Sampling & Data Acquisition: An Overview

Dr Maria Anastasiadi

m.anastasiadi@cranfield.ac.uk

13th January 2025

www.cranfield.ac.uk

Contents

1. SAMPLING

Importance
Key considerations

2. SEPARATION

Introduce sample purification techniques

3. ANALYSIS

Introduce analytical techniques for metabolite analysis/identification

Metabolomics: Workflow

1. Study

- Biological question
- Experimental Design

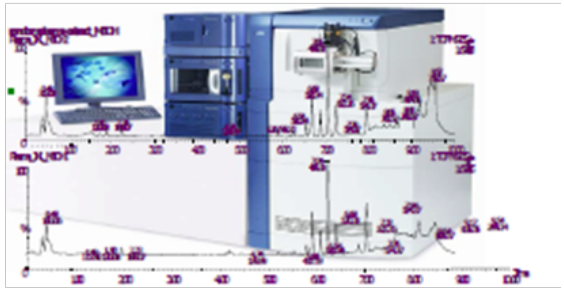
2. Sampling

- Sample collection
- Sample Storage

3. Sample preparation

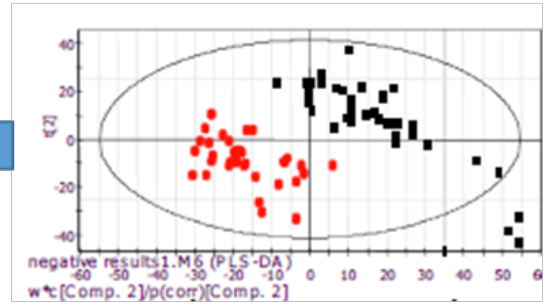
- Sample preparation
- Pre-treatment

4. Metabolite profiling



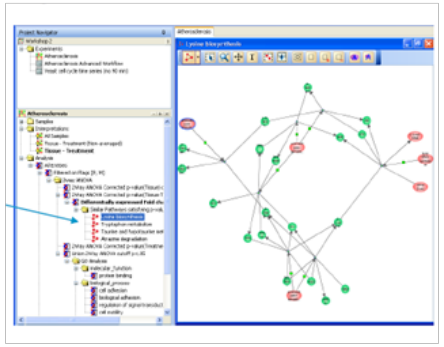
- Data acquisition
- Data pre-processing
- List of peaks/metabolites

5. Data mining



- Data analysis
- Modelling
- Biomarker discovery

6. Biological interpretation



- Database search
- Metabolic pathways

Metabolomics: Sampling

- **Sampling:** The process of collecting biological samples for metabolomic studies.
- First, we identify a specific population of interest for study.
- Key to minimizing sampling bias is ***specificity***.
- Sampling bias can also arise in experimental studies due to “inadequate control.”
- Treatment and control samples should be observed simultaneously, not in sequence.

Metabolomics: Sampling

Three rules of good sampling:

- Everything/everyone has equal likelihood of being sampled (representative sample).
- 2. Take enough samples to reduce sampling error.
- 3. The efficiency is 100% (no handling errors).

Metabolomics: Sampling

Detailed sampling protocol

- How many samples to be taken
- How often the sampling process needs to be repeated
- How much sample is to be taken
- How the sample is to be labelled, stored and transported
- How the sample is to be processed prior to analysis
- How the progress of the sample through the system is to be recorded
- Ethics

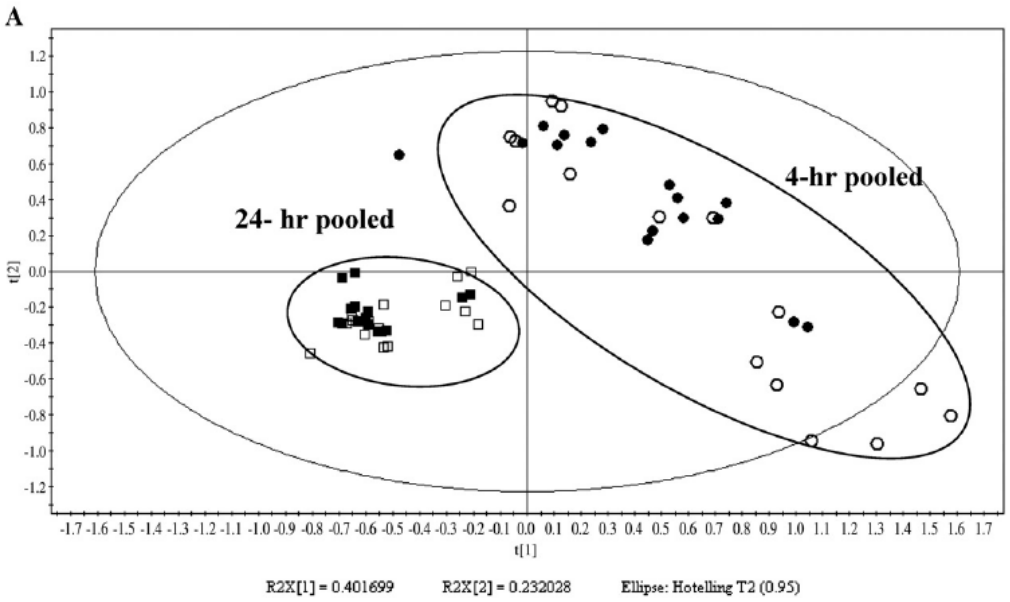
Metabolomics: Sampling

Journal of Bioscience and Bioengineering
VOL. 110 No. 4, 491–499, 2010
www.elsevier.com/locate/jbiosc

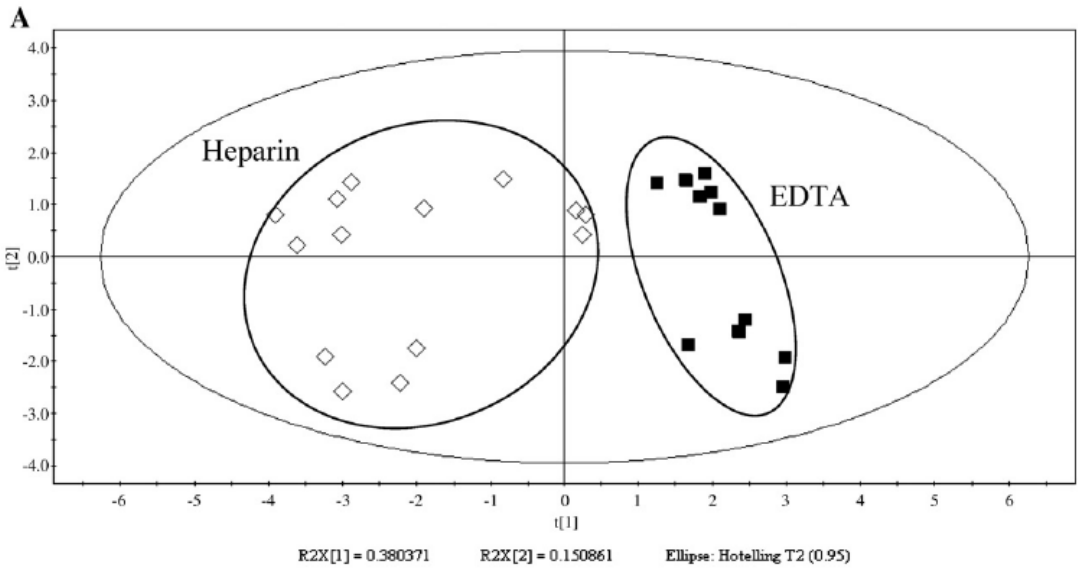
Influences of biofluid sample collection and handling procedures on GC–MS based metabolomic studies

Kiyoko Bando,[1,2] Rui Kawahara,[1] Takeshi Kunimatsu,[2] Jun Sakai,[3] Juki Kimura,[2] Hitoshi Funabashi,[2] Takaki Seki,[2] Takeshi Bamba,[1] and Eiichiro Fukusaki[1,*]

- The metabolic profiles of urine varied significantly when subjected to differing storage conditions (RT vs ice) and sampling duration (4h vs 24h).



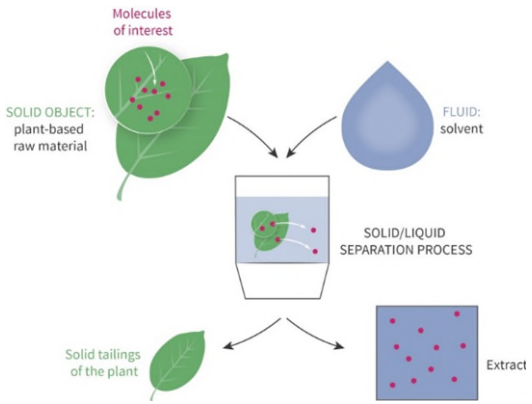
- The anti-coagulant used had a great impact on the GC-MS profiles of biofluids.

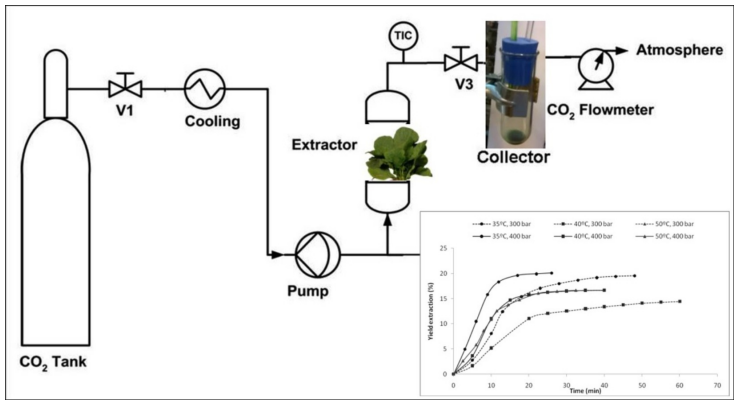


Metabolomics: sample preparation

Sample extraction methods:

- **Solvent extraction**
- **Supercritical extraction**
- **Head space sampling**

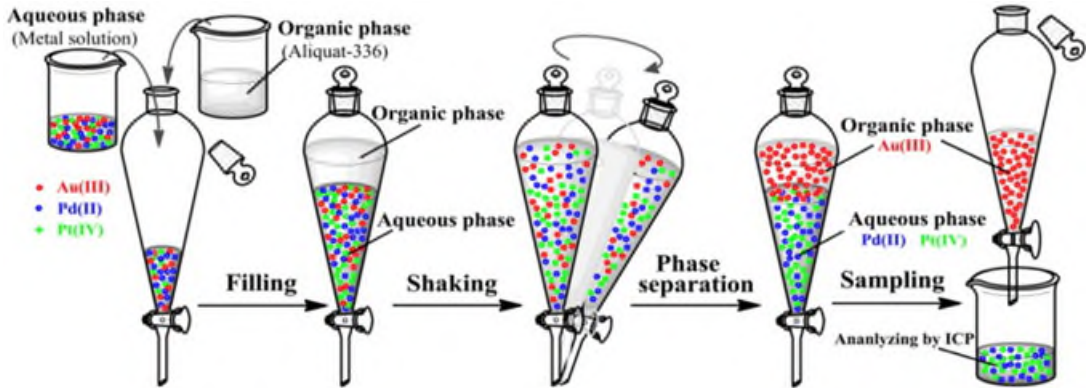




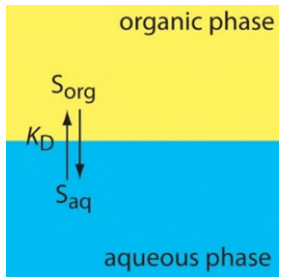
Metabolomics: sample preparation

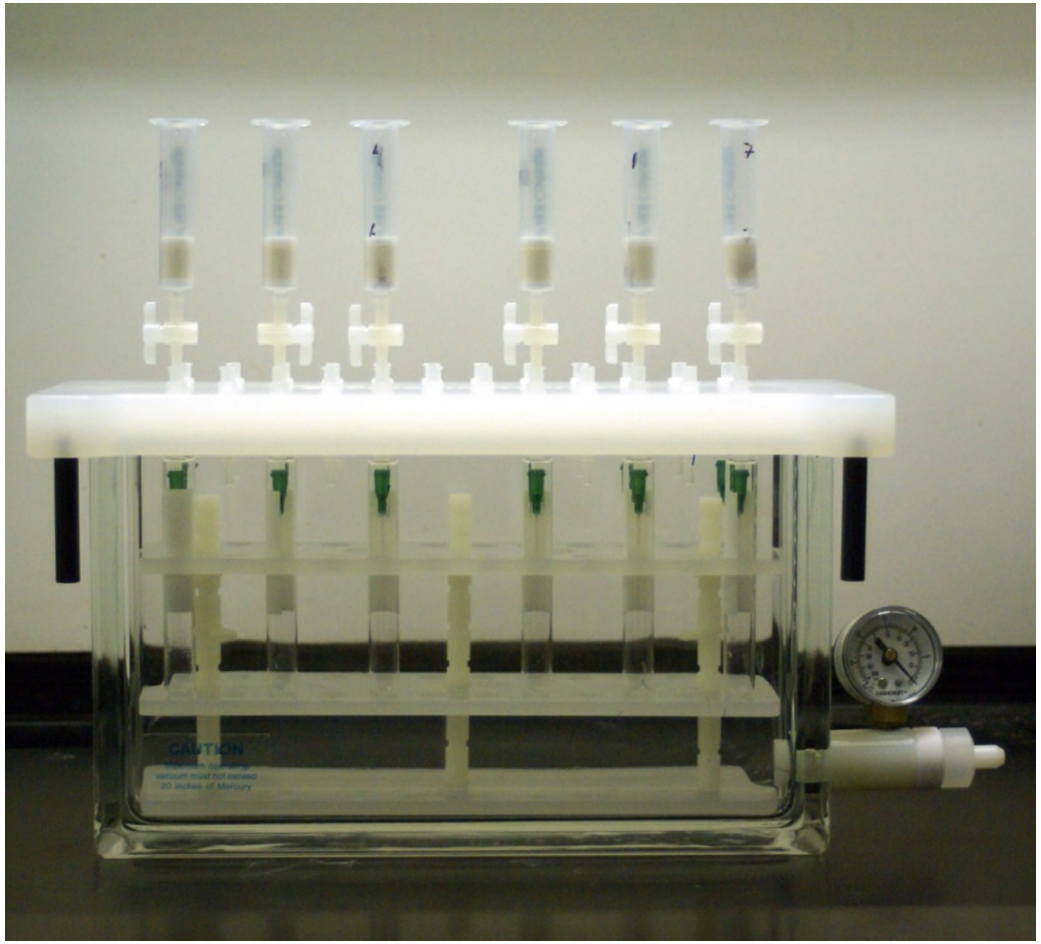
- **Solvent extraction**

Liquid-Liquid Extraction



Wei et al. 2016 *Journal of Molecular Liquids*



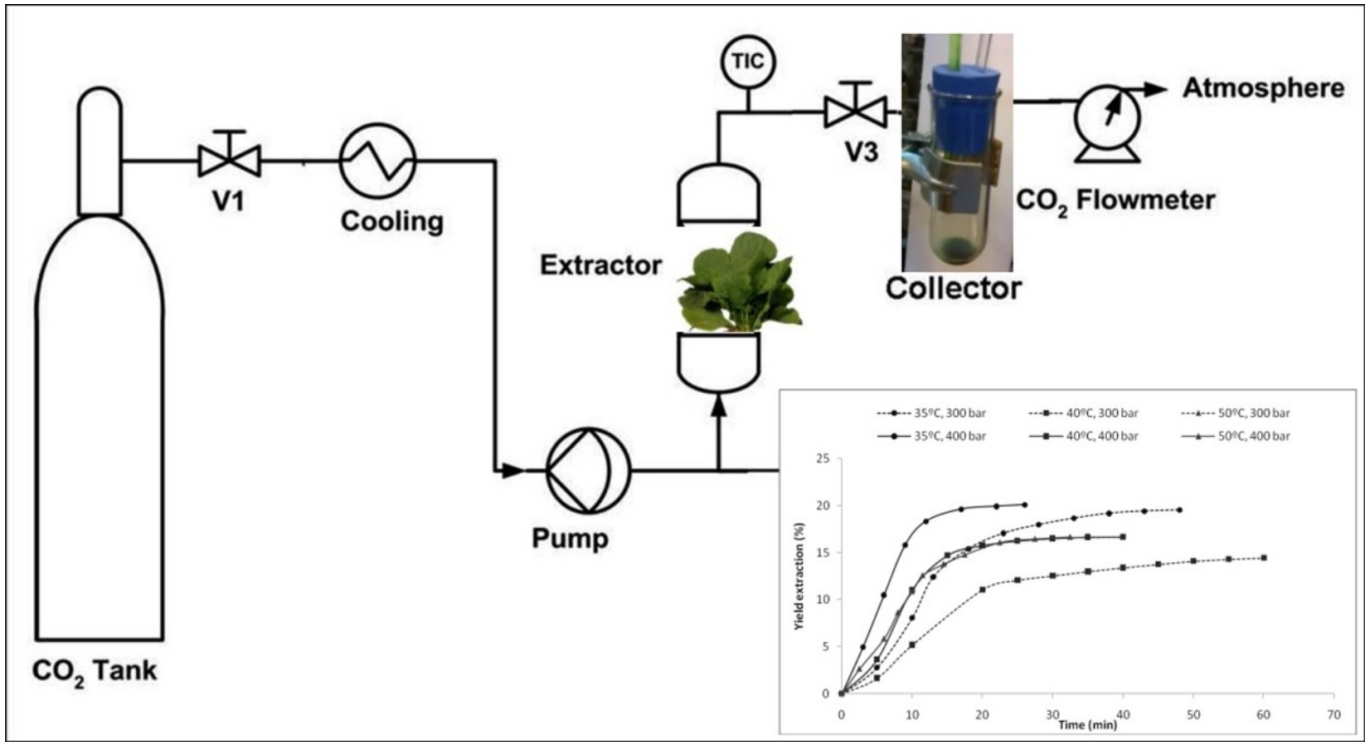


Metabolomics: sample preparation

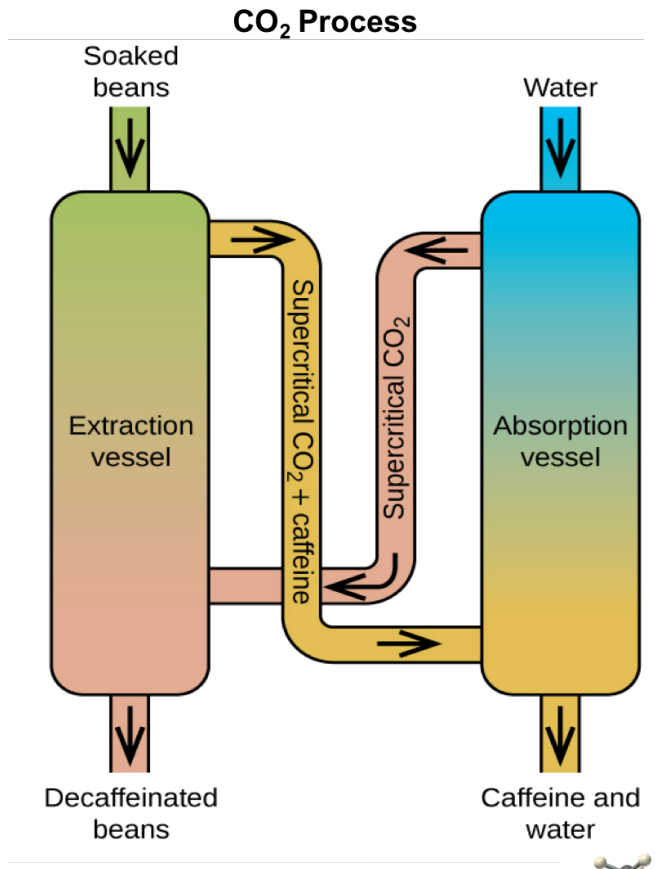
Decaf coffee

Supercritical Fluid Extraction (SFE)

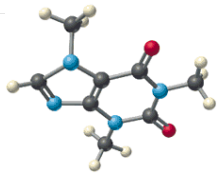
- SFE uses liquid CO_2 (at high pressure) as the mobile phase.



Goyeneche et al., 2018 The Journal of Supercritical Fluids



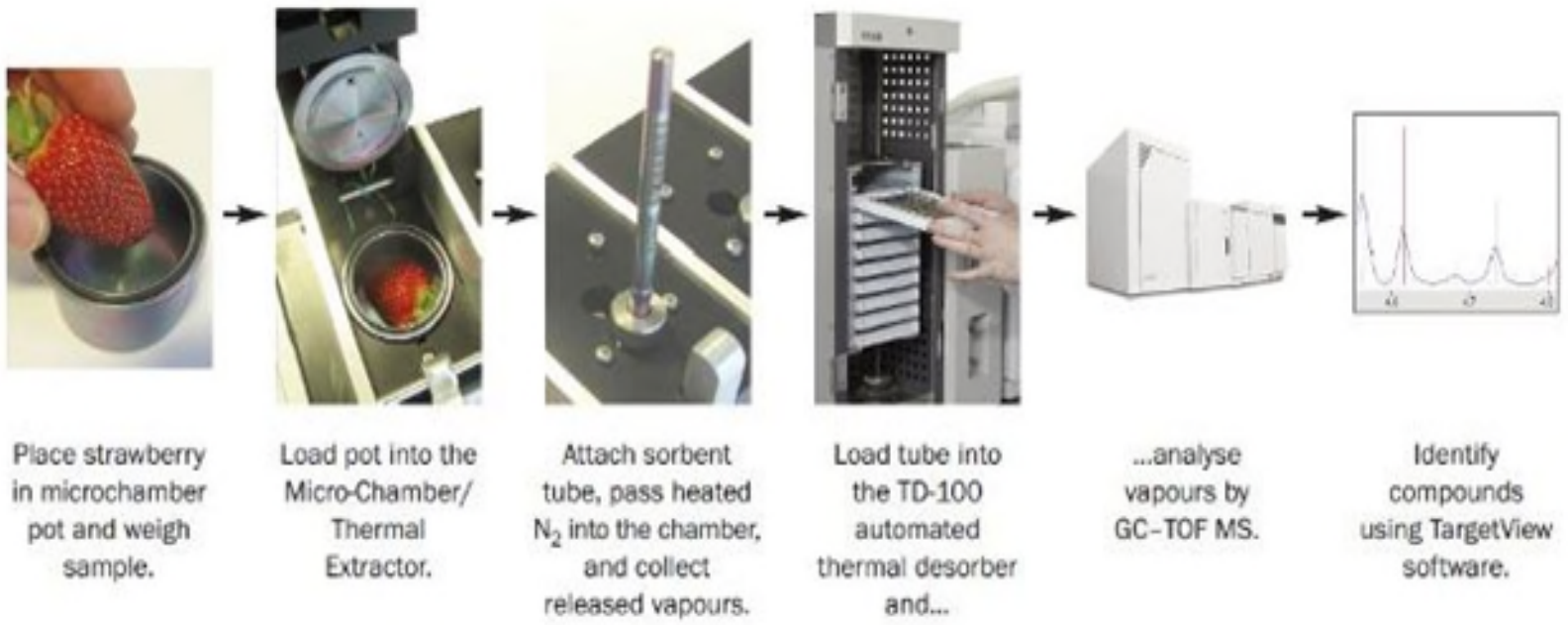
Source: https://cnx.org/contents/oFoO44pW@5/Phase-Diagrams

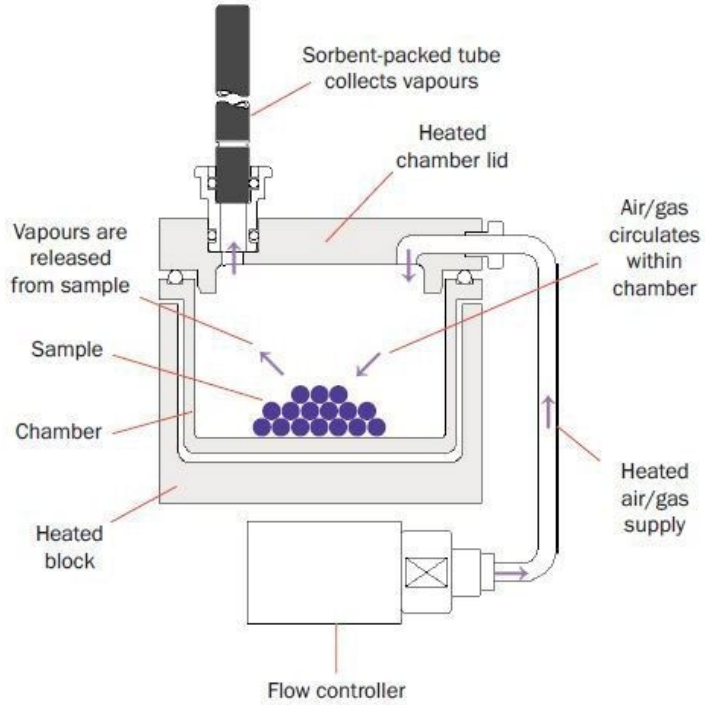


Metabolomics: sample preparation

Sampling of volatile compounds

Micro chamber thermal extractor

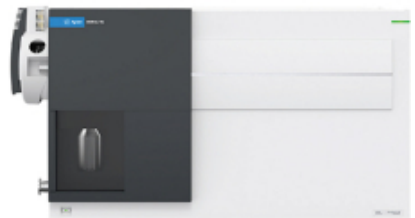




www.azom.com/article.aspx?ArticleID=11439 (last accessed 10/12/24)

Metabolomics: Sample Analysis

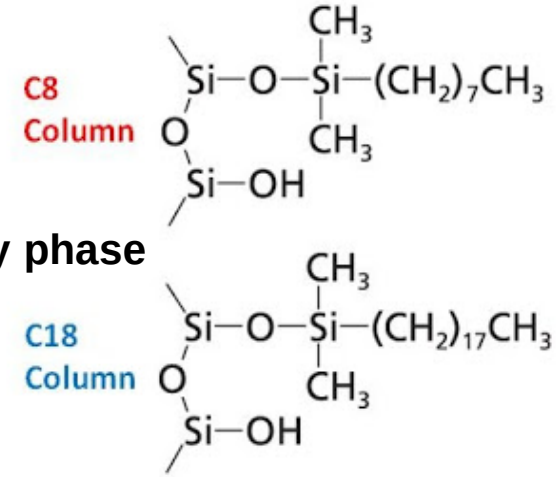
- Separation Techniques
  - Gas Chromatography (GC)
  - Capillary Electrophoresis (CE)
  - High Performance Liquid Chromatography (HPLC)
  - Ultra Performance Liquid Chromatography (UPLC)
- Detection Techniques
  - Nuclear Magnetic Resonance Spectroscopy (NMR)
  - Vis/NIR spectroscopy
  - Fluorescence spectroscopy
  - Mass Spectrometry (MS)
- Hyphenated Techniques
  - GC-MS
  - HPLC-MS

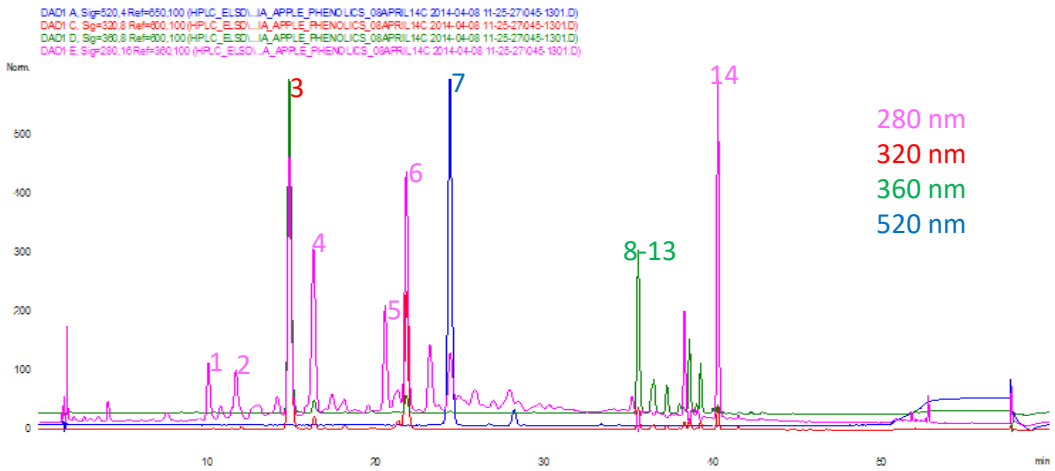


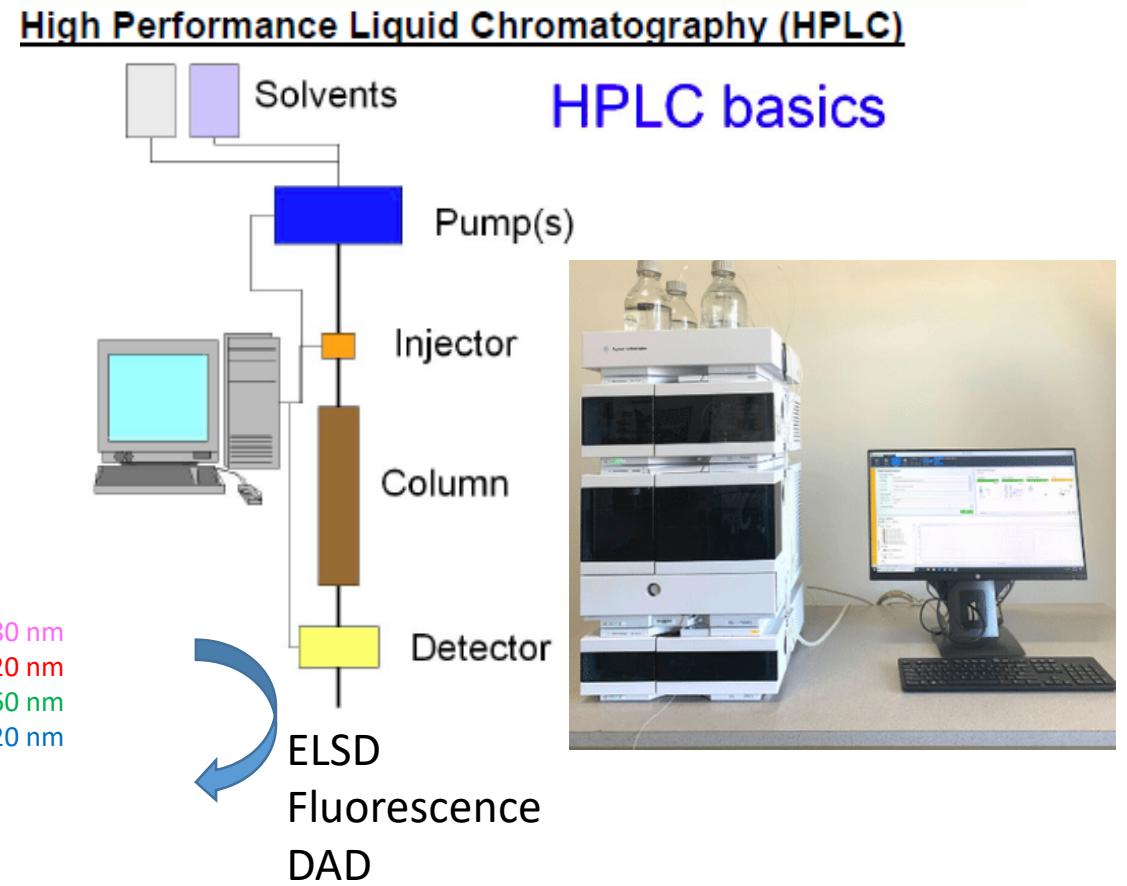
Metabolomics: Separation Techniques

High Performance Liquid Chromatography (HPLC)

Stationary phase





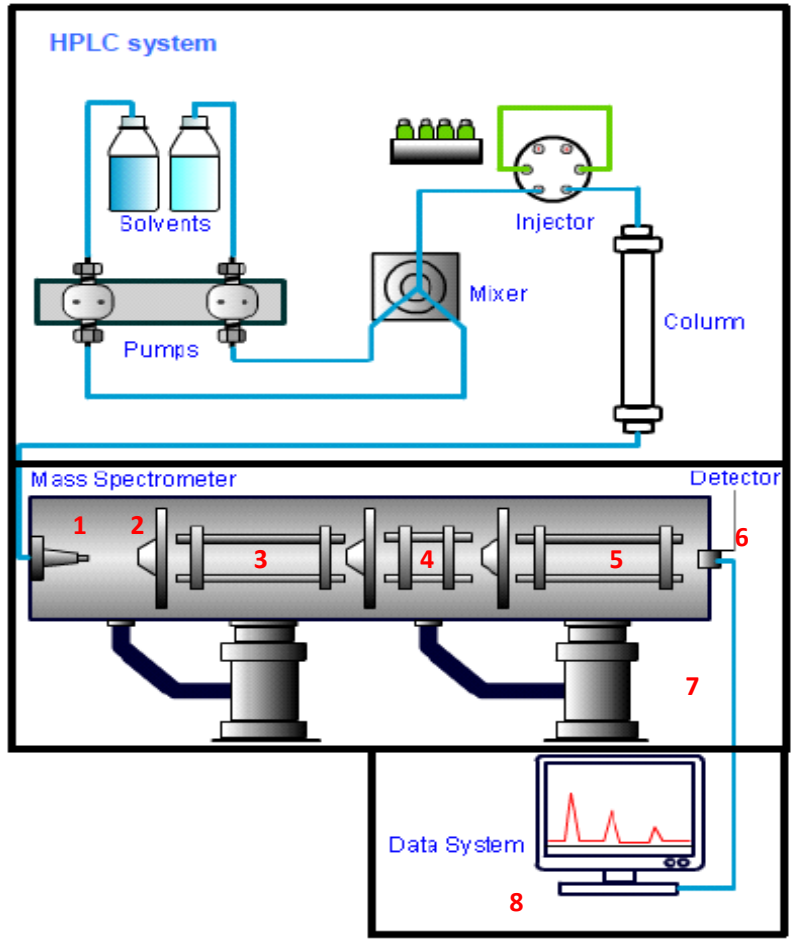


Metabolomics: Hyphenated Techniques

LC/MS basics

Analysis and identification of non-volatile low MW metabolites.

1. **Ion source**
2. **Skimmer cone**
3. **1st Quadrupole (Ion guide)**
4. **Collision Cell**
5. **2nd Quadrupole (for Triple Quad)**
6. **Detector**
7. **Vacuum system**
8. **Data acquisition**



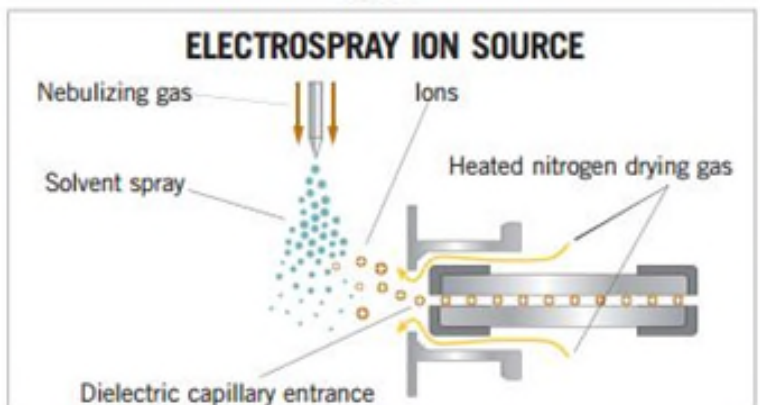
LC/MS diagram

ESI ("soft") ionisation

ESI produces molecular ions

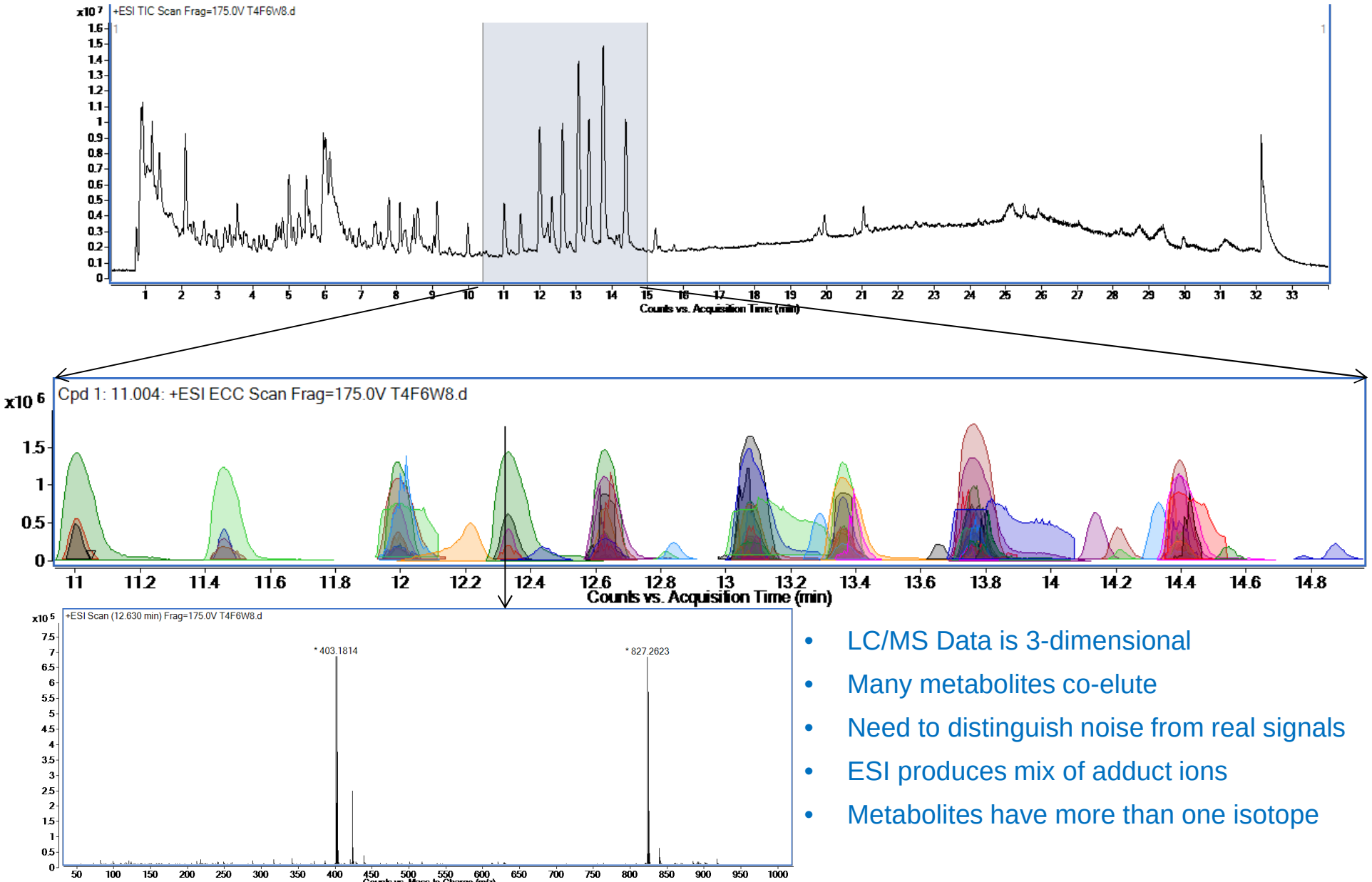
[M-H]+

[M-H]-



www.particle sciences.com

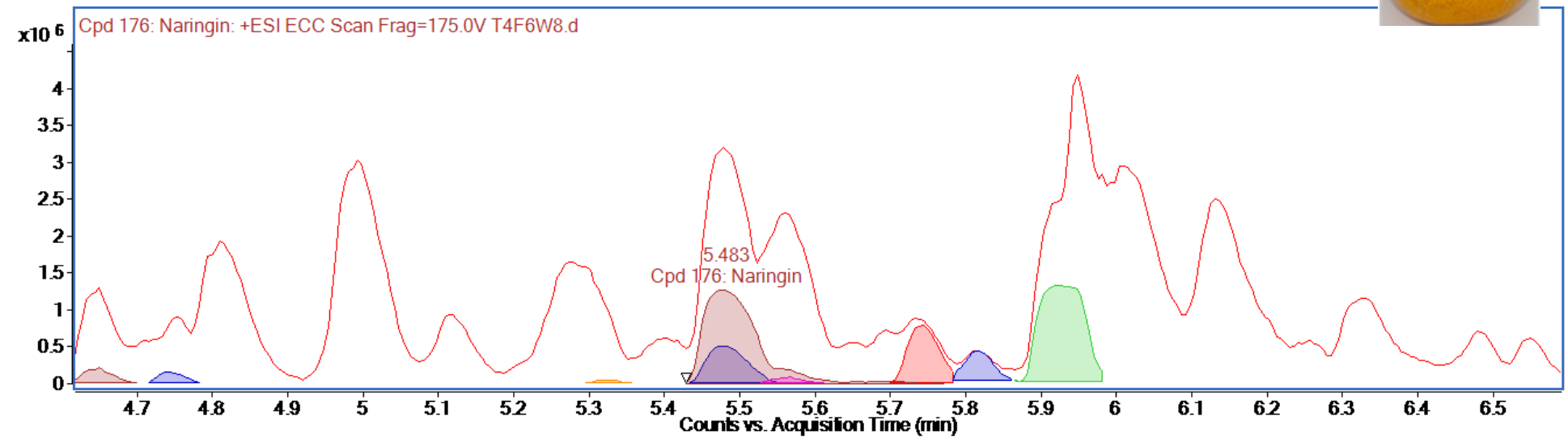
Metabolomics: LC/MS data acquisition

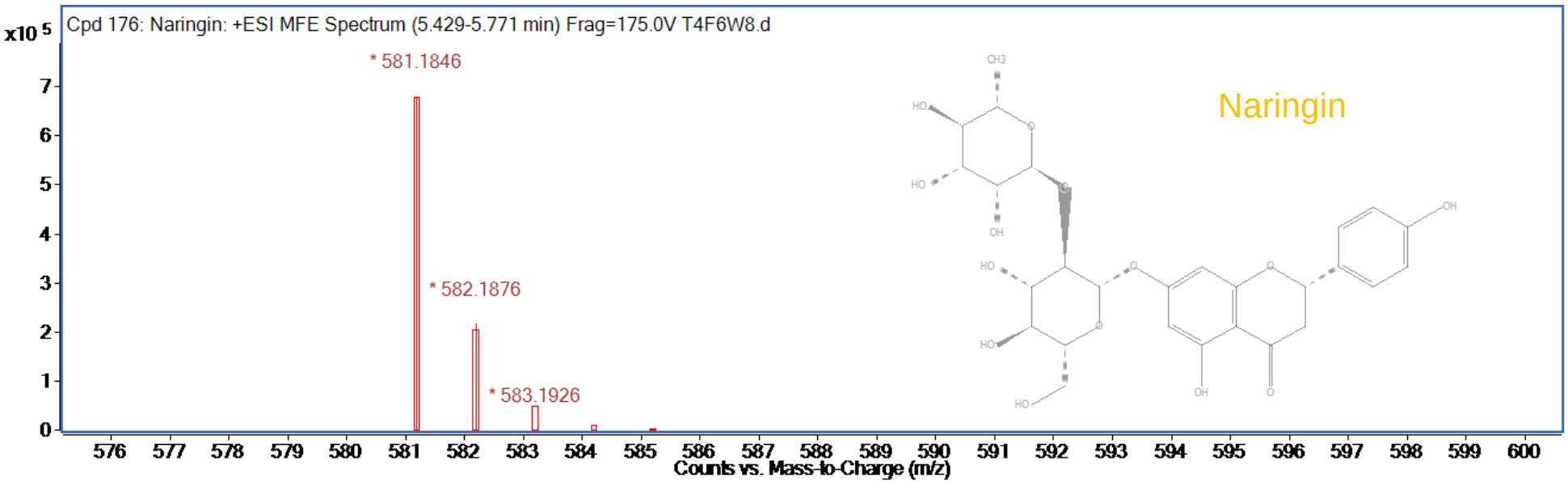


- LC/MS Data is 3-dimensional
- Many metabolites co-elute
- Need to distinguish noise from real signals
- ESI produces mix of adduct ions
- Metabolites have more than one isotope

Metabolomics: LC-MS applications

Clementine rind analysis by reversed phase ESI Positive Ion Mode



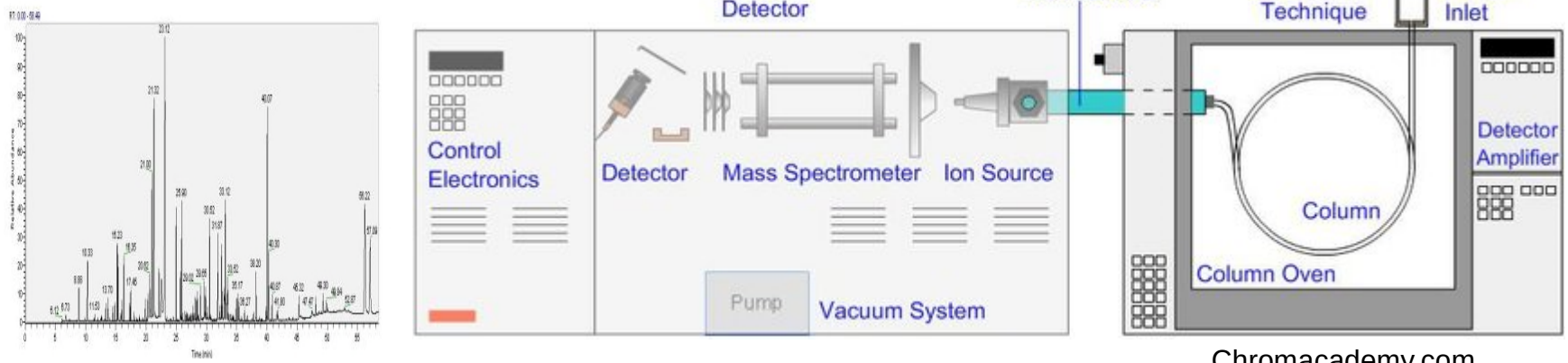


Metabolomics: Hyphenated Techniques

Gas Chromatography Mass Spectrometry (GC/MS)

GC/MS is used for the analysis of thermostable molecules separated in a chromatographic column ionised and detected based on their mass profile

- High Chromatographic resolution
- Separation of several hundred compounds per run
- Very sensitive method
- Databases available (NIST)
- Requires chemical derivatisation which could increase variance

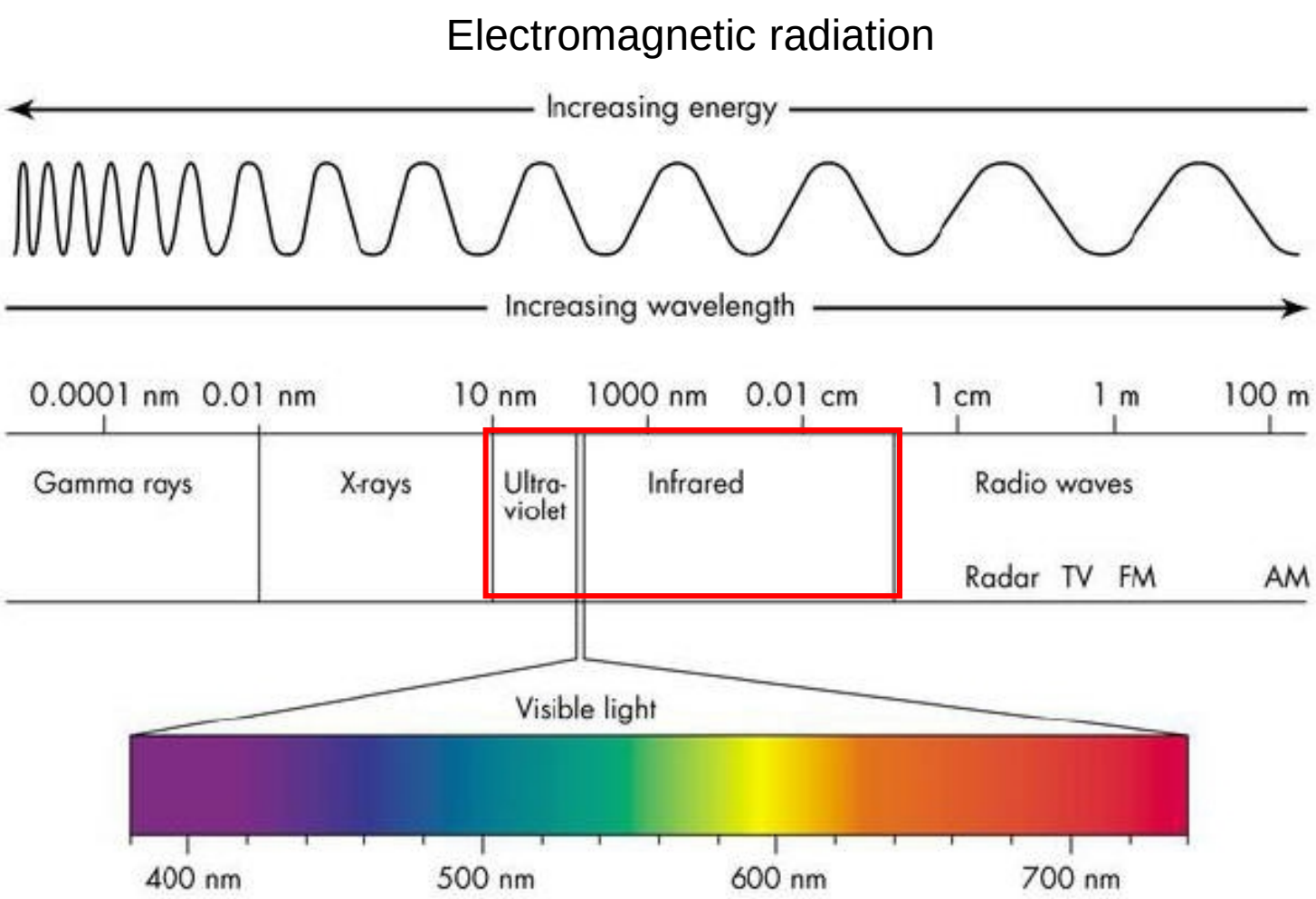


Chromacademy.com

Metabolomics: GC-MS data acquisition

Metabolomics: Detection Techniques

Non-destructive spectroscopic techniques

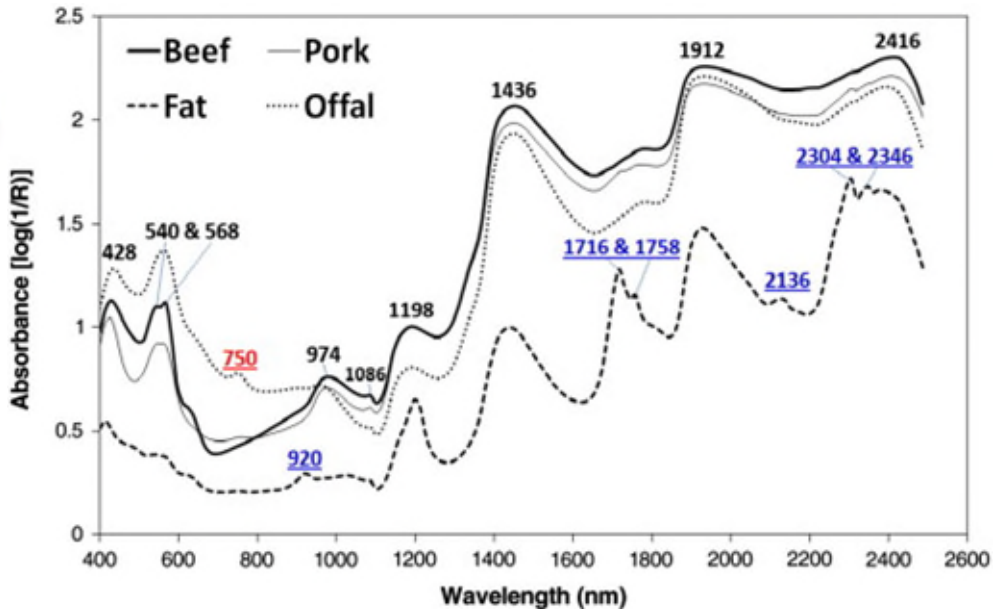


Near-IR (780-2500 nm),
Mid-IR (2500-25000),
Far-IR (25000-1000000 nm)

NIR spectroscopy

- NIR employs photon energy (750 to 2,500 nm) high enough to promote molecules only to their lowest excited vibrational states without promoting electron excitation.
- It provides qualitative/quantitative information for a complex sample based on the interaction of near-infrared electromagnetic waves with its constituents.

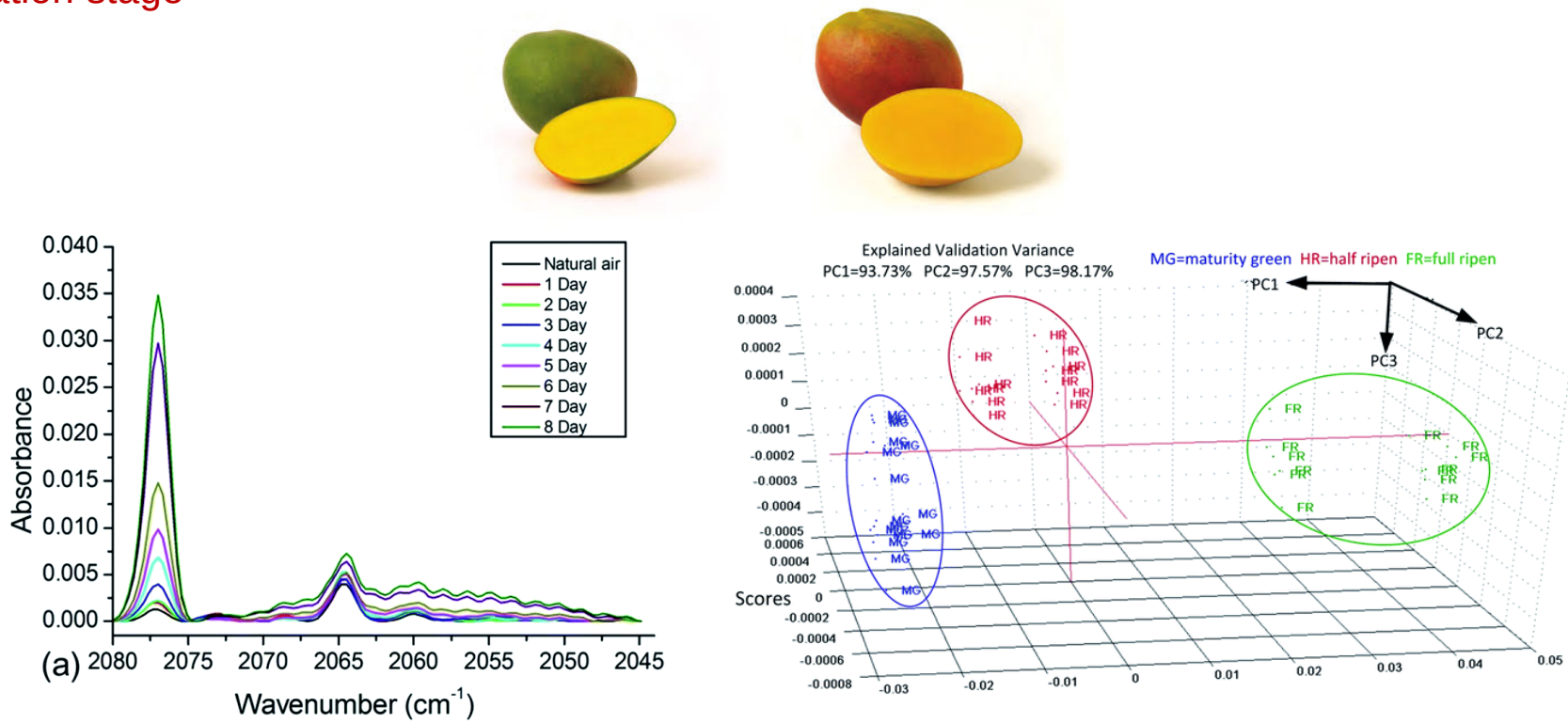
- Fast and non-destructive
- Minimal sample preparation
- Provides chemical and physical information of virtually any matrix (any molecule containing C-H, N-H, S-H or O-H bonds)
- Can be used to construct the metabolic fingerprint of a biological sample.
- Relatively insensitive



(Morsy & Sun, 2013)

FTIR spectroscopy

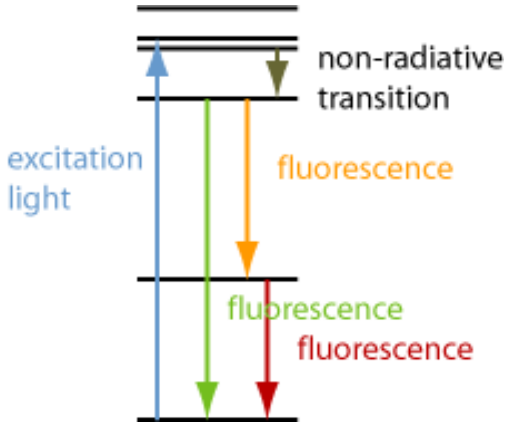
Application example: FTIR can be used to non destructively predict post harvest fruit maturation stage

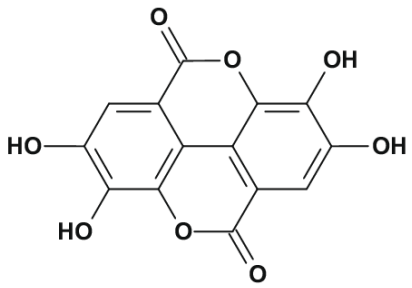


FTIR spectra of mango fruit at different maturity stages related to changes in volatile compounds (Left) and PLSDA scores plot for the prediction of maturity stage based on FTIR data (Right). (*Jiao et al., 2017*)

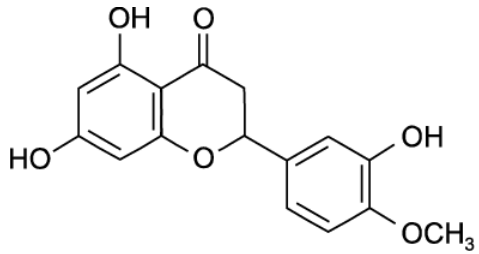
Fluorescence spectroscopy

- Fluorescence spectroscopy uses a beam of ultraviolet or visible light that excites the electrons in molecules of fluorescent compounds, and causes them to emit light at a higher wavelength (lower energy) than the absorption light.
- Fluorescent molecules contain chemical structures called fluorophores.
- Plants and plant-derived food matrices such as honeys contain numerous fluorophores such as polyphenols and amino acids.

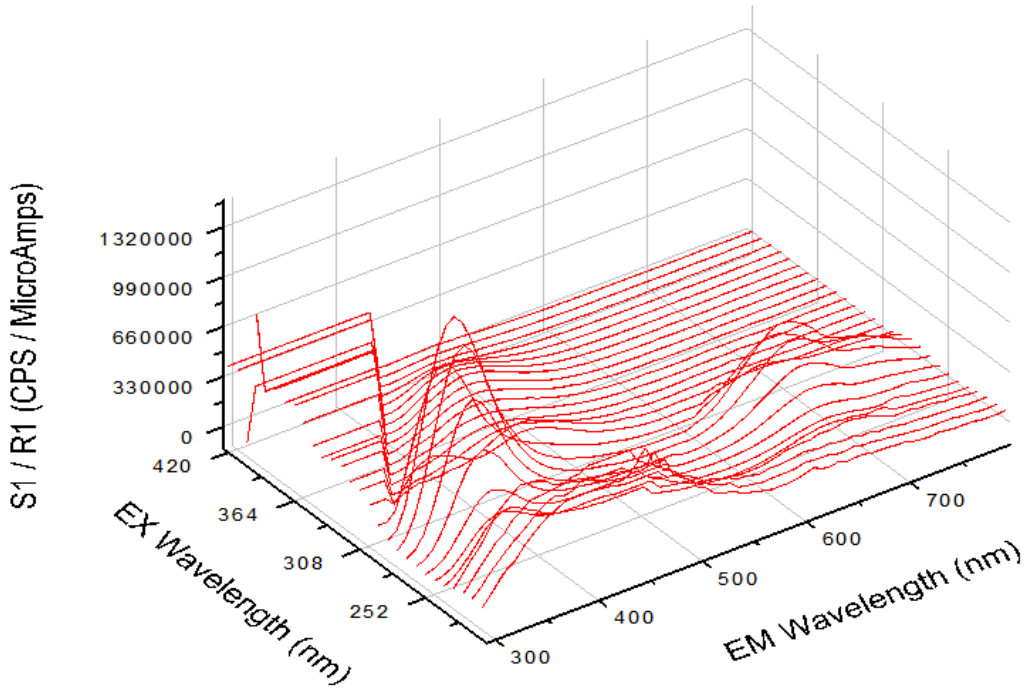




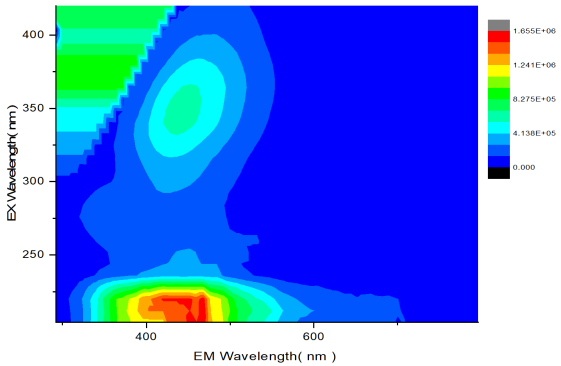
Ellagic acid



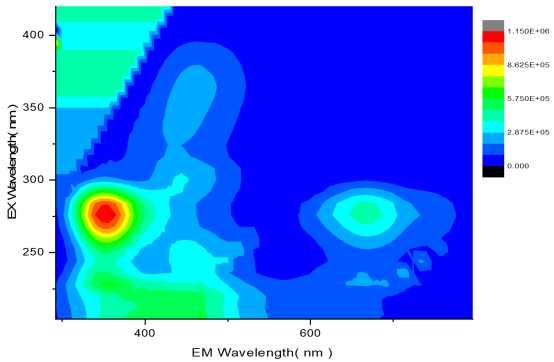
Hesperetin



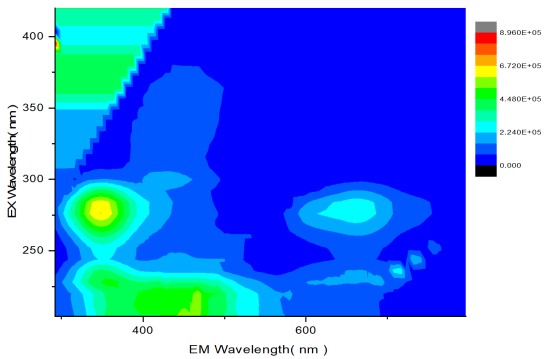
Photonics measurements



Heather honey



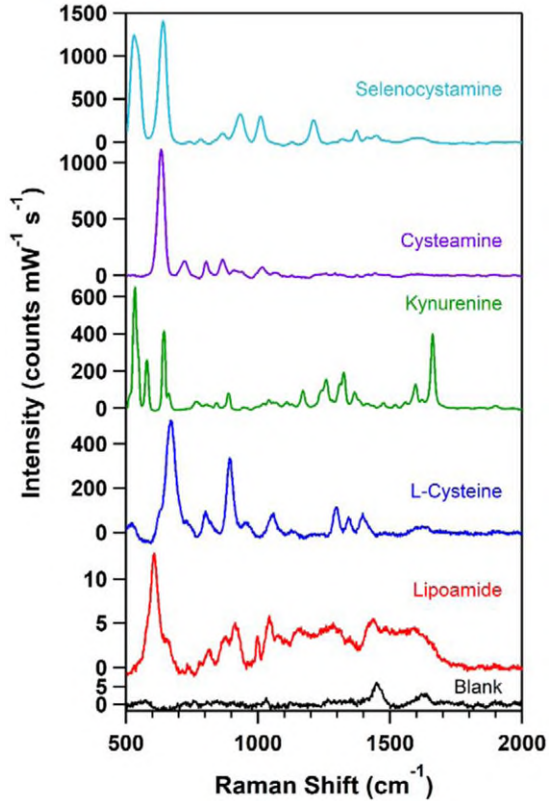
Multifloral honey



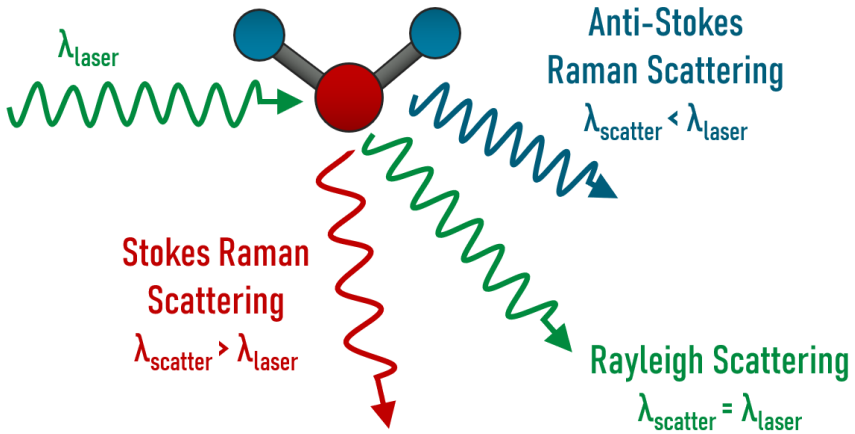
Borage honey

Raman spectroscopy

- Raman spectroscopy is a vibrational spectroscopy conveying rich chemical information on molecules in complex matrices.
- Raman scattering is a kind of inelastic scattering of light.
- After monochromatic light irradiates molecules, the interaction between light and chemical bond vibrations in molecules changes the energy of some of the scattered light, thus providing information on the chemical bond vibrations in molecules



https://doi.org/10.1016/j.talanta.2019.120645



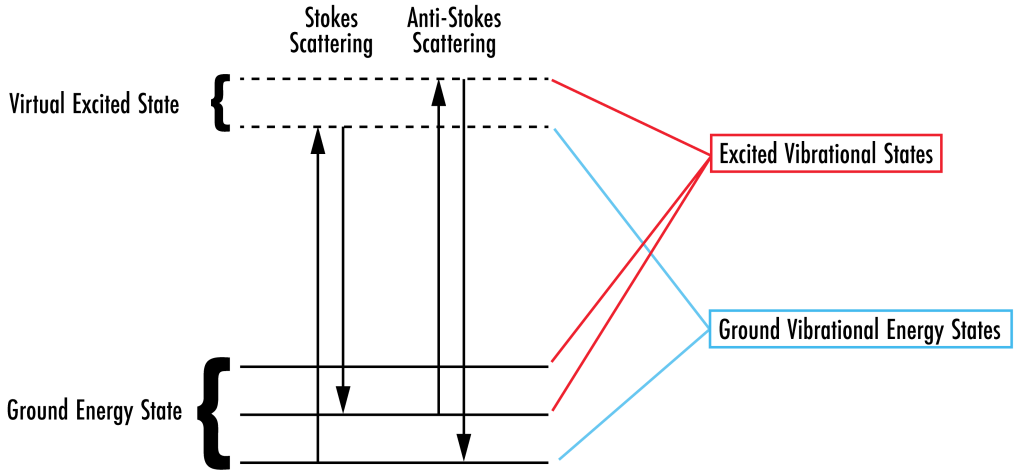
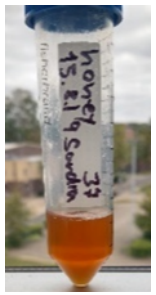


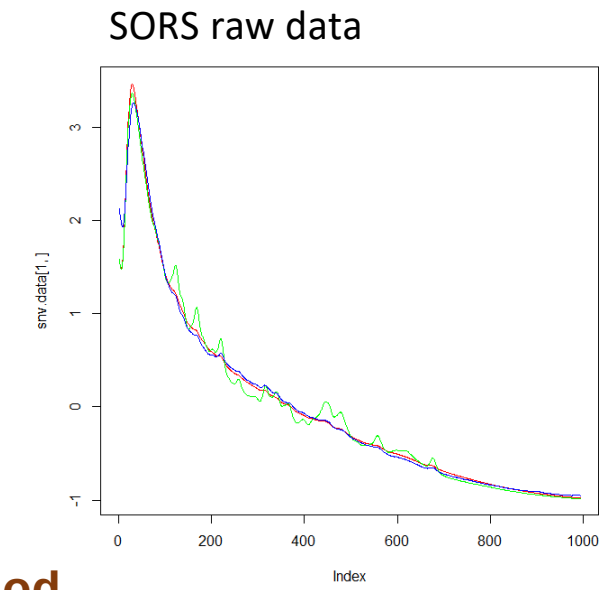
Image source \https://www.edmundoptics.co.uk/knowledge-center/application-notes/lasers/basic-principles-of-raman-scattering-and-spectroscopy/

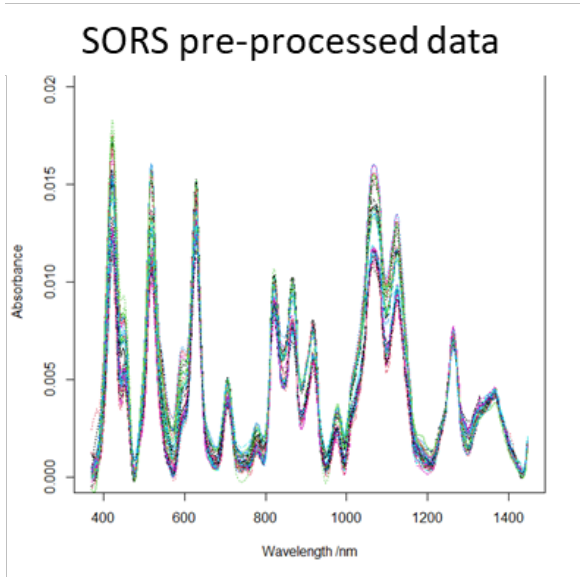
Spatial Offset Raman Spectroscopy (SORS) and Machine Learning

• Develop a SORS screening method for exogenous sugar detection

- Raman spectroscopy is suitable for chemical identification as the spectral information obtained can be used like a 'chemical fingerprint‘ and matched to a known chemical(s) from a spectral library.
- SORS is a new-generation technique capable of screening substances inside non-metallic containers such as plastic and glass bottles.
- It has already found applications in the detection of explosives, toxic industrial chemicals as well as in food authentication.







SORS: Rapid, Non-Invasive method

foods

MDPI

Article

Application of Spatial Offset Raman Spectroscopy (SORS) and Machine Learning for Sugar Syrup Adulteration Detection in UK Honey

Mennatullah Shehata [1], Sophie Dodd [1], Sara Mosca [2], Pavel Matousek [2], Bhavna Parmar [3], Zoltan Kevei [1] and Maria Anastasiadi [1,*]

BBC

NEWS

How to detect fake honey without opening the jar

Fake Honey Is Everywhere, and These Tests Can Prove It

Some studies estimate that half of commercial honey could be adulterated.

Article Published: October 1, 2024 | Leo Bear-McGuinness *speaking with* Dr. Maria Anastasiadi

Credit: iStock

Download Article

Summary

Sampling

Good practice

Protocols

Separation

Extraction & purification of metabolites

Liquid-liquid extraction

Solid-liquid extraction

Analytical techniques

HPLC

LC/MS

GC/MS

NIR

FTIR