Quantification of Glyphosate, Glufosinate, and AMPA in Food via In-vial Addition of Pairing Agent

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Glyphosate is currently one of the most common pesticides used worldwide. The analysis of glyphosate is challenging due to its high polarity. A new innovative method based on reverse phase liquid chromatography/tandem mass spectrometry is introduced here. The separation and the pesticides' quantification is possible due to the in-vial addition of a pairing agent. In this approach, the quantity of pairing agent may be as low as 125 nmol per injection, allowing the protocol to benefit from all the advantages of classical ion-pairing without the disadvantages.

35,000 tons per year – this is the amount of pesticides applied in the European Community, and there are many different types to look for. Pesticides are a large variety of substances, with multiple residues belonging to multiple classes. This makes the analysis a challenging task. It may require either liquid chromatography or SFC tandem mass spectrometry (LC-MS/MS or SFC-MS/MS) or gas chromatography tandem mass spectrometry (GC-MS/MS). These triple quadrupole mass spectrometers are the most widely used in pesticide screening due to their fast acquisition speed in selected reaction monitoring (SRM) allowing the screening of hundreds of pesticides simultaneously in one run with high sensitivity, selectivity, and a wide linear range.

The hazardous level of a pesticide depends on two factors: its toxicity and a person's exposure to that pesticide. Just a single exposure can have acute effects, such as impaired vision and motor disorders. Long-term, chronic exposure can lead to more serious illnesses and diseases, including blood and nerve disorders and even cancer. Because of these risks, the MRLs (Maximum Residue Limits) have been defined in the European Community for any food or feed where pesticides are applied correctly according to GAP (Good Agricultural Practices) in order to ensure the lowest consumer exposure. Commission Regulation (EC) No. 396/2005 lists 320 defined commodities for which more than 152,000 MRLs have been set [1].

The pesticide maximum residue levels are published by the EU Commission and regularly updated such as Regulation (EU) 2019/90 of 18 January 2019 [2].

The focus of this article is the analysis of Glyphosate and Glufosinate which are phytosanitary products widely used as weed killers, especially in cereal and vegetable crops. Glyphosate works by blocking the chain of synthesis of amino acid precursors essential for the functioning of the plant, especially for photosynthesis [3]. In 2015, the World Health Organization published a report concluding that Glyphosate, the most widely used herbicide, should be classified as probably carcinogenic to humans as well as its major metabolite Aminomethylphosphonic acid (AMPA) [4].

Analysis of Glyphosate, Glufosinate, and AMPA is difficult and expensive, because of their hydrophilic and ionic characteristics which prevent their analysis in a multi-residue method for monitoring the environment and food. Currently, different analytical techniques are used. Some analysis use derivatisation with active reagent such as FMOC (Fluorenylmethyloxycarbonyl), but this derivatisation step complicates their quantification. That's why many other approaches such as Anion exchange, Hydrophilic Interaction Liquid Chromatography (HILIC), Hypercarb, and mixed-mode columns to determine underivatised Glyphosate and other polar pesticides with LC-MS/MS in food matrixes have been tried. All these methods have limited success [5, 6, 7].

This study presents a new analytical approach allowing the pesticides' quantification and separation on reverse phase thanks to in-vial addition of a pairing agent. The classical ion-pairing technics are known to have some drawback like reducing sensitivity due to ionisation competition, contamination of LC-MS/MS system, necessity to increase system cleaning, and lack of volatility. These downsides are mainly due to the high quantity of pairing agent used in the mobile phase. This new strategy is using an ion-pairing reagent, but only by in-vial addition. In this way, the quantity used is very low, only 125 nmol by injection. So, in these conditions, the advantages of ion pairing are preserved without the disadvantages.

Method

The system configuration used for this type of analysis consists of the Shimadzu Nexera X2 liquid chromatograph with LCMS-8060 triple quadrupole mass spectrometer. Multiple Reaction Monitoring (MRM) in negative mode is performed with the transitions 167.9>62.9, 179.9>85.0 for Glyphosate, 110.0>62.9, 179.9>85.0 for AMPA, and 179.9>63.0, 179.9>85.0 for Glufosinate.

This setup, following the analytical conditions listed in Table 1 enables the quantification of Glyphosate, Glufosinate, and AMPA with a lower limit of detection below 50 µg/kg for fruits, and below 100 µg/kg for other matrices.

Calibration curve preparation

The calibration curves were prepared with a commercially available standard mixture solution of Glyphosate, Glufosinate and AMPA and the ion pairing agent Diamylammonium acetate (DAAA). A 20 µg/mL standard mixture solution was purchased from FUJIFILM Wako Pure Chemical (Osaka, Japan), and a commercially available standard ion pair solution of DAAA at 0.5 mol/L in water was sourced 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 of pesticides (SI) at 1000, 100, and 10 ng/mL were prepared in water. Then, these SI were diluted in methanol to obtain 8 solutions at 0.4; 1; 2; 4; 10; 20; 100 and 200 ng/mL. Finally, these solutions were diluted by 2 in 50 mmol/L DAAA solution.

Sample preparation

Four kinds of food were analysed: rice, flour, barley, and mandarin. These samples were prepared following the sample preparation described in Figure 1, with liquid extraction and dilution in the pairing agent. The rice, flour, and barley were spiked at 100 µg/kg, and the mandarin at 50 µg/kg. Each sample was extracted 3 times spiked, and 1 time non-spiked.

Table 1: Analyitcal Conditions

LC conditions	
Instrument	Nexera X2 (Shimadzu, Japan)
Analytical Column	Shim-pack Scepter Phenyl- 120 (100* 2.1 mm, 3 µm)
Mobile Phase	Water / Acetonitrile
Flow rate	0.4 mL/min
Column temperature	50 °C
Gradient (min)	0 - 0.2 (0 % B); 0.2 - 3.1 (0 - 100 % B); 3.1 - 4 (100 % B); 4 - 4.1 (100 - 0 % B); 4.1 - 7 (0 % B)
MS conditions	
Instrument	LCMS-8060 (Shimadzu, Japan)
Interface	Electrospray (ESI)
Neb gas	3 L/min
Drying gas	5 L/min
Heating gas	15 L/min
Desolvatation line	300 °C
Heat Block	500 °C
Interface	350 °C
CID	325 kPa
Interface Voltage	-5 kV

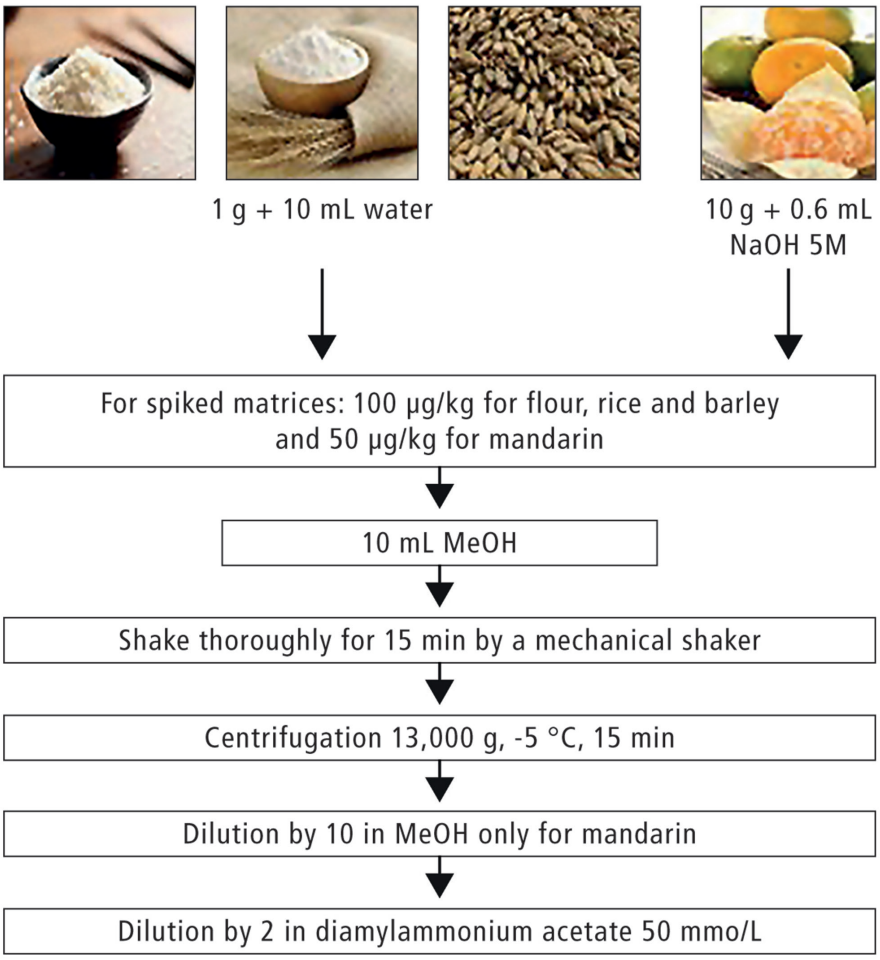


Figure 1: Sample preparation

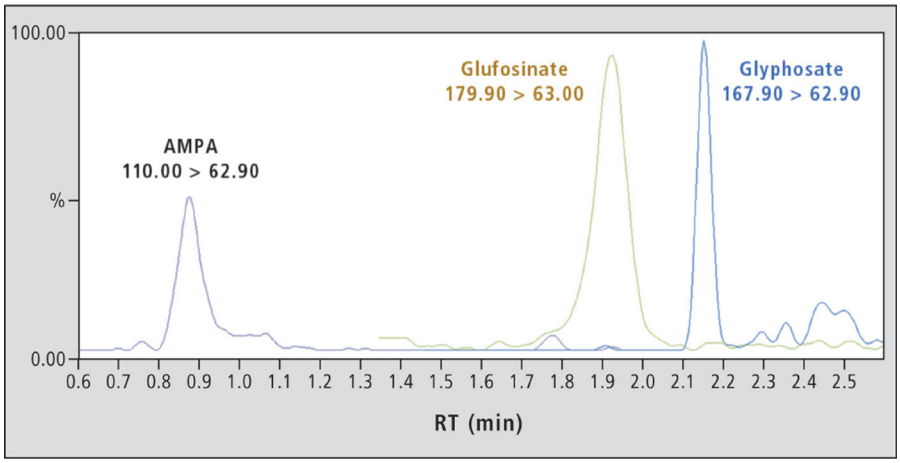
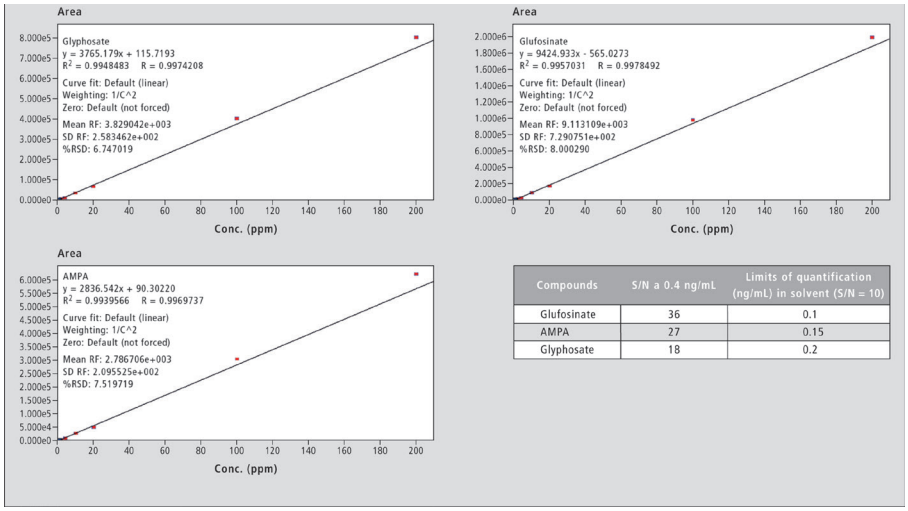


Figure 2: MRM chromatograms of 0,4 ng/mL of Glyphosate, AMPA and Glufosinate



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

Figure 3: Calibration curves and limits of quantification.

Results and discussion

Calibration data

The analysis of these pesticides, following the addition of pairing agent in vial, allows to obtain a good separation on phenyl column with reverse phase LC condition (see Figure 2).

The calibration curves, were prepared using the commercially available standard mixture solution of Glyphosate, Glufosinate and AMPA and Diamylammonium acetate (DAAA) solution as ion-pairing agent, all calibrations show good linearity (Figure 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 (Figure 3).

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

Extraction yield

To evaluate the extraction yield, the compound areas obtained on samples spiked before extraction and samples spiked after extraction are compared. Then, an average of the 3 extractions is calculated. independent of the compounds and matrixes variables, the extraction yield is always between 80 and 107%.

Repeatability

The area repeatabilities (RSD - relative standard deviation) were evaluated in matrix at the 100 µg/mL in flour, rice, and barley, and 50 µg/mL in mandarin. Each sample was extracted 3 times. Whatever the matrix, the RSD of Glyphosate, Glufosinate, and AMPA are between 0 and 11% independent as listed in Table 2.

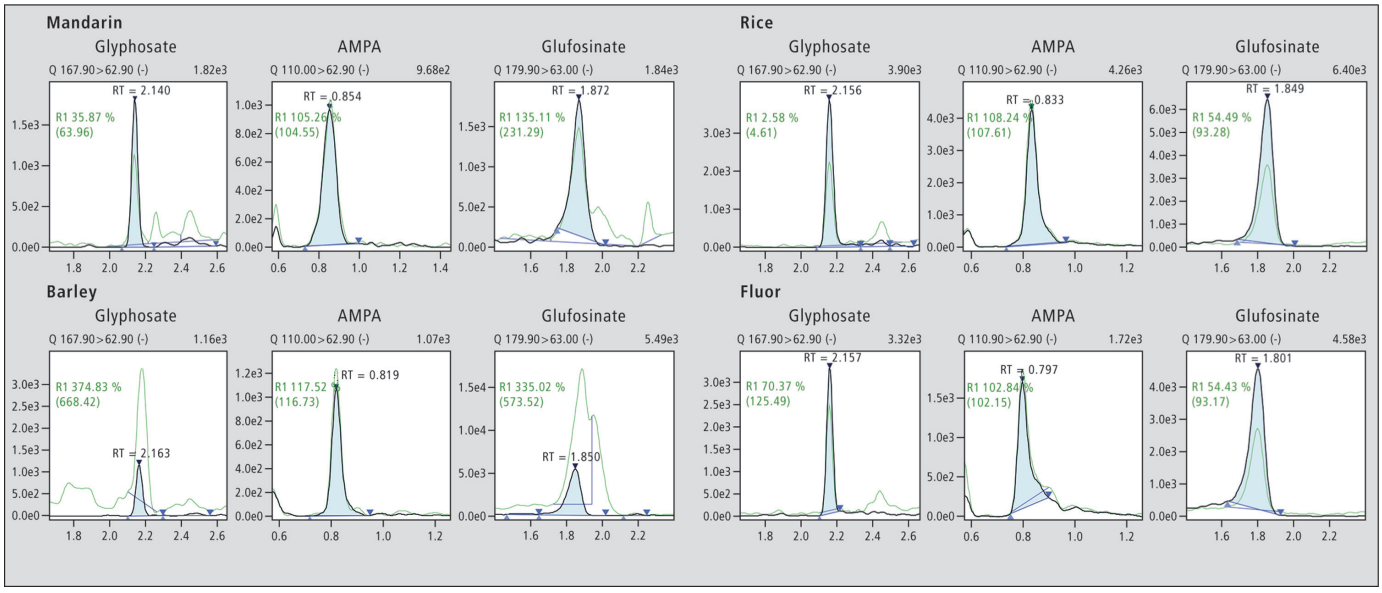


Figure 4: Chromatograms in matrix: rice, flour, barley 100 µg/kg and mandarin 50 µg/kg.

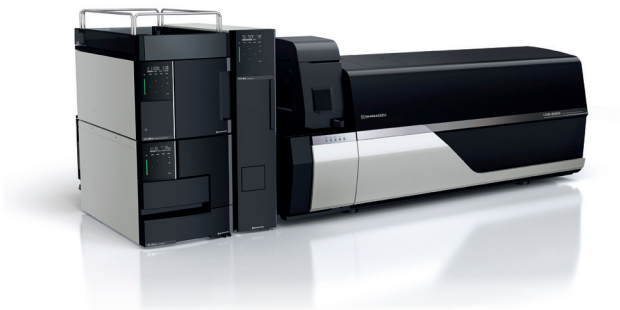
Summary and Conclusion

The Shimadzu LCMS-8060 allows the quantification of Glyphosate, Glufosinate, and AMPA in food. The new strategy of in-vial pairing agent addition provides a method that allows to achieving good retention, separation, and sensitivity with reverse phase conditions, and without the ion-pairing disadvantages.

A rapid method has been developed with a short runtime of only 7 minutes, easy sample preparation, and high sensitivity allowing the quantification below 50 µg/kg for fruit and 100 µg/kg for other matrices. This method shows a good repeatability, yield extraction, and robustness.

Table 2: Repeatability in different matrices (%).

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



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