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Determination of the Oregon Pesticide List in Cannabis Using a Simple Extraction Procedure With dSPE Cleanup and UPLC-MS/MS

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APPLICATION BENEFITS

- Sensitive and robust method for screening pesticides in cannabis per the Oregon Cannabis Pesticide Guide List
- Minimal sample preparation followed by rapid UPLC™ separation
- Automated UPLC-MS/MS method generation using the Quanpedia™ Database
- Ease of use with data analysis and reporting via MassLynx™ MS Software

WATERS SOLUTIONS

ACQUITY™ UPLC H-Class System

Xevo™ TQ-S micro Mass Spectrometer

XBridge™ BEH C_{18} ***XP*** Column

MassLynx MS Software

KEYWORDS

Cannabis testing, pesticides, multi-residue pesticide detection, LC-MS/MS

INTRODUCTION

The increased use of both medical and recreational cannabis in combination with its expanding legal acceptance in most US states[1] has led to rigorous cannabis safety and quality control testing. Pesticides are widely used in the cultivation of cannabis plants to safeguard against harmful insects and to promote better crop yields. The application of pesticides is regulated,[2] and their residues in cannabis products are closely monitored by state regulatory agencies. The number of pesticides and their action limits varies from state to state. In Oregon, 59 pesticides are monitored with action limits ranging from 100 to 2000 ppb. Therefore adopting a robust and rapid procedure for monitoring the Oregon pesticide list in cannabis products is critical.

Multi-residue pesticide detection is routinely performed using tandem quadrupole mass spectrometry (MS/MS) in combination with liquid chromatography (LC) and gas chromatography (GC). Both LC-MS/MS and GC-MS/MS are commonly used for multi-residue pesticide analysis as some pesticides are only amenable to either LC or GC. Tandem quadrupole MS is the detector of choice as it provides high sensitivity and selectivity for simultaneous analysis of hundreds of pesticides at low ppb (ng/g) levels in a single analysis.

In this application note we present the use of a simple sample extraction and dispersive solid phase extraction (dSPE) cleanup procedure followed by UPLC-MS/MS analysis for rapidly monitoring the Oregon Cannabis Pesticide Guide List[3] in cannabis matrix. With so many compounds to monitor, method generation can be a tedious task. In this study, the preexisting LC and MS methods from Waters™ Quanpedia Database were used to develop and implement a rapid solution for the Oregon pesticide list.

EXPERIMENTAL

Sample preparation

Standard compounds for the 59 pesticides monitored on the Oregon list were combined to produce a stock solution which was sequentially diluted to prepare the spiking solutions. Cannabis buds were first ground using a hand grinder. A 0.5 g portion of the ground material were weighed into 50 mL centrifuge tubes and spiked with 200 ppb of the acetonitrile spiking solutions. A 5-mL volume of acetonitrile was added and the samples were processed using a Geno Grinder (two stainless steel grinding balls, 11 mm) for 5 minutes (1500 rpm). The samples were then centrifuged at 5000 rpm for 5 minutes. For experiments where no further cleanup was performed, the supernatant was filtered using a 0.2 µm PTFE filter in preparation for analysis.

A 1 mL aliquot of the supernatant was added to a dSPE tube (2 mL centrifuge tube containing 150 mg $MgSO_4$, 50 mg PSA, 50 mg C_{18}, 7.5 mg graphitized carbon black), vortexed for 1 minute, centrifuged, and the supernatant transferred to a sample vial for analysis by UPLC-MS/MS.

Instrumentation and software

All separations were performed on the Waters™ ACQUITY UPLC H-Class System and the Xevo TQ-S micro Tandem Quadrupole Mass Spectrometer. MassLynx MS Software (v4.1) was used for data acquisition and processing. The Quanpedia Database can be used to automatically generate LC, MS acquisition, and TargetLynx™ data processing methods to reduce method setup times with minimal user interaction.

UPLC conditions

UPLC system:	ACQUITY UPLC H-Class
Separation mode:	Gradient
Column:	XBridge BEH C_{18} ***XP***, 130Å, 2.5 µm, 2.1 mm × 100 mm, P/N: 186006031
Solvent A:	5 mM Ammonium formate with 0.020% formic acid in water
Solvent B:	Methanol
Flow rate:	0.50 mL/min
Column temp.:	30 °C
Injection volume:	5 µL

Gradient conditions:

Time (min)	%A	%B	Curve
0.00	98%	2%	–
0.20	98%	2%	6
11.50	1%	99%	6
13.00	1%	99%	6
13.25	98%	2%	1
15.00	98%	2%	1

MS conditions

MS system:	Xevo TQ-S micro
Ionization mode:	ESI+/ESI-
Capillary voltage:	2.5 kV (+); 2.4 kV (-)
Cone voltage:	Various V
Collision energy:	Various eV
Desolvation temp.:	450 °C
Source temp.:	150 °C
Desolvation gas flow:	1000 L/Hr
Cone gas:	50 L/Hr

RESULTS AND DISCUSSION

METHOD DEVELOPMENT AND OPTIMIZATION

Quanpedia Database was used to automatically create the LC, MS, and data processing methods (Figure 1) for the various target pesticides monitored using the transitions listed in Table 1. Users can quickly generate pre-defined LC-MS/MS methods in three easy steps, which greatly reduces the potential for error and level of complexity involved in method development for large numbers of target analytes. As a result, it decreases the amount of work, time, and resources required for laboratories to set up methods.

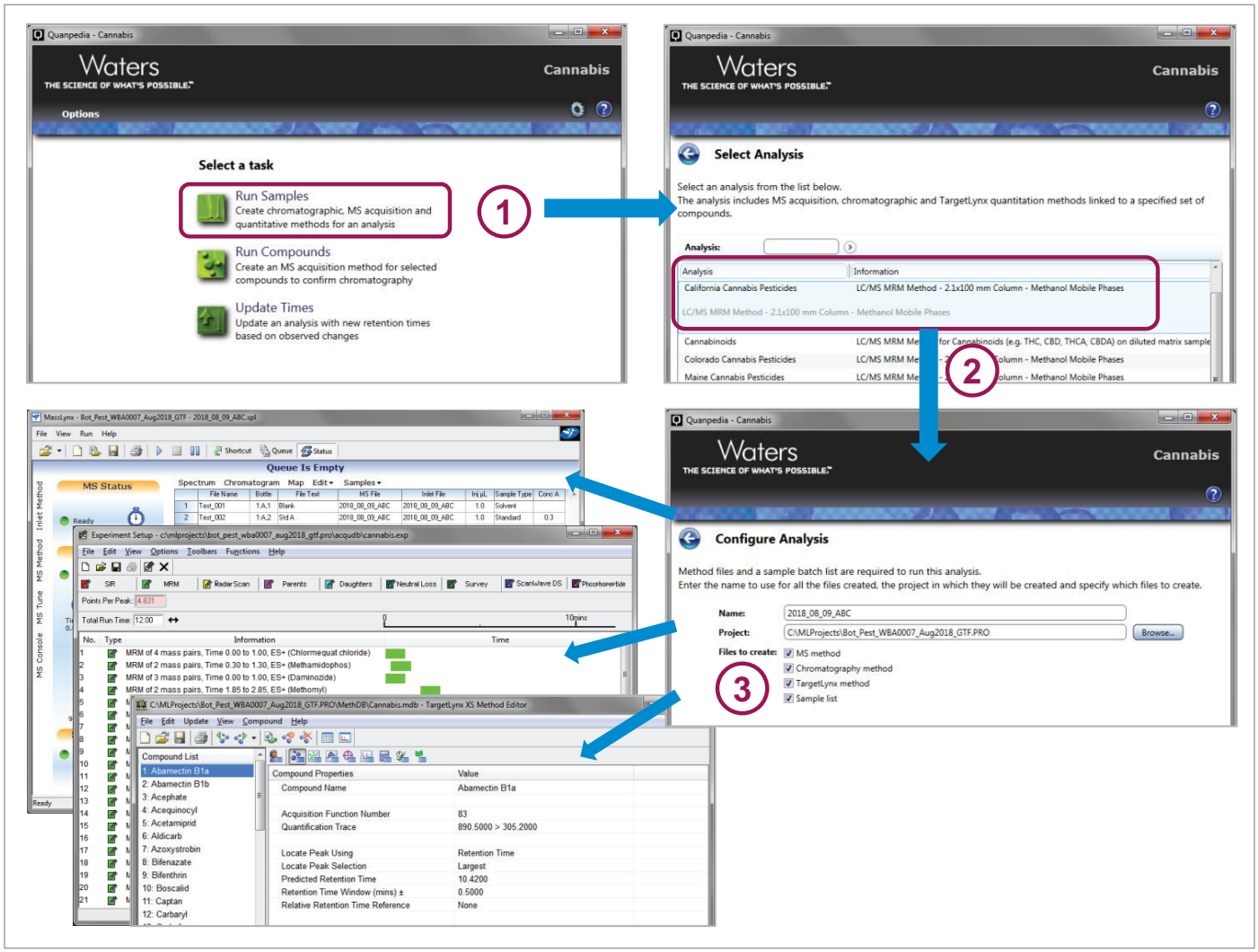

Figure 1. Rapid implementation of LC, MS, and data processing methods using the Quanpedia Database.

Table 1. Retention times, MRM transitions, and %Recovery for the Oregon pesticide list in cannabis matrix. Data based on four replicate measurements.

Pesticides	RT (min)	%Recovery	Quan trace	Qual trace
Abamectin	11.72	71	890.7>305.3	890.7>145.1
Acephate	2.40	85	184.1>143.1	184.1>95.1
Acequinocyl	12.71	82	343.2>189.1	343.2>115.0
Acetamiprid	5.06	90	223.1>126.1	223.1>56.1
Aldicarb	5.76	108	208.1>89.1	208.1>116.1
Azoxystrobin	8.10	95	404.1>344.1	404.1>372.1
Bifenazate	8.70	94	301.1>170.2	301.1>153.1
Bifenthrin	12.01	96	440.1>166.2	440.2>181.2
Boscalid	8.32	94	343.1>307.1	343.1>140.1
Carbaryl	6.86	92	202.1>145.1	202.1>127.1
Carbofuran	6.54	92	222.1>165.1	222.1>123.1
Chlorantraniliprole	7.83	90	481.9>283.9	481.9>450.9
Chlorfenapyr	10.42	85	409.2>59.0	409.2>379.1
Chlorpyrifos	10.82	92	351.9>124.9	351.9>199.9
Clofentezine	9.73	90	303.1>138.1	303.1>102.1
Cyfluthrin	11.25	114	451.1>191.1	453.1>193.1
Cypermethrin	11.43	90	433.1>191.0	435.1>193.1
Daminozide	0.59	53	161.1>143.1	161.1>61.1
Diazinon	9.46	95	305.1>169.1	305.1>153.1
Dichlorvos	6.41	90	221.1>109.1	221.1>79.1
Dimethoate	4.92	92	230.1>125.1	230.1>198.9
Ethoprophos	8.82	87	243.1>130.9	243.1>97.1
Etofenprox	11.91	92	394.3>177.1	394.3>106.9
Etoxazole	11.05	87	360.2>141.1	360.2>113.1
Fenoxycarb	9.20	96	302.1>116.1	302.1>88.1
Fenpyroximate	11.20	90	422.2>366.1	422.2>138.1
Fipronil	9.21	101	434.9>330.1	434.9>250.1
Flonicamid	3.67	96	230.1>203.1	230.1>148.1
Fludioxinil	8.38	99	247.2>126.1	247.2>180.2
Hexythiazox	10.87	87	353.1>228.1	353.1>168.1
Imazalil	7.54	48	297.1>159.1	297.1>69.1

Pesticides	RT (min)	%Recovery	Quan trace	Qual trace
Imidacloprid	4.65	87	256.1>175.1	256.1>209.1
Kresoxim-methyl	9.26	98	314.1>116.1	314.1>235.1
Malathion	8.42	98	331.1>127.1	331.1>285.1
Metalaxyl	7.50	90	280.2>220.1	280.2>192.1
Methiocarb	8.22	92	226.1>121.1	226.1>169.1
Methomyl	3.67	93	163.1>88.1	163.1>106.1
MGK 264	9.96	90	276.1>210.1	276.1>71.1
Myclobutanil	8.63	88	289.1>69.9	289.1>125.1
Naled	7.68	96	381.1>127.1	381.1>109.1
Oxamyl	3.47	93	237.1>72.1	237.1>90.1
Paclobutrazol	8.39	88	294.1>70.2	294.1>125.1
Parathion methyl	8.07	94	264.2>125.1	264.2>232.1
Permethrin	11.86	90	408.1>183.1	410.1>185.1
Phosmet	7.89	92	318.1>160.1	318.1>133.1
Piperonyl butoxide	10.60	84	356.2>177.1	356.2>119.1
Prallethrin	10.04	102	301.2>133.1	301.2>169.1
Propiconazole	9.50	80	342.1>69.1	342.1>158.9
Propoxur	6.45	92	210.1>111.1	210.1>168.1
Pyrethrin I	11.19	91	329.1>161.1	329.1>133.1
Pyrethrin II	10.13	94	373.2>161.1	373.2>133.1
Pyridaben	11.46	85	365.2>147.1	365.2>309.1
Spinosad A	9.82	43	732.6>142.1	732.6>98.1
Spinosad D	10.25	40	746.5>142.1	746.5>98.1
Spiromesifen	11.08	76	388.2>273.1	371.2>273.1
Spirotetramat	8.77	87	374.1>330.1	374.1>302.1
Spiroxamine	8.31	42	298.1>144.1	298>100.1
Tebuconazole	9.43	85	308.2>70.1	308.2>125.1
Thiacloprid	5.50	90	253.1>126.1	253.1>90.1
Thiamethoxam	3.92	92	292.1>132.1	292.1>211.2
Trifloxystrobin	10.12	96	409.1>186.1	409.1>145.1

Figure 2 shows an overlay chromatogram of 59 pesticides analyzed by UPLC-MS/MS. MRM chromatograms of selected pesticides in cannabis matrix are shown in Figure 3.

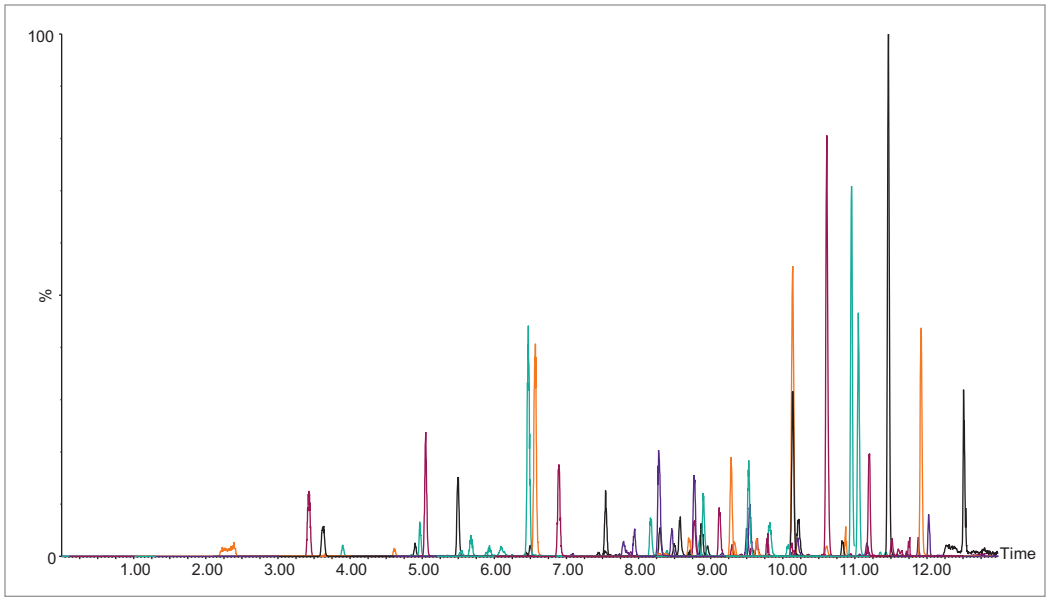

Figure 2. UPLC-MS/MS chromatogram overlay of 59 pesticides spiked at 200 ppb in the cannabis matrix.

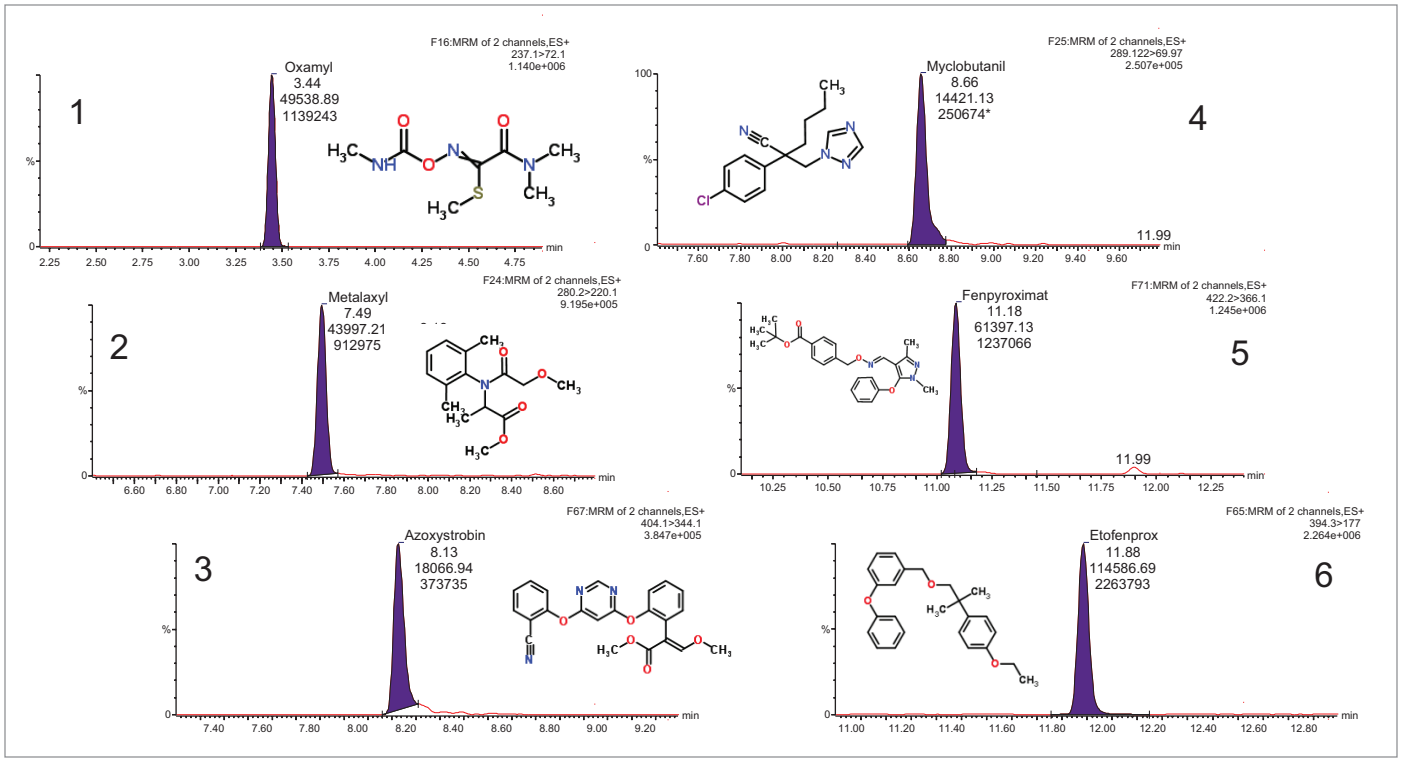

Figure 3. Representative MRM chromatograms for 1. oxamyl, 2. metalaxyl, 3. azoxystrobin, 4 myclobutanil, 5. fenpyroximat, and 6. etofenprox spiked at a level of 200 ppb and extracted using the sample preparation protocol reported.

LINEARITY

An example of the quantitation curve for methomyl and propoxur are shown in Figure 4. Linear calibration curves (R^2>0.990) for each pesticide were obtained over the range tested 6.25 to 1000 ppb in matrix. Table 2 highlights the limit of quantitation (LOQ) and action limits per the Oregon Cannabis Pesticide Guide List.[3]

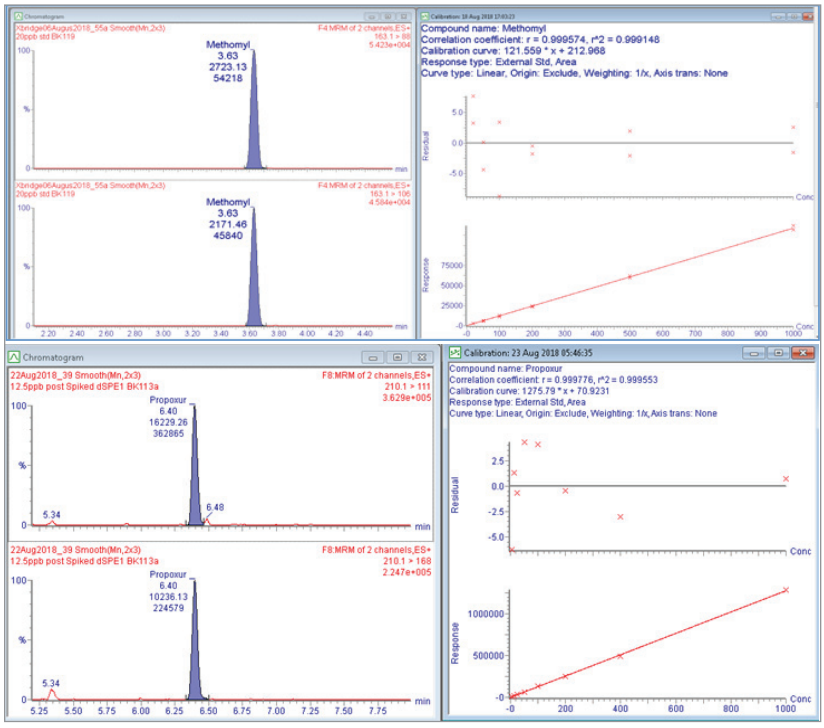

Figure 4. Representative example of quantitation curves for methomyl and propoxur analyzed with a linearity range of 6.25 to 1000 ppb.

RECOVERY AND MATRIX EFFECTS

Method recovery was assessed by spiking pesticides at the 200 ppb and 1000 ppb levels in a cannabis flower matrix and comparing the response to that observed from spiked matrix blanks (matrix-matched standards). As shown in Figure 5, the recoveries observed for most of the pesticides were in the range of 80% to 120%. Matrix suppression was determined at the 200 ppb level by comparing the response observed in matrix-matched standards to the response observed in the solvent standards. Matrix suppression data is presented in Figure 6. Those compounds that co-eluted with the cannabis resin constituents (retention times from 9 to 12 minutes) showed the greatest suppression before dSPE cleanup. The dSPE cleanup provided a significant reduction of suppression for most of the compounds.

Table 2. Limit of quantitation (LOQ) for pesticide analytes and their action levels in the Oregon Cannabis Pesticide Guide List.

Pesticides	LOQ (ppb)	Action levels (ppb)
Abamectin	<200	500
Acephate	<200	400
Acequinocyl	<100	2000
Acetamiprid	<100	200
Aldicarb	<200	400
Azoxystrobin	<100	200
Bifenazate	<100	200
Bifenthrin	<100	200
Boscalid	<100	400
Carbaryl	<100	200
Carbofuran	<100	200
Chlorantraniliprole	<100	200
Chlorfenapyr	<500	1000
Chlorpyrifos	<100	200
Clofentezine	<100	200
Cyfluthrin	<200	1000
Cypermethrin	<200	1000
Daminozide	<1000	1000
Diazinon	<100	200
Dichlorvos	<100	100
Dimethoate	<100	200
Ethoprophos	<100	200
Etofenprox	<200	400
Etoxazole	<100	200
Fenoxycarb	<100	200
Fenpyroximate	<100	400
Fipronil	<100	400
Flonicamid	<200	1000
Fludioxinil	<200	400
Hexythiazox	<100	1000

Pesticides	LOQ (ppb)	Action levels (ppb)
Imazalil	<100	200
Imidacloprid	<100	400
Kresoxim-methyl	<100	400
Malathion	<100	200
Metalaxyl	<100	200
Methiocarb	<100	200
Methomyl	<100	400
MGK 264	<100	200
Myclobutanil	<100	200
Naled	<100	500
Oxamyl	<100	1000
Paclobutrazol	<100	400
Parathion methyl	<100	200
Permethrin	<100	200
Phosmet	<100	200
Piperonyl butoxide	<100	2000
Prallethrin	<100	200
Propiconazole	<100	400
Propoxur	<100	200
Pyrethrin	<200	1000
Pyridaben	<100	200
Spinosad	<100	200
Spiromesifen	<200	200
Spirotetramat	<100	200
Spiroxamine	<100	400
Tebuconazole	<100	400
Thiacloprid	<100	200
Thiamethoxam	<100	200
Trifloxystrobin	<100	200

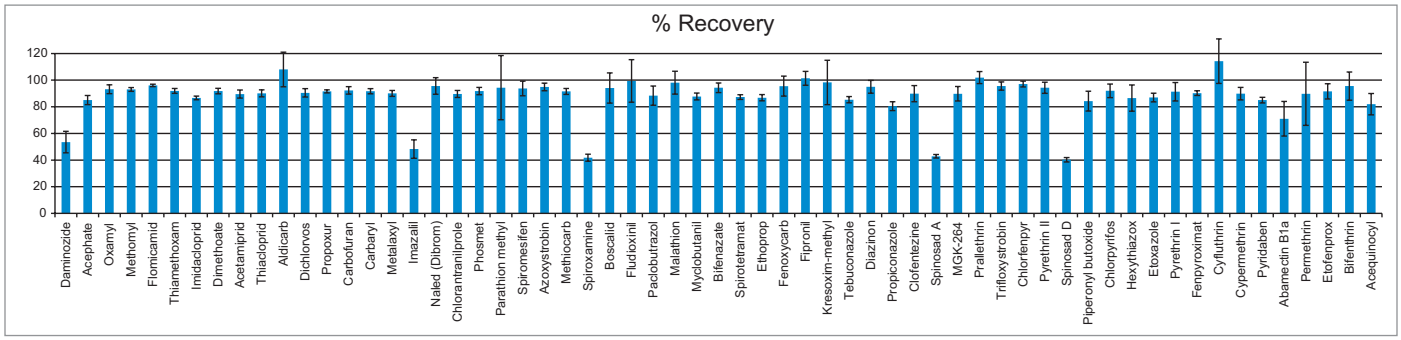

Figure 5. %Recovery of pesticides from the cannabis matrix (n = 4). Compounds are presented in order of retention (from 2.9 min for acephate to 12.8 min for acequinocyl). Error bars indicate the standard deviation observed for each compound. The combined recovery of spinosad A and D components is close to 85%.

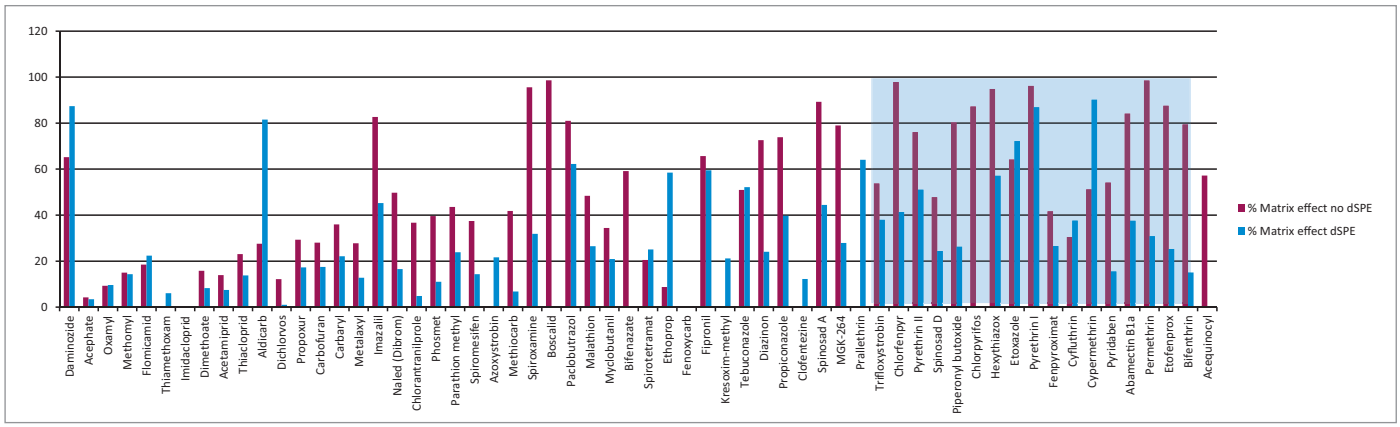

Figure 6. Matrix suppression at the 200 ppb level; the red bars indicate suppression observed without dSPE and the blue bars indicate suppression after dSPE cleanup. The shaded area indicates the compounds that co-eluted with the cannabis resin constituents.

CONCLUSIONS

This simple sample extraction and dSPE cleanup method followed by UPLC-MS/MS analysis using the ACQUITY UPLC H-Class System coupled to the Xevo TQ-S micro Tandem Quadrupole Mass Spectrometer provides a rapid, sensitive, and robust method for determination of the Oregon Cannabis Pesticide Guide List in a challenging cannabis matrix. Matrix suppression was significantly reduced by dSPE cleanup for many of the pesticides; thereby improving the data quality. This method is capable of meeting the MRLs for Oregon's pesticide list in cannabis matrix.

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