

INITIAL DEVELOPMENT WORK FOR THE AUTOMATED DERIVATISATION OF GLYPHOSATE AND AMINOMETHYLPHOSPHONIC ACID IN WATER FOLLOWED BY LC-QQQ ANALYSIS

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INTRODUCTION

Glyphosate is a herbicide commonly used by farmers and households alike to kill off weeds and other plants that can compete with desirable crops. Once applied, glyphosate breaks down into aminomethylphosphonic acid (AMPA). This degradation product can be used to indicate where glyphosate has been applied. Glyphosate has been heavily reported on recently, with many countries restricting future use due to potential carcinogenic effects. Therefore testing for this compound is likely to remain important in the future.

Current methods involve derivatisation followed by solid phase extraction (SPE) as a sample clean up and concentration step before injection, usually LC-MS being the most common analysis approach. When performed manually, this approach can be lengthy, prone to error and consumable intensive as many steps are involved. Using automation for this workflow both reduces analyst time and chemical use.

This application note describes the derivatisation of both glyphosate and AMPA using 9-fluorenylmethylchloroformate (FMOC-Cl) prior to sample injection. Unlike previous work, this method does not use an SPE step for sample clean up and so this reduces method time and use of consumables even further.

INTRUMENTATION

Autosampler: GERSTEL Dual Head MultiPurpose Sampler Robotic/Robotic Pro
Agilent HPLC coupled to 6460 QQQ



Figure 1a. Agilent HPLC coupled to 6460 QQQ

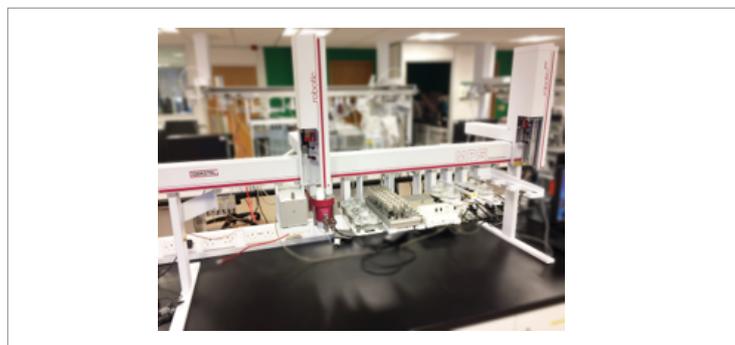


Figure 1b. GERSTEL MPS Robotic

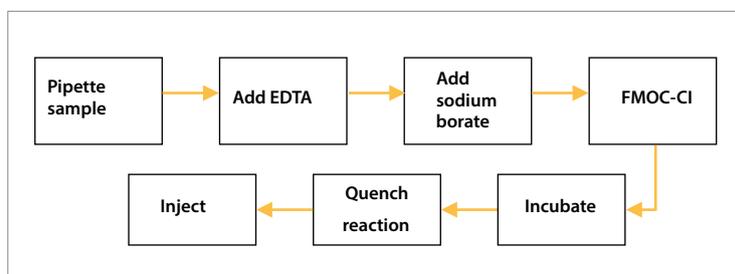


Figure 2. Sample preparation flow diagram

METHOD

Sample Preparation

Prior to preparation, samples were manually pipetted into a vial followed by addition of standards. The rest of the steps were automated using the GERSTEL MPS autosampler. An aliquot of ethylenediaminetetraacetic acid disodium salt (EDTA) was added to reduce matrix effects followed by addition of sodium borate to increase alkalinity of the solution prior to the derivatisation. FMOC-Cl in acetonitrile was added, which upon addition turns the solution cloudy. The samples were then incubated for 30 minutes after which the reaction was quenched using an aliquot of formic acid. Figure 2 depicts the flow diagram for the sample preparation. The samples were then transferred into 2ml autosampler vials ready for injection. Samples were analysed using direct injection with detection performed on an Agilent 6460 QQQ.

A full calibration range was prepared in duplicate, 0ng/L up to 120ng/L, which covers the range which may be expected.

Replicates were also prepared at 10, 20 and 100ng/L to assess repeatability. Once prepared samples were sent to customer's site for analysis.

RESULTS

Figure 3 shows the calibration curves produces which shows good linearity across the range with an R2 of 0.995 for glyphosate, 0.998 AMPA.

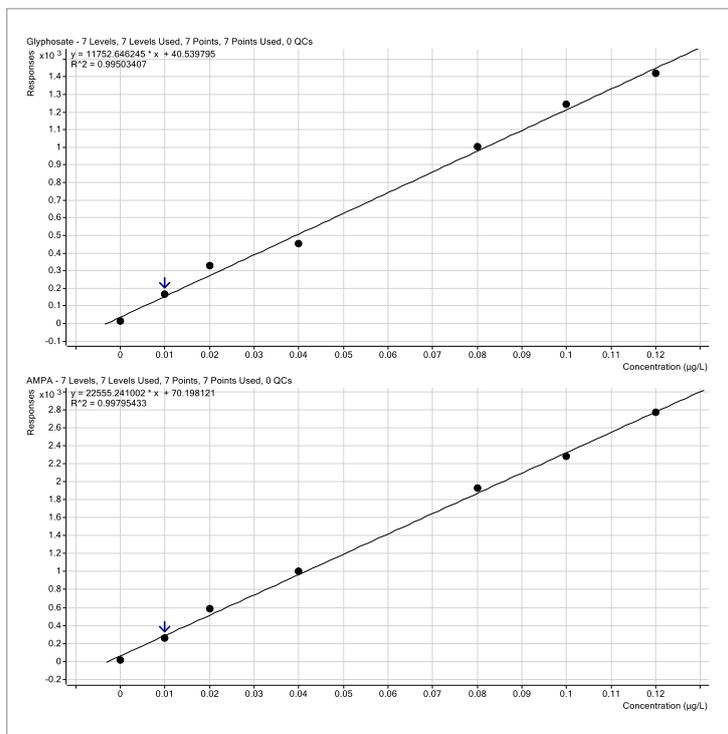


Figure 3. Calibration curves for glyphosate (top), AMPA (bottom)

Three replicates were prepared at levels that may expect to be found in water samples and also at the maximum allowed regulatory level, 100ng/L. Very good % RSD and recovery can be seen across the whole range although one outlier is present for glyphosate at 10ng/L. Chromatograms for both analytes at the lowest level can be seen in figures 4 and 5.

AMPA			
Level	0.01ug/L	0.02ug/L	0.1ug/L
Rep 1	0.0072	0.0216	0.0992
Rep 2	0.0072	0.0175	0.0997
Rep 3	0.0075	0.0172	0.0967
Mean	0.007	0.019	0.099
% RSD	2.37	13.10	1.63
% Recovery	73.00	93.83	98.53

Table 1. Replicate for AMPA

Glyphosate			
Level	10ng/L	20ng/L	100ng/L
Rep 1	0.0069	0.0231	0.1019
Rep 2	0.0115	0.0219	0.1043
Rep 3	0.0131	0.0195	0.0943
Mean	0.011	0.022	0.100
% RSD	30.65	8.53	5.21
% Recovery	105.0	107.5	100.2

Table 2. Replicate data for glyphosate

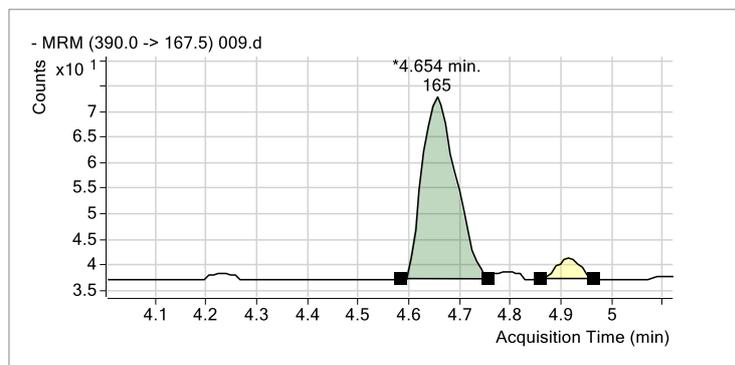


Figure 4. Glyphosate at 10ng/L

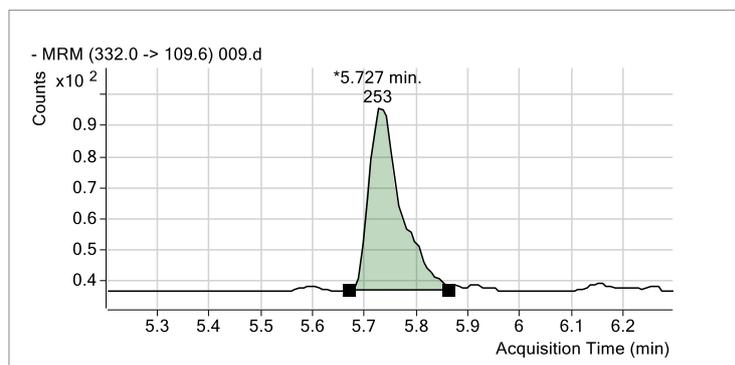


Figure 5. AMPA at 10ng/L

CONCLUSIONS

This work has shown that the sample preparation for glyphosate and AMPA analysis can be fully automated with direct injection into the LC negating the need for solid phase extraction steps thus reducing the analysis cost of this method. Further investigation would be needed to assess if further samples clean up is needed. Future work in this project will involve the addition of an LC valve to the MPS to allow for online injection. Further work and optimisation is needed to fully assess the precision and bias data and derivatisation across a range of water types. For this development work, internal standard was not used, using an appropriate internal standard would further improve the precision of the method.

ACKNOWLEDGEMENTS

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