

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 μ m.) @ 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"

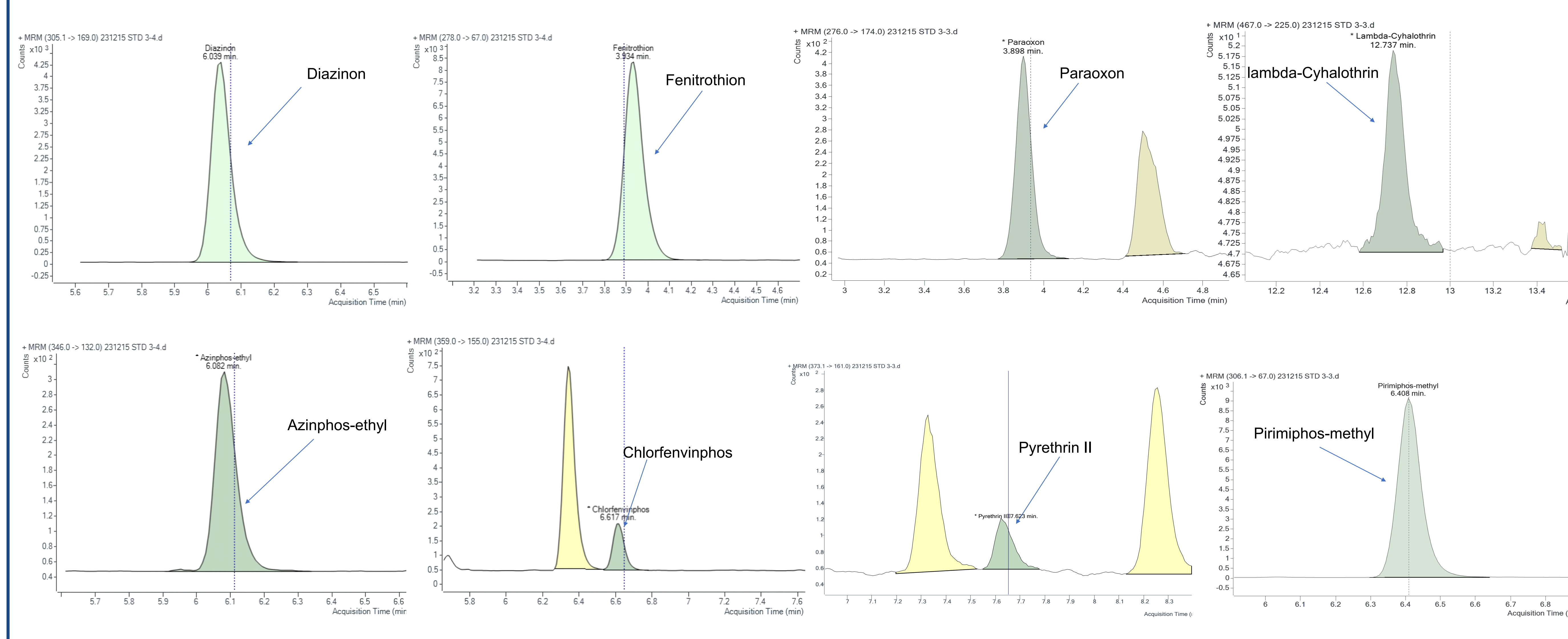
GC-MS/MS

- Injector (MMI): 180°C initial for 0 min, 400 °C/min to 280°C
- Column 1: Agilent DB-35MS Ultra Inert, 15 m x 0.25 mm, 0.25 μ m film thickness connected to an Agilent Purged Ultimate Union
- Column 2: Agilent HP-5MS Ultra Inert, 15 m x 0.25 mm, 0.25 μ m 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

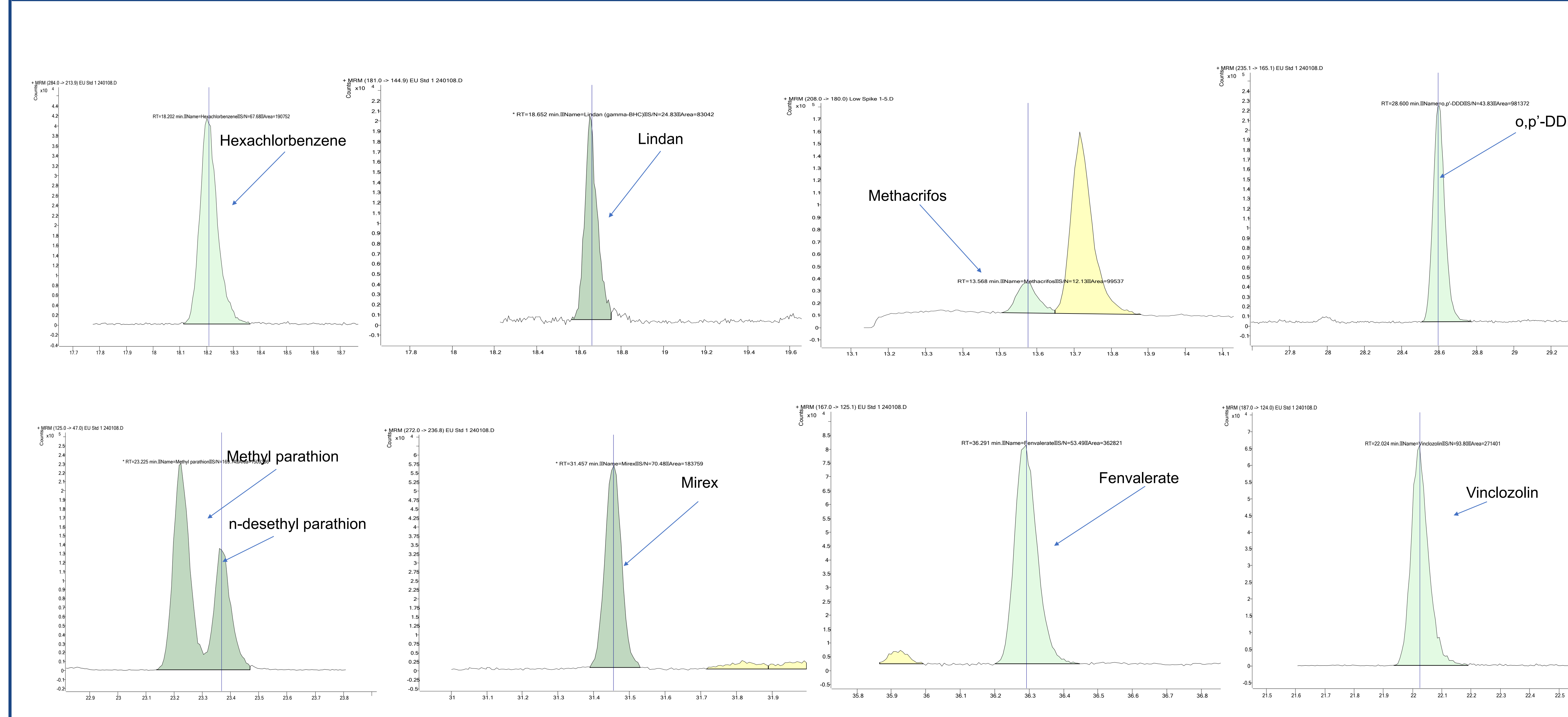
Sample Preparation

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) |
|---|---|---------------------|--------------------|------------------|
| 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 |
| Erimphos | 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 |
| t-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 |
| 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 |
| Chlorpyrifos-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 |
| Fenithion (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 |
| Pentachloranisole | 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.