

Mass-Spec methods in Forensic and Analytical Toxicology

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Methods in toxicology
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Table of Contents

- Definition
- Chromatography
- Ionization technics
- Mass Analyzer
- Detectors
- Examples

What is forensic analytics?

Forensic analytic:

- Detection of toxic compounds (police/court)
- Examples:
 - Narcotic substances (drivers)
 - Combination of alcohol and medicine (criminal assault)
 - Cyanide
 - Carbon Monoxide
 - Steroids

• Forensic toxicology:

- How drugs/poisons affect humans
- Ingest of a drug/poison
 - Absorbed into blood-stream
 - Circulates throughout the body
 - Where it can effect certain causes

What does a forensic toxicologist?

- **Determine the identity of all drugs/poisons present in a body**
- **Determine the quantities**
- Determine the metabolites
- Determine interactions
- Determine history and patterns of drug use

Examples



NO, I HAVEN'T SEEN YOUR PILLS....



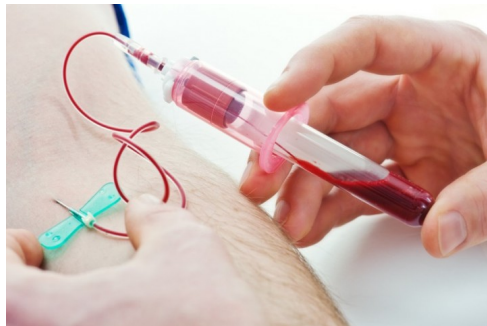
Hair analysis

- ~10 mg hair (control + test sample)
- Cut into 2cm parts
- Washed with isopropanol and twice with water
- Dry hair samples
- Extraction: precelly tube → add to stainless steel beads → extract. Medium + IS mixtures solution → shake
- Incubation/drying of the extracts → dilute in mobile phase
- Filtration (SPE) → LC

Metal intoxication → ICP-MS!

- 1-2 mL blood / Plasma
 - Mix gently
 - 1:1 dilution of blood sample with Triton-X-100 (0.02%) + IS
 - 20% (V/V) ammonia solution added
 - Urine
 - Dilution 1/10 (V/V) in a solution containing (1% HNO₃, IS, 1% (V/V) Ethanol)
 - Tissue (liver)
 - Dissolved in 2 mL 32% (V/V) nitric acid + IS
- LC-Separation

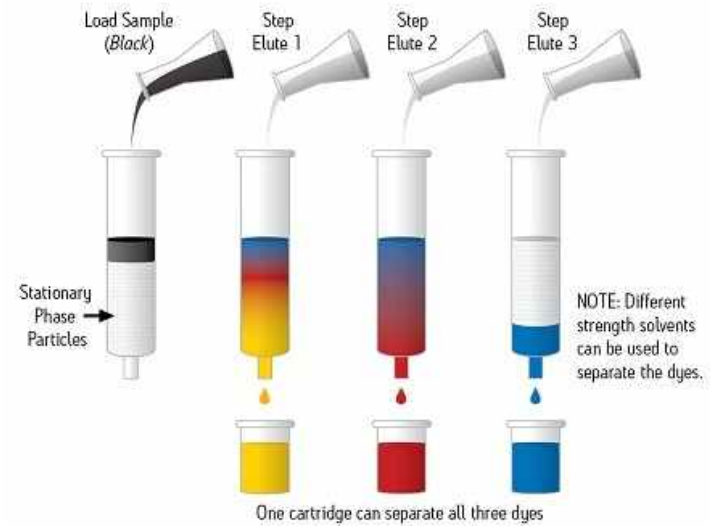
The Procedure



<http://medlinkscostcontainment.com/blood-draws-from-central-lines/>



<https://www.geneticistinc.com/blood-samples>

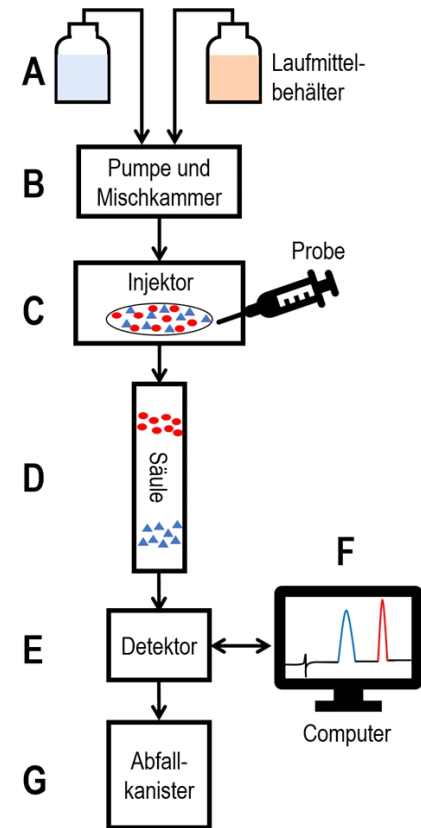
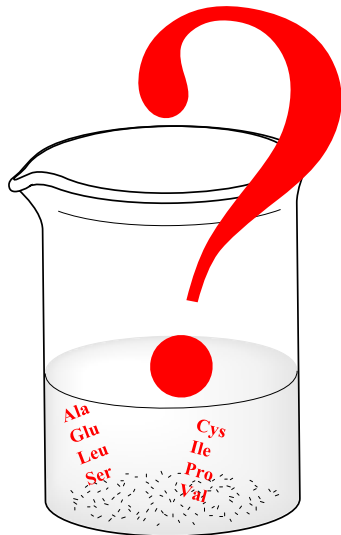


http://www.waters.com/waters/de_DE/Solid-Phase-Extraction-SPE-Guide/nav.htm?cid=134721476&locale=de_DE

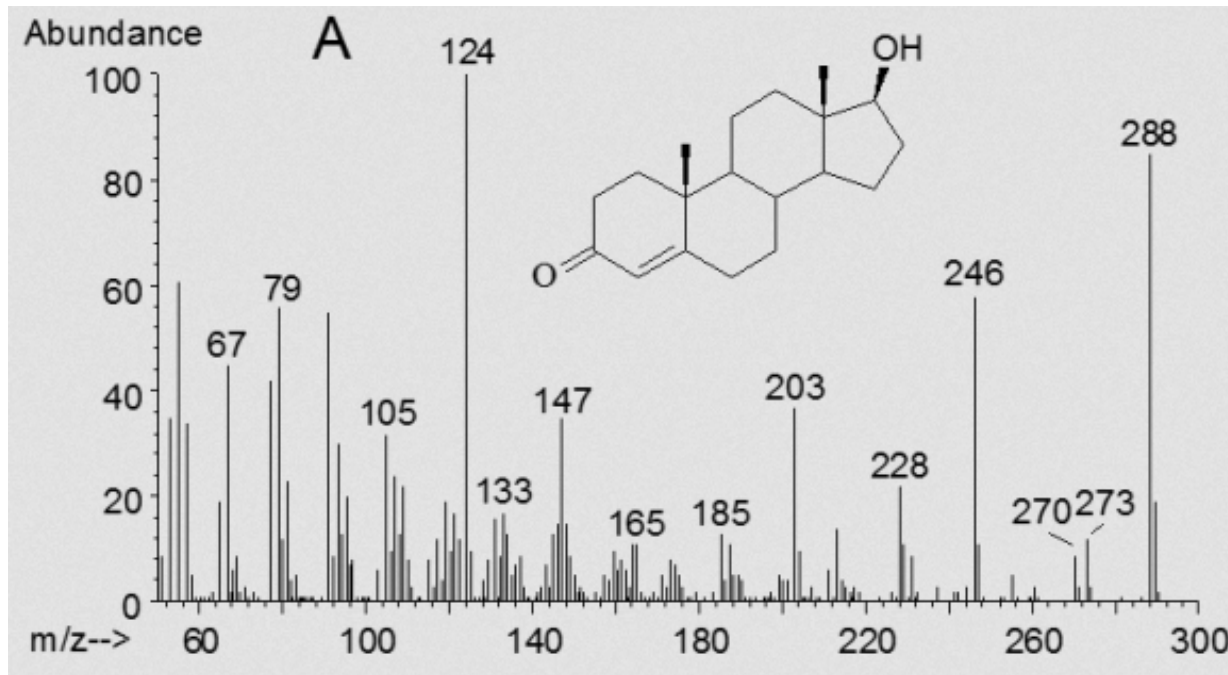
Important facts for sampling

- Contaminations → false-positive results
 - Disinfect skin before withdrawing blood for alcohol determination
- Taking several samples
 - Adding NaF to Bloodsamples → works against in vitro decomposition
 - And freeze the samples if you don't need them

Chromatography

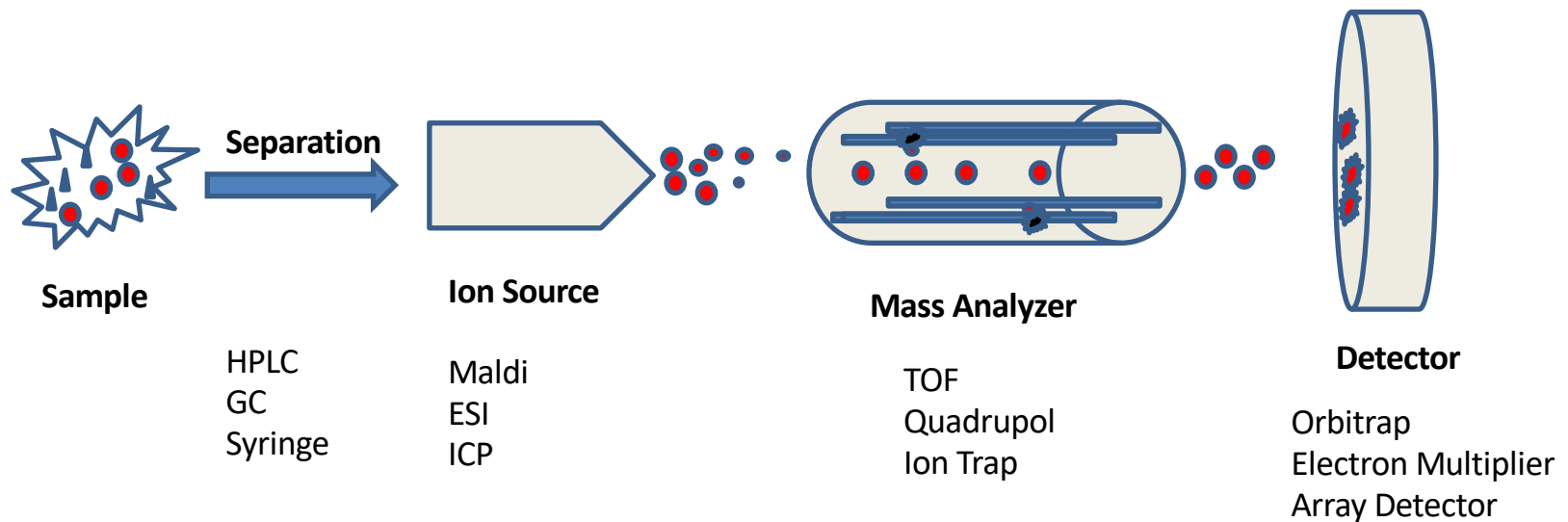


What can we see here?



<https://www.dshs-koeln.de/institut-fuer-biochemie/analyse-methoden/testosteron-nachweis/>

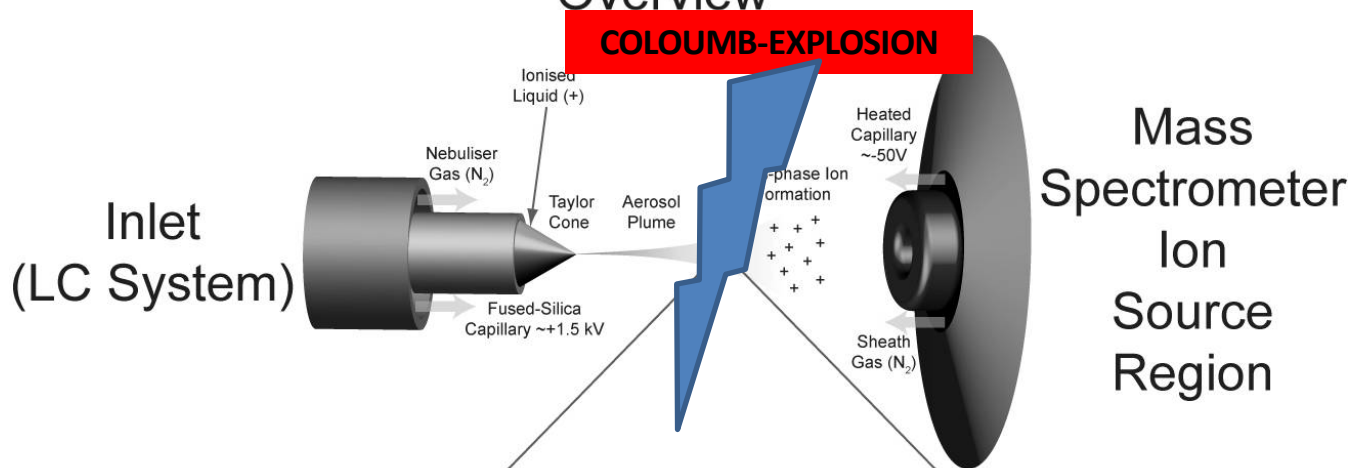
What do we need to get a spectrum like this?



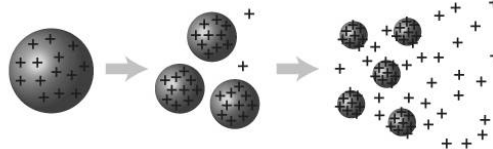
Ionization – Electrospray Ionization

Electrospray Ionisation (ESI) and Ion Source

Overview

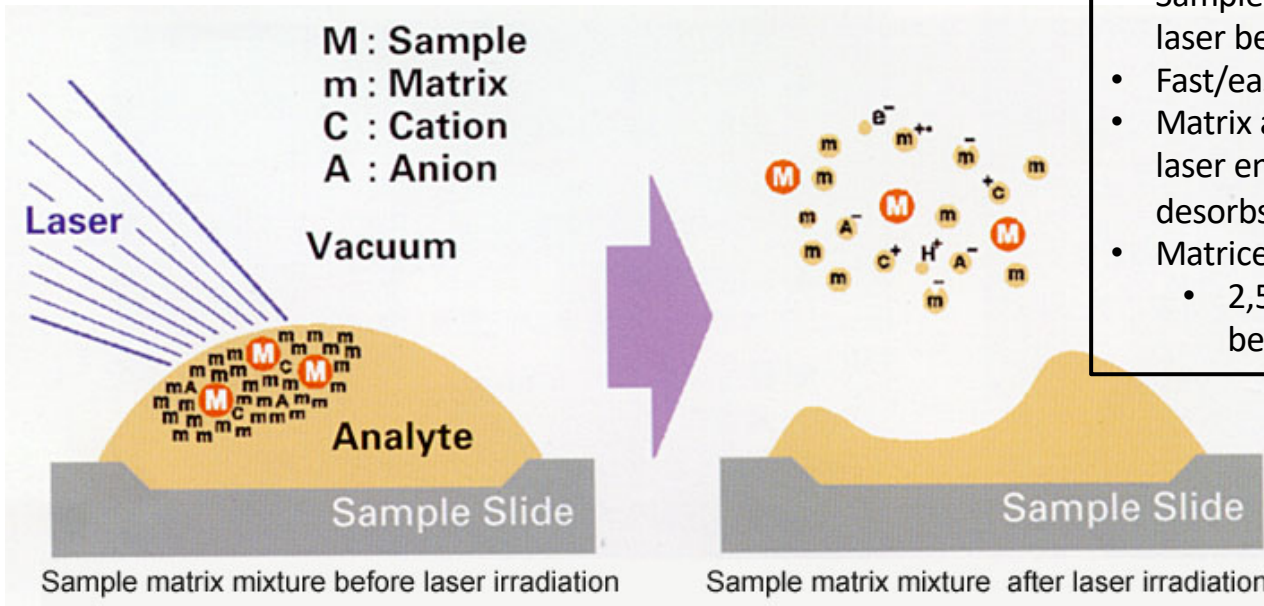


ESI Combination Model



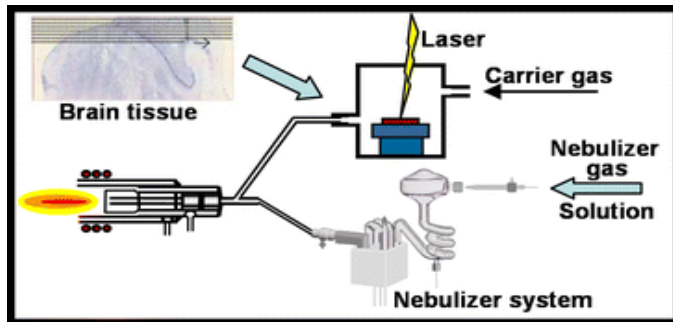
- At needle mm-sized droplets
- Via drying gas/heated capillary → solvents evaporated → **Coloumb Explosion**

Ionization- MALDI



- Sample ionization by laser beam
- Fast/easy/robust
- Matrix absorbs the laser energy and desorbs the samples
- Matrices:
 - 2,5-dihydroxy benzoic acid

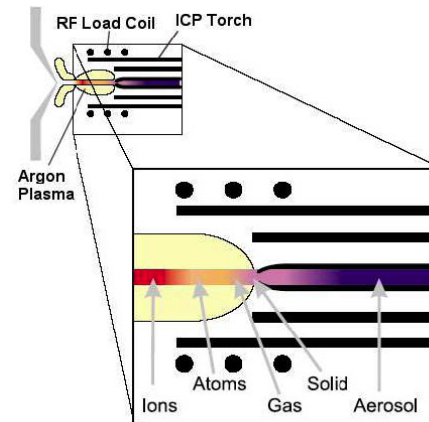
Ionization- ICP (inductively coupled plasma)



Bioimaging of metals in thin mouse brain section by laser ablation inductively coupled plasma mass spectrometry: novel online quantification strategy using aqueous standards, [J. Anal. At. Spectrom.](#), 2010, 25, 1739-1744

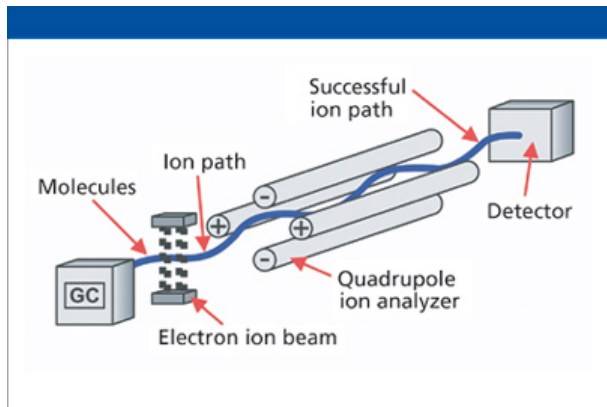
ICP:

- Combination of high-temperature ICP with MS (5000-10000°K)
- Converts atoms of the sample-elements into ions
- Mostly used for metal speciation



<https://crustal.usgs.gov/laboratories/icpms/intro.html>

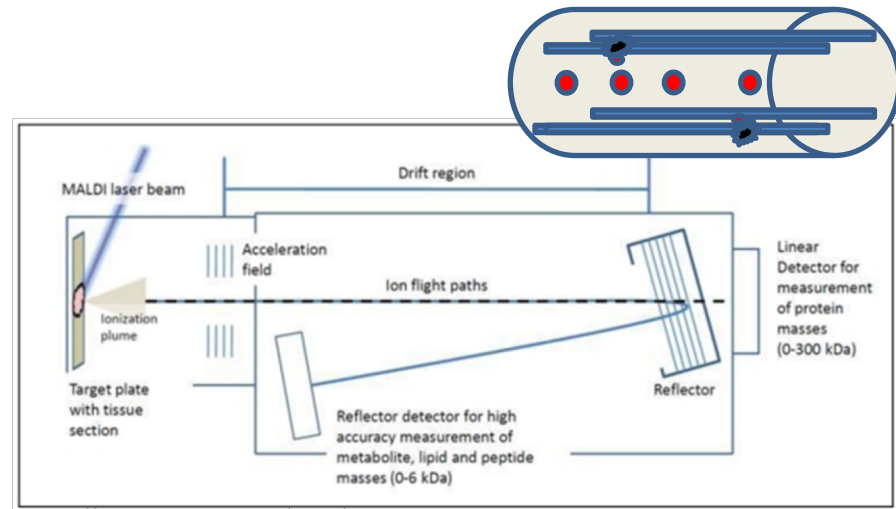
Other Mass analyzer



<http://www.chromatographyonline.com/comparing-capabilities-time-flight-and-quadrupole-mass-spectrometers-0>

Quadrupol

- only ions with certain m/z ratio can pass through the quadrupole
- to detect other ions change voltage



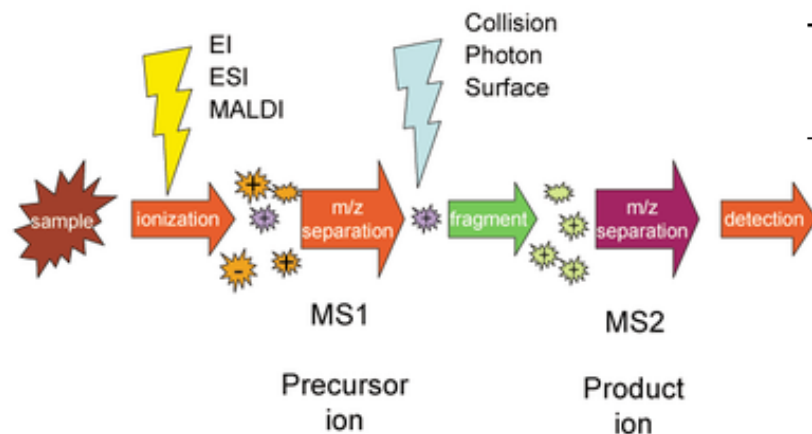
https://www.researchgate.net/figure/Basic-principles-of-matrix-assisted-laser-desorption-ionisation-MALDI-time-of-flight_fig1_49854071

Time-of-flight (ToF)

- Separation of ions through the different mass-to-charge-ratios
- small high charged ions faster than tall low charged

Recapitulation

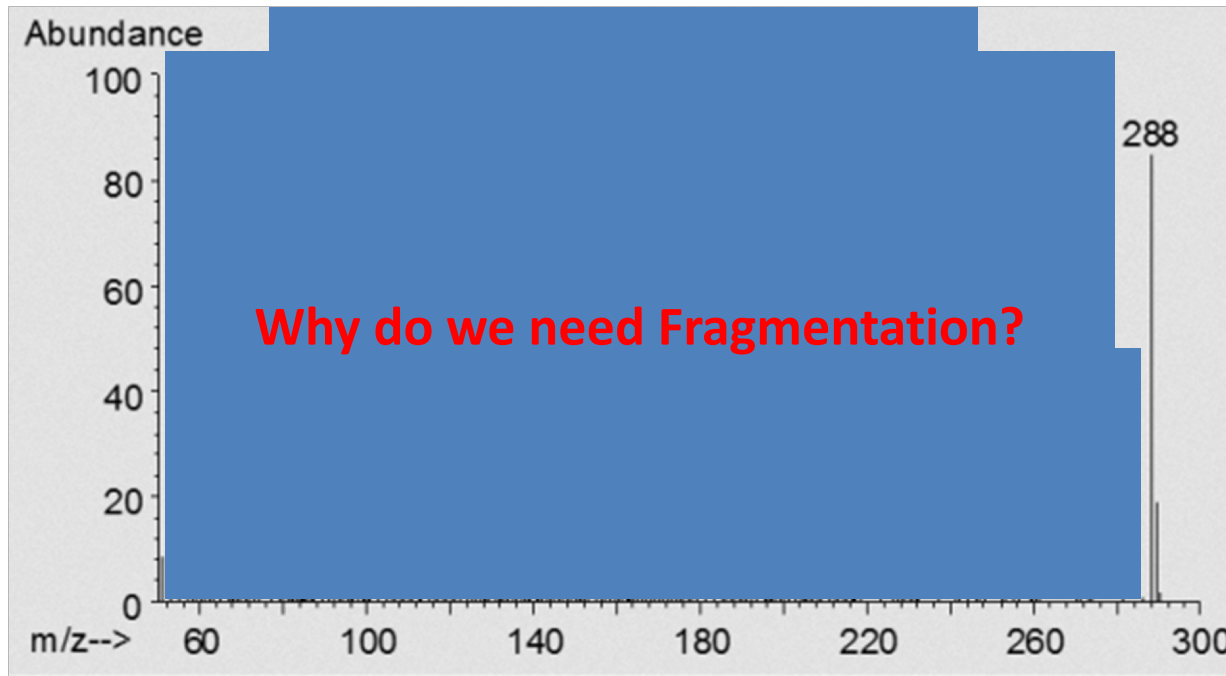
- What do you remember from Tandem MS? What is a parent/precursor ion?



Tandem MS:

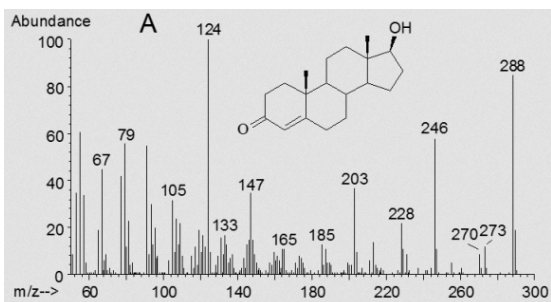
- physically Separation of the elements
- MRM (Multi Reaction Monitoring)
- SRM (Selected Reaction Monitoring)

Evaluation



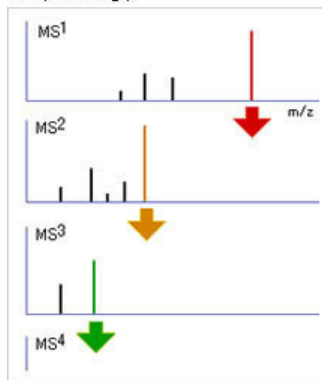
<https://www.dshs-koeln.de/institut-fuer-biochemie/analyse-methoden/testosteron-nachweis/>

This is why we need Fragmentation!

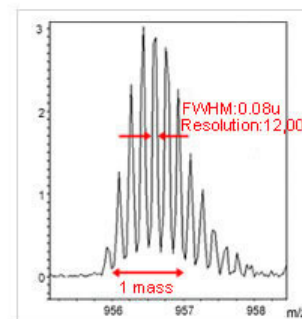


<https://www.dshs-koeln.de/institut-fuer-biochemie/analyse-methoden/testosteron-nachweis/>

MSⁿ measurement:
 One peak acquired by MS¹ is performed MS², and one peak acquired by MS² is performed MS³. LCMS-IT-TOF can perform by MS¹⁰. This function supports structural analysis strongly.



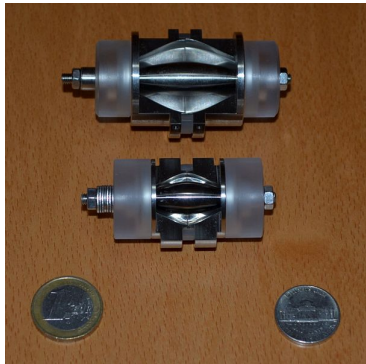
High resolution and accuracy
 This data shows a Mass spectra of Insulin Hexavalent Ion. Resolution of >12,000 was achieved. 6 peaks are separated clearly in one mass difference.



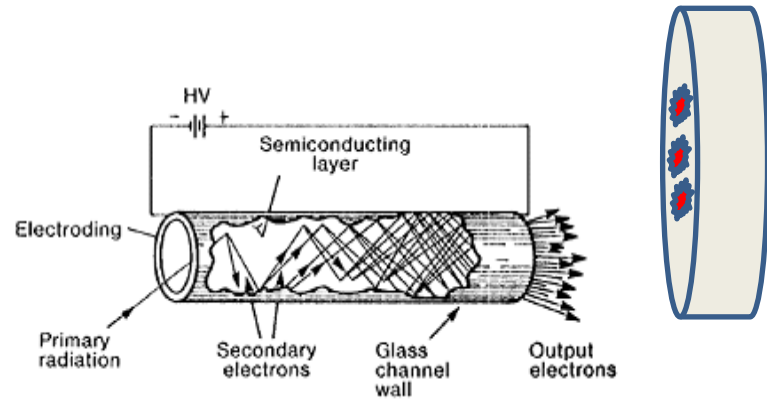
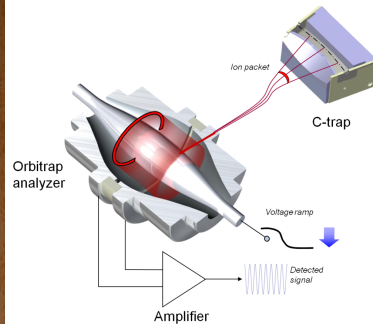
Main unit: 1685mm , LC unit (by module): 260mm

<https://www.shimadzu.de/lcms-it-tof%E2%84%A2>

Detectors



<https://en.wikipedia.org/wiki/Orbitrap>



https://attic.gsfc.nasa.gov/huygenscms/MS_Detector_1.htm

Orbitrap:

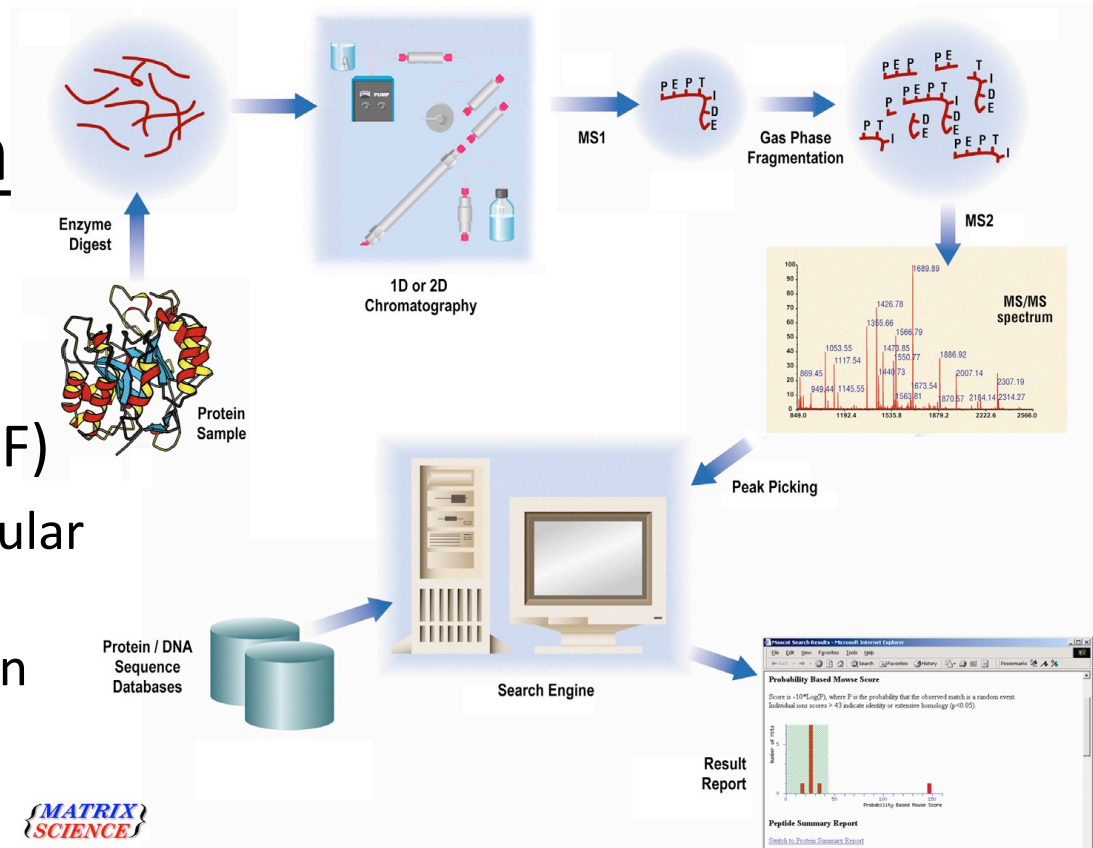
- Axial oscillations of ion rings around the central electrode (and two outer ones)
- Different ions have different oscillate frequencies
- Measuring the oscillation frequencies

Electron Multiplier:

- Detect every ion of the selected mass passed the analyser
- Basic physical process:
 - Secondary electron emission
 - Charged particle → surface → secondary electrons (number of secondary electrons depends on type/energy/characteristics of charged particle)

Data for Protein Identification

- Peptide Mass Fingerprint (PMF)
 - peptide molecular masses from a digested protein



Click for protein detail view

Protein Score

Queries Matched

1. [gi|1351907](#) Mass: 69328 Score: **180** Queries matched: 5
 Serum albumin precursor (Allergen Bos d 6) (BSA)

Check to include this hit in error tolerant search or archive report

Rank order of protein matches

Ion Scores

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
1	1283.70	1282.69	1282.70	-0.01	0	(21)	17	3	HPEYAVSVLLR
<input checked="" type="checkbox"/> 2	1283.70	1282.69	1282.70	-0.01	0	37	0.38	1	HPEYAVSVLLR
<input checked="" type="checkbox"/> 4	1439.90	1438.89	1438.80	0.09	1	20	30	1	RHPEYAVSVLLR
<input checked="" type="checkbox"/> 5	1479.80	1478.79	1478.79	0.00	0	47	0.048	1	LGEYGFQNALIVR
<input checked="" type="checkbox"/> 6	1567.80	1566.79	1566.74	0.06	0	75	6.3e-05	1	DAFLGSFLYEYSR

Click query number for peptide MS/MS details

Observed and Predicted Peptide Masses

Number of missed trypsin cleavage sites

Frequency this match would occur by chance.

Proteins matching the same set of peptides:
[gi|30794280](#) Mass: 69358 Score: **180** Queries matched: 5
 albumin [Bos taurus]

The Mascot Score is a statistical score for how well the experimental data match the database sequence.



THANK YOU FOR YOUR ATTENTION

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