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Determination of Anionic Polar Pesticides in Grapes using UPLC-MS with Anionic Polar Pesticide Column

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

Polar pesticides have recently gained significant public interest due to warnings of their potential carcinogenicity. They are classified under herbicides, fungicides and plant growth regulators. The analysis of highly polar anionic pesticides is challenging due to insufficient retention on reversed phase LC columns, surface interactions and the low levels required by legislation. This study evaluates the performance of the Waters' Anionic Polar Pesticide column, the ACQUITY UPLC I-Class PLUS System and the Xevo TQ-XS Tandem Quadrupole Mass Spectrometer for the separation, identification and quantification of polar pesticides and their metabolites to the 0.001 mg/kg level in grapes. Combining the retention of the Anionic Polar Pesticide column, the separating power of the ACQUITY UPLC I-Class PLUS System and the sensitivity of the Xevo TQ-XS Tandem Quadrupole Mass Spectrometer allowed the regulatory needs for routine polar pesticide analysis in food safety laboratories to be surpassed.

Benefits

· Successful retention and separation of polar pesticides without the need for derivatization

· Quantification of anionic polar pesticides in grapes to levels ≥0.001 mg/kg

· Method meets the regulatory needs for routine polar pesticide analysis in food safety laboratories

Introduction

India is one of the major exporters of grapes to many countries and especially to the European Union. Regulations are in place for monitoring polar pesticides in various foods. It is mandatory to perform preharvest testing on grapes before export to European Union countries.[1] The list of regulated agrochemicals can be found in Annexure-9.[2] Due to the physiochemical properties of highly polar, anionic compounds such as glyphosate and ethephon (Figure 1), standard analytical methods using reversed phase chemistries such as C_{18} are not applicable due to insufficient retention. Some of the other challenges include their ionic nature, surface interaction, solution instability, and detection of metabolites. Several methods have been recently developed, which can provide improvements in chromatographic retention and separation while avoiding the need for derivatization, but they require multiple single residue methods (SRMs). A method for the determination of underivatized anionic polar pesticides has been presented previously using Waters Anionic Polar Pesticide Column.[3] Here we present a single multi-residue method using this column, the ACQUITY UPLC I-Class PLUS System and the Xevo TQ-XS Tandem Quadrupole Mass Spectrometer for the separation, identification, and quantification of polar pesticides and their metabolites to achieve limit of quantification (LOQ) levels ≥0.001 mg/kg in grapes.
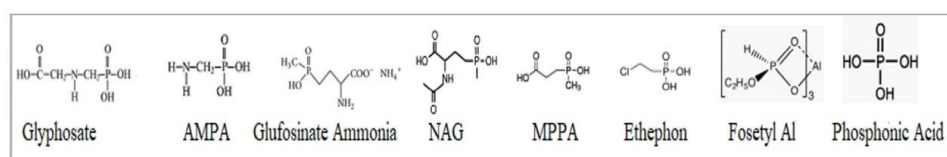

Figure 1. Chemical structures of all polar pesticides.

Results and Discussion

The method for the determination of underivatized anionic polar pesticides has been presented previously using Waters Anionic Polar Pesticide Column 2.1 x 100 mm, 5 µm (p/n: 186009287).[3] To achieve the required retention and separation for these compounds, a non-derivatized HILIC based method was used. The column stationary phase consisted of ethylene bridged hybrid (BEH) particles with tri-functionally bonded diethylamine (DEA) ligands. The combination of the hydrophilic surface and the anion-exchange properties of the ligand provides chromatographic characteristics well suited to the retention and separation of polar anionic compounds (Figure 2). The proposed methodology provides excellent chromatographic separation and retention of polar pesticides using a single multiresidue method. A panel of eight pesticides (glyphosate and its metabolite AMPA, glufosinate and its metabolites MPPA & NAG, ethephon, fosetyl Al, phosphonic acid) were analyzed in grape matrix using ESI positive and negative modes. Glufosinate in ESI negative ionization mode shows lower sensitivity as compared to ESI positive ionization mode, as shown in (Figure 3).
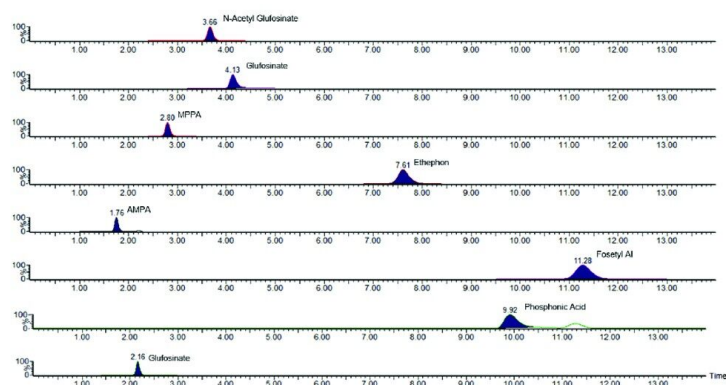

Figure 2. Representative chromatogram at 0.100 mg/kg in grape matrix.
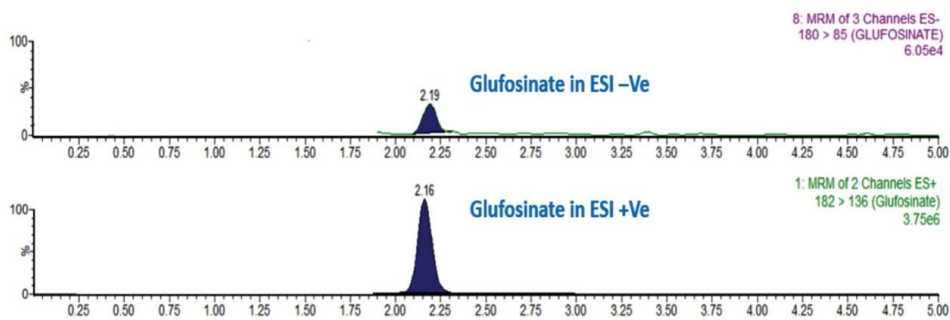
Figure 3. Glufosinate sensitivity in positive and negative ionization mode at 0.010 mg/kg in matrix.

Sample extraction was done using QuPPe EU SRM method.[4] Commercially available grapes samples were homogenized in a blender and approximately 10.0 ± 0.1 g of homogenized sample was taken. Recovery samples were also processed as per the extraction procedure mentioned in (Figure 4) by spiking mixed standard prior to the addition of extraction solvent. The recovery study was done at 0.010 and 0.050 mg/kg for phosphonic acid and 0.001, 0.0025, and 0.010 mg/kg for the rest of the analytes (n=3). Due to the presence of analyte in the blank matrix, the recovery was done from 0.010 mg/kg for phosphonic acid. Potassium phosphonate is often used as a plant strengthener which results in residual detection of phosphonic acid in trace amounts.[5]
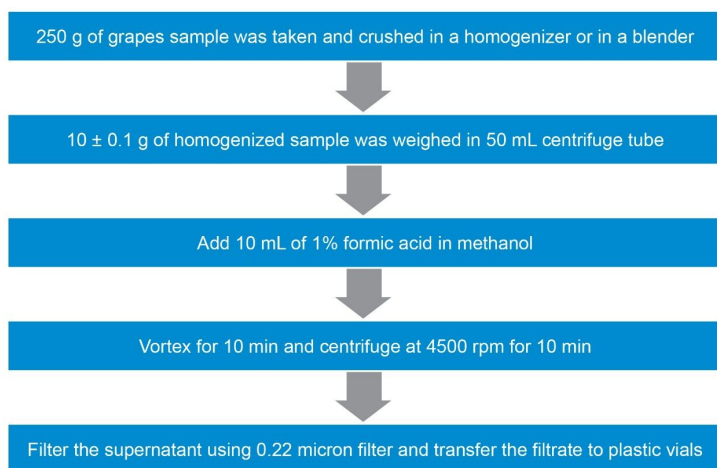

Figure 4. Sample preparation protocol.

The analytical method used here was Method B from the Anionic Polar Pesticides startup guide.[6] All the samples were injected using the ACQUITY UPLC I-Class PLUS System coupled to the Xevo TQ-XS Tandem Quadrupole Mass Spectrometer. Spiked samples were quantified against procedural standards using TargetLynx XS. Calibration curves were constructed by acquiring data from 0.0005 to 0.100 mg/kg and show excellent coefficients of determination ($R^2 \geq 0.99$) and all residuals were

within ±20% without using internal standards (Figure 5). This method gives retention time stability of ±0.1 min and ion ratio tolerance of ±30%. Trueness (%) and precision (%RSD) are within the acceptable range represented in (Figure 6), meeting the requirements of SANTE/12682/2019.[7]
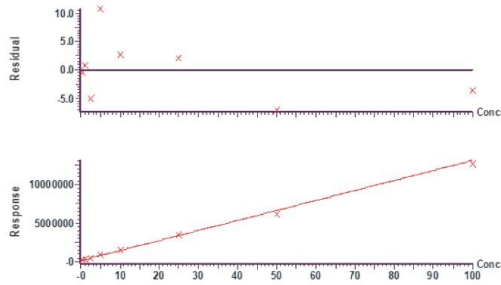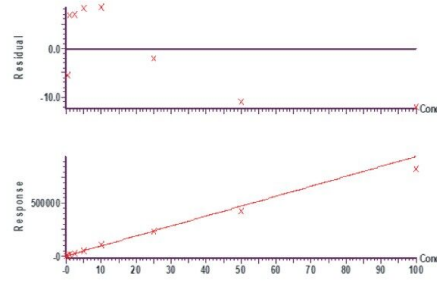

Figure 5. Representative examples of procedural calibration curves for fosetyl aluminum and glufosinate with their respective residuals.
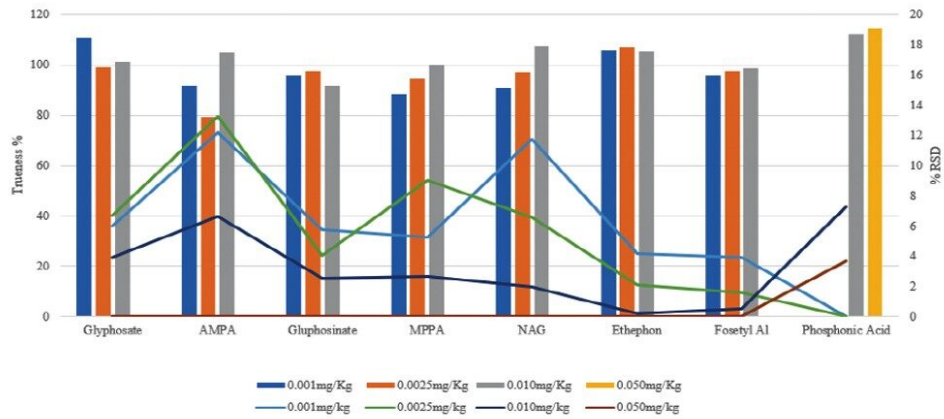

Figure 6. Trueness percentage (bar) and precision %RSD (line) at 0.001, 0.0025, 0.010 and 0.050mg/kg in grape matrix.

Conclusion

The analysis of highly polar anionic pesticides at levels 50-fold lower than the regulatory limits on ACQUITY UPLC I-Class PLUS and Xevo TQ-XS has been described in this application brief. Grape samples were spiked with a mixture of eight highly polar anionic pesticides, including their metabolites, to demonstrate appropriate sample recovery. Use of Waters Anionic Polar Pesticide Column provides excellent chromatographic retention and separation between parent analytes and their metabolites. The use of Waters ACQUITY UPLC I-Class PLUS System coupled to Xevo TQ-XS provides excellent sensitivity, coefficient of determination and repeatability for these analytes. The use of ESI positive and negative ionization modes provide maximum sensitivity for all the analytes. At a glance, combining the retention of the anionic polar pesticide column, the resolution of the ACQUITY UPLC I-Class PLUS System and the sensitivity of Xevo TQ-XS Tandem Quadrupole Mass Spectrometer surpasses the regulatory needs for routine polar pesticides analysis in food safety laboratories.

References

1. http://apeda.gov.in/apedawebsite/Grapenet/procedureforexportofgrapes2017-18.pdf

2. https://apeda.gov.in/apedawebsite/Grapenet/Annexure_9.doc_22.10.2019.pdf

3. Determination of Anionic Polar pesticides in High water food stuffs, Waters Technology brief no:720006645EN, January 2020.

4. https://www.eurl-pesticides.eu/userfiles/file/EurlSRM/meth_QuPPe-PO_EurlSRM.pdf

5. https://n-bnn.de/sites/default/dateien/bilder/Downloads/FactSheet_phosphonic_acid_en_Mai_2017.pdf

6. Method Startup guide for Anionic Polar pesticides Column, waters Manual guide no: 720006689EN Rev A, September 2019.

7. https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_mrl_guidelines_wrkdoc_2019-12682.pdf

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