

Sampling, analysis and reporting of glyphosate and AMPA in environmental samples

egeis

European Glyphosate Environmental Information Source

Important Notes to users:

This document is part of a toolbox which provides independent information on the sustainable use of Glyphosate. It cannot however be definitive and users must ensure that they assess local factors and particularly take account of any national or regional legislative requirements. At the end of the document reference sources used in its preparation and links to other relevant documents are provided.

Summary

Glyphosate and its major soil metabolite, AMPA, are difficult to analyse at residue level by simple and straightforward methods. The most common analytical method involves a direct derivatisation of a water sample with FMOCCI followed by high performance liquid chromatography (HPLC) determination using a fluorescence detector. More sensitive and robust methods became available recently. Guidance documents for sampling and data reporting were assembled under the monitoring activities of the Water Framework Directive and represent the current state of the art guidelines for water monitoring.

Detailed information

Introduction

Because of their unusual physico-chemical properties, glyphosate and its major soil metabolite, AMPA, are difficult to analyse at residue level by simple and straightforward methods :

- Non volatile compounds of relatively low molar mass (glyphosate 169 g/mol, AMPA 111 g/mol) with two to three functional groups (carboxylic and phosphonic acid, amine)
- Highly polar compounds, largely soluble in water and almost insoluble in organic solvents

Thus gas chromatographic (GC) and high pressure liquid chromatographic methods (HPLC) can only be used after derivatisation of the compounds to achieve chromatographic separation and improve its detectability. Stalikas *et al* (2001) reviewed numerous methods that were developed to analyse phosphonic and amino group containing pesticides (including glyphosate and AMPA) in different matrices. This summary will focus on the current methods for analysing of glyphosate and AMPA in water and soil. It also provides links to guidance documents for conducting valid water monitoring studies and results reporting.

Sampling and data reporting

Using the sampling protocol is an important aspect of monitoring studies to ensure consistent and reliable analytical results. The sampling strategies related to specific monitoring objectives are discussed in many papers and books, but recently the working group dealing with chemical monitoring activities under the EU Water Framework Directive (WFD) has assembled general and specific guidance documents. These documents reflect the current state of the art guidelines for water monitoring activities and can be found on the public library of the EU Circa website. They include:

- A general guidance document for monitoring under the WFD (guidance 07)
- A guidance document for groundwater monitoring (guidance 15)
- Surface water chemical monitoring (guidance 19)
- Reporting under the WFD (guidance 21).

Analysis of water

Water is a matrix that typically requires little clean-up, but this advantage is counterbalanced by the low quantification limit that is usually required.

The most common analytical method involves a direct derivatisation of a water sample with FMOCCI followed by high performance liquid chromatography (HPLC) determination using a fluorescence

detector (Le Fur *et al* (2000)). The typical quantification limit is around 0.05 – 0.1 µg/L for this procedure, which has the advantage to be simple and rapid, but lacks specificity, and can thus yield false positives. These shortcomings can be addressed by crosschecking the results using another derivatisation agent (Colin *et al* (2000)) or by confirming the results through mass spectrometry (LC/MS/MS) (Vreecken *et al* (1998)). This method is not suitable for other matrices, unless a substantial clean-up step is added before derivatisation. Other analytical methods in use in European laboratories involve a clean up step using chelating and/or ion exchange resins. The clean-up steps are followed by either HPLC and post-column derivatisation with o-phthalaldehyde (DFG method 405, DIN 38407-22) or by derivatisation with TFAA prior to GC/MS/MS analysis (Alferness *et al* (1994), Royer *et al* (2000), Börjesson *et al.* (2000)). Compared to the FMOCCI method described above, these methods are more time consuming and are more complicated and expensive, but are less subject to interferences. Typical quantification limits are in the range of 0.05 – 0.1 µg/L. An inter-laboratory validation (Reichert (2005)) study was performed in 2004 across 25 European laboratories. The laboratories were asked to analyse 12 blind fortified samples of groundwater and 12 of surface water using their routine method. The results showed that whilst glyphosate and AMPA are adequately detected by all types of detection method, some labs need to improve their routine method. In this study, the labs using the FMOCCI method largely underestimated the concentration in groundwater, whilst the labs using the methods involving an ion exchange clean-up were able to appropriately quantify the glyphosate present in these types of groundwater samples. In fact, glyphosate was bound to cations present in the water and could be released with the ion exchange clean-up step, but not by the FMOCCI method involving direct analysis of water. Freuze *et al* (2007) studied the influence of complexation phenomena on the analysis of glyphosate and AMPA in water. A recent publication by Hanke *et al* (2008) describes a highly sensitive method which includes a short clean-up, a step to minimize the complexation with cations, a refinement of the FMOCCI derivatisation and determination by liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS). Although slightly longer and more complicated, this method will likely be more robust and reliable than the standard FMOCCI procedure, and provide an advantageous (cost and complexity) alternative to the actual methods involving tedious and expensive ion exchange clean-up procedures. The authors claim good recoveries (> 90 %) on surface and groundwater samples fortified at 0.001 – 0.002 µg/L and a detection limit (LOD) of 0.2 ng/L, comparable to the LODs of other active ingredients of plant protection agents.

Analysis of soil

Due to their strong binding capacity to the soil matrix, glyphosate and AMPA are difficult to extract and best efficiency is achieved by an extraction under caustic conditions (0.5 M KOH or 0.5 M NH₄OH). The resulting extract is complex and requires a thorough clean-up before analysis. The methods involving ion exchange clean-up procedures are suitable for the analysis of soil (DFG 405, Alferness, *et al* (1994), Börjesson *et al.* (2000)). Typical quantification limits are around 0.01 mg/kg.

Reference for further detailed information:

1. Alferness P.L., Iwata Y. (1994), Determination of glyphosate and (amino methyl)phosphonic acid in soil, plant and animal matrices and water by capillary gas chromatography with mass selective detection *J. Agric. Food Chem* 42, 2751-2759
2. Börjesson, E., Torstensson, L.(2000), New methods for the determination of glyphosate and (amino methyl)phosphonic acid in water and soil, *Journal of Chromatography A*, 886, 207 – 216
3. Colin R., Le Fur E., Charreteur C., Dufau C., Perron J-J (2000) Determination of glyphosate herbicide and aminomethylphosphonic acid (AMPA) in water by liquid chromatography and fluorescence detection. Part II: Direct determination using pre-column derivatisation with NBDCI, *Analysis* 28, 819-824
4. DFG 405 (1989), Rückstandsanalytik von Pflanzenschutzmitteln, VCH Verlagsgesellschaft mbH, Weinheim, Band II, 10. Lieferung, Methode 405
5. DIN 38407-22, German standard methods for the examination of water, waste water and sludge – jointly determinable substances (Group F) – Part 22 : Determination of glyphosate and

aminomethylphosphonic acid (AMPA) by high performance liquid chromatography (HPLC), post-column derivatisation and fluorescence detection (F22)

6. Freuze I., Jadas-Hécart A., Royer A., Communal P-Y (2007) Influence of complexation phenomena with multivalent cations on the analysis of glyphosate and aminomethylphosphonic acid in water, *Journal of Chromatography A*, 1175, 197-206
7. Hanke I., Singer H., Hollender J. (2008), Ultra-trace level determination of glyphosate, aminomethylphosphonic acid and glufosinate in natural waters by solid phase extraction followed by liquid chromatography and tandem mass spectrometry : performance tuning of derivatisation, enrichment and detection, *Anal. Bioanal. Chem* 392, 2265-2276
8. Le Fur E., Colin R., Charrêteur C., Dufau C, Peron J-J (2000) Determination of glyphosate herbicide and aminomethylphosphonic acid in natural waters by liquid chromatography using pre-column fluorogenic labeling. Part I : Direct determination at the 0.1 µg/L level using FMOOC, *Analysis* 28, 813-818
9. Reichert N (2005) European round-robin study for glyphosate residues (glyphosate and its metabolite AMPA) in surface water and groundwater, Unpublished report, Study IF-04/00162935
10. Royer A., Beguin, S., Tabet J.C., Hulot, S., Reding, M-A, Communal P-Y, (2000) Determination of glyphosate and aminomethylphosphonic acid residues in water by gas chromatography with tandem mass spectrometry after exchange ion resin purification and derivatisation. Application on vegetable matrices. *Analytical Chemistry* 72, 3826-3832
11. Stalikas C.D. , Konidari C.N. (2001) Analytical methods to determine phosphonic and amino acid group-containing pesticides, *Journal of Chromatography A*, 907, 1-19
12. Vreecken R.J., Speksnijder P., Bobeldijk-Pastorova I., Noij Th.H.M. (1998), *Journal of Chromatography A*, 794, 187-199
13. WFD public library- sampling and reporting guidance : ([Click here](#))

See also:

- Environmental fate and behaviour of glyphosate and its main metabolite
- Monitoring results for glyphosate and AMPA in surface and groundwater

Document status:

Authors	Document status	
Marie-Anne Reding, Monsanto Europe Eberhard Zeitz, SGS Institut Frezenius	Final	February 2010

Disclaimer

All reasonable steps have been taken to ensure that the information provided in this document is accurate but neither EGEIS nor the authors can be held responsible for any use to which it is put.