A Fast, Robust Approach to Measuring Pesticides and Mycotoxins in Dry Cannabis Flower and Concentrates

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Trusted Answers

Combine triple quadrupole LC/MS and GC/MS analysis for up to 97 pesticides and five mycotoxins regulated by the U.S. and Canada

The recent and upcoming legalization of cannabis products in various North American regions requires highly regulated safety testing. These residual pesticide and mycotoxin tests require analytical systems that provide appropriate and productive coverage of regulatory requirements.

Advantages of a multiplatform approach

- Run more samples and increase lab profitability
- Reduce downtime and maintenance costs
- Decrease potential for errors through cross-validation
- Meet the current regulatory requirements of all jurisdictions, especially California and Canada
- Rapidly adapt to a changing regulatory environment

The key to successful pesticide residue analysis in cannabis products is understanding and controlling the background matrix. With a reduced matrix load in the final extract through solid phase extraction (SPE) and sample dilution, GC/MS/MS and LC/MS/MS methods are more sensitive, have fewer potential interferences, and require less instrument maintenance. Combined, these factors deliver a more efficient laboratory analysis by increasing sample throughput and reducing downtime due to maintenance.

Incorporating a multiplatform approach that includes GC/MS/MS and LC/MS/MS allows your laboratory to use the best and most sensitive instrument to quantitate each residual pesticide and meet the current regulatory requirements of any jurisdiction in North America. Conveniently, both mass spectral platforms use Agilent MassHunter software. Using two instrument platforms also allows the lab to orthogonally confirm and quantitate analytes amenable to both platforms. Dividing samples between two independent analytical instruments increases sample throughput by at least 2x compared to sequential independent methods on one instrument. Finally, multiple analytical platforms ensure that a laboratory is prepared to adapt to a fast-changing regulatory environment.

Eliminate the time required for method development

Quantitative evaluation of large numbers of pesticides and mycotoxins in cannabis matrices, as regulated by the different North American regions, is a challenging application. Agilent has developed complete pesticide and mycotoxin analytical workflows, designed for productivity and robustness on a day-to-day basis, meeting requirements in the United States and Canada.

Our workflows include:

- Sample preparation protocols for dry cannabis matrix and concentrates for both GC/MS and LC/MS platforms.
- Verified LC/MS/MS method parameters that are compatible with Agilent 1260 and 1290 Infinity II UHPLC sytems in tandem with Agilent 6470 and Agilent Ultivo quadrupole mass spectrometry systems. Sample-to-sample analysis cycle time: 12 minutes.
- Verified GC/MS/MS method parameters, including midcolumn backflush and JetClean self-cleaning ion source features, that are compatible with the Agilent 7890B GC system in tandem with the Agilent 7010 quadrupole mass spectrometry system. Sample-to-sample analysis cycle time for an extended list of analytes such as that required in Canada: 22 minutes.

For labs running a reduced GC/MS/MS analyte list (as in many U.S. jurisdictions), the analysis cycle times can be reduced by approximately 50 %, allowing for even higher sample throughput.

- Custom report templates or output formats to lab information systems.
- Optional consulting with a fully trained chemist at point of implementation for quick and successful system setup.

Sample preparation

- Simplified extraction technique increases throughput
- Higher dilution factors reduce matrix inferences
- Improved quantitative specificity and method robustness

Sample preparation and cleanup is the major aspect of our workflow. This allows your laboratory to reach the right balance between sensitivity, productivity, and robustness. We have recently simplified the sample prep procedure and increased throughput. Briefly, the sample prep consists of SPE cleanup of an acetonitrile extract from dried flower or concentrates using an Agilent SampliQ C18 EC 6 mL 500 mg SPE cartridge (p/n 5982-1365) followed by dilution in solvent prior to analysis.

LC/MS analysis of pesticide and mycotoxin extracts

LC/MS/MS analysis can be performed on a 1260 or 1290 Infinity II HPLC system in tandem with either a 6470 or Ultivo quadrupole mass spectrometry system. Injecting samples (250x total dilution) onto an Agilent InfinityLab Poroshell 120 Phenyl-Hexyl column (100 × 3 mm, 2.7 µm) using a nine-minute gradient and two-minute re-equilibration provides excellent analyte and matrix separation across the entire analysis. The sample-to-sample analysis cycle takes 12 minutes. Peak shapes are maintained by using an injector program during sampling, which inserts and mixes aqueous mobile phase on either side of the injected sample, effectively diluting the organic content to approximately 25 %. Pesticide and mycotoxin detection limits satisfy all current North American action levels.

Agilent CrossLab
From Insight to Outcome

Sample preparation | Separation | Analysis | Results | Services

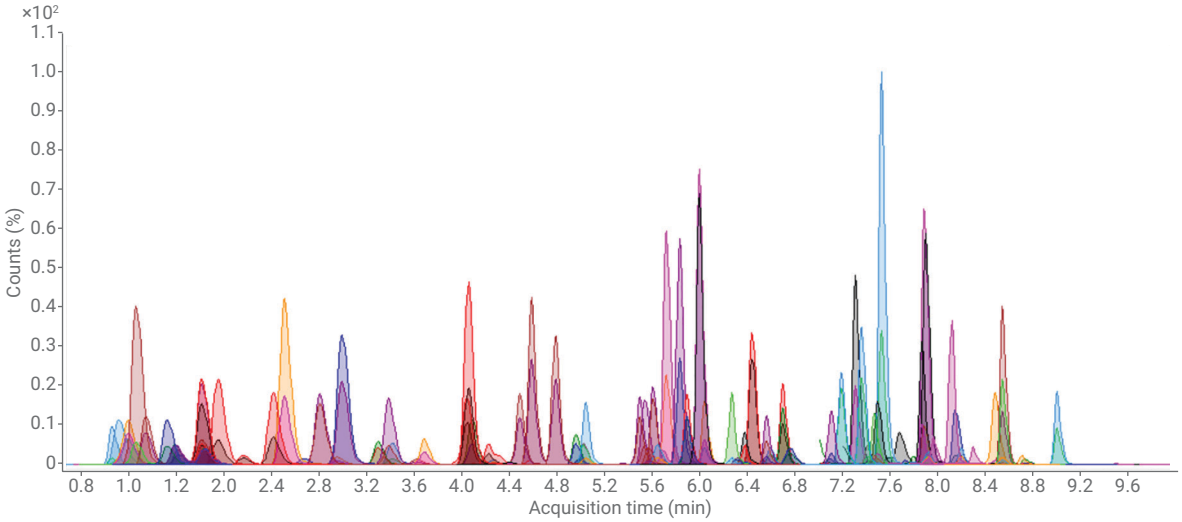


Figure 1. LC/MS/MS chromatogram of 89 pesticides and five mycotoxins analyzed in dry cannabis flower extracted matrix. The 94 analytes are (in elution order): daminozide, acephate, dinotefuran, oxamyl, methomyl, flonicamid, thiamethoxam, clothianidin, mevinphos, imidacloprid, dimethoate, acetamiprid, aldicarb, thiacloprid, dichlorvos, pirimicarb, propoxur, carbofuran, aflatoxin G2, carbaryl, thiophanate-methyl, imazalil, aflatoxin G1, dodemorph, aflatoxin B2, metalaxyl, cyantranilipole, azadirachtin, aflatoxin B1, spiroxamine, naled, fensulfothion, fludioxonil, chlorantraniliprole, methiocarb, paclobutrazol, fluopyram, boscalid, ethoprophos, myclobutanil, malathion, azoxystrobin, phosmet, methyl-parathion, cyprodinil, bifenazate, fipronil, tebufenozide, spirotetramat, tebuconazole, fenoxycarb, propiconazole, ochratoxin, diazinon, benzovindiflupyr, tetrachlorvinphos, kresoxim methyl, MGK-264, spinosad, chlorfenapyr, trifloxystrobin, buprofezin, spinetoram, allethrin, coumaphos, piperonyl butoxide, spiromesifen, etoxazole, abamectin, teflubenzuron, tetramethrin, pyrethrins, chlorpyrifos, methoprene, spirodiclofen, fenpyroximate, hexythiazox, iprodione, resmethrin, pyridaben, permethrin, cypermethrin, phenothrin, fenvalerate, deltamethrin, bifenthrin, etofenprox, cyfluthrin, and acequinocyl.

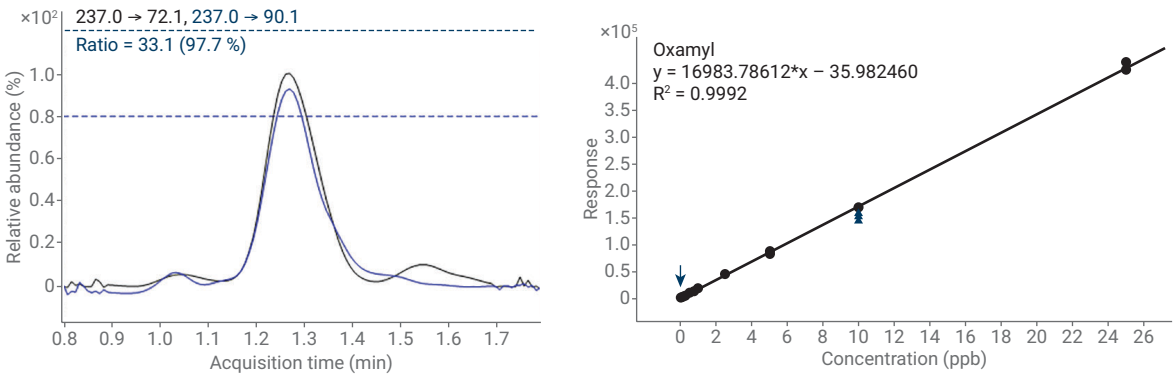


Figure 2. Typical calibration curves displaying linearity and sensitivity. Oxamyl, LC/MS/MS, lowest level on curve is 0.01 ppb, equivalent to 2.5 ppb from original plant matrix.

GC/MS analysis of pesticide extracts

While LC/MS/MS is the most common analytical platform for quantifying residual pesticides in cannabis, many pesticides are commonly, and more successfully, analyzed using GC/MS/MS with electron ionization.

Using GC/MS/MS for pesticides:

- Lowers the maintenance downtime for the LC/MS/MS system by allowing higher sample dilutions
- Increases lab productivity by at least 2x compared to sequentially independent methods on one instrument
- Facilitates critical cross-validation of many pesticides for confident analyte identification and reporting
- Allows the testing laboratory to rapidly adapt to a fast-changing regulatory environment

GC/MS/MS analysis can be performed on a 7890 GC system in tandem with a 7010 quadrupole MS system with a high efficiency source (HES).The GC system is configured with a multimode inlet (MMI), and uses Agilent Purged Ultimate Union (PUU) capillary flow technology to allow for column backflushing. The MMI is capable of fast temperature ramping, allowing for a cool inlet injection. Combining the cool inlet condition with a splitless injection helps reduce degradation of thermally labile compounds in the GC inlet. To improve peak shape for more-polar analytes, the PUU is placed at the midpoint of two Agilent analytical columns of different stationary phases (column 1: DB-35ms, 15 m × 0.25 mm, 0.25 µm df; column 2: HP-5ms Ultra Inert, 15 m × 0.25 mm, 0.25 µm df). The backflush is configured to begin post run as determined by the retention time of the last compound exiting the PUU, and designed to flush nonvolatile compounds from the injection port. Injecting samples with a 125x total dilution onto this GC/MS/MS system results in a sample-to-sample analysis cycle of 22 minutes, and provides excellent analyte and matrix separation across the entire analysis. For labs running a reduced GC/MS/MS analyte list (as found in many U.S. jurisdictions), the analysis cycle can be shortened by approximately 50 %, allowing even higher sample throughput. Pesticide analyte detection limits satisfy all current North American action levels.

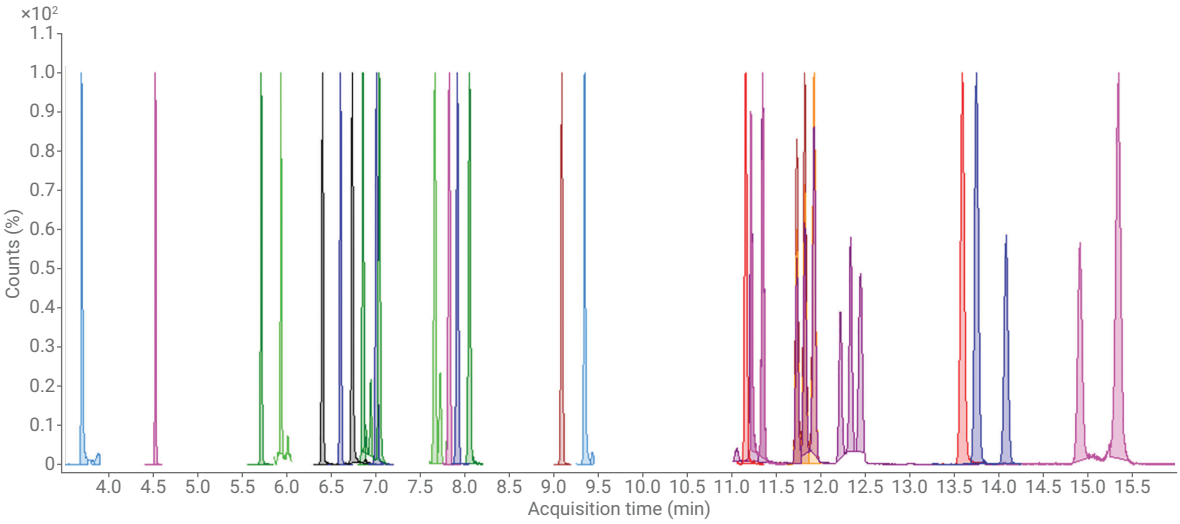


Figure 3. GC/MS/MS chromatogram of 21 pesticides analyzed in dry cannabis flower extracted matrix. The 21 analytes, some with multiple resolved isomers, are (in elution order): novaluron, etridiazole, quintozene (PCNB), kinoprene, methyl-parathion, chlorpyrifos, MGK-264 I, fenthion, MGK-264 II, pyrethrins, chlordane-*cis*, chlordane-*trans*, endosulfan-*alpha*, captan, endosulfan-*beta*, bifenthrin, cyfluthrins, cypermethrins, boscalid, fenvalerate, and deltamethrins.

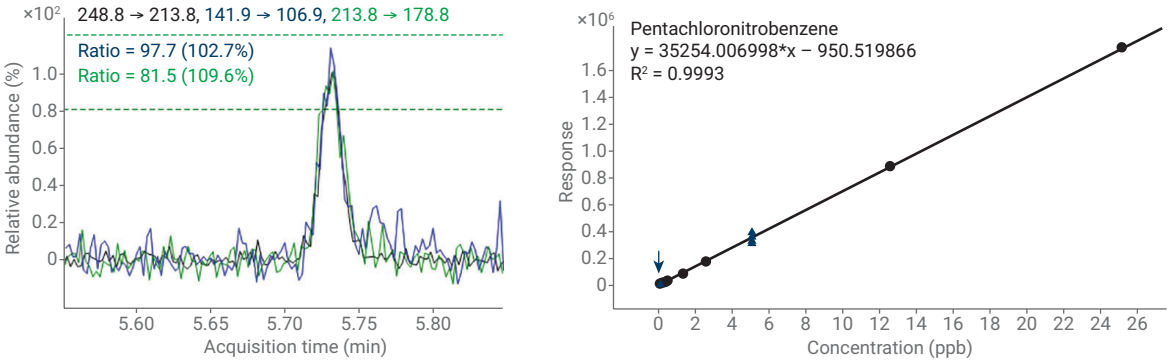


Figure 4. This figure depicts typical calibration curves displaying linearity and sensitivity. Pentachloronitrobenzene (PCNB, quintozene), GC/MS/MS, lower level on curve is 0.05 ppb, equivalent to 6.25 ppb in matrix.

Review and reporting

Agilent LC/MS/MS and GC/MS/MS instruments use the same MassHunter Quantitation software for data review and reporting, optimizing lab productivity and operator ease-of-use. To enable review by exception, MassHunter quantitation software allows quick and efficient batch processing using outlier settings per analyte. This approach automatically flags any sample or individual analyte, and draws the reviewer's attention to anything that may not be within designated limits. Figure 5 illustrates these flags; red indicates that the value is above accepted outlier limits.

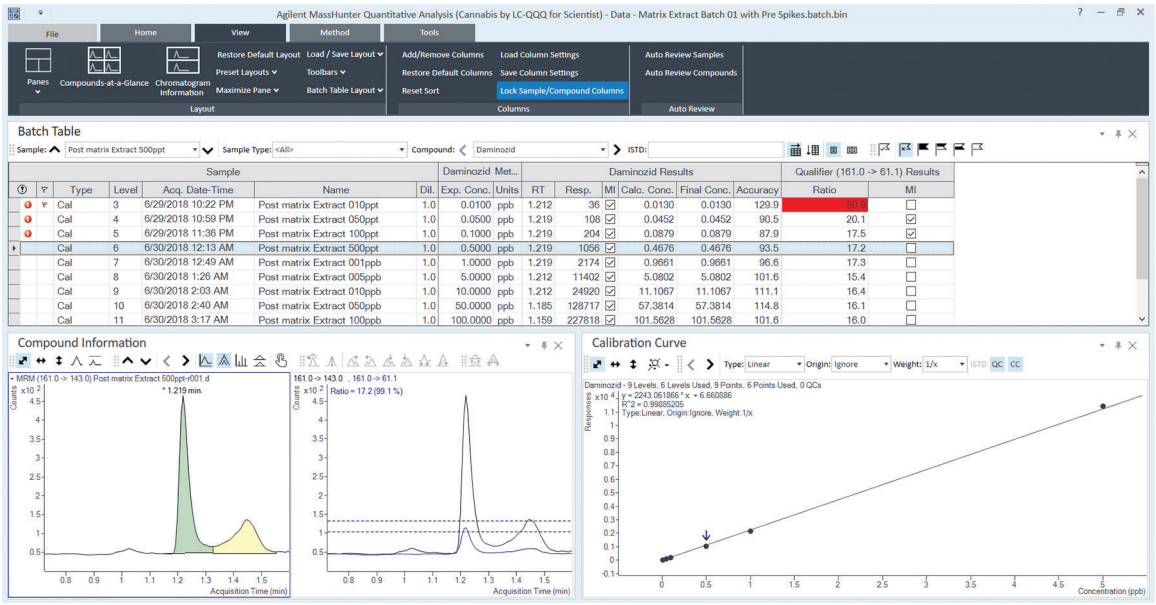


Figure 5. Example of the Cannabis Scientist (GUI) for LC/MS/MS data analysis.

To address an instrument operator's method development or compliance permissions and experience, MassHunter Quantitation graphical user interface (GUI) choices are also available. There are two main LC/MS or GC/MS Cannabis GUI choices. The Scientist GUI has complete method setup, batch review, and reporting capabilities. The Analyst GUI has a simplified and uncluttered interface, for use in the daily production environment, with batch review and report generation only allowed from predefined data review criteria, methods, and templates. By using these different GUI choices, a laboratory can more easily control how data are processed and reported. Custom report templates specifically designed for the cannabis analysis requirements of each geographic region are also available as an integral element of the MassHunter Quantitation software.

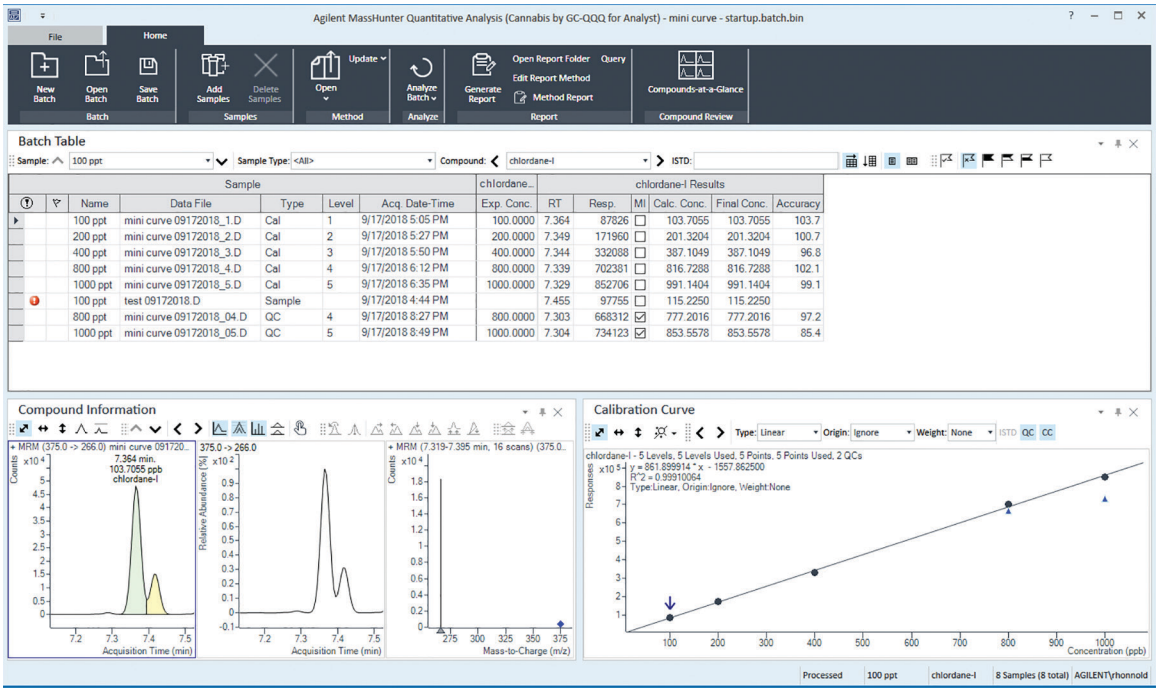
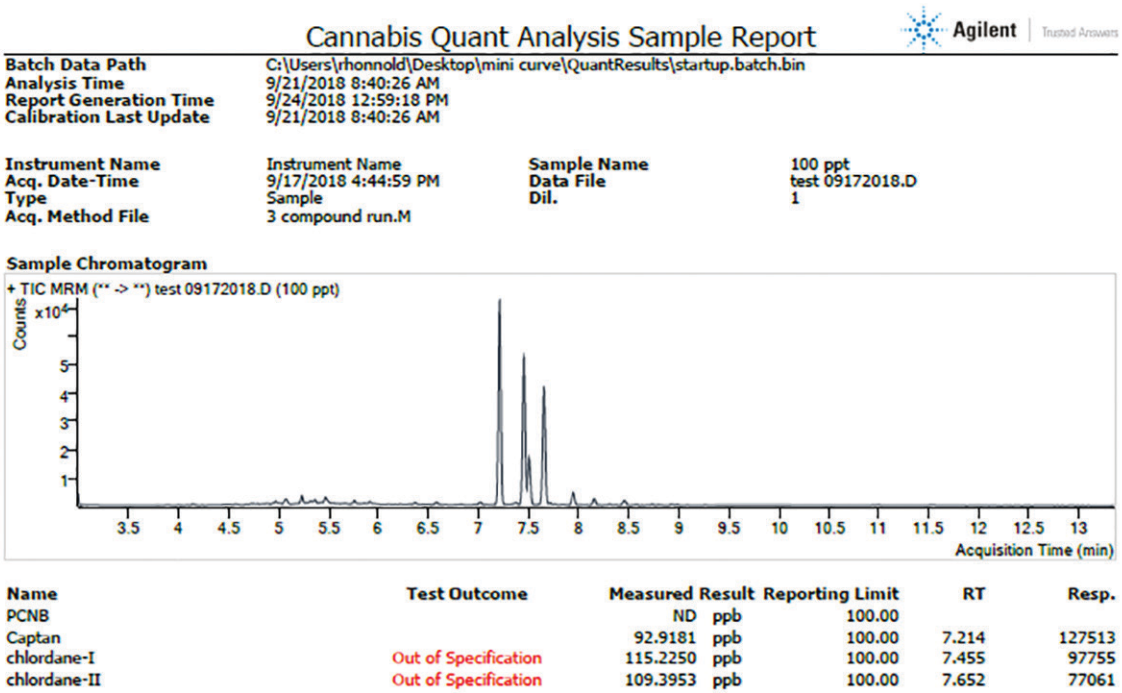


Figure 6. Example of the Cannabis Analyst (GUI) for GC/MS/MS data analysis.



Cannabis Quant Analysis Sample Report

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Batch Data Path	C:\Users\rhonnold\Desktop\mini curve\QuantResults\startup.batch.bin
Analysis Time	9/21/2018 8:40:26 AM
Report Generation Time	9/24/2018 12:59:18 PM
Calibration Last Update	9/21/2018 8:40:26 AM

Instrument Name	Instrument Name	**Sample Name**	100 ppt
Acq. Date-Time	9/17/2018 4:44:59 PM	**Data File**	test 09172018.D
Type	Sample	**Dil.**	1
Acq. Method File	3 compound run.M		

Sample Chromatogram

Name	Test Outcome	Measured Result		Reporting Limit	RT	Resp.
PCNB		ND	ppb	100.00		
Captan		92.9181	ppb	100.00	7.214	127513
chlordane-I	Out of Specification	115.2250	ppb	100.00	7.455	97755
chlordane-II	Out of Specification	109.3953	ppb	100.00	7.652	77061

Figure 7. Example Cannabis Sample Report for GC/MS/MS data.

Contact your local Agilent representative for more specific details and scientific methodologies representative of your North American region.

Agilent products and solutions are intended to be used for cannabis quality control and safety testing in laboratories where such use is permitted under state/country law.

For more information on cannabis testing visit:

www.agilent.com/en/promotions/cannabis

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