



Contaminant analysis

a targeted overview of analytical tools

Environmental contaminants Dioxins Heavy metals

Pesticides PCBs Process residues PAHs

Production contaminants Mycotoxins

Solvents Forbidden dyes

Process contaminants Migration residue

ITX Antibiotiques

A tooth ? Shards of glass in a food can ?

Unexpected contaminants...

Anything unwanted

U.V. spectroscopy

I.R. spectroscopy

Nuclear Magnetic Resonance

X-Ray

Radioactivity

Titration

Gas Chromatography

High Performance Chromatography

Electrophoresis

Absorbtion/Emission spectroscopy

Various microscopies

etc.

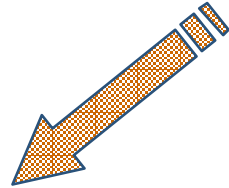
Any combination of those

Let's be reasonable...

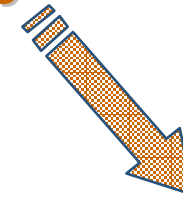
**...and keep to
classical chemical contaminants...**

**...that can be analysed by the most
widespread analytical tools...**

95% of contaminant analysis methods involve chromatographic systems



Gas Chromatography (GC)

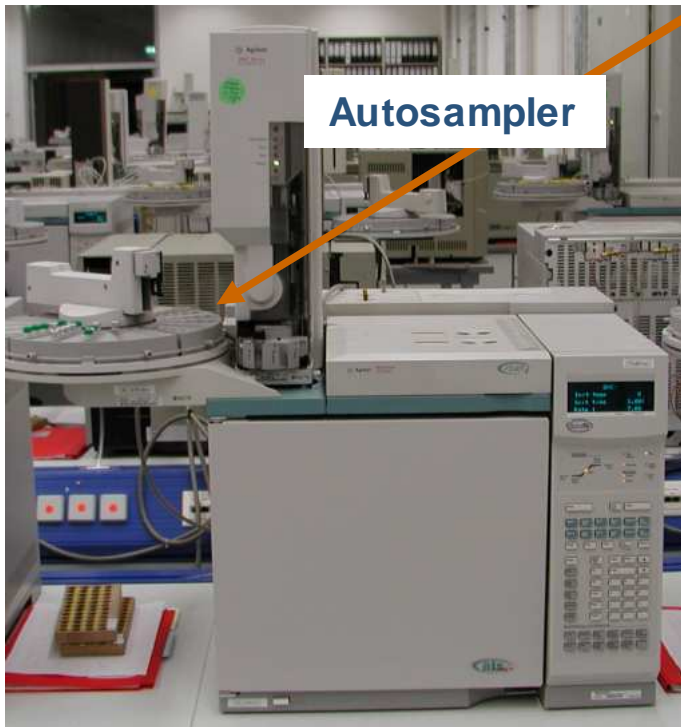


Liquid Chromatography (LC)



What is a chromatographic system ?

Gas Chromatography (GC)



Autosampler



Injection of a sample extract

Liquid Chromatography (LC)



Autosampler

Separation of the analytes from each other on a time scale

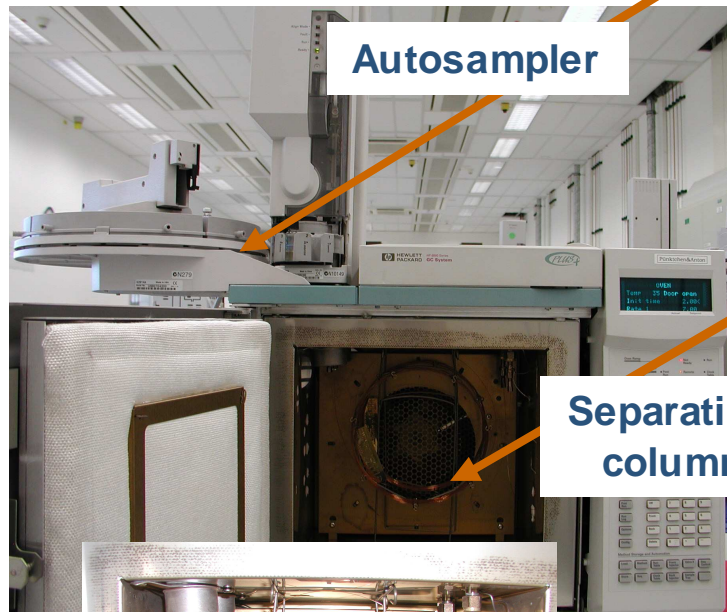
What is a chromatographic system ?

Gas Chromatography

Liquid Chromatography



Injection of a sample extract



Autosampler

Separation column

Column length: 10 - 100 m

Separation of the analytes from each other on a time scale



Column length: 2 - 250 mm

Autosampler

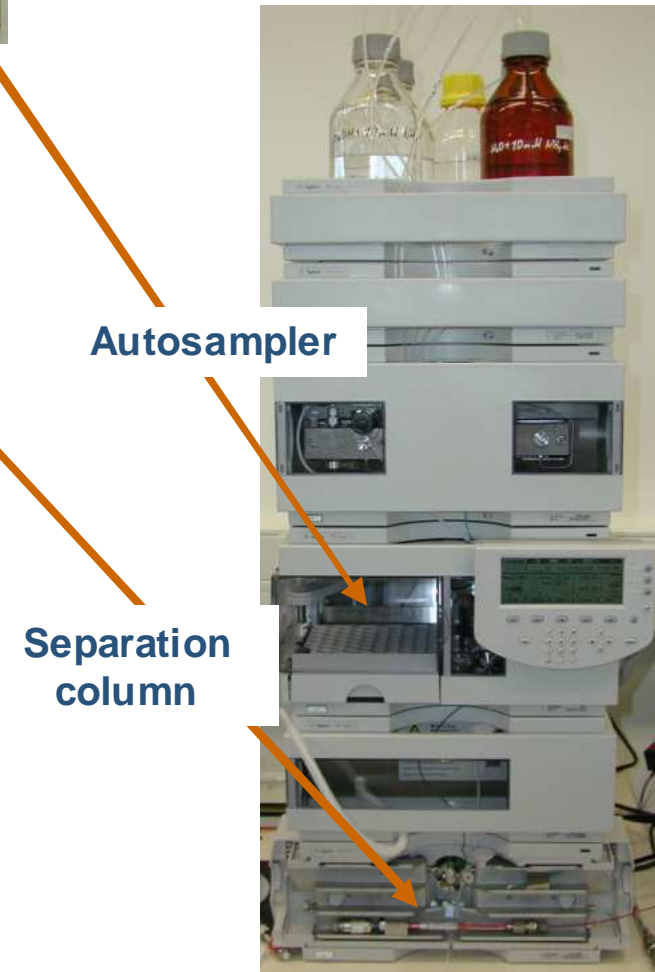
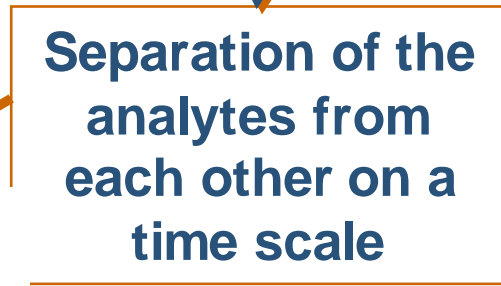
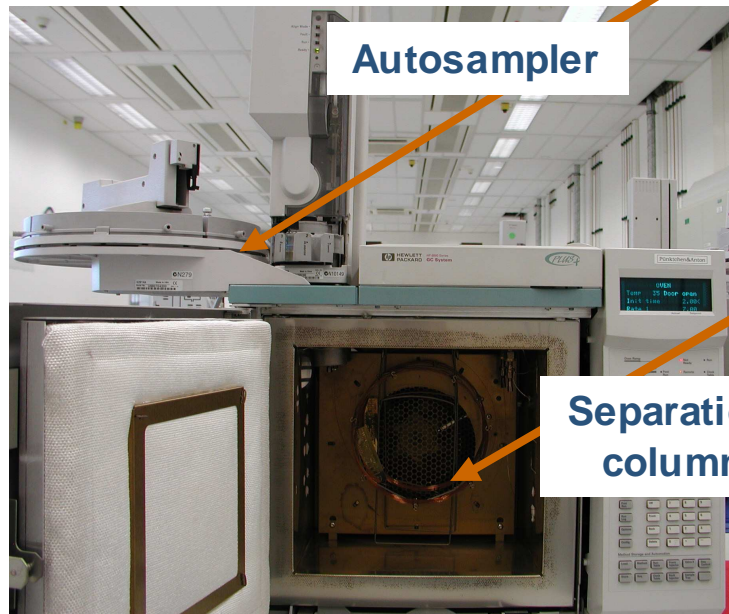
Separation column



What is a chromatographic system ?

Gas Chromatography

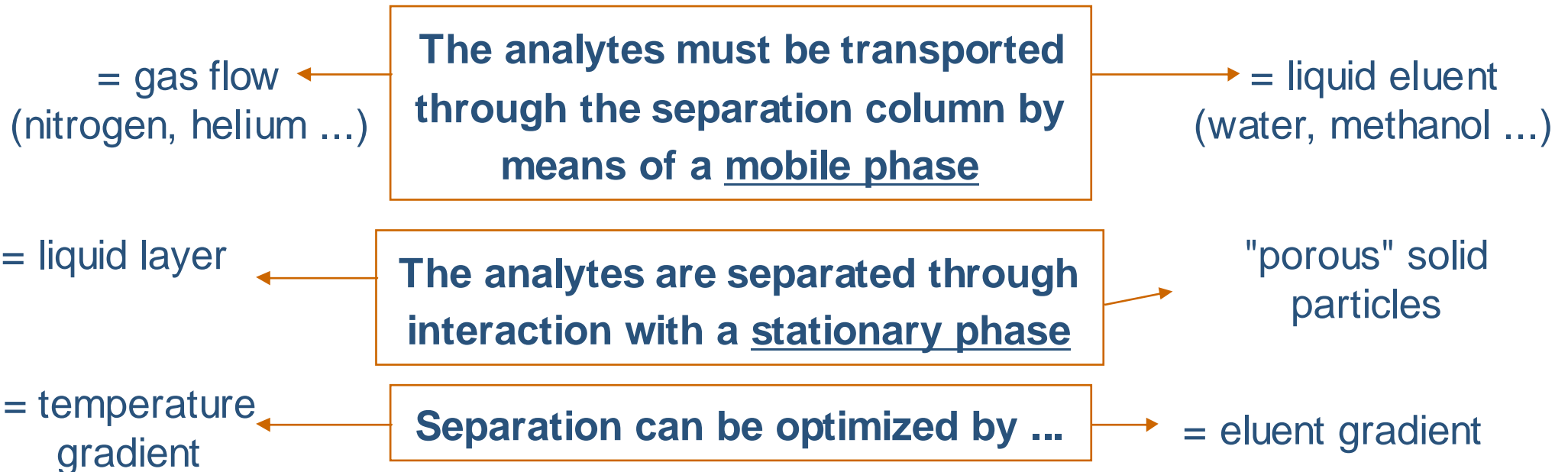
Liquid Chromatography



What are the differences between GC and LC ?

Gas Chromatography

Liquid Chromatography



GC is preferred for separation of analytes that are of:

- unpolar (lipophilic) character
- lower molecular weight

LC is preferred for separation of analytes that are of:

- polar to ionic (hydrophilic) character
- higher molecular weight

How can the analytes be detected ?

Gas Chromatography

- **ECD = Electron Capture Detector**

mostly used for analytes with halogen atoms

- **FPD = Flame Photometric Detector**

mostly used for analytes with phosphor and sulphur atoms

- **NPD = Nitrogen Phosphor Detector**

mostly used for analytes with nitrogen and phosphor atoms

Depending on the chemical and physical properties of the analyte different specific detector types can be applied

Universal detector types cover a wide range of different analyte classes

Liquid Chromatography

- **UVD = Ultra Violet Detector**

mostly used for analytes with a chromophore

- **FD = Fluorescence Detector**

- Refractor
- Electrochemical
- ...

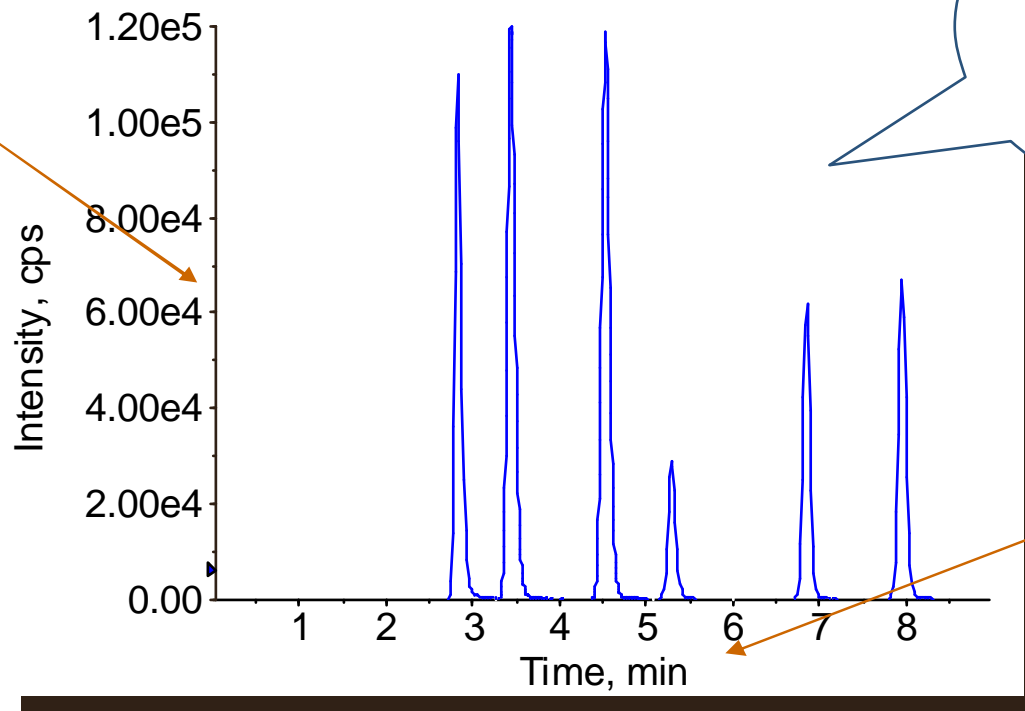
- **MSD = Mass Spectrometric Detector**

analytes are not only identified by their retention time but also by their specific molecular mass

What information does the detector give ?

All detectors finally produce chromatograms with each peak signal representing one single analyte (normally)

Intensity scale:
the concentration of each analyte can be determined from its intensity



In this chromatogram 6 analytes were determined

Time scale:
each analyte can be identified by its specific retention time

How sensitive can the analytes be determined ?

Final concentrations are mostly expressed in mg/kg

e.g. 0.05 mg Imidacloprid in 1 kg of tea
equivalent to 0.05 parts per million (ppm)
or 50 parts per billion (ppb)

To give you an impression ...



Boeing 747 Jumbo Jet
Maximum takeoff weight: 400 tons



Pilot captain
Maximum takeoff weight: 80 kg
= concentration of 200 mg/kg
equivalent to 200 ppm



Pilot's hat
Weight: 400 g
= concentration of 1 mg/kg
equivalent to 1 ppm



Bee on the cockpit window
Weight: 400 mg
= concentration of 1 μ g/kg
equivalent to 1 ppb



Rice grain in the lunch menu
Weight: 400 μ g
= concentration of 1 ng/kg
equivalent to 1 ppt

What is LC-MS/MS ?

A liquid chromatographic system (LC) is coupled to a mass spectrometric detector (MS)

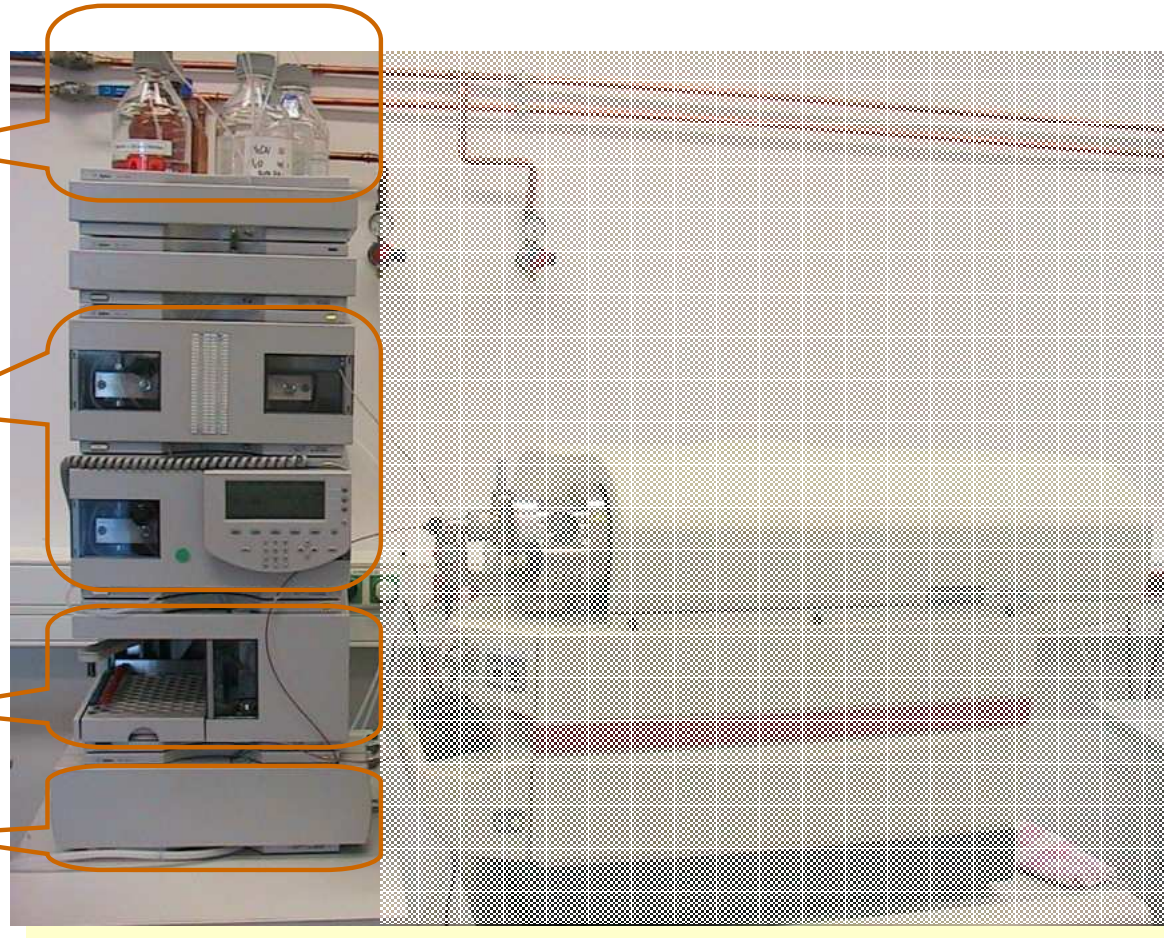
consisting of ...

Liquid mobile phase
(water and methanol)

Pumps for the
mobile phase

Autosampler for
injection

Compartment
for the separation
column



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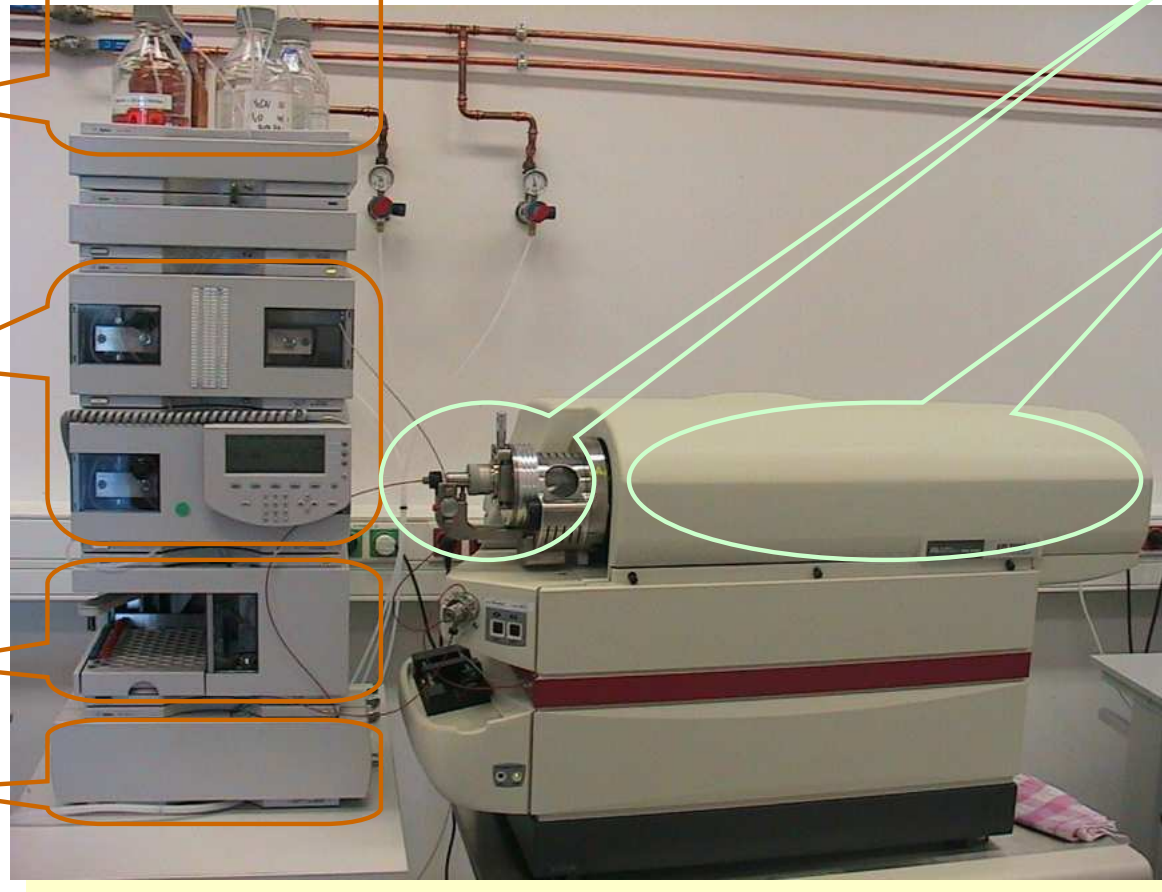
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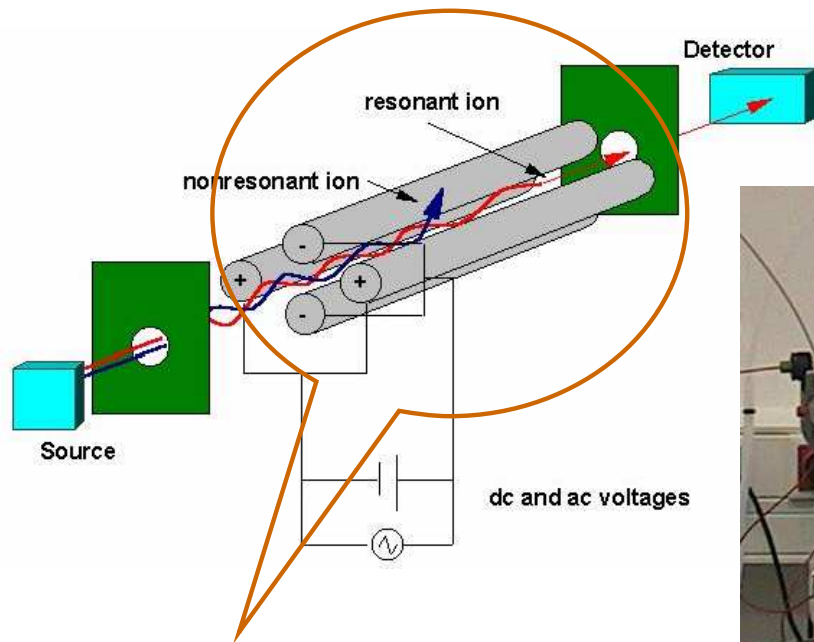


MS interface where
analytes are ionized

Ion pathway where
the ionized analytes
are separated
according
to their molecular
mass

Why are the terms MS/MS in double?

The separation of the analytes according to their molecular masses is done in the ion pathway by so-called quadrupole



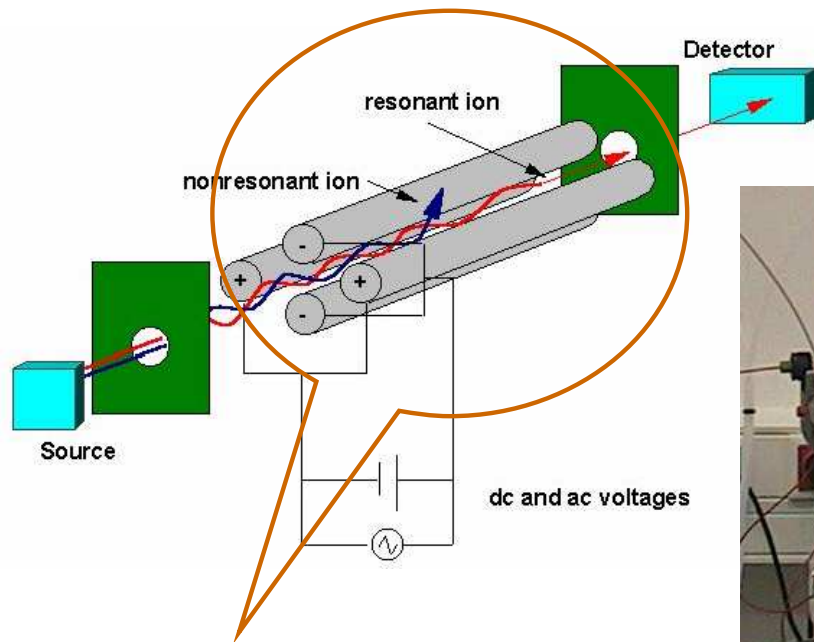
Quadrupole consisting of 4 metallic rods



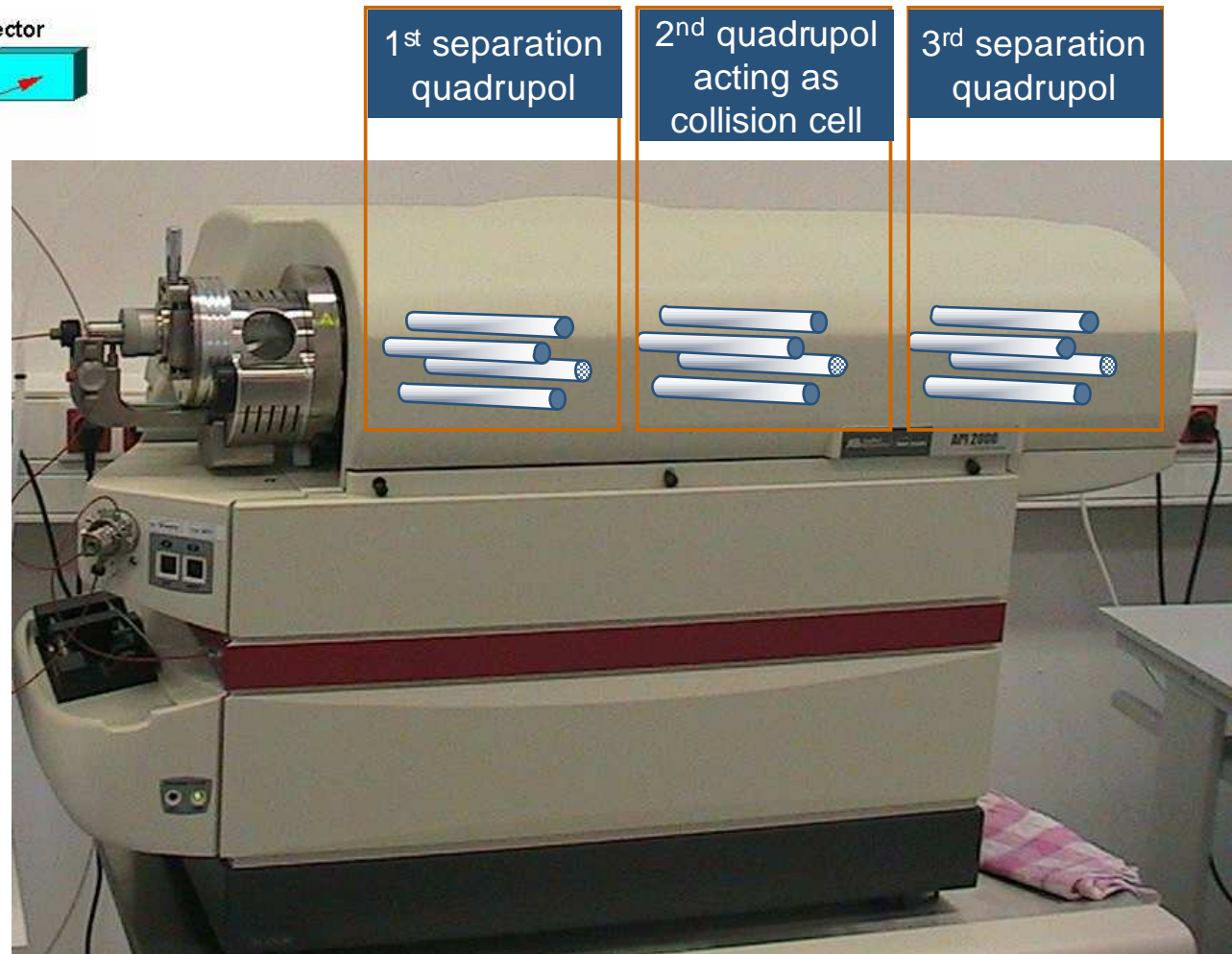
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➔ LC-MS/MS

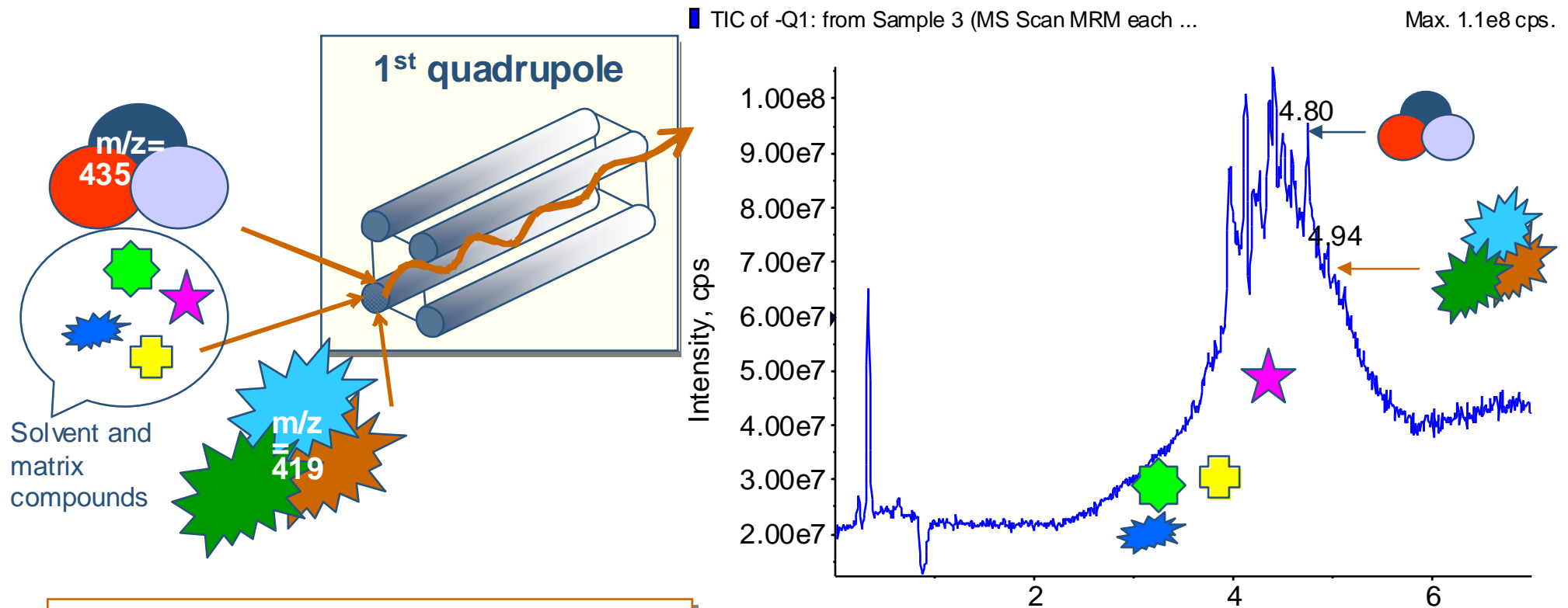


Quadrupole consisting of 4 metallic rods



LC-MS = single quadrupole MS

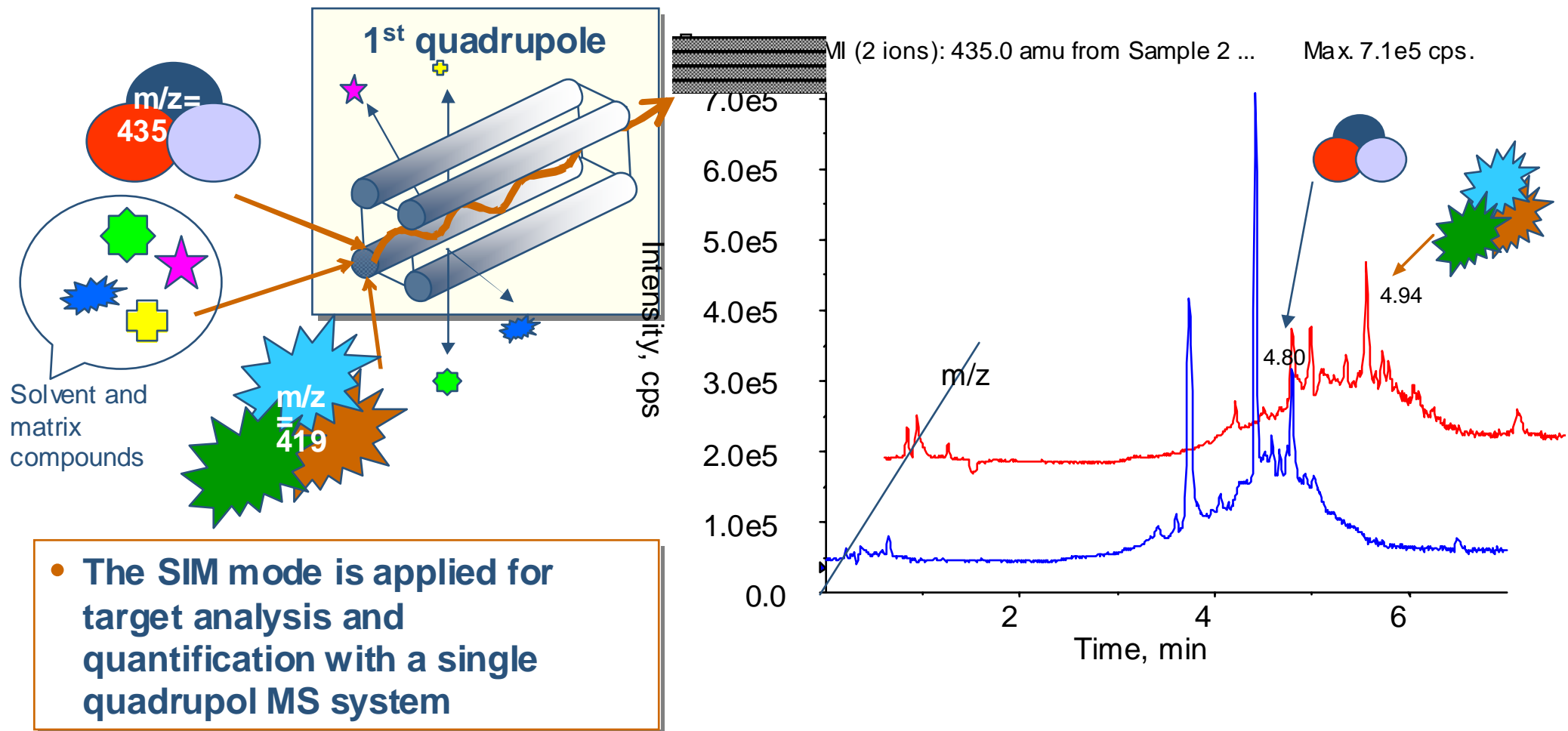
operated in scan mode



- The scan mode is only applied for general screening for unknown compounds

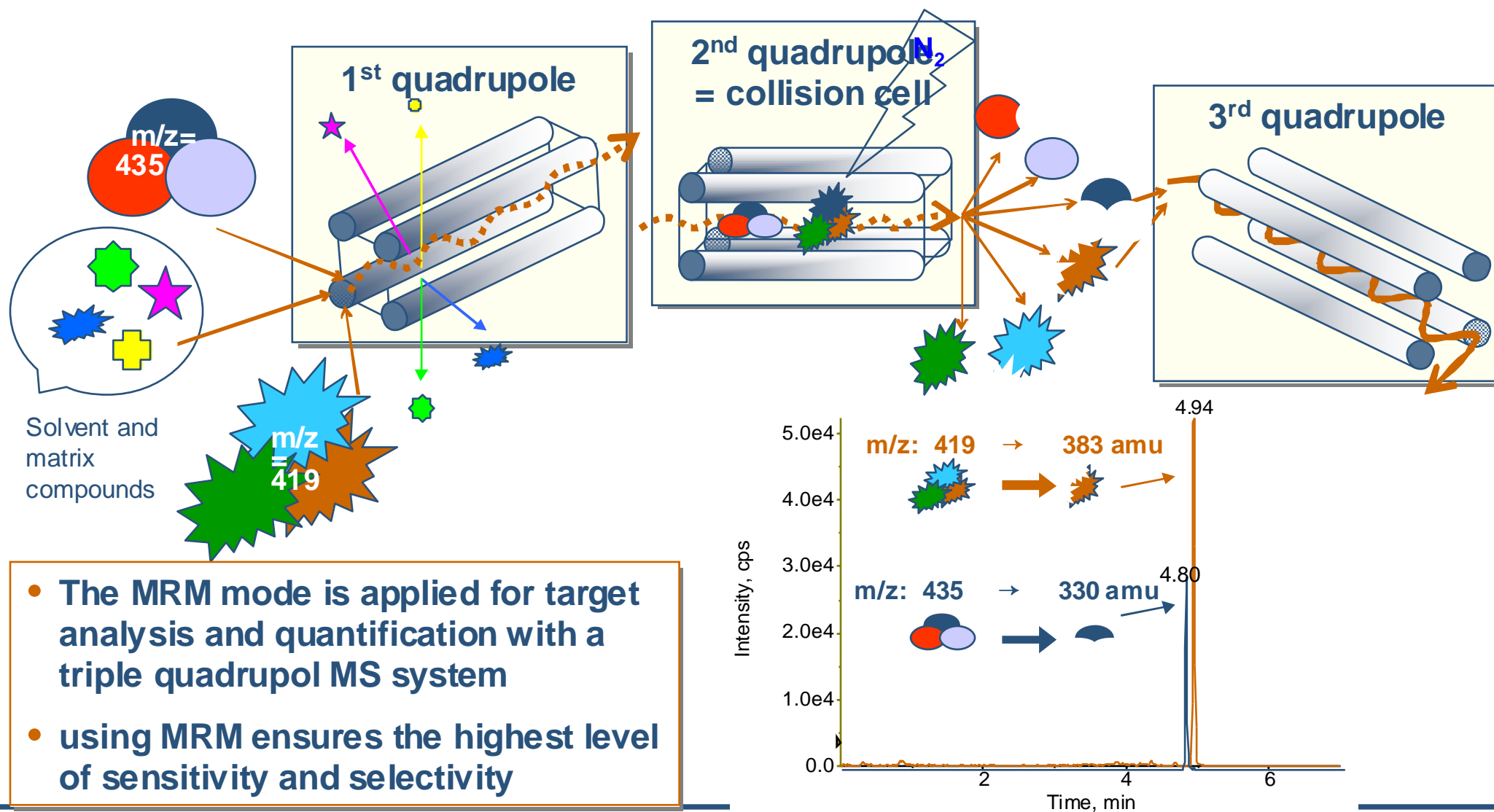
LC-MS = single quadrupole MS

operated in selected ion monitoring mode (SIM)



LC-MS/MS = triple quadrupole MS = tandem mass spectrometry

operated in multiple reaction monitoring mode (MRM)

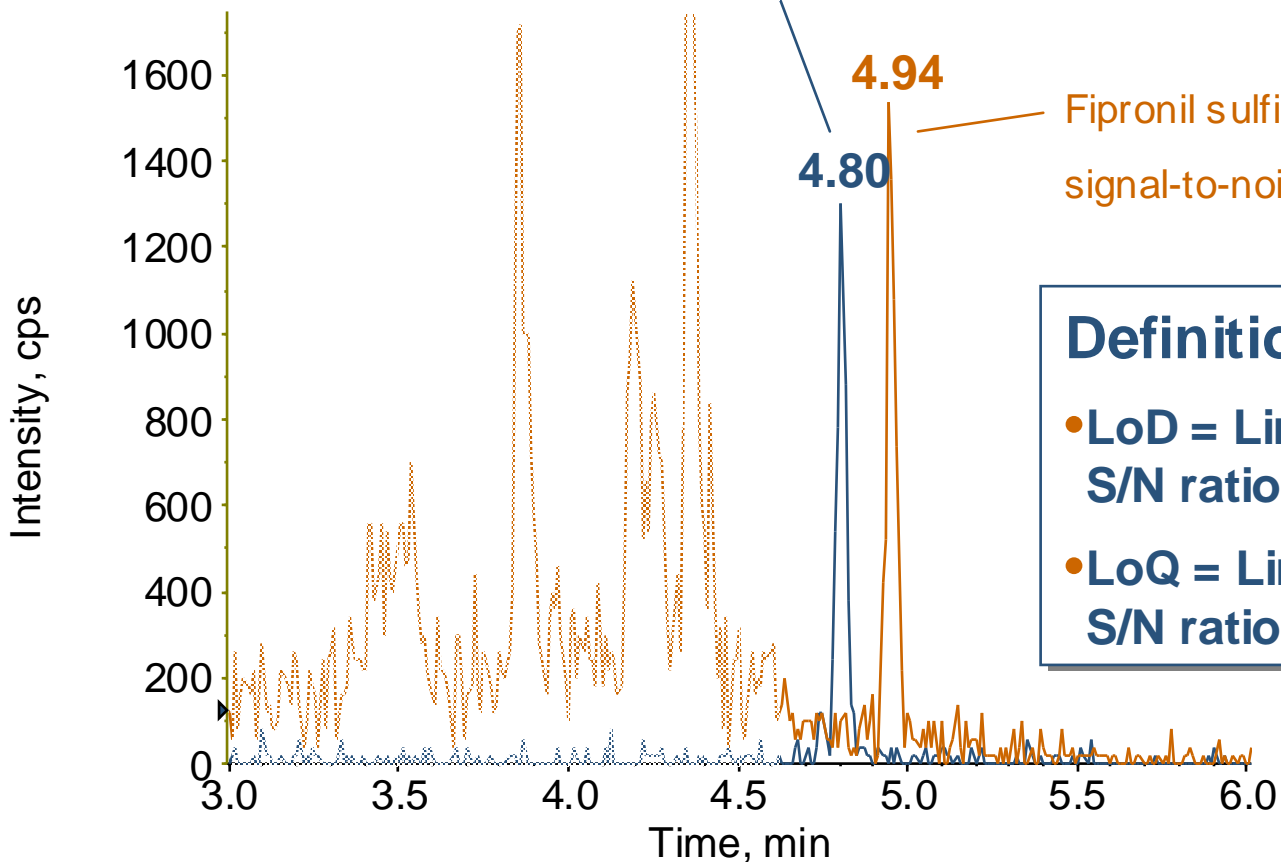


- The MRM mode is applied for target analysis and quantification with a triple quadrupole MS system
- using MRM ensures the highest level of sensitivity and selectivity

Achieving the maximum of sensitivity with LC-MS/MS in MRM mode

Fipronil:

signal-to-noise ratio = 16:1 at 2.5 pg/mL concentration



Fipronil sulfide:

signal-to-noise ratio = 10:1 at 2.5 pg/mL concentration

Definition of LoD and LoQ:

- **LoD = Limit of Detection**
S/N ratio should be at least 3:1
- **LoQ = Limit of Quantitation**
S/N ratio should be at least 6:1 (= 2 x LoD)

How do we know that an analytical method is working well ?

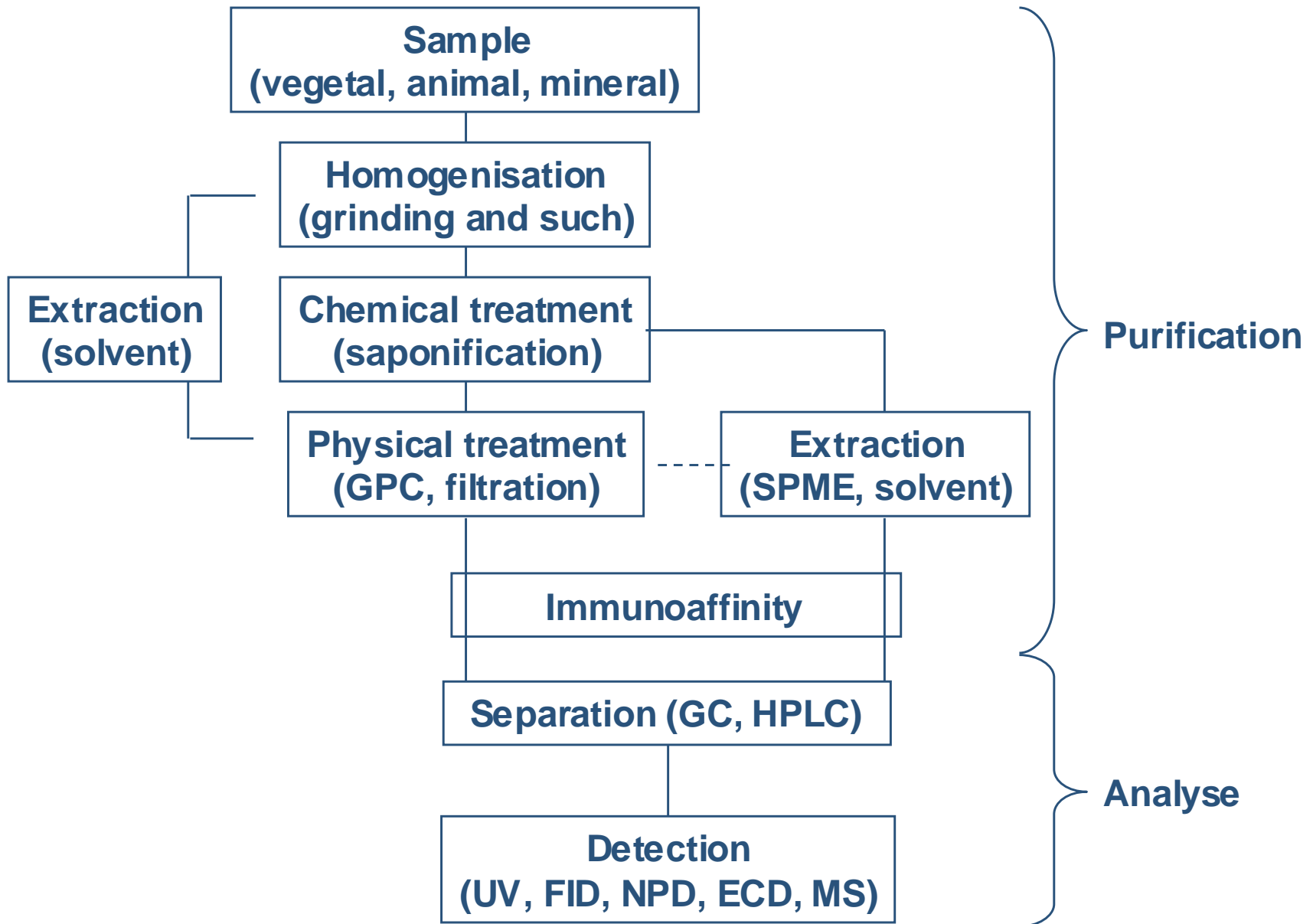
- Before the analytical method is offered to our customers it must be validated in-house
- Aim of the validation is to show that the pesticides can be recovered from the specified matrix with good results

Validation procedure

- ① Selection of representative samples that are free of any pesticide residues
- ② Working-up of 2 blank samples to prove the absence of any interferences
- ③ Spiking of each 5 identical blank samples with a pesticide mix solution at the requested Maximum Residue Level (MRL) and the tenfold MRL, respectively
- ④ Analysis of the 12 samples and statistical evaluation of the percentage recoveries found for each pesticide that was spiked



The method is valid if the mean recovery is between 70% and 110% and relative standard deviation is less than 20%



To Dr. Thomas Anspach

VERY

our attention