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

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1. Introduction

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].

Table 1: Examples of MRL´s for pesticides in various commodities as published by EU Commission   Regulation (EU) 2019/90

Pesticide	Commodity	Parts per Million
Bromuconazole	Root and tuber vegetables	0.01
Bromuconazole	Bulb vegetables	0.01
Bromuconazole	Fruiting vegetables	0.01
Bromuconazole	Lettuce and salad plants	0.01
Carboxin	Citrus fruits	0.03
Carboxin	Tree nuts	0.05
Carboxin	Pome fruits	0.03
Carboxin	Stone fruits	0.03
Fenpyrazamine	Citrus fruits	0.01
Fenpyrazamine	Tree nuts	0.01
Fenpyrazamine	Pome fruits	0.01
Fenpyrazamine	Root and tuber vegetables	0.01

This poster describe a new innovative approach for 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].

2. Method

This application describes the analysis of Glyphosate, Glufosinate and AMPA in food with in-vial addition of pairing agent. This method allows to achieve good retention, separation, and sensibility with reverse phase conditions, without the ion-pairing disadvantages. This application 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 transitions 167.9>62.9, 179.9>85.0 and for Glyphosate, 110.0>62.9, 179.9>85.0 for AMPA and 179.9>63.0, 179.9>85.0 for Glufosinate.

2.1 Analytical 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 ℃
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 ℃
Heat Block	500 ℃
Interface	350 ℃
CID	325 kPa
Interface Voltage	-5 kV

2.2 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 obtained 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.

2.3 Sample Preparation

Four 4 kind of food were analysed, rice, flour, barley and mandarin. These samples were prepared following the sample preparation below. The main steps were described Fig.1, with liquid extraction and dilution in the pairing agent. The rice, flour and barley were doped at 100 µg/kg and the mandarin at 50 µg/kg. Each sample was extracted 3 times spiked and 1 times non-spiked.
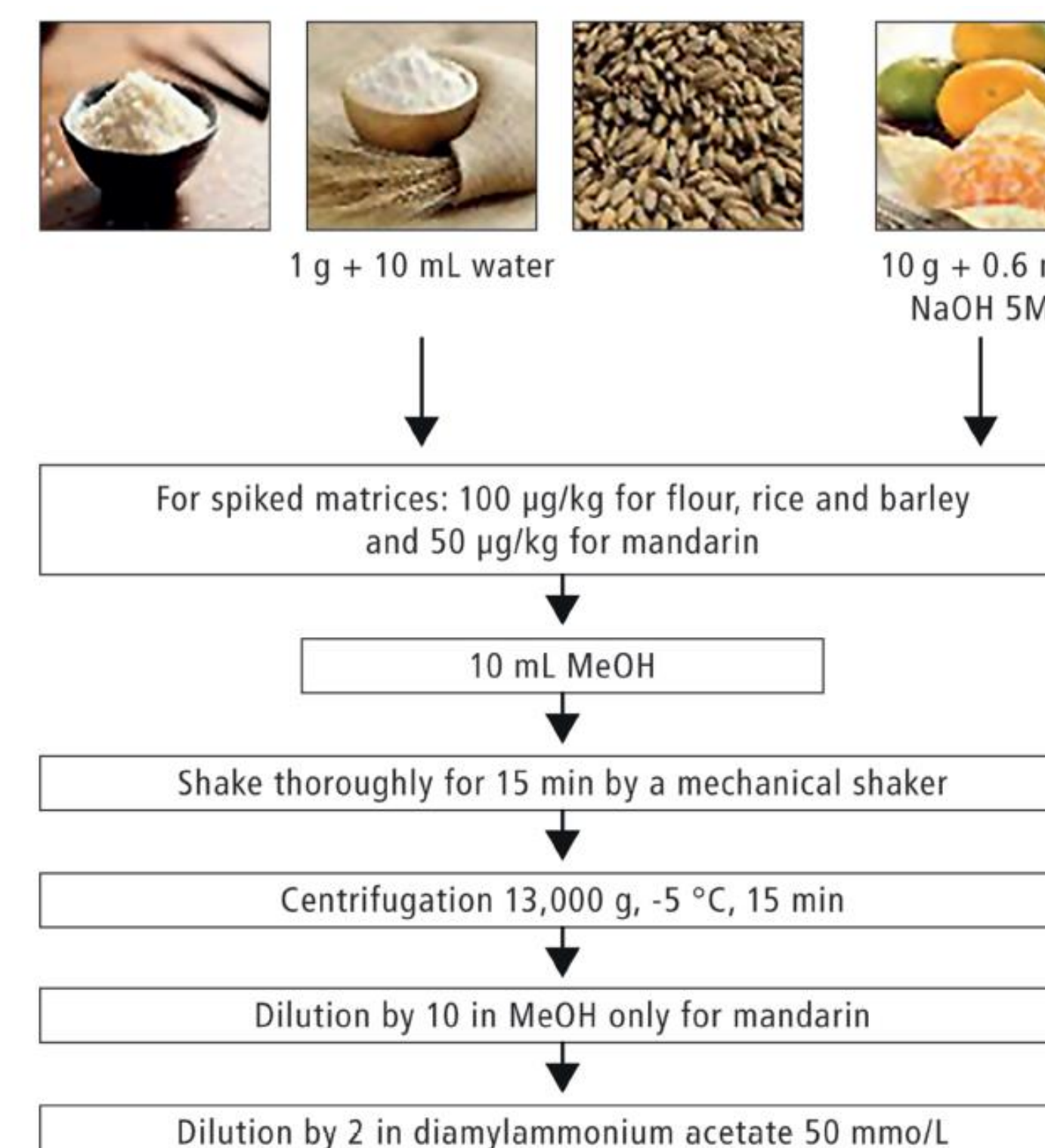

Figure 1: Sample Preparation

3 Results and Discussion

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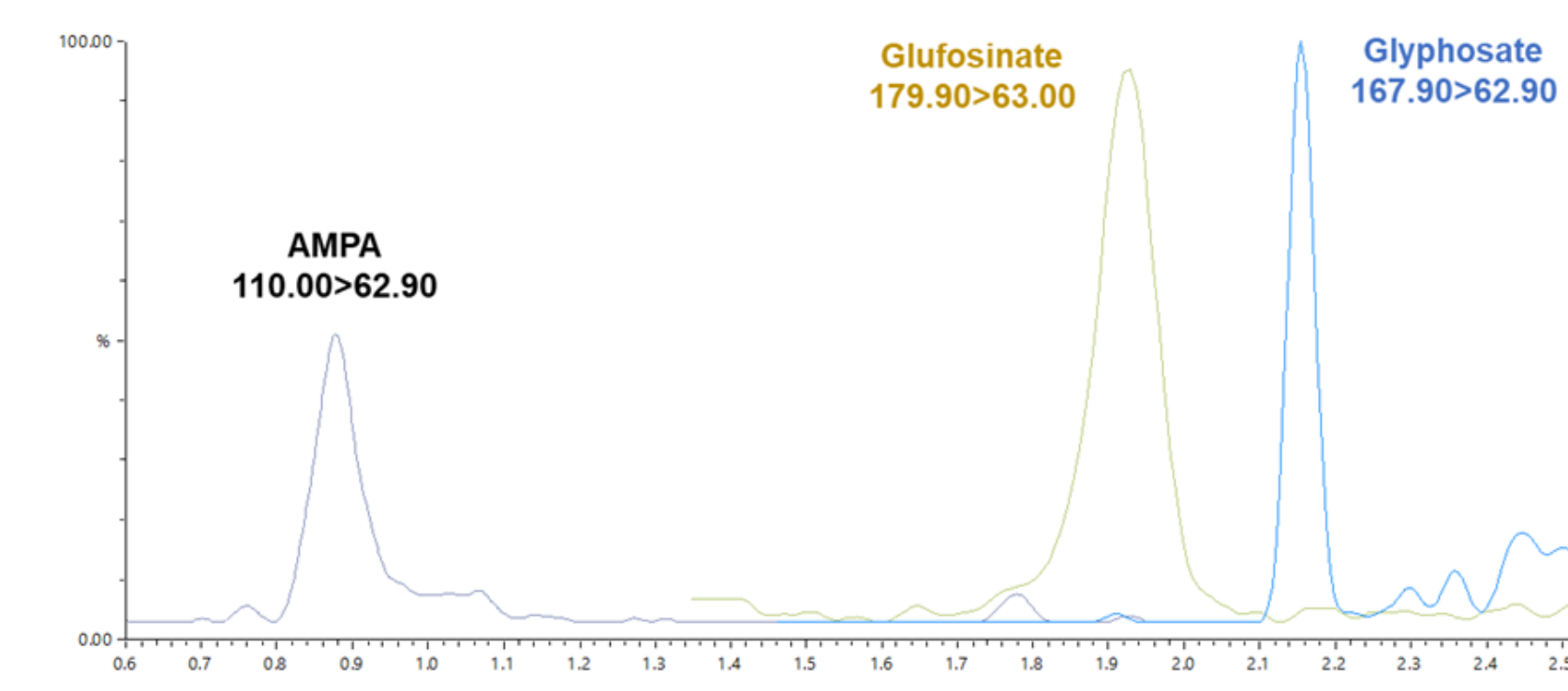

Figure 2: MRM chromatograms of 0,4 ng/mL in solvent of Glyphosate, AMPA, Glufosinate

3.1 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 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 mandarin.
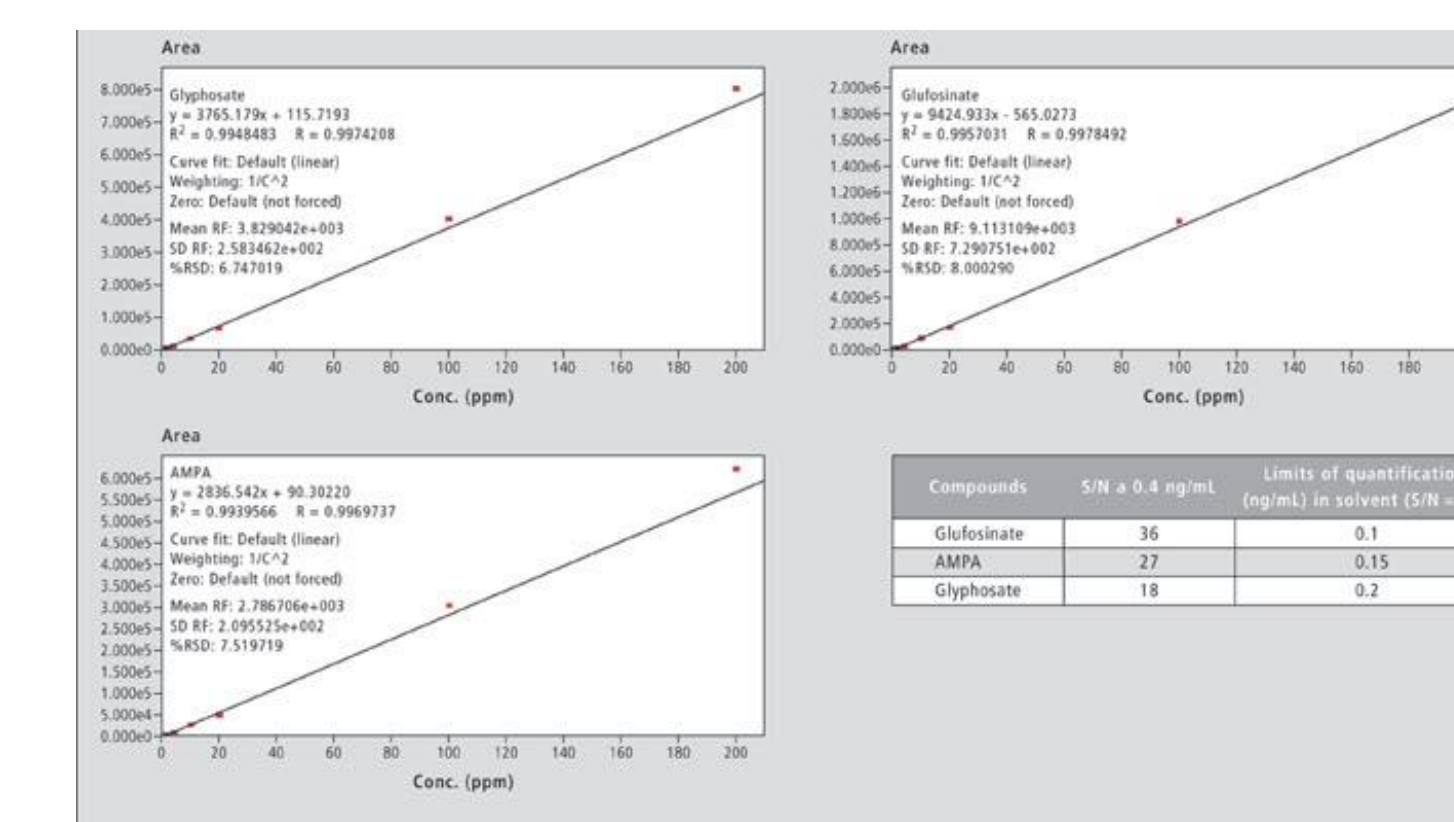

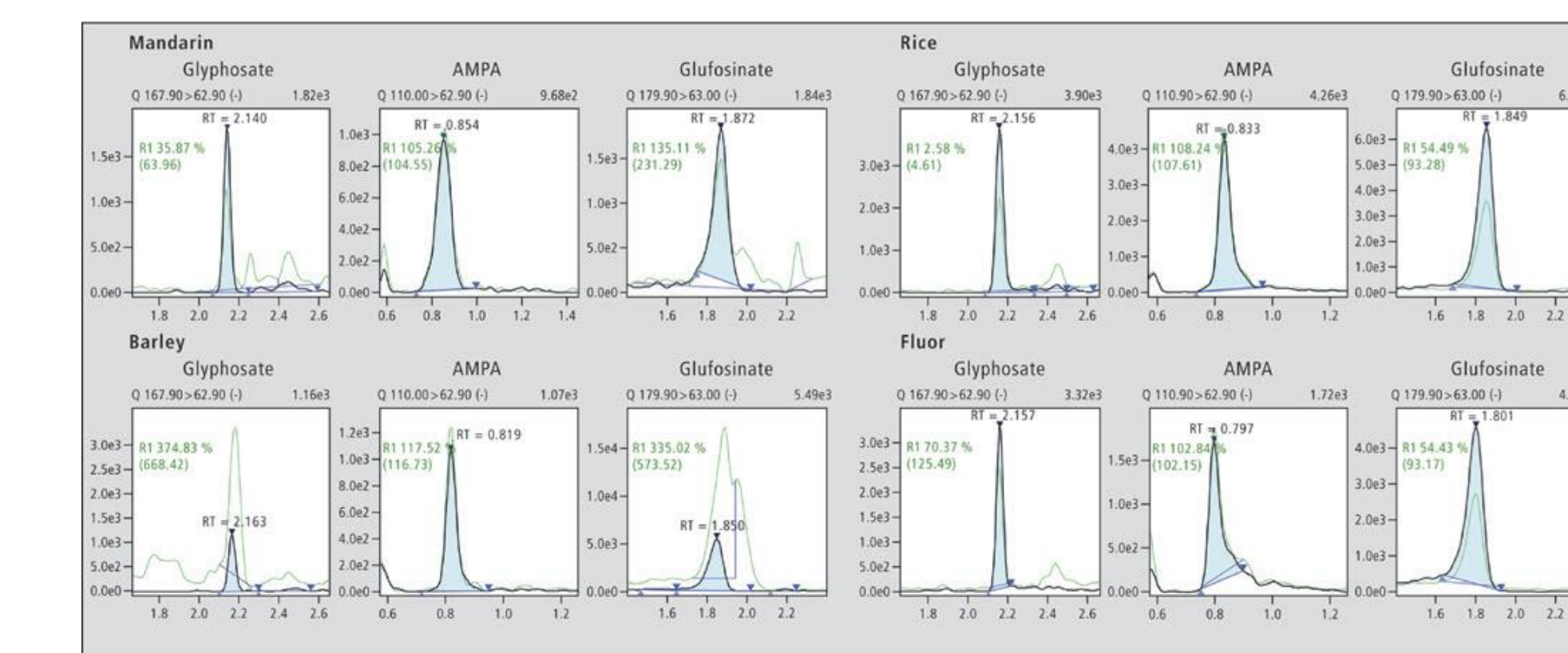Figure 3: Calibration curves and limits of quantification

Figure 4: Chromatograms in matrix

4 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

4 References

[1] Regulation (EC) No 882/2004 of the EU Parliament, 29 April 2004
[2] COMMISSION REGULATION (EU) 2019/89, https://eur-lex.europa.eu/legal-content/EN/TXT/?qid=1549378625609&uri=CELEX:32019R0089
[3] Gomes, M.P. et al.: J. exp. Botany, Vol. 65, Iss. 17, p. 4691–4703 (2014)
[4] http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/Specs/Glyphosate_2016_02_10.pdf