Quick and Real-Time Determination of Potency in Cannabis Extracts using FTIR Spectroscopy

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Agilent
Trusted Answers

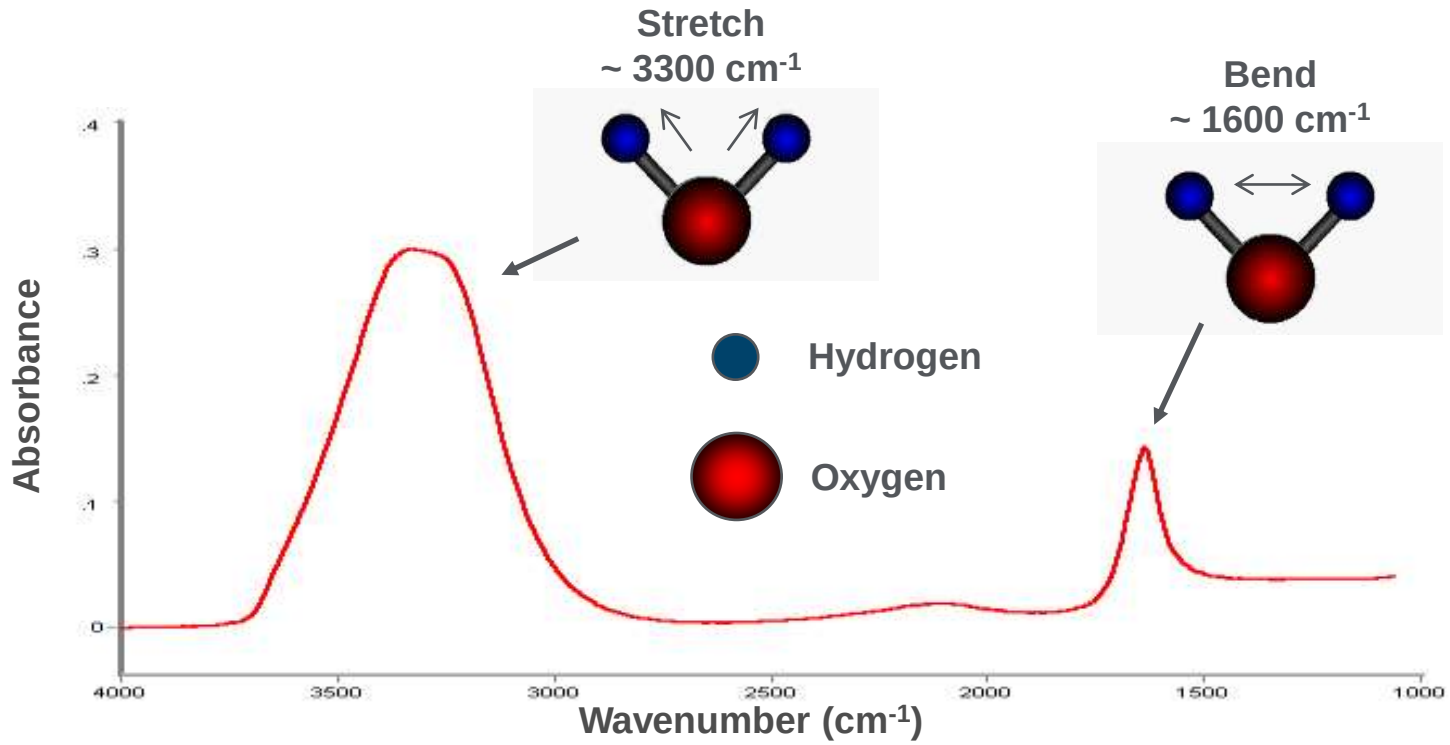
Agilent products and solutions are intended to be used for cannabis quality control and safety testing in laboratories where such use is permitted under state/country law.

Outline

1. FTIR Introduction
2. Cannabis Overview
3. Agilent Testing Capabilities
4. FTIR positioning in the process and production arena
5. Sampling considerations
6. Final product testing
7. Summary

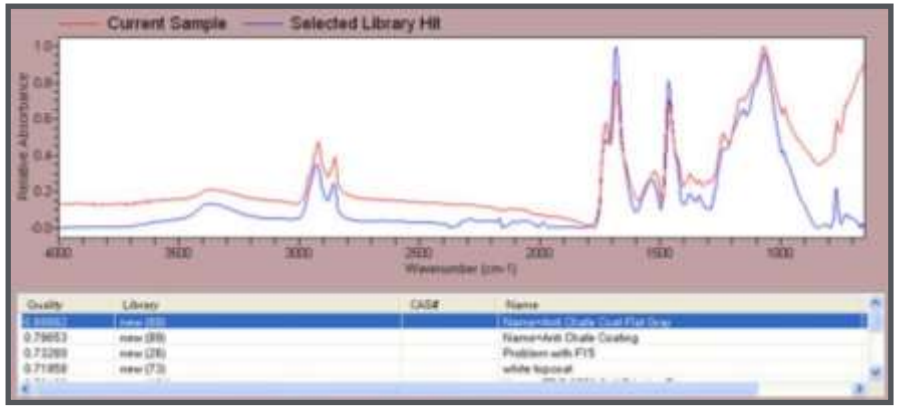
How does Infrared Spectroscopy work?

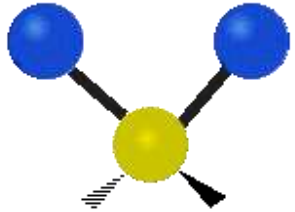
- Infrared spectroscopy is the study of how infrared light (heat) is *absorbed* by the bonds between the atoms of a *molecule.*
- And that pattern is unique for every molecule resulting in a *'spectral fingerprint'*



IR Spectroscopy Provides

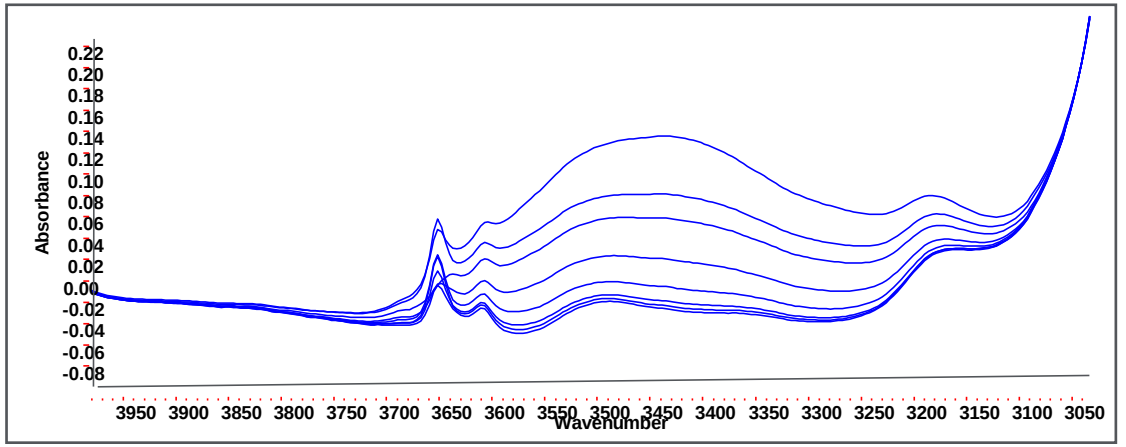
Each structural characteristic of a molecule has a unique vibrational frequency that can identify the functional group present and generates the unique spectrum that can be the source of spectral identification through a library search or quantitative analysis by univariate or multivariate techniques.



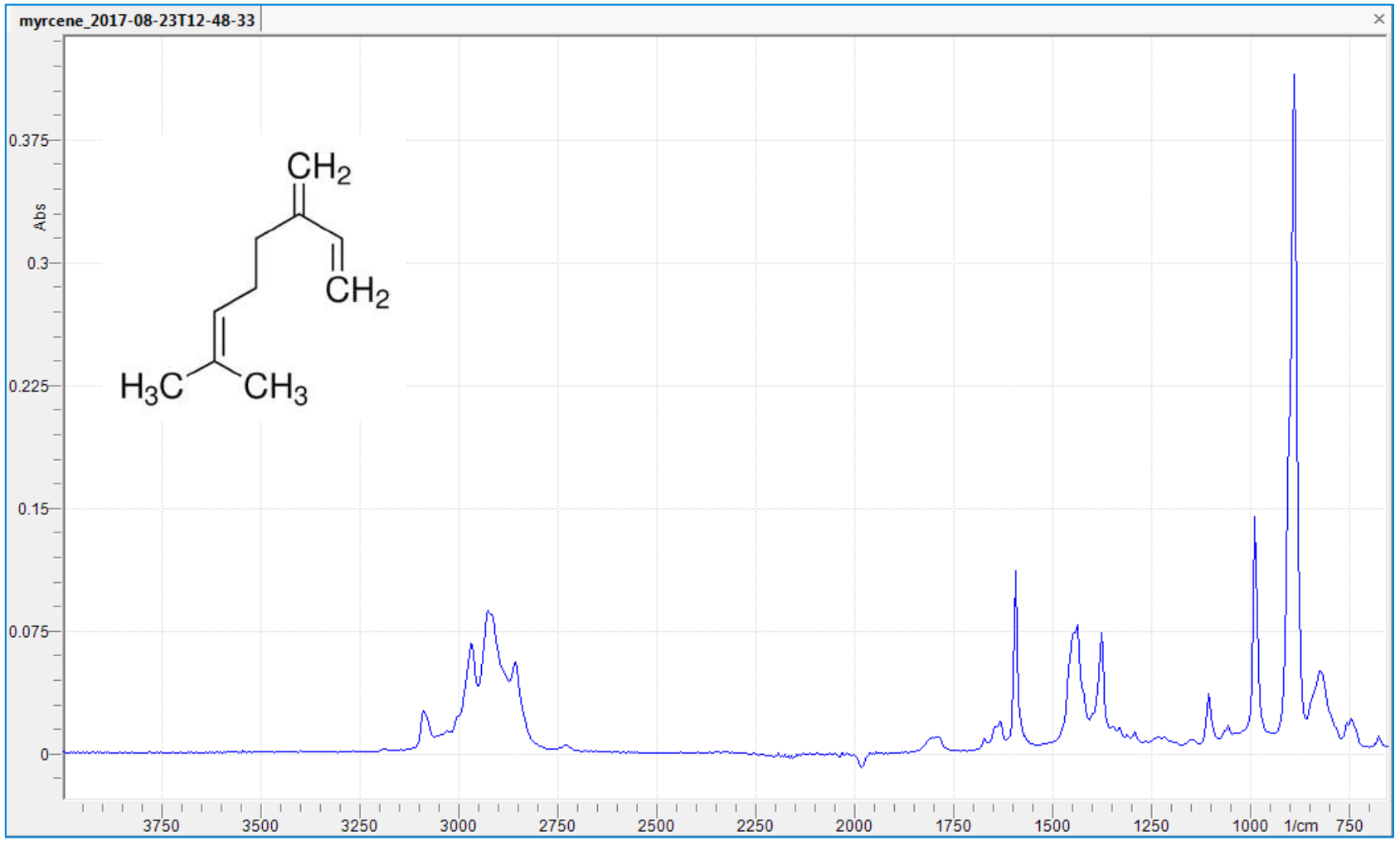


Quantitative analysis of sample components

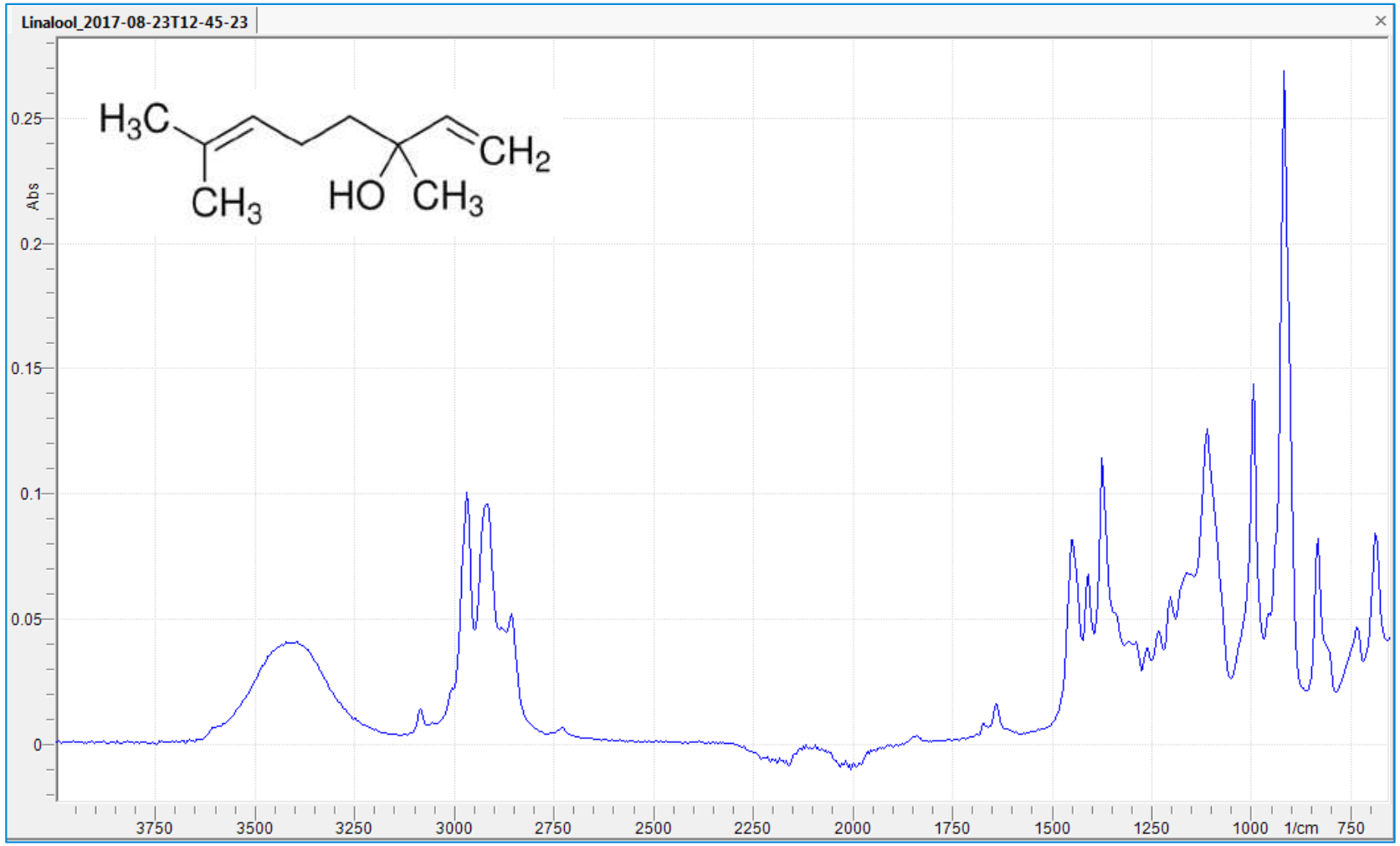
Spectral Identification of Unknowns



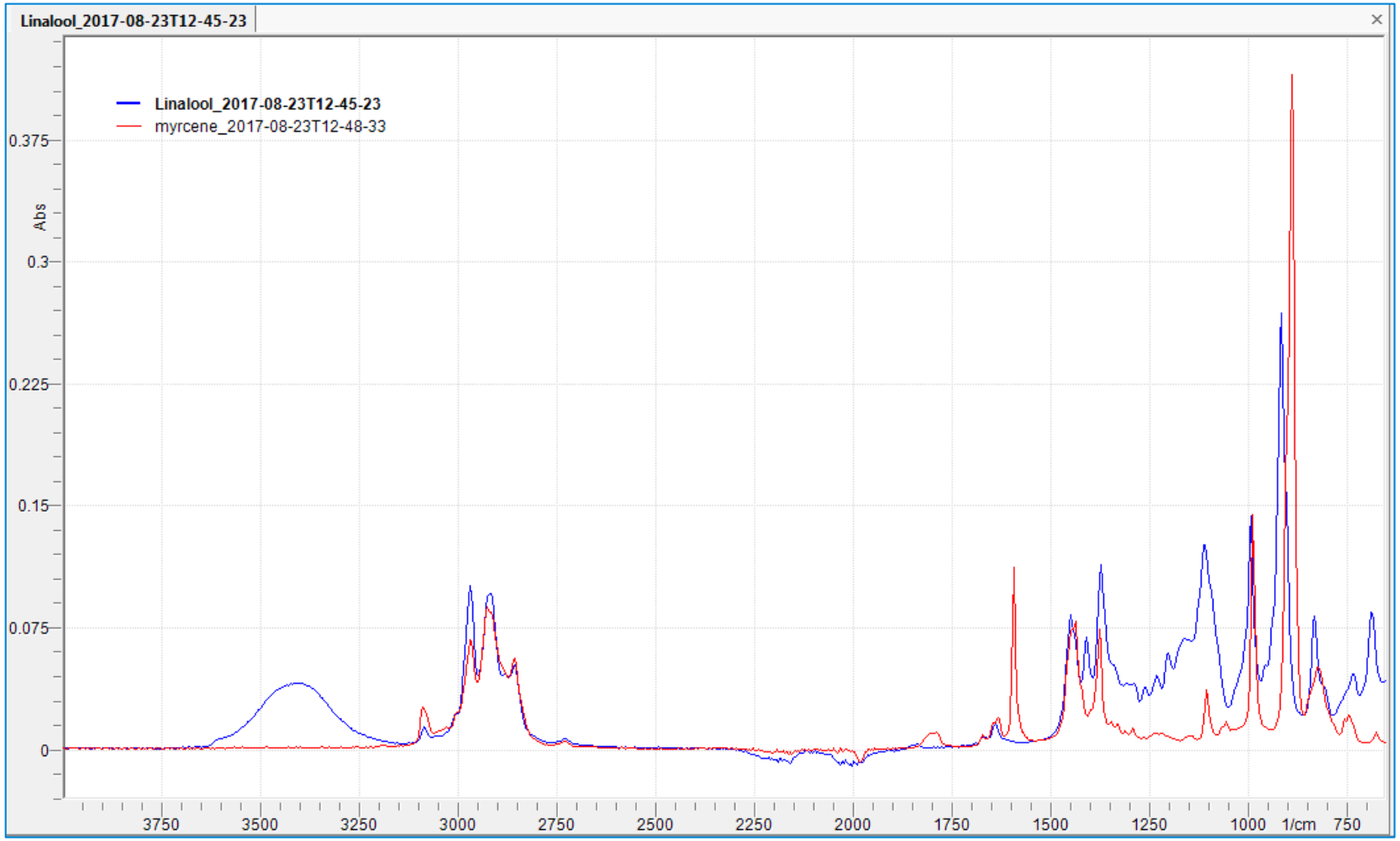
Terpenes: Myrcene



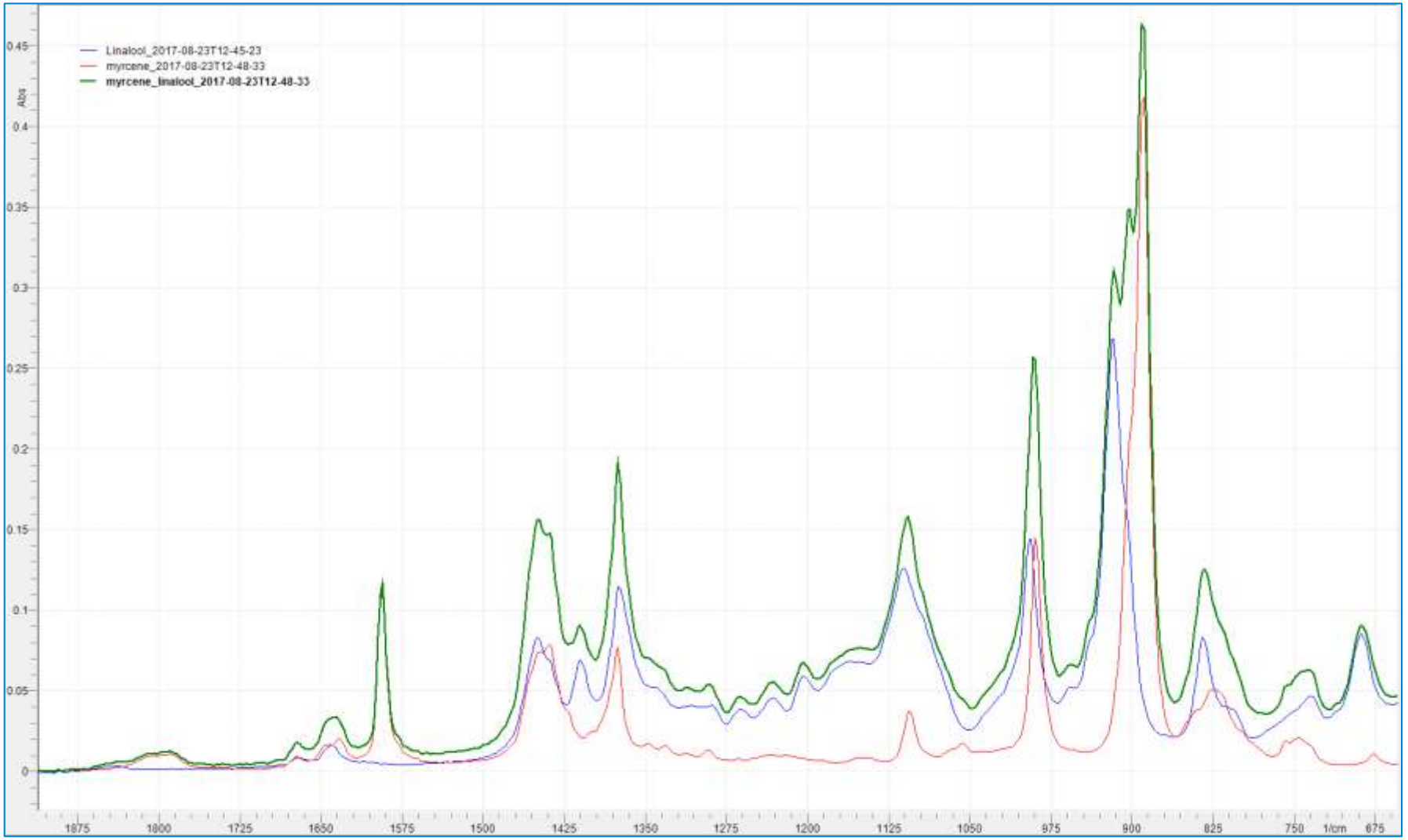
Terpenes: Linalool



Terpenes: Linalool and Myrcene Spectra Overlayed

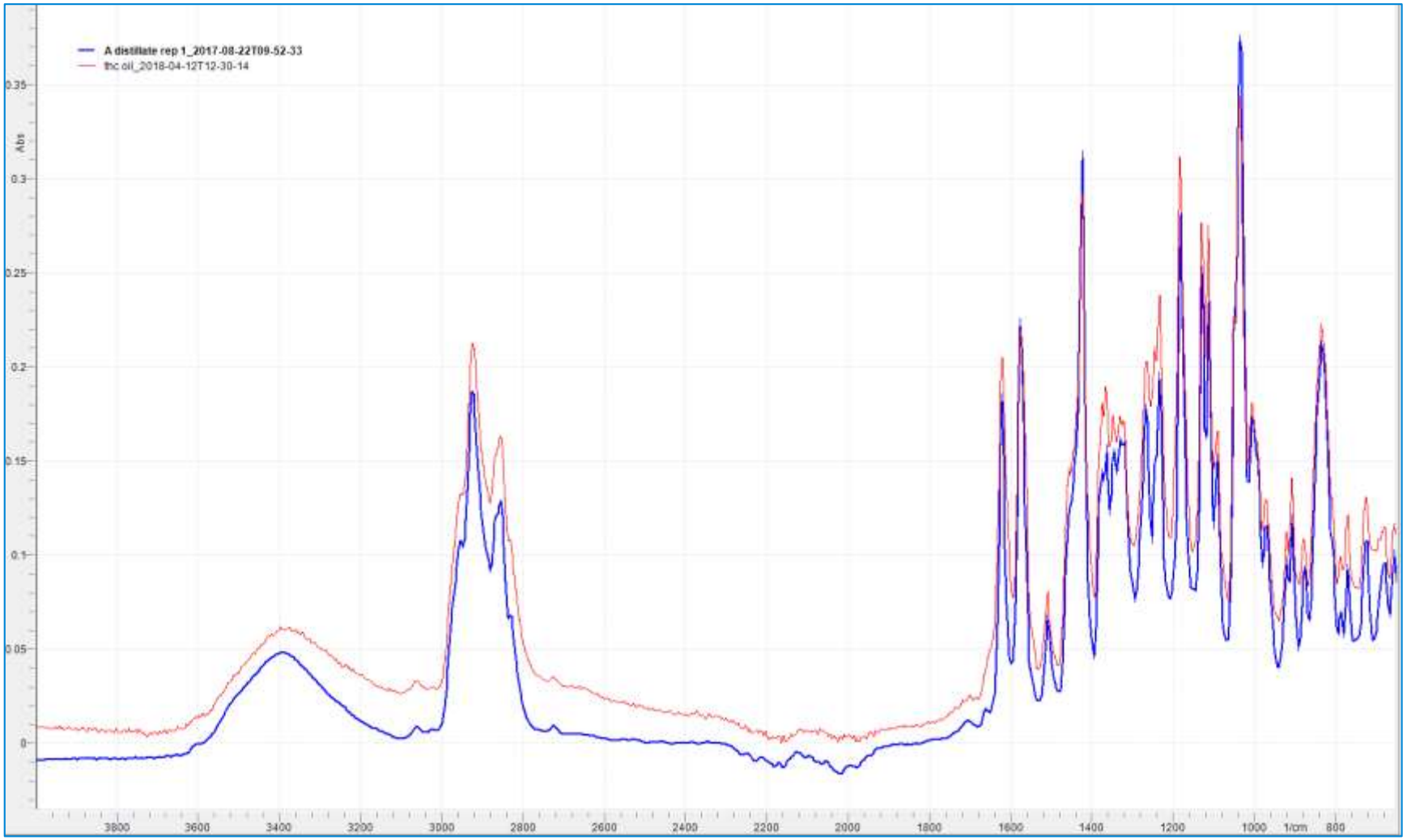


Linalool and Myrcene Spectra Added Then Overlayed



Spectrum of Wax

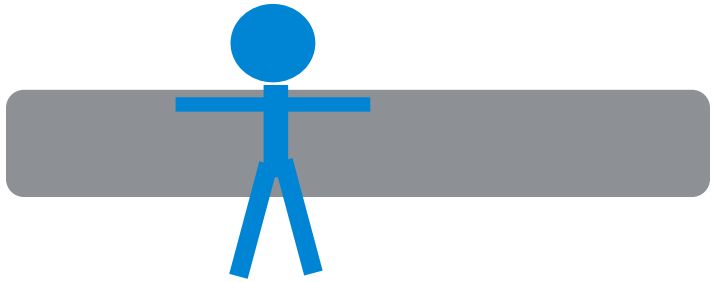
Comparison of CO2 Extraction Samples -- from Different Facilities: High THC and Terpenes



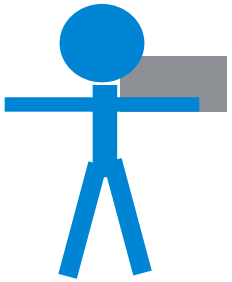
Instrument Evolution

Spectroscopy Measurements Continue to Move Directly to the Sample

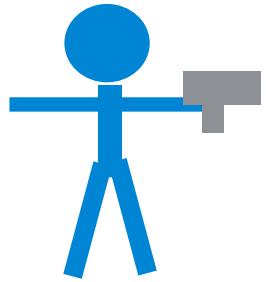
Laboratory (1970's)
120 kg



Portable (2008)
6.8 kg



Handheld (Today)
2 kg



The Smallest, Fully Functional FTIR Bench Spectrometer

- Cary 630 with 1 bounce Diamond ATR accessory is shown here
- Full spectral range (4000 – 650 cm^{-1})
- Internal DTGS detector
- MicroLab Method Driven Software

Cary 630 Sampling Flexibility --- for any sample

DialPath

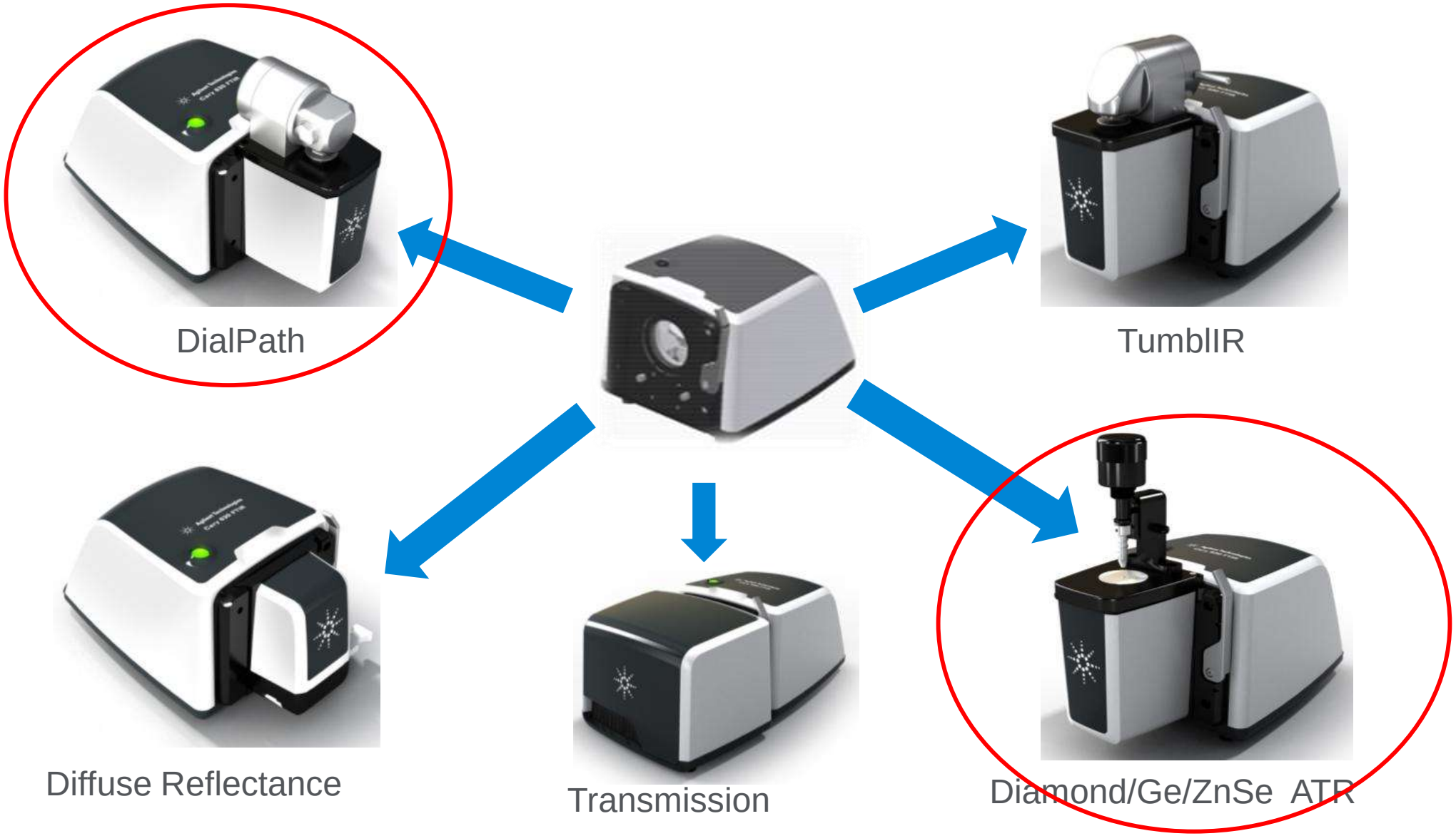
TumblIR

Diffuse Reflectance

Transmission

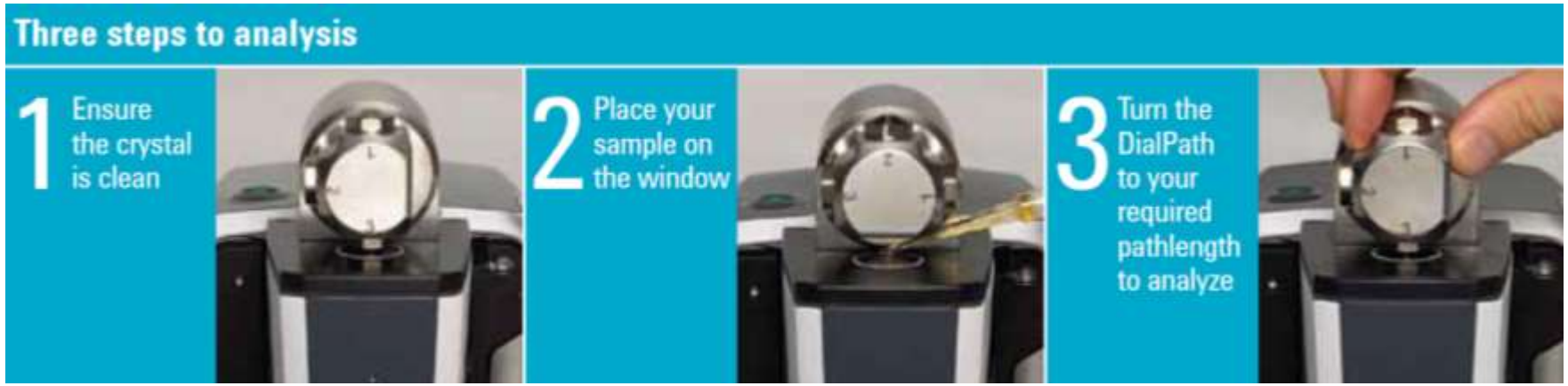
Diamond/Ge/ZnSe ATR

Cary 630 Sampling Flexibility --- for any sample



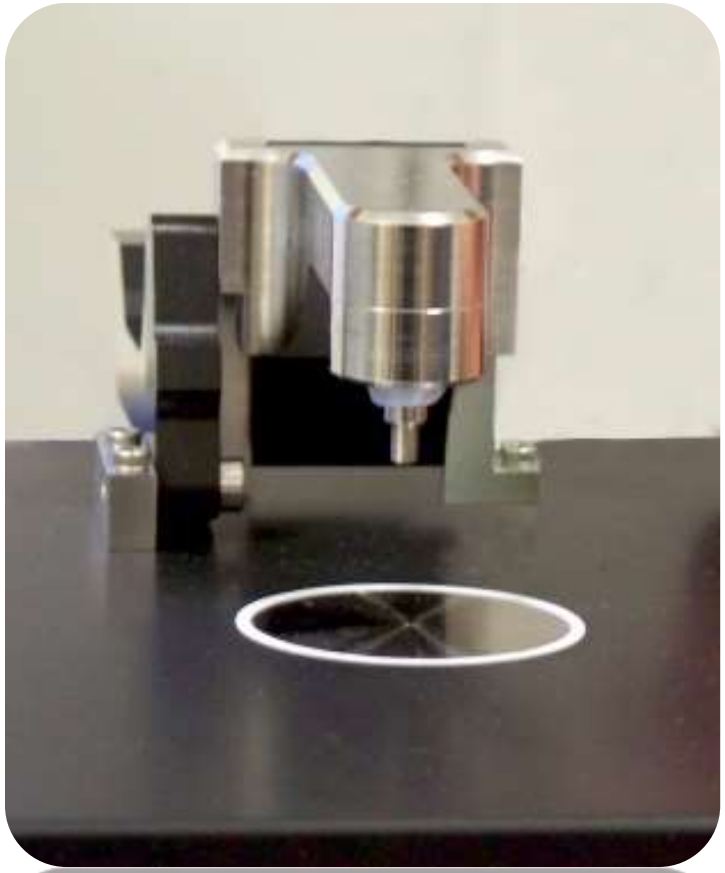
INNOVATION: Cary 630 with DialPath Sampling Technology

- Agilent-exclusive DialPath technology features an “open cell” transmission design for qualitative and quantitative FTIR analyses of liquids.
- As easy for liquid measurement as using ATR, and with the added benefit of variable and extended pathlength
- Where ATR pathlengths are typically less than 10 um, the Dialpath can be set from 25 um to 1000 um, allowing for a lower concentration range to be measured and quantified.

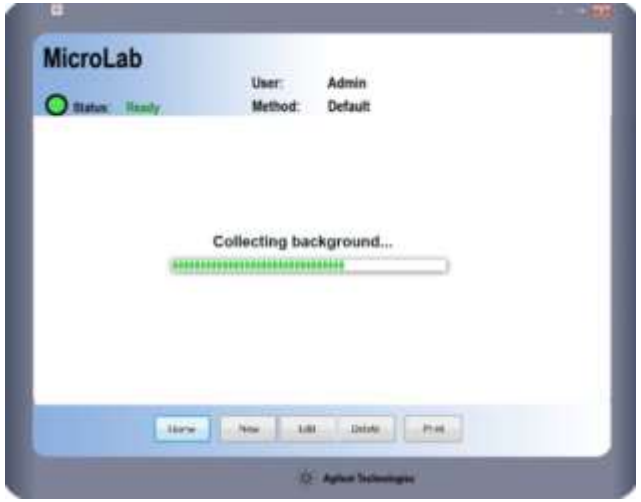


FTIR ATR Interface

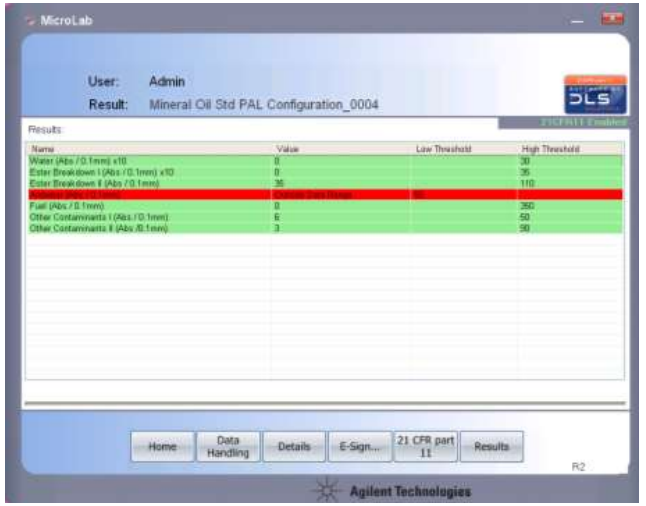
- Simple, easy to use
  - <2mg sample placed on sensor,
  - pressure device ensures contact,
  - answer provided in <30 secs.
- Short path length ~2 µm
  - Library match, product identification
- Uses Diamond sensor
  - Chemical and scratch resistant
  - Internal reflection



Routine FTIR with flexible, simple to use (intuitive) software

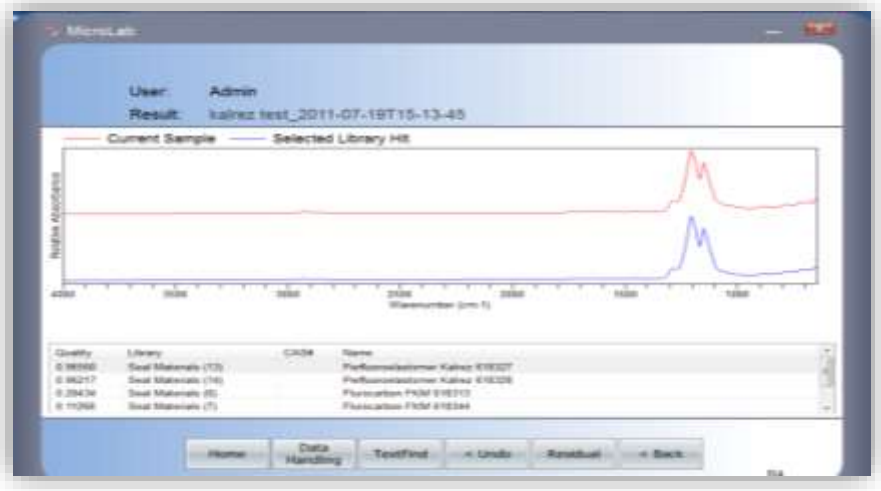


Software guides the user through the selected method. Also recognizes the correct sampling interface to guide proper sampling techniques and cleaning.



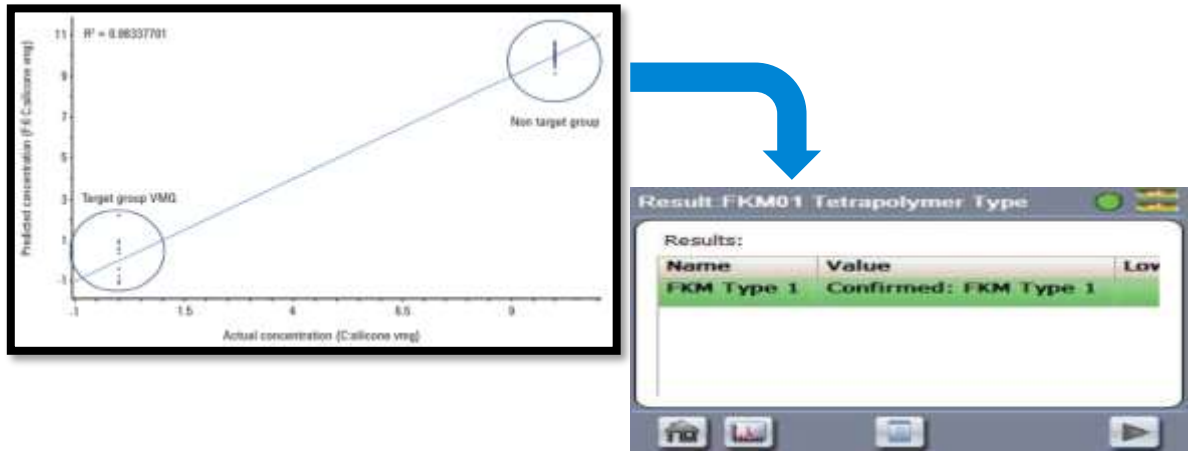
Data Analysis Methods

Identification
(Traditional)



- Easy to identify coatings with a simple library search
  - Good to total unknowns
  - Sensitivity ~ 5 – 10 % variation
  - Quality control and first article inspection

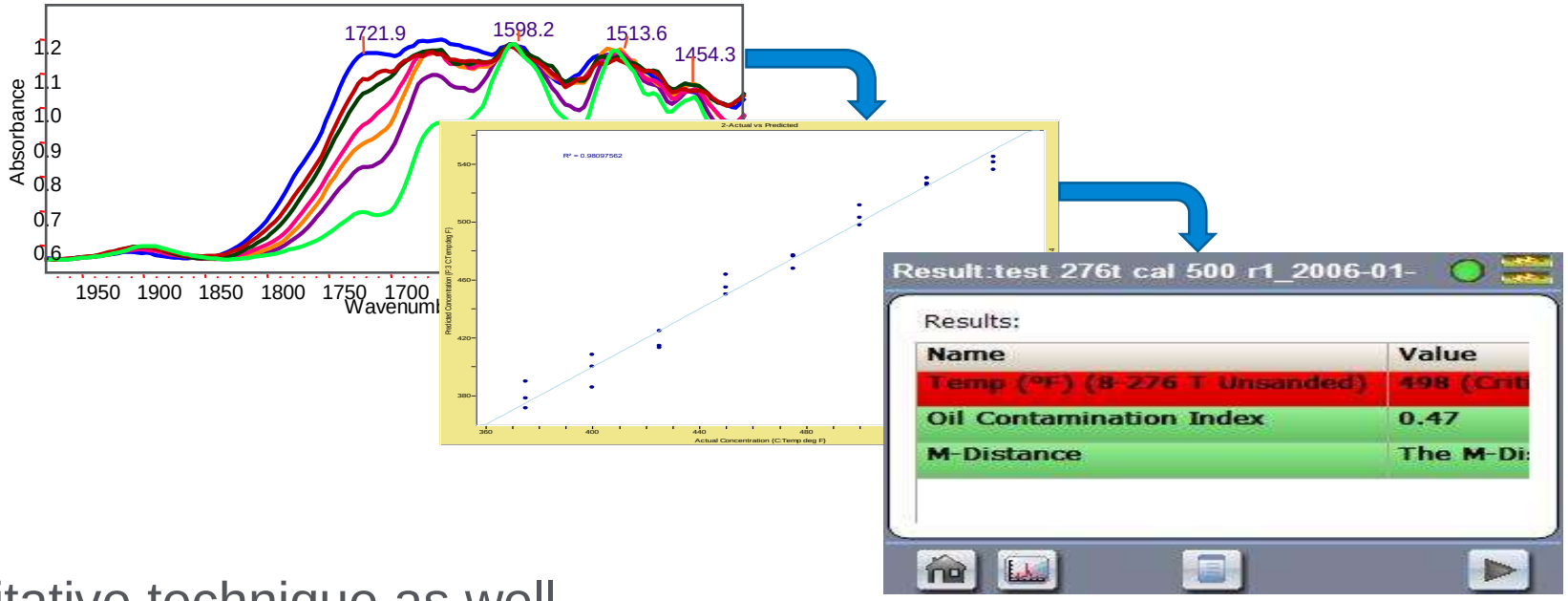
Classification
(Advanced)



- Discriminant analysis allows statistical separation of chemically relevant groups
- Turns the analysis into a yes / no question
  - Sensitivity ~0.5 – 5 % variation
  - Classify specific formulation within a category

Data Analysis Methods

Quantitation (Advanced)

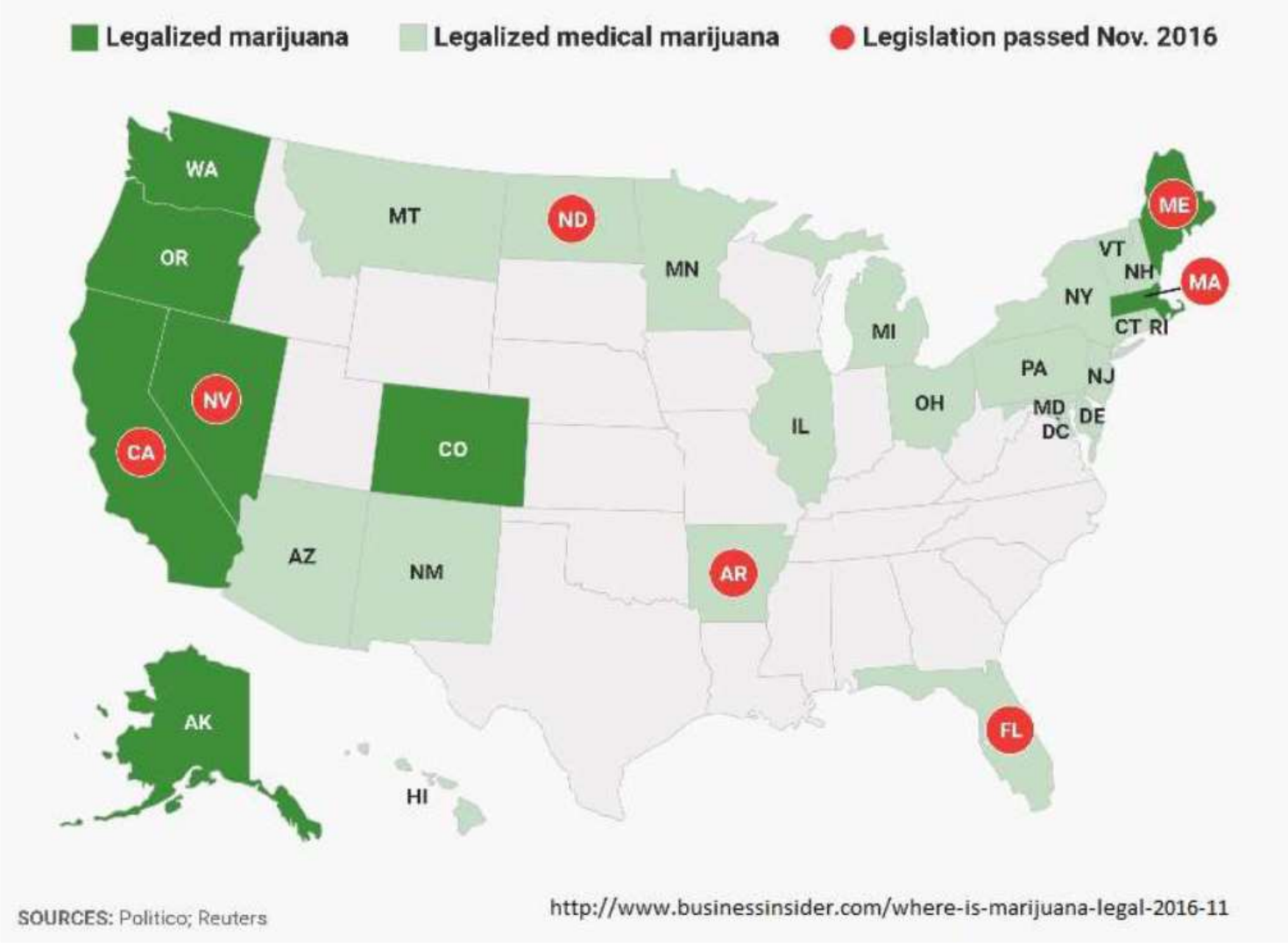


- FTIR is a quantitative technique as well
- Spectra can be correlated to quantitative chemical change in the samples.
- Useful for variety of applications
  - Weathering, cure, mix ratio, surface preparation

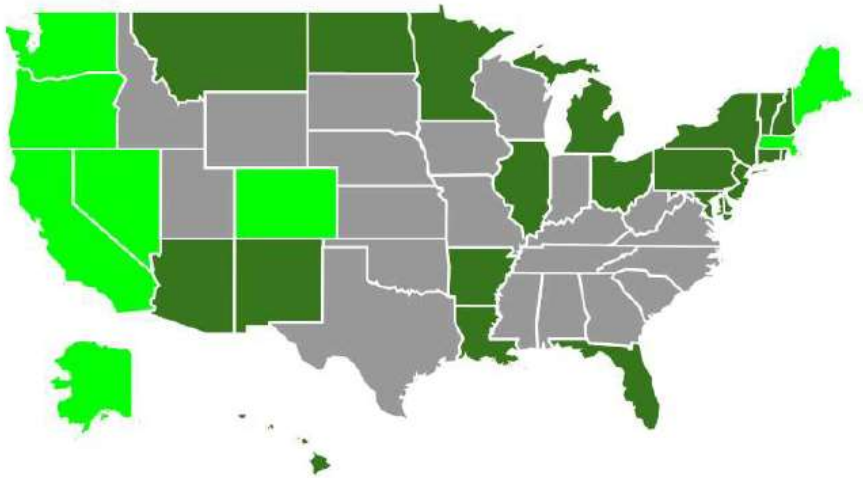
The Complicated World of Cannabis

- Legal Situation
  - Federal
  - State
- Classification
  - Plant
  - Food
  - Drug
- Testing Protocols
  - Regulatory
  - Research

States where Marijuana is legalized



More Than Just the US in North America



Mexico legalized medicinal marijuana in June, 2017
Canada expected to fully legalize in 2017

Analytical Challenges

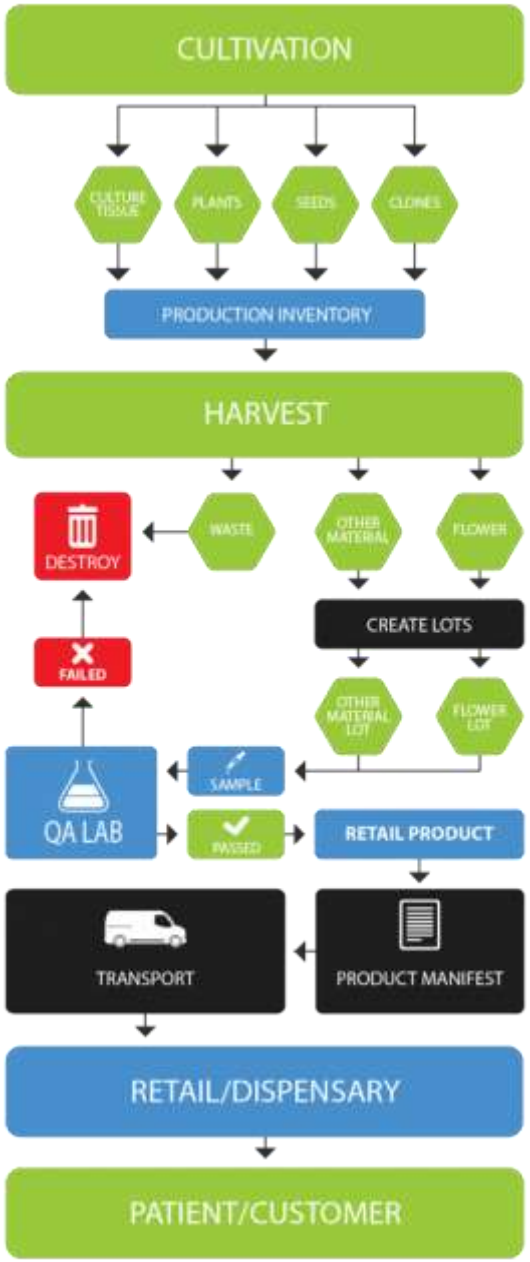
Matrix

- Raw plant material
- Extracts
- Food
  - Edibles

Seed to Sale (1): Cannabis is a Complex Matrix needing a full range Analytical Solutions

1. Species of Interest -- THCs and CBDs
2. Metals -- in Soil
3. Pesticides
4. Molds

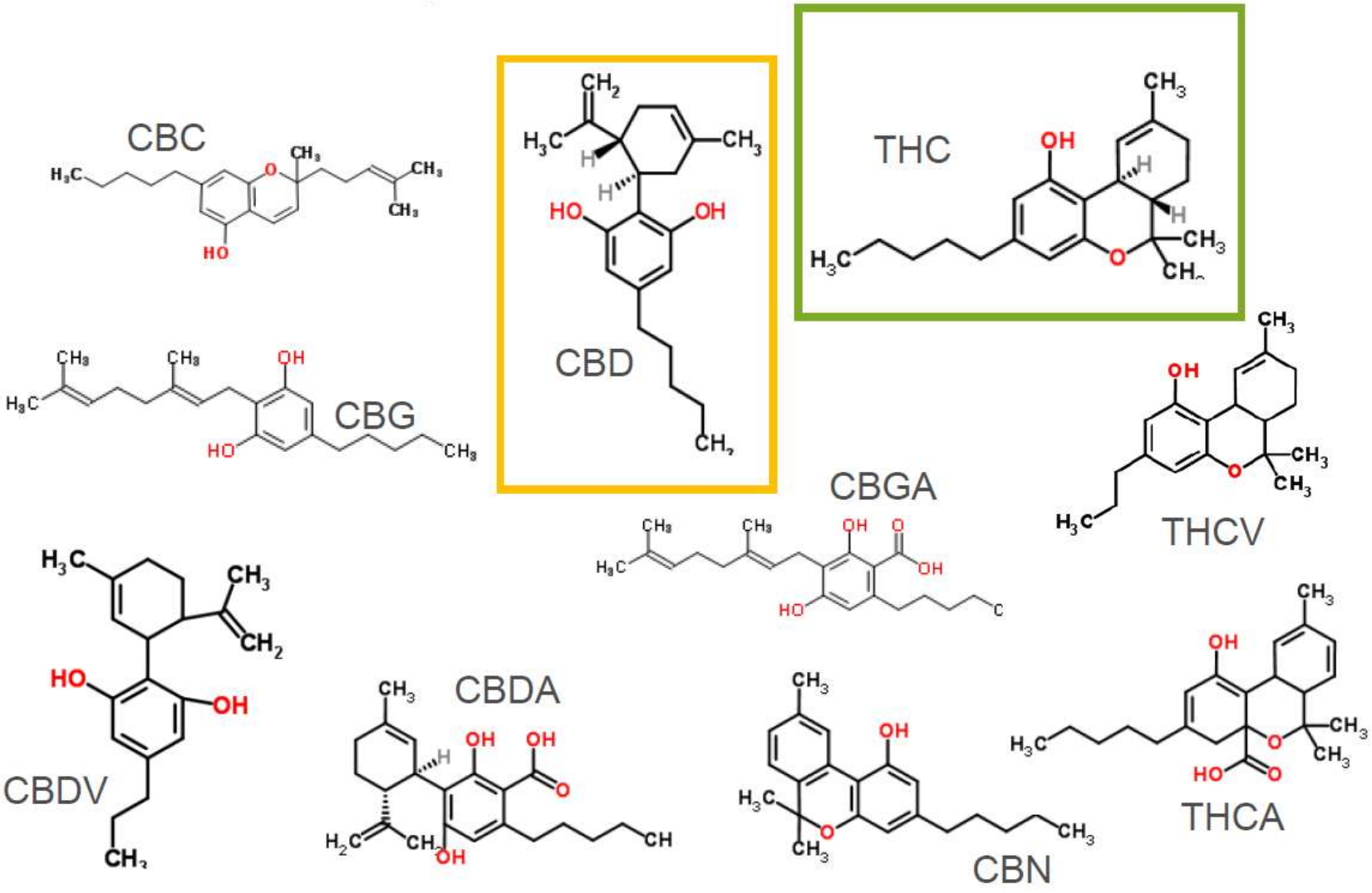
Agilent has a full complement of Instrumentation to provide answers in each testing area of need.



Testing

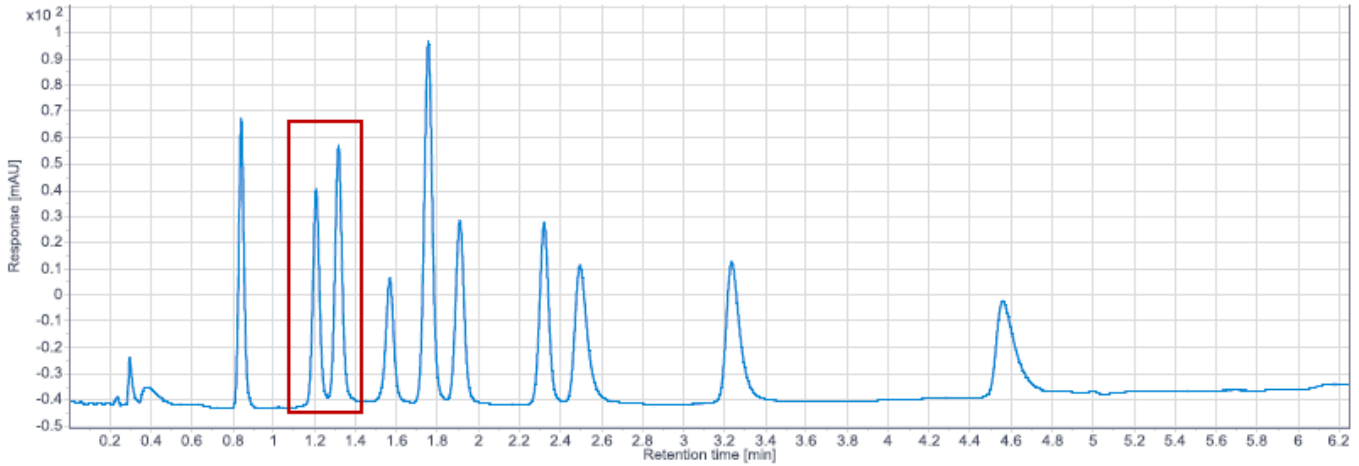
- All methods should be consistent with FDA, ELAP, USP, and EPA guidelines.
- Unfortunately, all states are not yet uniform in testing requirements.
- It's not enough to know that the product has been tested; the science behind it must be reliable.
- For analytical testing to be reliable, established methods must be used and available for scientific scrutiny.
- Detailed testing procedures should be available.

Chemical Structures of Top 10 Cannabinoids



http://herb.co/2016/02/06/top-10-cannabinoids/

Fast HPLC Analysis of cannabinoids --10 Compounds in 5 minutes



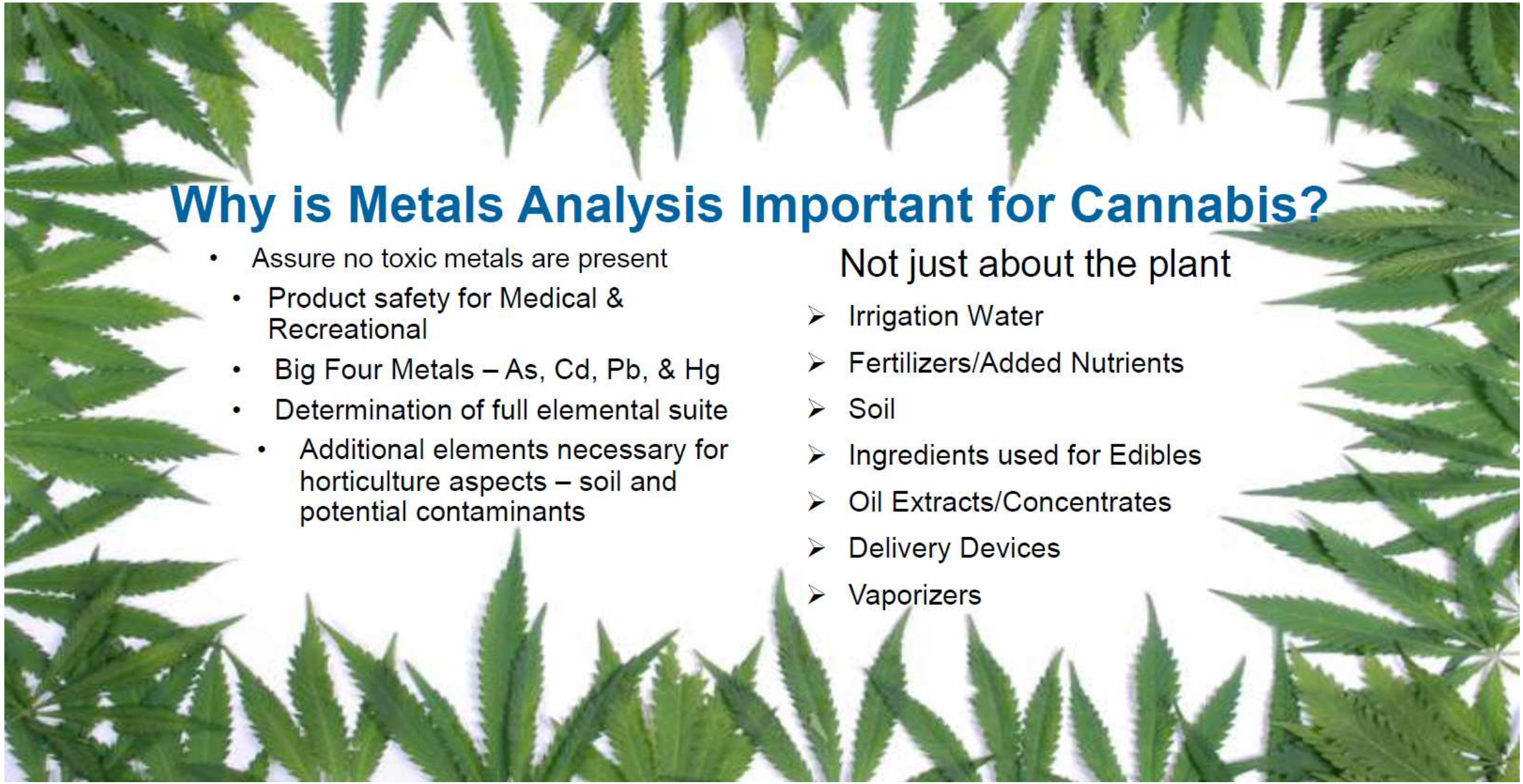
HPLC Conditions for short method (5 minutes)

Agilent 1290 Infinity II UHPLC series Quaternary Pump, Multisampler with wash , Multi Column Themostat, DAD

Column: Zorbax Bonus RP 2.1 x 50 mm, 1.8 µm or Poroshell 3.0 x50 2.7um

Column temperature:	50°C
Injection volume:	0.05 µL
Autosampler temp:	23 °C
Needle wash:	3.5 s Flush Port (25:25:50) (H_2O:IPA:MeOH)
DAD-UV	254 nm
Mobile phase:	A = Water **Binary pump - A is buffer** B= Methanol C= 0.1% CH_2O_2 + 2.2 ml 5M NH_4formate in H_2O
Flow rate:	0.5 mL/min
Gradient:	Time (min) %B %C 0.0 72 5 6.25 95 5
Stop time:	5.00 min.
Post time:	1.0 min.
Overall run time	6.0 minutes (incl. re-equilibration)

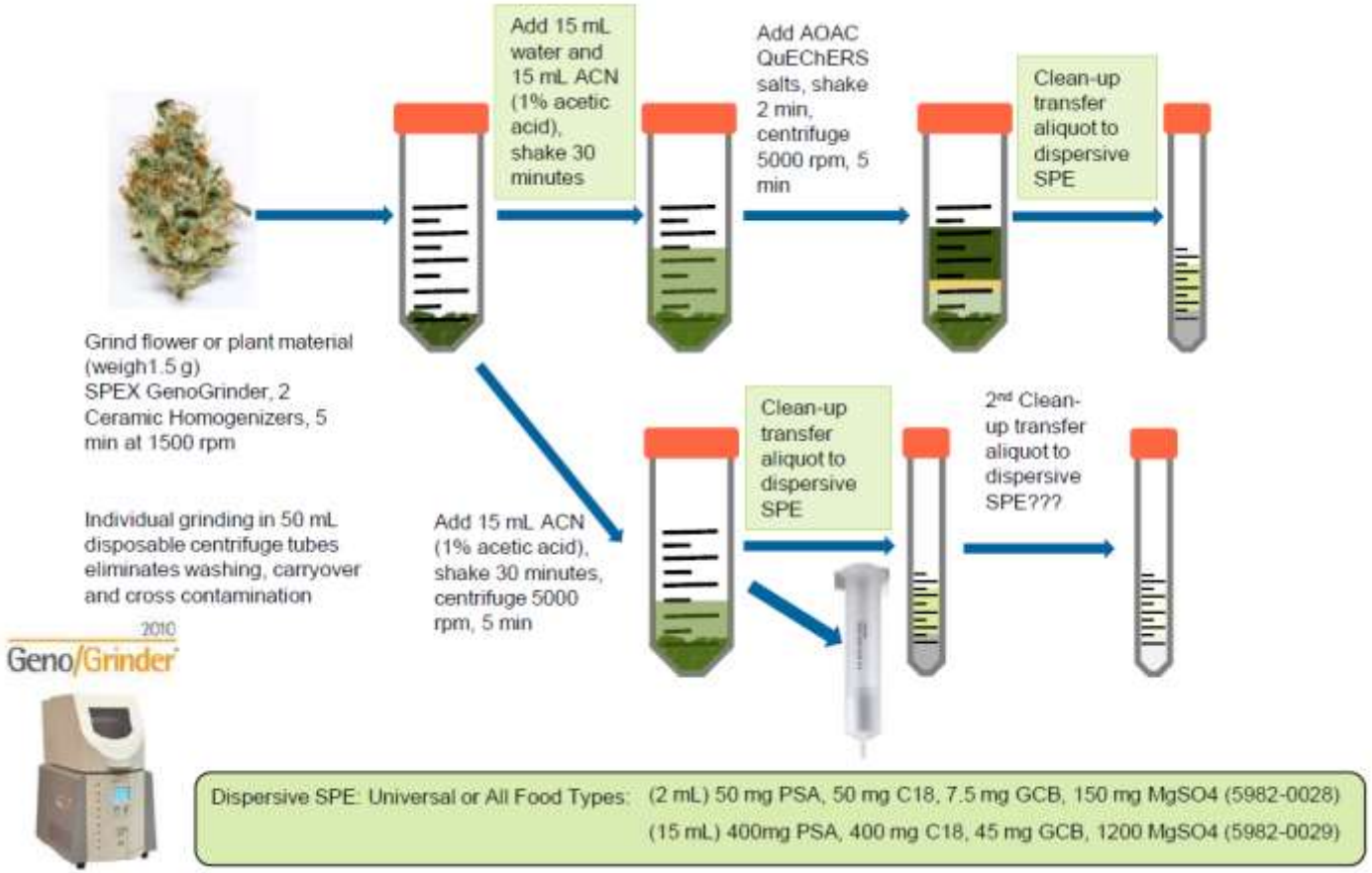
Tetrahydrocannabivarin (THCV)
(–)-trans-Δ9-tetrahydrocannabinol (THC)
Cannabidiol (CBD)
Cannabigerol (CBG)
Tetrahydrocannabinolic acid (Δ9-THCA)
Cannabidiolic Acid (CBDA)
Cannabinol (CBN)
Cannabigerolic acid (CBGA)
Cannabichromene (CBC)
Cannabidivarin (CBDV)



Cannabis and Cannabis-Based Products: Pesticide Analysis

No tolerances have been established for marijuana, because of its illegal federal status and because the pesticide companies have yet to embark on the lengthy and expensive process of testing their products on cannabis……*Cannabis Now Issue 19*

Basic Protocol: Pesticide Analysis

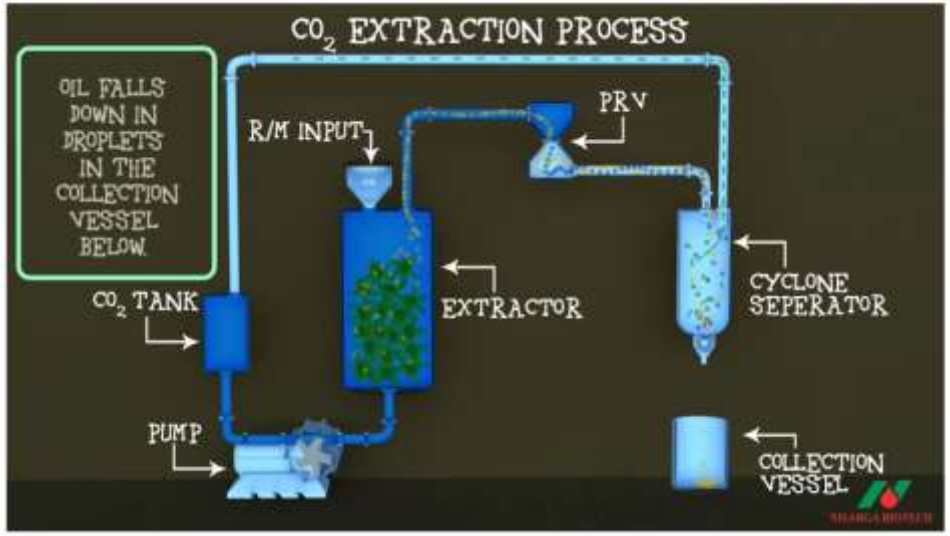


Seed to Sale (2): Production Processing and Current Opportunity

Areas of application:

1. Extraction Processes
2. Monitoring the process
3. Saving cost/ saving time.

This is where FTIR value becomes apparent



Cannabinoid Extraction Methods

Dry Sieve

This is a natural method of extracting compounds by sifting trim and shake through a fine-weave screen filter. This solvent-free process does not always produce the cleanest or most effective results.

Water

This method uses ice, screens of different micron sizes, and agitation to remove trichomes. While slightly more effective than dry sieve, there is a danger of mold growth if the extract is not dried thoroughly.

Carbon Dioxide

This is a very effective, although expensive, extraction method that does not use solvents and creates a clean and pure product.

Isopropyl Alcohol

Due to the ability to dissolve waxes, Isopropyl alcohol is best used as a quick wash to increase the purity of the cannabis extract.

Ethanol

The two-wash Quick Wash Ethanol, or QWET, process is probably the most common and easily executed extraction process. The first wash can extract 75 to 80% of the oil, the second extracts most of the balance.

Butane/Propane

When performed safely in a laboratory, butane and propane extractions are inexpensive and create clean, potent products. Either solvent is easily purged.

Hexane

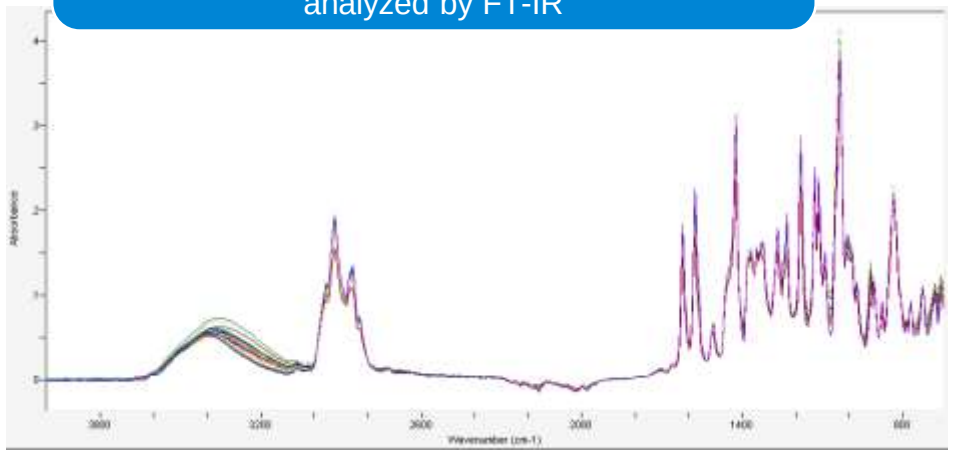
Although an effective solvent, hexane is more toxic than butane, and is insoluble in water, extremely flammable, and potentially explosive.

Butane Extraction:
Instrument and Experimental Condition

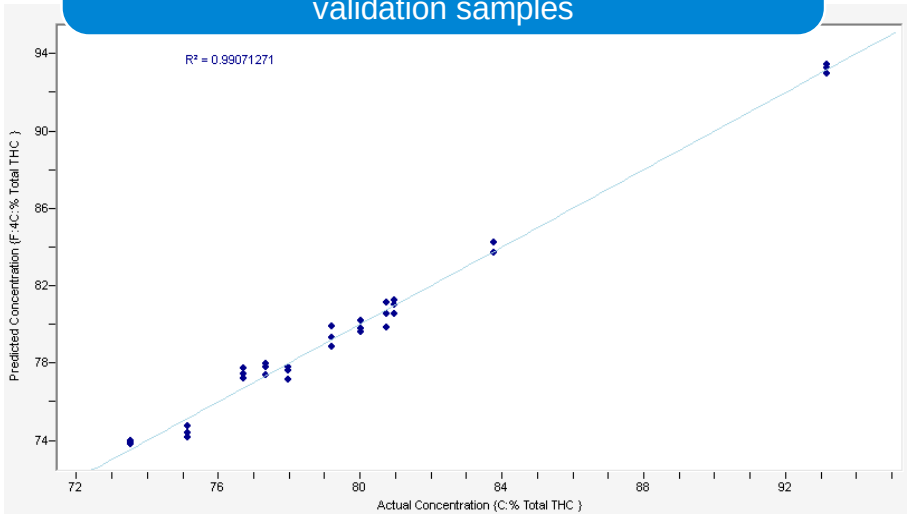
- Cary 630 with 1 bounce Diamond ATR accessory
- 4 cm^{-1} resolution, 32 scans
- 4000 – 650 cm^{-1} full spectral range
- Co ncentrate potency calibration for THC, THCA, and total THC content [HPLC measurement as reference value]
  - Concentrates [hydrocarbon extraction] = shatter, wax, budder, sugars (HC extraction)
- Sample type
  - Leaves and Trim

FTIR Cannabis Workflow

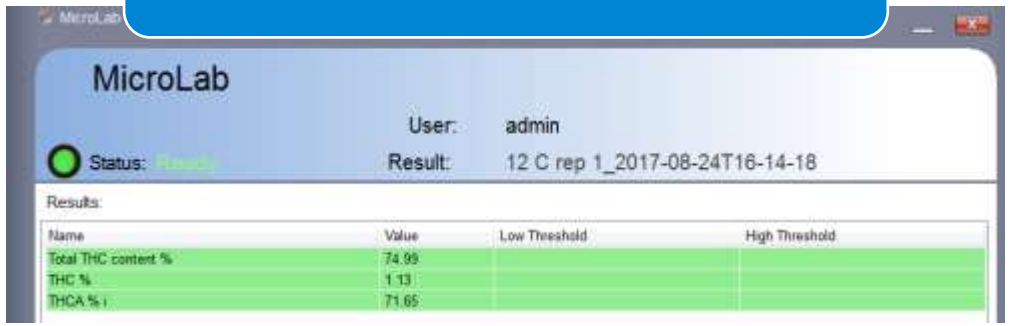
1. Measure FT-IR spectra of each sample in training set and measure the potency value from reference method for each sample analyzed by FT-IR



2. Build a multivariate calibration model correlating the FT-IR spectra with the reference value. Validate the model using the validation samples



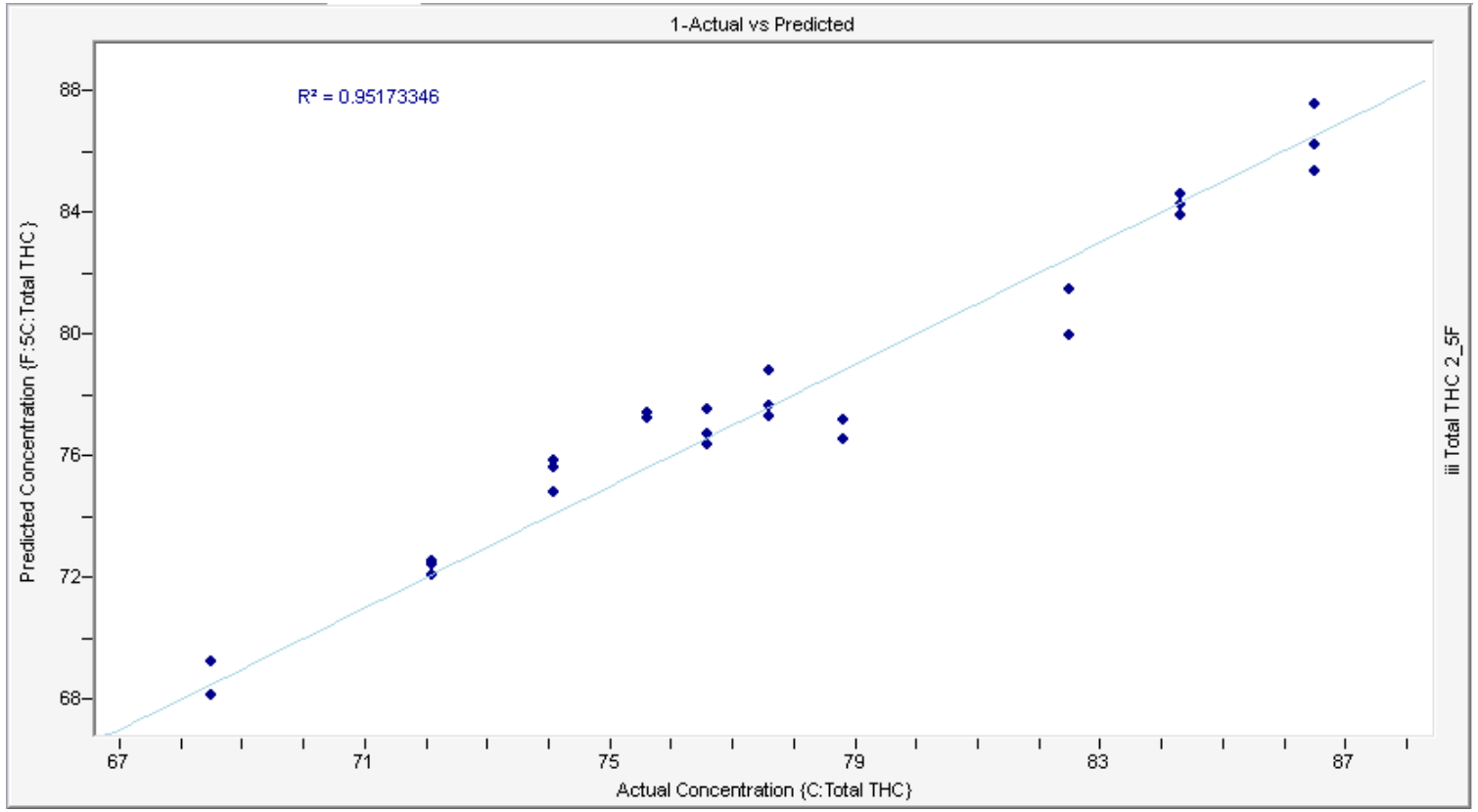
3. Use the calibration model in a MicroLab method to predict the unknown samples



FT-IR potency validation data			
Cannabinoid Model (PLS-1)	**R^2**	**Potency range (wt. %)**	**RMSEP %**
Distillates: Total THC	0.99	74 - 94 %	1
Concentrates: Total THC	0.95	68 - 87 %	6
Concentrates: THCA	0.95	63 - 83 %	6

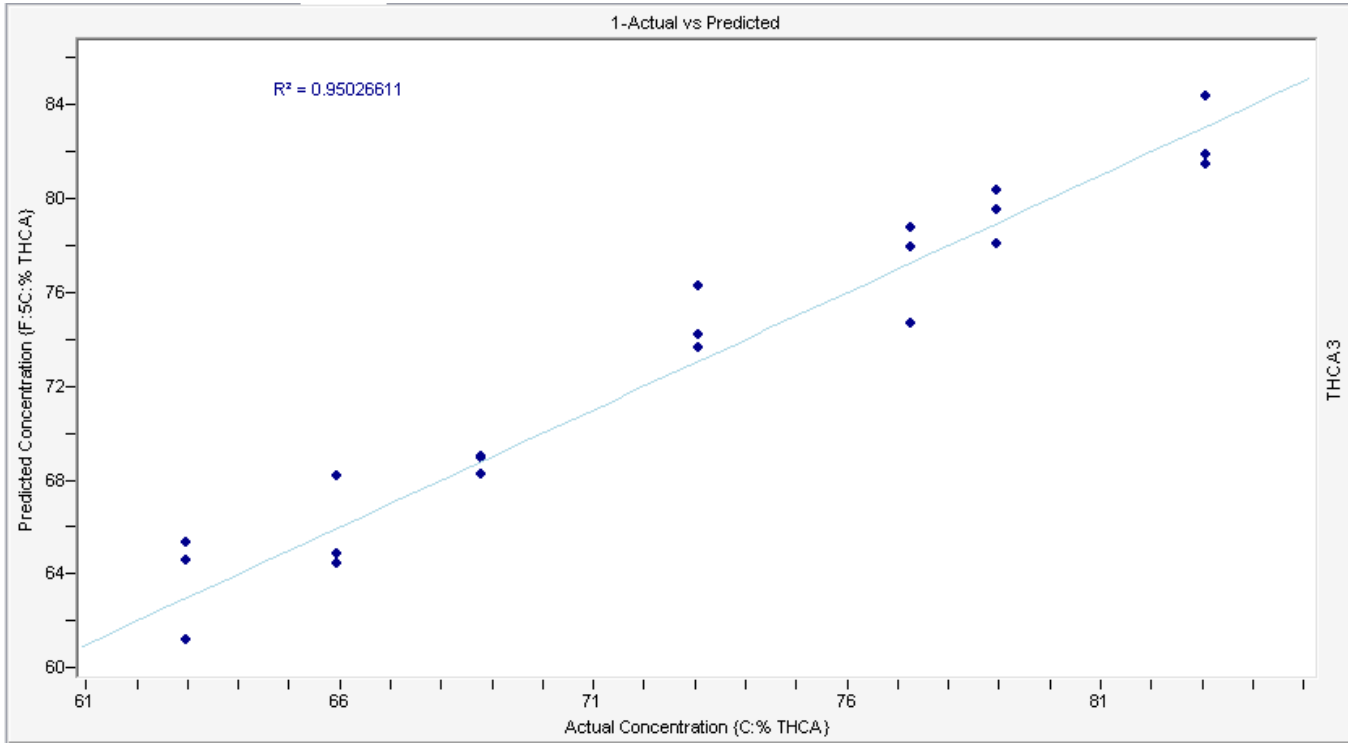
Butane Extraction: Concentrates Calibration

- Total THC content
- Calibration range = 68.5 % - 86.5 %
  - Correlation R^2 = 0.95, RMSEP = 6 %, 5 factors PLS-1 model



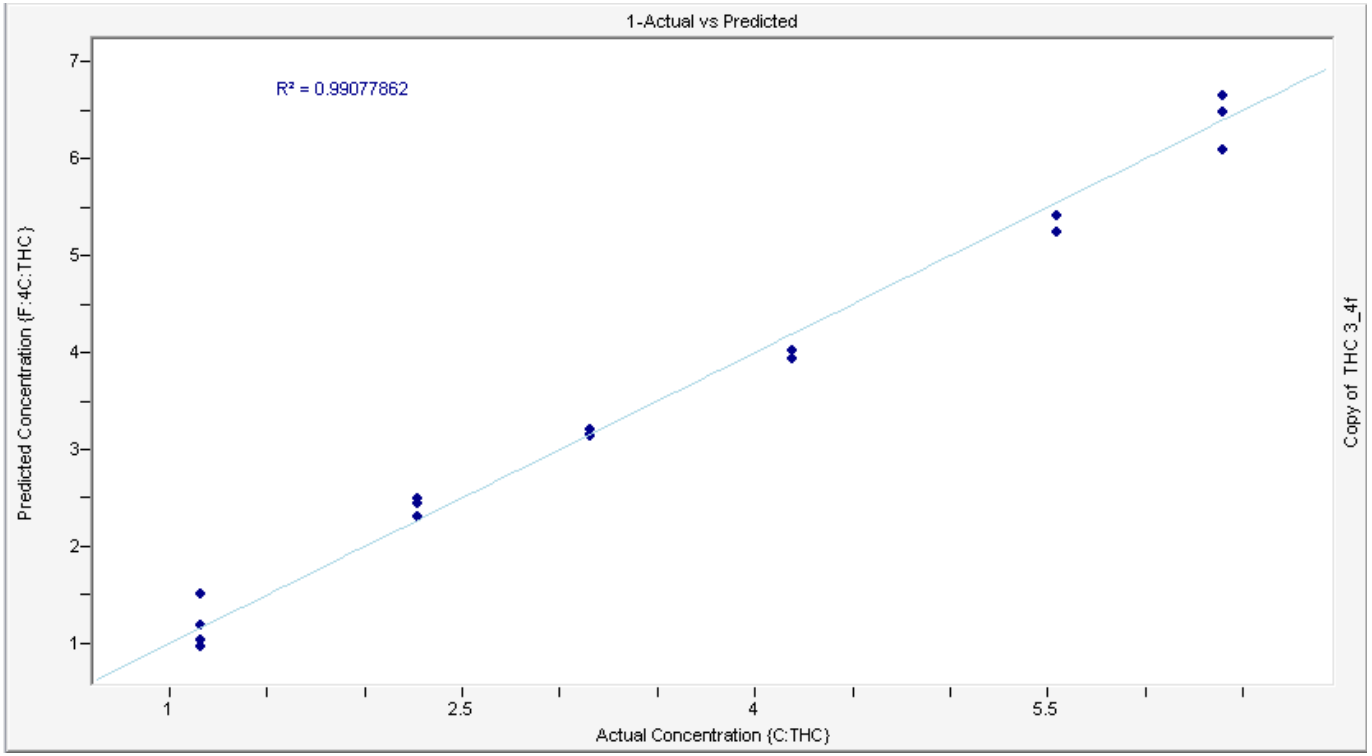
Butane Extraction: THCA Content

- THCA content
  - Calibration range = 62.96 % - 83.09 %
  - Correlation R^2 = 0.95 , RMSEP = 6 %, 5 factors PLS-1



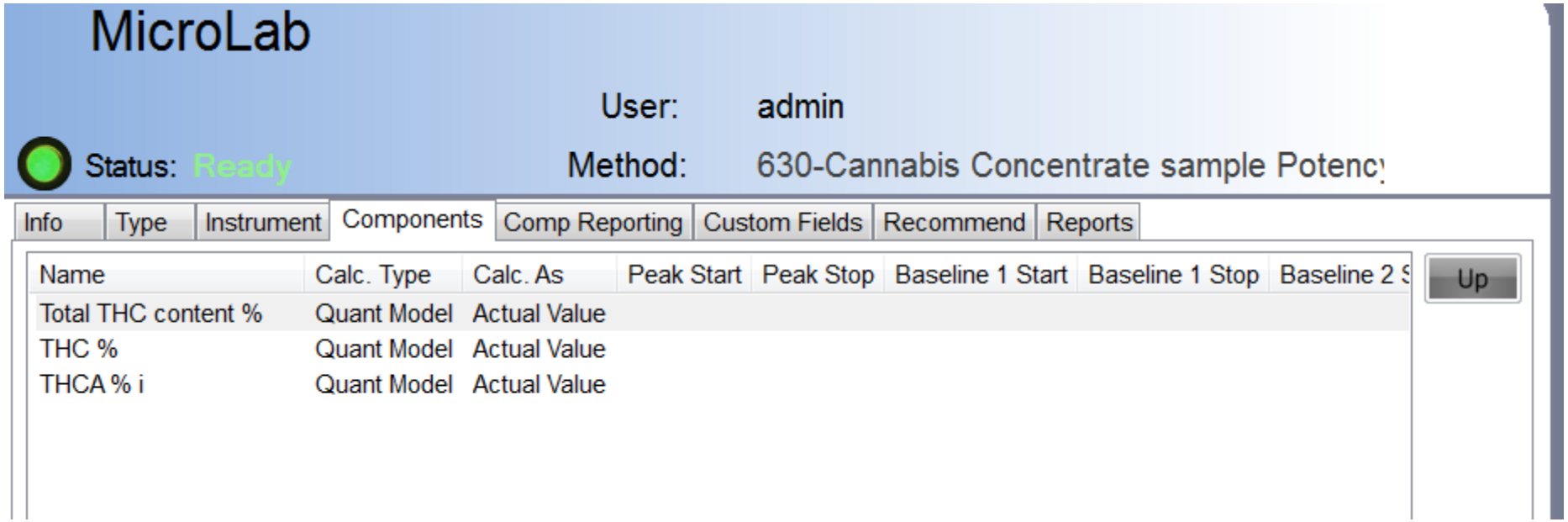
Butane Extraction: THC content

- THC content
  - Calibration range = 1.16 – 6.4 %
  - Correlation R^2 = 0.99, RMSEP =0.81, 4 factors PLS-1 model



MicroLab Method and Results Screen

- 630-Cannabis Concentrate sample Potency Analysis



Butane Extraction: Summary

The Cary 630 FTIR is used to monitor THC concentration during the extraction process. The spot measurement allows extractors to more cheaply, easily and efficiently track the process to the desired completion point, with accuracy that is comparable to that provided by chromatographic techniques.

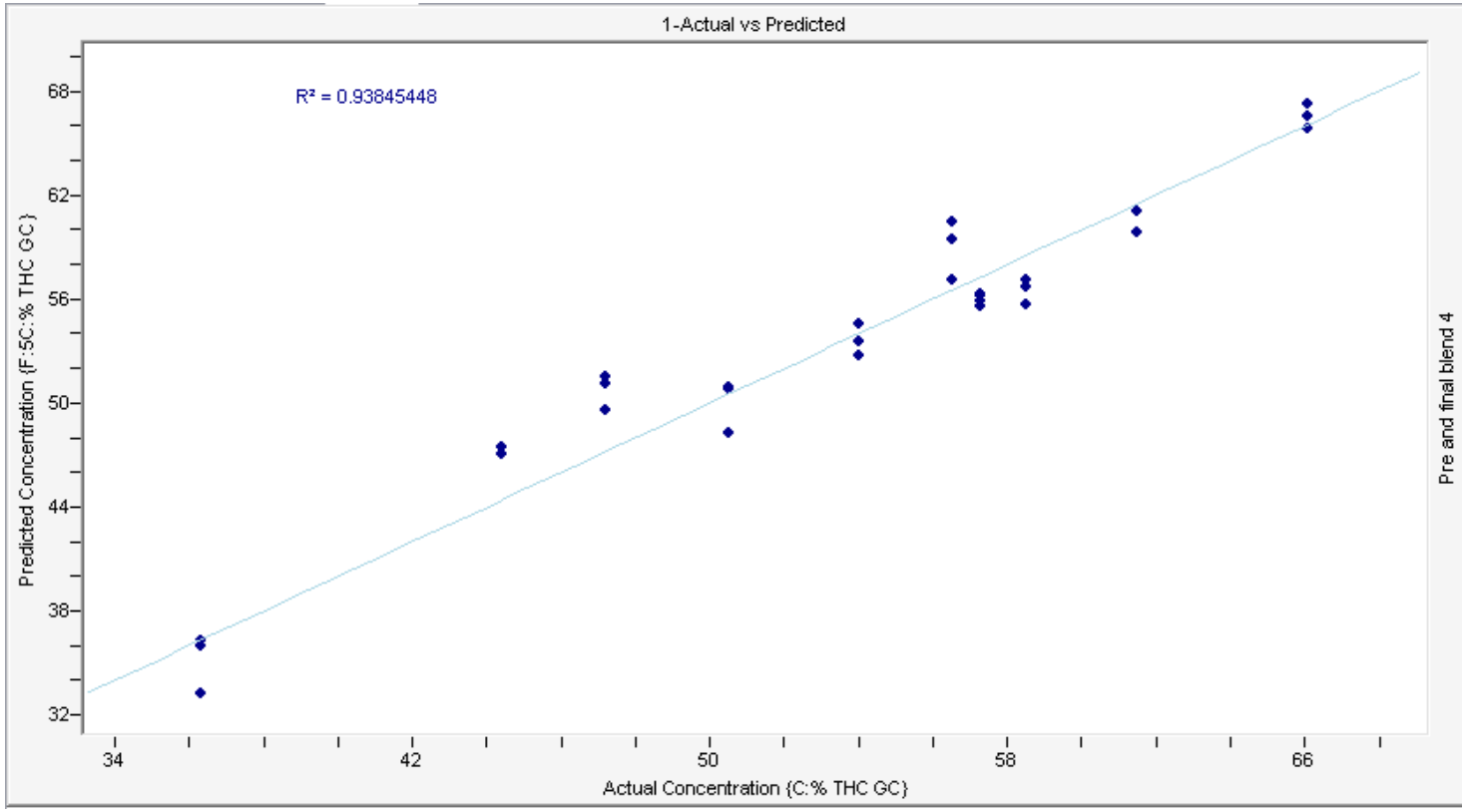
- The expected accuracy of FT-IR prediction (RMSEP) for total THC content is 6 % and
  - Covers the calibration range of 36.3 – 66.1 for CO_2 extracted pre-extracts and final products
  - Covers the calibration range of 68.5 – 86.5 % for hydrocarbon extracted concentrates
- The calibration model for CO_2 extract was built by encompassing both pre- extract and final product sample, and can be used to predict the THC content of those samples prepared in a similar manner as the calibration sample
- The calibration model for hydrocarbon concentrate extract was built by including samples of shatter, wax, budder, and sugars. The model can be used to predict total THC, THC, and THCA content of similar kind of samples prepared in similar manner as calibration samples.

CO2 Extraction:
Instrument and Experimental Conditions

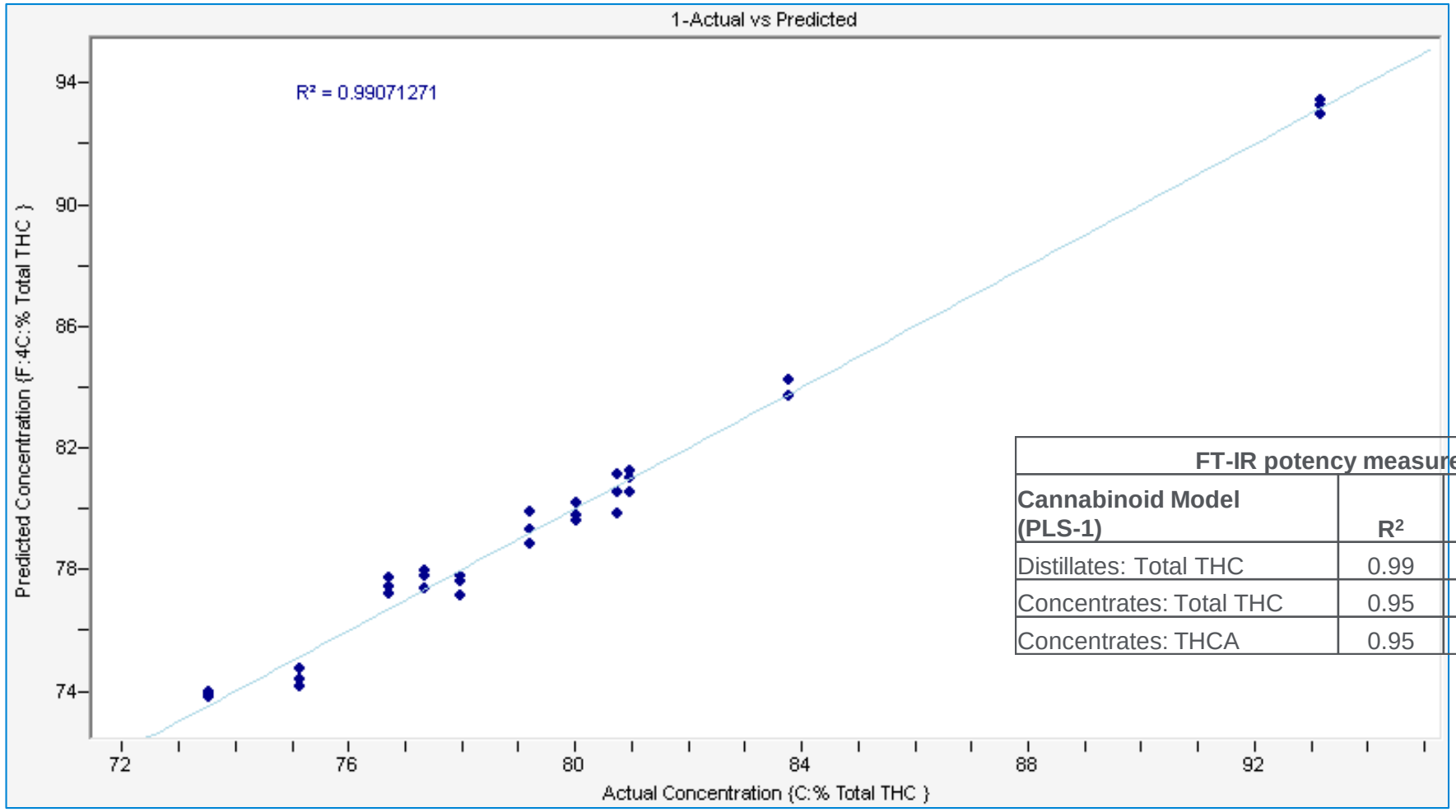
- Cary 630 with 1 bounce Diamond ATR accessory
- 4 cm-1 resolution, 64 scans
- 4000 – 650 cm-1 spectral range
- Concentrate potency calibration for THC (GC measurement as reference value)
- Sample type measured
  - Concentrates = Pre Extract and final product

CO2 Extraction: Concentrates Calibration

- Total THC content [Pre-extract and final products]
- Calibration range = 36.3 % - 66.1 %
  - Correlation R^2 = 0.94, RMSEP = 5.5 %, 5 factors PLS-1 model



CO2 Extraction: Distillates calibration



FT-IR potency measurement results			
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  - Covers the calibration range of 68.5 – 86.5 % for hydrocarbon extracted concentrates
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- The calibration model for hydrocarbon concentrate extract was built by including samples of shatter, wax, budder, and sugars. The model can be used to predict total THC, THC, and THCA content of similar kind of samples prepared in similar manner as calibration samples.

Cary 630 with 1-Bounce Diamond ATR accessory

Major benefits of FTIR analysis

- Real-time potency value
- Sample measurement in seconds
- Non-destructive sample analysis
- No consumables required
- Portable, easy-to-use instrument
- Small laboratory footprint
- Method-driven software guides the user in each step of the analysis

Introduction

Cannabis products that are currently on the market consist of either the dry material, such as flower buds; the plant concentrates, including waxes and distillates; or infused products, such as foods and candies. The main active ingredients in these products are the group of chemical compounds that provide either a medicinal or recreational effect on the consumers. Of the many different cannabinoids that have been isolated from the cannabis plant, the THC, THCA, CBD, and CBDA compounds are generally those of greatest interest.

As different cannabis products have differing potency levels, knowing the potency of a product is important for determining the correct dosage. At present, a chromatographic methodology is approved by regulatory agencies for testing the potency of cannabis products.

The FTIR spectroscopy technique has long been used to analyze complex spectra and matrices using chemometric methodology. Unique chemical signatures from each compound are used to develop a multivariate calibration model. This is then used to calculate the potency value or quantitate other species of interest.

Agilent Technologies

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Application Note

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Quick and Real-Time Potency Determination of Cannabinoids Using FTIR Spectroscopy

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Introduction

Cannabis products that are currently on the market consist of either dry material such as flower buds, concentrates such as shatter, budder, waxes, oils, extracts, and distillates, or infused products such as food and candies. The main active ingredients in these products are chemical compounds called cannabinoids, which provide either a medicinal or recreational effect to consumers. Of the many different cannabinoids that have been isolated from cannabis plants, tetrahydrocannabinol (THC), tetrahydrocannabinolic acid (THCA), cannabidiol (CBD), and cannabidiolic acid (CBDA) compounds are generally those of greatest interest and the most prevalent found in commercial cannabis plants.

The cannabinoid profile of most commercial cannabis cultivars is either enriched with THCA content or CBDA content. THCA is the acidic form of THC. THCA gets decarboxylated as the samples gets dried or exposed to heat, converting THCA to THC (the psychoactive component). Therefore, to measure the real potency, both THCA and THC should be considered. The total THC content refers to the addition of THCA and THC potency value, with the correction factor of 0.877 for THCA value before adding to the THC value. The correction factor is due to the molecular weight difference between THC and THCA.

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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.

Conclusions

- The Agilent Cary 630 FTIR (Fourier transform infrared spectroscopy) provides a fast, easy, and economical system for accurate potency measurements of cannabis products.
- FTIR spectroscopy has long been used to analyze complex spectra and matrices using chemometric methodology. The unique chemical signatures from each compound are used to develop a multivariate calibration model. This is then used to calculate the potency value or quantitate other species of interest.
- Importantly FTIR, is considered an excellent screening tool as the sample analysis is largely non-destructive, needing little to no sample preparation or consumable requirements.
- The Agilent Cary 630 and mobile 4500 FTIR bring the power of spectral analysis to potency measurements of cannabis products either in the lab or in the field.
- The benefits of FTIR for cannabis analysis include guided software methods to assist the user at each step of the process for near real-time potency determination from samples measured in seconds.
- FTIR has demonstrated the ability to achieve measurement accuracy on par with the chromatographic standard methods when appropriate chemometric analysis is applied, expanding the capabilities of spectroscopic analysis in this rapidly growing area.

Thank You