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Comparing Diode Array Detectors, with MS, and MS/MS for the Analysis of Plant Hormones in Plant Extracts

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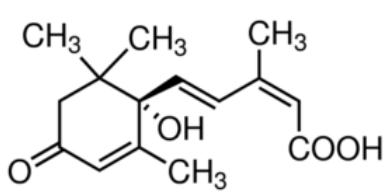
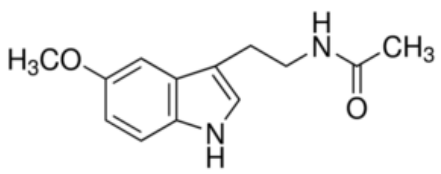
Introduction

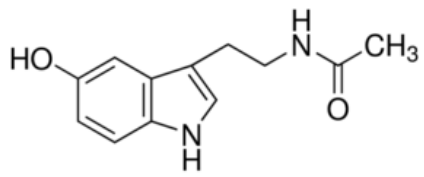
Tryptamine, serotonin, and melatonin are metabolites of tryptophan through the tryptophan-dependent IAA biosynthetic pathway. Abscisic acid (ABA) is an isoprenoid signaling hormone for a plant's response to pathogenic insults and environmental stressors. ABA is synthesized in the plastidial MEP pathway which interestingly is also a pathway for phytocannabinoid biosynthesis in *Cannabis spp*. N-acetylserotonin is a precursor for melatonin and is synthesized as a condensation product of serotonin and acetic acid.



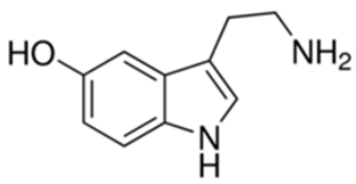
Abscisic acid



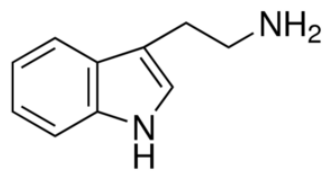
Melatonin



N-Acetylserotonin



Serotonin



Tryptamine

Structures of Analytes

Plant hormones can range in endogenous concentrations from low pg/g to µg/g levels. Accurately measuring this wide concentration range with selectivity can be difficult in plant matrices. In this study, hempseed oil was selected as the sample matrix. Five targeted compounds were spiked into hempseed oil. LC-DAD with LC-MS and LC-MS/MS were compared to evaluate how each system provides selective and sensitive identification and quantification.

The Agilent 1290 Binary HPLC with DAD, the Agilent 1290 Binary / iQ MSD LC-MS, and the Agilent 1290 Binary / 6470 LC-MS/MS systems were used. All five analytes were readily identified on all systems. However, LOQ in matrix markedly different: LC-MS/MS < LC-MS << HPLC-DAD. Selectivity was greatly affected by matrix interferences in the HPLC-DAD data thus significantly increasing the LOQ compared to mass spectrometry. SIM LC-MS vastly improved both selectivity and sensitivity. LC-MS/MS however demonstrated superior selectivity and sensitivity for the plant hormones in spiked matrix.

Experimental

Chromatographic Conditions

UHPLC: Agilent 1290 Infinity II
Column: Agilent Zorbax SB-AQ 150 x 2.1 mm, 1.8 µm
pn: 859700-914
Column oven temperature: 45 ± 2°C
Injection volume: 2 µL
Autosampler: 5 ± 2°C
Flow rate: 0.25 mL/min

Mobile Phase A: 0.1% Formic Acid in Water
Mobile Phase B: 0.1% Formic Acid in Acetonitrile

Gradient:

Time, min	%A	%B
0	75	25
4.0	5	95
4.5	5	95
4.6	75	25
6.0	75	25

MS Conditions-Agilent MSD IQ Single Quadrupole/Agilent 6470 Triple-Quadrupole

Parameter	
MS acquisition	SIM/Dynamic MRM
Ion source	Electrospray ionization/ Agilent Jet Stream electrospray ionization (AJS ESI positive)
Drying gas temperature	190 °C
Drying gas flow	9 L/min
Nebulizer	40 psi
Sheath gas heater	390 °C (LC-MS/MS only)
Sheath gas flow	12 L/min (LC-MS/MS only)
Capillary	4500 V
Nozzle voltage	0 V

Sample Preparation

- ✓ Weigh 1 g of hemp oil into a 15 mL centrifuge tube
- ✓ Add 2 mL ACN, shake for 5 mins
- ✓ Centrifuge at 3500 RPM for 6 mins
- ✓ Inject the top ACN layer with injection program to minimize the solvent effect

Experimental

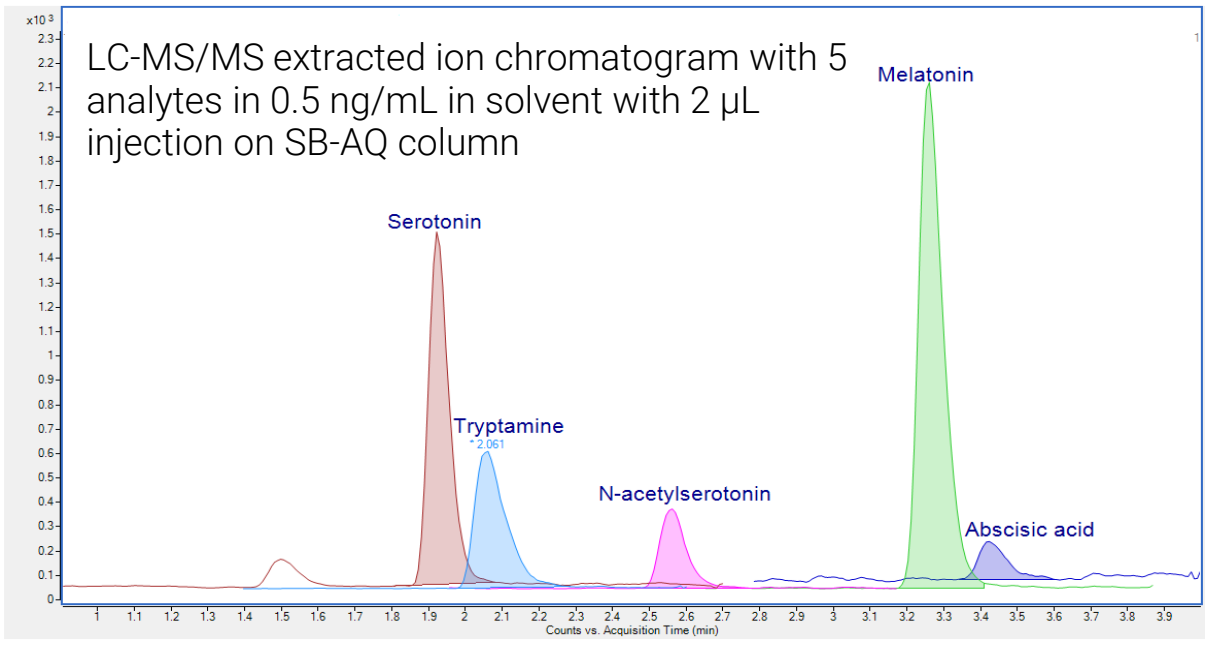
Compound Specific Conditions

Compound Name	Precursor Ion (m/z) SIM	Product Ion (m/z) MRM	Fragmentor (V)	Collison Energy (V)	UV Wavelength (nm)
Abscisic acid	265.2	*247.1, 187.1, 135.1, 91	88	4, 12, 24, 52	254
Melatonin	233.1	174.1, *159.1, 131.1	88	16, 32, 40	254
N-acetylserotonin	219.1	*160.1, 132.1, 117, 115	88	16, 28, 40, 40	280
Serotonin	177.1	160.1, *117, 115, 77.1	60	8, 32, 32, 60	280
Tryptamine	161.1	144.1, 117.1, *115, 91.1	60	8, 28, 44, 44	280

*Primary transitions

Results and Discussion

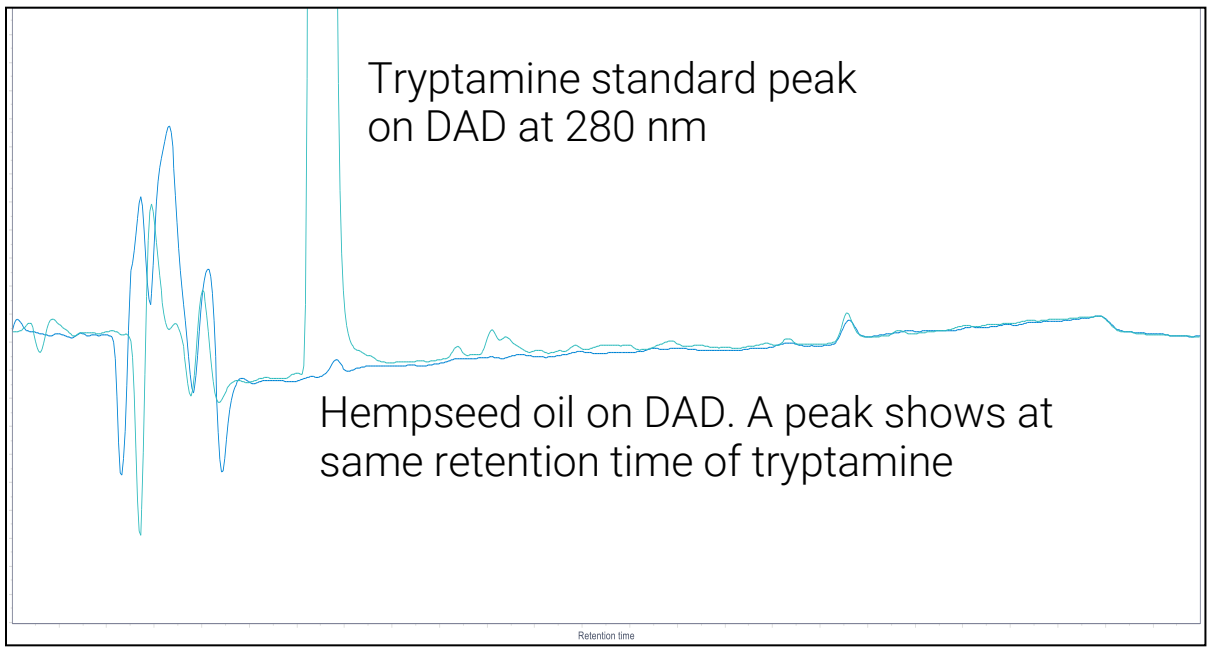
Elution Profile of 5 Plant Hormones

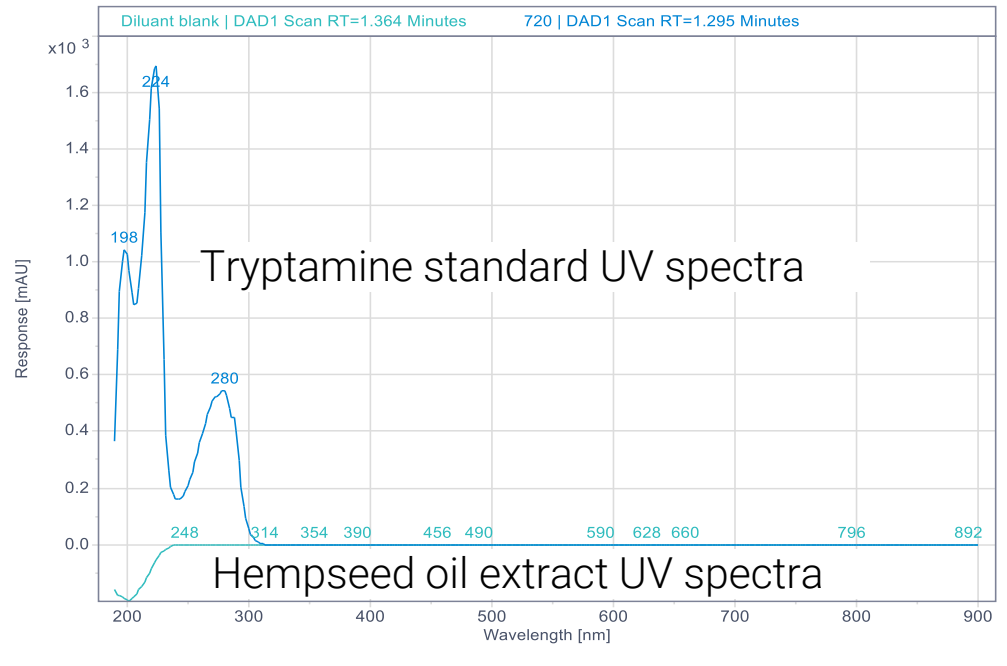


LOQ on DAD/SIM in Hempseed Oil

Compound name	LOQ on DAD (ppb)	LOQ on SIM (ppb)
Abscisic acid	420,000	0.42
Melatonin	440,000	0.44
N-acetylserotonin	515,000	0.51
Serotonin	410,000	0.41
Tryptamine	720,000	0.70

Sample Matrix Interferences on the DAD Detection-Tryptamine as an Example



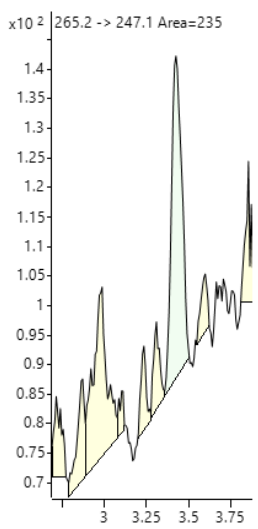


Hempseed oil has a UV component coeluting with the DAD signal of tryptamine, which gave false positive signal.

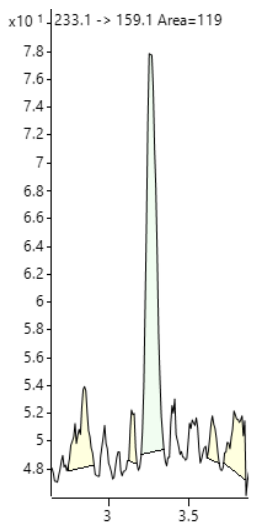
Results and Discussion-Identification and Quantitation with MRM

Response at ILOD (fg), S/N≥3

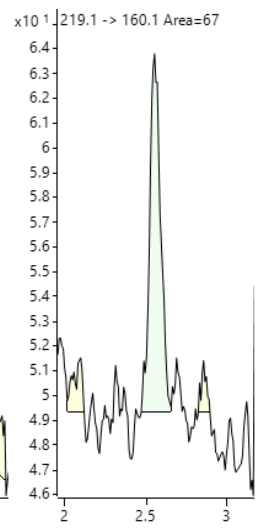
Abscisic acid at 200 fg



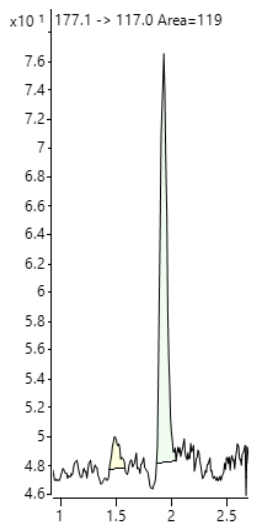
Melatonin at 4 fg



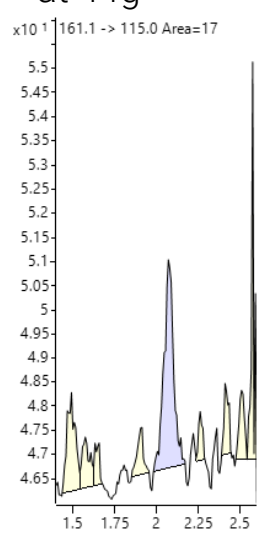
N-acetylserotonin at 4 fg



Serotonin at 100 fg



Tryptamine at 4 fg

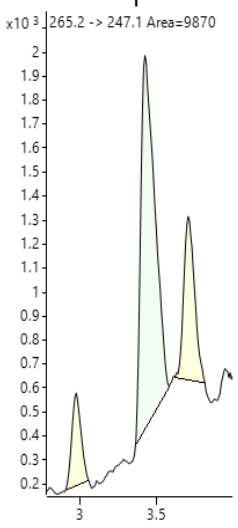


Linearity and Instrument LOD (ILOD)

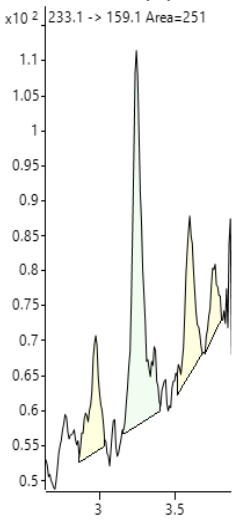
Compound name	Linear range, ng/mL	ILOD on column 2 µL injection volume	R^2
Abscisic acid	0.1-50	200 fg	0.997
Melatonin	0.002-50	4 fg	0.999
N-acetylserotonin	0.002-50	4 fg	0.999
Serotonin	0.05-50	100 fg	0.997
Tryptamine	0.002-50	4 fg	0.998

Response at LOQ in Hempseed Oil (ppb)

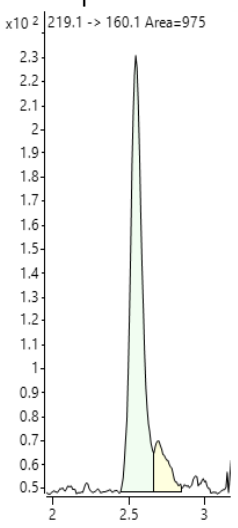
Abscisic acid at 16.6 ppb in sample



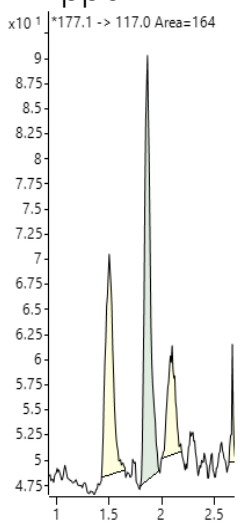
Melatonin Spiked at 0.01 ppb



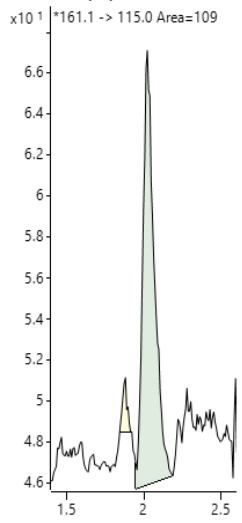
N-acetylserotonin at 0.098 ppb in sample



Serotonin spiked at 1 ppb



Tryptamine spiked at 0.1 ppb



Criteria to Accept the Quantitation Results

- ✓ Ion ratio for sample matches that of mean of all standards within the range of ± 30%
- ✓ The calibration curve constructed from external points has a coefficient of determination (r^2) of ≥ 0.99
- ✓ LOQ in sample determination
  - Extraction efficiency 50-150%
  - S/N ≥ 10
  - Post-matrix spike used for matrix effect correction

Sample Results and LOQ in Hemp Oil Determination Approach

Compound name	Hemp oil amount (g)	Dilution factor (mL)	Amount in sample (ppb)	Extraction efficiency at 0.01 ppb (%)	Matrix** effect (%)	Extraction efficiency at 0.1 ppb (%)	Matrix effect, (%)	Extraction efficiency at 1 ppb (%)	Matrix effect (%)	Extraction efficiency at 10 ppb (%)	Matrix effect (%)	LOQ, ppb
Melatonin	1	2	<LOQ	104	56	63	62	101	78	87	83	0.01
Abscisic acid	1	2	16.592	-	-	-	-	-	-	108	78	*0.4 (ILOQ)
Serotonin	1	2	<LOQ	-	-	37.1 (failed)	57	60	26	118	24	1
N-acetylserotonin	1	2	0.098	-	-	72.2	78	93.7	85	105	94	*0.01 (ILOQ)
Tryptamine	1	2	<LOQ	-	-	61	52	60	54	89	46	0.1

*Can't determine LOQ in sample due to the presence in sample. ILOQ was determined
** Matrix effect =100, no matrix effects; Matrix effect < 100, ion suppression; Matrix effect > 100, ion enhancement

Conclusions

- ✓ DAD has limited sensitivity and selectivity to identify/quantify the analytes in the sample
- ✓ SIM has good sensitivity but limited selectivity

- ✓ MRM has great sensitivity and selectivity for rapid sample prep, detection and accurate quantification for plant hormones in matrices

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