SHIMADZU

Excellence in Science

We strive to contribute to people's health and well being since our inception in 1875, by consistently challenging ourselves to develop state of the art technology that creates new values in a variety of fields.

Our corporate philosophy, 'contributing to society through science and technology', and our core management principle, 'for the well being of both mankind and the earth', is enshrined in our values

Nigel Grieves
Sales & Operations Manager

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Agenda

- Who is Shimadzu
- TGA Guidelines For Cannabis Analysis
- What is Chromatography
- Raw Product Testing Of Cannabis
  - Mycotoxin/aflatoxin
  - Moisture content
  - Pesticide screening
  - Heavy metals

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Who is Shimadzu

Corporate Philosophy:

Contributing to Society through Science and Technology

Management Principle:

Realizing Our Wishes for the Well-being of Mankind and the Earth

Established: *1875* *(144 years)*

Formation of Limited Company: *1917*

Number of Employees: *~ 10,000*

Consolidated Sales: *> AU$4 B*illion

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Who is Shimadzu

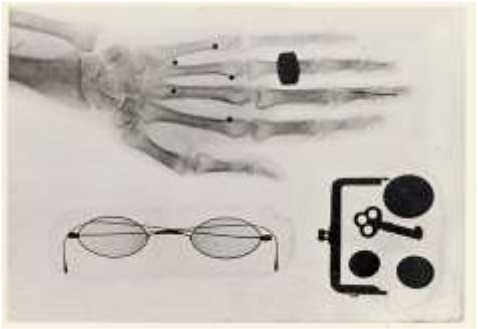
SHIMADZU HISTORY

1875	Established in Nijo area of Kyoto's Kiyamachi district Started manufacture and sales of physical and chemical instruments
1877	Succeeded in Japan's first manned balloon flight
1896	Succeeded in taking radiographs
1897	Started production of storage batteries
1909	Built Japan's first medical X-ray apparatus

Koichi Tanaka

2002 Nobel Prize for Chemistry
Development of MALDI
Shimadzu

Successful balloon flight (1877)



Early X-ray radiographs (1896)

Founder
Genzo Shimadzu

Genzo Shimadzu Jr.

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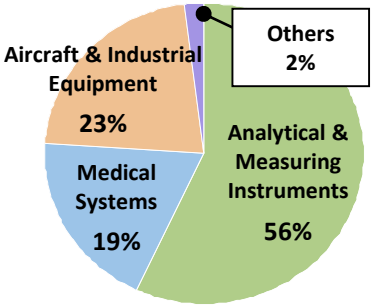
Who is Shimadzu

SHIMADZU BUSINESS DIVISIONS

Industrial Machinery and Equipment



Analytical and Measuring Instruments

Aircraft Equipment

Medical Systems

Who is Shimadzu

Shimadzu Australasia

Established: 1991

Scientific and Medical Subsidiaries

Staff > 100

Sales > $60 Million

Physical offices with local sales, technical and service support in all major cities

Application labs in Sydney, Melbourne & Brisbane

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  - **Heavy metals**

TGA Guidelines for Cannabis Testing

- Therapeutic Goods Administration (TGA) regulate the access to therapeutic goods and the quality of therapeutic goods
- Growers, manufactures and importers must be licenced and listed with the Office of Drug Control
- Therapeutic Goods Order No. 93 Is the Standard for Medicinal Cannabis in Australia
- This standard specifies the minimum quality requirements for medicinal cannabis product
- Unapproved medicinal cannabis products imported into and supplied or manufactured in Australia must conform with this standard.

TGA Guidelines for Cannabis Testing

- The tests in Schedule 1 are standard pharmacopoeia tests applied to the cannabis plant used in the manufacture of medicinal products.
- You do not have to use the methods specified in Schedule 1 as long as you use an equivalent method.
- Every batch of medicinal cannabis product must be tested and meet the requirements of Schedule 1
- The limits are report on a dried basis.
- However you may be able to perform reduced or rotational testing for non-critical tests.
- The majority of methods use some form of *chromatography*.

TGA Guidelines for Schedule 1 Testing

Schedule 1 Testing Requirements for Cannabis Plant Testing

1. aflatoxins
2. foreign matter
3. heavy metals (arsenic, cadmium, lead and mercury)
4. ochratoxin A
5. pesticides
6. total ash

TGA Guidelines for Schedule 1 Testing

Schedule 1 Testing Requirements for Cannabis Plant Testing

1. **aflatoxins**
2. foreign matter
3. **heavy metals (arsenic, cadmium, lead and mercury)**
4. **ochratoxin A**
5. **pesticides**
6. total ash

One Tool or Multiple Tools in the Tool Box

Shimadzu offers reliable turn-key solutions for all aspect of cannabis testing and applications support for tailored workflows.

Complete Analytical Solution:

- ✓ Potency
- ✓ Terpenes
- ✓ Pesticides/Fungicides
- ✓ Residual Solvents
- ✓ Heavy Metals
- ✓ Mycotoxins/aflatoxins
- ✓ Moisture Content

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What is Chromatography?

A way of separating stuff to find out about its constituents.

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What is Chromatography?

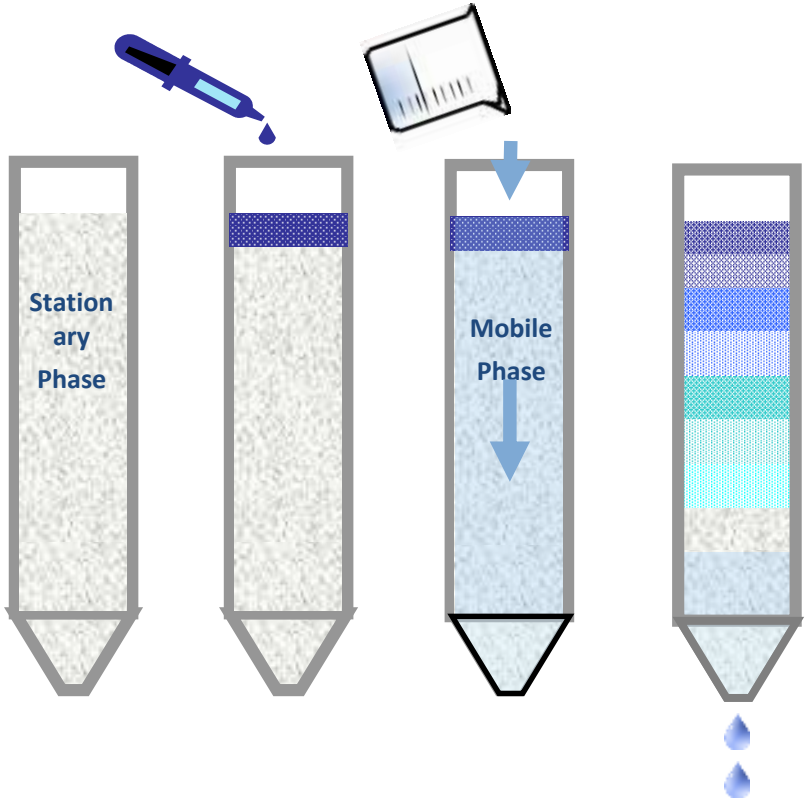
Chromatography is a technique for separating mixtures into their constituent parts.

Chromatography is the interaction between a mobile phase which carries the mixture being separated and a stationary phase which performs the separation.

What is Chromatography?

Mikhail Tsvet

Russian botanist who invented chromatography in 1906 when using liquid solid adsorption to separate plant pigments using calcium carbonate as a stationary phase and petroleum ether/ ethanol as mobile phase.



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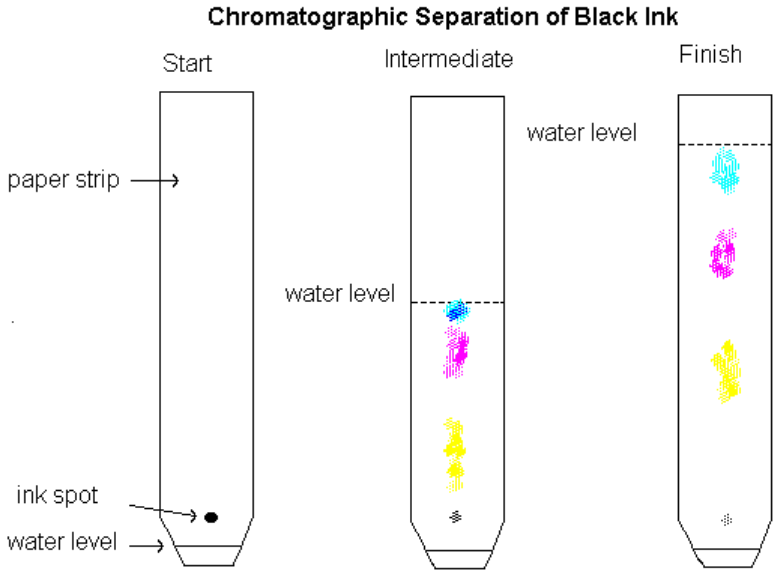
What is Chromatography?



- The compounds in our mixture interact with the paper in their own way.
- We push them across the paper with a moving solvent.
- The compound that forms the weakest interaction with the paper or has the highest solubility in the solvent will move the biggest distance.

Paper chromatography

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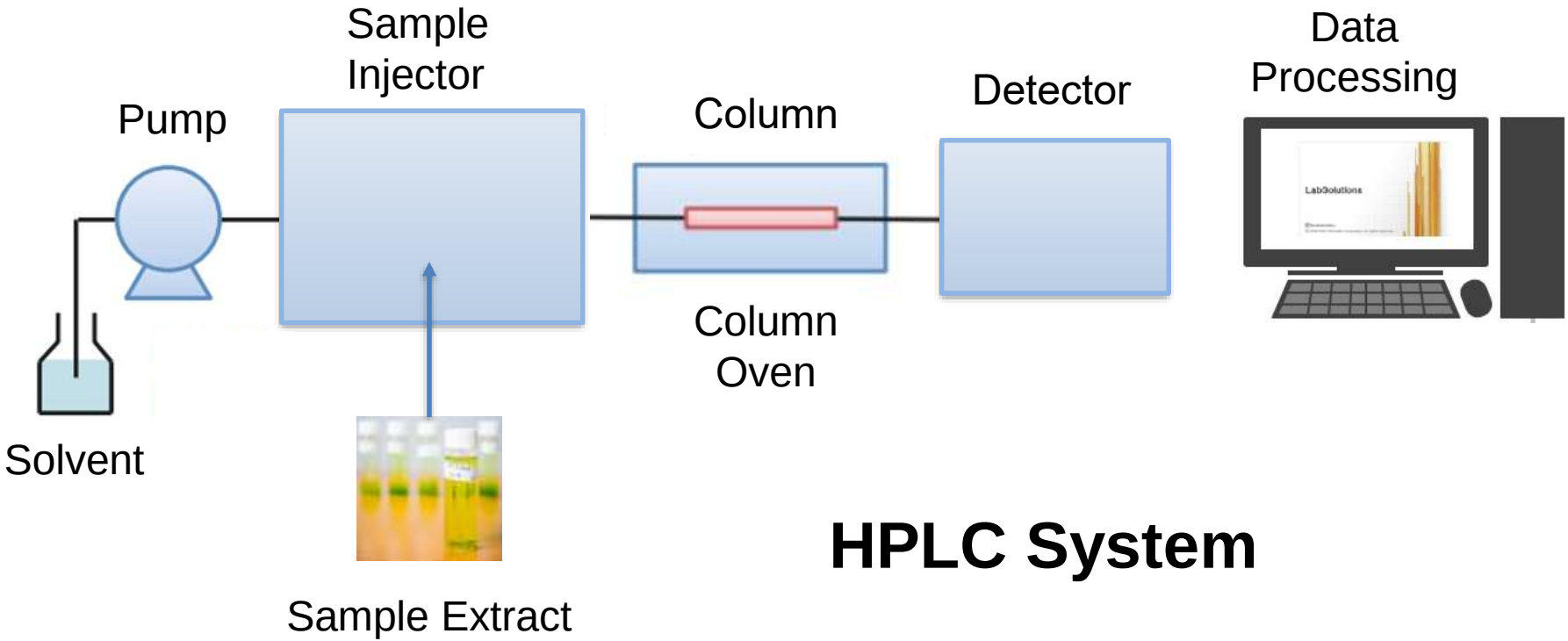
What is Chromatography?



HPLC System

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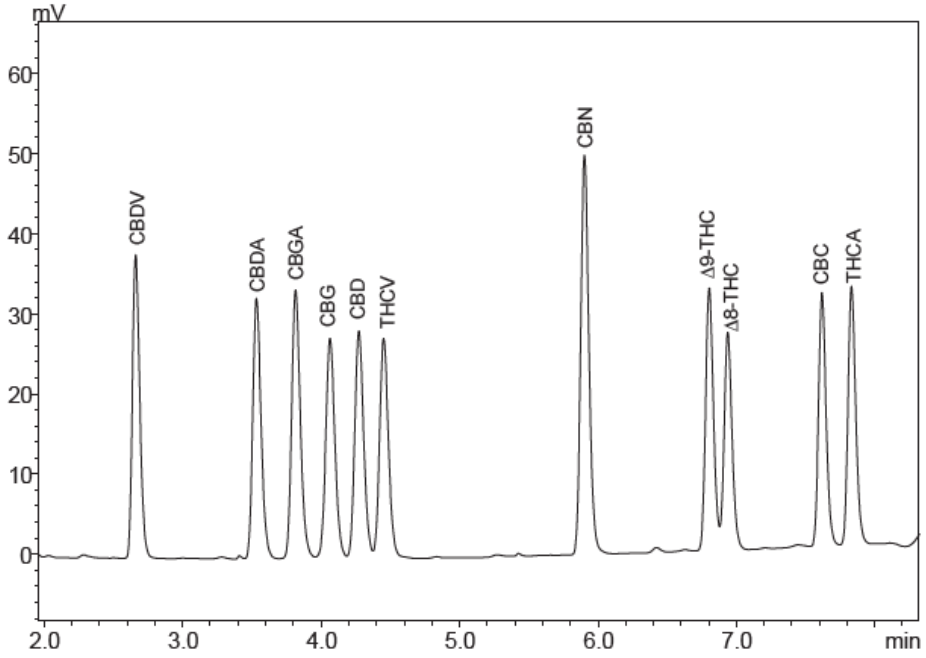
What is Chromatography?

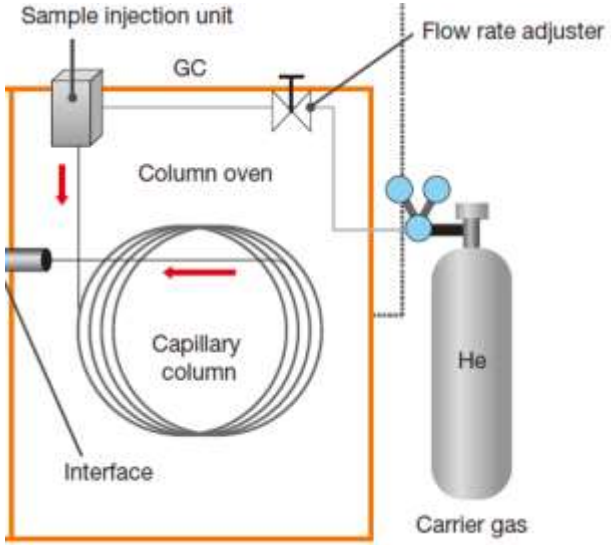


Chromatogram

What is Chromatography?

GC System

Detector



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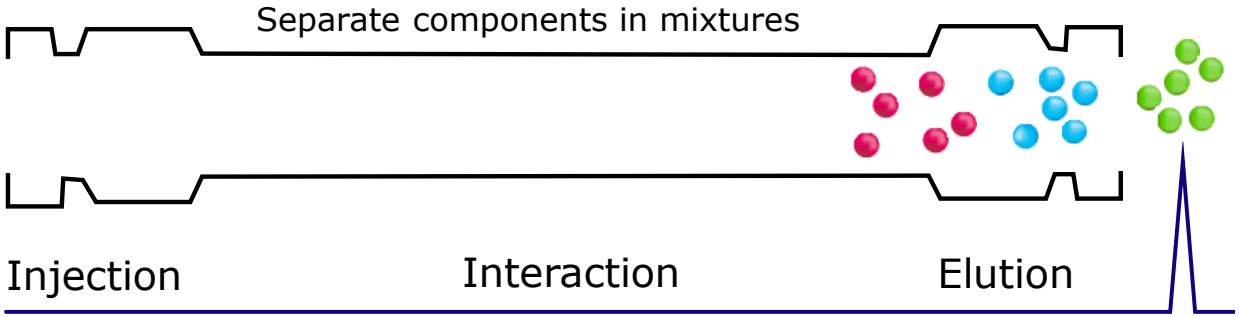
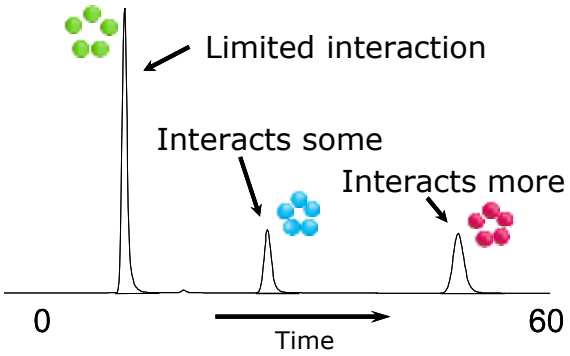
What is Chromatography?





The separation process

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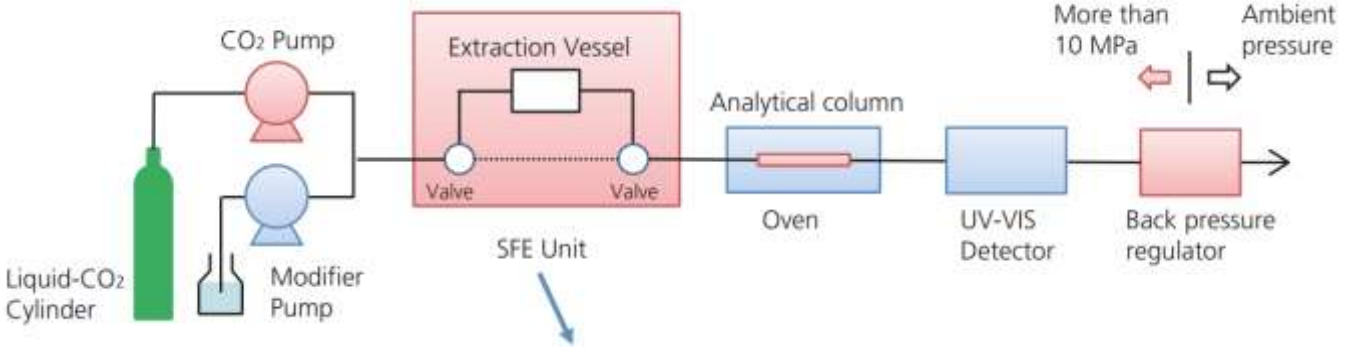
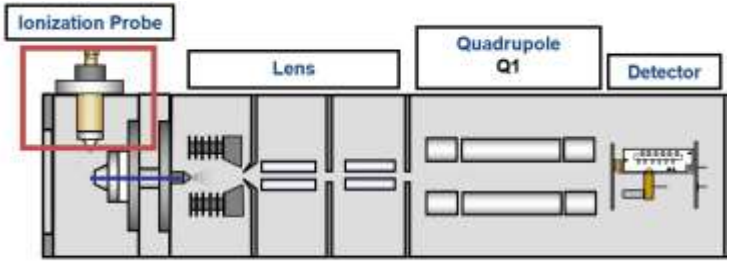
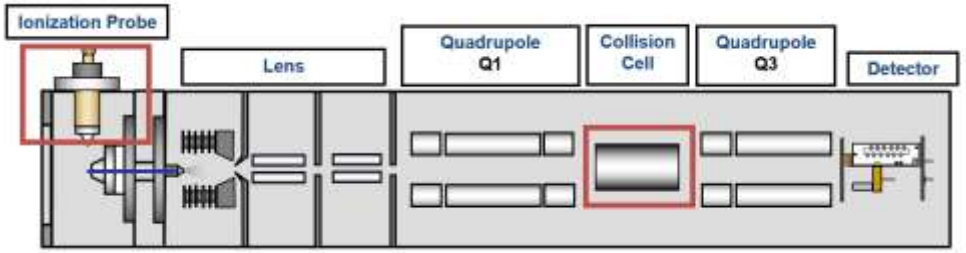
What is Chromatography?



SFE/SFC System

Detection Method



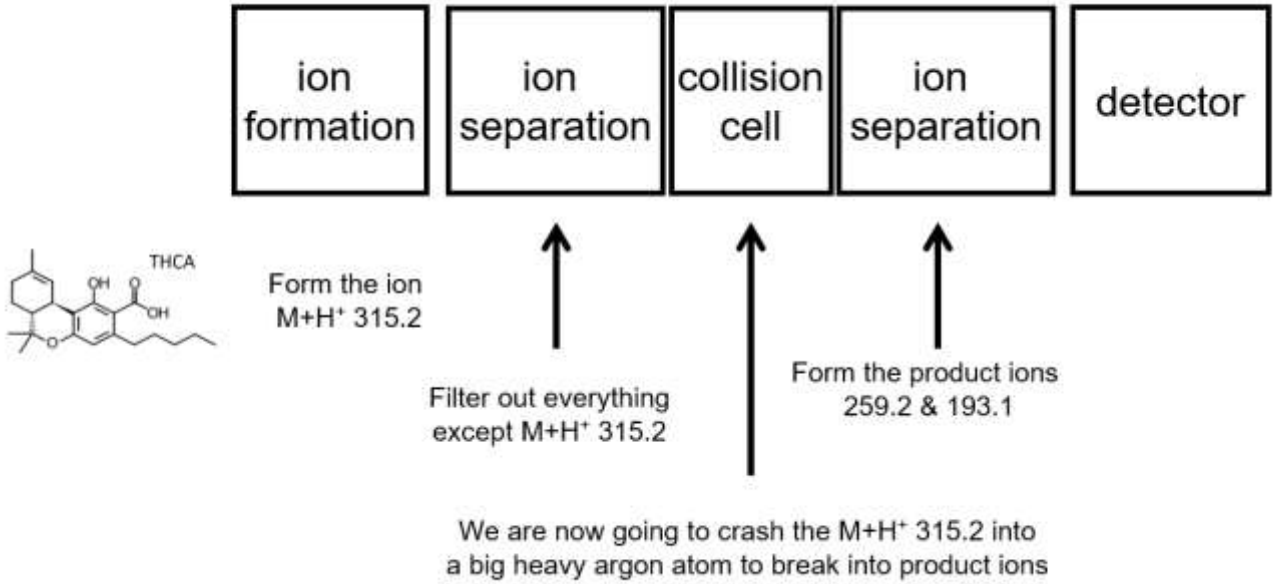


LCMS

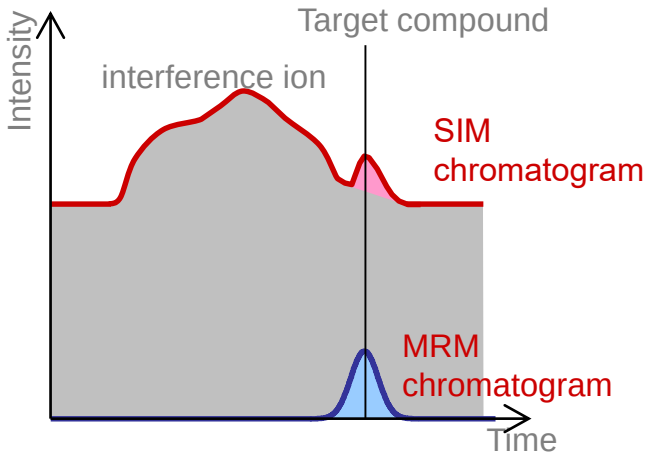
LCMSMS

Detection Method

MSMS



Detection Method



- With MRM, ion intensity is reduced, but chemical noise is reduced more, so S/N ratio is enhanced
- MRM is highly sensitive and extremely selective for quantitation
- MRM detection limit is lower than SIM by 10x
- Linear range extended to lower concentrations

MS Compared to MSMS

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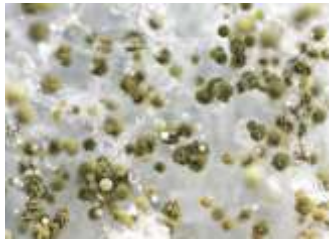
Analogy for Reporting Units

- The major distinction between analytical techniques is their sensitivity

- Analogy:
  - 1mg/kg or 1 ppm ~ 1 drop in a kitchen sink
  - 1ug/kg or 1 ppb ~ 1 drop in a swimming pool
  - 1ng/kg or 1 ppt ~ 1 drop in a small lake

Aflatoxins & Ochratoxin A

- Cannabis's high moisture content combined with long-term storage of the material can be a source of mold growth.
- The mold itself can also cause diseases such as lung infection.
- Aflatoxins are a toxic secondary metabolite of mold.
- Aflatoxins are found in soil and decaying vegetation.
- Aflatoxins G1, G2, B1, B2, and Ochratoxin A, are immunosuppressive, carcinogenic, neurologically toxic, and hepatotoxic.
- TGO 93 Schedule 1 states not more than 2ug/kg of aflatoxin B1 and not more than 4ug/kg for sum of aflatoxin B1, B2, G1,G2, Ochratoxin A not more than 20ug/kg



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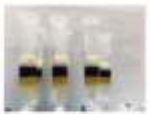
Aflatoxins & Ochratoxin A

- Aflatoxins & Ochratoxin can be analysed by HPLC with fluorescence detection.
- Due to the complexity of the cannabis plant the most sensitive method for aflatoxins is on LCMSMS.
- QuEChERS method for sample prep.
- Can be combined with pesticide screen on LCMSMS.


Homogenize Sample


QuEChERS Extract (top layer)


Extract Before SPE Cleanup


Cartridge SPE Manifold


Final Extract Ready

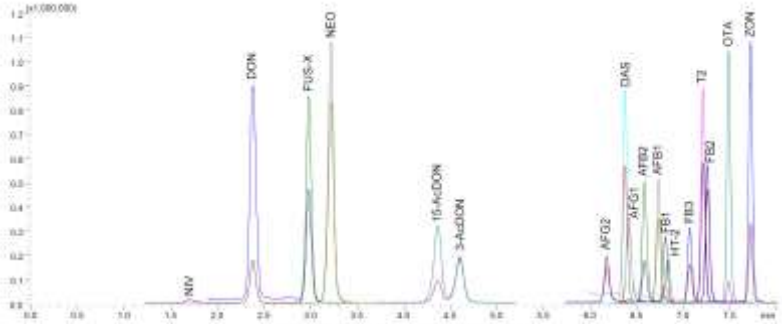


Fig 1 MRM chromatograms of 18 mycotoxins

AFB1 (aflatoxin B1; 1µg/kg), AFB2 (aflatoxin B2; 1µg/kg), AFG1 (aflatoxin; 1µg/kg), AFG2 (aflatoxin G2; 1µg/kg), OTA (ochratoxin A; 1µg/kg), FB1 (fumonisin B1; 100µg/kg), FB2 (fumonisin B2; 100µg/kg), FB3 (fumonisin B3; 100µg/kg), 15-AcDON (15-acetyldeoxynivalenol; 100µg/kg), 3-AcDON (3-acetyldeoxynivalenol; 100µg/kg), DON (deoxynivalenol; 100µg/kg), DAS (diasteoxyscirpanol; 100µg/kg), FUS-X (fusarenon-X; 100µg/kg X), HT-2 (100µg/kg), T-2 (100µg/kg), NEO (neosolaninol; 100µg/kg), NIV (nivalenol; 100µg/kg), ZON (zearelenone; 100µg/kg).

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Moisture Content

- Dried cannabis typically has a moisture content of 10-12%.
- A moisture content above 12% is prone to fungal growth (mold).
- The best way to analyze the moisture content of any product is the thermo-gravimetric method.
- This process involves placing the sample of cannabis into the sample chamber and taking an initial reading. Then, the moisture balance will heat up until all the moisture has been evaporated out of the sample. A final reading is then taken to determine the percent weight of moisture that was contained in the original sample

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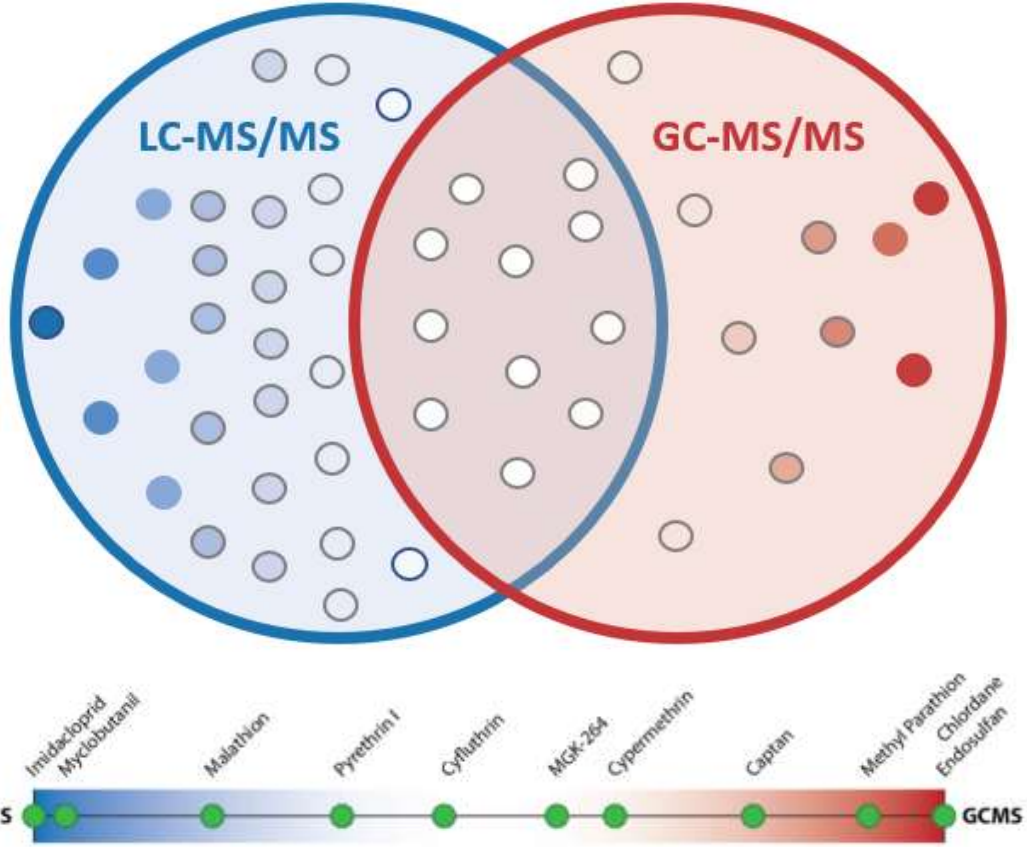
Pesticides

- Pesticides are used commercially on cannabis crops to kill many pests that thrive on the plants and in greenhouses.
- Many pesticides and especially lipophilic ones are slow to degrade and therefore persistent in the environment. This means even neighboring crops can be contaminated due to rainfall, irrigation run off or wind.
- Testing for pesticides is one of the more problematic analyses due to the difference in chemical properties of different classes of pesticides.
- Not all pesticides can be run on GCMS or LCMS. You require both techniques to cover all classes of pesticides.
- Therefore the best solution is to run certain classes of pesticides on GCMS or GCMSMS for better specificity and detection limits of LCMSMS.

Pesticides



Pesticides Analysis in Cannabis by Instrumentation

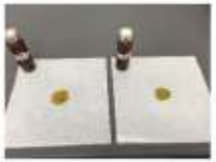
Pesticides

- TGO 93 Schedule 1 states pesticide levels must be less than listed in EU Pharmacopoeia Version 9.6 Chapter 2.8.13 (Table 2.8.13)
- If the presence of a pesticide is suspected for any reason that is not listed in the above table, refer to Regulation (EC) No.396/2005
- Shimadzu's GCMS Cannabis Method Package can detect all pesticides except dithiocarbamates in a single run.
- Typically analysis would be done on GCMS or for greater sensitivity GCMSMS.
- Shimadzu also offers an LCMSMS Cannabis Method Package for pesticides that covers over 200 pesticides.

Pesticides

- Method Package for GCMS or GCMSMS
- QuEChERS method for sample prep

.

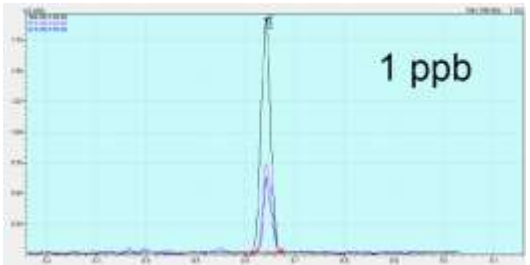

Homogenize Sample


QuEChERS Extract (top layer)


Extract Before SPE Cleanup

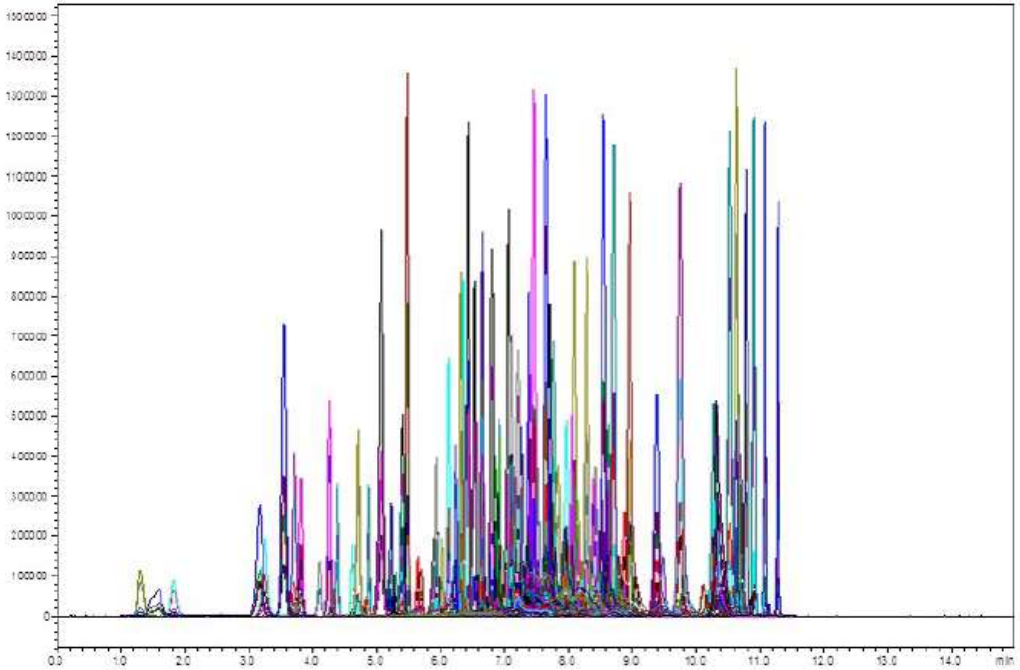

Cartridge SPE Manifold


Final Extract Ready For GC-MS/MS



Pesticides

LC-MS/MS Analysis of 211 Pesticides



High sensitivity LC-MS/MS analysis of 211 pesticides in cannabis dry product in under 12 minutes using a Shimadzu LCMS-8040 triple quad mass spectrometer.

Polarity Switching is Vital as Some Compounds only Work in Negative Mode

Shimadzu LCMS-8060

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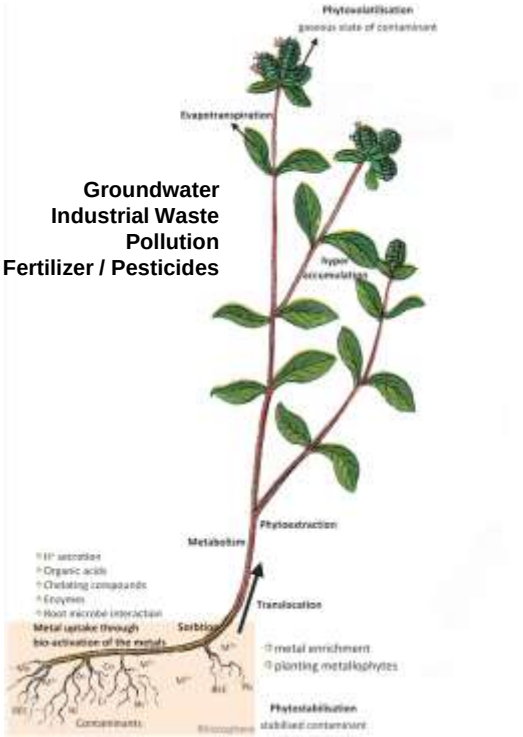
Heavy Metals



Why worry about heavy metals?

- As plants grow, they bioaccumulate metals from the soil and surrounding environment into their plant tissue.
- Often times, these elements can be beneficial / nutritional, such as iron in legumes or potassium in almonds.
- Sometimes, however, plants can store potentially toxic and/or harmful elements, such as As, Cd, Hg, and Pb.

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Heavy Metals

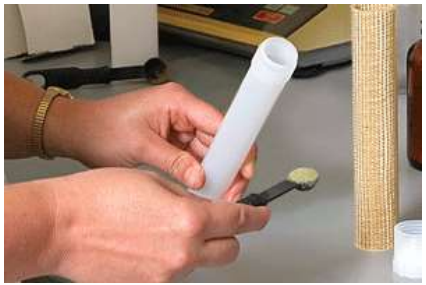
- TGO 93 Schedule 1 state the following requirements for metals;
  - Arsenic not more than3.0ppm
  - Cadmium not more than 0.5ppm
  - Lead not more than 5.0ppm
  - Mercury not more than 0.5ppb

- ICP-MS is the instrument of choice for the analysis of heavy metals in cannabis.
- ICP (Inductive couple plasma) is an electrical flame at 10,000 degrees which ionizes the metals to be detected by the mass spectrometer.
- This instrument can analyse more than 70 elements simultaneously within a minute.

Heavy Metals

Sample Preparation

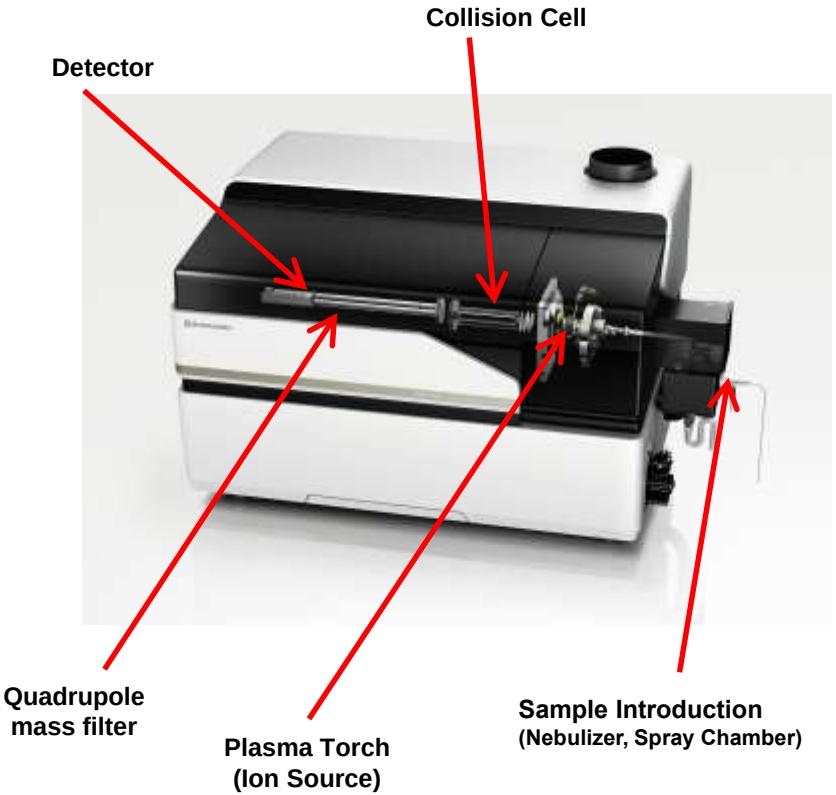
- Samples are analyzed in solution – that means that they need to be dissolved.
- The most common method is microwave digestion.
- Sample of known mass is added to digestion vessel with reagents (acids) and heated/pressurized until they are entirely in solution.
- Also, can do hot plate / hot block digestions



Heavy Metals

ICP-MS for Elemental Analysis

- **I**nductively **C**oupled **P**lasma **(ICP)** to ionize samples
- Quadrupole filters analyte ions to generate **M**ass **S**pectra **(MS)**
- *Only* for elemental analysis – not analyzing molecules, fragments, or derivatives.



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THANK YOU

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Thank You.