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Application News

No. C220A

Liquid Chromatography Mass Spectrometry

New Strategy for Glyphosate, Glufosinate, and AMPA quantification in food: In-vial addition of pairing agent

Introduction

Glyphosate and Glufosinate are phytosanitary products that are commonly used as weed killers, particularly in cereals and vegetable crops. Glyphosate works by blocking the chain of synthesis of amino acid precursors which are essential for the functioning of the plant, especially for photosynthesis.

In 2015, the World Health Organization published a report concluding that Glyphosate should now be classified as a probable human carcinogen along with its major metabolite, Aminomethylphosphonic acid (AMPA). *1

The analysis of Glyphosate, Glufosinate, and AMPA is difficult and expensive. Indeed, their hydrophilic and ionic characteristics prevent their analysis in a multi-residue method for monitoring the environment and food.

Currently, different analytical techniques are used. Some analysis used derivatization with active reagents such as FMOC, however, this derivatization step complicates their quantification. For this reason, many other solutions, such as Anion exchange, Hydrophilic Interaction Liquid Chromatography (HILIC), porous graphite carbon, and mixedmode columns were used to determine the underivatized Glyphosate and other polar pesticides with LC-MS/MS in food matrixes. However, each of these methods had limited success. *2

This study presents a new analytical approach allowing for the quantification and separation on the reverse phase thanks to the in-vial addition of a pairing agent.

Classical ion-pairing technics are known to have certain drawbacks such as reducing sensitivity due to ionization competition, contamination of LC-MS/MS system, and necessity to increase system cleaning. These drawbacks are mainly due to the high quantity of pairing agents used in the mobile phase.

This new strategy uses an ion-pairing reagent—but only by in-vial addition. In this way, the quantity used is extremely low, only 125 nmol by injection. Therefore, in these conditions, the advantages of ion pairing are preserved without the disadvantages.

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*1 http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/Specs/Glyphosate_2016_02_10.pdf
*2 https://www.eurl-pesticides.eu/userfiles/file/EurlSRM/meth_QuPPe_PO_V11(1).pdf

Method

This application describes the analysis of Glyphosate, Glufosinate, and AMPA in food with in-vial addition of a pairing agent. This method allows us to achieve good retention, separation, and sensibility with reverse phase conditions, without the ion-pairing disadvantages.

These applications have the objective to allow their quantification with a low limit below 50 µg/kg for fruits, and below 100 µg/kg for other matrices.

Liquid chromatography Nexera™ X2 and mass spectrometry LCMS™-8060 are used. A Multiple Reaction Monitoring (MRM) in negative mode is performed with the tranisitions 167.9>62.9, 167.9>78.9 and for Glyphosate, 110.0>62.9, 110.0>78.8 for AMPA and 179.9>63.0, 179.9>85.0 for Glufosinate.

Table 1 **Analytical conditions**

Chromatography liquid	
Column	: Shim-pack Scepter™ Phenyl-120 Metal Free (100 mm × 2.1 mm I.D., 3 µm) (227-31093-02)
Mobile phase A	: Water
Mobile phase B	: Acetonitrile
Flow rate	: 0.4 mL/min
Column temp.	: 50 °C
Injection volume	: 5 µL
Gradient (min)	: 0 %B (0.0-0.2 min)- 100 %B (3.1-4.0 min)- 0 %B (4.1-7.0 min)
Mass spectrometry	
System	: LCMS-8060
Interface	: Electrospray (ESI)
Neb gas	: 3 L/min
Drying gas	: 5 L/min
Heating gas	: 15 L/min
Desolvation line	: 300 °C
Heat block	: 500 °C
Interface	: 350 °C
CID	: 325 kPa
Interface Voltage	: -5 kV

Calibration curve preparation

The calibration curves were prepared with a commercially standard mixture solution of Glyphosate, Glufosinate, and AMPA and Diamylammonium acetate (DAAA) solution.

A 20 µg/mL mixture standard solution was purchased from FUJIFILM Wako Pure Chemical (Osaka, Japan) and a commercially standard solution of DAAA at 0.5 mol/L in water was purchased from TCI (Tokyo, Japan). The DAAA solution was diluted by 10 in acetonitrile to obtain a final concentration of 50 mmol/L.

Three intermediate solutions (SI) of pesticides at 1000, 100, and 10 ng/mL were prepared in water. Then, the SI were diluted in methanol to obtain 8 solutions at 0.4, 1, 2, 10, 20, 100 and 200 ng/mL. Finally, these solutions were diluted by 2 in 50 mmo/L DAAA solution.

Samples preparation

Four kinds of food were analyzed—rice, flour, barley, and mandarins. These samples were prepared following the sample preparation described below. The main steps are described in Fig. 1, with liquid extraction and dilution in the pairing agent. The rice, flour, and barley were doped at 100 µg/kg and the mandarins at 50 µg/kg. Each sample was extracted spiked 3 times and non-spiked once.

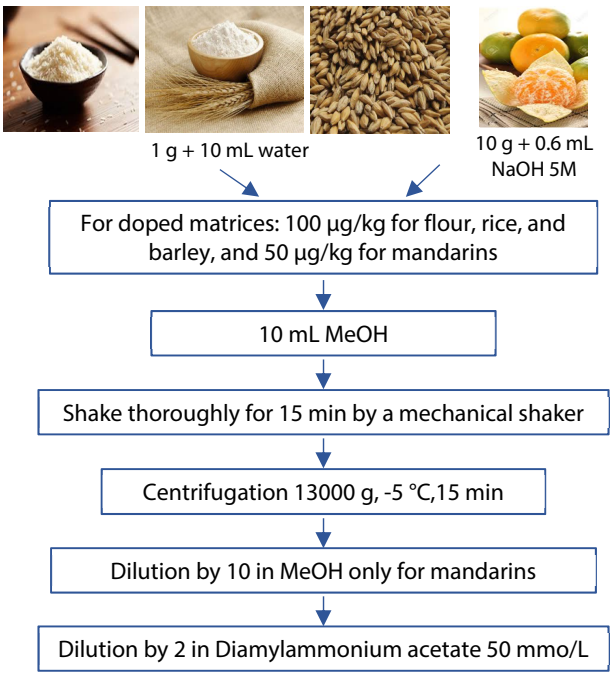


Fig. 1 **Sample preparation**

Results and discussion

Calibration data

The analysis of these pesticides, following the addition of a pairing agent in-vial, allow us to obtain a good separation on phenyl column with reverse phase LC condition, as seen in Fig. 2. The calibration solutions analysis allows us to obtaina good linearity, as seen in Fig. 3. The regression factor is greater than 0.99, and the accuracies obtained are between 85 % and 115 %.

Limits of quantification

The limits of quantification (LOQ), in solvent, are estimated at 0.1, 0.15 and 0.2 ng/mL, respectively for Glufosinate, AMPA and Glyphosate (Fig. 3).

The matrices analysis at 100 and 50 µg/kg allow us to obtain peaks with a good intensity (Fig. 4). Thus, the LOQs could be less than 100 µg/kg for flour, rice, and barley, and less than 50 µg/kg for the mandarins.

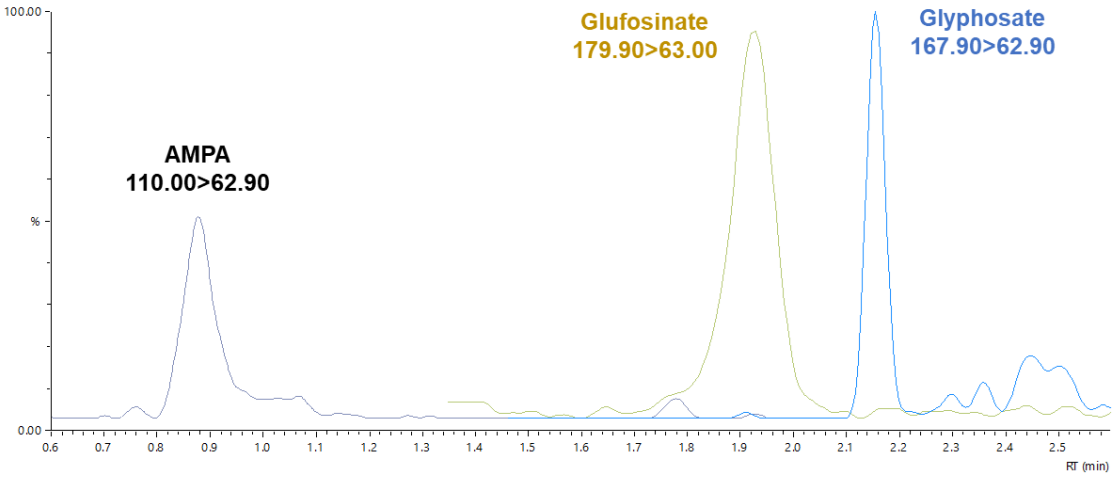
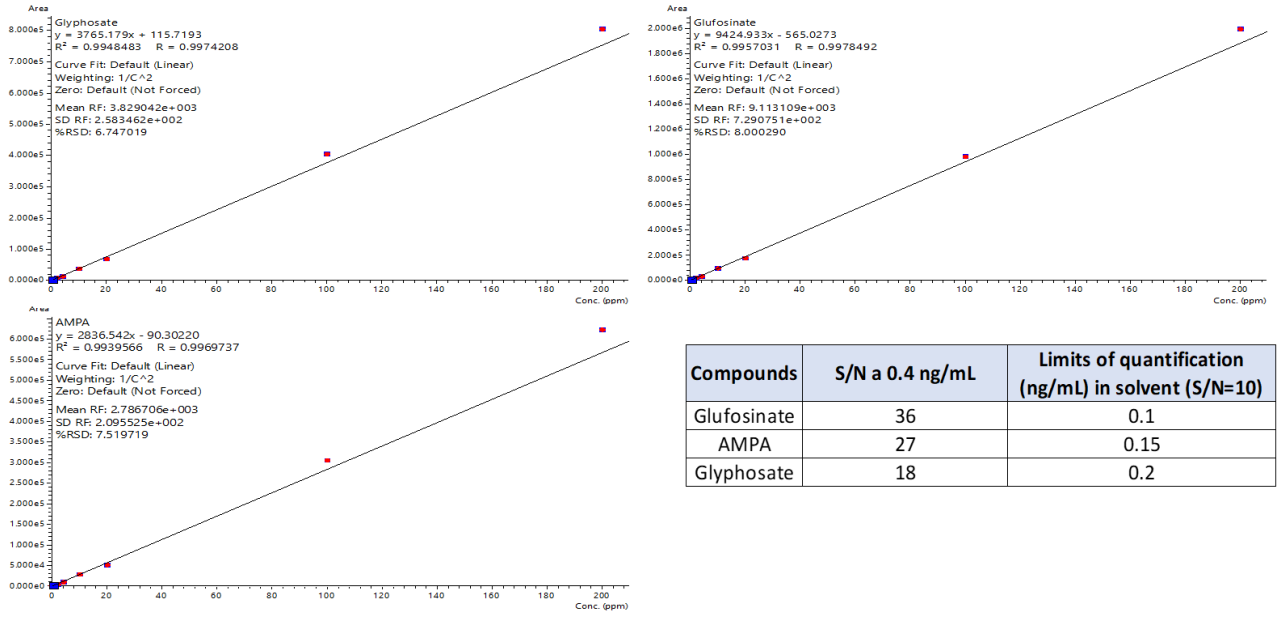


Fig. 2 **MRM chromatograms of 0.4 ng/mL in solvent of Glyphosate, AMPA, Glufosinate**



Compounds	S/N a 0.4 ng/mL	Limits of quantification (ng/mL) in solvent (S/N=10)
Glufosinate	36	0.1
AMPA	27	0.15
Glyphosate	18	0.2

Fig. 3 **Calibration curves and limits of quantification**

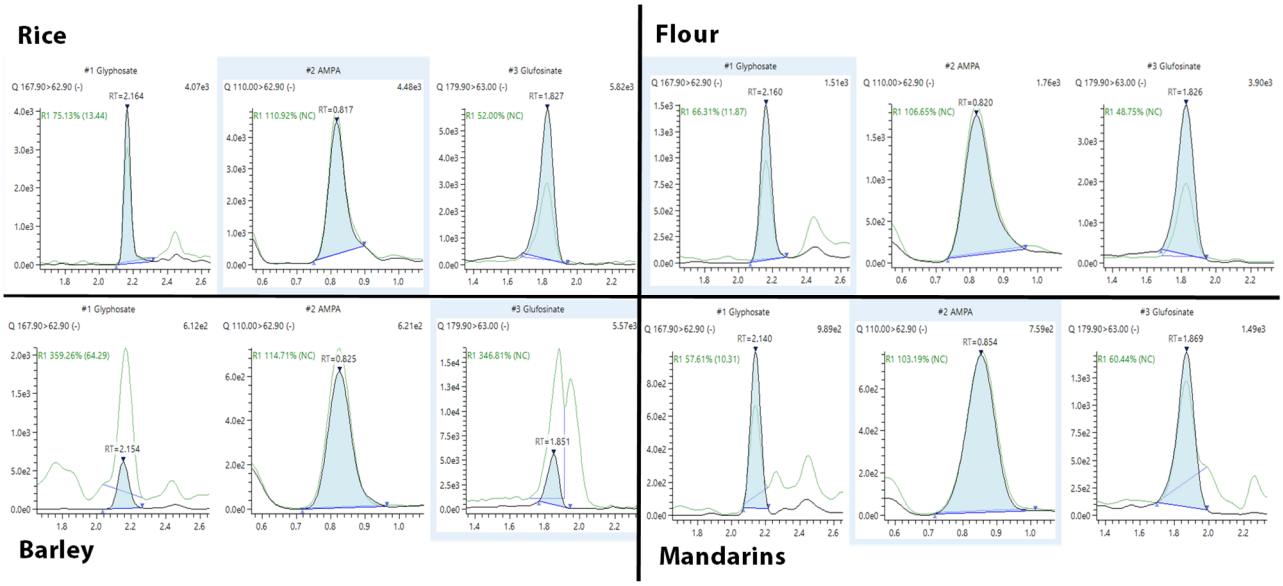


Fig. 4 **Chromatograms in matrix: rice, flour, barley 100 µg/kg, and mandarins 50 µg/kg.**

Extraction yield

To evaluate the extraction yield, the compound areas obtained on samples spiked before extraction and after extraction are compared. Then, an average of the three extractions are calculated. Regardless of the compounds and matrices, the extraction yields are between 80 % and 107 %.

Repeatability

The area repeatability (RSD) were evaluated in matrix at the 100 µg/mL in flour, rice, and barley and 50 µg/mL in mandarins. Each sample was extracted three times. Whatever the matrix, the RSD of Glyphosate, Glufosinate, and AMPA are between 0 % and 11 %.

Table 2 **Repeatability in matrice (%)**

Compounds	Flour RSD (%)	Rice RSD (%)	Barley RSD (%)	Mandarins RSD (%)
Glufosinate	0	5	2	3
AMPA	8	6	11	3
Glyphosate	2	5	7	6

Summary and conclusion

The Shimadzu LCMS-8060 allows the quantification of Glyphosate, Glufosinate, and AMPA in food.

This new strategy of in-vial pairing agent addition provides a method that allows us to achieve good retention, separation, and sensibility with reverse phase conditions, and without the ion-pairing disadvantages.

A rapid method is set up with a 7-min run and easy sample preparation. This sensitivity allows the quantification below 50 µg/kg for fruits and 100 µg/kg regardless of the matrix. This method shows good repeatability, yield extraction, and robustness.

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