Agilent 6460 Triple Quadrupole LC/MS System with an Agilent 1290 Infinity LC For Multi-Plant Growth Regulator Analysis in Grapes

Application Note

Food Safety

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Abstract

This document describes an effective, sensitive triple quadrupole LC/MS method with a simple, fast and economic sample preparation technique for the quantitation of 12 multi-plant growth regulators (PGR) in grape samples.

Plant hormones or phytohormones are chemicals that regulate plant growth. Manmade compounds, or PGRs are used to regulate the growth of cultivated plants, weeds, and in vitro growth plants and plant cells. Synthetic PGRs are also used in various techniques such as cutting, grafting, and micropropogation.

PGRs activate cellular responses such as cell death. Some have the ability to adversely affect human cancer cells. Because the European Union has listed these compounds, they are required to be measured.

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Experimantal

PGRs detection in positive mode

1. Chlormequat
2. d4-chlormequat (ISTD)
3. Daminozide
4. Zeatin
5. Kinetin
6. Indol acetic acid (IAA)
7. 6 Benzyl adenine
8. Indol Butyric acid (IBA)
9. Forchlorfenuron
10. Paclobutrazol

PGRs detection in negative mode

1. Gibbrelic acid
2. Abscisic acid
3. 2, 4 Dichlorphenoxy acetic acid (2, 4- D)

New Method

- Easy to use
  Rugged UHPLC method and very robust interfacing with Agilent 6460 Triple Quadrupole LC/MS with Jet Stream ESI source
- Sample Prep
  Easy, fast and economic dispersive C18 sample cleanup
- Robustness
  Significantly improved method stability. Ion suppression effects are reduced to minimum
- Sensitivity
  Chlormequat detected at 0.05 ppb level with good peak response and an S/N ratio that is 1% of the required limit specified by EU (for example, 50 ppb).

Methods and Operations

This method for the analysis of PGRs is based on LC/MS with a triple quadrupole system. The sample preparation is done by a simple dispersive C18 cleanup process followed by direct injection. Separation is based on fast and quantification is done by ESI – LC/MS/MS in MRM mode.

Analysis steps

1. Liquid phase extraction followed by dispersive C18 cleanup.
2. The samples and standards were spiked with internal standard d4-chlormequat.
3. UHPLC analysis was performed in gradient mode.
4. Detection by Jet Stream ESI-LC/MS/MS in MRM mode.

Sample preparation steps

A 5-g amount of homogenized grape sample was added to 40 mL extraction solvent Methanol :Water (80:20) at ambient temperature and the mixture was extracted with ambient shaking for 60 mins using Rotaspin at 50 rpm. 1 ml supernatant transferred to 1.7 ml centrifuge tube and centrifuge at 14000 rpm for 5 mins. A 0.8-ml amount of supernatant liquid was mixed with 200 mg of C18 ODS SPE bulk, vortexed and centrifuged. A 0.4-mL amount of the sample was diluted to 1 mL with methanol and 2 µL were injected for LC/MS/MS analysis.

LC/MS/MS Method

The total run time required to determine 10 PGRs in positive mode is 16 min. A 2 µL amount of the sample extract was injected for LC/MS/MS. Method details in positive mode are noted below:

Column	Agilent ZORBAX Extend C18 2.1 mm × 100 mm, 1.8 µm
Flow rate	0.2 mL/min,
Column temp	40 °C
Mobile phase A	Water (0.5% formic acid)
Mobile phase B	Methanol

Time	% Mobile Phase A	% Mobile Phase B
0	95	05
7	10	90
9	10	90
12	95	05

Mass spectrometer settings

Name	Retention time (min)	Fragmentor voltage (V)	Precursor ion (m/z)	Production (m/z)	Collision energy (eV)
Chlormequat	1.28	75	122.1	58.1 63.0	22 22
d4-Chlormequat	1.28	75	126.1	58.1 67	22 25
Daminozide	1.41	75	161.1	143.1 61.1	10 10
Zeatin	2.11	75	220.1	135.9 148.1	13 13
Kinetin	3.81	75	216.1	81.1 148.1	17 17
6-Benzyladenine	5.31	75	226.1	91.0 65.0	19 19
IAA	6.01	75	176.1	130.1 144.9	6 6
IBA	7.25	75	203.1	186.1 168.0	9 9
Forchlorofenuron	7.91	75	248.1	129.1 154.9	15 15
Paclobutrazol	8.69	75	294.1	70.1 60.2	17 17

Ten PGRs in positive polarity

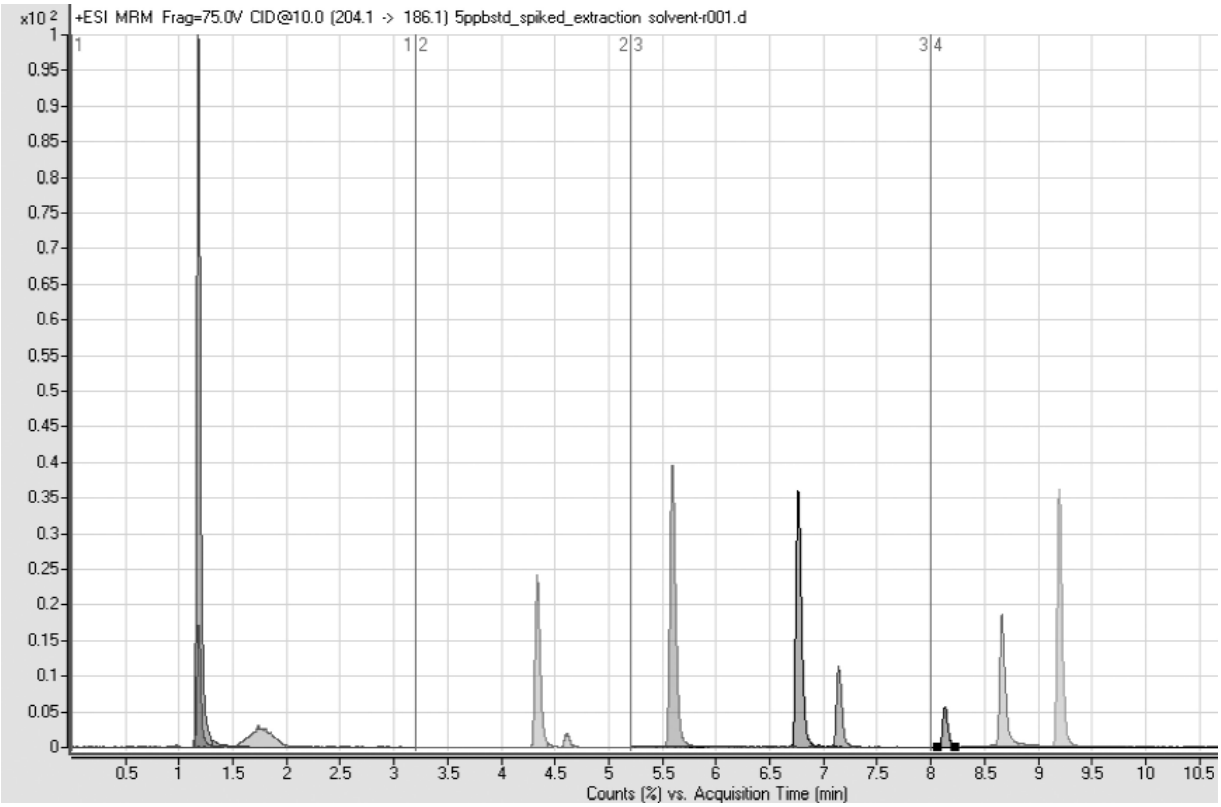

Figure 1. Typical chromatogram at 5 ppb concentration. Elution pattern: chlormequat, d4-chlormequat, daminozide, zeatin, kinetin, 6-benzyladenine, IAA, IBA, forchlorfenuron, paclobutrazol.

The total run time required to determine three PGRs in negative mode is 6 min. Two microliters of the sample extract were injected for triple quadrupole LC/MS. Method details in negative mode are noted below:

Column	Agilent ZORBAX Eclipse Plus Rapid Resolution High Definition C18 2.1 mm × 100 mm, 1.8 µm
Flow rate	0.2 mL/min
Column temp	40 °C
Mobile phase A	Water (0.5% formic acid)
Mobile phase B	Methanol

Time	% Mobile Phase A	%Mobile Phase B
0	81	19
2.5	10	90
4.5	10	90
6	81	19

Mass spectrometer settings

Name	Retention time (min)	Fragmentor voltage (V)	Precursor ion (m/z)	Production (m/z)	Collision energy (eV)
Gibbrellic Acid	1.81	75	345.1	143.2	21
				221.2	21
Abscisic Acid	2.41	75	263.1	152.9	11
				219.0	11
2-4-Dichlorphenoxy Acetic Acid	2.81	75	218.9	160.9	13
				125.0	13

Three PGRs in negative mode

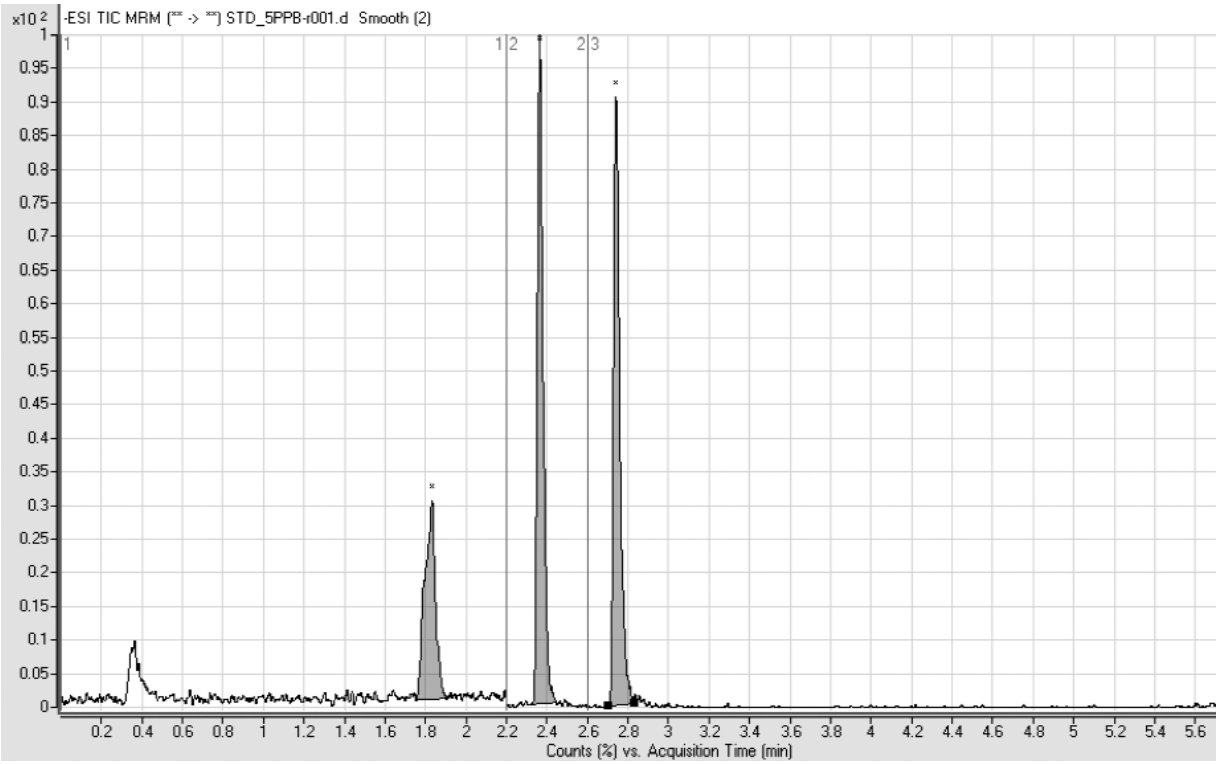

Figure 2. Typical chromatogram at 5 ppb concentration. Elution pattern: gibbrellic acid, abscisic acid and 2-4-D.

Results

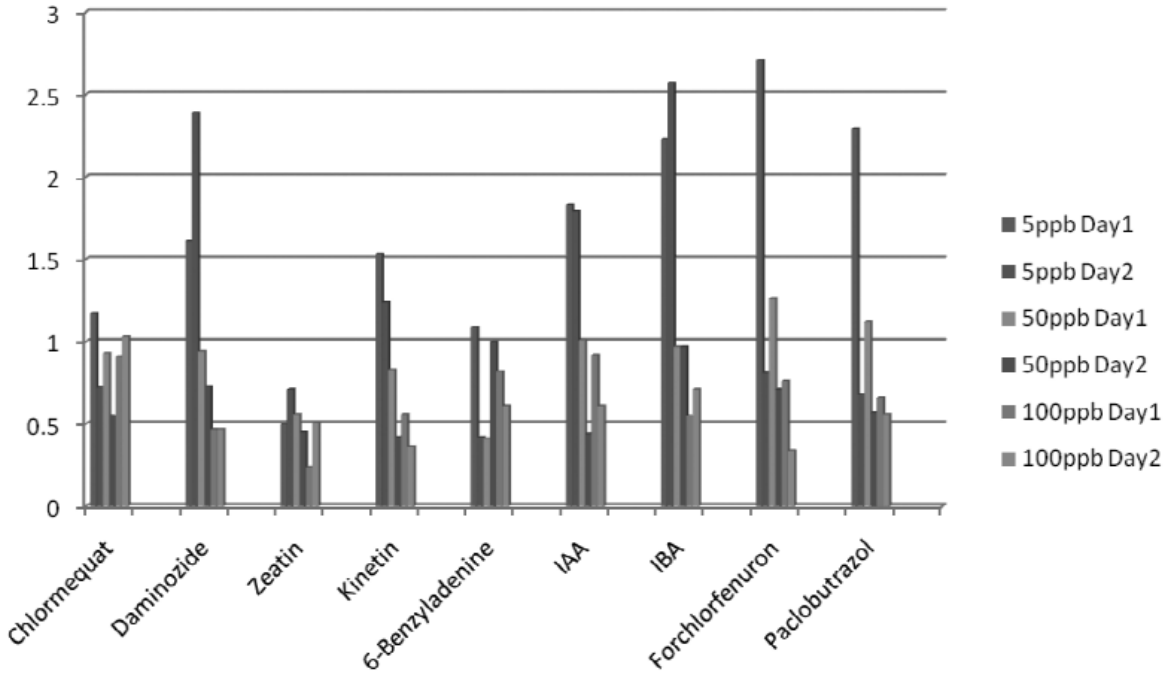

Figure 3. Summary of repeatability for nine PGRs spiked and extracted from grape samples in positive mode.

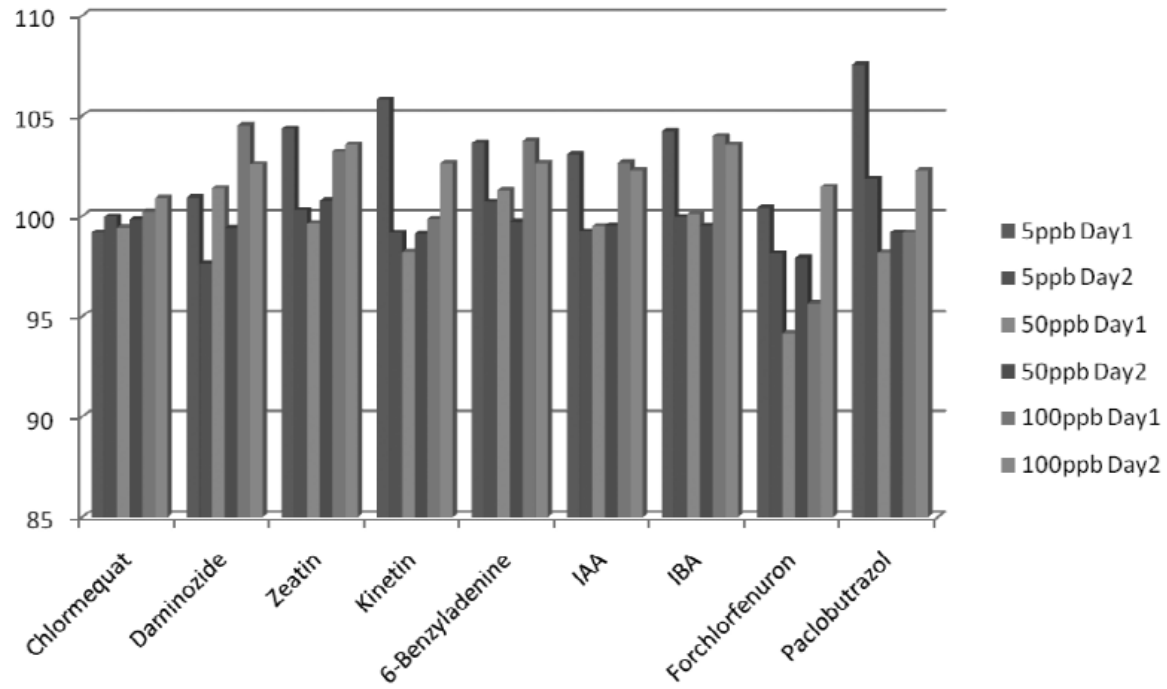

Figure 4 Summary of recovery for nine PGRs spiked and extracted from grape samples in positive mode.

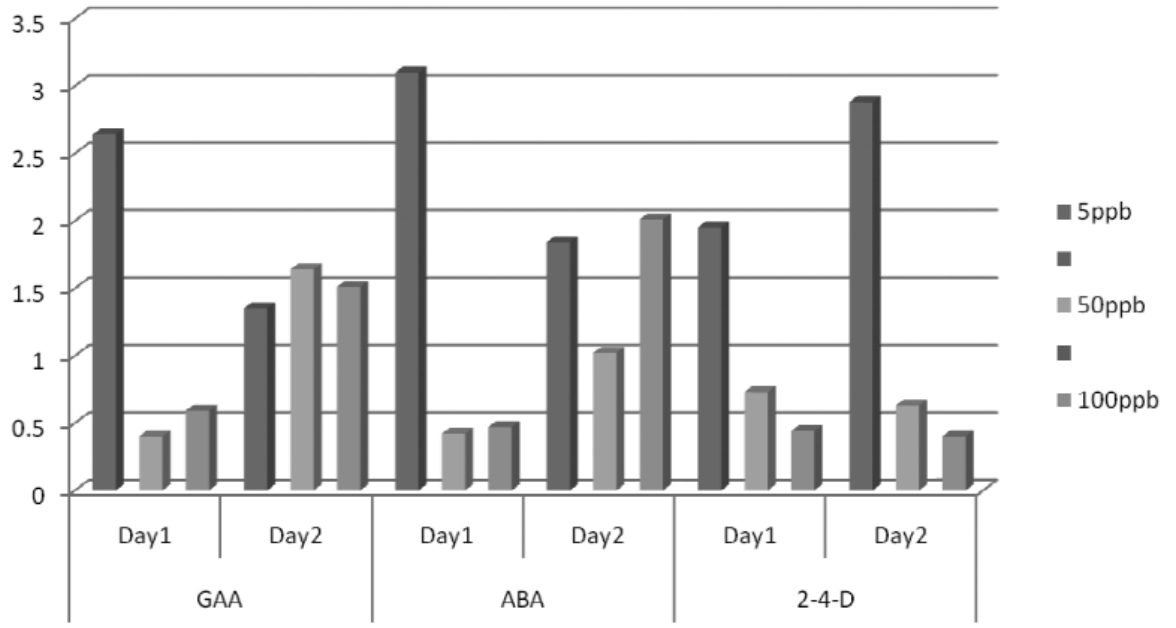

Figure 5. Summary of repeatability for three PGRs spiked and extracted from grape sample in negative mode.

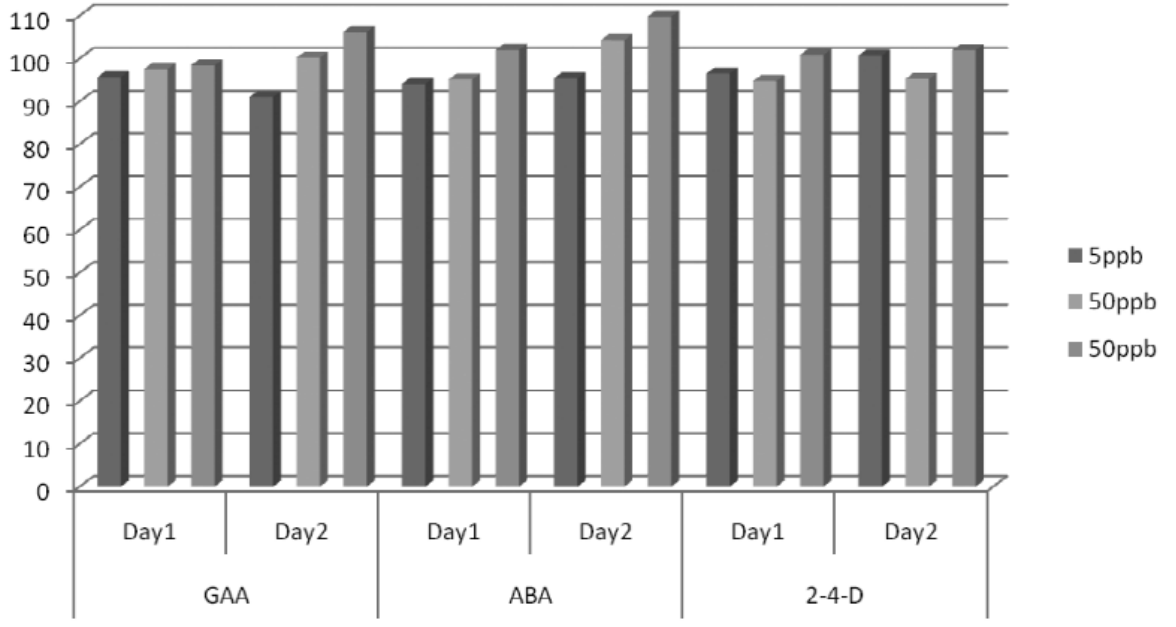

Figure 6. Summary of recovery for three PGRs spiked and extracted from grape samples.

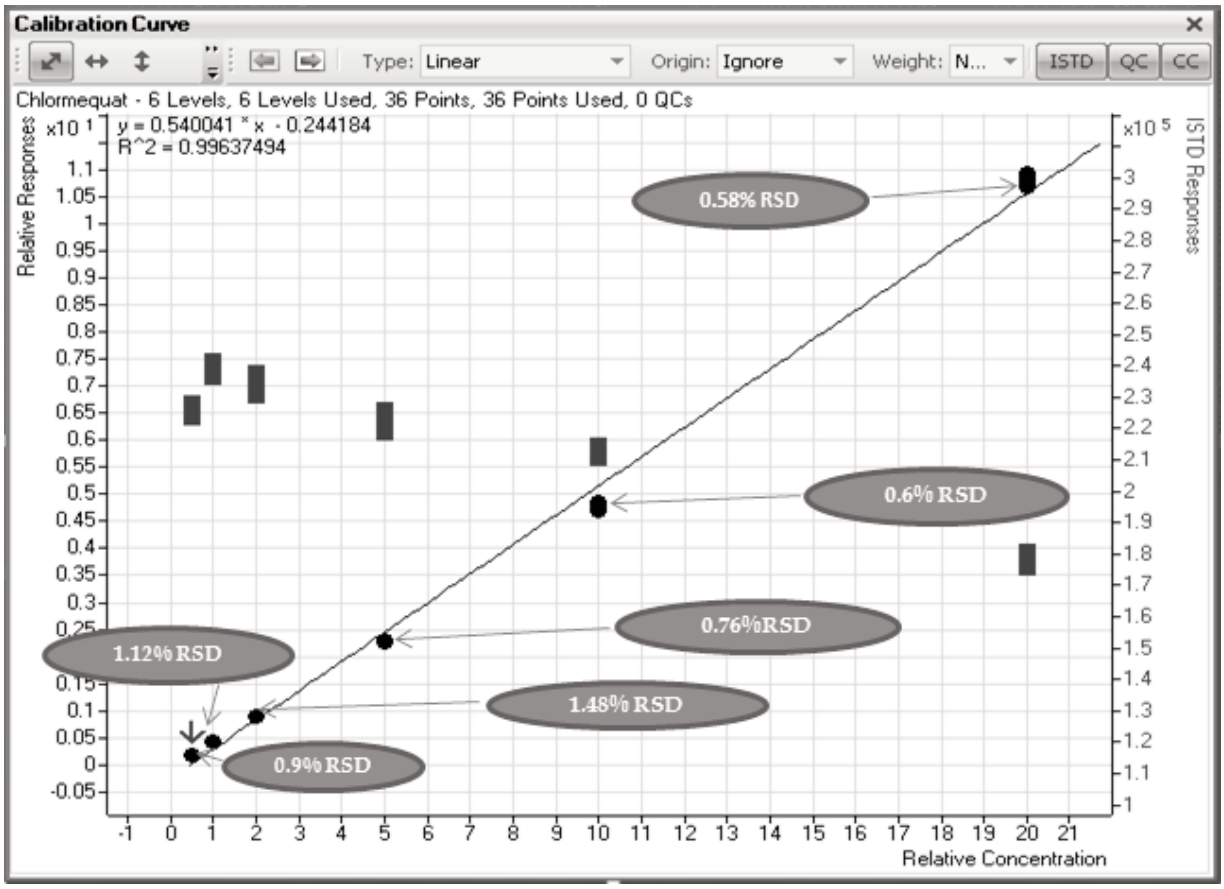

Figure 7. Calibration curve for chlormequat standard in positive mode.

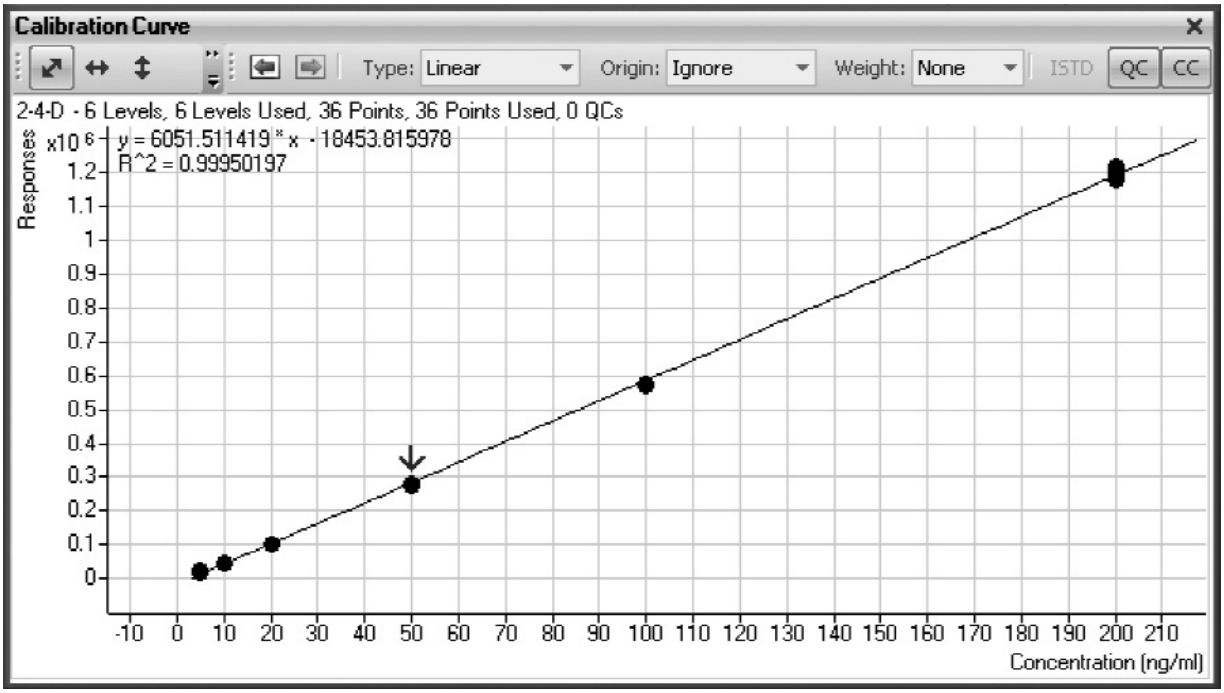

Figure 8. Calibration curve for 2-4 Dichlorphenoxy acetic acid in negative mode.

Conclusion

The Agilent 6460 Triple Quadrapole with Jet stream ESI ion source coupled with 1290 Infinity UHPLC and Dispersive C18 ODS sample clean up shows excellent sensitivity, linearity and recoveries for quantitation of multi class PGRs in Grapes.

Acknowledgement

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References

1. Peter Stone, Yang Chen and Jack Cappozzo, "Sensitive Femtogram Determination of Aflatoxins B_1, B_2, G_1, G_2) in Food Matrices using Triple Quadrupole LC/MS", 5990-6894EN 2010.

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