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Quantitative Determination of Plant Growth Regulators (PGRs) in Grape Matrix Using the Xevo TQD

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Using a simple acetonitrile dilution followed by liquid-liquid extraction, all pesticides were successfully extracted from the grape matrix and analyzed in an 8.0 minute run.

GOAL

Develop a sensitive LC-MS/MS method for the robust analysis of some plant growth regulators (PGRs) in grape matrix without the need of derivatization.

BACKGROUND

Food safety laboratories primarily focused on pesticide residue quantification typically employ multi-residue LC-MS/MS methods for the quantification of target pesticides. However, the analysis of certain pesticides is extremely challenging due to their physico chemical properties e.g. amphoteric nature, high boiling point, and high molecular mass. In many of the reported methods derivatization has been used to increase chromatographic retention of these pesticides using a reverse phase column.

The WHO CODEX and EU guidelines have established Maximum Residue Limits (MRLs) for abamectin at 0.01 mg/kg, azadirachtin at 1.00 mg/kg, mepiquat at 0.30 mg/kg, chlormequat chloride at 0.05 mg/kg, nereistoxin at 0.01 mg/kg, thiocyclam at 0.01 mg/kg, 6-benzyl adenine at 0.01 mg/kg, and cartap hydrochloride at 0.01 mg/kg in grape commodity.

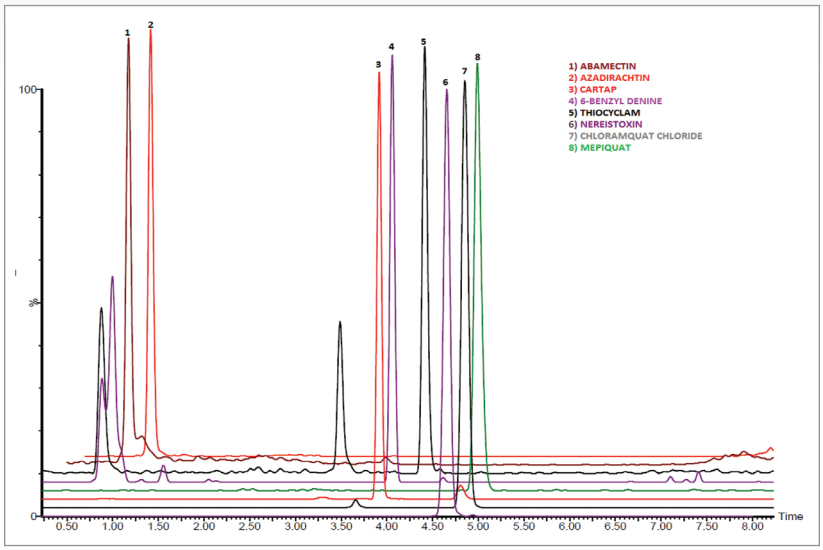

Figure 1. UPLC chromatograms for eight PGRs at 0.001 mg/kg in grape matrix.

THE SOLUTION

In this technology brief, eight different classes of synthetic plant growth regulators were investigated using Waters® tandem quadrupole Xevo® TQD Technology. A pentflurophenyl bonded phase column was selected for HILIC separation with an MS friendly mobile phase consisting of 0.1% aqueous formic acid with ammonium acetate and an acetonitrile gradient with electrospray positive ionization mode.

The Xevo TQD coupled with the ACQUITY® UPLC® I-Class System achieves mg/kg detection limits for thiocyclam, abamectin, azadirachtin, cartap hydrochloride, chlormequat chloride, 6 benzaldehyde, mepiquat, and nereistoxin, as shown in overlay chromatogram in Figure 1.

By using a simple acetonitrile dilution followed by a liquid-liquid extraction procedure, all pesticides were successfully extracted from the grape matrix and analyzed in an 8.0 minute run. System robustness, in terms of precision was observed from the analysis of spiked extracts of grape matrix. The extraction procedure was simplified by using acidified acetonitrile aqueous extraction followed by further cleanup of the acetonitrile layer with a non polar solvent, filtered, and injected into the ACQUITY UPLC I-Class System coupled to the Xevo TQD. A summary of the results acquired showing replicate injections at LLOQ levels from single batch is provided in Table 1.

Table 1. Summary of precision data (%RSD) from the analysis of extracts from grape matrix, spiked at 5.0 ppb.

Compound name	%RSD at LLOQ level (n=6 injections)
Abamectin	3.22
Azadirachtin	2.35
Cartap hydrochloride	3.14
Chloramquat chloride	1.52
6-Benzaldhyde	0.97
Mepiquat	3.10
Nereistoxin	1.42
Thiocyclam	1.12

Linearity was evaluated for all analytes in grape matrix and neat solution. Excellent correlation and residuals were achieved using linear fit with 1/x2 weighing factor. An example calibration curve is shown in Figure 2, with an $R^2 \geq 0.998$ and residuals of <10% for all concentration levels (0.001 to 50 mg/kg). When comparing the slopes of the solvent and matrix calibration curves, significant matrix effect were observed for cartap.

Table 2. MRM transitions.

Compound name	Quantifier	Qualifier	Retention time
Mepiquat	114.00>98.00	114.00>58.00	4.66
Chlormequat chloride	122.00>59.00	122.00>58.00	4.62
Neresitoxin	150.00>105.00	150.00>61.00	4.57
Thiocyclam	181.92>136.88	181.92>73.00	4.76
6-Benzyladenine	226.01>90.94	226.01>147.85	3.83
Cartap	238.10>72.94	238.10>115.94	4.18
Azadirechtin	742.89>724.80	NIL	0.72
Abamectin	895.46>751.22	895.46>182.90	0.68

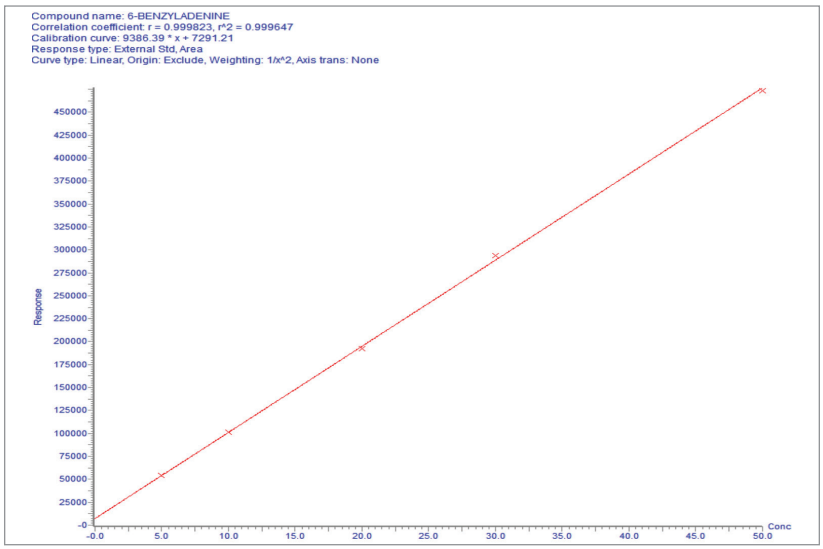

Figure 2. Example of the calibration achieved for 6-benzaldehyde in grape matrix from 0.001 mg/kg.

SUMMARY

A fast and simple LC-MS/MS method has been demonstrated for the analysis of eight multi-class plant growth regulators in grape extract. The Xevo TQD provides good limits of detection, suitable for monitoring MRL compliance of 0.01 mg/kg for these eight challenging PGR's, and delivers good quantitative performance in terms of precision and calibration characteristics, even in the absence of internal standards.

References

1. http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=pesticide.residue.selection&language=EN
2. http://apeda.gov.in/apedawebsite/Grapenet/Annexure9grapetestreport_1st-Feb-2016.pdf
3. http://www.fao.org/fao-whoCodexalimentarius/standards/pestres/commodities-detail/en/?c_id=113

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