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 for food. For pesticides, there is 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 (LC-MS/MS or SFC-MS/MS) or gas chromatography tandem mass spectrometry (GC-MS/MS).

Two 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].

2. Method
This application describes the analysis of Glyphosate, Glufosinate and AMPA in food via an in-vial addition of pairing agent. This method allows to achieve good retention, separation, and sensitivity with reverse phase conditions, without the ion pairing disadvantages. This application have the objective to test their quantification with a low limit below 50 µg/kg for fruits, and below 100 µg/kg for other matrices. Liquid chromatography Nexum X2 and mass spectrometry LCQD8400 are used. A Multiple Reaction Monitoring (MRM) in negative mode is performed with the transitions 167.0->62.0, 179.8->65.0, and for Glufosinate, 110.0->52.0, 179.8->45.0 for AMPA and 173.6->13.0, 173.6->38.5 for Glufosinate.

2.1 Analytical Conditions

2.2 Calibration Curve Preparation
The calibration curves were prepared with a commercially standard solution of Glyphosate, Glufosinate and Ammonium acetate (AAOA) solution. A 20 µg/L mixture standard solution was purchased from FUJIFILM Wako Pure Chemical (Osaka, Japan) and commercially standard solution with 0.5 µL/mL in water was purchased from TCI (Tokyo, Japan). The AAOA solution was diluted by 10 in acetonitrile to obtain a final concentration of 50 µL/mL. Three intermediate solutions of pesticides (SI) at 100, 10 and 5 ng/mL were prepared in water. Then, these SI were diluted in methanol to obtain 8 solutions at 0.1, 0.2, 1, 2, 10, 20, 100 and 300 ng/mL. Finally, these solutions were diluted by 2 in 50 mL AAOA solution.

2.3 Sample Preparation
Four 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 diluted 1000 and the mandarin 100 µg/kg and the mandarin at 50 µg/kg. Each sample was extracted 3 times spiked and 1 times non-spiked.

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 allow to get a good linearity Figure 3. The regression factor is greater than 0.99 and the accuracies obtained are between 85% and 115%.

3.1 Limits of Quantification
The limits of quantification (LOQ), in sorven, are estimated at 0.1, 0.15 and 0.2 ng/mL, respectively for Glufosinate, AMPA and Glyphosate. Fig. 4. The matrix analysis at 100 and 50 µg/kg allow to obtain peaks with a good intensity Fig. 4. Thus, the LODs could be less than 100 ng/kg for flour, rice and barley and less than 50 ng/kg for mandarin.

4 Summary and Conclusion
The Shimadzu LCQD8400 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 achieve good retention, separation, and sensitivity with reverse phase conditions, and without the ion pairing disadvantages. This method has been developed with a short runtime of only 7 minutes, easy sample preparation, and high sensitivity allowing the quantification below 50 ng/kg for but and 100 µg/kg for other matrices.

References
[5] Uwe Oppermann, Stephanie Moreau, Darienne Toinoir Shimadzu Corporation, Kyoto, Japan The products and applications in this presentation are intended for Research Use Only (RUO), not for use in diagnostic procedures.