APPLICATION NOTE

Liquid Chromatography/ Mass Spectrometry

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Direct Analysis of Glyphosate in Wine with No Sample Preparation Using the QSight 220 LC-MS/MS System

Introduction

Glyphosate is an organophosphate herbicide that is used on crops to kill weeds and grasses. Its usage has multiplied with the introduction of transgenic crops made resistant to glyphosate. Because of its rampant use, it is not surprising that glyphosate has been detected in variety of foods. Recently, the International Agency for Research on Cancer classified glyphosate as "probably carcinogenic in humans". In lieu of regulatory bodies setting limits on glyphosate in food, it has become imperative to develop robust and sensitive analytical methods for glyphosate detection. Since glyphosate is a very polar molecule, it does not retain well on a traditional reverse phase column, making it very difficult to chromatographically separate from other components and detect. Methods involving derivatization with a hydrophobic moiety can help retain glyphosate on column, but, it also makes the process labor intensive and tedious. We present a study that involves direct analysis of glyphosate in wine on a mixed mode column with no sample dilution or extraction using a PerkinElmer QSight® 220 triple quadruple mass spectrometer with a patented StayClean™ source, consisting of a hot surface induced desolvation (HSID)™ interface and a Laminar Flow Ion Guide™. Both the HSID and ion guide prevent any contaminants from entering the mass spectrometer, keeping it at its highest performance level and, thereby, maintenance free.

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Experimental

Wine samples were filtered through a nylon filter (0.2 µm) and spiked with known concentrations of glyphosate and its degradation product, amino methyl phosphonic acid (AMPA), to set up a calibration curve. The samples were directly injected onto an LC column using a UPLC system fitted with the PerkinElmer QSight 210 series mass spectrometer. Table 1 shows the LC conditions and the MS parameters are described in Table 2.

Results and Discussion

The glyphosate spiked in red and white wine samples was analyzed in multiple reaction monitoring (MRM) mode and was easily detectable at five parts per billion (ppb) with no sample preparation in both red (Figure 1) and white wine. The signal to noise (S/N) of 200 obtained for the 5 ppb glyphosate spike in red wine suggests we should be able to detect the analyte at concentrations lower than 5 ppb.

Table 1. LC conditions.

Column	Acclaim Trinity Q1 100 x 3 mm 3 µm
Mobile Phase	*Solvent A:* 50 mM ammonium formate, pH adjusted to 2.9 with formic acid *Solvent B:* Acetonitrile (ACN)
Gradient Conditions	3 min of 95% A/5% B mobile phase followed by 3 min of wash with Acetonitrile. Equilibrate for 7 min with initial conditions
Oven Temp.	Ambient conditions
Injection Volume	20 µL

Table 2. MS source conditions.

Ion source	ESI negative mode
ElectroSpray/V	-5000
Source Temp/°C	450
HSID Temp/°C	320
Dry Gas	100
Nebulizer Gas	150

Table 3. MS/MS parameters.

Experiment (0 – 1.3 min)	MRM Transitions			
	Quantifier Ion	Qualifier Ion	CE(V)	Dwell Time (msec)
AMPA	109.7/78.8		36	70
AMPA		109.7/62.8	26	70
Experiment (1.3 – 13 min)	**MRM Transitions**			
	Quantifier Ion	Qualifier Ion	CE(V)	Dwell Time (msec)
Glyphosate	167.5/63		35	70
Glyphosate		167.5/78.8	57	70

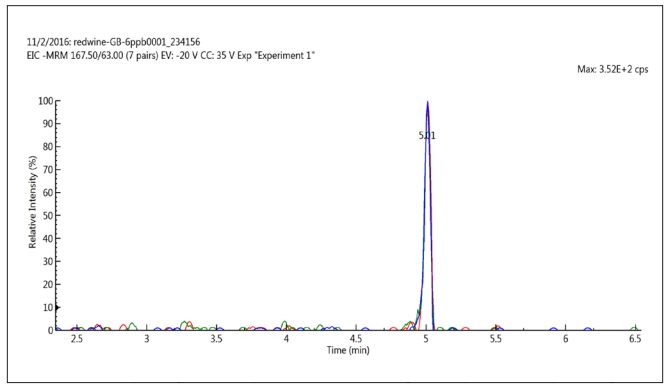

Figure 1. Overlay of MRM chromatograms of a triplicate analysis of 5-ppb glyphosate spike in a red wine sample.

The calibration curves of glyphosate and AMPA spiked in red (Fig. 2a) and white wine (Fig. 2b) showed good linearity ($r^2 > 0.99$).

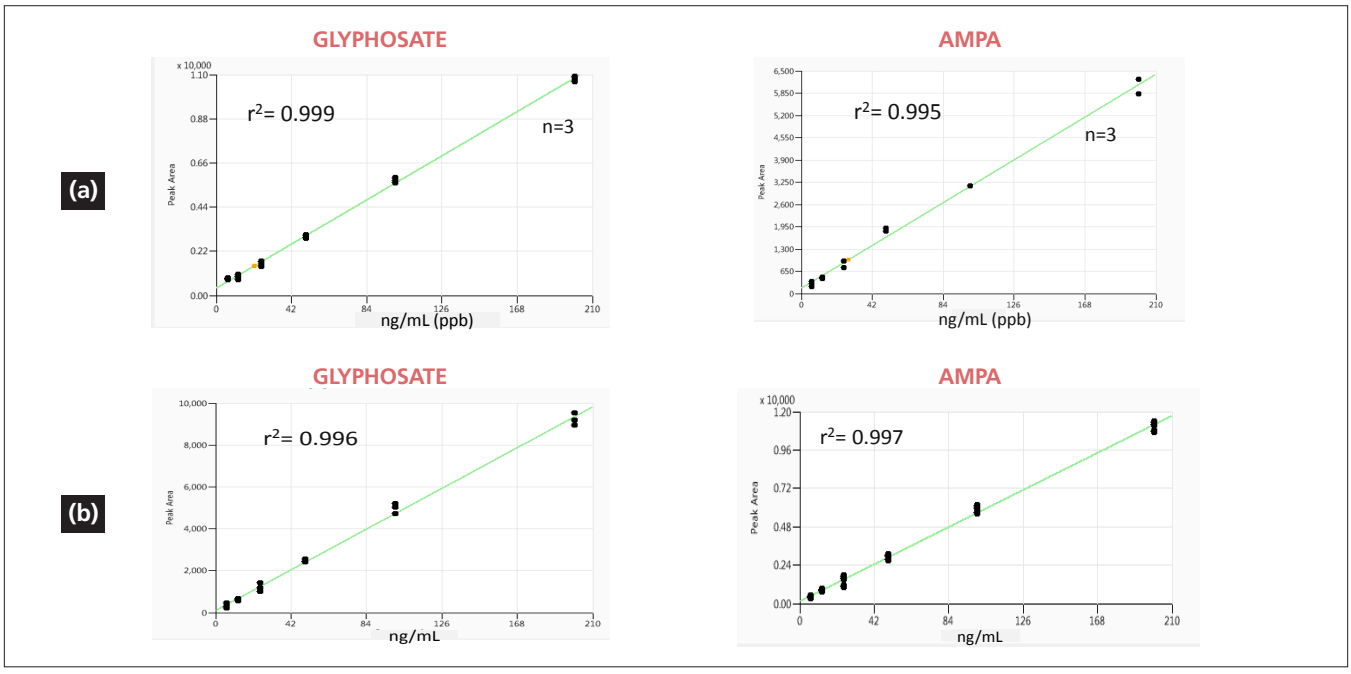

Figure 2. (a) Matrix matched calibration curves in red wine for glyphosate and AMPA. (b) Matrix matched calibration curves in white wine for glyphosate and AMPA.

Overlay of the matrix matched calibration curves for glyphosate shows the peak area intensity was similar for the analyte in both varieties of wine, suggesting that red wine, in spite of being heavily pigmented compared to white wine, did not suppress/ interfere with the signal (Figure 3).

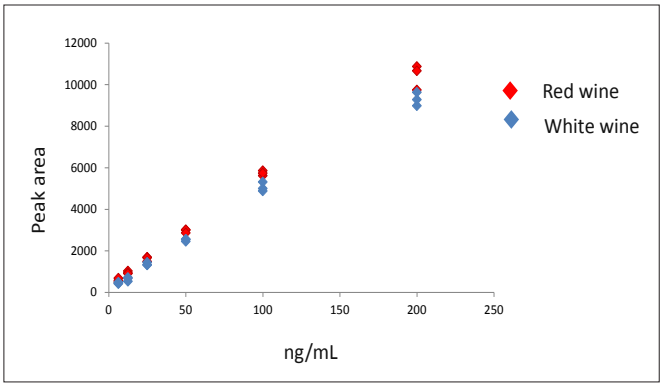

Figure 3. Overlay of glyphosate matrix matched calibration curves in red and white wine.

The robustness of the "Stay clean" source was tested by analyzing nearly 300 injections (20 µl each) of red wine. As seen in Figure 4, there was minimal loss of glyphosate signal (RSD ≤ 10%), indicating the source was unaffected by contamination from dirty matrices.

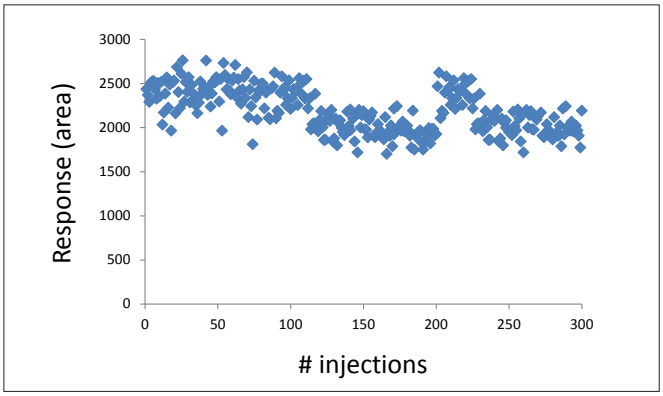

Figure 4. Shows minimal loss in response of glyphosate spiked (50 ppb) in red wine (RSD<10%).

The robustness of the source can be attributed to the HSID source and the laminar flow ion guide present in the QSight. The HSID is a multi-orthogonal interface (directly heated to 300 °C) that is present immediately after the sampling orifice in the source and connects the orifice to the laminar flow ion guide of the QSight mass spectrometer. Unlike traditionally designed interfaces, the HSID, with its orthogonal multi-channels, produces turbulent and laminar flow and disrupts the free jet expansion of the sample ions. The orthogonal channels prevent neutrals from entering the mass spectrometer, reducing chemical noise. A solvated, charged clusters entering the HSID are entrained and desolvated in the hot flow of gas, further contributing to the reduction in chemical noise.

The ions from the HSID interface are gently transferred by gas flow to the Laminar flow ion guide, which is not subject to the traditional axial fields, but kept at zero potential. The ion guide has multiple pumping stages to generate several pressure regions from the sample interface to the mass analyzer. In these regions, pressure gradually drops, creating a well-defined flow pattern along the ion path, enabling ions to be gently extracted into the analyzer. Both the HSID and laminar flow ion guide prevent accumulation of contaminants along the ion path, making the QSight more maintenance free. The many benefits of the HSID interface include high sensitivity, due to an inherent reduction in chemical background, and the ability to perform analysis at high LC flow rates (up to 3 mL/min) without reduction in signal.

Conclusion

The QSight triple quadrupole mass spectrometer is fitted with a unique, low maintenance source, allowing for minimal sample preparation and resulting in increased productivity.

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