Rapid and reliable detection of glyphosate in pome fruits, berries, pulses and cereals by flow injection – Mass spectrometry

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Abstract

A flow injection – mass spectrometry method for rapid glyphosate detection in food commodities was developed and validated. The sample preparation protocol included a simple and rapid extract purification step through polymeric solid phase extraction cartridges followed by addition of isotopically labeled glyphosate to the final test sample. The optimized method was subjected to intra-laboratory validation (spiking range 0.5–100 mg/kg) in chickpeas, grapes and apples, as representatives of three different commodity groups as defined in SANTE/11813/2017 guidelines. Recoveries were in the range 60–111%, repeatability and within laboratory reproducibility were ≤17%.The trueness of the results generated with the developed method was evaluated by analysis of a set of incurred chickpea and wheat samples (glyphosate range 0.5–36 mg/kg) and comparison with the reference method (Quick Polar Pesticides Method), confirming the method fitness-for-purpose of rapid compliance testing.

Keywords

Glyphosate, Polar pesticides, Mass spectrometry, Rapid detection, Food safety

1. Introduction

Glyphosate (IUPAC name N-(phosphonomethyl)glycine), is one of the most widely applied herbicides sprayed against emerged annual, perennial and biennial weeds in all crops including root and tuber vegetables, pulses, oil seeds, and cereals. A recent report on worldwide glyphosate application showed a considerable increase of glyphosate agricultural uses after the development of glyphosate-resistant genetically modified crops (Benbrook, 2016). On the basis of these data the frequency and levels of glyphosate in a variety of foods are expected to increase, and more refined dietary-risk assessments should be carried out.

Glyphosate was re-discussed by the European Food Safety Authority (EFSA) (2015) after the publication of the Monograph 112 by the International Agency for Research on Cancer (IARC) regarding the potential carcinogenicity of glyphosate or glyphosate containing plant protection products (International Agency for Research on Cancer, 2015). EFSA concluded that the epidemiological evidence was very limited and insufficient for glyphosate classification with regards to its carcinogenic potential. At the end of November 2017, the DG SANTE Appeal Committee on Phytopharmaceuticals – Plant Protection Products – Legislation with EU Member State representatives voted in favor of the proposal by the EU Commission granting a 5-year re-approval period for this herbicide (European Parliament, 2016, European Parliament, 2017).

In contrast to the EU common harmonization strategy, the responsibility for further possible restrictions on the use of glyphosate was passed to the Member States because of intense discussions during the re-approval process. The major focus of the public debate was on the conflicting assessments of carcinogenicity by the IARC and EU authorities as well as other national authorities and institutions of the World Health Organization (Tarazona et al., 2017).

Maximum residue levels (MRL) for glyphosate in foodstuffs are currently enforced within the European Union and range from 0.05 to 20 mg/kg (European Commission, 2013), whereas the Codex Alimentarius Commission and the US Environmental Protection Agency established MRLs in the range of 0.05–40 mg/kg (Codex Alimentarius, 2019). Glyphosate is therefore subjected to regular assessments by national and international regulatory agencies. However, its high polarity, low molecular weight and lack of a chromophore or fluorophore make glyphosate analysis historically difficult (Raina-Fulton, 2014, Valle et al., 2018). The direct determination by liquid chromatography-tandem mass spectrometry (LC-MS/MS) after extraction by water adjustment and addition of acidified methanol (Quick Polar Pesticides method, QuPPe) is the method suggested by the EU Reference Laboratory for pesticides requiring Single Residue Methods (EURL-SRM) for very polar pesticides that are not amenable to QuEChERS (Quick Easy Cheap Effective Rugged and Safe) multi-residue methods (Anastassiades et al., 2019). One of the commonly recognized drawbacks of the QuPPe method coupled to Hypercarb™ Porous Graphitic Carbon LC column (suggested for glyphosate analysis) is the variation in retention times of polar pesticides and the need of performing several “priming” injections with spinach extract prior to obtaining reproducible chromatography.

Alternative approaches for direct (without derivatization) LC-MS determination of glyphosate alone or in combination with other polar pesticides have been proposed so far. A selection of main MS/MS methods available in the literature over the last 10 years is reported in Table 1. Some of the proposed methods foresee a clean-up step to reduce matrix effects and improve detection limits. However in some cases in spite of a careful optimization of chromatographic conditions and/or a clean-up procedure, consistent matrix effects (up to 50% ion suppression) are still observed. Most of the proposed methods use isotopically labeled glyphosate as internal standard (IS) to compensate for matrix effects. Interestingly, when comparing analytical performances of methods listed in Table 1 in terms of recoveries and quantification limits, it is evident that the flow injection – tandem mass spectrometry approach (FI-MS/MS), might represent a promising alternative for rapid glyphosate detection.

Table 1. Overview of main methods available in the literature (2009–2019) for the determination of glyphosate in food commodities, and relevant analytical performances.

Target analyte/food matrix	Extraction	Clean up	LC-MS detection	LOQ (mg/kg)	Recovery range, %	Matrix effects (SSE, %)	References
Glyphosate, chlorate, perchlorate, fosetyl-aluminum, aminomethylphosphonic acid (AMPA), phosphonic acid, *N*-acetyl AMPA, and *N*-acetyl glyphosate/Fruits and Vegetables	Methanol	NO	IC-MS/HRMS IS	0.01	92%	*Not reported*	Rajski, Diaz-Galiano, Cutillas, & Fernández-Alba, 2018
Glyphosate, AMPA, *N*-acetyl-AMPA, glufosinate, 3-methylphosphinicopropionic acid, *N*-acetylglufosinate, ethephon, chlorate, perchlorate, fosetyl-Al, phosphonic acid/Cereals (oatflour) Grapes	Water and acidified methanol	NO	IC-MS/MS IS	0.01	89–112%	97–135%	Adams et al., 2017
Glyphosate/Rice Maize Soybean	Water (rice) water and dichloromethane (maize/soybean)	NO (rice) polymeric SPE (maize/soybean)	LC-MS/MS IS	0.02–0.4	77–100%	80% (rice) 50% (maize/soybean)	Botero-Coy et al., 2013
Glyphosate, glufosinate, AMPA/soybean, corn	Water containing Na_2EDTA and acetic acid	Polymeric SPE	LC-MS/MS IS	0.042–0.045	100–107%	95–101%	Chamkasem & Harmon, 2016
glyphosate, glufosinate, AMPA, 3-methylphosphinicopropionic acid, *N*-acetylglufosinate/beer, barley, tea, malt, corn	Water	Anion exchange SPE	IC-MS/MS matrix-matched calibration	0.010	99–113%	*Not reported*	Nagatomi, Yoshioka, Yanagisawa, Uyama, & Mochizuki, 2013

Target analyte/food matrix	Extraction	Clean up	LC-MS detection	LOQ (mg/kg)	Recovery range, %	Matrix effects (SSE, %)	References
Glyphosate, AMPA/soybean	Water and dichloromethane	NO	LC-MS/MS matrix-matched calibration	0.30	80–109%	*Reported but not quantified*	Martins-Junior, Lebre, Wang, Pires, & Bustillos, 2009
Glyphosate, AMPA/Fruit and vegetables	Water	NO	LC-MS/MS matrix-matched calibration	0.005	75–110%	≤60%	Chen, Cao, Jiang, & Zhu, 2013
glyphosate, Maleic Hydrazide, Fosetyl-Al, Ethephon/Grapes	Water/methanol containing Na_2EDTA and acetic acid	Polymeric SPE	LC-MS/MS IS	0.019	87–111%	89–99%	Chamkasem, 2017
Glyphosate, AMPA, glufosinate/Milk	Protein precipitation with diluted acid and Na2EDTA	polymeric SPE	LC-MS/MS IS	0.025	81–107	97%	Chamkasem et al., 2015
Glyphosate, AMPA, glufosinate/edible oils	Water, 1% formic acid	NO	LC-MS/MS standard calibration	0.01	82–110	≤13%	Chiarello et al., 2019
chlorate, ethephon, fosetyl aluminium, glufosinate, glyphosate, *N*-acetyl AMPA, *N*-acetylglyphosate, perchlorate and	Water and methanol	NO	IC-MS/MS matrix-matched calibration	*Not reported*	70–120	*Not reported*	Melton, Taylor, & Flynn, 2019

Target analyte/food matrix	Extraction	Clean up	LC-MS detection	LOQ (mg/kg)	Recovery range, %	Matrix effects (SSE, %)	References
phosphonic acid/fruit and vegetables							
Bromide, chlorate, ethephon, fosetyl, glufosinate, glyphosate, *N*-acetyl AMPA, *N*-acetylglyphosate, *N*-acetylglufosinate, HEPA, MPPA, perchlorate and phosphonic acid/grapes, lettuce, orange, oat and soya beans	Water and acidified methanol	NO	LC-MS/MS IS	0.02–0.05	83–113	≤29%	Herrera López, Scholten, Kiedrowska, & de Kok, 2019
Amitrole, chlormequat, cyromazine, daminozide, diquat, ethephon, fosetyl-Al, glufosinate, glyphosate, AMPA, maleic hydrazide, mepiquat and paraquat/apple, lettuce, wheat flour	Water	NO	FI-MS/MS IS	0.1	23–		

IC: ion chromatography.

IS: isotopically labelled internal standard.

SSE: signal suppression/enhancement.

SPE: solid phase extraction.

Advantages and drawbacks of quantitative analysis by flow injection-mass spectrometry (FI-MS) in comparison with chromatography-MS have been extensively discussed by Nanita and Kaldon (2016). Indeed the recent trend in investigating the potential of FI-MS for high throughput analysis has been driven by the continuous improvement in sensitivity and selectivity of benchtop MS instrumentation. Nevertheless, to develop rugged and fit-for-purpose FI-MS methods there are critical parameters to be evaluated which are mainly related to a proper knowledge and management of matrix effects. The applicability of FI-MS approaches for the determination of highly polar pesticides has been recently investigated by Mol and van Dam (2014). Results obtained in this study indicated that accurate

quantitative determination of polar pesticides was possible using isotopically labelled standards. However since the method covered a range of residues, some compromises were made in the optimization of the analytical procedure, resulting in low recoveries for glyphosate in some commodities.

Aim of the present work was to set up and validate a FI-MS/MS method for the quantitative determination of glyphosate in a range of food commodities. A simple and rapid extract purification step and the addition of isotopically labeled glyphosate to the final test sample were included in the optimized sample preparation protocol to improve selectivity, ruggedness and consequently the fitness-for-purpose of the developed method. A comparison with the QuPPe method (Anastassiades et al., 2019) was also performed on a set of incurred samples to critically evaluate the reliability of the proposed approach, advantages and drawbacks with respect to the reference method.

2. Materials and methods

2.1. Chemicals

Glyphosate standard and 100 ng/μL 1,2-$^{13}C_2{}^{15}N$ glyphosate in water were purchased from LGC standards (Milan, Italy). Methanol (HPLC grade) was purchased from VWR International (Milan, Italy), whereas ammonium acetate was from Sigma-Aldrich (Milan, Italy). Ultrapure water (18MΩ) was produced by a Millipore Milli-Q system (Millipore, Bedford, MA, USA). Oasis® HLB columns (3 cc, 60 mg) were purchased from Waters (Milan, Italy). Whatman GF/A glass microfiber filters were obtained from Whatman International Ltd. (Maidstone, UK). Syringe filters (0.22 μm PTFE) were from Aisimo Corporation (London, England).

2.2. Samples

Representative food commodities belonging to three different commodity groups (as defined in the guidance document SANTE/11813/2017) (European Commission Directorate General for Health and Food Safety, 2017) were selected for method validation (Table 2). Fruits and pulses used for single laboratory validation were from the local market. Incurred chickpea and wheat samples, used to compare FI-MS/MS and QuPPe methods, were selected among samples from official controls of imported products performed by the Istituto Zooprofilattico Sperimentale of Umbria and Marche Regions. In particular, chickpeas and wheat samples were from outside EU (Canada, Ukraine, Iran, Russian Federation, USA) and were collected in different ports (year 2017) in central and southern Italy. All samples were cryogenically milled into a powder using dry ice, sieved (≤500 μm), and stored at −20 °C until the analysis.

Table 2. FI-MS/MS method performance parameters obtained by single-laboratory validation in the selected commodity groups and relevant representative commodities as defined in the guidance document (European Commission Directorate General for Health and Food Safety, 2017).

Commodity groups	Representative commodity	Spiking level (mg/kg)	Recovery (%)	RSD*r* (%)	RSD_{WLR} (%)
1. High water content	Apples	50 (high)	78	17	17
		5.0 (low)	84	6.6	8.5
		0.5 (LOQ)	62	6.9	–
2. High acid content and high water content	Grapes	50 (high)	80	13	13
		5.0 (low)	86	15	16
		0.5 (LOQ)	60	10	–
5. High starch and/or protein content and low water and fat content	Chickpeas	100 (high)	111	9.8	9.8
		10 (low)	97	7.6	8.8
		2.0 (LOQ)	62	10	–

2.3. Sample preparation for FI-MS analysis (extraction and clean-up)

Milled samples (10 g group 1 and 2, 2.5 g group 5) were extracted with 10 mL of deionized water by 30 min shaking. Then the sample was centrifuged (15 min, 2500×*g*). The supernatant was passed through a glass microfiber filter to obtain a clear extract. One milliliter of the filtered extract (corresponding to 1 g matrix equivalent for groups 1 and 2, and 0.25 g for group 5) was passed through the Oasis® HLB column (activated and conditioned according to manufacturer instructions) and collected in a 3 mL glass vial. The purified extract was then diluted with distilled water. Dilution factors of 1:20 and 1:5 were applied for samples of group 1–2 and group 5, respectively. The diluted extract was filtered through a 0.22 µm filter. The final test sample was then prepared by adding 40 µL of IS (1,2-$^{13}C_2{}^{15}N$ glyphosate) solution to 200 µL of filtered extract.

2.4. Standard solutions and calibrant solutions

Glyphosate stock solution (1 mg/mL) was prepared in water and stored at 4 °C until use. Glyphosate working solutions (0.25–0.50–0.62–1.25–1.81–2.5–3.0–6.25–12.5 µg/mL) in water were prepared from the stock solution just before use. Two IS (1,2-$^{13}C_2{}^{15}N$ glyphosate) solutions were prepared by dilution with water of the commercial stock solution, to obtain IS concentrations of 2.50 and 3.75 µg/mL to be added to samples fortified at LOQ (limit of quantification) or higher levels, respectively. Standard and matrix-matched calibrant solutions were prepared by adding 40 µL of each

working solution and 40 µL of IS solution (3.75 µg/mL) to 200 µL of LC mobile phase or 200 µL of blank matrix extract prepared according the above described procedure.

2.5. Validation design

Method validation was performed according to the guidance document SANTE/18813/2017 (European Commission Directorate General for Health and Food Safety, 2017). According to these guidelines, validation needs to be performed for the target analyte for at least one commodity from each of the commodity groups in the scope of the method. In the present work, method validation was performed for apples (representative commodity for group 1), grapes (for group 2) and chickpeas (for group 5).

To evaluate recoveries, repeatability (RSD_r) and within laboratory reproducibility (RSD_{WLR}), the following sample set was prepared and analyzed: i) calibration standards (in neat solvent); ii) 1 blank (not spiked) sample; iii) 5 samples spiked at the LOQ (0.5 mg/kg groups 1-2, 2 mg/kg group 5); iv) 5 samples spiked at low level (5 mg/kg groups 1-2, 10 mg/kg group 5); v) 5 samples spiked at high level (50 mg/kg groups 1-2, 100 mg/kg group 5). The design was repeated twice in two different days (over a time period of 3 weeks). The obtained results were subjected to one-way ANOVA to calculate RSD_r and RSD_{WLR}.

2.6. FI-MS/MS analysis

All FI-MS/MS analyses were carried out by injecting each sample 5 times. To ensure system stability and reliability a relative standard deviation of replicate injections ≤5% was considered acceptable. The flow injection MS/MS instrumental set up consisted of an Acquity UPLC system (binary pump and microautosampler, Waters, Milford, MA, USA) interfaced to an API 5000 mass spectrometer (AB Sciex, Foster City, CA, USA) equipped with an Electrospray (ESI) source. The autosampler was directly connected to the source by a 50 cm × 0.13 mm ID peek capillary. The test sample (2 µL) was injected into the carrier solvent (water/methanol 10/90 by vol, containing 0.1% ammonia) delivered at 300 µL/min. ESI conditions were as follows: polarity, negative; capillary temperature, 450 °C; curtain gas (nitrogen), 20 (arbitrary units); nebulizer gas (air), 60 (arbitrary units); heater gas (air), 40 (arbitrary units); ion spray voltage, −4500 V. Glyphosate and 1,2-$^{13}C_2$,^{15}N glyphosate were detected in multiple reaction monitoring, using the following transitions: 168 – 150, 168 – 63 (glyphosate), 171 – 153, 171 – 63 (1,2-$^{13}C_2$,^{15}N glyphosate)

2.7. Quick polar pesticides (QuPPE) method

The LC-MS/MS reference method (QuPPE method) was accredited ISO 17025 at the Istituto Zooprofilattico Sperimentale of Umbria and Marche, Italy.

2.7.1. Sample preparation for LC-MS/MS analysis

To 5.0 ± 0.05 g of sample, 10 mL of deionized water were added followed by 100 µL of IS stock solution (1,2-$^{13}C_2$, ^{15}N glyphosate) at 100 µg/mL in water. The sample was then vortexed and shaken for 60 min on orbital shaker. After adding10 mL of extraction solution (methanol, 1% formic acid), the sample was shaken again for 60 min, then centrifuged (15 min, 3000×*g*). The supernatant was placed in a centrifuge tube and cooled down for 3 h at −20 °C, then centrifuged (15 min, 3000×*g*). To prepare the final test sample, an aliquot (e.g. 2–3 mL) of the supernatant was withdrawn using a syringe and filtered through a 0.22 µm PTFE syringe filter directly into a plastic auto-sampler vial.

To compensate for matrix effects, matrix-matched calibration standards were prepared adding volumes of glyphosate working solutions to suitable aliquots of blank extract (containing IS).

2.7.2. LC-MS/MS analysis

The LC-MS/MS instrumental set up consisted of an Nexera X2 UPLC system (binary pump, column oven and autosampler, Shimadzu, Kyoto, Japan) interfaced to an API 3200 QTrap mass spectrometer (AB Sciex, Foster City, CA, USA) equipped with an ESI source. The chromatographic separation of the analyte was conducted at 40 °C using a Hypercarb™ (100 × 2.1 mm; 5 µm) column and a guard column (10 × 2.1 mm; 5 µm) both from Thermo Scientific, Waltham, MA, USA. Before use it was mandatory to prime the column and the precolumn by injection of 50 µL of spinach extract (prepared according to the above reported procedure) to improve sensitivity and peak shape then priming was repeated prior to each analytical batch or when a decrease of peak intensity or shape was observed. The mobile phase was a time programmed gradient composed of mobile phase A (water, 1% formic acid, 5% methanol v/v/v) and mobile phase B (methanol, 1% formic acid v/v). The injection volume was 5 µL. ESI conditions were as follows: polarity, negative; capillary temperature, 550 °C; curtain gas CUR (nitrogen), 40 (arbitrary units); ion source gas GS1 (nitrogen), 60 (arbitrary units); ion source gas GS2 (nitrogen), 60 (arbitrary units); ion spray voltage, −4500 V. Glyphosate and 1,2-$^{13}C_2$,^{15}N glyphosate were detected in Scheduled MRM mode, monitoring two transitions (Q1, Q3): 168 – 63, 168 – 124, (glyphosate); 171 – 63, 171 –126, (1,2-$^{13}C_2$, ^{15}N glyphosate).

Quantitative determination was performed by matrix–matched calibration prepared as follows. Two glyphosate working solutions (WS1 at 10 and WS2 at 100 µg/mL water) were prepared from the stock solution just before use. Matrix-matched calibrant solutions were prepared by adding 10 µL and 50 µL of WS1 and WS2 to 990 or 950 µL of blank matrix extract, prepared according the above described procedure, to obtain four solutions at final concentrations of 0.1; 0.5; 1.0 and 5.0 µg/mL, respectively. IS concentration was 0.5 µg/mL.

3. Results and discussion

3.1. Set up of the sample preparation procedure

Flow injection analysis (FIA) has several advantages with respect to conventional chromatographic methods, such as very short analysis times, lower costs due to the reduced amount of organic solvents and consumables (no LC column needed). Indeed, skipping the chromatographic separation, the FIA sensitivity and selectivity may be affected by stronger matrix effects, and interferences due to co-injected matrix compounds. However, if properly managed this technique may achieve adequate accuracy and sensitivity.

The simplest strategy to reduce co-injected matrix compounds is to apply high dilution factors to the final test sample and/or to work with low injection volumes when the MS detector sensitivity allows. However, sample clean-up is still an important and necessary step in many FI-MS methods to improve selectivity, sensitivity and ruggedness by reducing matrix interferences (Nanita & Kaldon, 2016). Consequently, FI-MS is often coupled to extract pretreatments to purify the analytes and decrease the complexity of the sample prior to instrumental analysis. QuEChERS based procedures are the most commonly used for sample preparation for pesticide analysis in food (Anastassiades et al., 2019), however the non volatile inorganic salts employed (NaCl and $MgSO_4$) in this method can lead to residual levels in the extracts and the ion source affecting its compatibility with FI-MS analysis.

Solid phase extraction (SPE) using polymeric Oasis® HLB cartridges has been proven to be effective in glyphosate purification from complex food matrices prior to LC-MS/MS analysis (Table 1) (Botero-Coy et al., 2013, Chamkasem and Harmon, 2016, Chamkasem, 2017, Chamkasem et al., 2015). In the present work, a quick (about 1 min) pass through procedure using Oasis® HLB cartridges was applied to remove part of matrix compounds, prior to injection.

For a more accurate quantification, the isotopically labeled internal standard was added to the final test sample. Fig. 1 shows a comparison of calibration curves obtained by injecting calibrants in neat solvent and in chickpea extract purified through the optimized procedure. The full overlap of the two regressions (which was verified also for the other food commodities considered for method validation) confirmed that the use of isotopically labeled glyphosate as internal standard properly compensated for matrix effects.

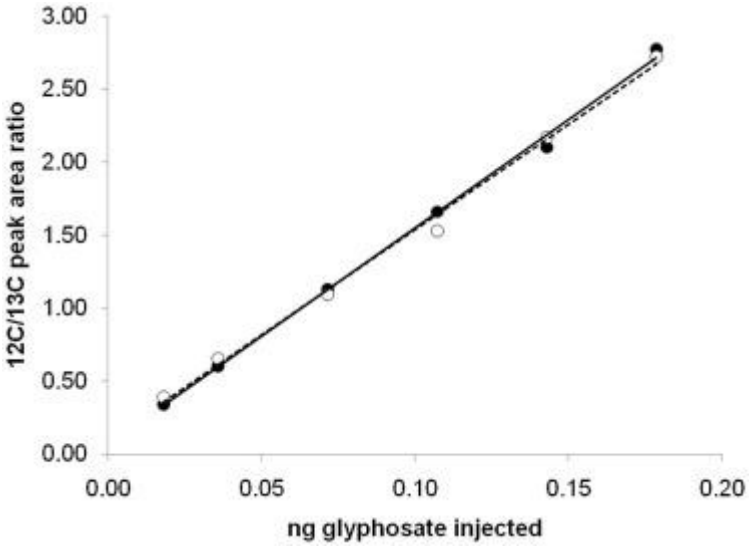

Fig. 1. FI-MS/MS calibration curves obtained by plotting the peak area ratio glyphosate/13C15N glyphosate in FI carrier solvent and chickpea extract purified through Oasis® HLB column. The injected amounts are relevant to 0.08 mg injected matrix, and an injection volume of 2 μL.

3.2. FI-MS/MS method validation

To evaluate quantitative performances of the proposed method, a validation study was performed according to the guidance document SANTE/18813/2017 (European Commission Directorate General for Health and Food Safety, 2017) for representative commodities of three commodity groups, namely apples (group 1), grapes (group 2) and chickpeas (group 5). Results are summarized in Table 2. Recoveries, repeatability and within laboratory reproducibility at low and high level were within the acceptability range (70–120% for recoveries, ≤20% for RSD_r and RSD_{WLR}) for all tested commodities.

Recoveries lower than 70% were obtained at the lowest spiking levels, i.e. 2 mg/kg for chickpea (61% recovery) and 0.5 mg/kg for apples (62% recovery) and grapes (60% recovery). However, according to SANTE guidelines, they could be considered acceptable being $RSD_r \leq 20\%$ in all cases. Besides quantitative validation aspects, the identification parameters were also assessed, in particular the ion ratio, and all samples resulted to be compliant with identification requirements (European Commission Directorate General for Health and Food Safety, 2017). Based on these data the lowest

tested spiking levels could be considered a reliable estimation of quantification limits of the FI-MS/MS method in the relevant commodities.

Quantitative performances of glyphosate determination by FI-MS/MS in a working range (0.2–1 mg/kg) close to the lowest spiking levels considered in this study were previously investigated (Mol & van Dam, 2014), obtaining recovery values of 75% and 39% at 0.2 mg/kg and 1 mg/kg, respectively, in apple ($RSD_r \leq 9$), and of 85% and 56% at 2 mg/kg and 1 mg/kg, respectively, in wheat ($RSD_r \leq 14$). The overall improvement of FI-MS/MS performances obtained in the present work could be mainly attributed to the use of the isotopically labeled internal standard instead of external matrix assisted calibration.

Validation data for commodity group 5 were available also for the QuPPE method and were therefore compared with performances of the FI-MS/MS method. Quantitative performances of glyphosate determination by LC-MS/MS by isotope dilution were assessed by analysis of 6 replicate wheat samples (per each spiking level) obtaining recovery values of 74% and 76% at 0.5 mg/kg and 10 mg/kg, respectively, with RSD_r of 2.9 and 6.2% respectively. The lowest spiking level (0.5 mg/kg) was considered as LOQ. Therefore, with respect to the reference method, the FI method showed similar accuracy (Table 2), and, as expected, a higher quantification limit probably due to a higher ion suppression since missing the LC separation.

3.3. Analysis of incurred samples

The trueness of the data generated with the developed method was evaluated by analysis of a set of incurred wheat and chickpea samples (both belonging to commodity group 5) either by FI-MS/MS, after water extraction and purification through Oasis® HLB column, and by LC-MS/MS according to sample preparation and analysis required by the QuPPe reference method (extraction with acidified methanol and no clean-up). Results and relevant uncertainty values are reported in Table 3.

Table 3. Results of the analysis of chickpea and wheat (commodity group 5) incurred samples obtained by the FI-MS/MS method and the QuPPE reference method.

Sample	Empty Cell	**FI-MS/MS**	**FI-MS/MS relative ion ratio**[a]	**QuPPE**
Chickpea	A	15.9 ± 3.4	0.5%	21.3 ± 4.3
	B	24.0 ± 4.8	0.3%	37.7 ± 7.0
	C	29.3 ± 5.6	−2.1%	35.9 ± 6.7
	D	26.7 ± 5.1	−3.6%	36.3 ± 6.7
	E	36.3 ± 6.7	0.5%	42.7 ± 7.8
Wheat	1	1.8 ± 0.5	2.4%	1.4 ± 0.4

Sample	Empty Cell	FI-MS/MS	FI-MS/MS relative ion ratio[a]	QuPPE
	2	1.8 ± 0.5	0.3%	1.9 ± 0.6
	3	2.0 ± 0.6	28%	1.3 ± 0.4
	4	3.4 ± 0.9	34%	2.9 ± 0.8
	5	1.8 ± 0.5	26%	1.7 ± 0.5
	6	0.4[b] ± 0.1	–	0.3 ± 0.1

a

Relative ion ratio: the ion ratio (qualifier transition/quantifier transition) shall be within ±30% (relative) of the average calibration standard from the same sequence (European Commission Directorate General for Health and Food Safety, 2017).

b

Contamination level below the estimated LOQ.

Given the lack of interlaboratory validation, proficiency test data and data from the analysis of reference materials at the time of the study, a preliminary estimation of the uncertainty was calculated using the Horwitz equation as suggested by Codex Alimentarius Commission Guidelines (Codex Alimentarius Commission, 2006), alternatively a default value of 50% for the expanded uncertainty could be adopted (Codex Alimentarius Commission, 2006, European Commission Directorate General for Health and Food Safety, 2017).

The good agreement between the two result sets indicated the fitness-for-purpose of the proposed method as a rapid and reliable alternative approach for glyphosate determination, at levels above 0.5 mg/kg. It is worth mentioning that glyphosate levels in most of the wheat samples resulted to be close to the estimated LOQ (2 mg/kg for commodity group 5), confirming that the FI-MS/MS method provides reliable quantitative estimation at these levels. Table 3 also reports relative ion ratios (qualifier transition/quantifier transition) obtained for the FI-MS/MS analysis of incurred samples. Ion ratios met the identification criteria (+30% of average calibration standards from the same sequence) for all analyzed samples but one which is slightly above the tolerance level. Fig. 2 shows a comparison between FI-MS/MS peaks and LC-MS/MS peaks obtained for incurred samples wheat 4 and chickpea A. The figure also includes the FI-MS/MS profiles of blank wheat and chickpea samples to show method selectivity.

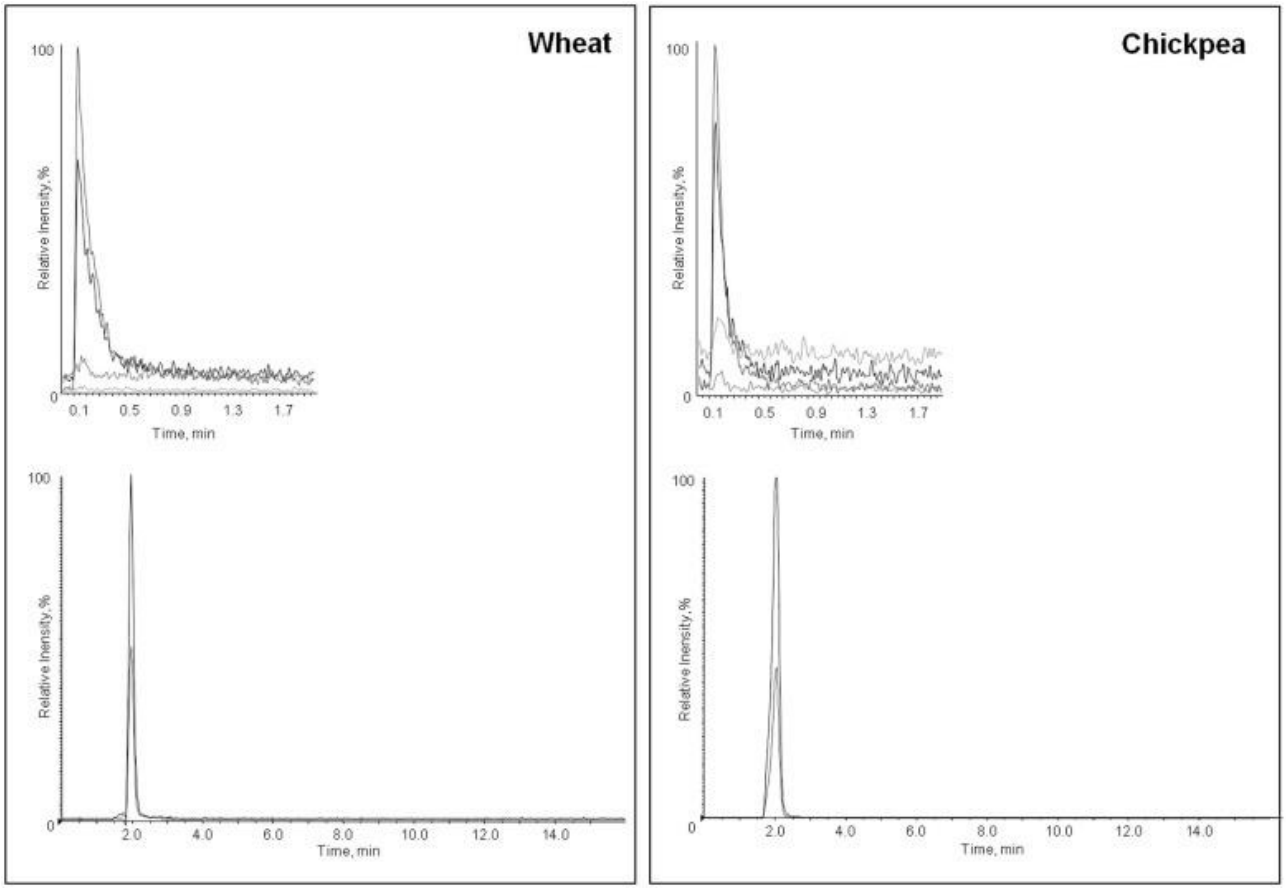

Fig. 2. Comparison between FI-MS/MS peaks and LC-MS/MS peaks obtained for incurred samples wheat 4 (2.9 mg/kg glyphosate) and chickpea A (21.3 mg/kg glyphosate). FI-MS/MS traces of a blank wheat sample and a blank chickpea sample are overlaid to traces of incurred samples to show method selectivity.

For the two sample groups a unique standard calibration was used for FI-MS/MS analysis. These data therefore indicated that the additional costs due to the use of the isotopically labeled internal standards, can be fully compensated in routine analysis eliminating the need to perform a specific matrix assisted calibration for each analyzed commodity.

Indeed, in a critical comparison of the two analytical approaches, it should be taken into account that the FI-MS method does not include any glyphosate metabolite. Some practical considerations could be made according to the available data on glyphosate and related metabolites incidence. In a recent report by the EURL-SRM, results obtained for many QuPPe amendable pesticides and metabolites including glyphosate, AMPA, N-Acetyl glyphosate and N-Acetyl AMPA were evaluated (EURL-SRM, 2018). In particular, 2061 samples belonging to different commodity categories (as defined in Annex A of SANTE/11813/2017) were analyzed over the year 2018, and none of the above mentioned glyphosate metabolites was detected. Therefore, with the view of data from real incurred samples the proposed method can still be considered fit-for-the purpose of fast glyphosate screening.

4. Conclusions

In this study an extensive validation was carried out to evaluate the quantitative performances of the FI-MS/MS approach for the rapid determination of glyphosate in selected food commodities. To deal with matrix effects, and to improve quantitative performances and sensitivity, making the method applicable to benchtop MS detectors, a pass through extract purification step and the addition of isotopically labeled glyphosate to the final test sample were included in the optimized sample preparation protocol. When subjected to intra-laboratory validation the method showed recoveries and precision values in compliance with acceptability criteria, including identification criteria for MS detection. A good agreement of the results with those obtained with the reference method confirmed its fitness-for-purpose of rapid compliance testing, even if more sensitivity is needed to meet regulatory requirements for apples. Overall, the data obtained in this work indicate FI-MS/MS as a valuable alternative for rapid glyphosate detection, ensuring high quality of the generated data, and therefore appearing suitable for broad monitoring programs as well as risk assessment purposes.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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