Analytical Methodology for EU Target List Residual Pesticides in Cannabis

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Abstract

Identification and quantification of the European Union (EU) target list of residual pesticides in Cannabis is of interest for cannabis products allocated for sale in Europe, based on guidance from the Committee on Herbal Medicinal Products (HMPC). There are limited reports of analytical methods or strategies that include the EU target list of pesticides in dry cannabis flower. In this study, a comprehensive strategy was developed and validated to identify and quantify the pesticides and respective isomers from the EU target list in dry cannabis flower samples. Analysis of these pesticides required two independent analytical methods from one sample extract: a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for about half of the compounds and a gas chromatography-tandem mass spectrometry (GC-MS/MS) method for the other half of the compounds using matrix-matched calibration standards. Samples are extracted with acetonitrile and C18 SPE cartridges. The validation data from both analytical methods show correlation coefficients of greater than 0.99, good recoveries (between 70% - 130%), and method LOQs at the European Pharmacopoeia limit for each pesticide. Additionally, precision and specificity (resolution and tailing) met the validation criteria. This strategy promises accurate identification and quantification of the EU target list of residual pesticides in dry cannabis flower samples, which are required for exporting cannabis products to EU countries.

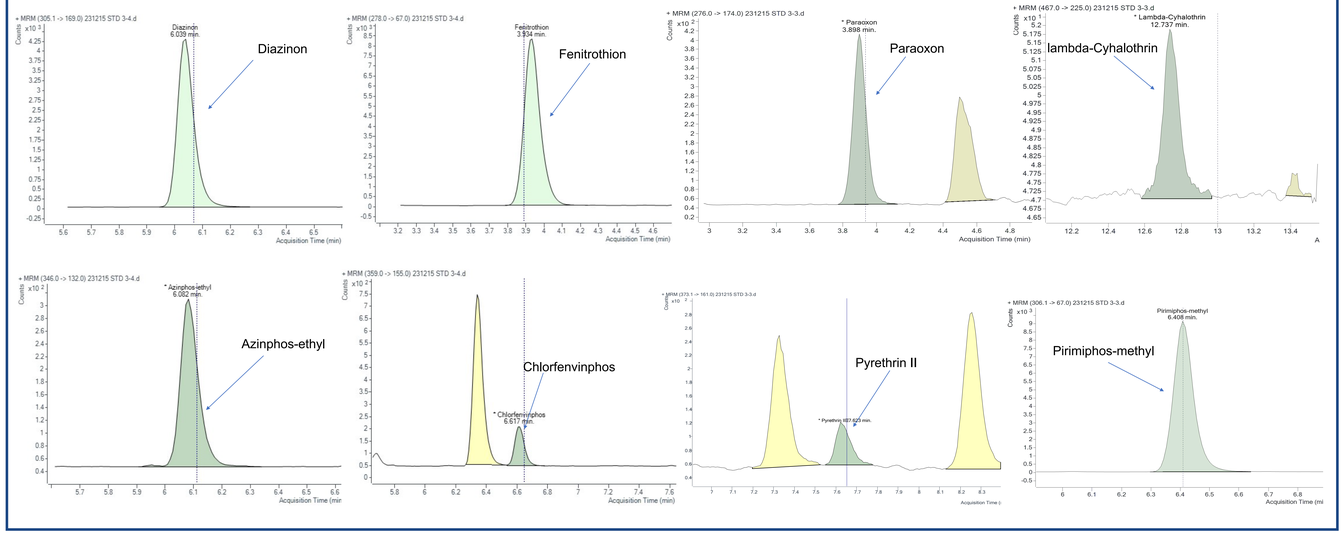
Instruments

- **LC-MS/MS**
  - Column (Infinity Poroshell 120 Phenyl Hexyl, 3.0 x 100 mm, 2.7 µm.) @ 55°C
  - Flow rate: 0.5 mL/min; Gradient: 50% A for 1 min, then to 30% A at 4 min, 25% A at 12 min, 20% A at 16 min, 17% A at 19 min, 0% A at 19.01 and held for 2 min
  - Solvents: 5 mM ammonium formate + 0.1 % formic acid in water “A”, 0.1 % formic acid in methanol/acetonitrile (9:1) “B”
- **GC-MS/MS**
  - Injector (MMI): 180°C initial for 0 min, 400 °C/min to 280°C
  - Column 1: Agilent DB-35MS Ultra Inert, 15 m × 0.25 mm, 0.25 µm film thickness connected to an Agilent Purged Ultimate Union
  - Column 2: Agilent HP-5MS Ultra Inert, 15 m × 0.25 mm, 0.25 µm film thickness
  - Column flow: 1.0 mL/min for column 1, 1.4 mL/min for column 2.
  - Oven program: 70°C for 1 min, 60°C/min ramp to 240°C , 4 °C/min to 255°C, 30°C min ramp to 300°C and a hold of 6.9 min. Total run time 15 min

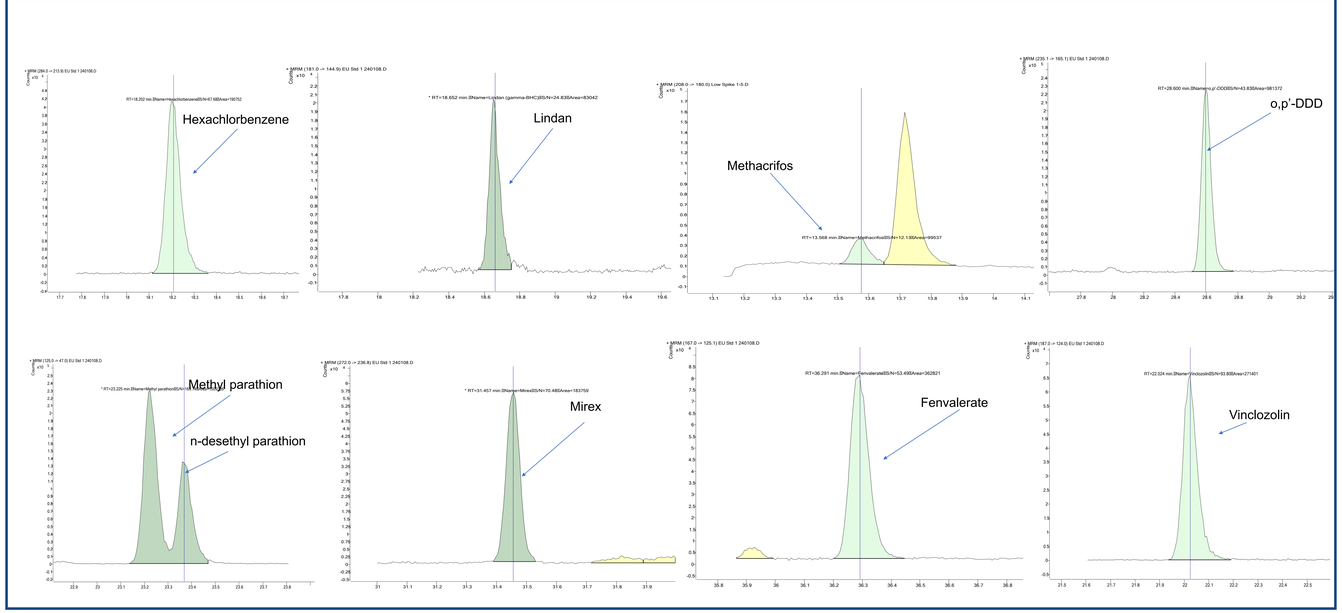
Sample Preparation

A ground cannabis sample is accurately weighed and mixed with acetonitrile. The sample is cleaned up with a C18 solid-phase extraction cartridge. The sample is then analyzed by either GC-MS/MS or LC-MS/MS.

LC-MS/MS Chromatograms



GC-MS/MS Chromatogram



Results

	Compound	Correlation coefficient (R^2)	% Recoveries at LOQ	Method LOQ (mg/kg)	EU Limit (mg/kg)
LC-MS/MS	Acephate	0.999	100.0	0.10	0.10
	Alachlor	0.999	94.1	0.05	0.05
	Azinphos-ethyl	0.999	94.6	0.10	0.10
	Azinphos-methyl	0.999	94.9	1.00	1.00
	Bromopropylate	0.999	100.3	3.00	3.00
	Chlorfenvinphos	0.999	96.7	0.50	0.50
	Chlorpyrifos	0.9999	91.8	0.20	0.20
	Cypermethrin and isomers (sum of)	0.9976	107.0	1.00	1.00
	Deltamethrin	0.9982	114.0	0.50	0.50
	Diazinon	0.9993	103.0	0.50	0.50
	Dichlofluanid	0.997	107.7	0.10	0.10
	Dichlorvos	0.9991	105.0	1.00	1.00
	Dimethoate and Omethoate (sum of)	0.9987	97.9	0.10	0.10
	Ethion	0.999	93.9	2.00	2.00
	Etrimphos	0.999	96.5	0.05	0.05
	Fenitrothion	0.999	79.1	0.50	0.50
	Fenpropathrin	0.999	88.8	0.03	0.03
	Fensulfothion (sum of)	0.999	91.3	0.05	0.05
	τ-Fluvalinate	0.999	79.2	0.05	0.05
	lambda-Cyhalothrin	0.999	99.3	1.00	1.00
	Malaoxon and Malathion (sum of)	0.999	92.6	1.00	1.00
	Mecarbam	0.999	92.8	0.05	0.05
	Methamidophos	0.999	100.3	0.05	0.05
	Methidathion	0.999	94.0	0.20	0.20
	Monocrotophos	0.999	91.9	0.10	0.10
	Pendimethalin	0.999	93.8	0.50	0.50
	Phosalone	0.999	95.7	0.10	0.10
	Phosmet	0.9991	94.5	0.05	0.05
	Piperonyl-butoxide	0.999	81.4	3.00	3.00
	Primiphos-ethyl	0.9995	79.8	0.05	0.05
	Pirimiphos-methyl and N-desethyl-pirimiphos-methyl (sum of)	0.9984	103.8	4.00	4.00
	Profenofos	0.9992	92.2	0.10	0.10
	Pyrethrin (sum of cinerin I, cinerin II, jasmolin I, jasmolin II, pyrethrin I and pyrethrin II)	0.999	89.6	3.00	3.00
	Quinalphos	0.999	93.6	0.05	0.05
GC-MS/MS	Aldrin	0.999	80.4	0.05	0.05
	Bromophos-ethyl	0.999	94.4	0.05	0.05
	Bromophos-methyl	0.999	93.0	0.05	0.05
	Chlordane (sum of)	1.000	101.5	0.05	0.05
	Chlorpyriphos-methyl	0.999	94.1	0.1	0.1
	Chlorthal-dimethyl	0.997	102.7	0.01	0.01
	Cyfluthrin (sum of)	1.000	90.4	0.1	0.1
	DDT(sum of o,p'-DDE, p,p'-DDE, o,p'-DDT, p,p'-DDT, o,p'-TDE and p,p'-TDE)	0.999	94.7	1	1
	Dicofol	0.999	96.9	0.5	0.5
	Endrin	0.999	93.5	0.05	0.05
	Endosulfan (sum of)	0.997	80.2	3	3
	Fenchlorophos (sum of)	0.999	95.1	0.1	0.1
	Fenthion (sum of)	0.999	96.4	0.050	0.050
	Fenvalerate	0.999	86.9	1.500	1.500
	Flucythrinate	0.996	76.9	0.05	0.05
	Fonofos	0.999	98.5	0.05	0.05
	Heptachlor (sum of)	0.998	98.6	0.05	0.05
	Hexachlorbenzene	0.999	96.0	0.1	0.1
	Hexachlorocyclohexane (sum of α-, β-, δ- and ε)	0.999	87.1	0.3	0.3
	Lindan (γ-hexachlorocyclohexane)	0.999	90.2	0.6	0.6
	Methacrifos	0.999	96.6	0.05	0.05
	Methylparathion & Paraoxon methyl (sum of)	0.998	95.5	0.2	0.2
	Paraoxon and Parathion (sum of)	0.998	97.2	0.5	0.5
	Pentachloranisol	0.999	94.1	0.01	0.01
	Permethrin and isomers (sum of)	0.999	109.5	1	1
	Procymidone	0.999	97.0	0.1	0.1
	Prothiophos	0.999	95.5	0.05	0.05
	Quintozene (sum of)	1.000	99.4	1	1
	S-421	0.996	85.3	0.02	0.02
	Tecnazene	0.999	90.5	0.05	0.05
	Tetradifon	1.000	89.1	0.3	0.3
	Vinclozolin	0.999	94.8	0.4	0.4

Summary & Conclusions

In support of EU import requirements for residual pesticide testing, we have developed and validated an LC-MS/MS and a GC-MS/MS method for pesticides and respective isomers listed under European Pharmacopoeia 9.6 section 2.8.13. The two methods had limits of quantitation at the EU limit for each pesticide, were validated with good accuracies, and will be used for routine testing of dry cannabis flower samples.