POLAR PESTICIDE ANALYSES: VALIDATION OF GLYPHOSATE DETERMINATION IN SOIL BY LC-MS/MS

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Abstract

Although glyphosate is a heavily applied herbicide worldwide, the risk of environmental contamination through transport mechanisms of this substance is still not well documented and the methods for its analysis are usually very complex and nor sensitive enough. In the measurement procedure with LC-MS/MS, a negative mode was used. FMOC-Cl derivatized glyphosate ions were identified by the precise determination of their ion mass. The LOQ was 0.01 mg/kg, with recovery of 101.4±4.49% and linearity over 0.99. The obtain method demonstrates the sensitivity, which enables measurements of glyphosate soil as a matrix. The success of the new extraction method was confirmed by measuring spiked soil samples to control recovery, linearity, LOD and LOQ.

Introdustion

Glyphosate (n-phosphonomethyl glycine) is a polar herbicide widely used in agriculture, horticulture, and silviculture applications but also commonly used around homes and gardens [1]. Its usage was approved by European Commision in 2002. This herbicide is among the largest-selling single crop-protection chemical products on the market [2]. Commercial glyphosate formulations are frequently used as herbicides in agriculture, due to their good efficacy on most weed species and their relatively low cost but recently discussed due to the re-evaluation process by the European Union. As a most frequently used herbicide, it is the one of several hundred active substances that have been assessed by Member States and the European Food Safety Authority (EFSA) in recent years.

The Member States – in the Standing Committee on Plants, Animals, Food and Feed during the meeting of national experts, recently, voted in favor of a proposal by the European Commission to restrict the conditions of use of glyphosate in the EU. These conditions include a ban of a co-formulant (POE-tallowamine) from glyphosate-based products, obligations to reinforce scrutiny of pre-harvest use of glyphosate as well as to minimise the use in specific areas (public parks and playgrounds). The proposal was made in parallel with the extension of the approval of the active substance for the limited time. The measures will apply for the duration of the extension untilEuropean Chemical Agency (ECHA) issues its opinion. The Commission adopted the extension of the current approval of glyphosate for a limited period until the ECHA has concluded its review - since Member States failed to take responsibility (no qualified majority was reached at either the Standing Committee or the Appeal Committee) [3].

For a long time many polar pesticides like glyphosate has been excluded from the routine scope of laboratories due to the lack of sample methods[4].Direct analyses usually show low sensitivity, high method detection limits, poor chromatographyc separation, etc. Derivatization with FOMC-Cl (fluorenylmethyloxycarbonyl chloride), represents a solution of some drawbacks of direct

methods; however this step makes the determination more expensive and time consuming [4]. Polar pesticides represents compounds of middle or high polarity and low molecular mass which makes glyphosate is analytically called as "SRM (single residue method) analyte" and is not possible to analyse with pesticide multiresidues method. Variety of SRMs are published for glyphosate.

In this work derivatization with FMOC-Cl purification step followed by liquid chromatography tandem-mass spectrometry (LC-MS/MS) were optimizes in order to decrease detection limit for residue analyses of glyphosate in soil. Method was validated according to SANTE/11945/2015.

Experimental

Chemicals and apparatus

The acetonitrile and methanol were of HPLC quality.The KOH, H_3BO_3, KCl, NaOH, H_3PO_4, FOMC-Cl, NH_4HCOOH and EDTA were analytical grade and obtained from Merck (Darmstadt, Germany). The water was deionized and formic acid was concentrated (>95%). The certified pesticide analytical standard of glyphosate (99.0%) was purchased from Dr. Ehrenstorfer (Augsburg, Germany). Glyphosate-D3 was used as an internal standard (ISTD, c=1 µg/mL).

For LC analysis, an Agilent 1200 (Agilent Technologies, USA) HPLC system with a binary pump was used. This was equipped with a Zorbex Eclipse Plus C18 (3.5 µm, 2.1×100 mm) (Agilent Technology). The mobile phase was 10 mM NH_4HCOOH (pH 9, solvent A in methanol) and 10 mM NH_4HCOOH (pH 9, solvent B in water) in gradient mode, with the flow rate of 0.2 mL/min. The elution program was started with 70% B. It was linearly decreased to 10% B in 15 min. The stop time was 15 min with the post run of 5 min. The 5 µL of sample was injected.

For the mass spectrometric analysis, an Agilent 6460 Triple-Quad LC/MS system was applied. Agilent MassHunter B.04.00 software was used for the data acquisition and processing. The analysis was performed in the negative ion mode. The ESI source values were as follows:drying gas (nitrogen) temperature 325 °C, drying gas flow rate 5 L/min, nebulizer pressure 45 psi and capillary voltage 2000 V. The detection was performed using the multiple reactions monitoring mode (MRM).

Validation parameters

The evaluation of the calibration curve`s linearity was done based on the injections of standard solutions prepared in the mobile phase and also in the extract of blank soil samples, at the concentrations of 0.01, 0.025, 0.05 and 0.1 µg/mL.

The LOD was estimated from the chromatogram of the lowest level of calibration using the Agilent MassHunter software (Agilent Technologies, Data Acquisition for Triple Quad B.04.00) for those concentrations that provide a signal to noise ratio of 3:1. The LOQ was based on the accuracy and precision data, obtained via the recovery determinations and was defined as the lowest validated spike level which meets the requirements of a recovery within the range of 70 – 120% and a RSD ≤ 20 % (SANTE/11945/2015). The LOQ was determined at 0.01 mg/kg.

The main goal of the recovery experiments was to determine the method accuracy via the comparison of the real concentration of a glyphosate, measured by performing the complete procedure, with the known pesticide concentration initially added to the matrix [4]. The method precision is expressed as the repeatability (RSD, 4.49 %) of the recovery determinations at three different spiking levels (0.025, 0.05 and 0.10 mg/kg).

Glyphosate extraction

10 g sample + 9mL d. H_2O + 1mL KOH
↓ Shake vigorously for 5 min and centrifuge for 5 min at 4000 rpm
1 mL of aliquot + 100 µL ISTD + 1 mL botate buffer + 0.5 mL FMOC-Cl Shake immediately for 1 min
↓ Storage in dark for 2 hours
100 µL 2% H_3PO_4 + 0.1 M EDTA
↓Shake vigorously for 1 min
LC-MS/MS

Results and discussion

Basic acquisition parameters (precursor ion – prec ion, product ion – prod ion, fragmentation energy – Frag, collision energy – CE and polarity) for glyphosate and glyphosate-D3 as an internal standard, were given below.

Compound	Prec Ion	Prod Ion	Frag (V)	CE (V)	Polarity
Glyphosate	390.2	168	100	5	Negative
	390.2	150	100	15	Negative
Glyphosate-D3	393.2	170.8	100	15	Negative

The calibration curves based on matrix-matched standards were obtained at the concentration levels from 0.01-0.10 µg/mL. The good linearity was achieved, with the coefficient of determination (R^2) better than 0.99 (Figure 1).

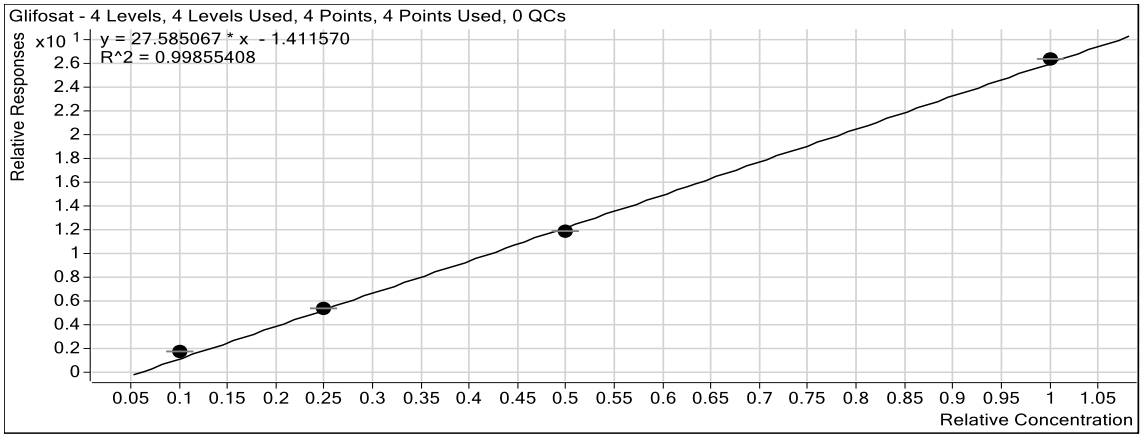

Figure 1. Calibration curve

The recovery studies were performed with the fortification experiments at the final mass concentrations of 0.025 to 0.10 mg/kg in three replicates. The average recovery was 101.4±4.49%. The precision was assessed in terms of repeatability at 0.10 mg/kg. A good repeatability (n = 6), with RSDs of 4.49% was obtained and it was calculated through the recovery.

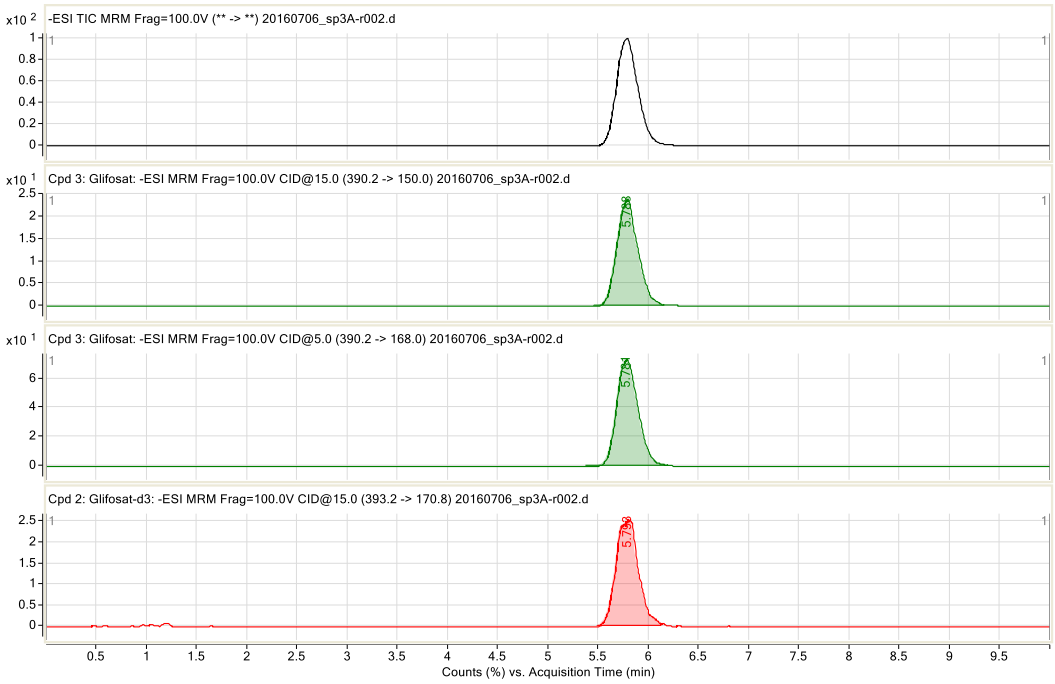

Figure 2. Chromatogram of spiked soil sample (level0.1 mg/kg)

Conclusion

The LC-MS/MS method is robust, can be applied to determine the glyhosate residues from soil as a matrix without any loss of sensitivity and assures a LOQ of 0.01 mg/kg. One sample can be processed with less than 2.5 hours. Quantifies man power is necessary to maintain very complex instrumentation in a good working condition.

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