Reliable determination of anionic polar pesticides in vegetables by using LC-MS/MS

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1. Overview

Glyphosate, one of the Anionic polar pesticide (APP) has become one of the world's most widely used herbicides due to its relatively low cost and high efficiency. APPs are a group of pesticides that have non-QuEChERS-amenable characteristics and can generally be characterized as having Log Kow values less than 1. These are one of the challenging pesticide molecules when it comes to developing an analytical method & require alternative conditions for extraction and LC retention/separation. Traditionally, these compounds have been managed with selective single-residue methods, which significantly increase costs. Laboratories are now aiming to extend the scope of methods to include a wider range of pesticides, metabolites, and other contaminants. Monitoring these polar ionic pesticides has been made mandatory by many regulators and is of significant interest to the food industry and contract testing labs globally.

2. Introduction

The QuEChERS methodology has been widely used for the extraction of many different classes of pesticides however, APPs are exception to this and are not efficiently recovered. Moreover, the analysis of APP by a single LC-MS method is extremely challenging due to the complexities associated with their retention, separation and detection. Conventional approaches for the analysis of APPs often involve the use of a single residue method or a small group of compounds with similar properties, these methods used, are often time-consuming with limited throughput. In 2008, the Quick Polar Pesticides (QuPPe) method was developed by the European Reference Laboratory for the simultaneous extraction of numerous APPs in foods[1]. This poster describes a partially validated method for determination of six APPs namely Glufosinate, MPPA, Ethefon, Fosetyl-aluminium, Glyphosate & N-acetyl glufosinate (NAG) (Figure 1) using an Ultra High Performance Liquid Chromatograph (UHPLC) Nexera™ X3 coupled with an LCMS-8060NX, a Triple Quadrupole Mass Spectrometer. (Figure 2)

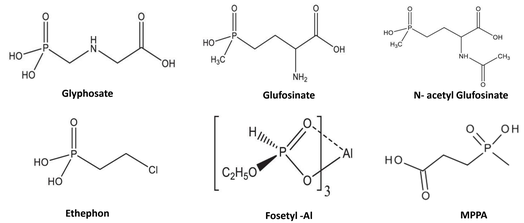

Figure 1. Structures of Anionic polar pesticides

3. Materials and method

The pesticides standards were procured from local vendor and standard solutions were prepared. From these standard solutions, 1000 ppb standard stock solution was prepared in Methanol for LC-MS analysis using the instrumental parameters described in table 1. Extracted protein powder matrix blank was used to prepare 50.0 ppb working stock which was further diluted to make matrix match linearity from 1.0 ppb to 35.0 ppb.

3-1. Sample preparation

❑ **Preparation of Samples and quality control (QC) samples**

Commercially available Fresh chilli sample was procured from local market. This sample was well homogenized with grinder-mixer and below mentioned sample preparation protocol was followed.

- Weighed 10 g of homogenized sample in a 50 mL centrifuge tube
- Sample spiked with pesticide standard solution to have spiking concentration of 10 ppb, 20 ppb and 50 ppb.
- Added acidified methanol to the sample tubes and the tube were vortexed for 5 min.
- Further, the sample tubes were centrifuged at 7000 rpm at 4 ^{0}C for 10 min.
- The supernatant was aliquoted in micro tubes and were again centrifuged at 12000 rpm at 4 ^{0}C for 5 min.
- 1 mL of above supernatant was collected and filtered through 0.45 µ nylon syringe filter in a micro centrifuge tubes
- 0.5 mL of above supernatant was diluted with acetonitrile in auto-sampler vial an analyze using LCMS-8060NX.

Figure 2: Shimadzu Nexera™ X3 UHPLC coupled with an LCMS-8060NX Triple quadrupole mass spectrometer

3-2. LC-MS/MS analysis

Table 1. LC-MS/MS Instrument parameters

UHPLC condition (Nexera X3)	
Column	Waters Taurus DEA column
Mobile Phase	A: 0.9 % formic acid in LC-MS grade water
	B: 0.9 % formic acid in LC-MS grade methanol
Flow Rate	0.5 mL/min
Column Temperature	40 ^{0}C
Elution Mode	Gradient B concentration in % : 0-1.5 min 80%, 10-13.6 min 0.5%, 13.6-14 min 00% 14.25-18 min 20%.
Mass Spectrometry Parameters	
MS Interface	Ion Focus
Desolvation Line Temperature	150 ^{0}C
Heating Block Temperature	350 ^{0}C
Interface Temperature	300 ^{0}C
Nebulizing Gas Flow	3.0 L/min
Drying Gas Flow	8.0 L/min
MS Mode	MRM mode with CID gas

4. Results

4-1. Linearity

A matrix match standard linearity ranging from 1.0 ppb to 35.0 ppb was plotted. All calibration levels showed linear response with the accuracy ranging from 80 to 120 % . (Figure 3)

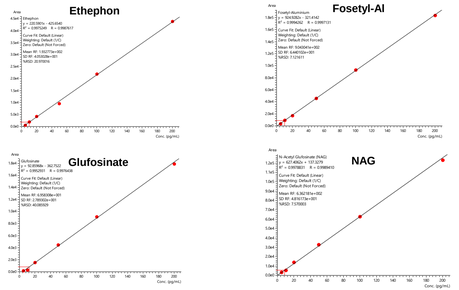

Figure 3. Calibration curve for Ethephon, Fosetyl-Al, Glufosinate and N-acetyl Glufosinate (Representative chromatograms of 4 compounds)

4-2. Recovery

Recovery was evaluated by analyzing six pre-spiked samples at 10.0 ppb 20.0 ppb and 50.0 ppb against matrix match calibration curve mentioned in section 3-1. Average recovery values of respective analytes were found to be within acceptance criteria of 70-120 % as per SANTE guidelines[2] (Figure 4 & 5).

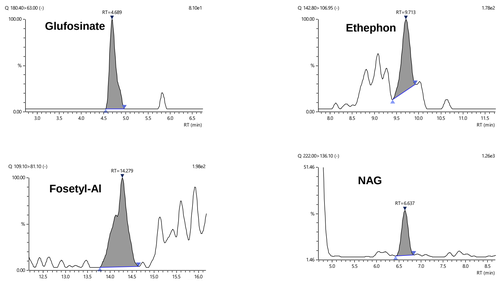

Figure 4. Chromatograms for Ethephon, Fosetyl-Al, Glufosinate and N-acetyl Glufosinate of pre-spiked Chilli sample (Representative chromatograms of 4 compounds)

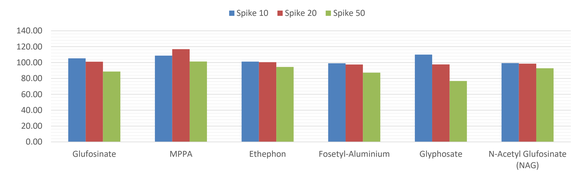

Figure 5. Graphical representation of anionic polar compounds recoveries in pre-spiked chilli sample

4-3 Reproducibility (RSD)

Three recovery samples, at 10 ppb, 20 ppb and 50 ppb level were injected and checked for reproducibility. LC-MS/MS which showed good precision (RSDr) with less than 20 % RSD which is within acceptance criteria as per SANTE guidelines (Figure 6)

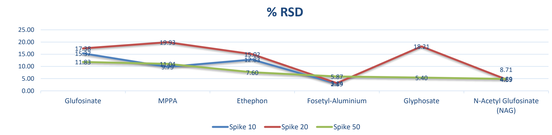

Figure 6. RSD of pre-spike samples analysed on LCMS-8060NX

5. Conclusion

- Average recovery values for anionic polar pesticides in chilli sample were found to be within the acceptance criteria as per the SANTE/12682/2019 guidelines.
- The results obtained at 10.0 ppb, 20 ppb and 50 ppb concentration levels were accurate, repeatable and reproducible with RSD less than 20 %.
- A simple QuPPe extraction method has been successfully developed and validated for the simultaneous quantification of 6 anionic polar pesticides in a single run.

6. References

1. Quick method for the analysis of highly polar pesticides in food involving extraction with acidified methanol and LC- or IC-MS/MS measurement - I. Food of plant origin (QuPPe-PO-Method) – version 8.1, March 2015.
2. Guidance document on analytical quality control and method validation procedures for pesticide residues and analysis in food and feed. (SANTE/11312/2021).