

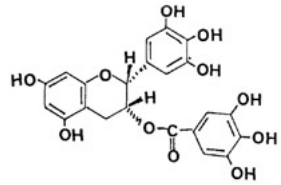


Advantages in Food Analysis Using LC/MS/MS Techniques – Applications for the Determination of Valuable and Harmful Compounds

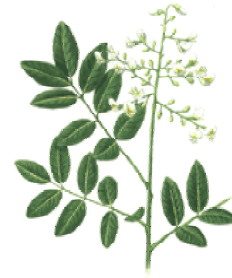
Dr. Jens Dahlmann, Applied Biosystems, Germany

Outline of talk

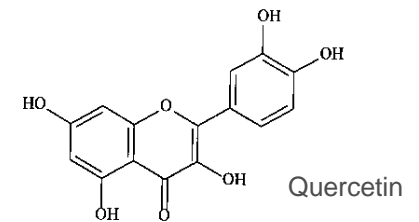
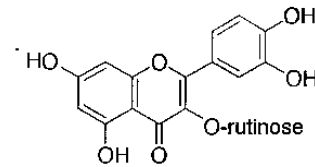
- ✓ Analysis of flavonoides
- ✓ Analysis of vitamins by LC/MS/MS
- ✓ Some examples of phospholipid analysis
- ✓ Determination of cyanobacterial toxins in health supplements and in drinking water
- ✓ Multi-Component analysis of pesticides in fruits
- ✓ Contamination of foodstuff by antibiotics



L-EGCG (Epigallocatechin Gallate)

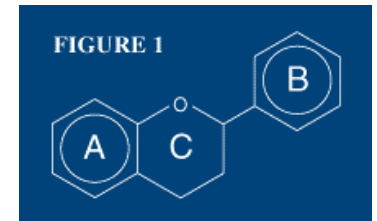


Analysis of flavonoides



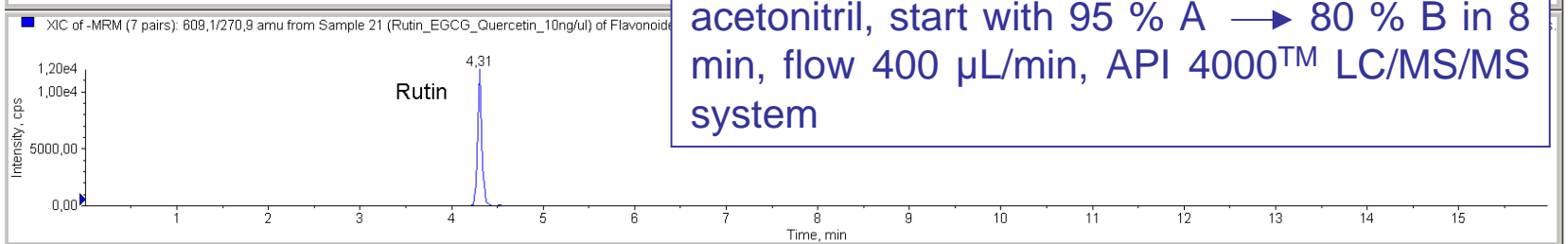
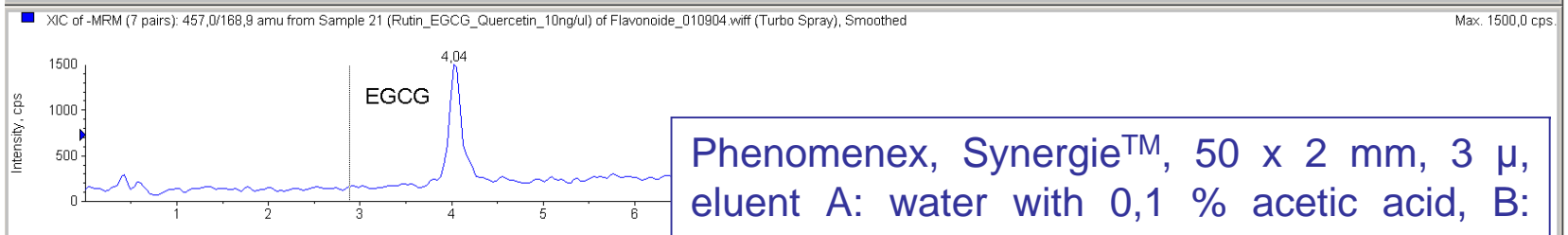
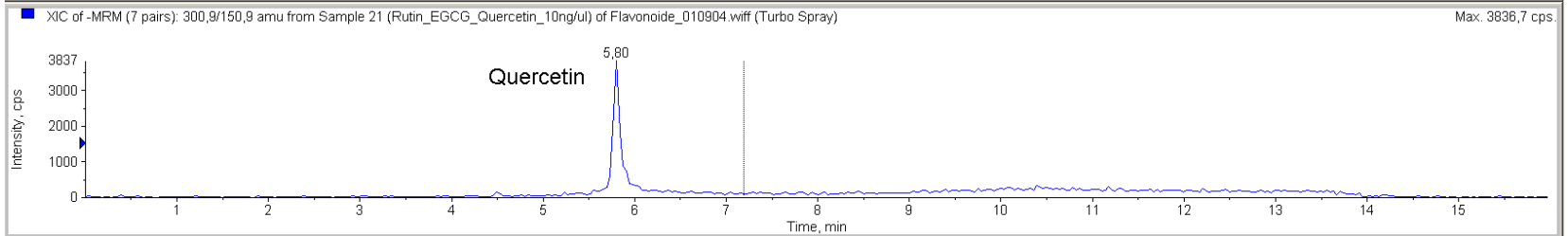
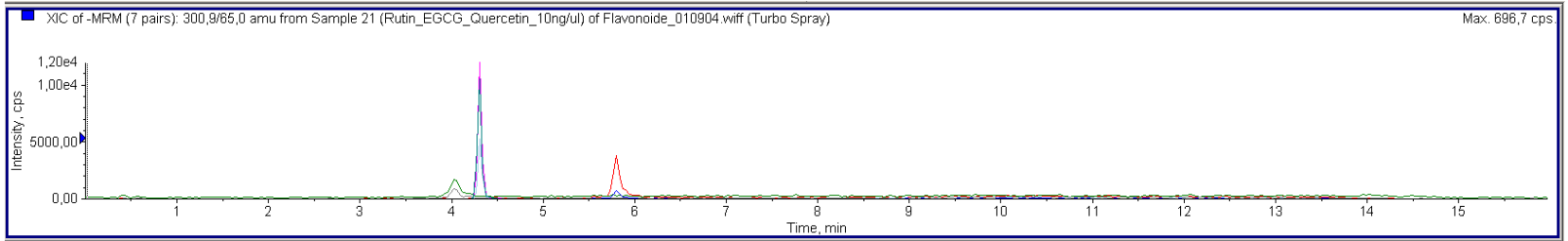
Information about flavonoids

- ✓ A ubiquitous group of polyphenolic substances which are present in most plants, concentrating in seeds, fruit skin or peel, bark, and flowers
- ✓ The structure includes two benzene rings on either side of a 3-carbon ring. Multiple combinations of hydroxyl groups, sugars, oxygens, and methyl groups attached to these structures create the various classes of flavonoids



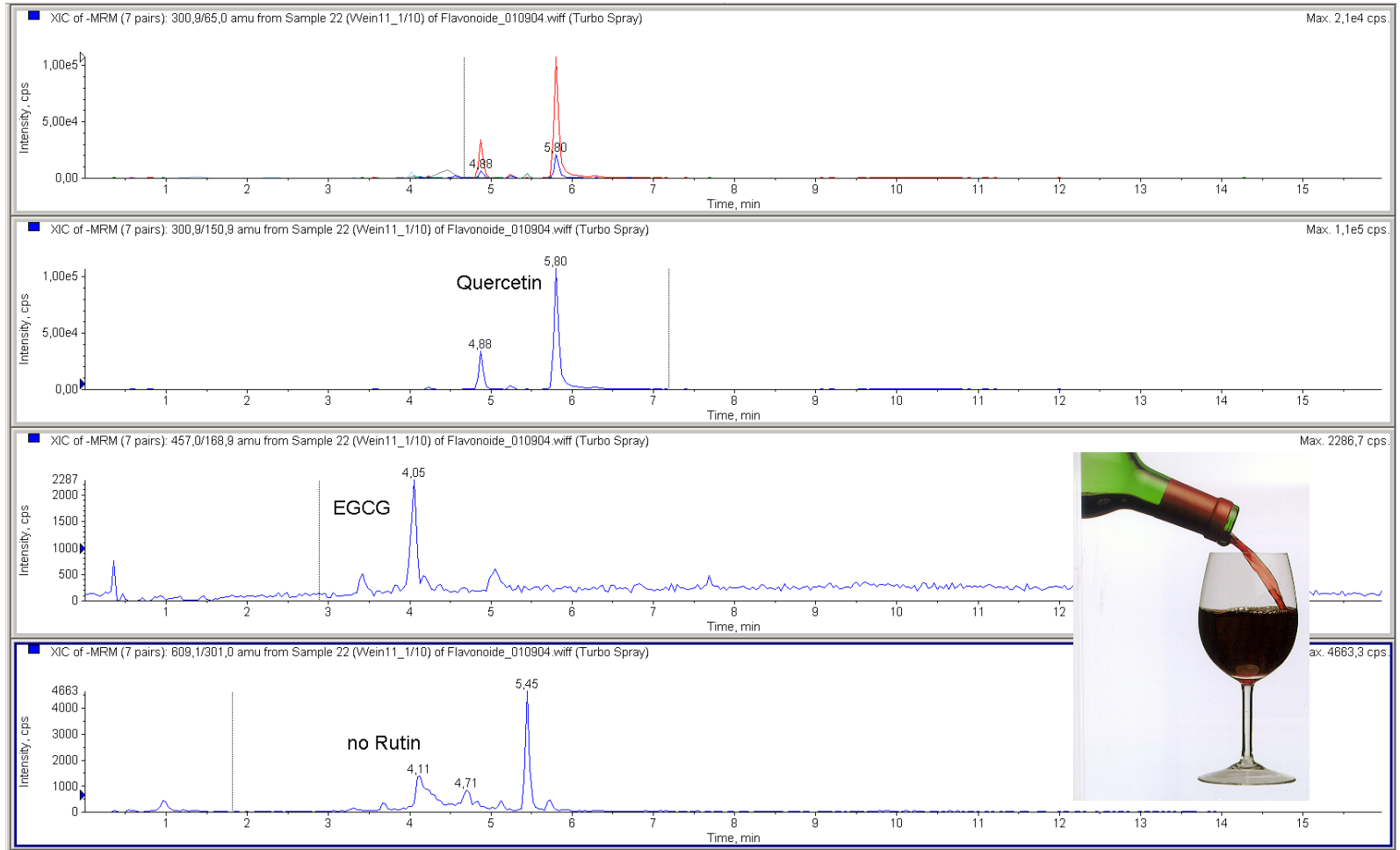
- ✓ Flavonoids have been shown in a number of studies to be potent antioxidants, capable of scavenging hydroxyl radicals, superoxide anions, and lipid peroxy radicals

LC/MS/MS chromatogram of selected flavonoid standards



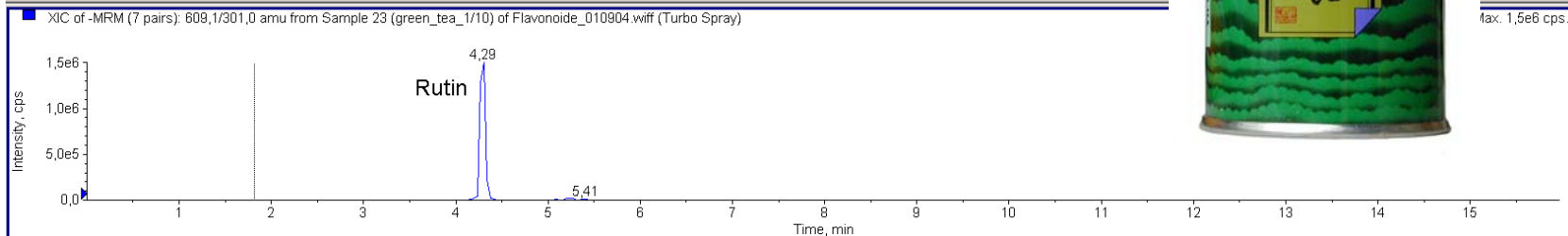
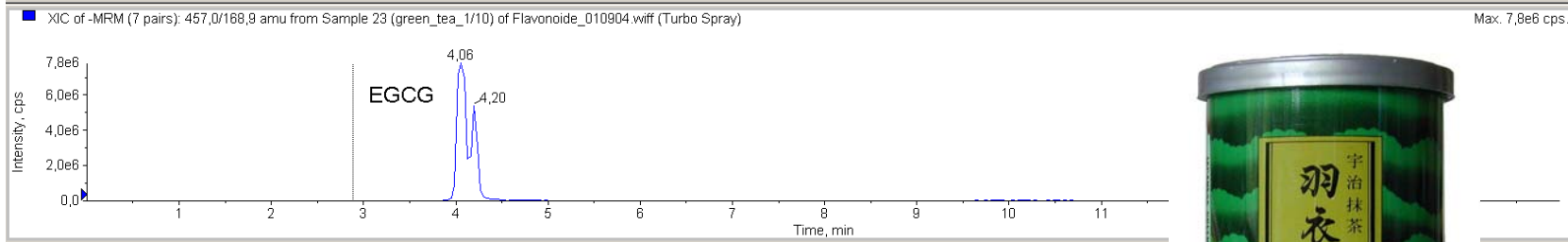
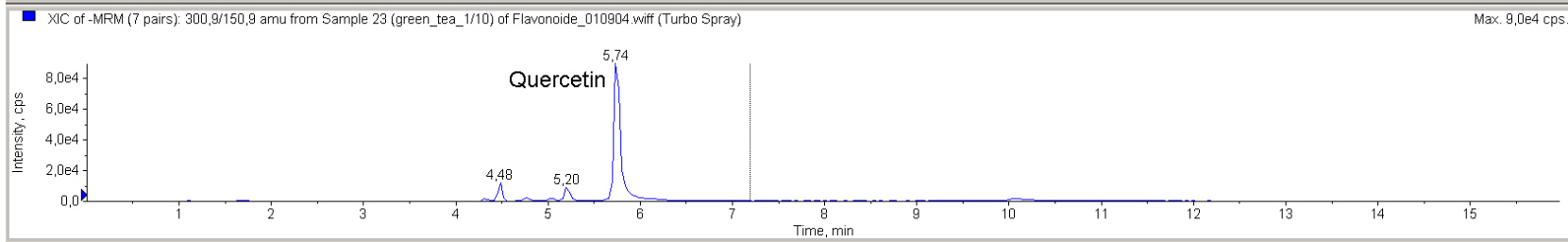
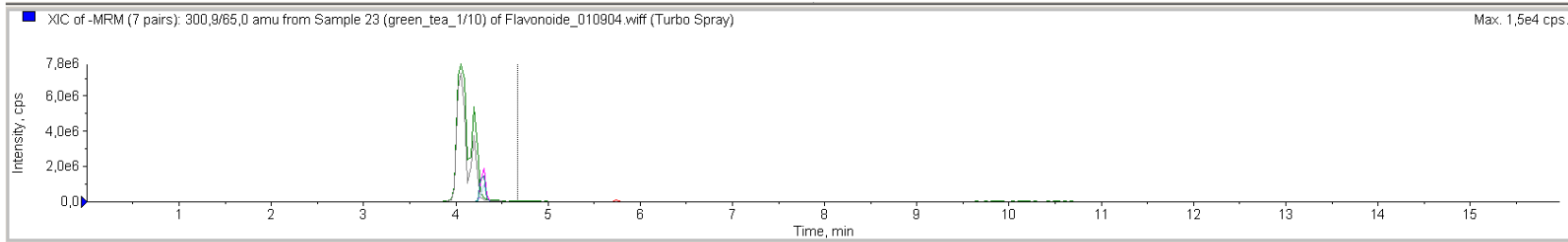
Phenomenex, Synergie™, 50 x 2 mm, 3 μ,
 eluent A: water with 0,1 % acetic acid, B:
 acetonitril, start with 95 % A → 80 % B in 8
 min, flow 400 μL/min, API 4000™ LC/MS/MS
 system

Quercetin, rutin and epigallocatechin gallate in red wine

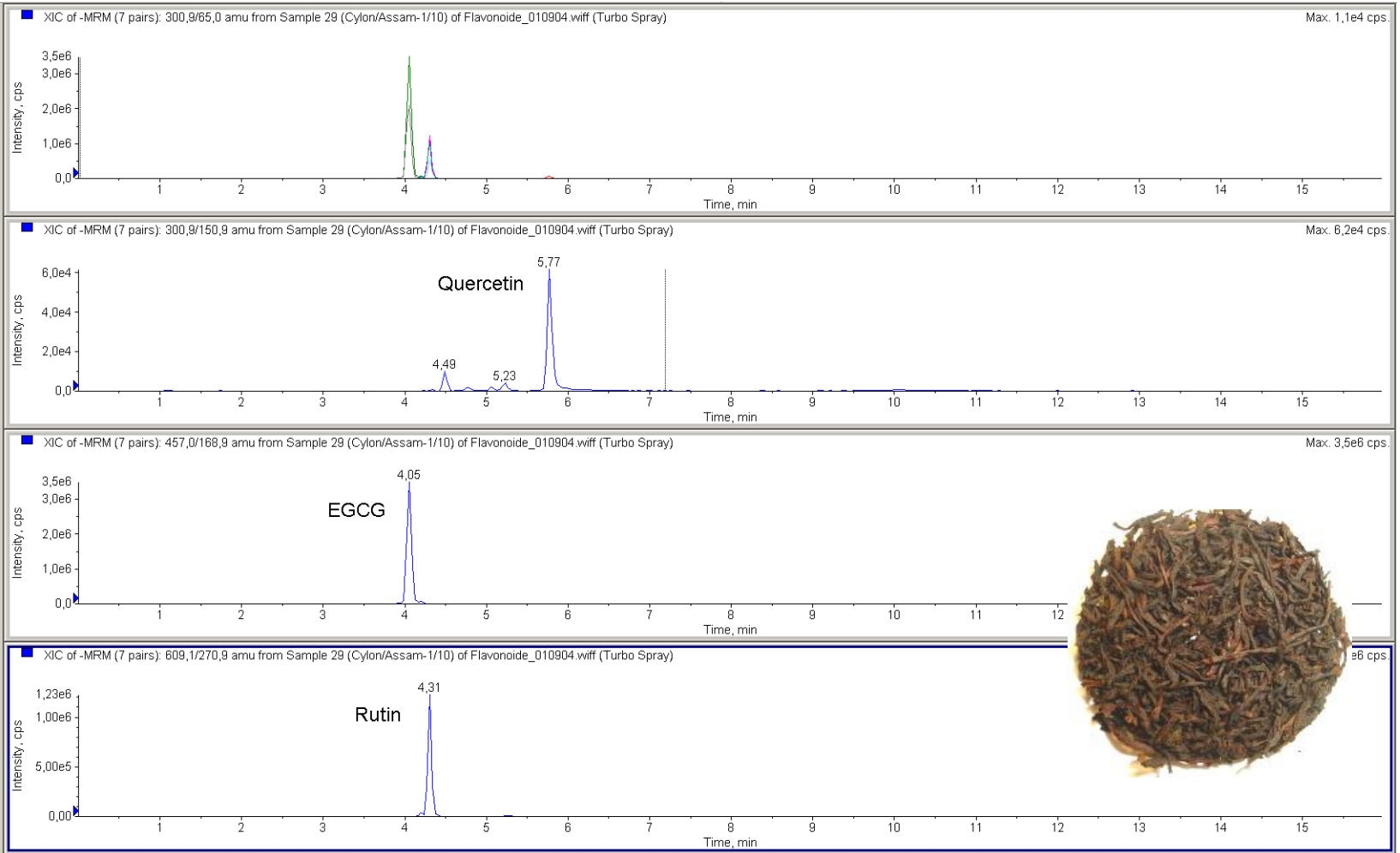




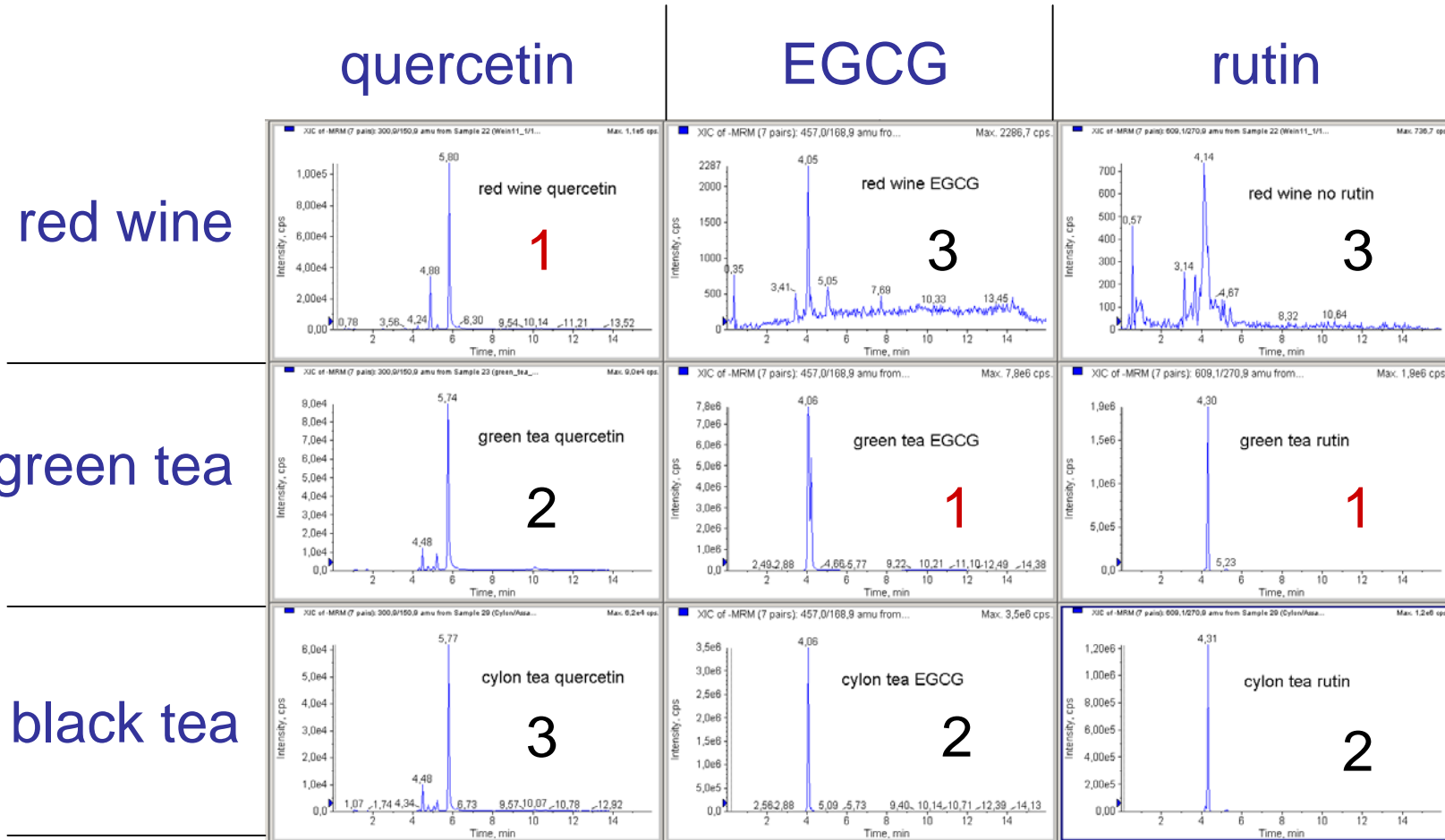
Quercetin, rutin and epigallocatechin gallate in green tea



Quercetin, rutin and epigallocatechin gallate in black tea



Different sources for the flavonoides quercetin, rutin and epigallocatechin gallate



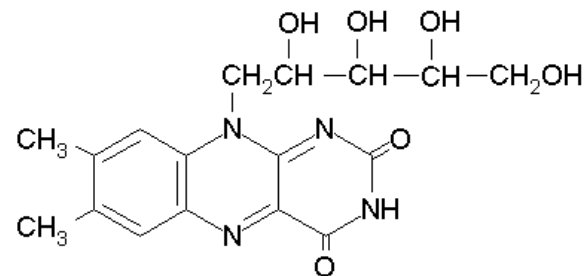


Analysis of vitamins by LC/MS/MS



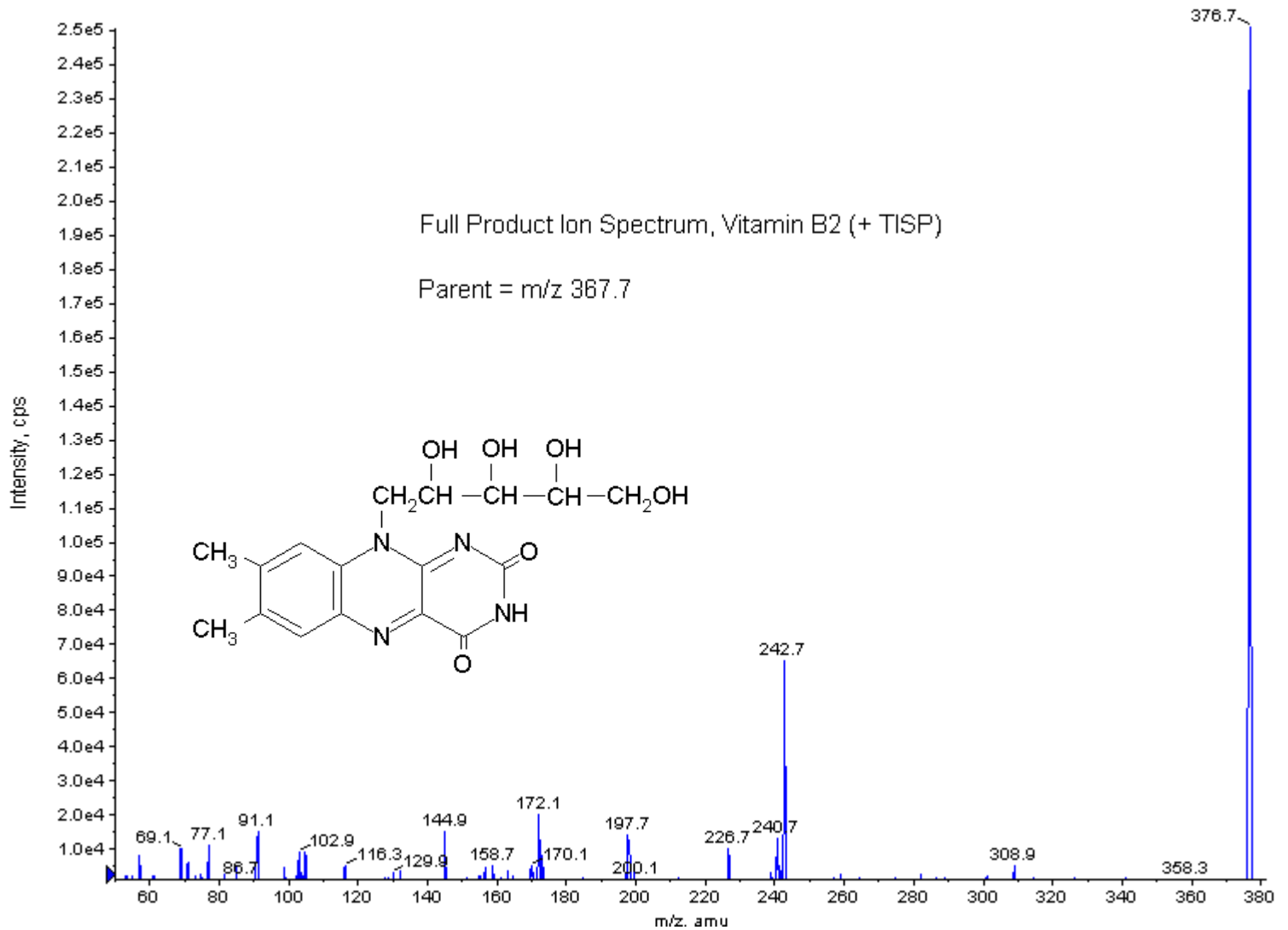
Vitamin B 2 - riboflavin

- ✓ An essential function of riboflavin is to metabolize carbohydrates, proteins (amino acids), and fats for use by the body
- ✓ Riboflavin is also necessary for red blood cell formation, antibody production, cell respiration, normal fetus growth, the development of body tissues (like hair, skin, and nails)
- ✓ Vitamin B 2 maintenance mucous membranes in the digestive tract



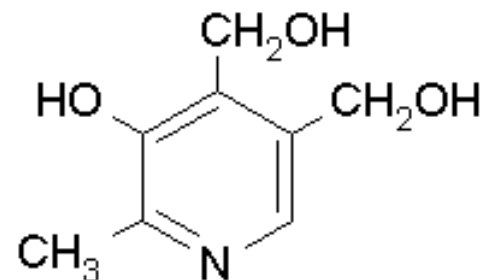


Vitamin B2, Positive TurbolonSpray (TIS)TM



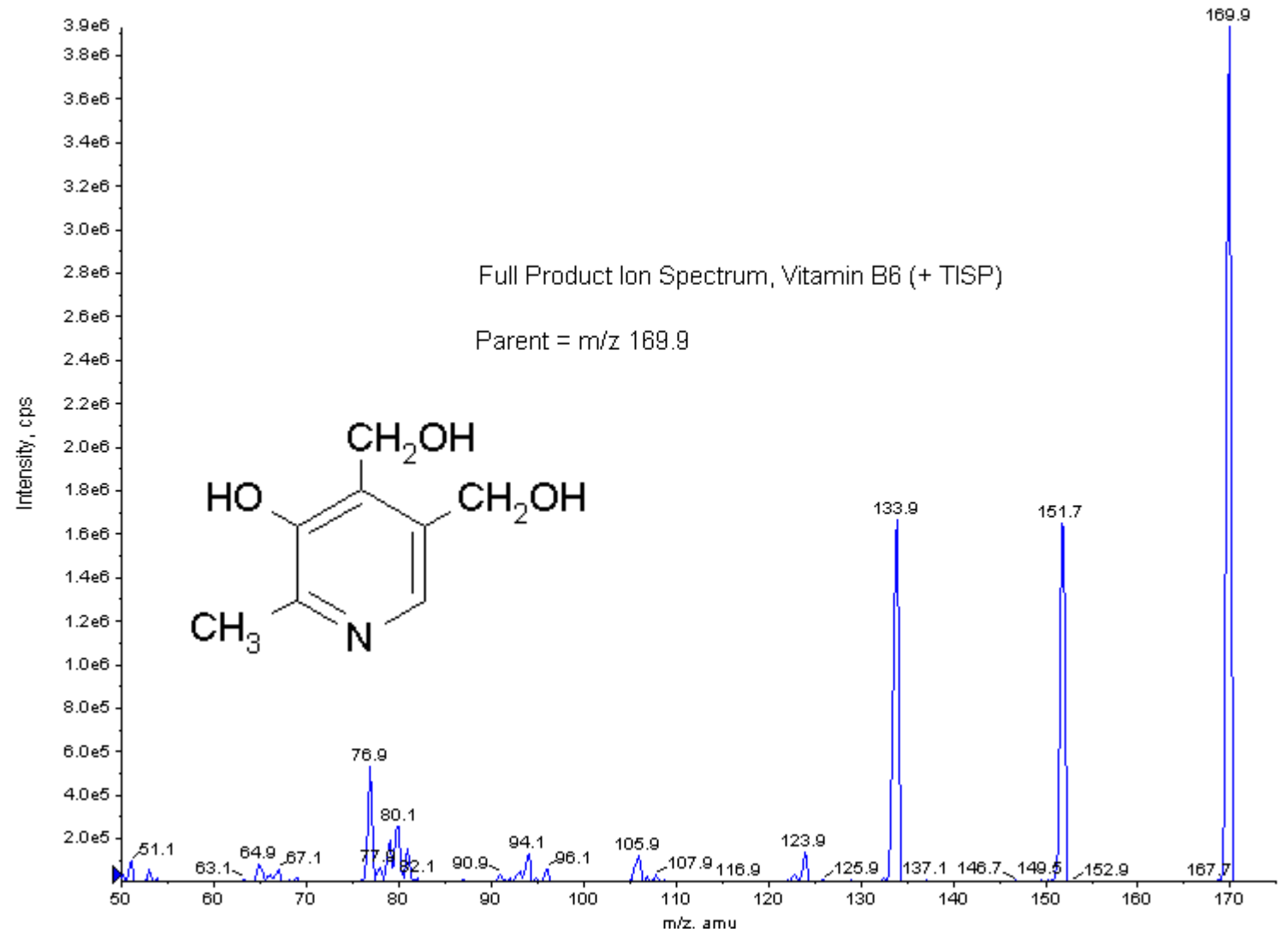
Vitamin B 6 - pyridoxine

- ✓ Vitamin B 6 plays a role in the synthesis of antibodies by the immune system, which are needed to fight many diseases
- ✓ It helps maintain normal nerve function and also acts in the formation of red blood cells
- ✓ Vitamin B 6 is also required for the chemical reactions needed to digest proteins



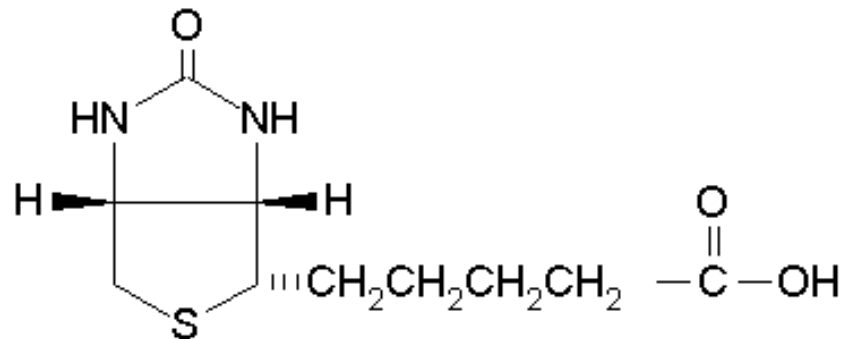


Vitamin B 6, Positive TurbolonSpray (TIS)TM



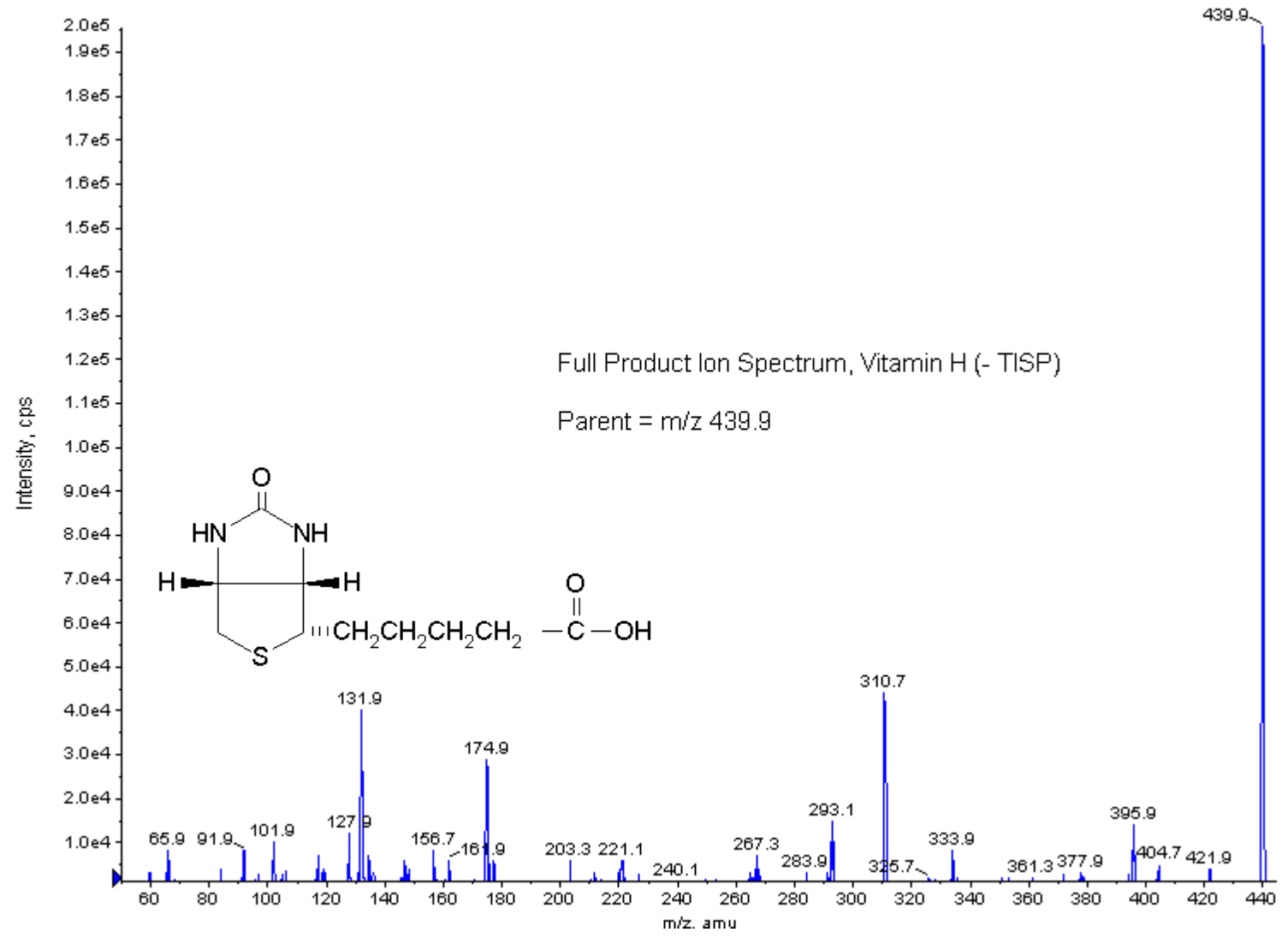
Biotin

- ✓ Biotin is used in cell growth, the production of fatty acids, metabolism of fats, and proteins
- ✓ Biotin not only assists in various metabolic chemical conversions, but also helps with the transfer of carbon dioxide
- ✓ Biotin is also helpful in maintaining a steady blood sugar level



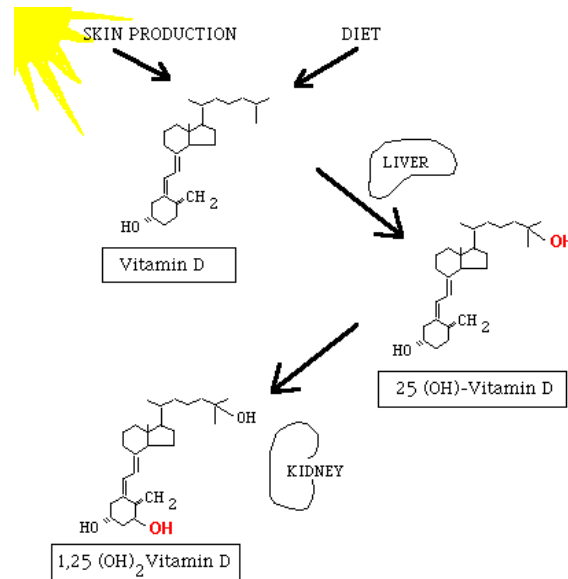


Biotin, Negative TurbolonSpray (TIS)TM

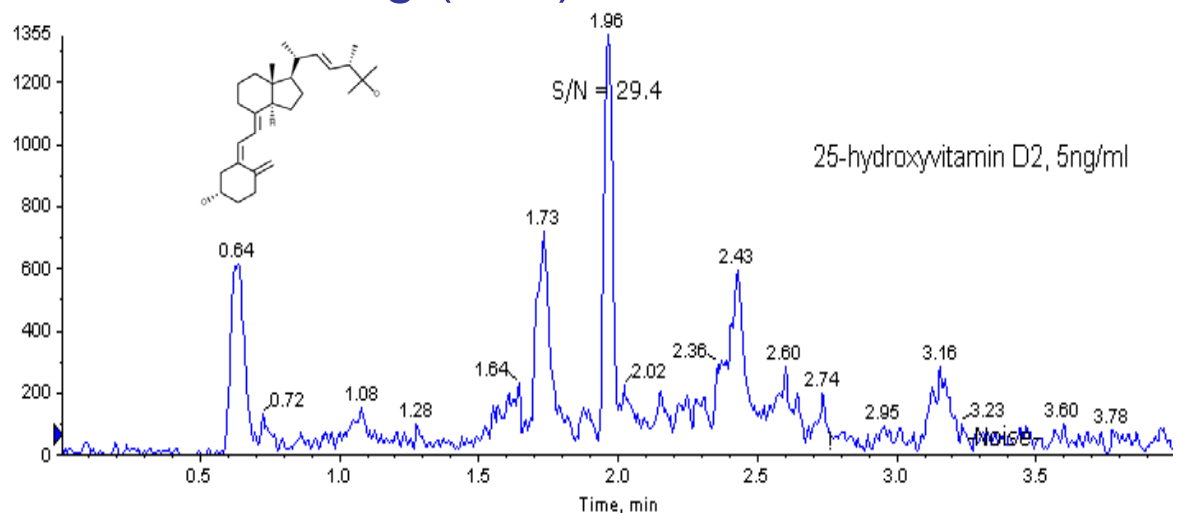


Vitamin D

- ✓ Vitamin D promotes the body's absorption of calcium, which is essential for the normal development and maintenance of healthy teeth and bones - calcium is also important to nerve cells
- ✓ Vitamin D also helps maintain adequate blood levels of calcium and phosphorus

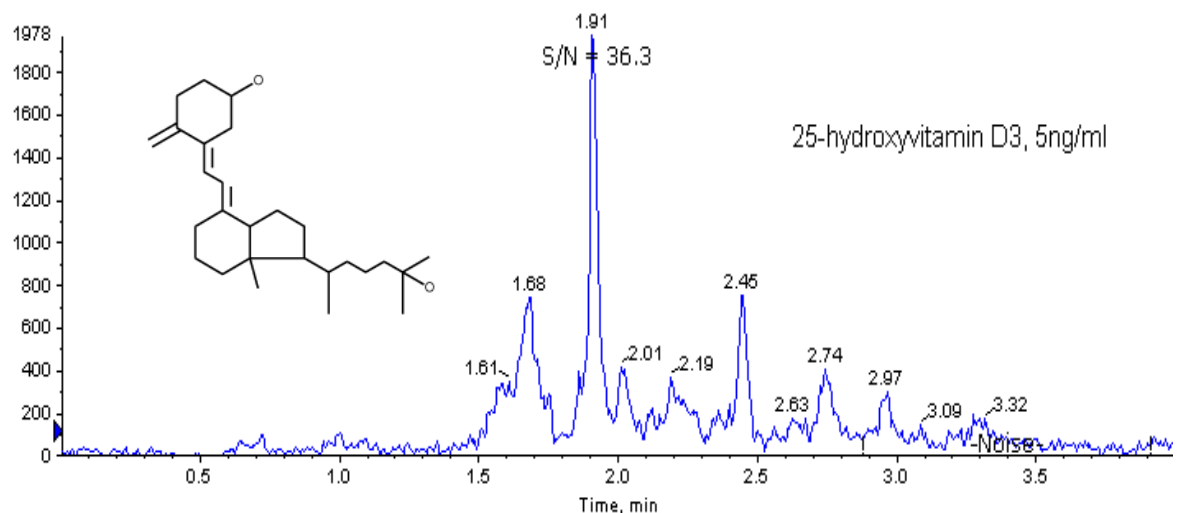


Selected Ion Monitoring (SIM) for D vitamins in human plasma



XIC of +MRM (2 pairs): 401.4/383.5 amu from Sample 3 (5ng/ml) of 150403 Samples.wiff (Turbo Spray...

Max. 1978.3 cf



HPLC Column: 50x2.1 mm, 3 μ , C-8 (ThermoHypersilKeystone)

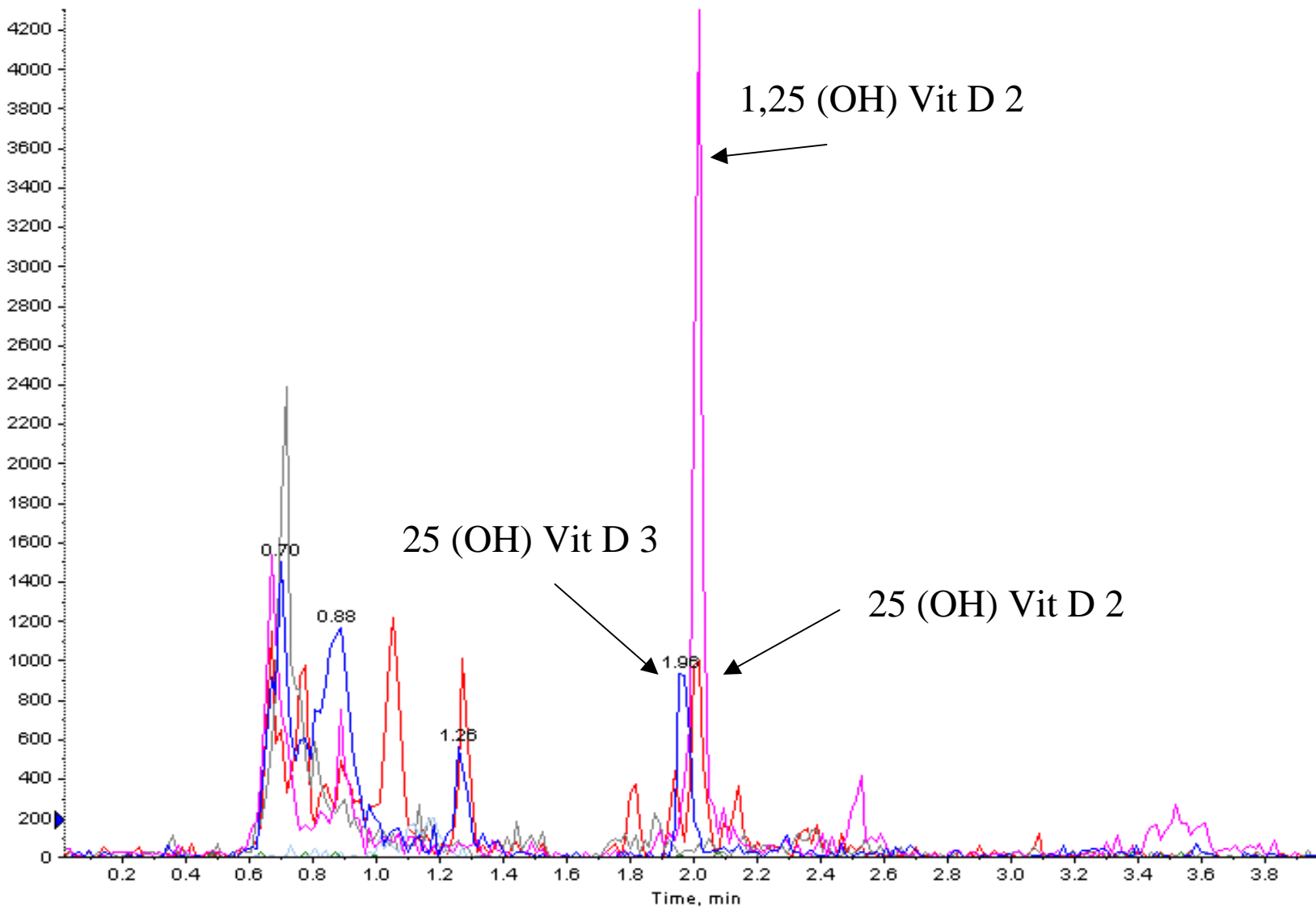
Mobile Phase A: 0.1% aqueous Formic Acid

Mobile Phase B: Methanol

Flow rate: 400 μ l/min, gradient method

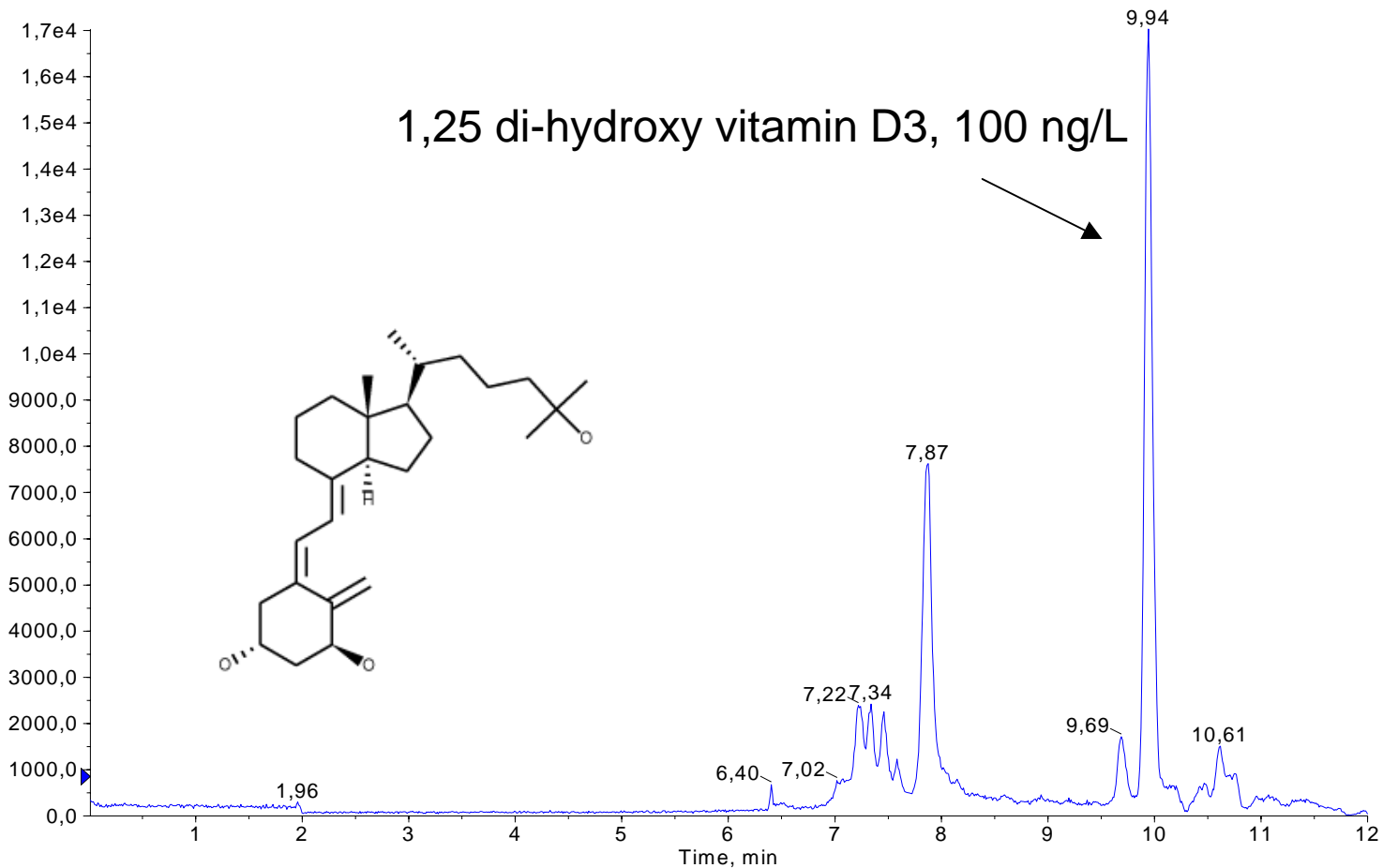


Extracted patient plasma sample showing full metabolite profile



XIC of +MRM (1 pair): 381,3/363,3 amu from Sample 1 (AcnEx+100ppb) of AcnEx+100ppb...

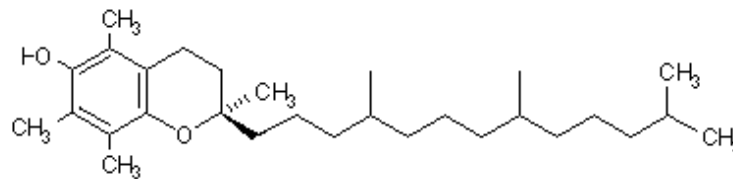
Max. 1,7e4 cps.





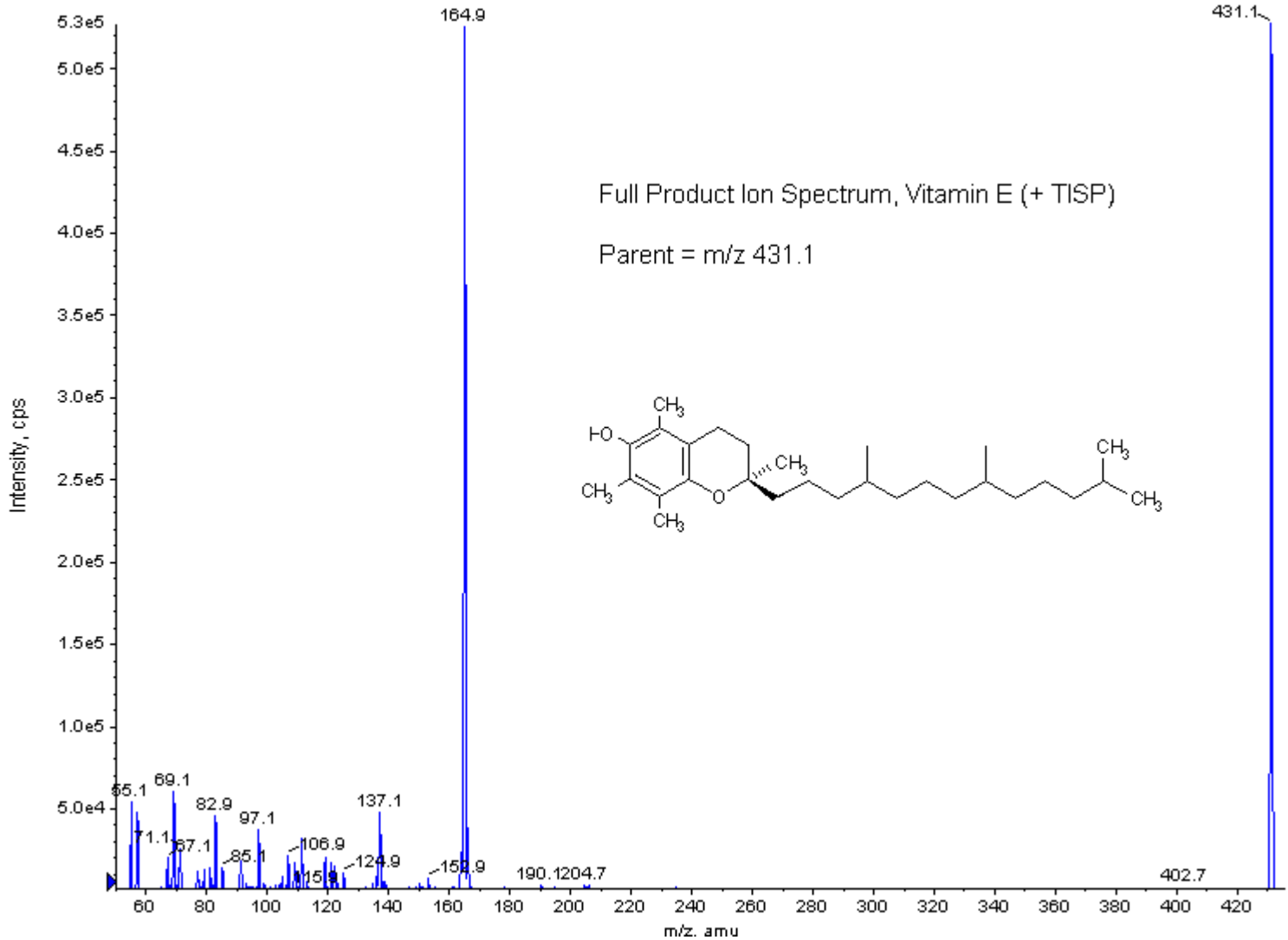
Vitamin E - *alpha*-Tocopherol

- ✓ Vitamin E is an antioxidant that protects body tissue from damage caused by free radicals. Free radicals can harm cells, tissues, and organs
- ✓ Vitamin E is also important in the formation of red blood cells and it helps the body to use vitamin K





Vitamin E, Positive TurbolonSpray (TIS)TM



Strategy for vitamin analyses by LC/MS/MS

- ✓ Vitamin B 2 & B 6 and rutin (water soluble), mobile phase: MeOH:water, possibly with 0.1% formic acid on a C-18 column with TIS™ ionization
- ✓ Vitamin D2 & D3 and E with PhotoSpray™ (or APCI), mobile phase: MeOH:acetonitrile 25:75 (isocratic)
- ✓ 25 (OH) Vit. D 2 and D 3 can be best ionized with TIS™
- ✓ D vitamins will lose water and should be optimized on this ion $[M+H-H_2O]^+$



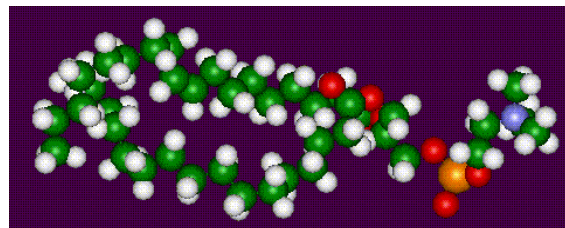
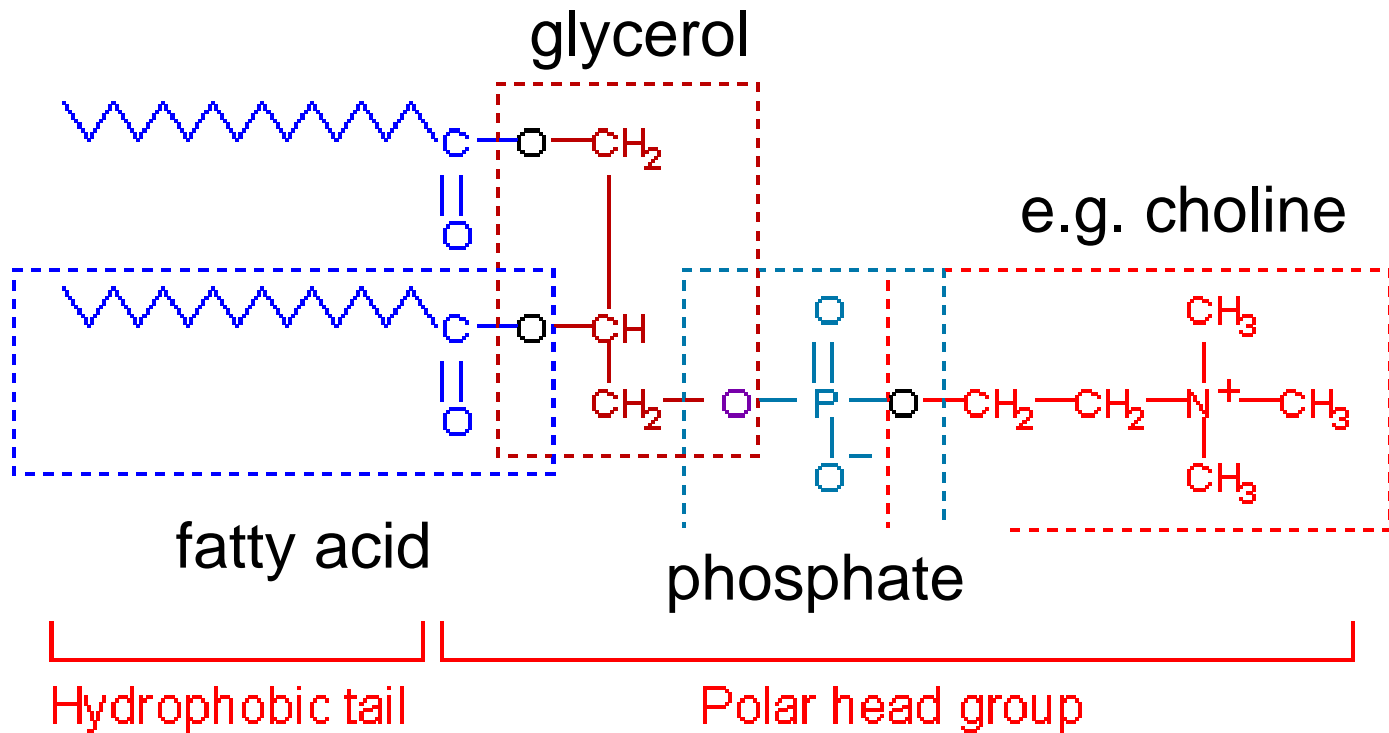
Some examples of phospholipid analysis



Facts about phospholipids

- ✓ Phospholipids are diglycerides that are covalently bonded to a phosphate group by an ester linkage. Amino groups can be additionally attached to the phosphate
- ✓ The hydrocarbon chains are hydrophobic. The phosphate and amino groups make that portion of the molecule hydrophilic. The result is an **amphiphilic molecule**
- ✓ Amphiphilic lipids are major constituents of cell membranes – polar heads are facing aqueous surroundings (e.g. cytosol) and hydrophobic tails facing each other
- ✓ This ideal combination of physical and chemical functionality makes phospholipids an indispensable component in cosmetic products

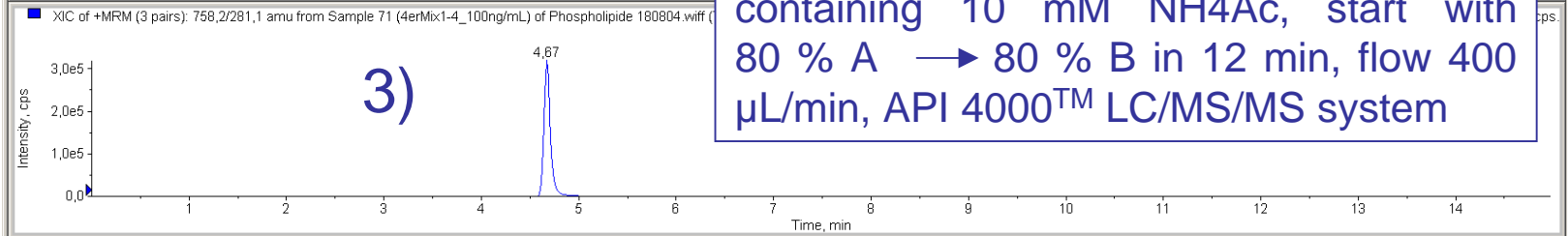
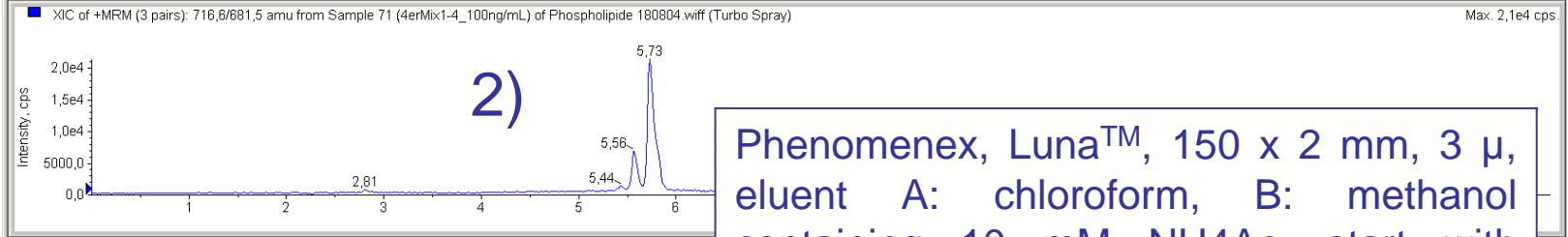
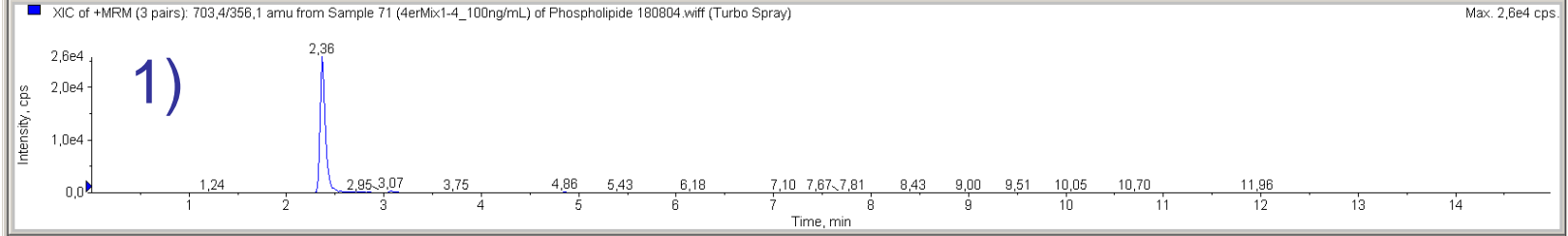
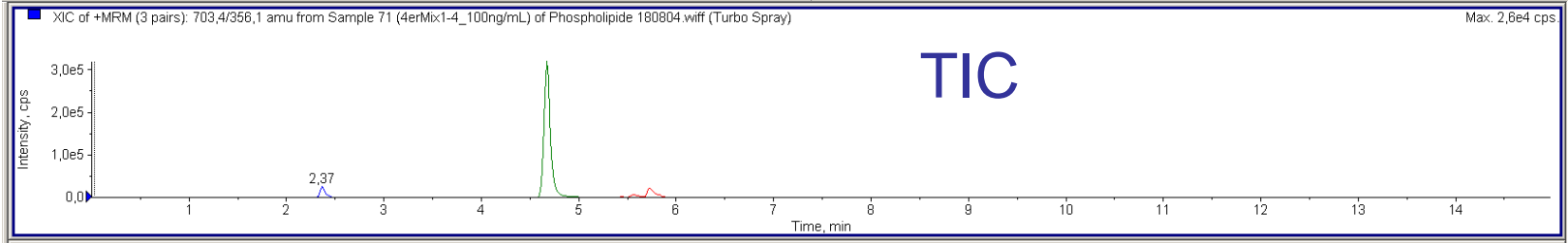
Basic structure of a phospholipid molecule





Chromatographic separation of:

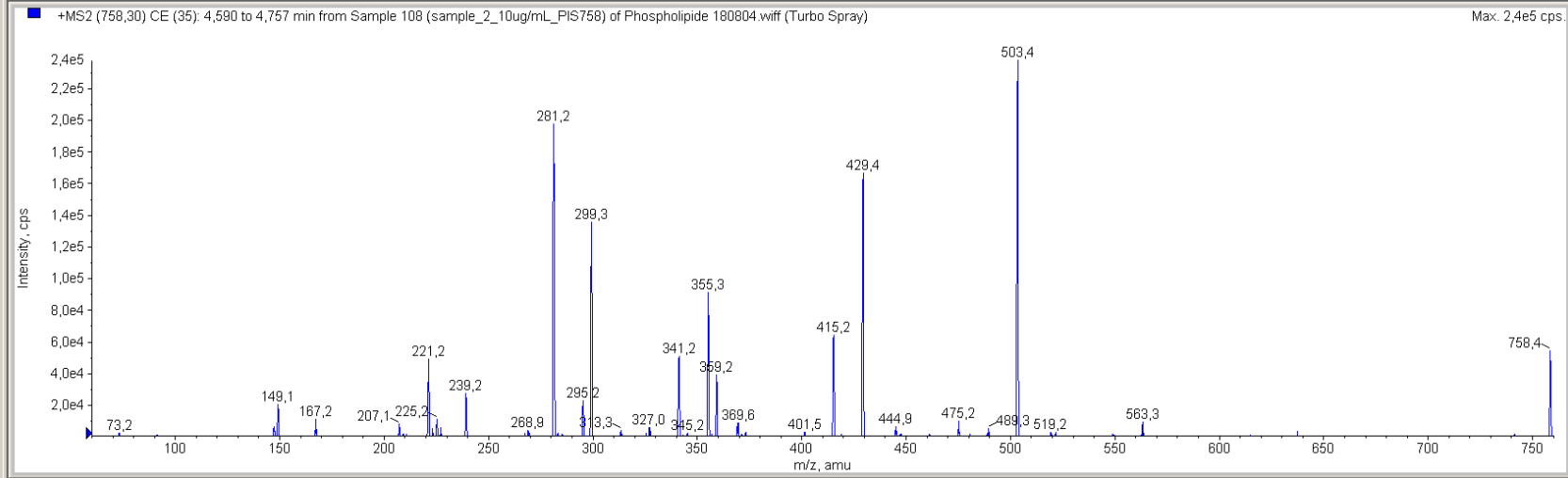
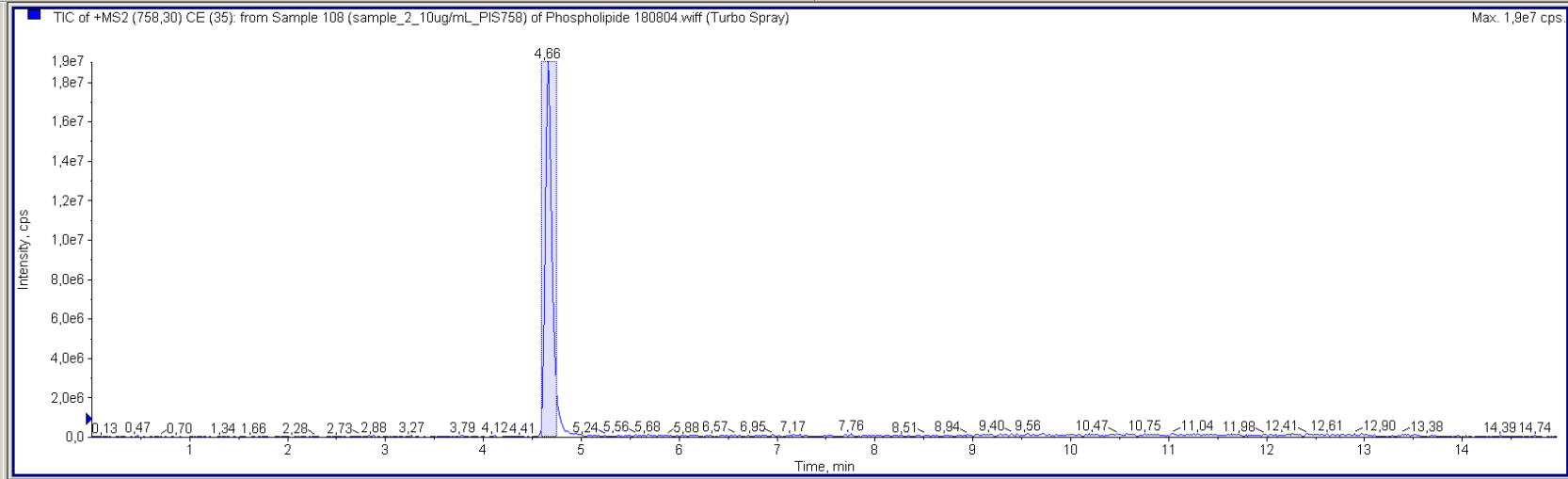
- 1) sphingomyelin,
- 2) phosphatidylethanolamin and
- 3) phosphatidylserin



Phenomenex, Luna™, 150 x 2 mm, 3 μ, eluent A: chloroform, B: methanol containing 10 mM NH4Ac, start with 80 % A → 80 % B in 12 min, flow 400 μL/min, API 4000™ LC/MS/MS system

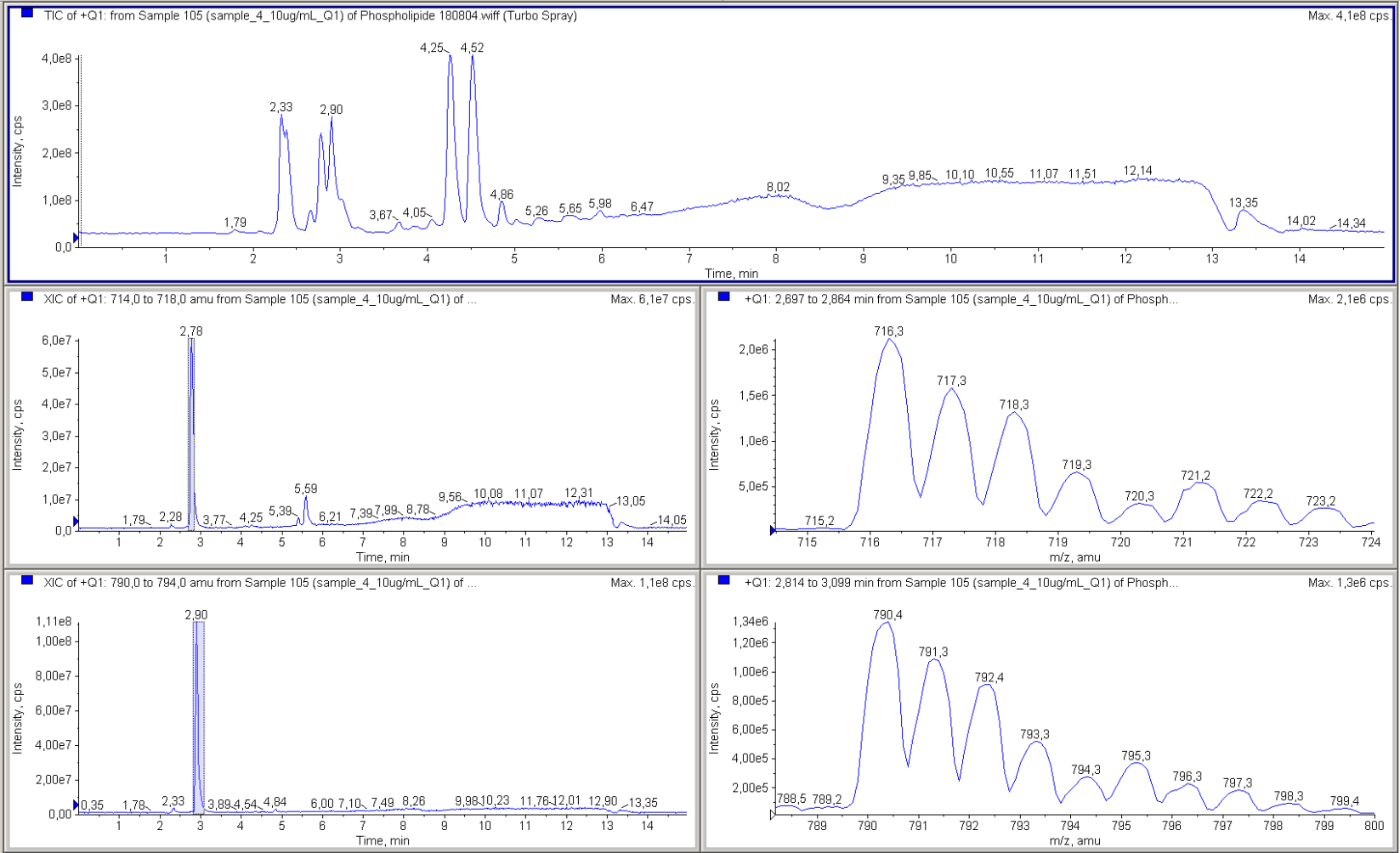


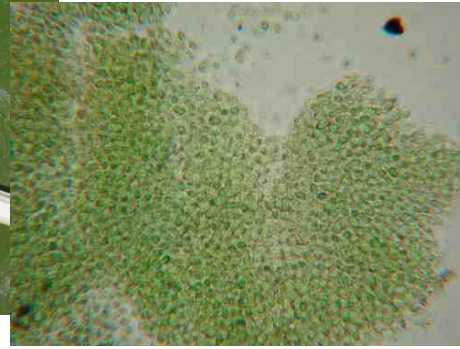
Product-Ion-spectrum of phosphatidylcholin, ESI





Q1 Scan of phosphatidylethanolamin





Determination of cyanobacterial toxins in health supplements and in drinking water





Cyanobacteria as a dietary supplement

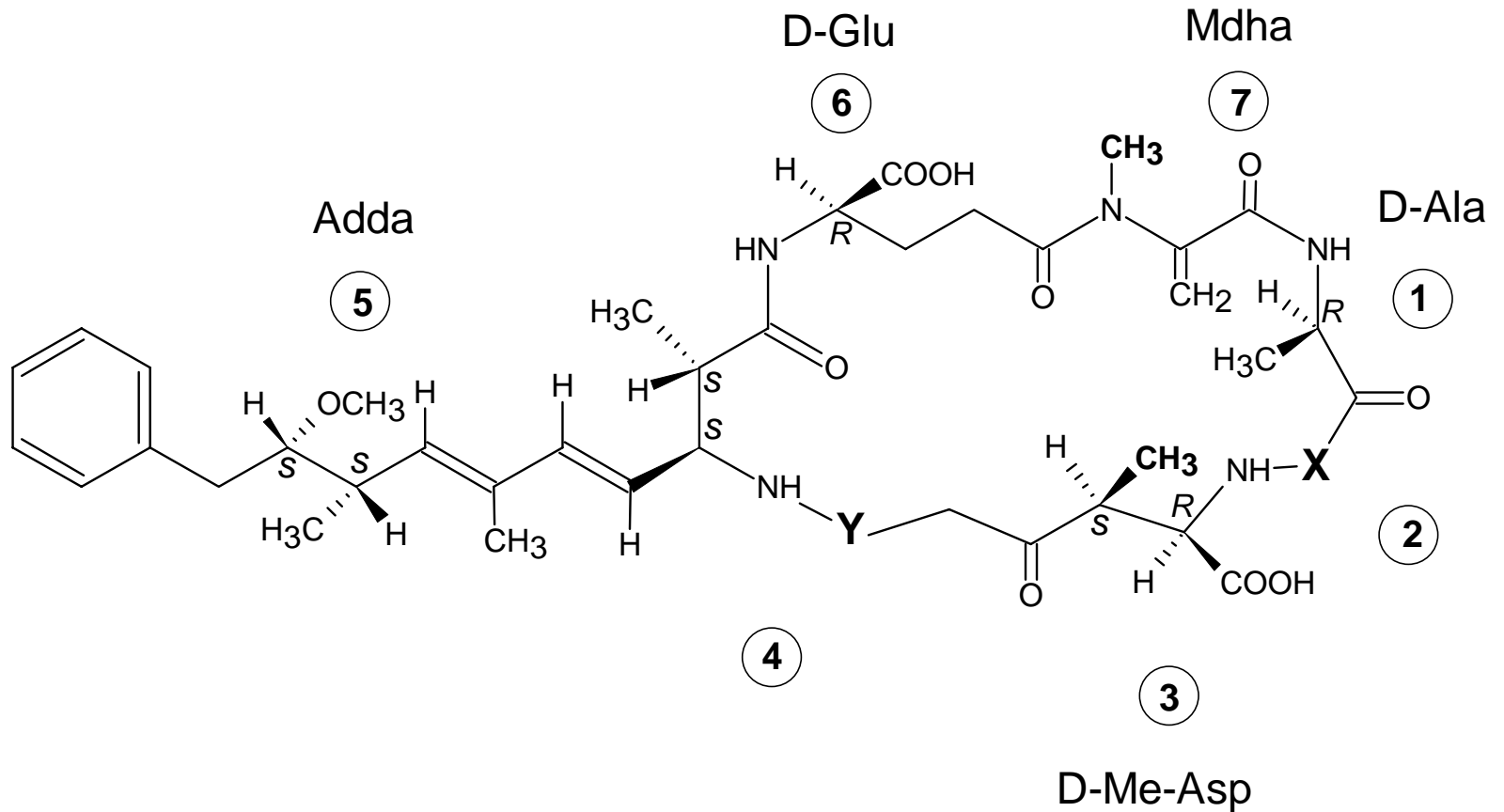
cyanobacteria are advertise as:

- ✓ having a high content of omega-3 fatty acids
- ✓ are an excellent source of amino acids
- ✓ supports the immune system by stimulating the production and movement of “natural killer cells”
- ✓ stimulate macrophage activity

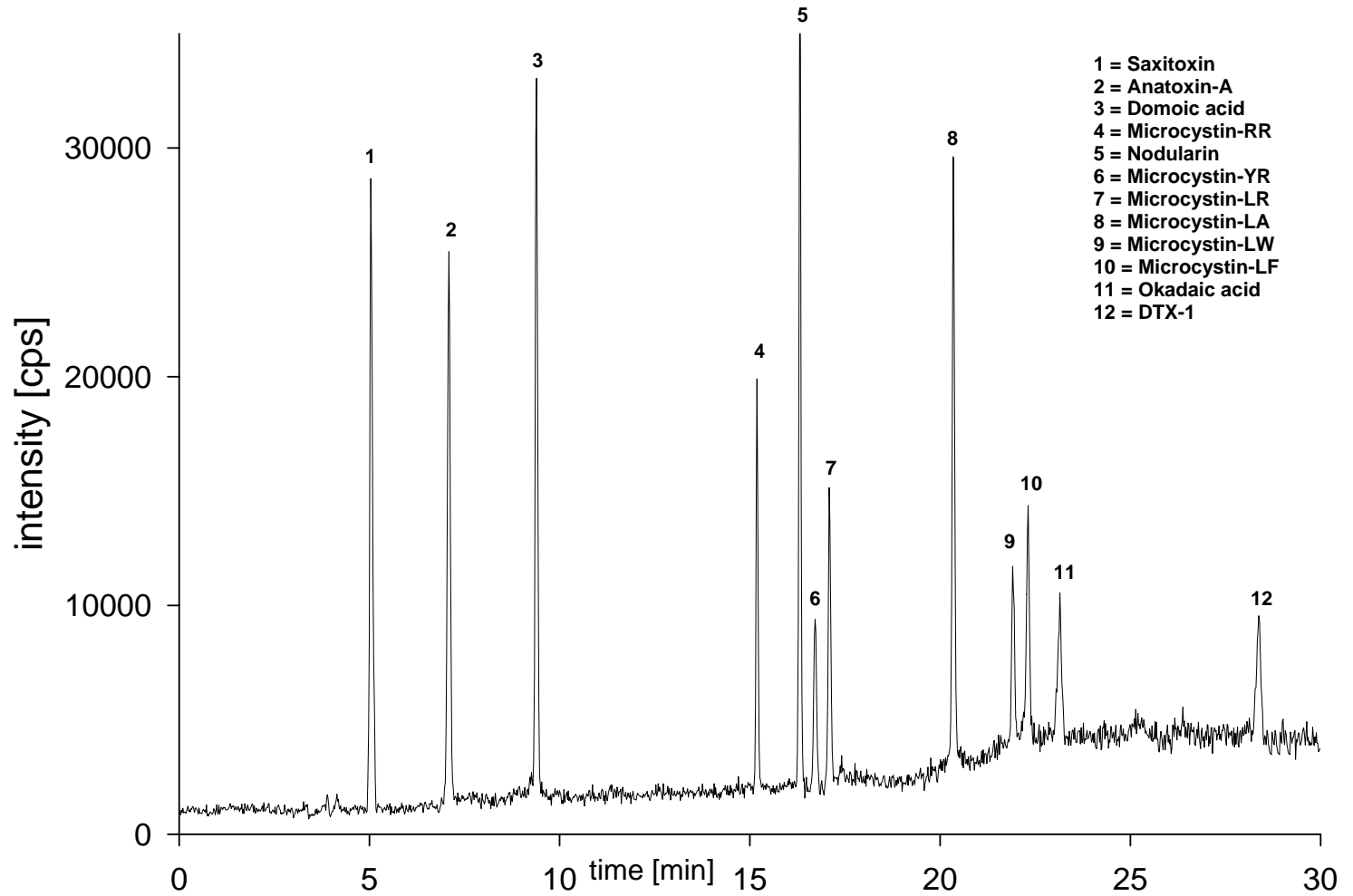
what most people don't know:

- ✓ some cyanobacteria produce toxins – these substances are tumor promoters as well as hepatotoxic

General structure of microcystins – one class of cyanotoxins

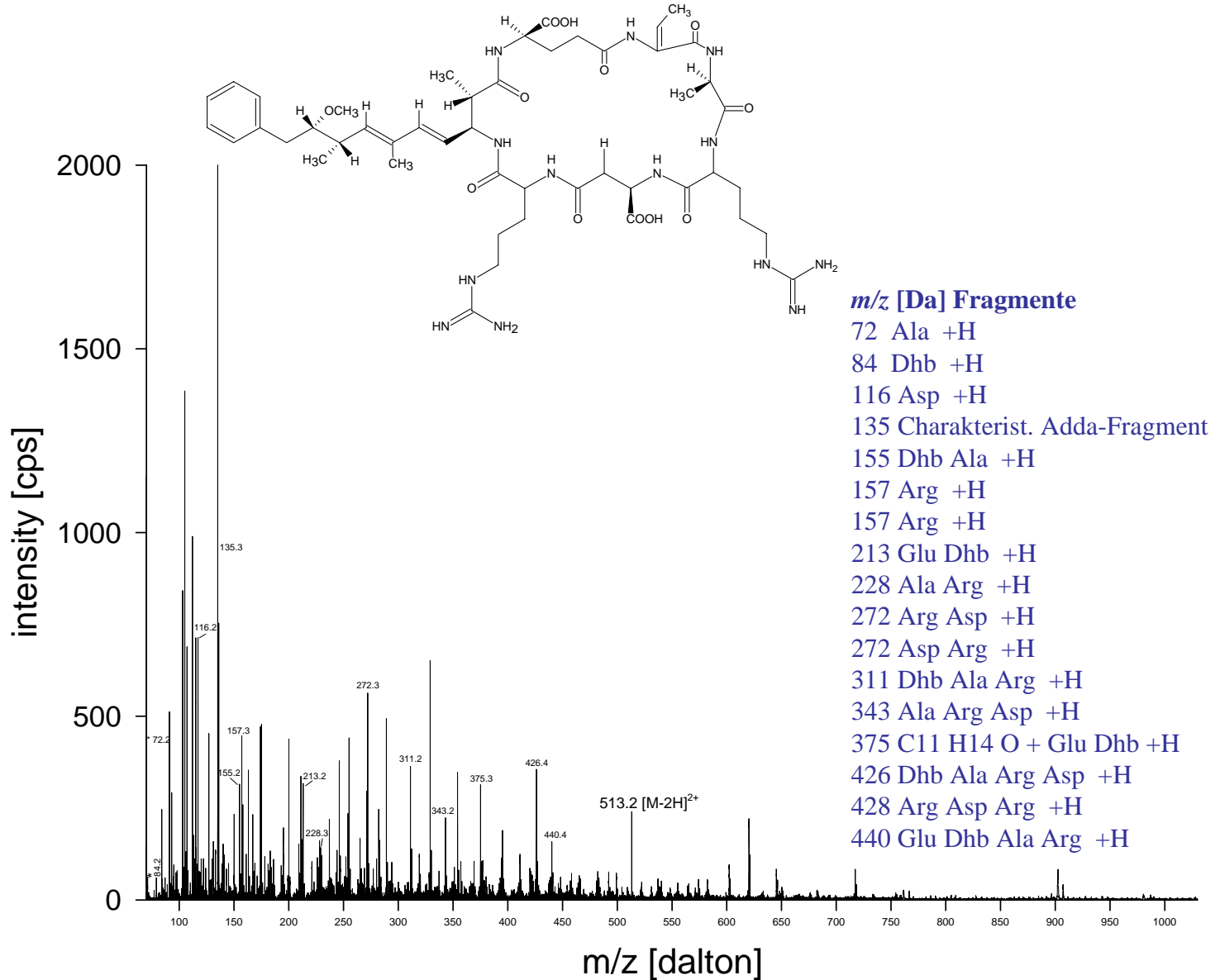


MID-chromatogram of various cyanobacterial toxins





Structure elucidation on an API 2000™ LC/MS/MS system





Bloom of *Planktothrix Rubescense* in „Lake Behlendorf“, Germany during spring 2003

cyanobacteria are often found in drinking water reservoirs



Multi-Component analysis of pesticides in fruits

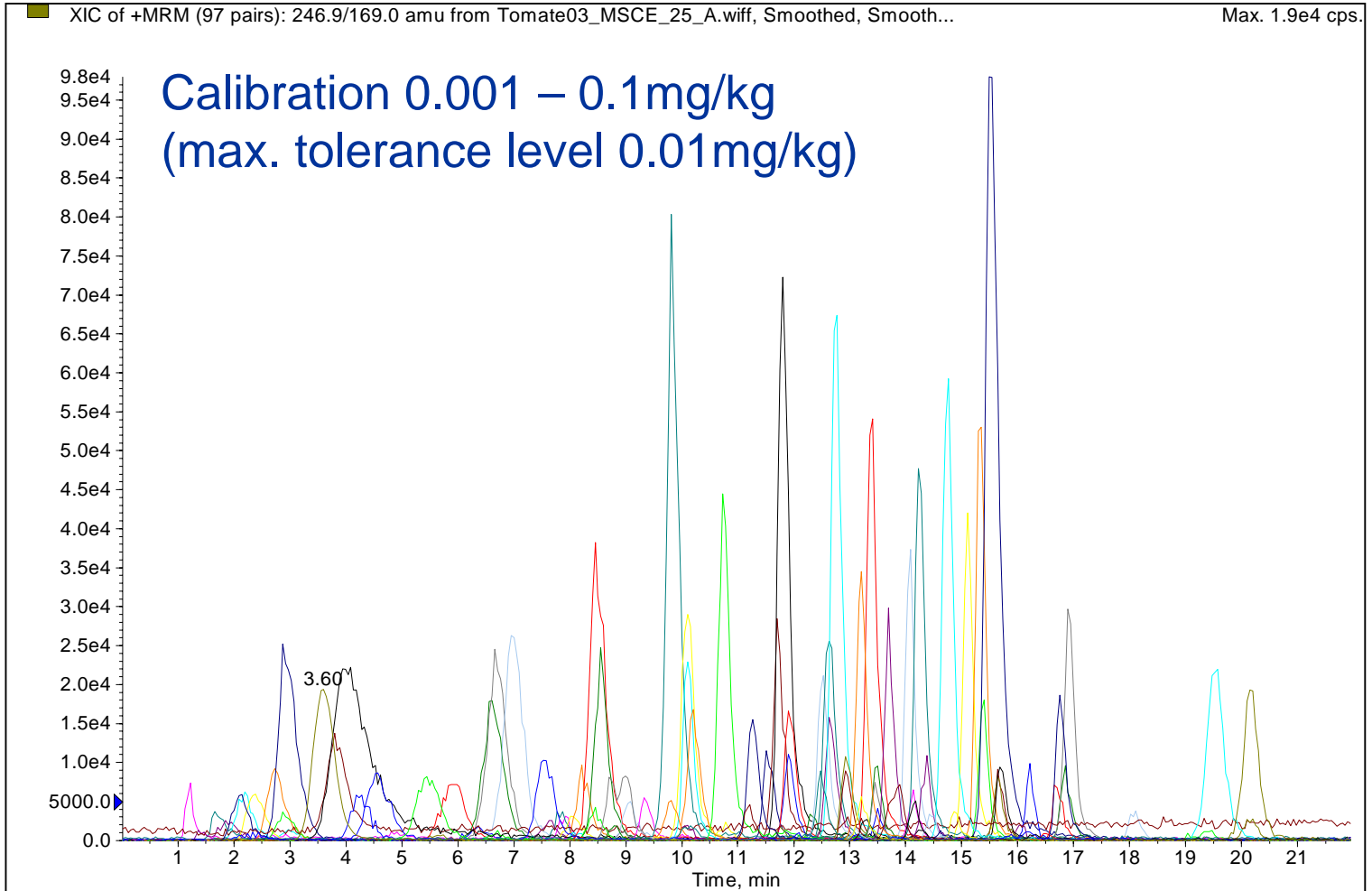


What are pesticides?

- ✓ Substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest (insects, animals, unwanted plants (weeds), fungi, or microorganisms)
- ✓ Pesticides can cause health problems, such as birth defects, nerve damage, and cancer
- ✓ World Health Organization (WHO) estimates one million pesticides poisoning cases and 20,000 deaths every year globally



Standard containing 100 pesticides



Analytical conditions

Column: Phenomenex Aqua™, 50x2 mm, 5 μm

Flow: 200 μL/min

Gradient: H₂O/CH₃OH + 5 mM NH₄form

API 2000™ LC/MS/MS system

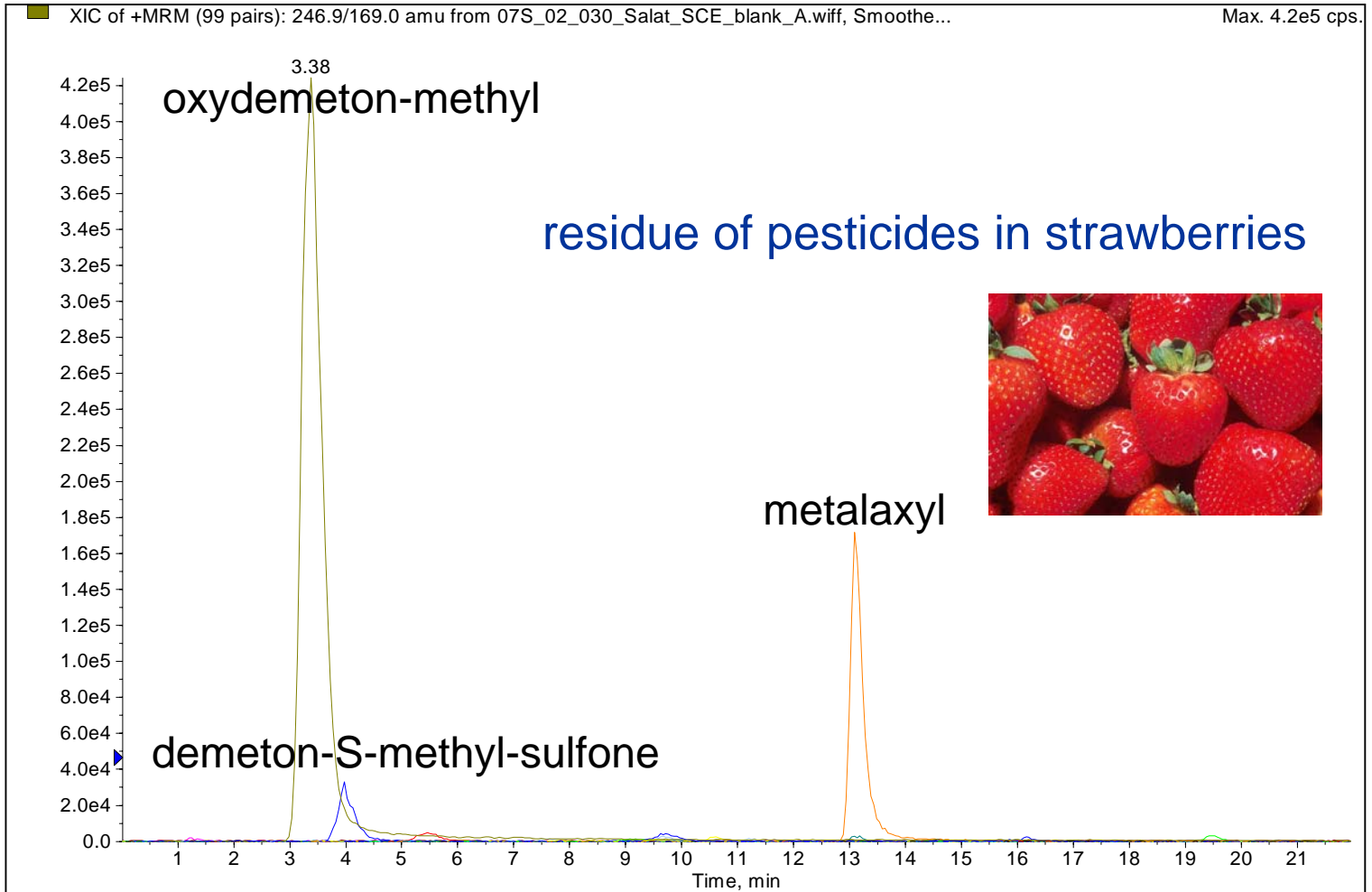
Positive TurbolonSpray™ source

Quantitation in MRM

Dwell time per MRM = 25msec

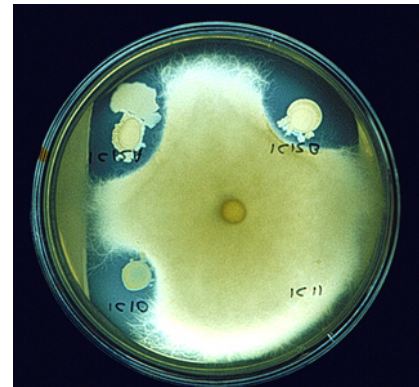
Extraction with ChemElut and Filtration

Positive real sample





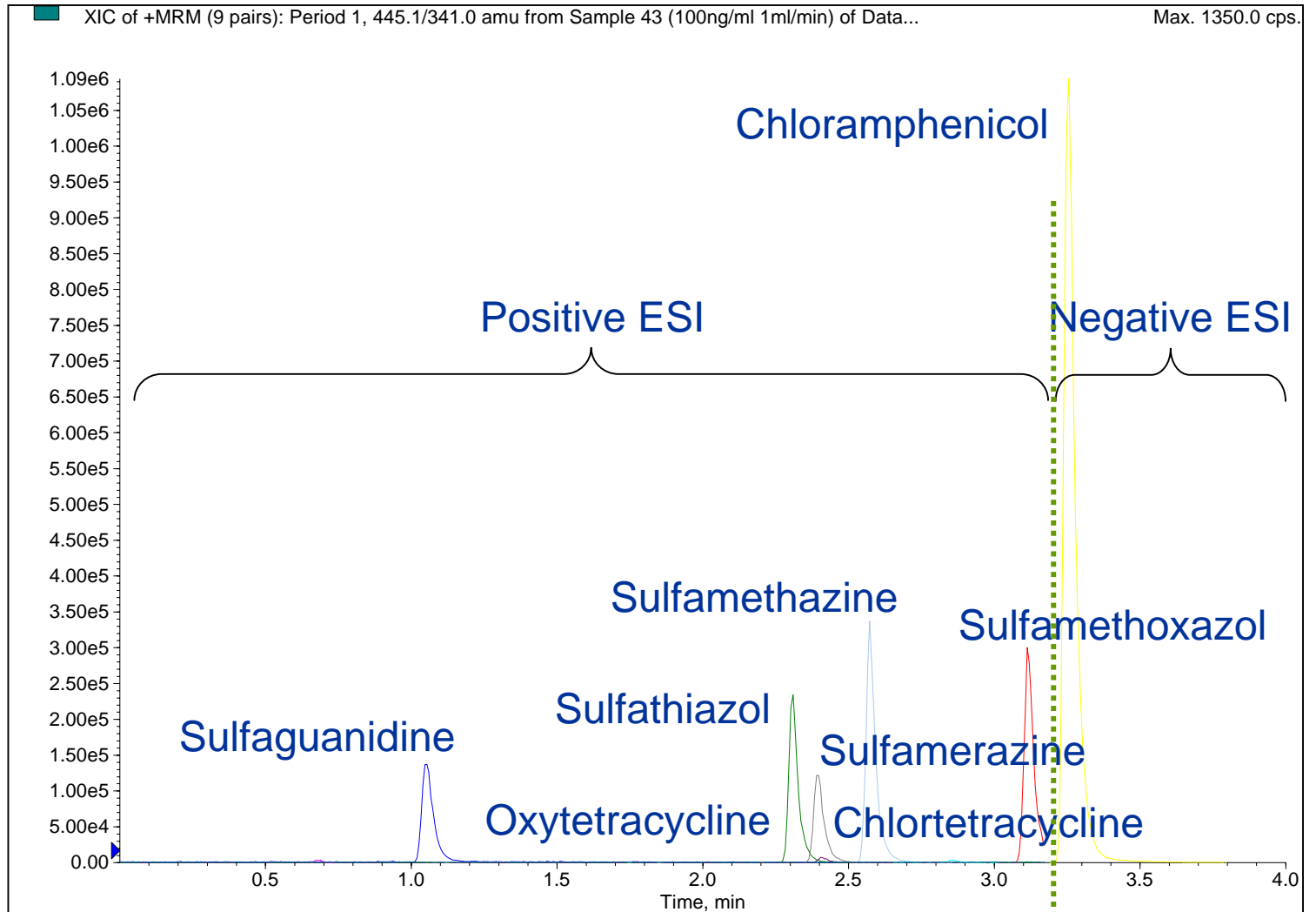
Contamination of foodstuff by antibiotics



What are antibiotics?

- ✓ Antibiotics are used in the treatment of infections caused by bacteria. It works by killing bacteria or preventing their growth
- ✓ Antibiotics should only be used for serious infections in which other medicines do not work
- ✓ Antibiotics may cause some serious side effects
- ✓ Antibiotics were found in e.g milk, honey, and shrimps

Selected standards of antibiotics





Simple sample preparation for the analysis in honey

- ✓ Dissolving of 1 g honey in 1 mL H₂O
- ✓ Adding 200 uL ISTD (CAP-D5, ca 0.1 µg/ml in methanol)
- ✓ Adding 4 mL of ethyl acetate
- ✓ Extraction in ultra sound bath
- ✓ Centrifugation
- ✓ Taking 1 mL of the upper phase
- ✓ Evaporating the solvent and dissolving the residue in 1 mL of water

Analytical conditions

Column: monolythic Chromolith SpeedROD
(Merck), RP18 (50x4.6mm)

Flow rate: 1mL/min - 5mL/min (no split API 4000™)

Gradient: H₂O/CH₃OH + 0.1% formic acid

API 4000™ LC/MS/MS system

Negative Turbo V™ source

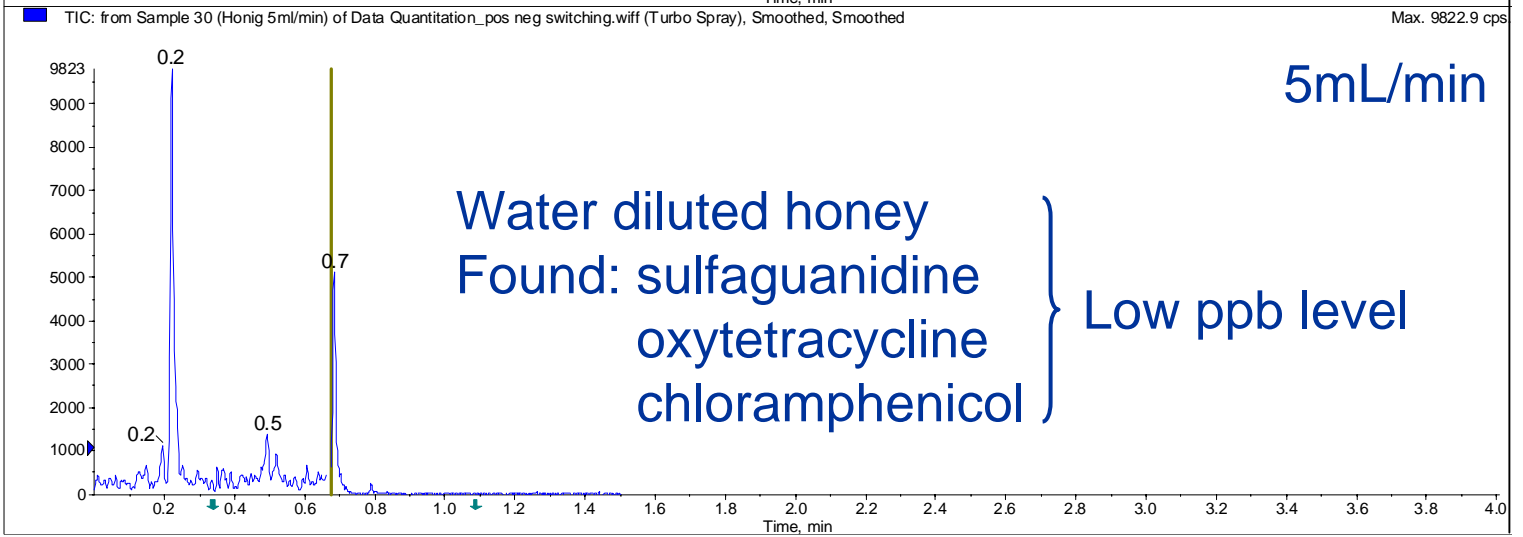
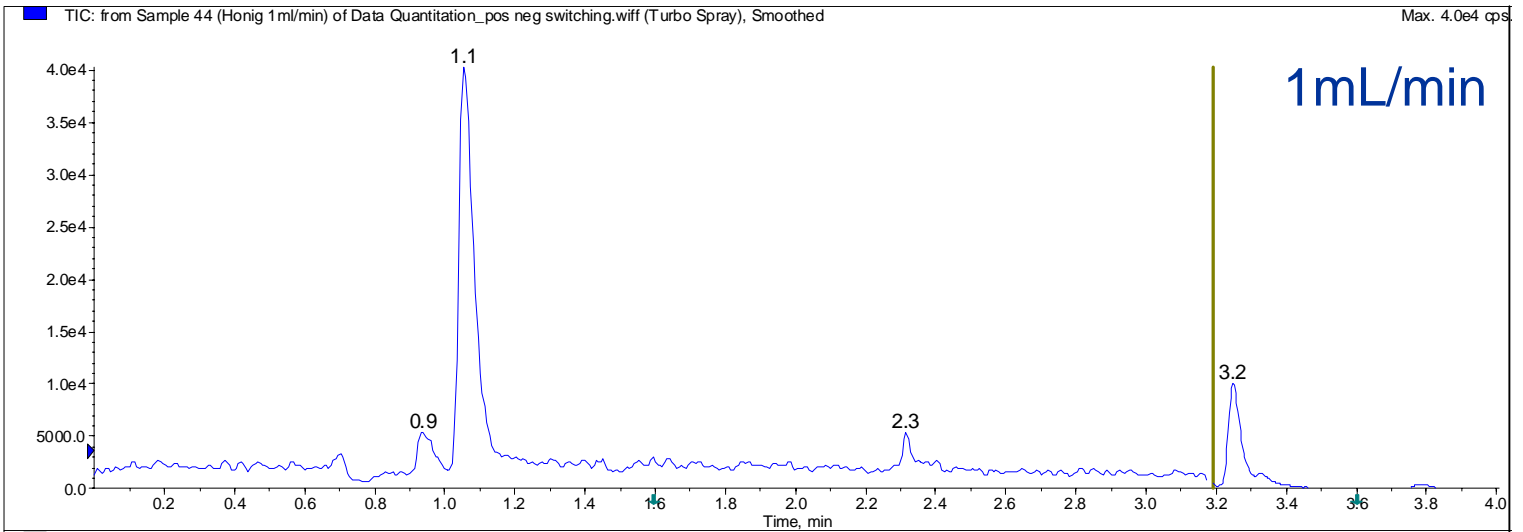
Quantitation in MRM

Mix of antibiotics:

Positive and negative ion mode by different periods



Contaminated real sample (honey)





Acknowledgements

- André Schreiber (Applied Biosystems, Germany)
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- Fans Schoutsen (Applied Biosystems, The Netherlands)
- Ales Merta (Applied Biosystems, Czech Republic)

Presented applications are available as hard copies or electronically

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Metabolomics using an electrochemical cell

