



# BENAKI PHYTOPATHOLOGICAL INSTITUTE

## Residue determination of glyphosate and AMPA in soils, after derivatization, by liquid chromatography coupled to tandem mass spectrometry

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#### INTRODUCTION

Glyphosate [N-(phosphonomethyl)glycine] is a broad spectrum, non-selective, post-emergence herbicide that control weeds by inhibiting their ability to synthesize amino acids. Aminomethylphosphonic acid (AMPA) is the major metabolite of glyphosate, found in plants, water and soil. Glyphosate is strongly adsorbed to most soils and thus, does not lead or run off appreciably. Then soil microorganisms break it down. The estimated half life of glyphosate in soil is approximately 60 days. After 360 days, residue levels were from 6 to 18% of the initial applied dose. Precipitation, soil composition, presence and absence of a soil constricting layer and drainage type may influence the leaching of glyphosate from soil. Glyphosate is an aminophosphonic analogue of the natural amino acid glycine.

The lack of adequate chemical groups in glyphosate molecule (e.g. chromophores or fluorophores) hampers its measurements by conventional detectors. Moreover, due to its ionic character, low volatility and low mass, its determination at the low concentrations required for residue analysis is very difficult. Even more difficult can result the residue determination in soil at low concentration level (e.g. below 0.1 mg/kg), due to complexity of this matrix sample. Because of the highly polar nature of this compound, extraction from environmental matrices with organic solvents is almost impossible. This has made its determination a difficult challenge.

An analytical method has been developed for the rapid and robust determination at low concentrations of glyphosate and its major metabolite AMPA in soil by LC-MS/MS.

The sample preparation procedure included a derivatization step with 9-fluorenylmethylchloroformate (FMOC-Cl), previous to their determination, in order to improve the chromatographic behaviour of both glyphosate and AMPA.

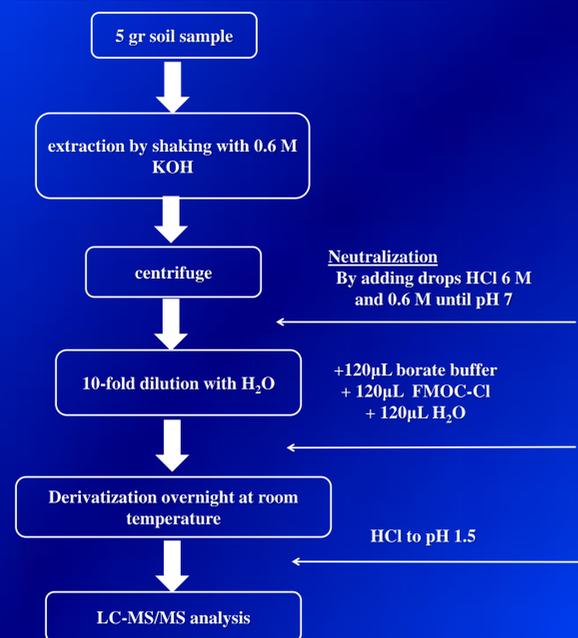
The method fulfilled the requirements of excellent sensitivity and unequivocal confirmation of the residues detected by using two MS/MS transitions for each compound.

#### EXPERIMENTAL

Analytical standards of glyphosate (99%) and AMPA (97,5%) were purchased from Dr Ehrenstorfer. Analytical reagent-grade disodium tetraborate decahydrate was obtained from Lachner. 9-fluorenylmethylchloroformate (FMOC-Cl) was purchased by Alfa Aesar. Reagent-grade hydrochloric acid (HCl) and potassium hydroxide (KOH) were obtained from Merck, whereas acetic acid (Hac) and ammonium acetate were purchased from Panreac. LC-MS grade water and acetonitrile were obtained from Fischer Scientific.

Standard stock solutions of glyphosate and AMPA (1000mg/L) were prepared in water and mix standard solutions at different concentrations (1 – 0.01 µg/mL) were prepared in methanol. Soil samples were allowed to dry at room temperature in the dark, sieved and frozen at -20 °C till extraction. The extraction procedure is described in Figure 1.

Figure 1: Extraction procedure in soil sample



The selected reaction monitoring (SRM) transitions chosen for glyphosate were: 392 → 88 for quantification and 392 → 214 for confirmation. In the case of AMPA the transitions selected were: 334 → 179 for quantification and 334 → 112 for confirmation.

#### RESULTS AND DISCUSSION

##### Method validation

The method developed for glyphosate and AMPA was validated, obtaining satisfactory recoveries and precision for both analytes. Method validation of the procedure was performed, included the study of: linearity, limit of detection and quantification, as well as recovery tests.

Calibration curves were obtained for both compounds in the range 0.01-1 µg/ml with correlation coefficients (R<sup>2</sup>) 0.9991 for glyphosate and 0.9995 for AMPA. The method was found to be precise as RSD% was found to be 12% for glyphosate at 0.1 µg/g (lowest) fortification level, and RSD% whereas was 9% for AMPA at the same fortification level. The accuracy of the method provided satisfactory recoveries between 65.5% - 98% for glyphosate and 67.9% - 118.8% for AMPA at 0.1 µg/g fortification level. Limits of quantification (LOQ) were established as the lowest concentration assayed and validated, which gave satisfactory recovery and precision as previously referred. The limits of detection (LOD), defined as the lowest concentration that the analytical process can reliably differentiate from background levels, were obtained when the signal was three times the average of the background noise in the chromatogram at the lowest analyte concentration assayed.

##### Fortification

For the determination of the recovery fortified samples were prepared by spiking blank soil samples, previously unfrozen at ambient temperature, with different known volumes of working mix standard solutions. They were homogenised by mechanically shaking for better analyte distribution and the bulk of the solvent was left to evaporate at ambient temperature and controlled by weight. This is a procedure able to mimic weather conditions. It should be mentioned that blank soil samples were previously analyzed to confirm the absence of the analytes. The recovery experiments were performed by adding appropriate volume of mixture standard solutions to blank soil sample in order to yield fortification levels of 0.2 µg/g dry soil and 0.1 µg/g dry soil. The last step of sample preparation is the dilution of soil extracts with LC-grade water which assayed as fast as and simple way to minimize matrix interferences. All samples were quantified with mix standard solutions due to the 10-fold dilution, which has minimized the matrix effects.

##### Application

The procedure was applied to the analysis of soil (approximately 84 samples) from Kopaida, county of Viotia (Greece), an important agricultural area where glyphosate is widely used in cotton crop. The samples were collected in four different periods of time (February, May, July and September) in order to monitor the spatial and periodical variation of their concentration. In Figure 2 is illustrated the total concentration profile measured for soil samples from six different sampling sites. The occurrence of glyphosate was expected, due to the presence of a large number of hard-to-kill perennial weeds during the monitoring period.

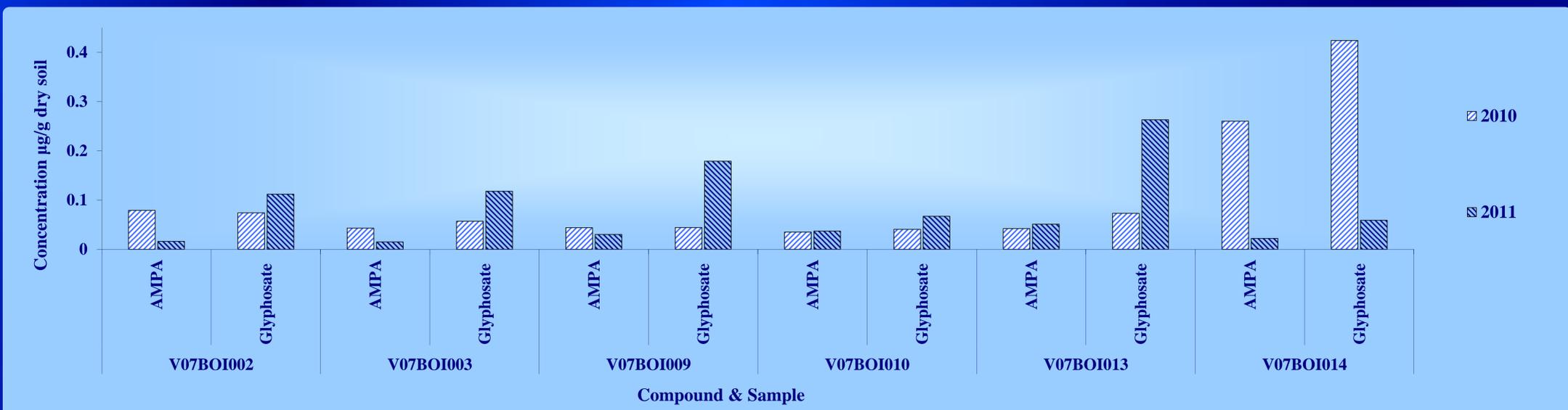
#### CONCLUSIONS

The problem of soil pollution in the pilot area due to pesticide residues is focused on herbicides, mainly glyphosate. The analytical method described shows the rapid and simple application of LC/ESI/MS/MS to the simultaneous analysis of glyphosate and its major metabolite AMPA. The concentration of glyphosate found depends on the type of soil, weather conditions and application practices used in the area.

##### Acknowledgments:

This study has been supported by the Life+ (LIFE07 ENV/GR/000266) Ecopest Environment Policy and Governance Programme, which is funded by the European Commission.

Figure 2: Levels of glyphosate and AMPA residues determined during the monitoring period 2010-2011 in six different cotton farms



To perform the chromatographic separation the mobile phase consisted of solvent A (water, ammonium acetate, acetic acid (pH 4.6)) and solvent B (acetonitrile) in the gradient presented in table 1. The injection volume was 5µl and the flow rate 0.2 ml/min.

According to the literature glyphosate and AMPA have been recorded in negative and in positive ion mode. In our study, both compounds were recorded in positive ion mode as sensitivity was found to be much higher.

Table 1: Gradient for LC separation

Time (min)	A %	B %
0:00	90	10
5:06	90	10
9:00	10	90
10:00	10	90
15:00	90	10
20:00	90	10

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