ASPEC® 271 System: Determination of Glyphosate and AMPA

TECHNICAL NOTE TN216

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Introduction

Since 1970, glyphosate has been used as a systemic, non-selective herbicide in public and residential areas and as a crop desiccant. With the development of genetically modified organism (GMO) crops resistant to glyphosate, its usage increased drastically with a 15–fold jump of the quantity used between 1995 and 2014[1], to become one of the world's most widely used herbicides.

In soil, microbial action degrades Glyphosate rapidly into AminoMethyl Phosphonic Acid (AMPA) as shown in Figure 1.

Figure 1
Glyphosate and AminoMethyl Phosphonic Acid (AMPA) General Structure

Due to its strong adsorption, relative stability in soils and sediments, and its high solubility in water, glyphosate and its main degradation compound AMPA are present at low level concentrations in multiple environmental matrices: plants, soil, water, and also in the food chain.[2,3]

In 2015, the World Health Organization's cancer agency, the IARC, declared glyphosate "probably carcinogenic to humans," (group 2A). This controversial and sensitive decision increased the interest in glyphosate and AMPA. Both molecules are under strong regulation, but the maximum tolerated values vary greatly between countries (Table 1).

Table 1

Gyphosate and AMPA limits in Drinking Water

DRINKING WATER	CUT-OFF VALUES
US	700 µg/L
Canada	280 µg/L
Europe	0.1 µg/L

Due to the disparity between the limits of quantification (LOQ), the techniques used for analysis are also different and can include ELISA, GC-MS, LC-UV, LC-FLUO, LC-MS/MS. Therefore, the choice of sample preparation methodology will not only depend on the sample type (matrix), but also on the analytical approach. Sample preparation techniques include: Liquid-Liquid Extraction, Filtration, Centrifugation, SPE (off-line/on-line), and may or may not require derivatization.

Automated Workflows

The most common workflows are listed in Table 2 with their relevant sample preparation technique.

Table 2

Norms and Sample Preparation Recommendations for Glyphosate and AMPA Analysis

ANALYTICAL TECHNIQUE	NORM	SAMPLE PREPARATION
HPLC with Fluorescence Detection	NF ISO 21458 (Drinking Water)	LLE + Pre-column Derivatization
	Food and Complex Matrix	Pre-column Derivatization + SPE or (LLE) + SPE + Post-column Derivatization
	EPA Method 547 (Drinking Water)	Post-column Derivatization
GC with MS Detection	AOAC 2000.05 (Crops)	SPE with SCX Polymeric Sorbent
HPLC with MS/MS Detection	ISO 16380 (Drinking Water)	Pre-column Derivatization + LLE (Optional SPE)

Gilson liquid handlers an be configured to automate all processes including Liquid-Liquid Extraction, SPE, and pre-column derivatization. Figure 2 shows an example of how Norm ISO 16308 could be automated using Gilson automated liquid handling. As a further example, we present data from the automation of NF ISO 21458.

Figure 2
Norm ISO 16308 for Drinking Water

Automation of NF ISO 21458

Glyphosate and AMPA in Drinking Water (Dissolved Fraction)

The method described in NF ISO 214584 involves a pre-column derivatization step with FMOC–Cl (Figure 3) and the quantification is performed by liquid chromatography and fluorescence detection.

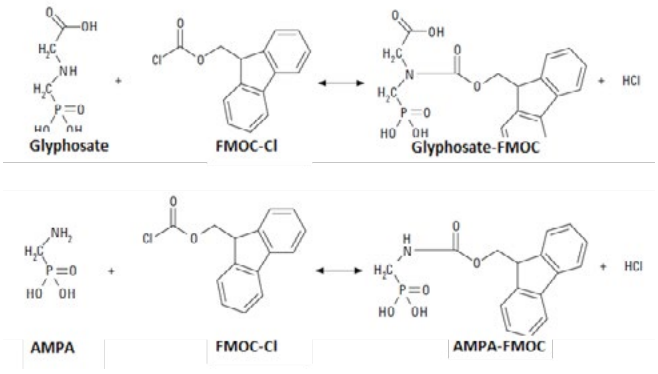

Figure 3
Glyphosate and AMPA Derivatization

The derivatization (nucleophilic substitution) in alkali conditions fixes a fluorophore on these compounds. This not only allows detection, but also decreases the polarity, and increases the retention of the compound in chromatography separation.

The sample preparation shown in Figure 4 is described in detail in the norm ISO 21458.

After filtering the samples (dissolved fraction) and adding 100 µL of internal standard to perform the derivatization step in optimal pH conditions, 500 µL of Tetra borate buffer are added (in case of hard water, rich in calcium or magnesium, EDTA has to be added also). To decrease the matrix effect and to remove organic interferents especially for the natural water, liquid-liquid extraction step with Diethyl Ether maintains a good specificity of the method.[5]

Glyphosate and AMPA present in the aqueous phase are derivatized by addition of an excess of FMOC (in acetonitrile, due to the poor polarity of this reagent in water).

After reaction time (60 min), a second liquid-liquid extraction will remove the excess of FMOC prior to the HPLC analysis and will avoid FMOC and AMPA peaks co-eluting.

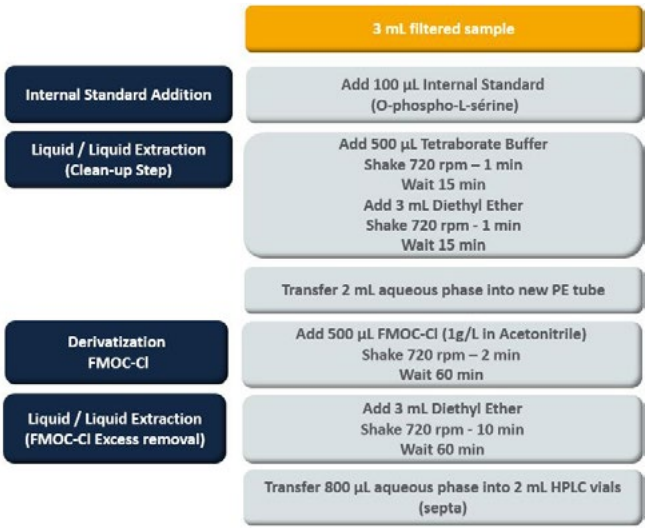

Figure 4
ISO 21458 – Preparation Method

This protocol can be automated with the Gilson ASPEC 271 as shown in Figure 5.

Figure 5
Norm NF ISO 21458 for water quality

The GX-271 Liquid Handler (Figure 6) is paired with a VERITY® 4260 Dual Syringe Pump with 10 mL and 1 mL syringes, so small volumes of internal standard and larger volumes of liquid/liquid solvents can be added precisely and simultaneously.

Figure 6
GX-271 Liquid Handler paired with a VERITY® 4260 Dual Syringe Pump and Orbital Shaker

The automated Orbital Shaker allows for solvent mixing to optimize the liquid-liquid extraction and the derivatization.

TRILUTION® LH Software controls all of the liquid transfers, the shaking, and the wait times.

Results of Automation of NF ISO 21458

The Glyphosate and AMPA are analyzed by HPLC with fluorimetric detection.

- Agilent 1200 LC system: 1 mL/min isocratic ACN 30% / 70% buffer KH2PO4 0.05 M - pH 5.4
- Column: Agilent Zorbax NH2 4.6 mm x 250 mm 5 µm
  Temperature: 35°C
- Fluoreometer set up:
  - Ex wavelength: 260 nm
  - Em wavelength: 310 nm

A typical chromatogram is shown in Figure 7.

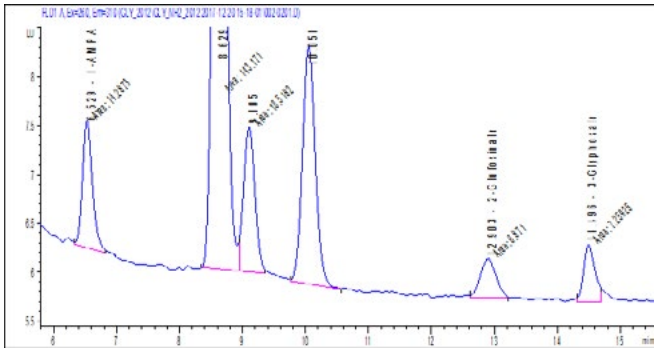

Figure 7
Results of Automation of NF ISO 21458

Figure 8 shows a Glyphosate and AMPA calibration curve with linearity for five points from 0.1 µg/L to 2 µg/L:

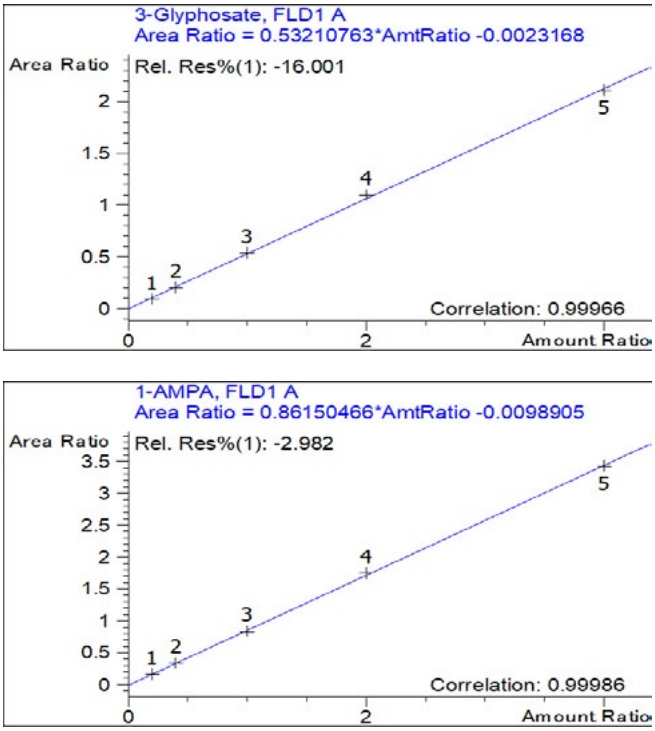

Figure 8
Glyphosate and AMPA Calibration Curve

The limit of quantification (LOQ) is 0.1 µg/L and has been validated following the NF T 90-210 guidance.

The validation has been established on different days, with several matrices (two drinking water, two surface water, and two underground water), at three levels of concentration (Figure 9).

	CONCENTRATION	
	0.20 µL	1.0 µG/L
AMPA	CVIF = 10.0%	CVIF = 8.0%
	Bias = 6.6%	Bias = 5.6%
GLYPHOSATE	CVIF = 7.2%	CVIF = 6.9%
	Bias = 2.2%	Bias = 8.4%

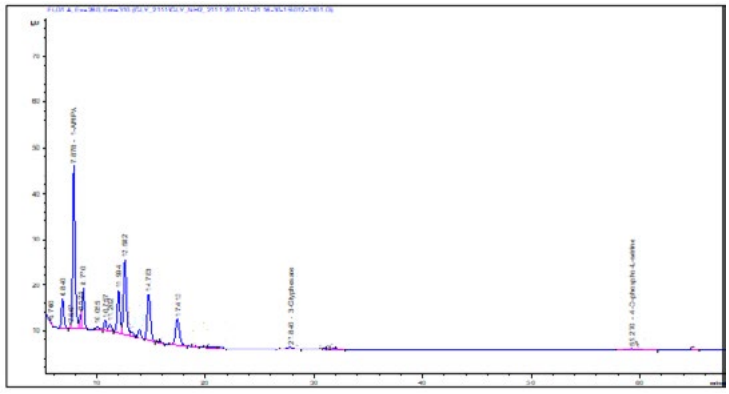

Figure 9
Example of Contaminated Surface Water

Conclusion

The Method NF ISO 21458: "Water quality — Determination of glyphosate and AMPA — Method using high performance liquid chromatography (HPLC) and fluorometric detection" can be fully automated on the ASPEC 271 with important benefits:

- Full automation = High throughput with 24h run possible
- Up to 35 samples unattended = Large capacity
- Orbital Shaker = Efficient and reproducible extraction and derivatization steps
- All samples are mixed at the same time with a controlled speed
- Dual Syringe Dilutor = High precision and accuracy for solvent, samples, internal standard and creation of calibration curve
- Predefined "Glyphosate & AMPA" Methods = Quick installation with limited training.
- Ability to transfer final derivatized samples into HPLC septa vials
- Upgradeable to solid phase extraction = Adaptable to future changes in regulation

Acknowledgments

We would like to give a special acknowledgment to Mrs. Moratelli and Mr. Baranek (Environmental Laboratory of Seine & Marne – France) who kindly shared their results with us.

Reference

[1]Environmental Working Group. "Monsanto's glyphosate now most heavily used weed-killer in history: Nearly 75 percent of all glyphosate sprayed on crops in the last 10 years." ScienceDaily, 2 February 2016; www.sciencedaily.com/releases/2016/02/160202090536.htm.

[2]M. Helander, I. Saloniemi, K. Saikkonen. (2012) "Glyphosate in northern ecosystems." Trends in Plant Science, 17(10), p569-574, "http://dx.doi.org/10.1016/j.tplants.2012.05.008" dx.doi.org/10.1016/j.tplants.2012.05.008

[3]V. Torretta, I.A. Katsoyiannis, P. Viotti, E.C. Rada (2018) Critical Review of the Effects of Glyphosate Exposure to the Environment and Humans through the Food Supply Chain. Sustainability, 10(4), 950; doi.org/10.3390/su10040950

[4]International Organization for Standardization (2008) ISO 21458:2008. Water quality -- Determination of glyphosate and AMPA -- Method using high performance liquid chromatography (HPLC) and fluorometric detection. www.iso.org/standard/40245.html

[5]R. Colin, E. Le Fur , C. CharrÍteur , C. Dufau , J.J. Péron Dosage du glyphosate et de l'AMPA dans l'eau Comparaison de deux fluorophores : FMOC et NBD-Cl, (2001), Actes du XXXIe congrès du Groupe Français des Pesticides, pp 8- 16; http://symposcience.lyon.cemagref.fr/exl-doc/colloque/ART-00000271.pdf

Ordering Information

PART NUMBER	DESCRIPTION
2614008	GX-271 ASPEC, DUAL 4260, WITH Z DRIVE
25025345	SYRINGE, 10ML, 215/235
25025343	SYRINGE, 1ML, 215/235
2644701	PLUMB PKG,GX241/GX271 ASPEC 10 mL
2644700	PLUMB PKG,GX241/GX271 ASPEC 5 mL
2644708	DUAL ADAPT KIT, GX271 ASPEC
2604706	SHIELD ASSEMBLY, GX27X
2604613	GUIDEFOOT, GX-271, 2.3MM
26034551	RINSE STATION,GX INSIDE 175MM
26034555	RINSE STATION,GX OUTSIDE 175MM FC
26041035	LOCATOR 3 200 SERIES, GX-271
251711	ORBITAL SHAKER
25045514*	RACK HGTNR,200 SERIES 48.2MM RH1
2504628	RACK,CODE 228 4 500/700ML REAGENT BOT
543701500	BOTTLE, SOLVENT, 500ML, 4/PK
2504607	RACK,CODE 207 75 16 X 100MM VACUTAINER
2504609	RACK,CODE 209 96 12 X 32MM 2ML VIALS
361832	508 INTERFACE MODULE, 110-220 VOLT
210630R30	TRILUTION LH MEDIA - V3.0
21063023	TRILUTION LH 3.0 LICENSE, LIFETIME

* QUANTITY 2 REQUIRED