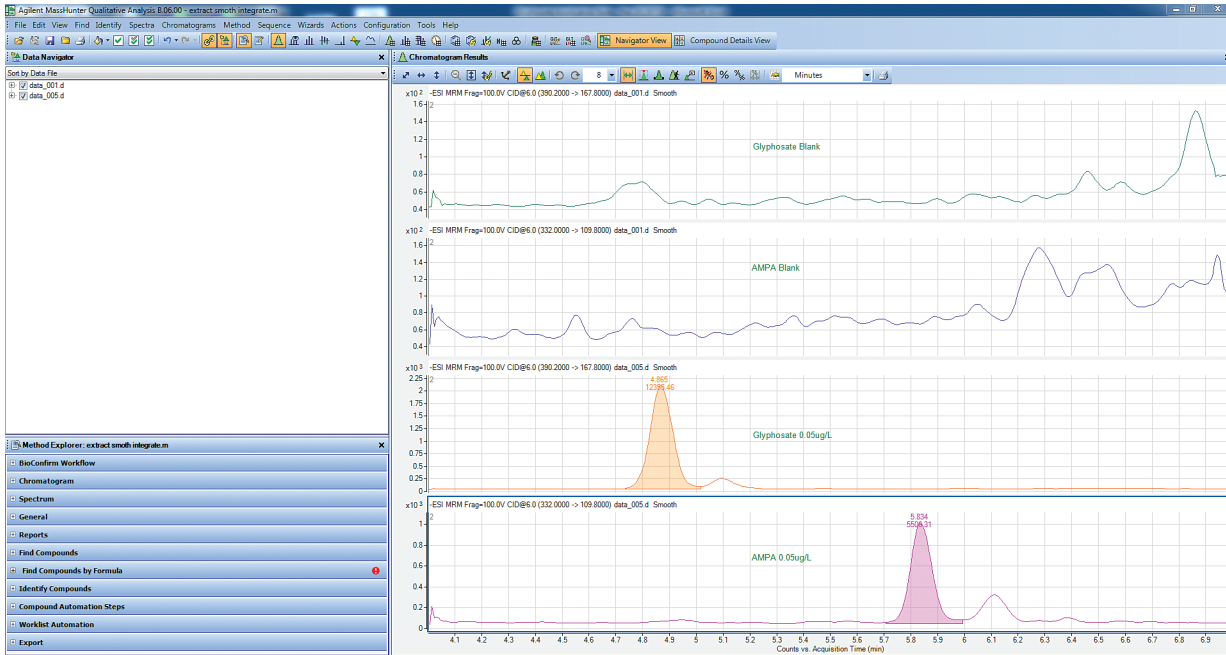


Figure 2: Blank (upper) and 0.05 µg/L (lower) Standard Chromatograms for the quantitation ions of derivitised Glyphosate and AMPA.

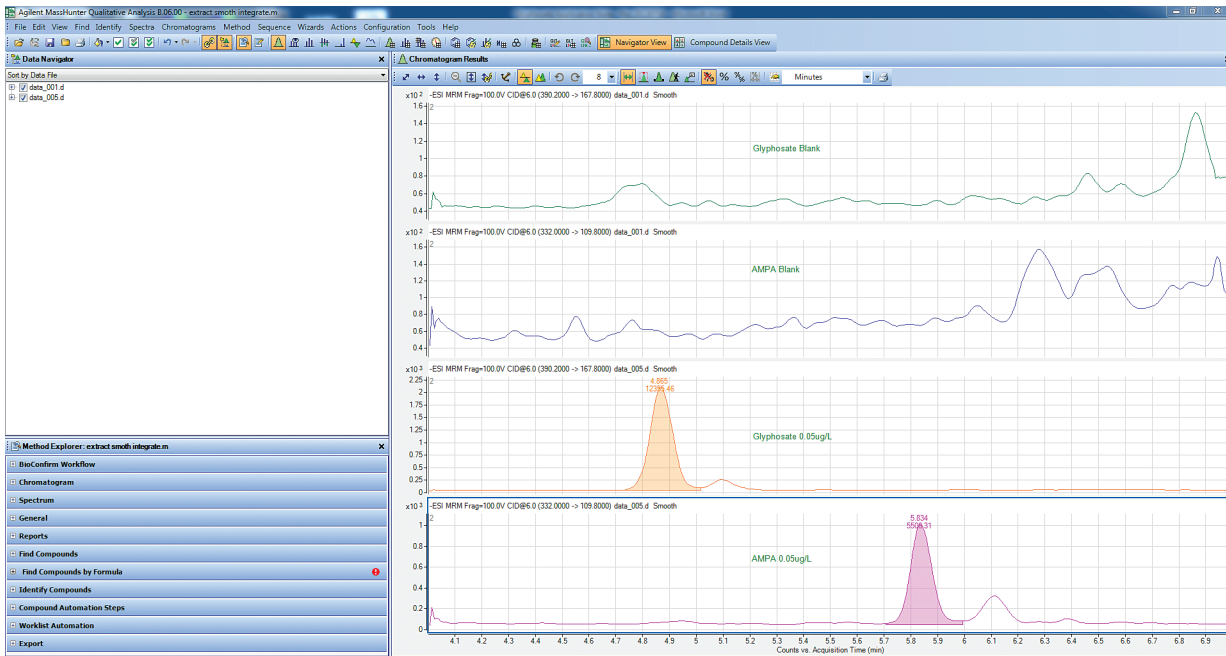


Figure 3: Glyphosate calibration with calibration standards at 0.025, 0.05 and 0.2 µg/L (Type: Linear, No Weighting).

Performance

Performance data was obtained by performing a validation to ISO/TS 13530:2009 specifications. This involved analysing the following:

- 0.01 µg/L, 0.10 µg/L and 0.50 µg/L standards
- Tap water & tap water spiked at 0.1 µg/L
- Borehole water & borehole water spiked at 0.1 µg/L
- River water & river water spiked at 0.1 µg/L

Analysis Time – A set of calibration standards, 20 samples, controls and blanks can be prepared and analysed within a 24 hour period.

| | 0.01 µg/L Standard | | 0.10 µg/L Standard | | 0.50 µg/L Standard | | LOD |
|------------|--------------------|----------|--------------------|----------|--------------------|----------|-------|
| | Mean, µg/L | SD, µg/L | Mean, µg/L | SD, µg/L | Mean, µg/L | SD, µg/L | µg/L |
| Glyphosate | 0.00964 | 0.00053 | 0.10347 | 0.00356 | 0.51065 | 0.01045 | 0.002 |
| AMPA | 0.01062 | 0.00075 | 0.09984 | 0.00305 | 0.49983 | 0.01328 | 0.002 |

| Water Type | Glyphosate Recovery, % | AMPA Recovery, % |
|----------------|---------------------------|---------------------|
| Tap Water | 98.8 | 100.7 |
| Borehole Water | 101.3 | 98.9 |
| River Water | 102.4 | 102.0 |

Conclusion

Agilent's 1200/6460 LC/QQQ with fully automated online enrichment package offers the ability to determine Glyphosate and AMPA in potable, borehole and river waters. This technique is capable of achieving LODs of less than 0.01 µg/L (10 ng/L) and offers the following advantages:

- Derivatisation Process – Produces derivatives that can be separated by reverse phase chromatography and detected by negative ion electrospray
- Online Enrichment – An injection volume of 900 µL increases the sensitivity in comparison to direct injection, LODs of less than 0.01 µg/L (10 ng/L) are achievable
- Robust Method – Online enrichment protects the analytical column from borate buffer and derivitising agent which might otherwise cause column degradation
- Internal Standards – Compensate for recovery variations in natural waters due to matrix effect.

Turn-key Package

Agilent's turn-key guaranteed analyser package for the measurement of Glyphosate and AMPA in drinking water comprises the following:

- Complete hardware set-up of the Agilent 1200/6460 LC/QQQ
- Standard Operating Procedure (SOP) with detailed descriptions of the analysis and validation procedures
- Method of analysis (a DVD/CD containing sample preparation and sample analysis methods, recommended consumables and materials)
- On-site Training & Full Agilent Support.

For further information please contact:
maps_agilent@agilent.com

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