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

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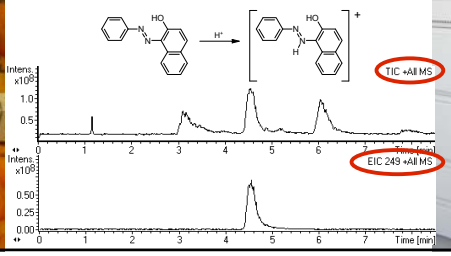


Outline

- 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 ppb ≡ 1 µg/kg ≡ 1 mg/t**

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)

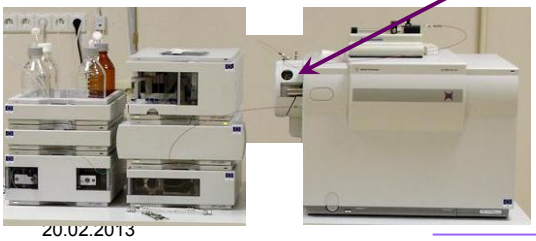
?

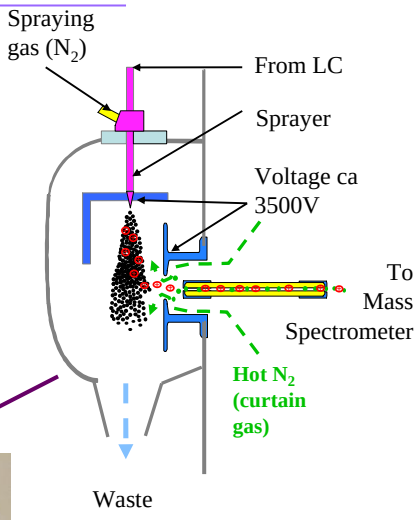
Coupling LC and MS

- 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 disperged by electric field into small droplets
- The analyte ions evaporate from droplets
- The ions are directed into MS entrance by the electric field





ESI in action (Positive ions)

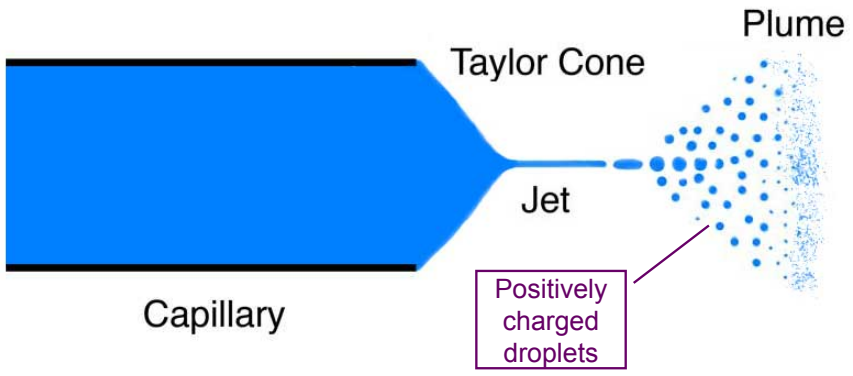
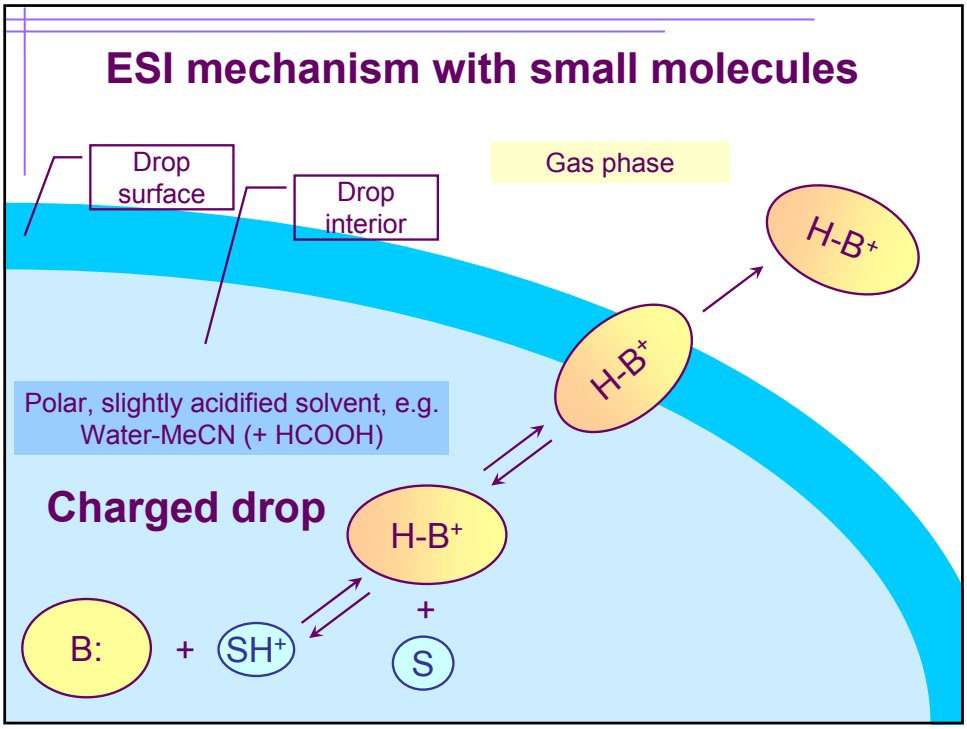


Image by K. K. Murray (via Wikipedia)

9

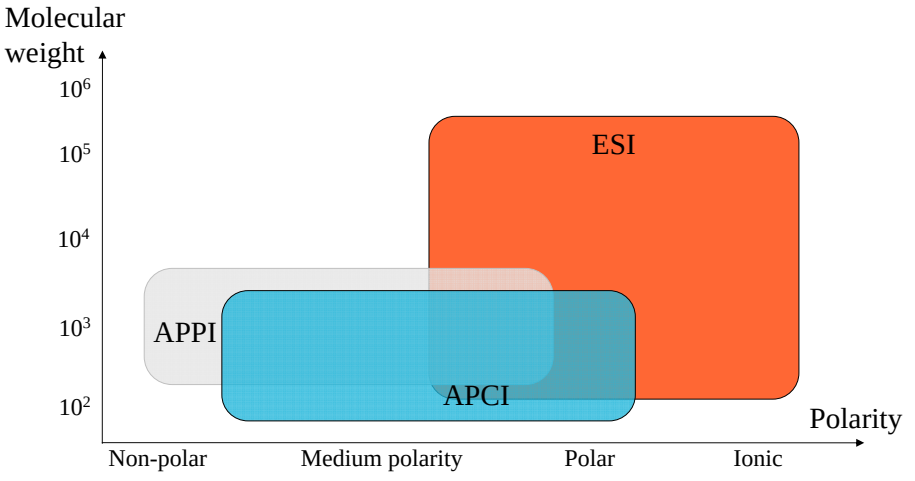
ESI mechanism with small molecules



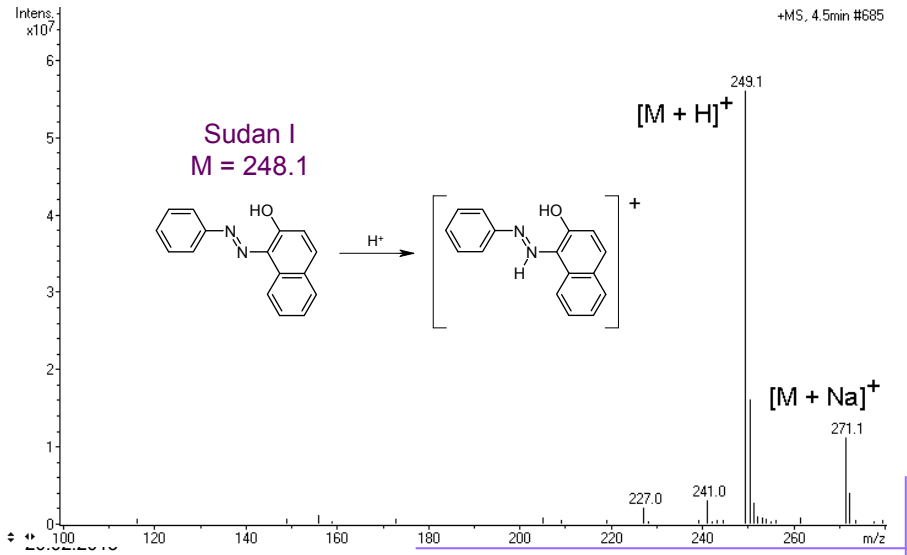
ESI Ionization

- 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

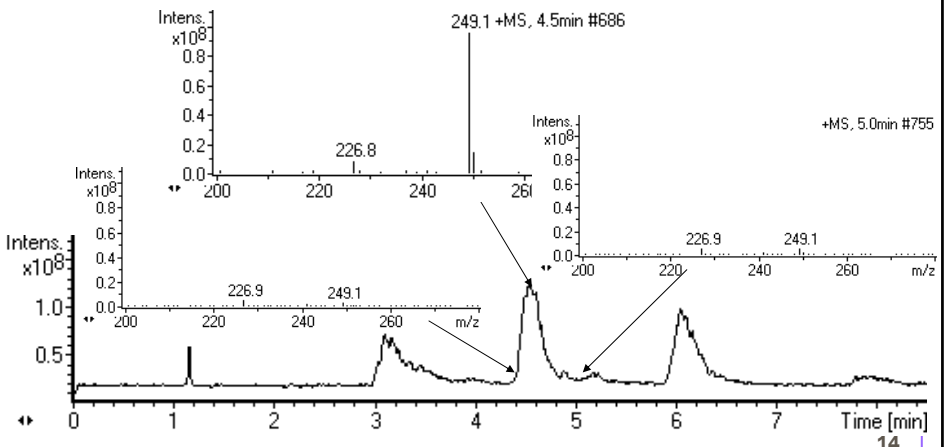


ESI-MS spectrum



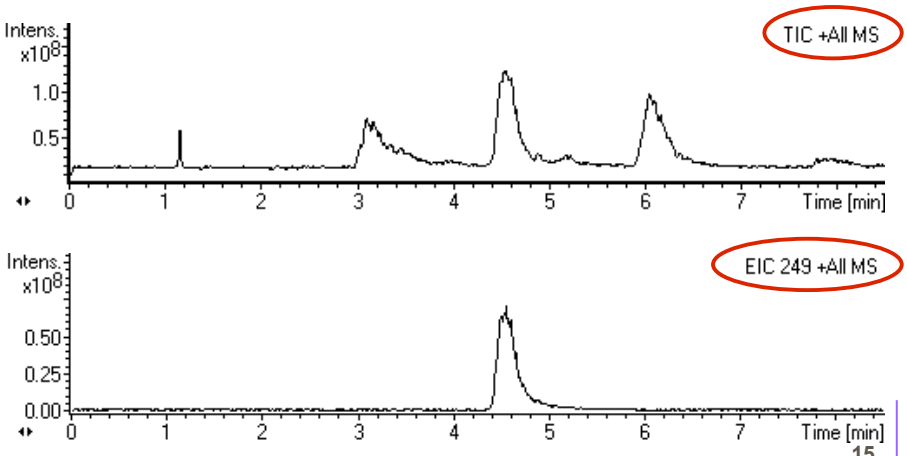
LC-ESI-MS: Mass-chromatogram (TIC)

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



LC-ESI-MS: Mass-chromatogram (EIC)

- MS acts as another separation technique!



Problems with LC-ESI-MS

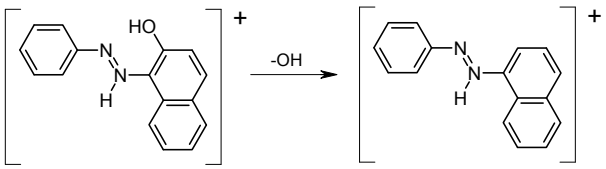
- ESI mass spectra are **not very characteristic**
  - ESI is soft and ions do not fragment extensively
  - Often just $[M+H]^+$
  - The lower the analyte levels the higher the probability that some interfering compound has the same retention time and gives ions with the same m/z
- ESI mass spectra are **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 → 232



- 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

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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

Thanks to all these people!

Thank you for your attention!

22