Pesticides in Cannabis
- A sample prep perspective

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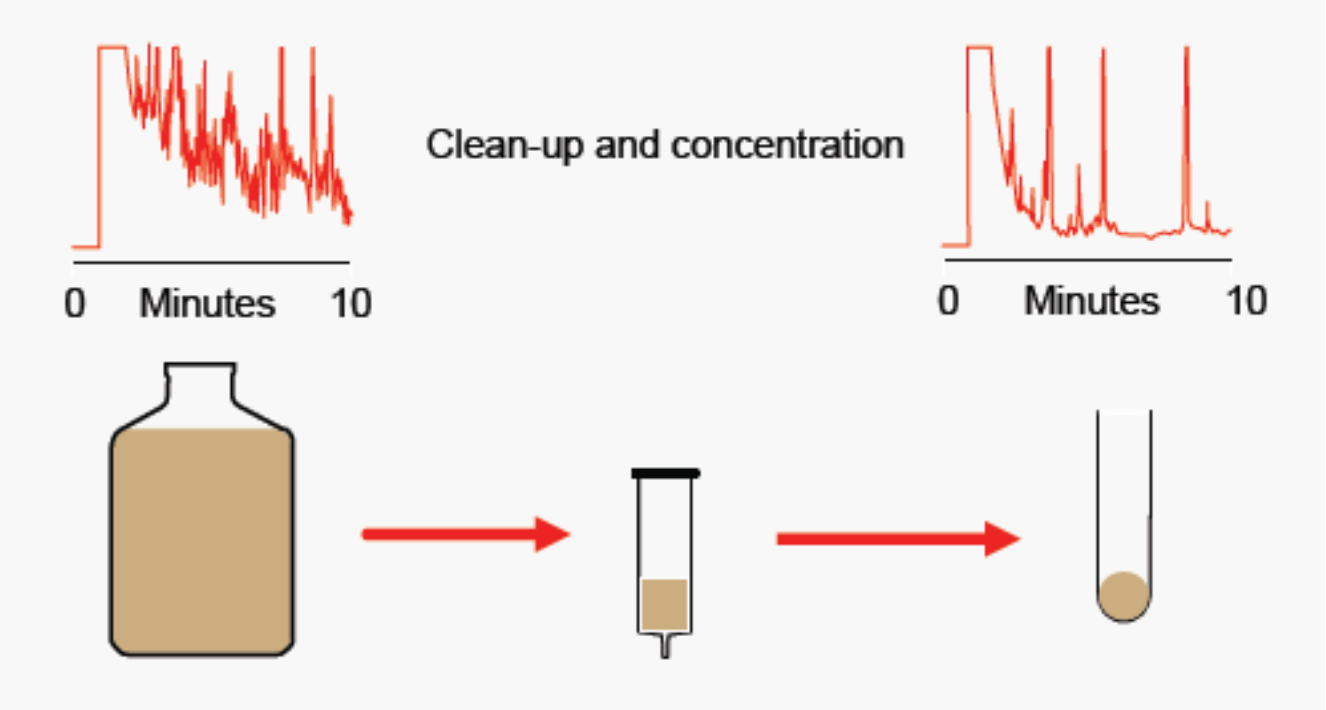
Agilent
Trusted Answers

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.

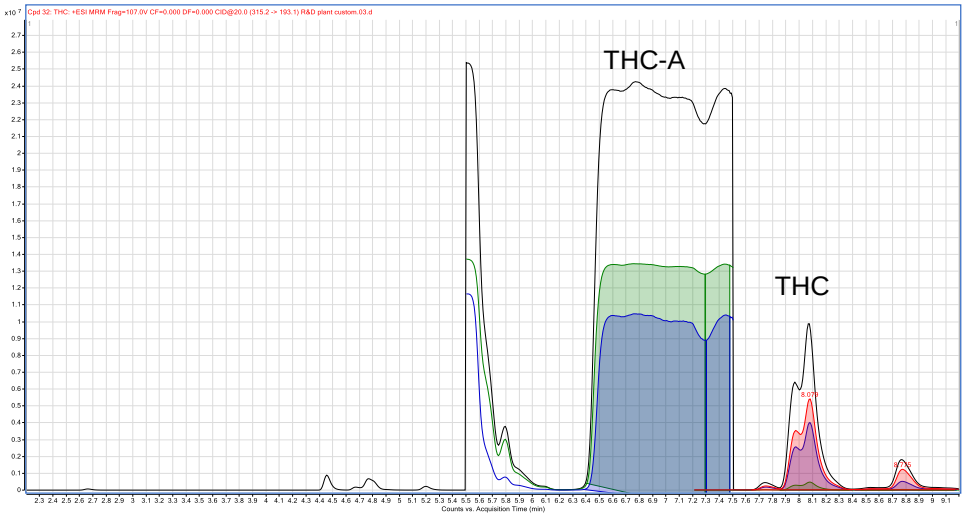
Traditional Reasons for Sample Prep



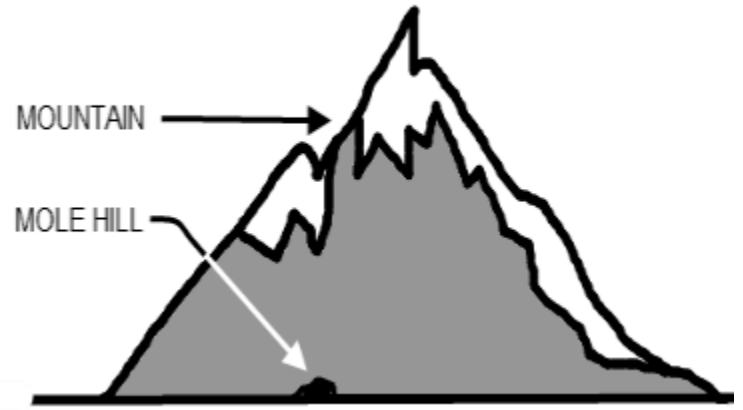
- Removal of interferences which would otherwise affect detection of analyte
- Concentration of an analyte to detectable levels
- Solvent switching into an analytically more compatible solvent

High Abundance Matrix Components Cause Suppression

- Cannabinoids (10-30% or 100,000-300,000 ppm), especially THC-A
- Non-cannabinoid plant components (10-5000 ppm) – terpenes, fatty acids, sterols
- Pesticides in 500 ppb amounts (0.00005%)

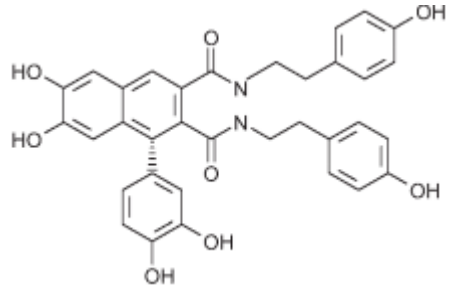


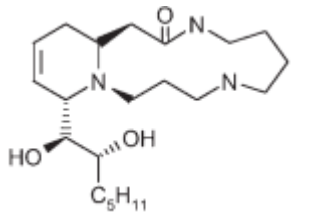
LC/MSMS analysis after QuEChERS and dSPE cleanup (universal)



Constituents of Cannabis Plants: Complex

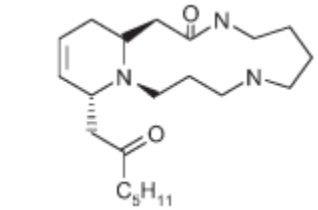
- **Nitrogen containing compounds (27 known)**
- Amino acids (18),
- Proteins (3)
- Glycoproteins (6)
- Enzymes (2)
- **Sugars and related compounds (34)**
- Hydrocarbons (50)
- Simple alcohols (7)
- Aldehydes (13)
- Ketones (13)
- Simple acids (21)
- **Fatty acids (22)**
- Simple esters (12)
- Lactones (1)
- Steroids (11)
- **Terpenes (120)**
- **Non-cannabinoid phenols (25)**
- **Cannabinoids (66)**
- **Flavonoids (21)**
- Vitamins (1) [Vitamin A]
- Pigments (2)
- Elements (9).

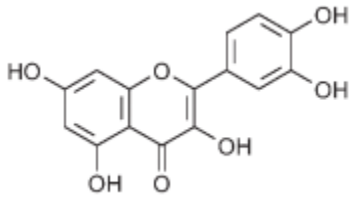


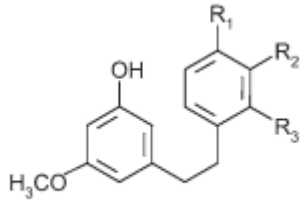


Structure of Terpenes

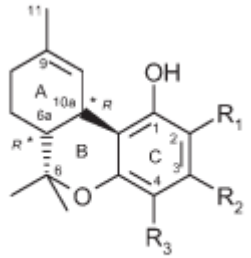
- Terpenes are composed of two or more isoprene units.
- The isoprene units will maintain its isopentyl, usually with modification of the isoprene double bonds.







R_1 = OH, R_2 = isoprenyl, R_3 = H



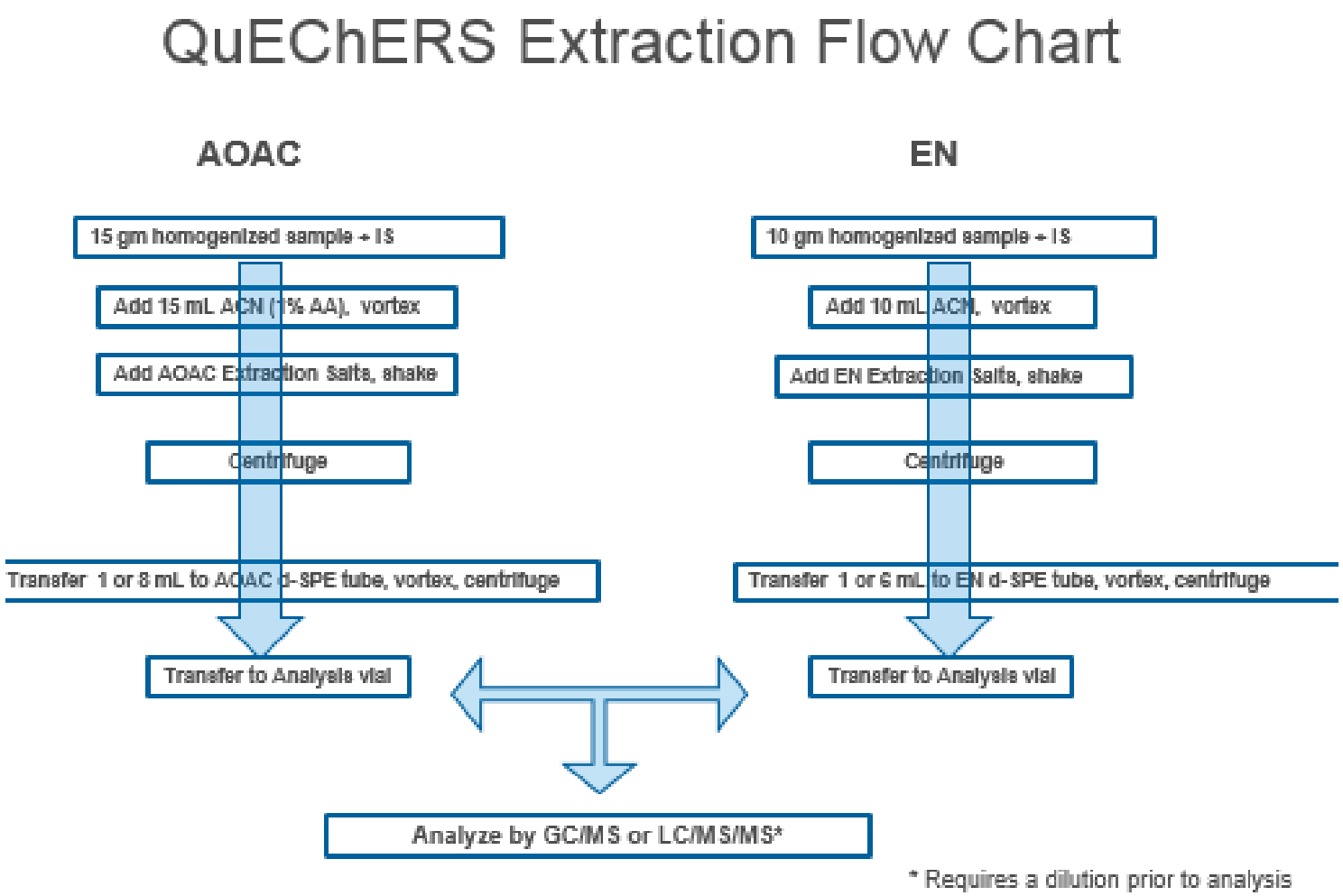
R_1 = COOH, R_2 = C_5H_{11}, R_3 = H

Some co-extract with Pesticides

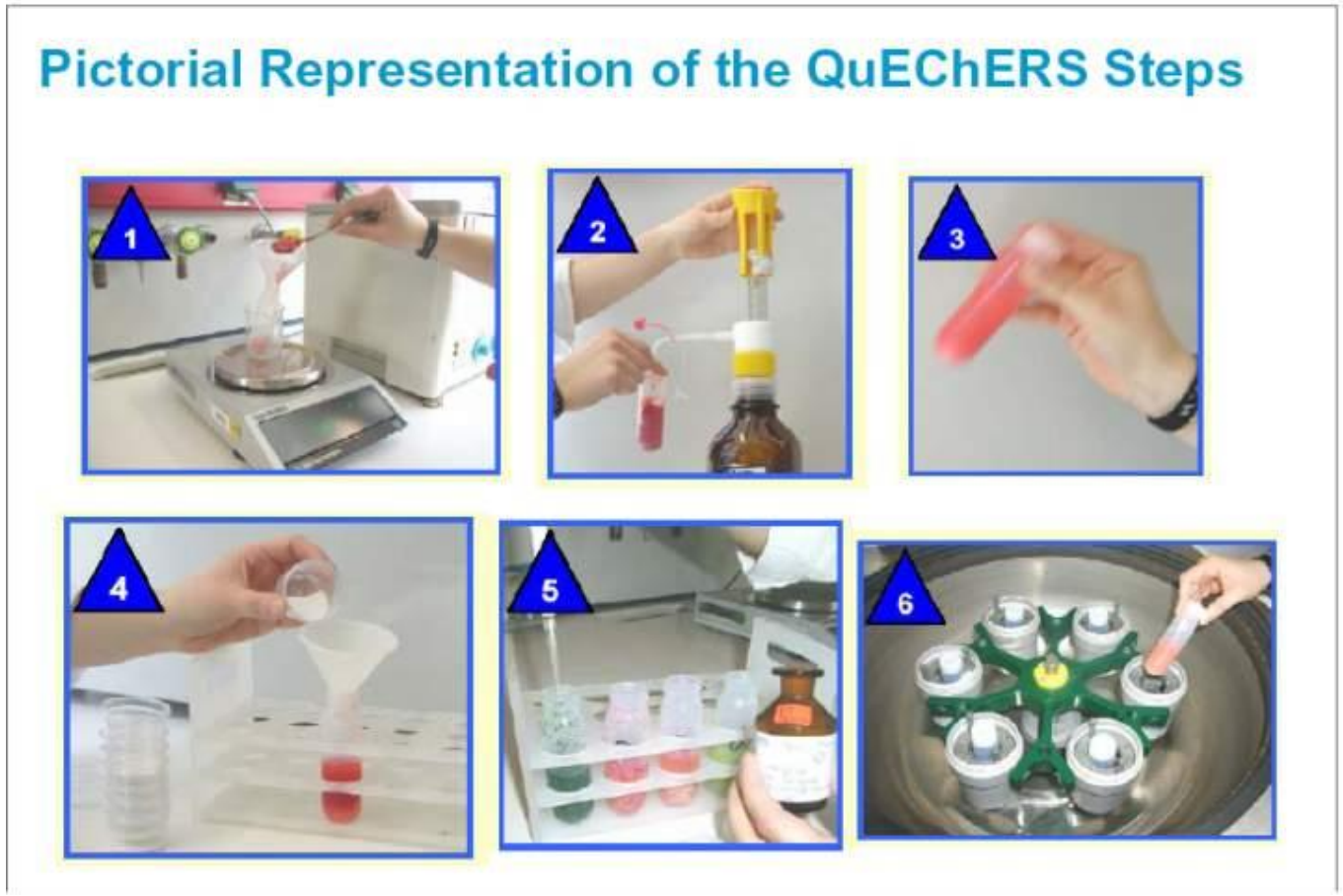
Many Sample Preparation Techniques Can Be Used

	More Specific ← **Instrument Separation and Detection Specificity** ← *Less Specific*								
	Less Specific → ***Sample Preparation Specificity*** → *More Specific*								
Sample Prep Technique / Interference Removed	**Dilute & Shoot**	**Filtration**	**Liquid/Liquid Extractions**	**Supported Liquid Extractions (SLE)**	**Dried Matrix Spotting**	**Precipitation**	**QuEChERS**	**Lipid Removal 'Hybrid' Filtration**	**Solid Phase Extraction**
Lipids	No	No	No	Some	No	No	Yes	Yes	Yes
Oligomeric Surfactants	No	No	No	No	No	No	No	Yes	Yes
Particulates	No	Yes	No	Some	No	Yes	Yes	Yes	Yes
Pigments	No	No	No	Some	No	No	Yes	No	Yes
Polar Organic Acids	No	No	Yes	Yes	No	No	Yes	No	
Proteins	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Salts	No	No	Yes	Yes	No	No	No	No	Yes
Suggested Agilent Product	Agilent Autosampler Vials	Captiva Syringe Filters		Chem Elut		Captiva	Bond Elut QuEChERS	Captiva EMR LIPIDS	Bond Elut Silica and Polymeric SPE
Agilent Captiva Filtration Products are recommended for use with any LC or LC-MS method									

QuEChERS – The Universal Sample Prep for Pesticides?

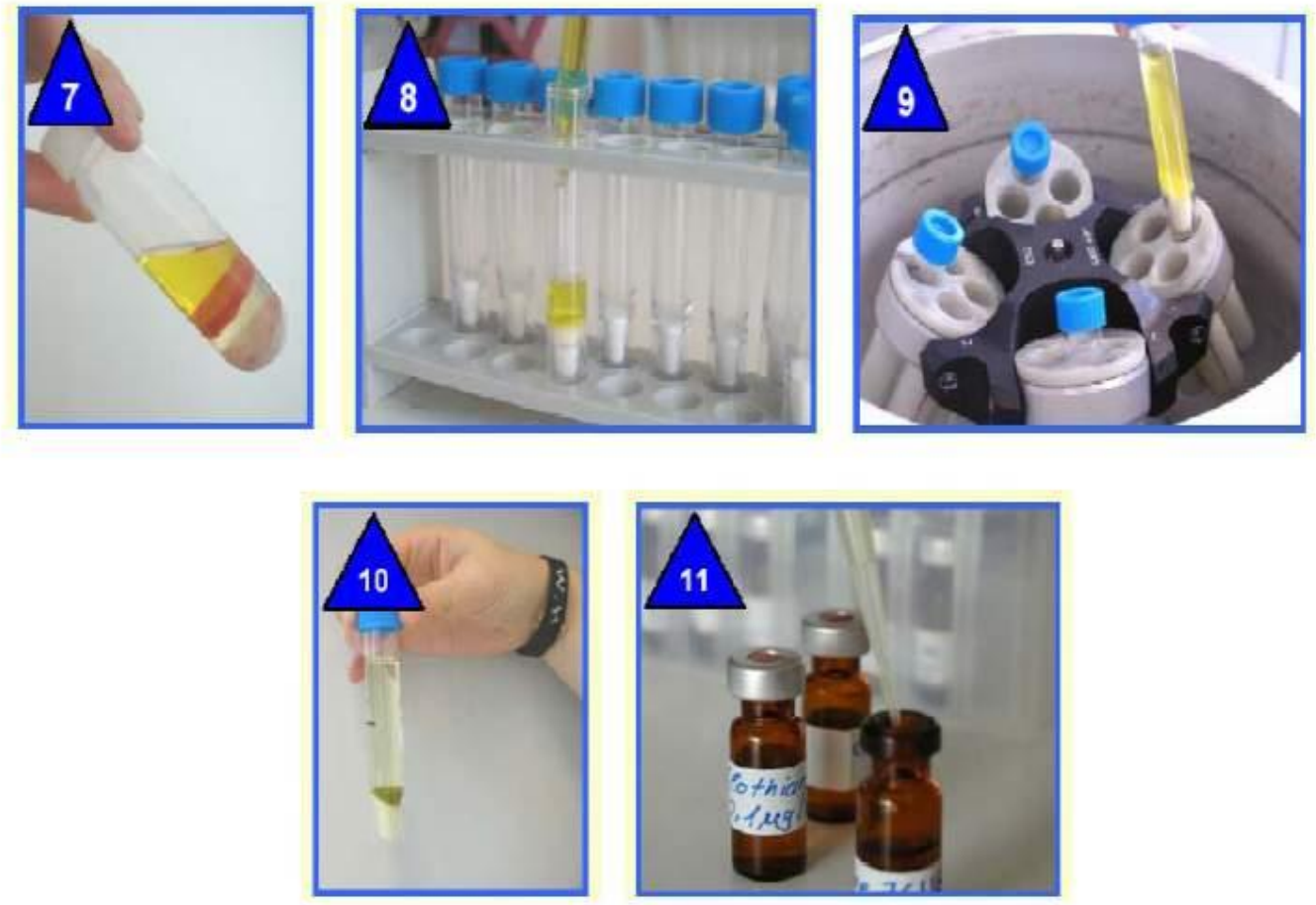


QuEChERS
First Step: Extraction



1) Weigh sample, add water if needed, spike
2) Add 10ml ACN
3) Vortex
4) Add salt packet
5) Shake 1 minute
6) Centrifuge at 4,000 rpm for 5 minutes

Second Step: Dispersive SPE

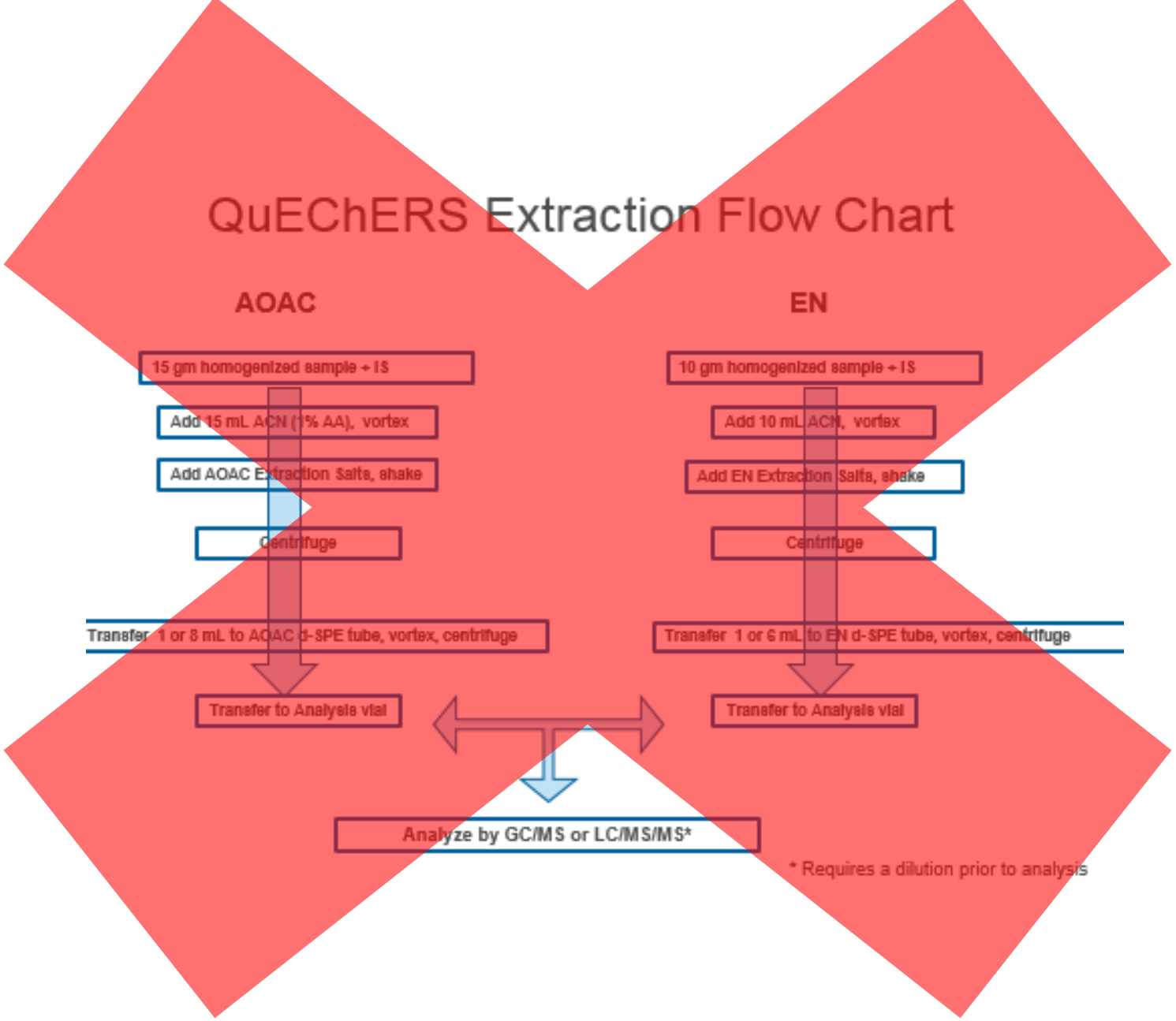


7) Choose d-SPE kit based on matrix characteristics

8) Transfer 1-8ml aliquot, vortex 1 minute

9) Centrifuge

10) Analyze by GC/MS or LC/MS



- No endogenous water to eliminate
- Adding water increases pH, problem with base labile pesticides
- dSPE does not offer enough capacity
- PSA co-scavenges acidic pesticides (MeOH fix)
- GCB co-scavenges planar pesticides (toluene fix)

Pesticide Analytes and Their Action Levels in Oregon

Analyte	Chemical Abstract Services (CAS) Registry number	Action level ppm
Abamectin	71751-41-2	0.5
Acephate	30560-19-1	0.4
Acequinocyl	57960-19-7	2
Acetamiprid	135410-20-7	0.2
Aldicarb	116-06-3	0.4
Azoxystrobin	131860-33-8	0.2
Bifenazate	149877-41-8	0.2
Bifenthrin	82657-04-3	0.2
Boscalid	188425-85-6	0.4
Carbaryl	63-25-2	0.2
Carbofuran	1563-66-2	0.2
Chlorantraniliprole	500008-45-7	0.2
Chlorfenapyr	122453-73-0	1
Chlorpyrifos	2921-88-2	0.2
Clofentezine	74115-24-5	0.2
Cyfluthrin	68359-37-5	1
Cypermethrin	52315-07-8	1
Daminozide	1596-84-5	1
DDVP (Dichlorvos)	62-73-7	0.1
Diazinon	333-41-5	0.2
Dimethoate	60-51-5	0.2
Ethoprophos	13194-48-4	0.2
Etofenprox	80844-07-1	0.4
Etoxazole	153233-91-1	0.2
Fenoxycarb	72490-01-8	0.2
Fenpyroximate	134098-61-6	0.4
Fipronil	120068-37-3	0.4
Flonicamid	158062-67-0	1
Fludioxonil	131341-86-1	0.4
Hexythiazox	78587-05-0	1

Analyte	Chemical Abstract Services (CAS) Registry number	Action level ppm
Imazalil	35554-44-0	0.2
Imidacloprid	138261-41-3	0.4
Kresoxim-methyl	143390-89-0	0.4
Malathion	121-75-5	0.2
Metalaxyl	57837-19-1	0.2
Methiocarb	2032-65-7	0.2
Methomyl	16752-77-5	0.4
Methyl parathion	298-00-0	0.2
MGK-264	113-48-4	0.2
Myclobutanil	88671-89-0	0.2
Naled	300-76-5	0.5
Oxamyl	23135-22-0	1
Paclobutrazol	76738-62-0	0.4
Permethrins*	52645-53-1	0.2
Phosmet	732-11-6	0.2
Piperonyl_butoxide	51-03-6	2
Prallethrin	23031-36-9	0.2
Propiconazole	60207-90-1	0.4
Propoxur	114-26-1	0.2
Pyrethrins†	8003-34-7	1
Pyridaben	96489-71-3	0.2
Spinosad	168316-95-8	0.2
Spiromesifen	283594-90-1	0.2
Spirotetramat	203313-25-1	0.2
Spiroxamine	118134-30-8	0.4
Tebuconazole	80443-41-0	0.4
Thiacloprid	111988-49-9	0.2
Thiamethoxam	153719-23-4	0.2
Trifloxystrobin	141517-21-7	0.2

Range 0.1-1 ppm or 100-1000 ppb

* Permethrins should be measured as cumulative residue of cis- and trans-permethrin isomers (CAS numbers 54774-45-7 and 51877-74-8).

† Pyrethrins should be measured as the cumulative residues of pyrethrin 1, cinerin 1 and jasmolin 1 (CAS numbers 121-21-1, 25402-06-6, and 4466-14-2 respectively).

Customized Approach to Sample Preparation

Step 1: Solvent extraction of sample

- No water addition, no salting out
- ACN is most commonly used (adjust polarity as needed)

Step 2: Removal/Reduction of Cannabinoids

- Cartridge based clean up improves capacity
- Highly inert SPE material prevents analyte loss
- SampliQ C18 EC provides economic solution vs polymeric sorbents

Step 3: Removal of miscellaneous matrix components

- Different dSPE for LC/MS-MS vs. GC/MS-MS

Basic Protocol: Pesticide Analysis

AddACN
shake 10
minutes

centrifuge 5000
rpm, 5 min
Then process to
SampliQ C18EC
Rinse cartridge
2X ACN

If needed
Use dSPE
To improve
Signal/noise

Dilute further

Grind flower or plant material
(weigh1.0 g)
SPEX GenoGrinder, 2
Ceramic Homogenizers, 5
min at 1500 rpm

SampliQ C18EC
6ml 500mg
PN 5982-1365

Individual grinding in 50 mL
disposable centrifuge tubes
eliminates washing, carryover
and cross contamination

2010
Geno/Grinder

Dispersive SPE for GCMSMS and LCMSMS respectively:

GC:(2 mL) 50 mg PSA, 50 mg C18, 7.5 mg GCB, 150 mg MgSO4 (5982-0028)

LC:(2 mL) 100mg C18, 2.5 mg GCB (5982-0027)

dSPE Tips and Tricks

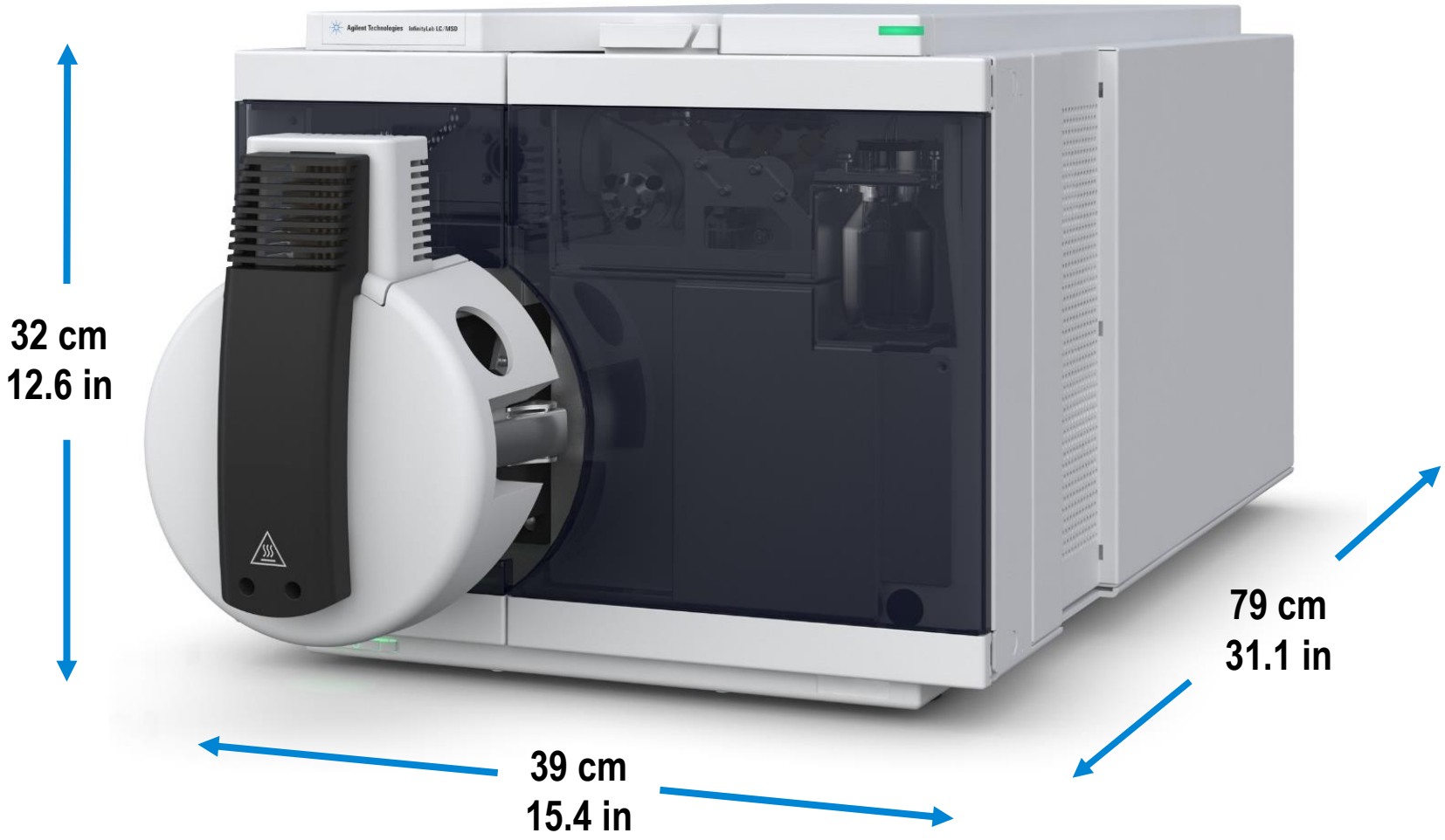
- PSA reduces recovery of acidic/heavily hydroxylated compounds – adjust to approx. 10-20% MeOH
- GCB reduces recovery of planar compounds – adjust to approx. 3% toluene

Dilution
is the
Solution
to
Pollution

A novel comprehensive strategy for residual pesticide analysis in cannabis flower

Printed in the USA, March 9, 2018
5991-9030EN

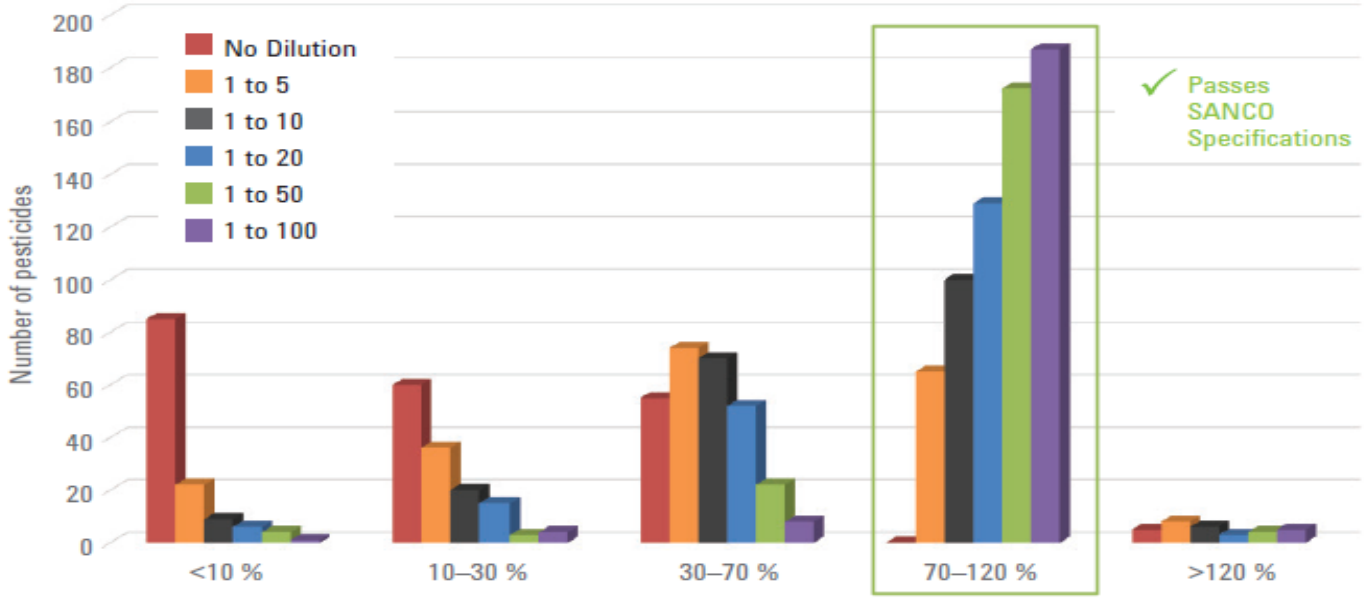
Ultivo: Robust and Low Maintenance LC/TQ



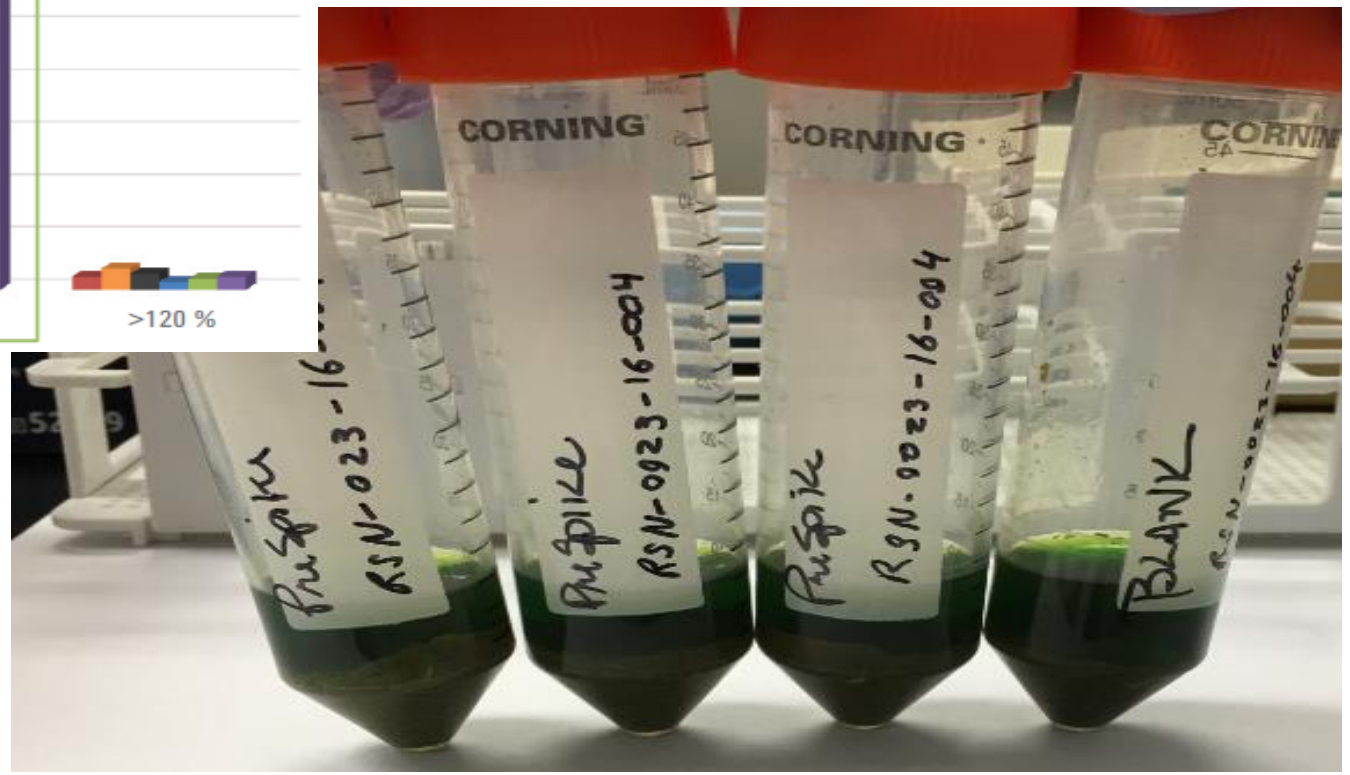
Typical LC/TQ Lab Layout

6 feet
1.83 meters

Dilution better for Quantitation & Recovery?



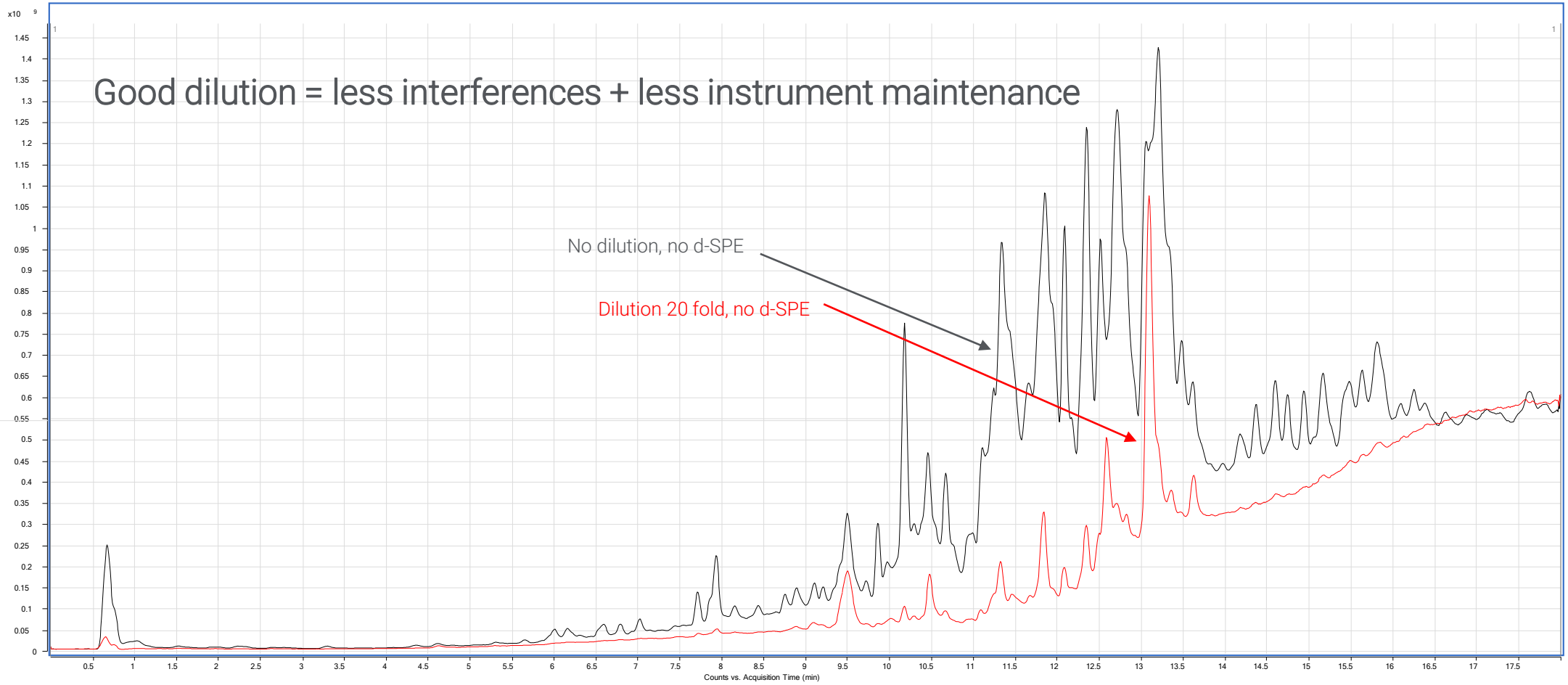
Compound recoveries in black pepper extremely complex matrix



Agilent 6495B: LC/MSMS

1:100 dilution required for acceptable recoveries >90% of the compounds

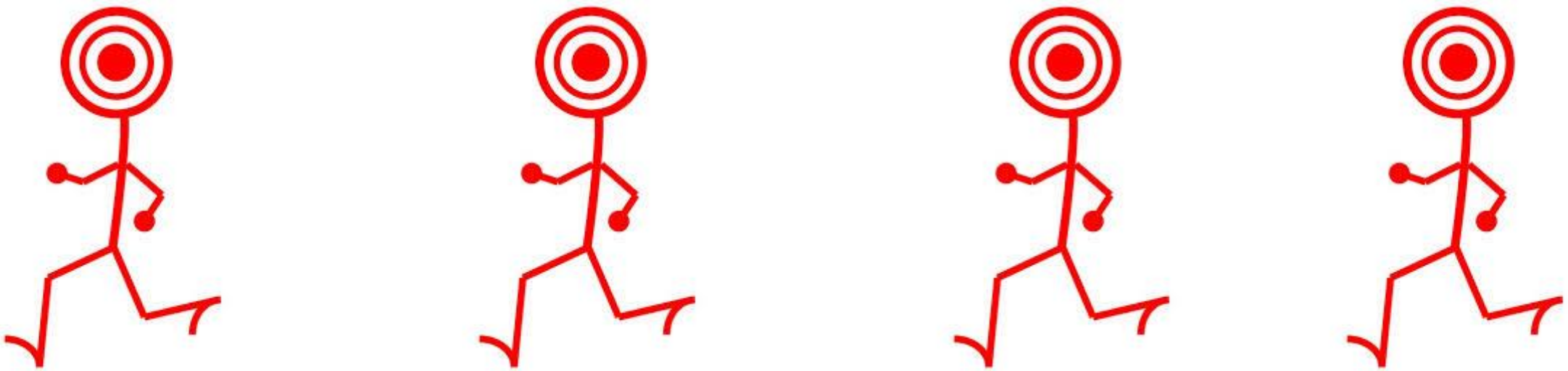
LCMS Full Scan of the Cannabis Mix Extracted with JASBC Protocol, 1 ppb



Hengel, M. J. Expanded Method Development for the Determination of Pesticides in Dried Hops by Liquid Chromatography with Tandem Mass Spectrometry. *J. American Society Brewing Chemists.* 69(3): 121-126, 2011.

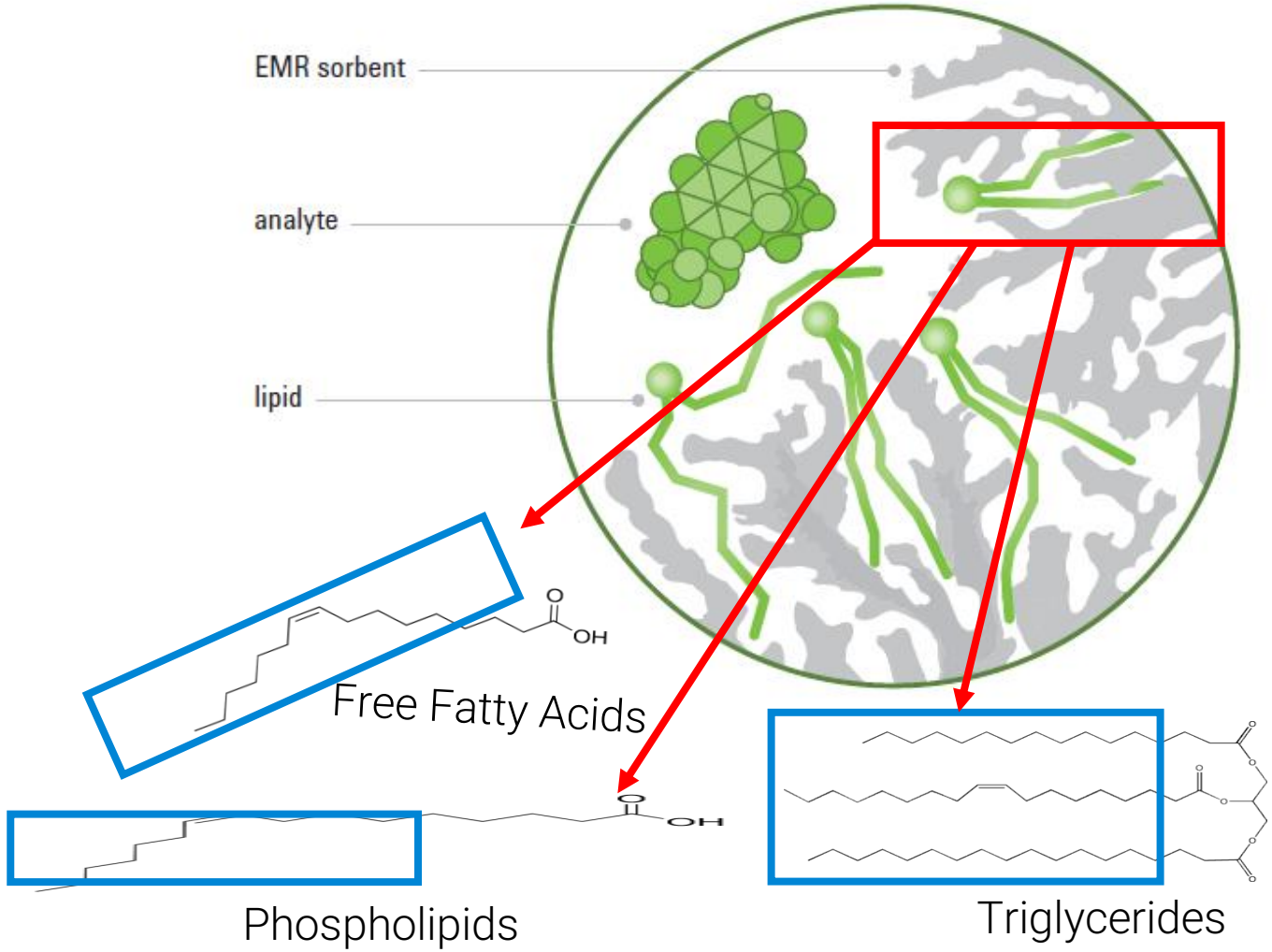
Need for Further Customization?

- State-specific list of analytes
- State-specific list of matrices (edibles!!)
- Pending and future regulatory changes



THC and CBD High Fat Products

Captiva EMR-Lipid: Mechanism of Lipid Removal

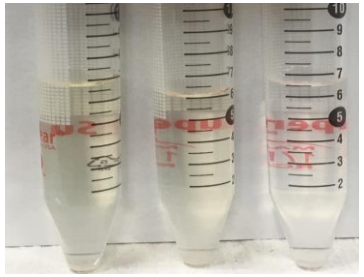


EMR sorbent technology effectively traps lipids through two mechanisms:

- **Size exclusion** – Unbranched hydrocarbon chains (lipids) enter the sorbent; bulky analytes do not
- **Sorbent chemistry** – Lipid chains that enter the sorbent are trapped by hydrophobic interactions

LC/MSMS
Extracts After d-SPE (C18/PSA) and New EMR Lipid Formulations Cleanup

Chocolate Chip Cannabis Cookies



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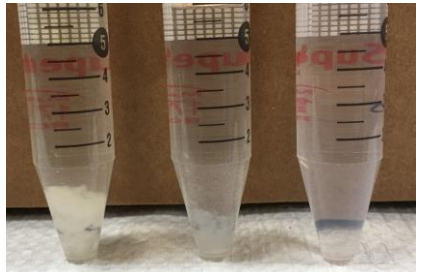

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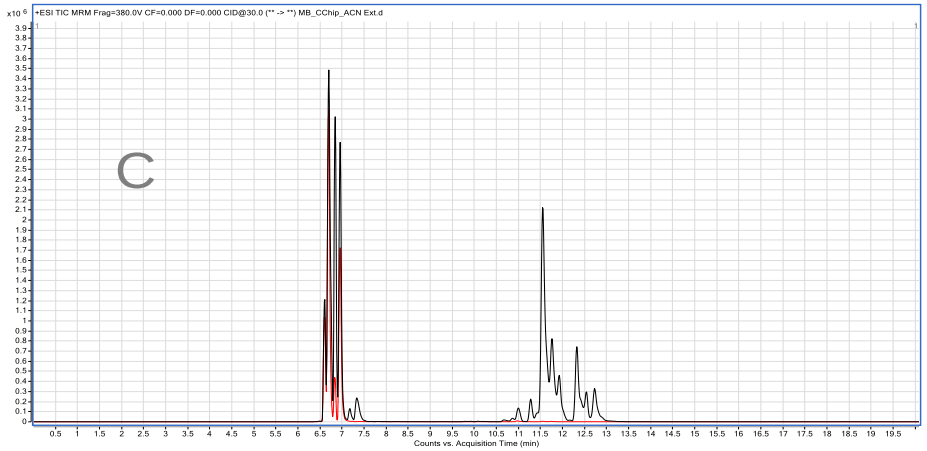
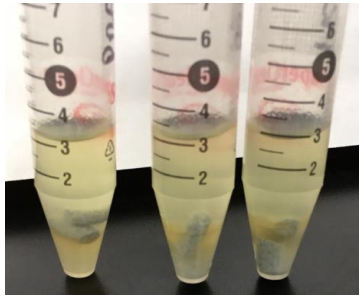


Cascadia Herbal Tincture



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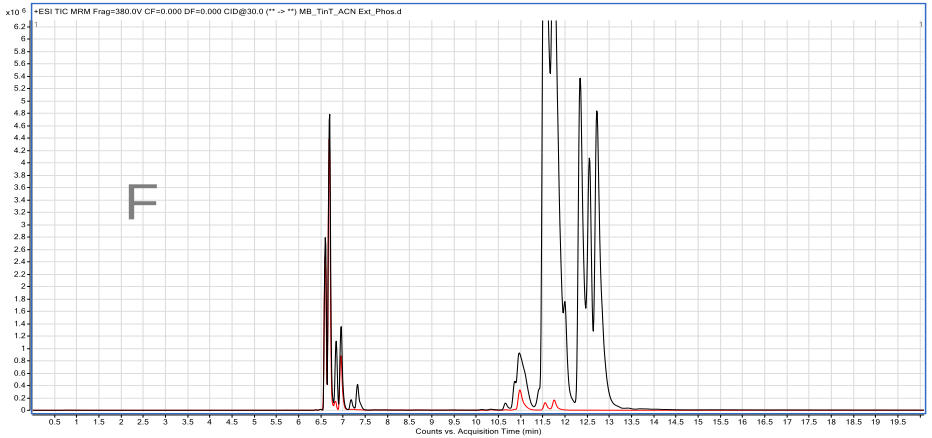
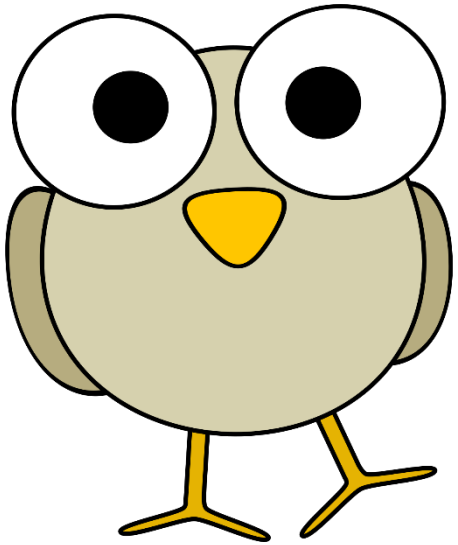


Insights and Observations

- Cannabis is a very unique matrix without scientific precedent
- Sample Preparation must accommodate diverse coextracting matrix components
- Importance of dilution
- Monitor background contamination (TIC/MS2 scan) to assess risk to analytical instrument
- Is the existing clean-up techniques enough for long term analysis?

Thank You!



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Happy Sample Prepping!