Liquid chromatography tandem mass spectrometry (LC-MSMS) - the primary tool for trace contaminant analysis

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Outline

• Trace analysis
• How to connect LC and MS?
• Electrospray (ESI) ion source
• LC-ESI-MS
• LC-ESI-MSMS
Trace contaminant analysis

- Samples: almost never pure compounds but *(very) complex mixtures* (matrixes)
  - Food, soil, biological samples, ...

- Analytes (compounds that we determine) often at *trace level*
  - pesticides, drug residues, mycotoxins, ...

- Contents often in the range of ppm and ppb
  - $1 \text{ ppb} \equiv 1 \mu g/\text{kg} \equiv 1 \text{ mg/t}$

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The central problem of trace contaminant analysis:

The analysis method has to find and reliably identify the trace analyte in the presence of a large number of main components

- Organic traces: chromatography coupled with mass spectrometry
LC-MS as analysis technique

• Combines two powerful techniques:

**LC** (liquid chromatograph) separates the analyte from other sample components

**MS** (mass spectrometer) detects and identifies the analytes and determines concentration

LC and MS: strong contrast

• LC separation is carried out in **liquid phase** (mobile phase)

• MS detection proceeds in **high vacuum** ($n \cdot 10^{-6}$ mbar)
Coupling LC and MS

- The connecting interface has to convert compounds in the liquid phase into ions in the gas phase
- Mobile phase must not get into MS
- Developing this interface has been the biggest challenge in development of LC-MS

Electrospray (ESI) ion source

- The liquid flow is dispersed by electric field into small droplets
- The analyte ions evaporate from droplets
- The ions are directed into MS entrance by the electric field

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Spraying gas (N₂)
From LC
Sprayer
Voltage ca 3500V
To Mass Spectrometer
Hot N₂ (curtain gas)

Waste
ESI in action (Positive ions)

Image by K. K. Murray (via Wikipedia)

ESI mechanism with small molecules

Polar, slightly acidified solvent, e.g. Water-MeCN (+ HCOOH)

Charged drop

B: + SH⁺ → H⁻B⁺ + S

H⁻B⁺
ESI Ionization

- The most widely applied ionization method in MS
  - Nobel prize in 2002 (Fenn, Tanaka, Wüthrich)
- Ionization via
  - Protonation
  - Deprotonation
  - Adduct formation
- Complex mechanism

LC-MS usability with different ion sources

Molecular weight

10^9
10^8
10^7
10^6
10^5
10^4
10^3
10^2

Polarity

Non-polar  Medium polarity  Polar  Ionic

ESI  APPI  APCI
LC-ESI-MS: Mass-chromatogram (TIC)

- Three-dimensional data: Mass spectra can be obtained for LC peaks

ESI-MS spectrum

Sudan I
M = 248.1

\[ \text{[M + H]}^+ \]

\[ \text{[M + Na]}^+ \]
LC-ESI-MS: Mass-chromatogram (EIC)

- MS acts as another separation technique!

Problems with LC-ESI-MS

- ESI mass spectra are **not very characteristic**
  - ESI is soft and ions do not fragment extensively
  - Often just \([M+H]^+\)
  - The lower the analyte levels the higher the probability that some interfering compound has the same retention time and gives ions with the same m/z

- ESI mass spectra are **quite noisy**
  - High LoD values
Solution: Tandem mass spectrometry

LC-ESI-MSMS

- **MSMS workflow:**
  - The main ion (usually $[M+H]^+$) is selected
  - All other ions are ejected
  - The selected ion is excited
    - By collision with inert gas molecules
  - The ion fragments
    - By ejecting some part of the molecule
  - This is controlled by the MS software
  - Needs triple quadrupole or ion trap mass analyzer
    - Cannot be done with single quadrupole

LC-ESI-MSMS with Sudan I

- Sudan I: main transition $249 \rightarrow 232$

![Chemical structure](image)

- Now identity is confirmed in **triplicate**
  - Retention time, m/z of parent ion, m/z of fragment ion
  - For higher reliability in identification several transitions can be monitored

*Journal of Chromatography A, 1160 (2007) 227–234*
LC-ESI-MSMS

- Almost all quantitative trace analysis is done in the MSMS mode
  - Better signal to noise ratio
  - Couple of orders of magnitude lower LoD
    - Few μl of injected solution with few ppb concentration is sufficient
  - Reliable identification by using different transitions

LC-ESI-MSMS: standing problems

- Different ionization efficiency of different compounds
  - Also some polar compounds do not ionize well
- Dependence of ionization efficiency on co-eluting compounds – matrix effect
- Poor retention of some ionic compounds in LC

See more at:
http://tera.chem.ut.ee/~ivo/Chrom_MS/
http://tera.chem.ut.ee/~ivo/LC-MS_Matrix_Effect_Toolbox/
### LC-MS vs GC-MS

**LC-MS “+”**
- Almost unlimited M
- No volatility needed
  - Ionic compounds OK
- Thermal stability is not needed

**LC-MS “-”**
- Expensive
- Not robust
- Predicting ionization is tricky

**GC-MS “+”**
- Better resolution
- Robust

**GC-MS “-”**
- Low to medium M
- Volatility needed
- Thermal stability needed

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Thanks to all these people!

Thank you for your attention!