THE ROLE OF LABORATORY MEDICINE FOR BIOPASSPORTIZATION IN SPORTS

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SYNOPSIS

- The flood of new technologies in Lab Medicine
- Principles & application of modern mass spectrometry
  - GC-MS, LC-QQQ MS, MALDI-TOF MS, Orbitrap MS
- Expanding role of MS in lab and sports medicine
  - Sport endocrinology, steroid profiling, microbiology
  - Clinical chemome as a new diagnostic tool
  - The “omics” revolution in lab and sports medicine

Technological Transfer in Lab Medicine

Today:

- the era of total laboratory automation
- flood of new technologies:
  - mass spectrometry
  - cell sorting platforms
  - genome assays.

The future:

- microfluidic & in vivo assay platforms
- “omic” research turns into “omic” diagnostics
- big data analysis and subject controlled care

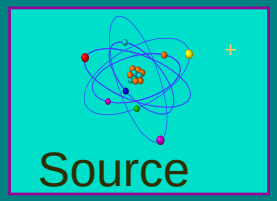
Bioathology – need for transformation

„The circumstances in our environment, the pace of innovation, the extraordinary flood of new technologies – these factors are driving our transformation.“

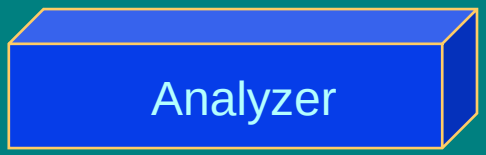
J.N. Schwartz, CAP TODAY 2009

A prominent answer:
Components of a Mass Spectrometer

Ionization / Desorption — Mass Sorting — Detection

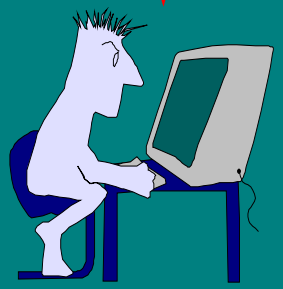


Form ions (charged molecules)

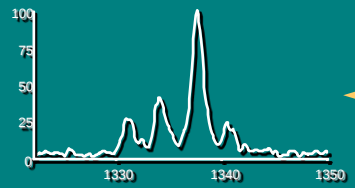


Sort Ions by Weight

Detect Ions



Data Analysis



- Solid
- Liquid
- Vapor

Sample Introduction

Method to vaporize sample

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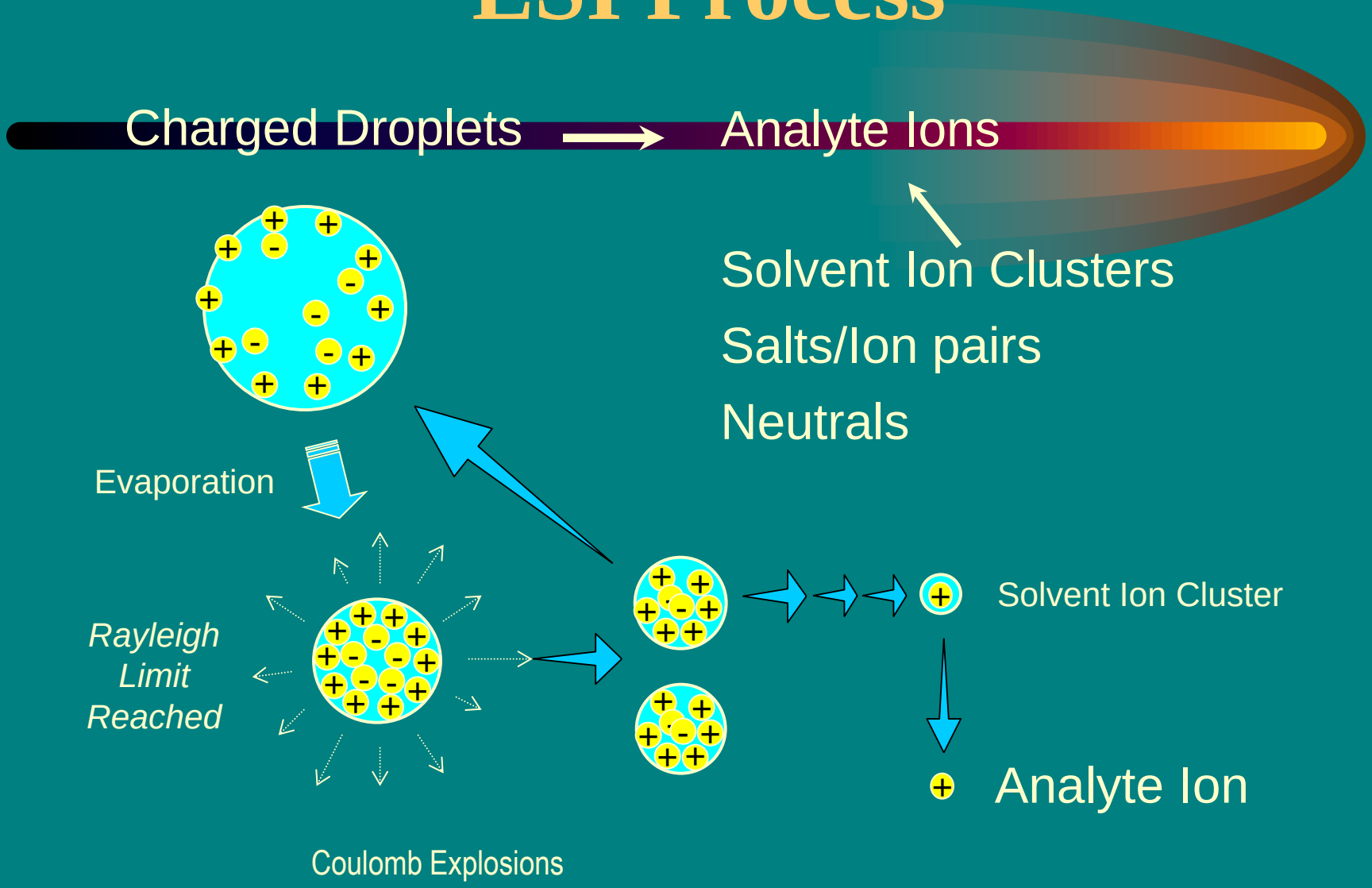
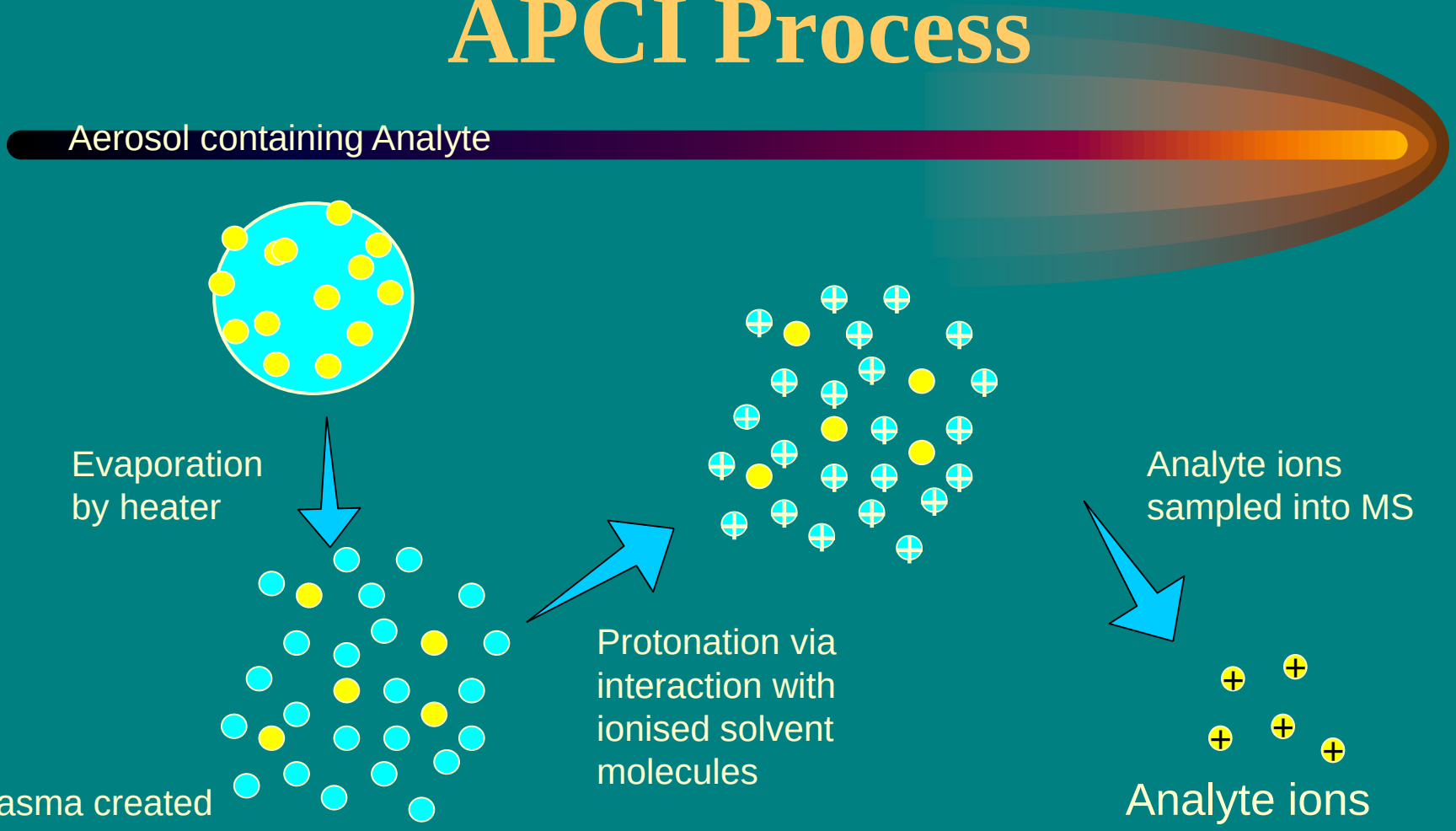
ESI Process

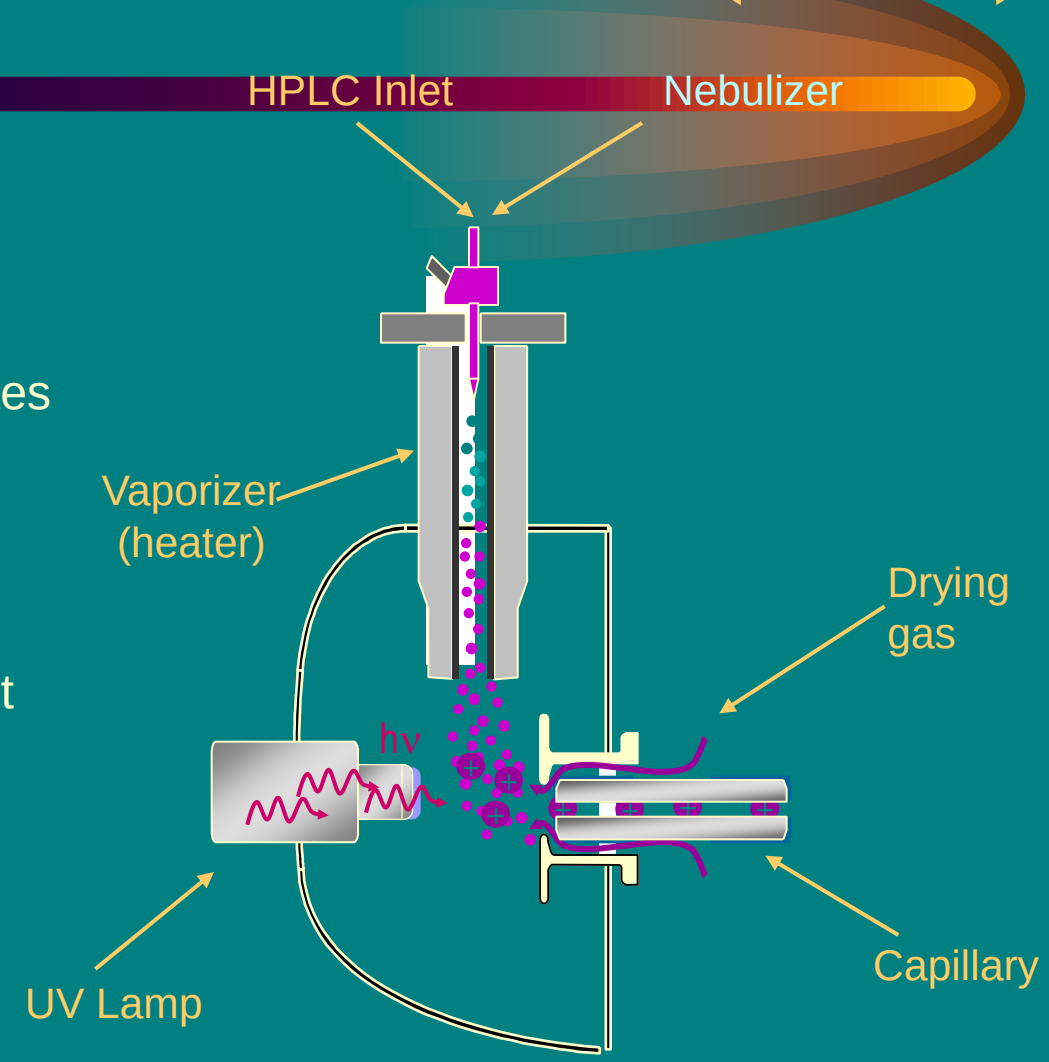

Charged Droplets
Analyte Ions
Solvent Ion Clusters
Salts/Ion pairs
Neutrals
Evaporation
Rayleigh Limit Reached
Coulomb Explosions
Solvent Ion Cluster
Analyte Ion

APCI Process



Atmospheric Pressure Photoionization (APPI)

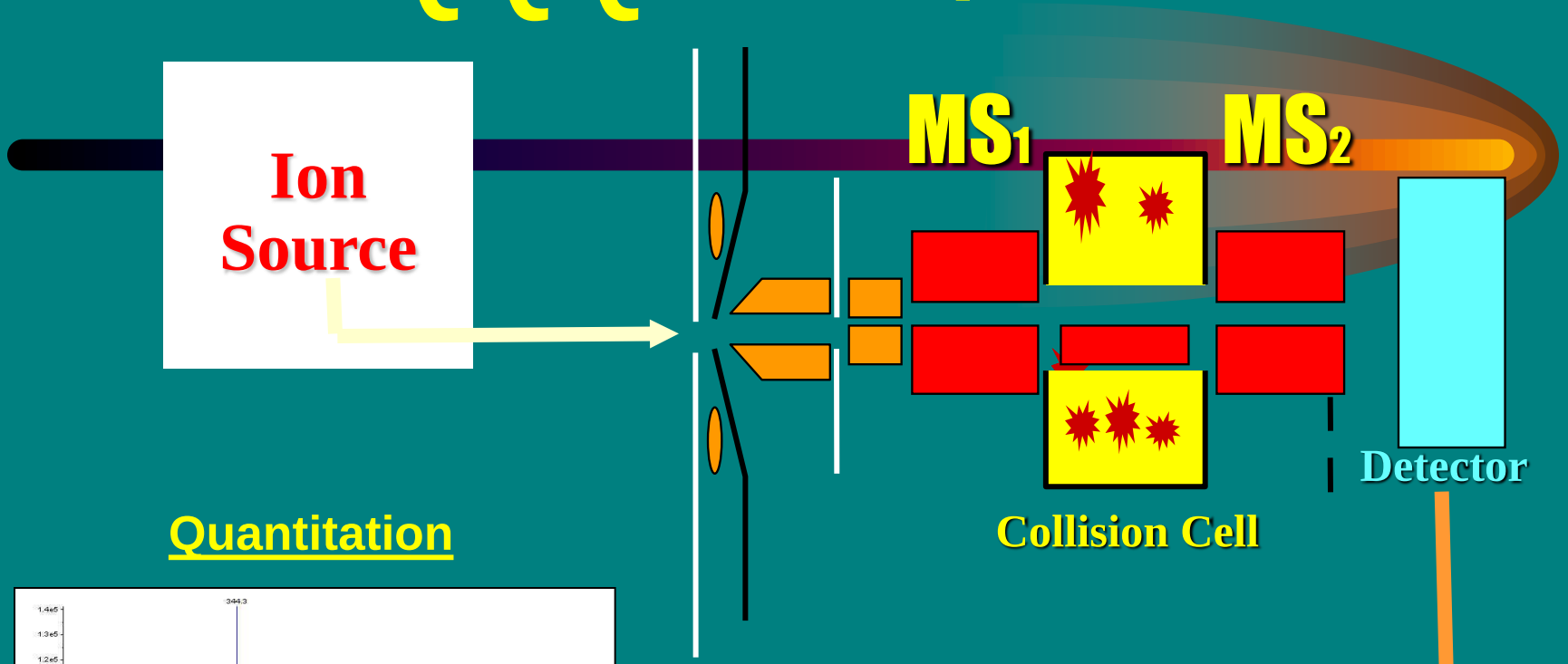
- A new atmospheric pressure ionization technique
- Ionizes gas phase analytes with light instead of a corona discharge
- Uses a high-output gas discharge tube for transmission of ultraviolet light
- Similar to PIDs for GC



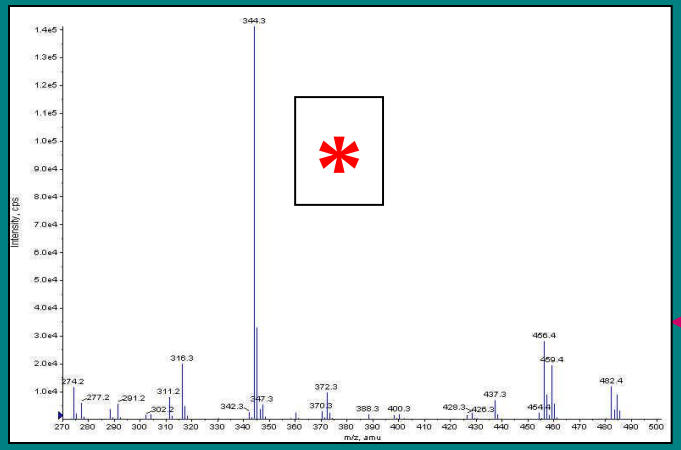
Direct sample introduction via:

- Matrix
- Assisted
- Lazer
- Desorption
- Ionization

QQQ – MS/MS



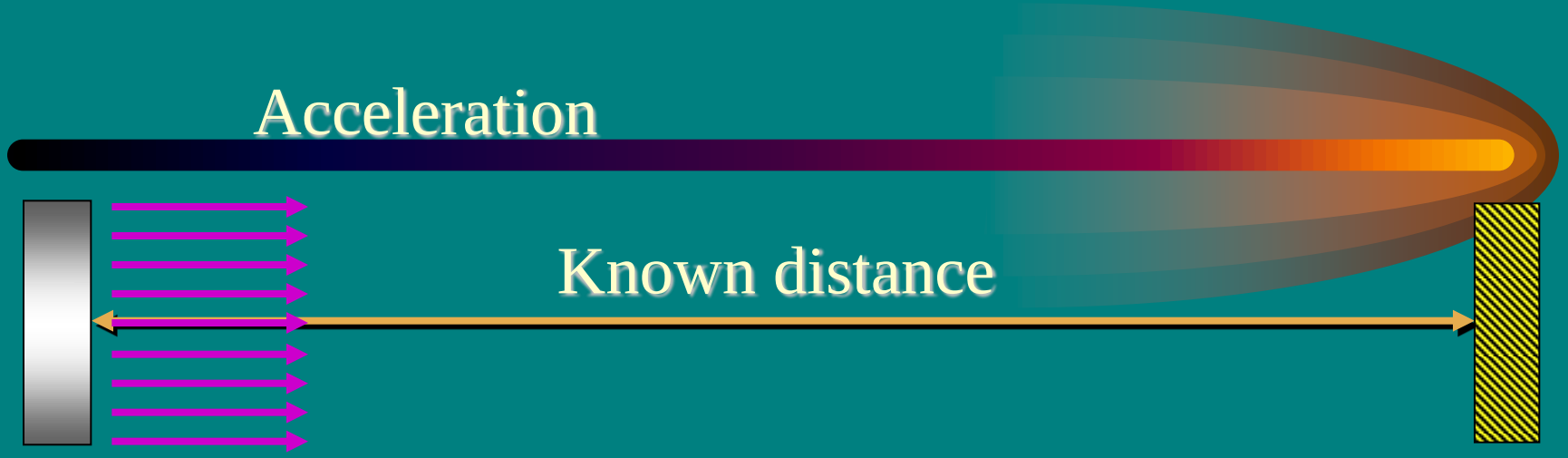
Quantitation



MRM (SRM)

Computer

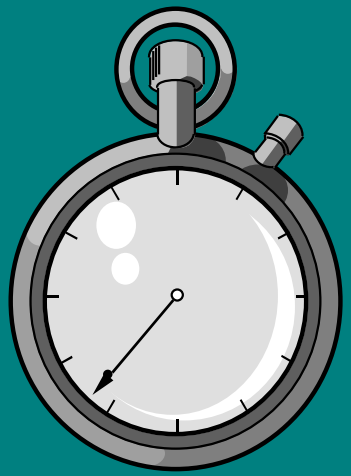
Hardware of ToF



Sample
(start point)

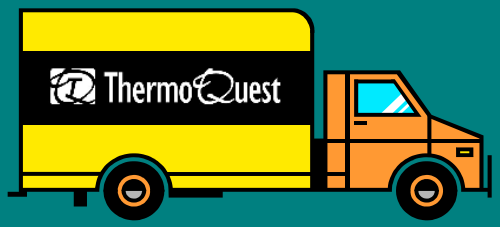
Detector

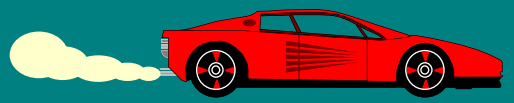
Timing
electronics



Underlying principle of ToF

- Given the same energy...



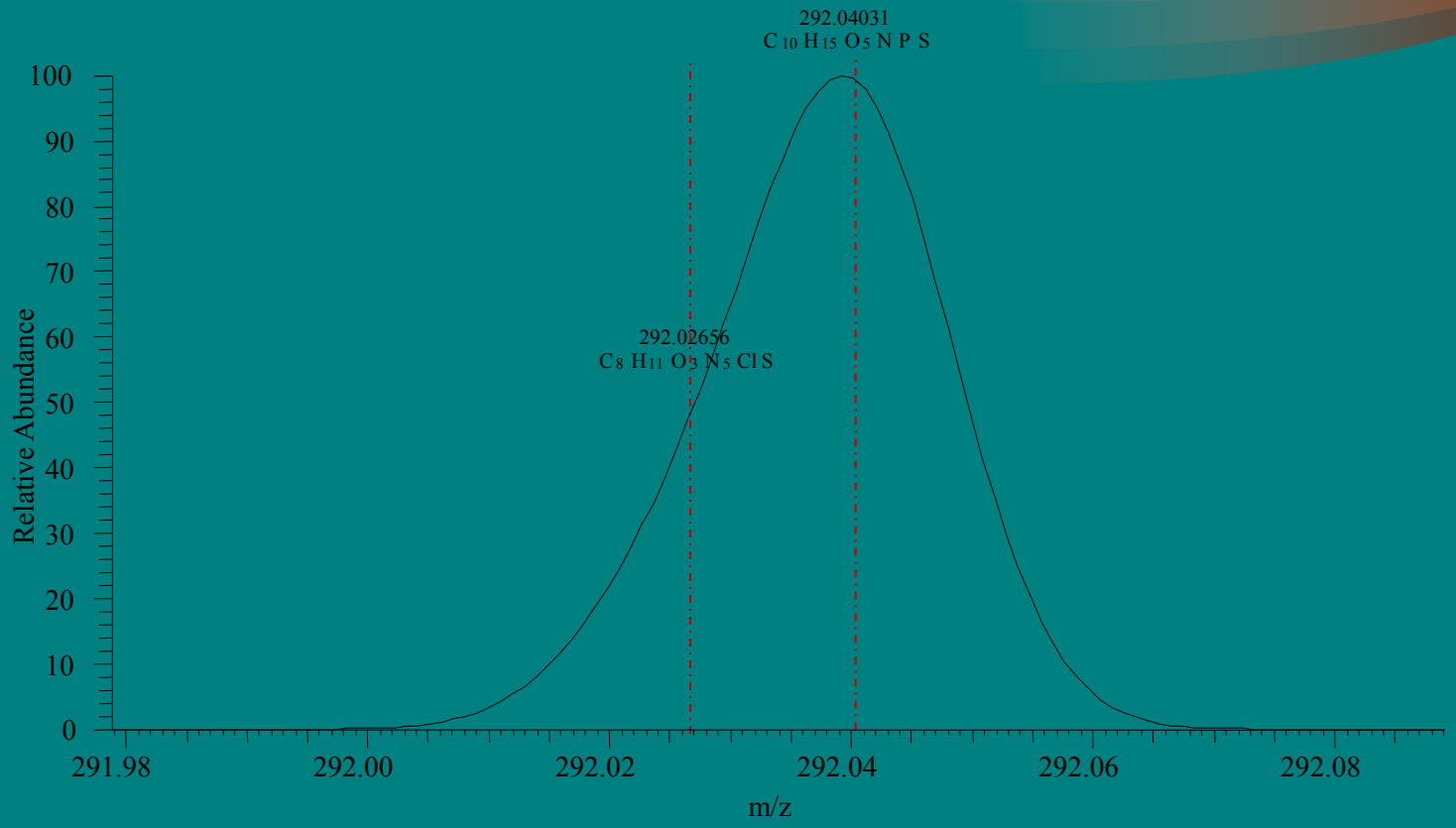


- …lighter is faster

Orbitrap™ Mass Spectrometers

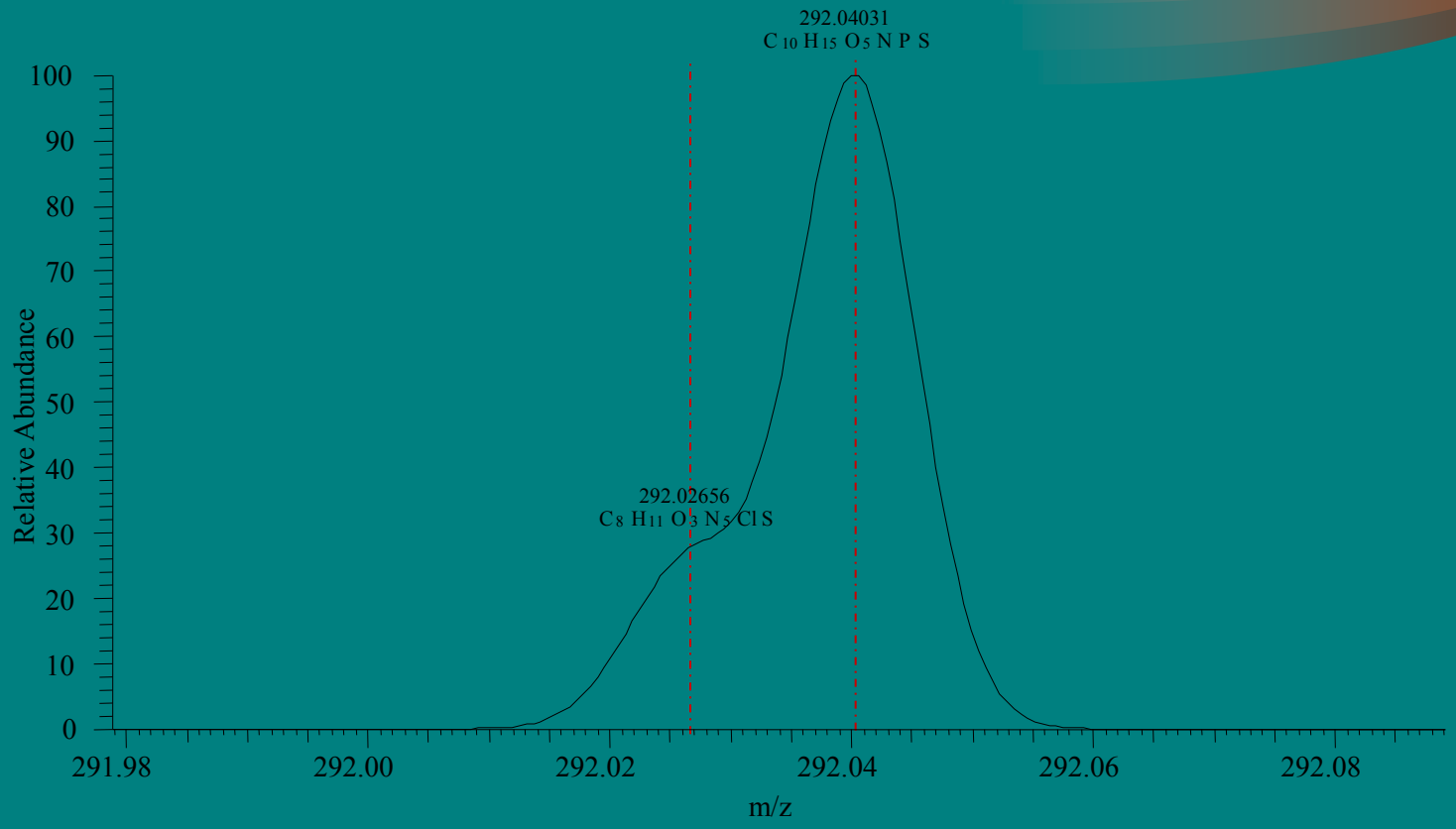
Simulated Resolution = 15,000 (Mix 1:3)

Resolution 15,000



Simulated Resolution = 25,000 (Mix 1:3)

Resolution 25,000



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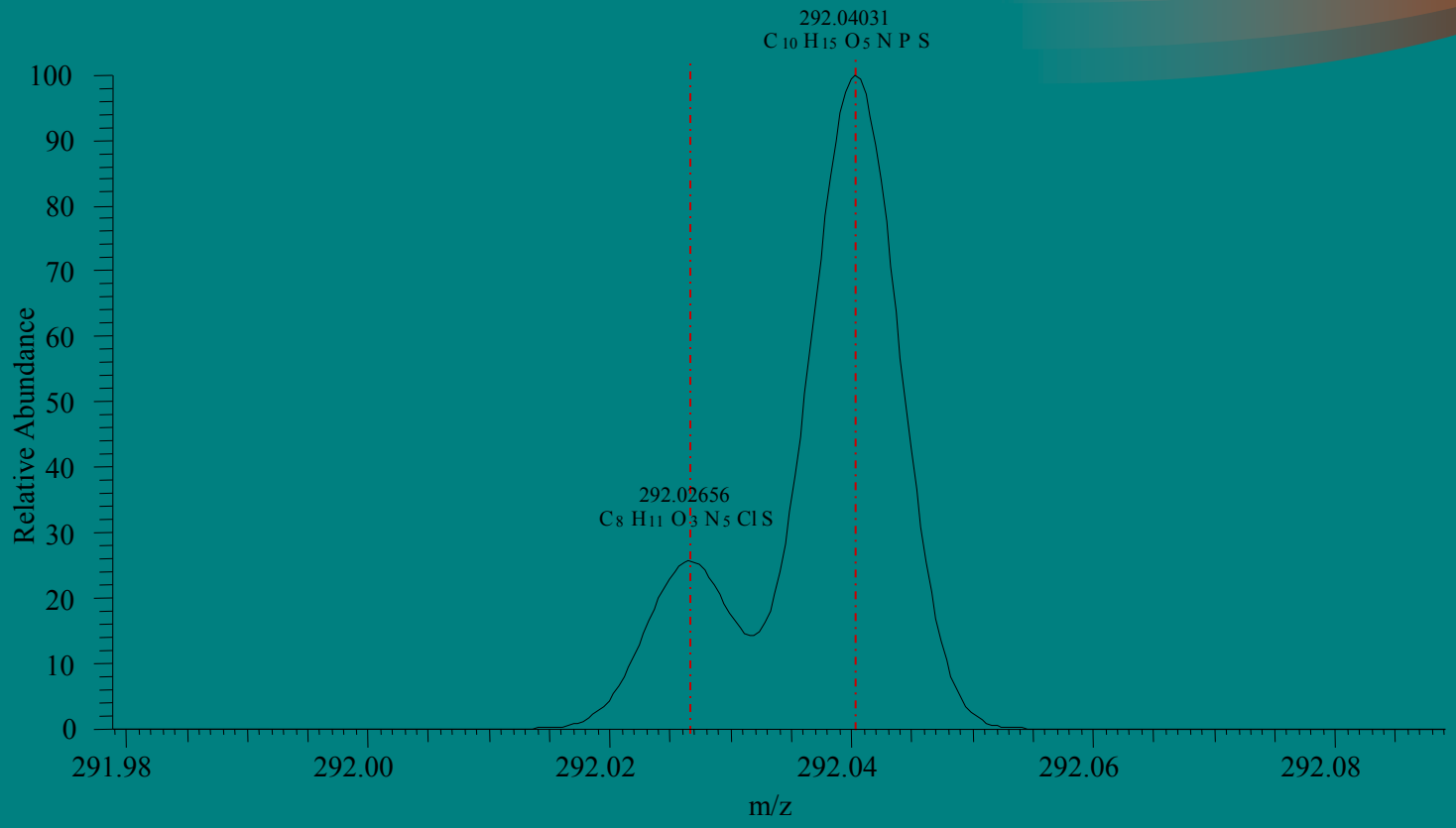
Simulated Resolution = 35,000 (Mix 1:3)

Resolution 35,000



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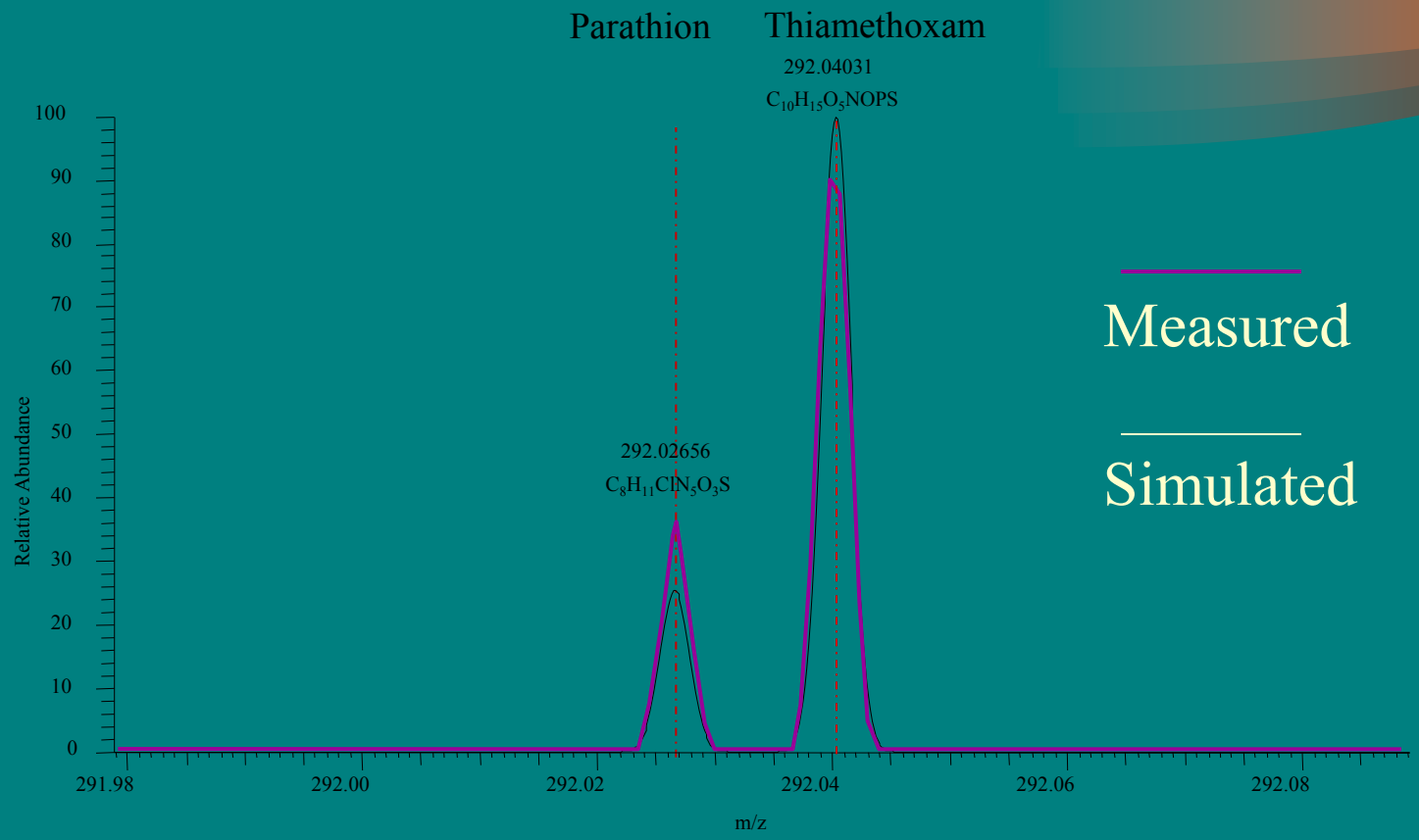
Measured vs. Simulated at 100,000 (Mix 1:3)



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Expanding role of mass spectrometry in lab and sports medicine

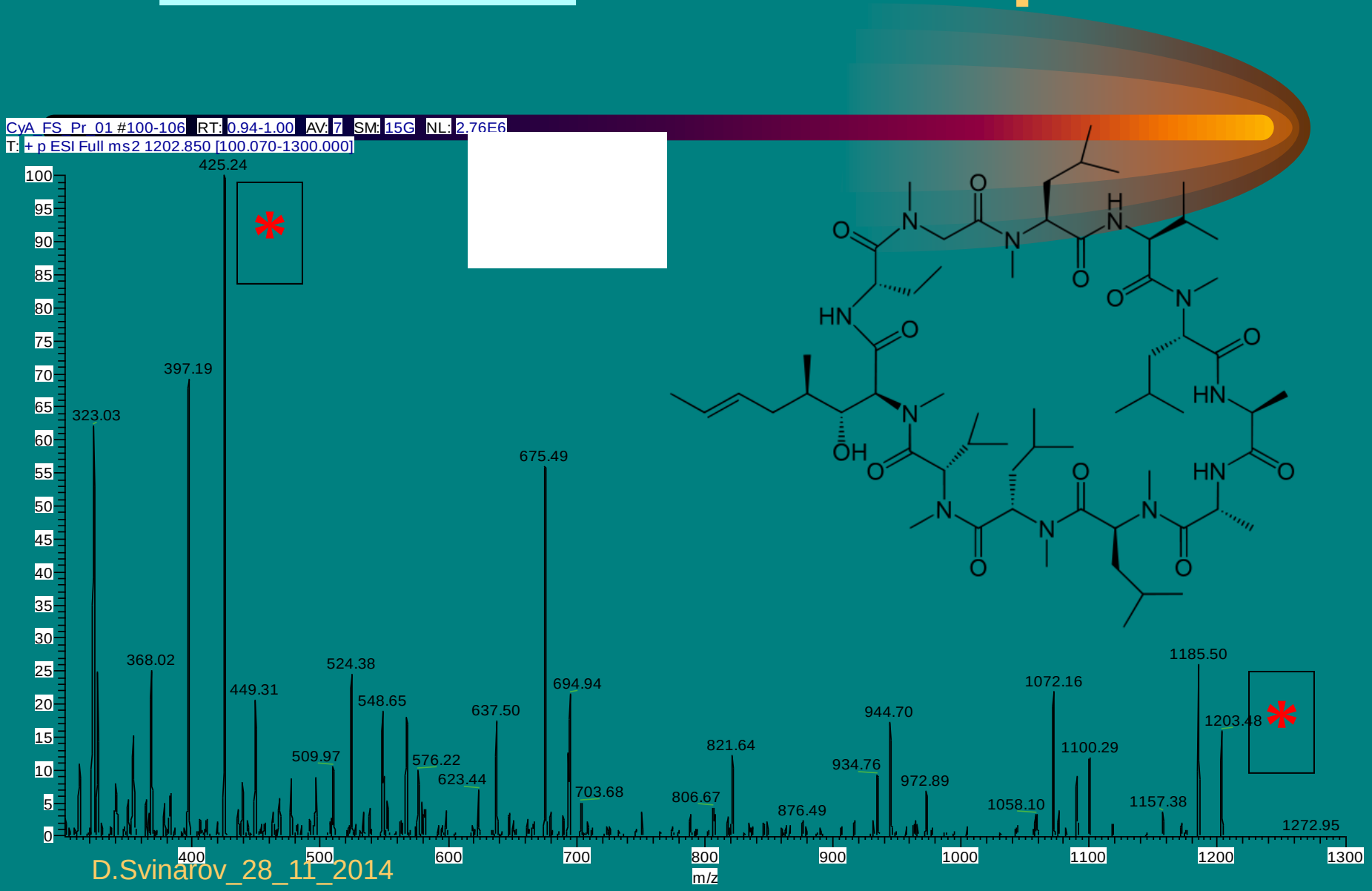
LC-MS/MS

- **TDM** (immunosuppressants, antiretroviral drugs, antidepressants, antipsychotics)
- **Drugs of Abuse, Clinical Toxicology & Doping Control**
- **Endocrinology** (steroid profiles, FT3, FT4, free metanephrines)
- **Newborn screening** (e.g. acylcarnitines, amino acids, steroids)
- **Vitamin D status** (25-OH-D2, 25-OH-D3)
- **Peptidomics** (EPO, Angiotensins, Oxytocin, ADH, hepcidine)

MALDI-TOF / ORBITRAP HD MACHINES

- **Proteomics** (EPO, Research – omics, Biomarker Discovery)
- **Medical Microbiology**

LC-MS/MS: Product Spectra



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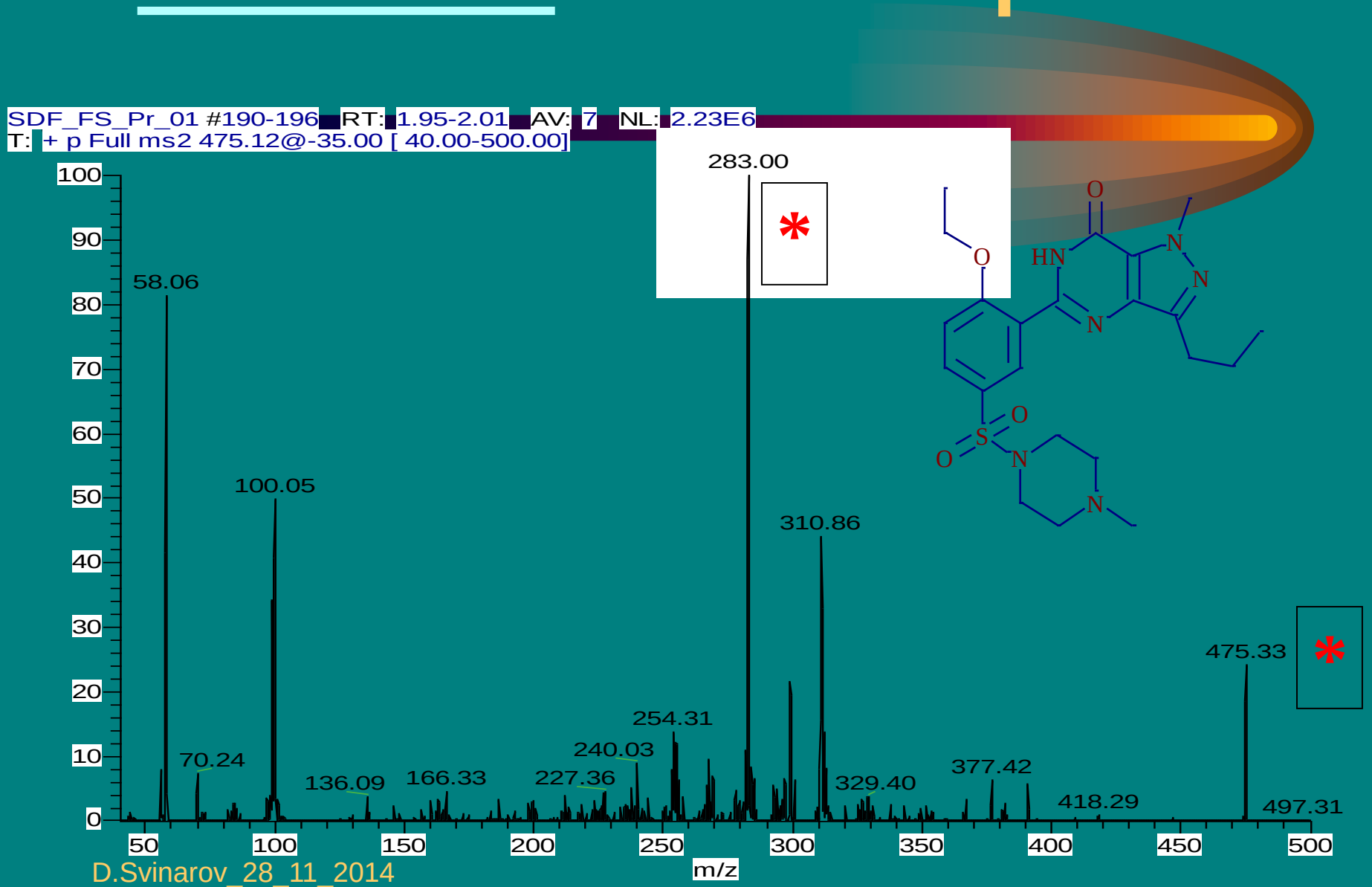
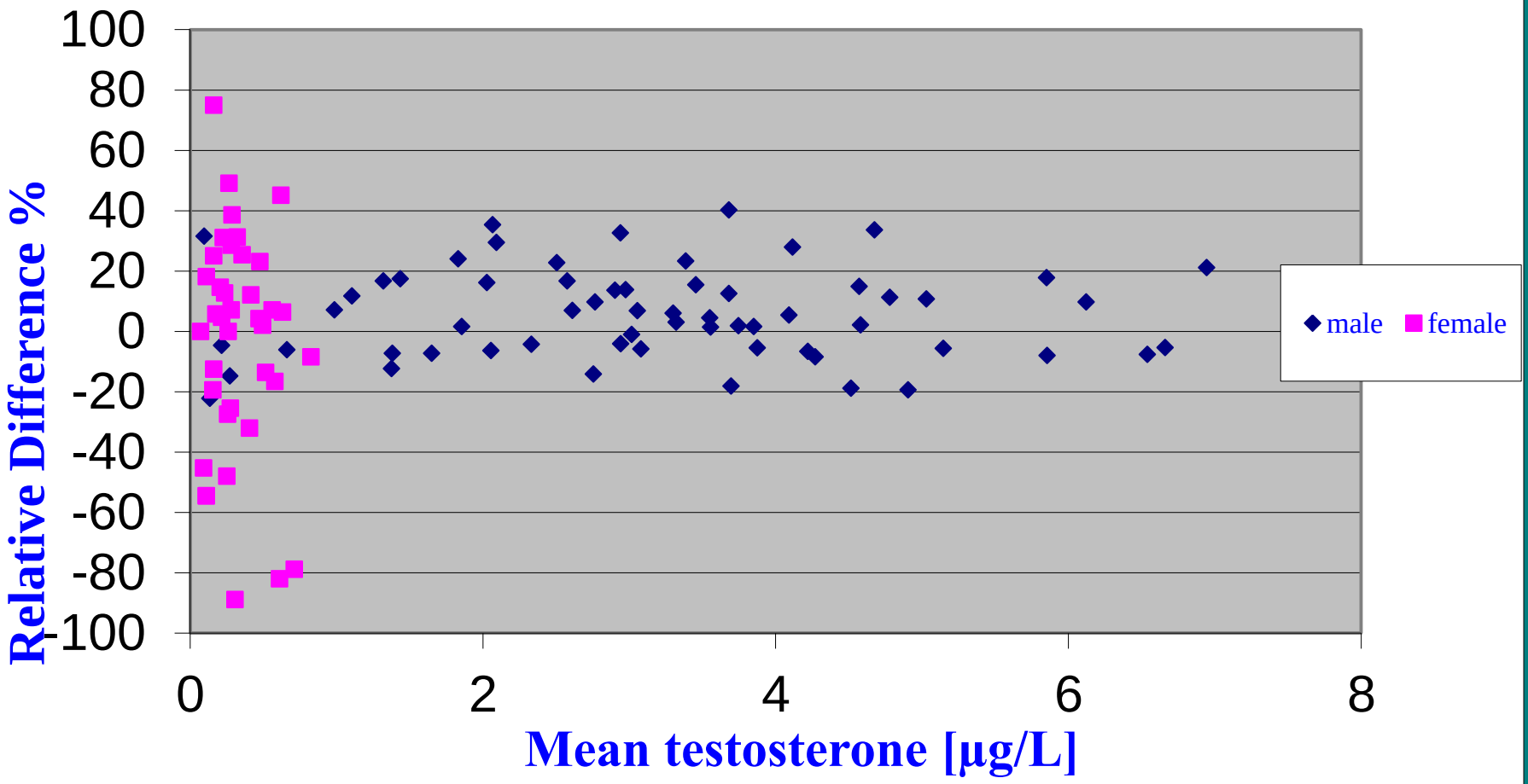
LC-MS/MS: Product Spectra



Testosterone measured with Immunoassay & LC-MS/MS



Streit et al (unpublished)

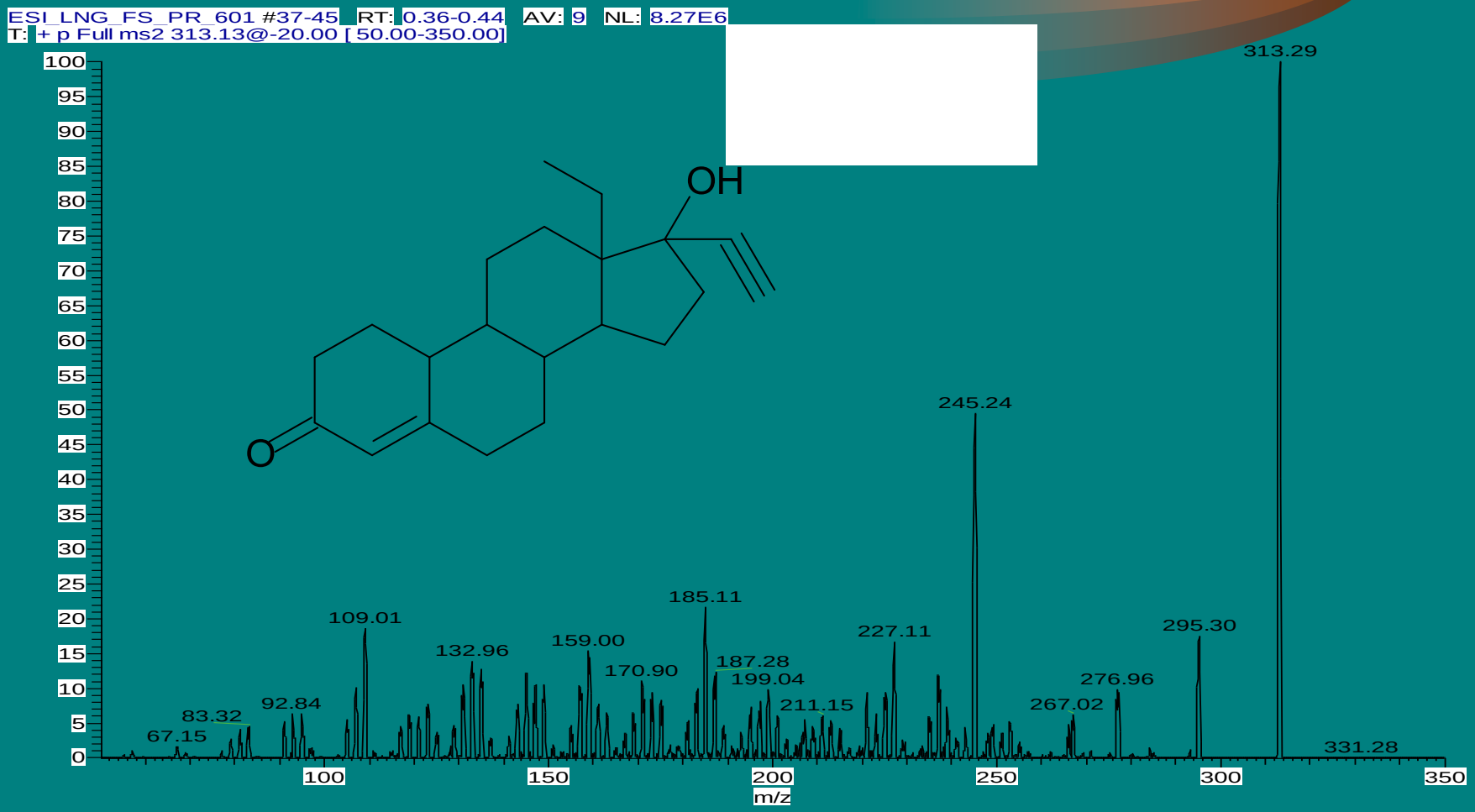
Sample preparation

- To an appropriate-sized plastic tube were applied:
  - 300 µL of plasma (calibration, control, unknown sample);
  - 100 µL of o-phosphoric acid : H_2O(1:2);
  - 50 µL of INTSTD solution (MTS in 50% methanol);
  - 2.0 mL of of Hexane:Cyclohexane (2:1)
- Tubes were extracted (30 min), organic layer was evaporated under vacuum, dry residue was reconstituted with 50 µL of mobile phase : H_2O, (2:1), and 20 µL were injected for analysis

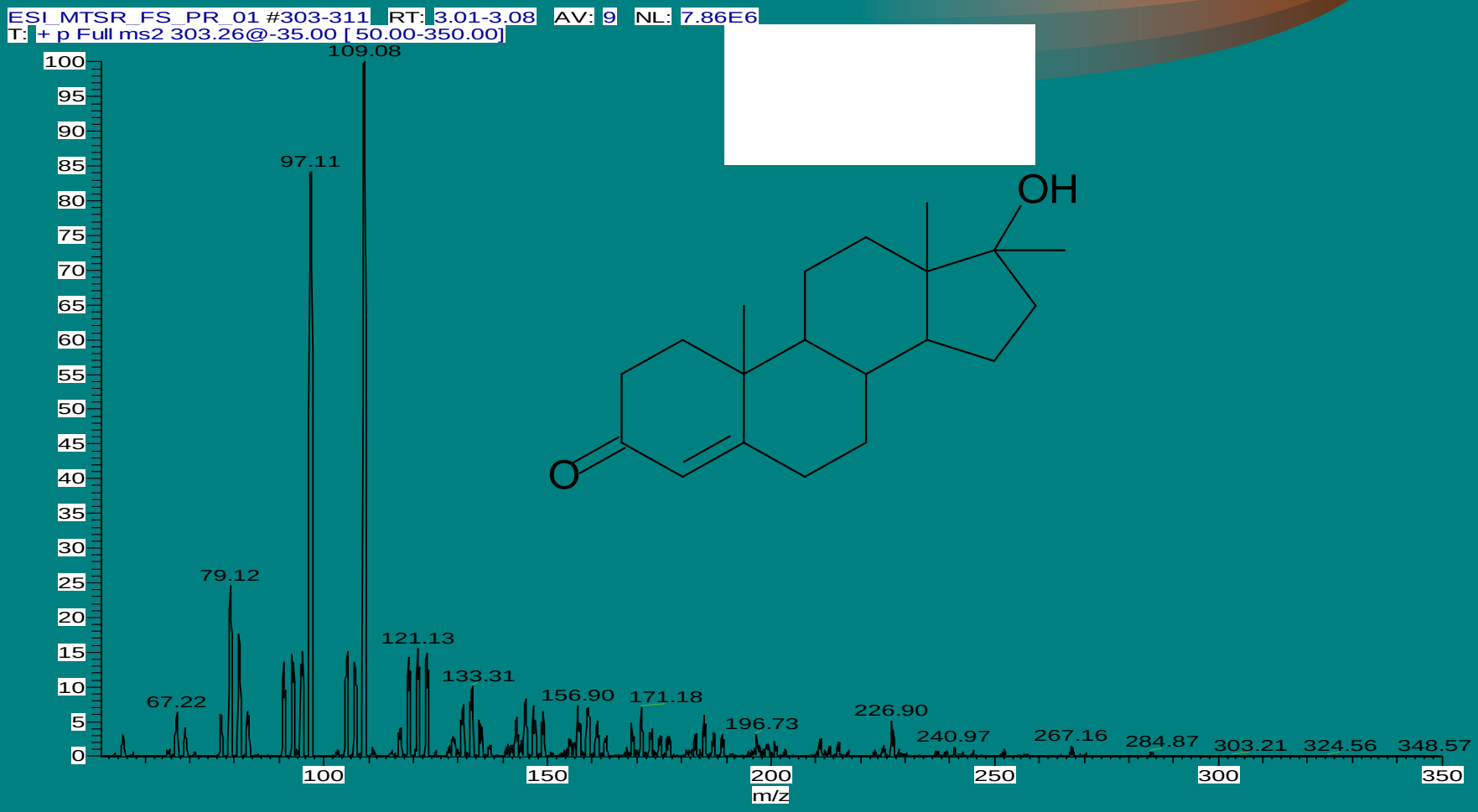
LC-MS/MS Conditions

- **Instrument:** TSQ Quantum Discovery Max (TermoFisher Sci)
- **Column:** RP C18, 100 x 2.1 mm, 3 µm particles (45°C)
- **Mobile phases:** A - 60% MeOH, 1mM AA, 0.1% FA; B – 95% MeOH, 1mM AA, 0.1% FA; flow 0.25 mL/min, pressure 50-100 bars; gradient – A-B-B-A/0-1-4-5-15 min
- **SRM @:** 2.5 KV spray voltage, gases (in arbitrary units): sheath 35, ion sweep 0, aux 5; capillary at 300°C; collision at 1.5mTorr and CE = 20 V at m/z 313⟶245 for LNG; CE=32 V at m/z 303⟶109 for MTS; scan width 0.500 m/z, scan time 0.5 s, pW 0.7 at Q1 and at Q3.

LNG, $C_{21}H_{28}O_2$, M = 312.446

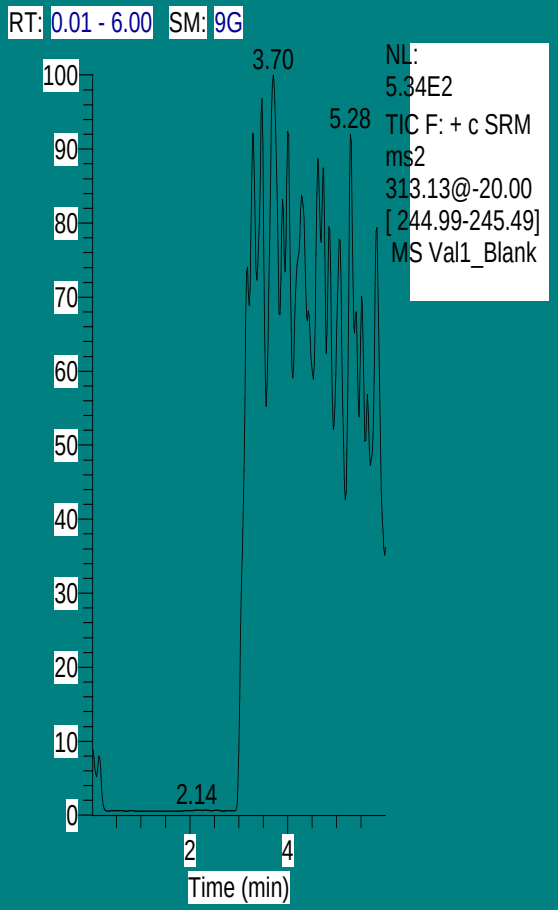


MTS, $C_{20}H_{30}O_2$, M = 302.451

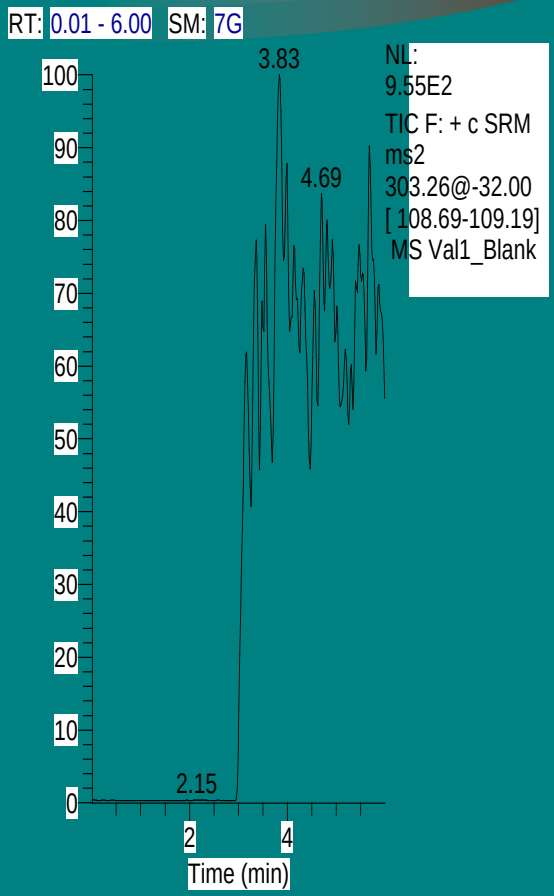


Blank Mass-spectrogram

LNG

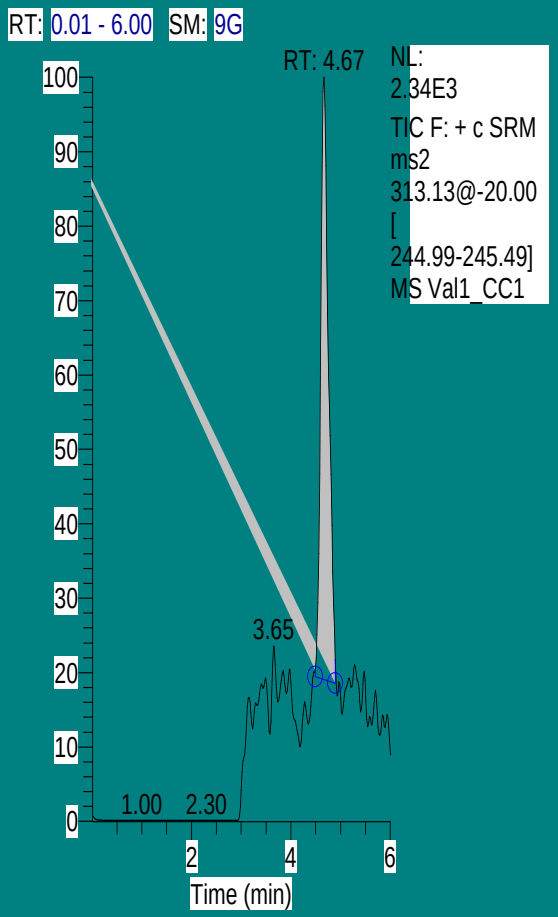


MTS

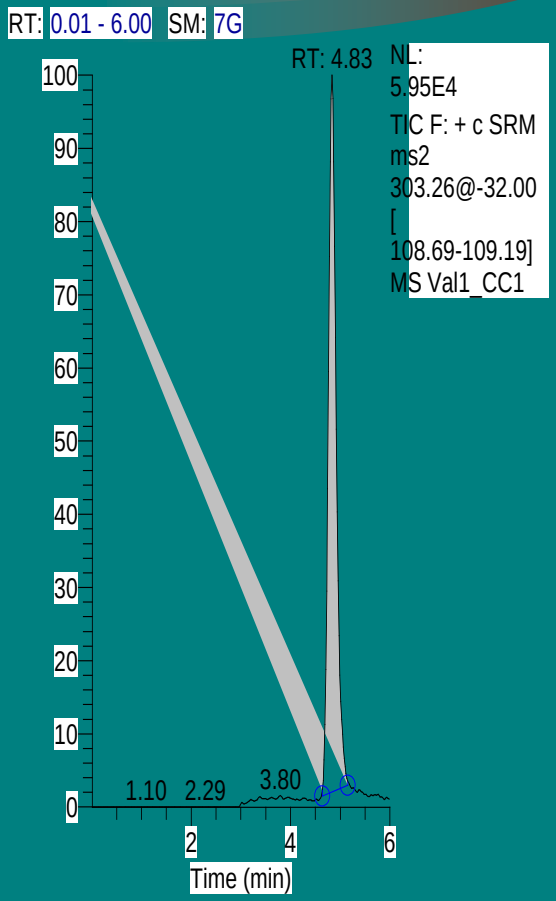


LLOQ Sample Mass-spectrogram

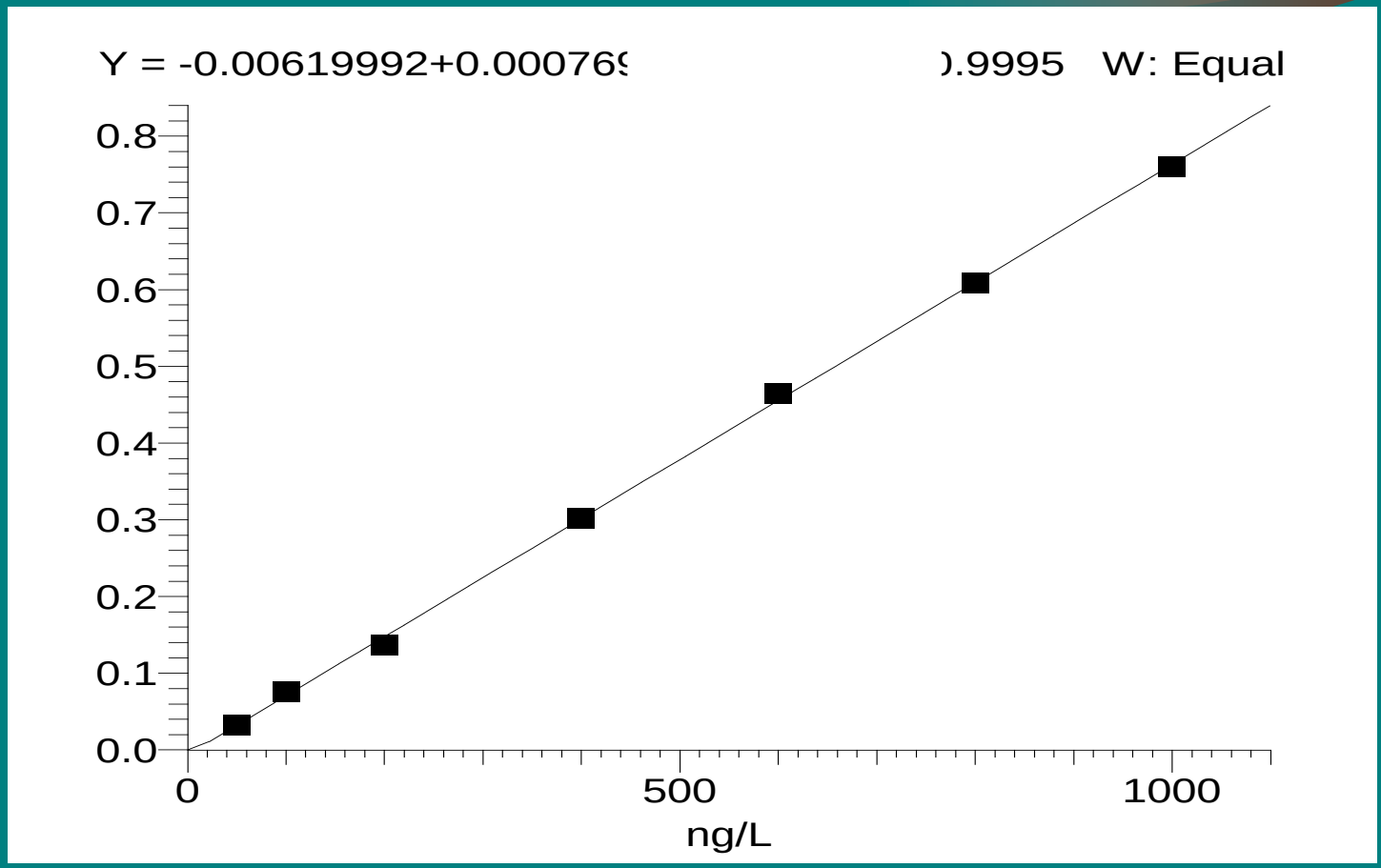
LNG (50.0 ng/L)



MTS



Example Calibration Curve



Validation parameters tested at LLOQ, QC-L, QC_M, and QC-H

- **Selectivity**: confirmed with matrix effect (ME) of 94-108 % for LNG and MTS, and relative ME of 101-105% for LNG, by analysis of 6 different plasma sources
- **Accuracy**: batch ± 2.3 %, between run ± 3.7% (d)
- **Precision**: batch < 3.0 %, between run < 6.4% (CV)
- **Recovery,%**: 56-61 (LNG), 60-64 (MTS)
- **Linearity,** ng/L: 50.0 (LLOQ) ÷ 1000.0; R>0.99
- **Stability**: freeze-thaw - 3 cycles of 24 h, post-preparative - 48 h, short-term – 4 h, daylight - 4 h, stock solution and long-term - 292 days @ -20^{o}C

MALDI-TOF MS in Medical Microbiology

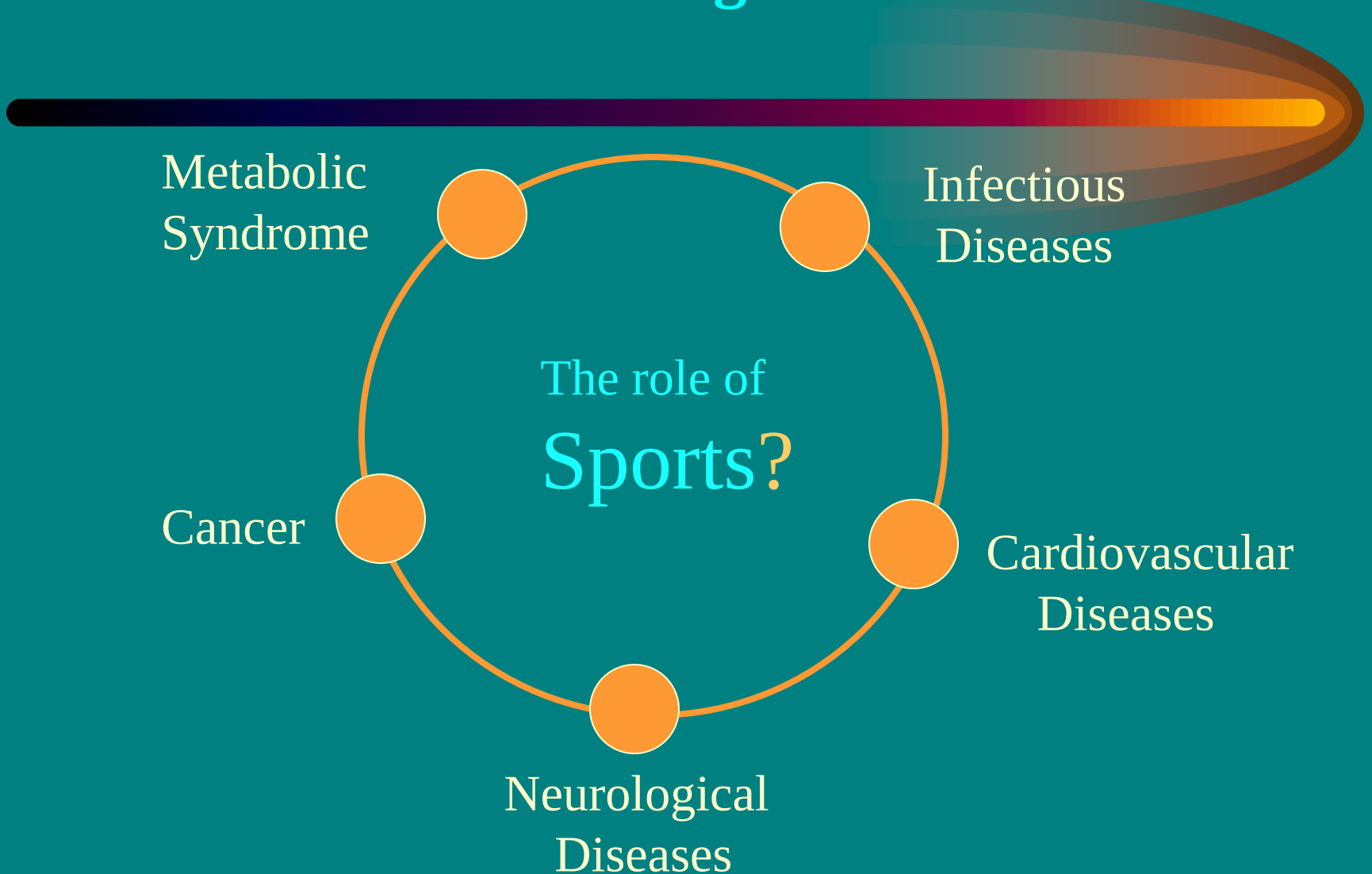
- Traditional methods require 48 – 72 h and are restricted regarding the number of microorganisms identified
- MALDI-TOF MS detects highly conserved microbial proteins and peptides (mainly ribosomal) and by matching the proteomic fingerprint from the sample to a known database, differentiates thousands of individual pathogens at a species level in a matter of minutes
  - Major limitation – cannot yet provide atibiotic succeptibilities
  - Future – identification of microbes directly from patient samples

Blood-based lipidomic biomarkers for preclinical detection of Alzheimer's disease

- Proof-of-concept study led by H.Federoff, MD, PhD
- By use of MS researchers identified a panel of lipids that could predict the onset of cognitive impairment 2-3 years ahead of clinical manifestation
- In the validation phase they found that a 10-lipid panel predicted the progression from normal to Alzheimer's disease with a sensitivity of 90% and a specificity of 90%

Cheema A et all, AACC 2014 Plenary & Nature Medicine, 2014

The Disease Challenges in Public Health



CLINICAL CHEMOME

nonenzymatic chemical changes of biomolecules

Major reasons:

aging and disease

- Mechanism :
  - FREE RADICAL DAMAGE
- Common Diseases :
  - Cancer
  - Diabetes
  - Cardiovascular
  - Neuro-degenerative

Major constituents:

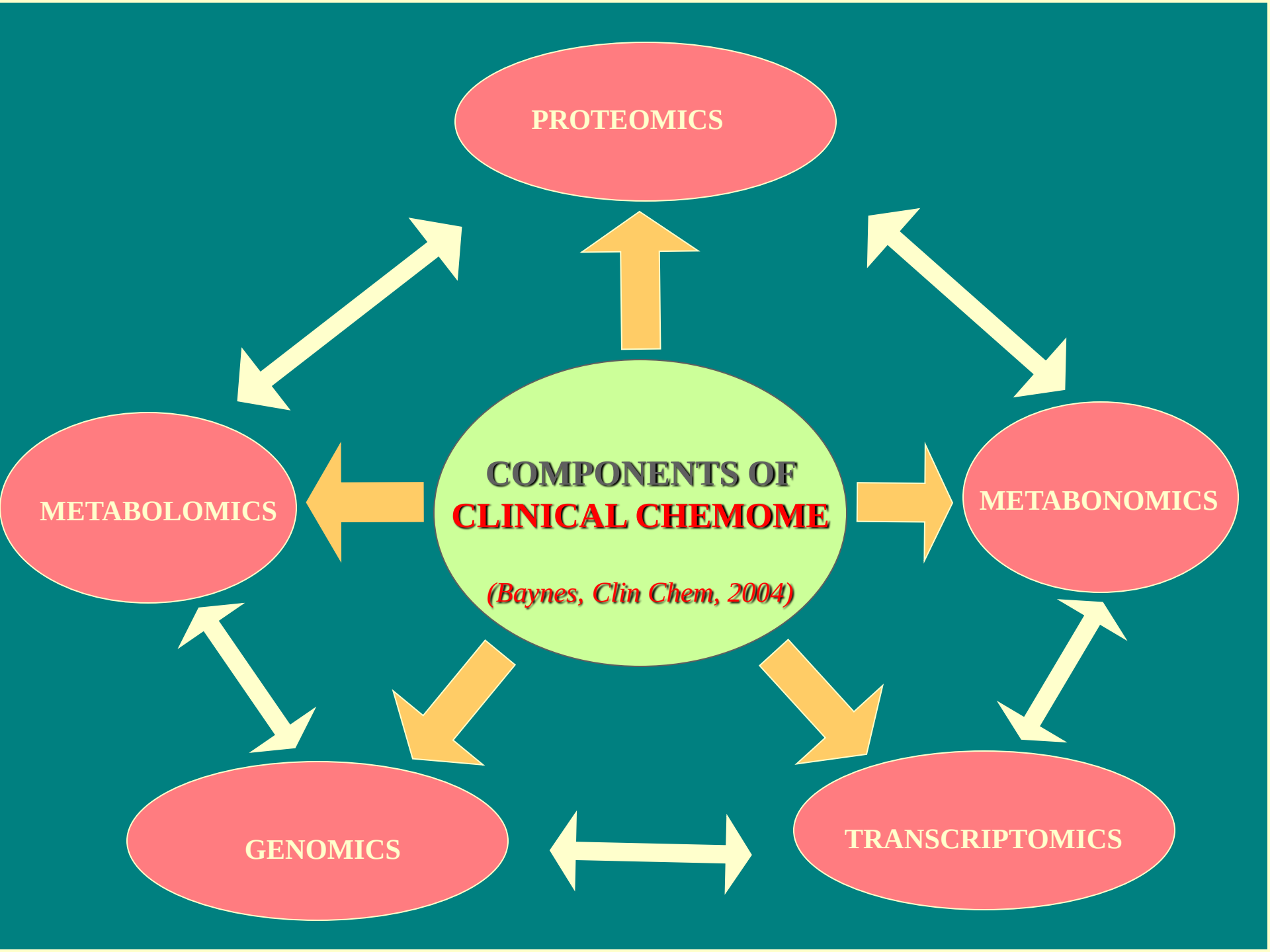
oxidized nucleotides, AAs, sugars and lipids

- Low molecular components of body tissues and fluids
- Modify proteins and DNA, forming advanced glyoxidation and lipoxidation products
- Examples: glycohemoglobin, isoprostanes, malondialdehyde, nitro and ortho-tyrosine, …

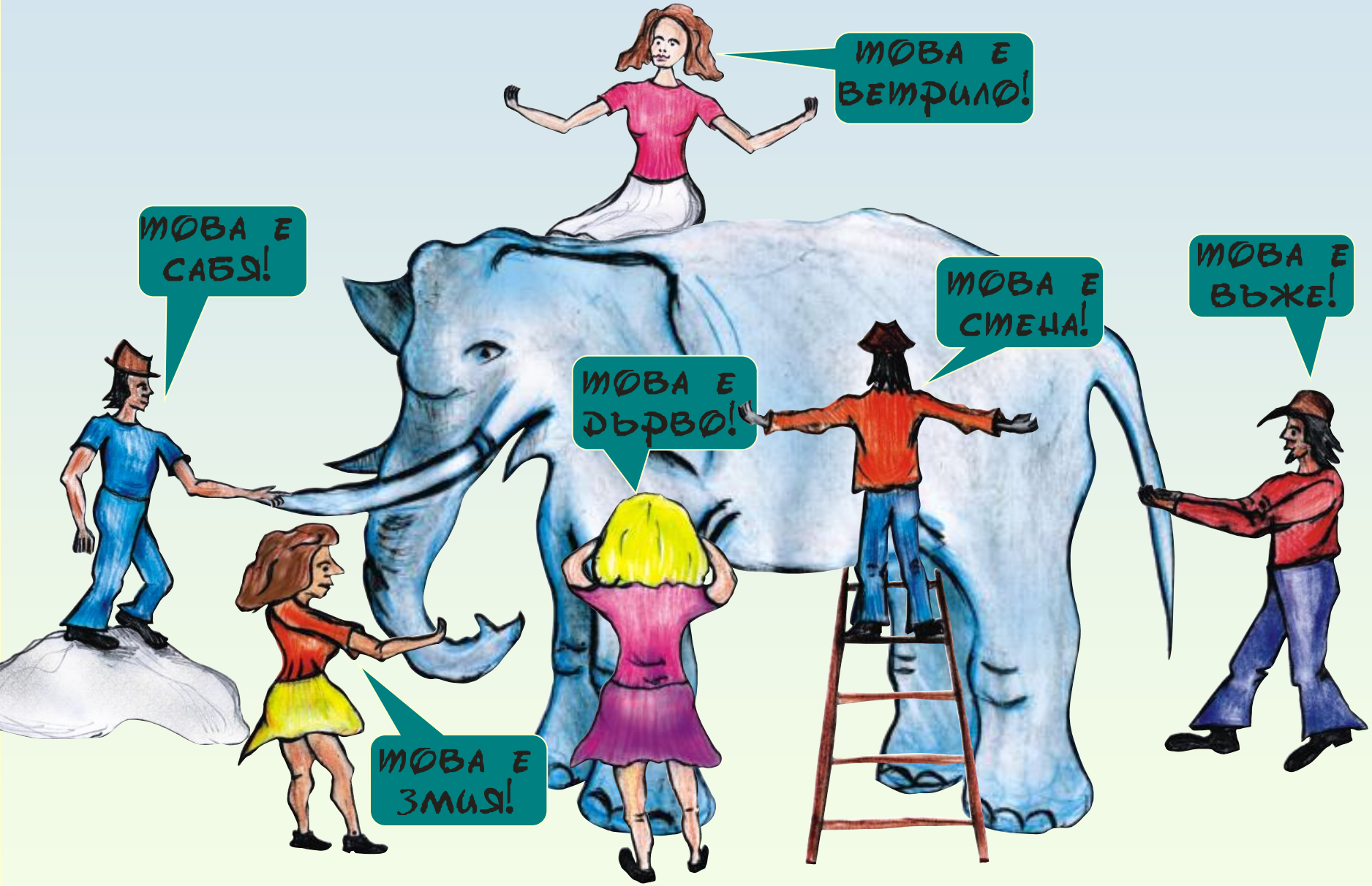
Methods for analysis:

LC/MS/MS coupled to bioinformatic system

- Provide new understanding for health, aging, disease , risk and response to therapy
- By revealing the “omics” signatures of disease
- Result - nonlinear technologic advance and management in clinical medicine


PROTEOMICS
METABOLOMICS
COMPONENTS OF
CLINICAL CHEMOME
(Baynes, Clin Chem, 2004)
METABONOMICS
GENOMICS
TRANSCRIPTOMICS

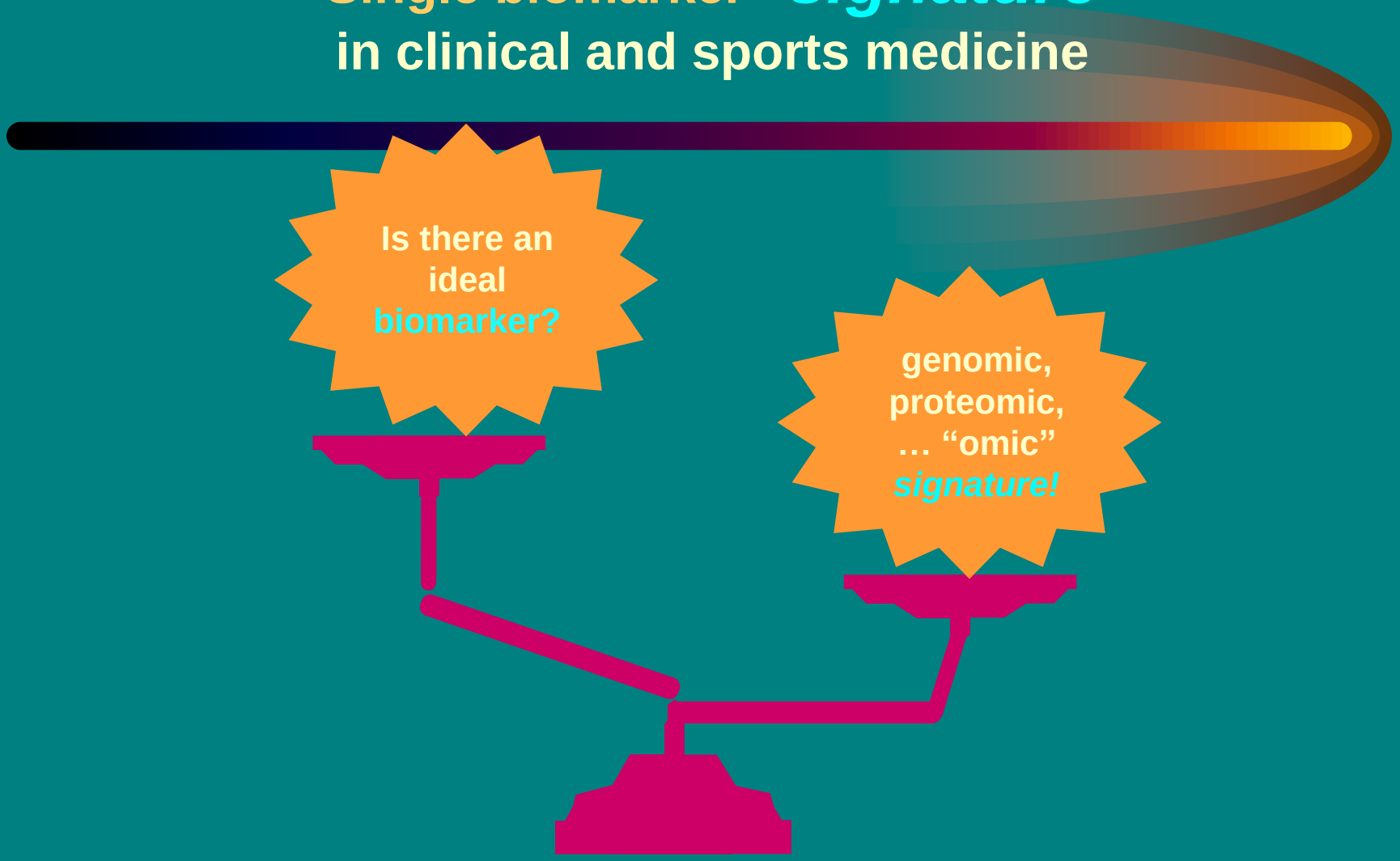
Какво е това?


това е ветрило!
това е слон!
това е дърво!
това е стена!
това е въже!
това е змия!

The ballance

Single biomarker –*signature*

in clinical and sports medicine



MAS SSPECTROMETRY – MEDICAL LABORATORY ANALYSER OF THE NEAR FUTURE

Mass spectrometry analysis of nucleic acids, proteins, low molecular metabolites provides dramatic advantages

High throughput:

Analysis of thousands of components in a drop of blood in several minutes >> hundreds of samples in a single batch

Absolute specificity:

Structural identification of known and unknown components >> direct analysis of PCR products!

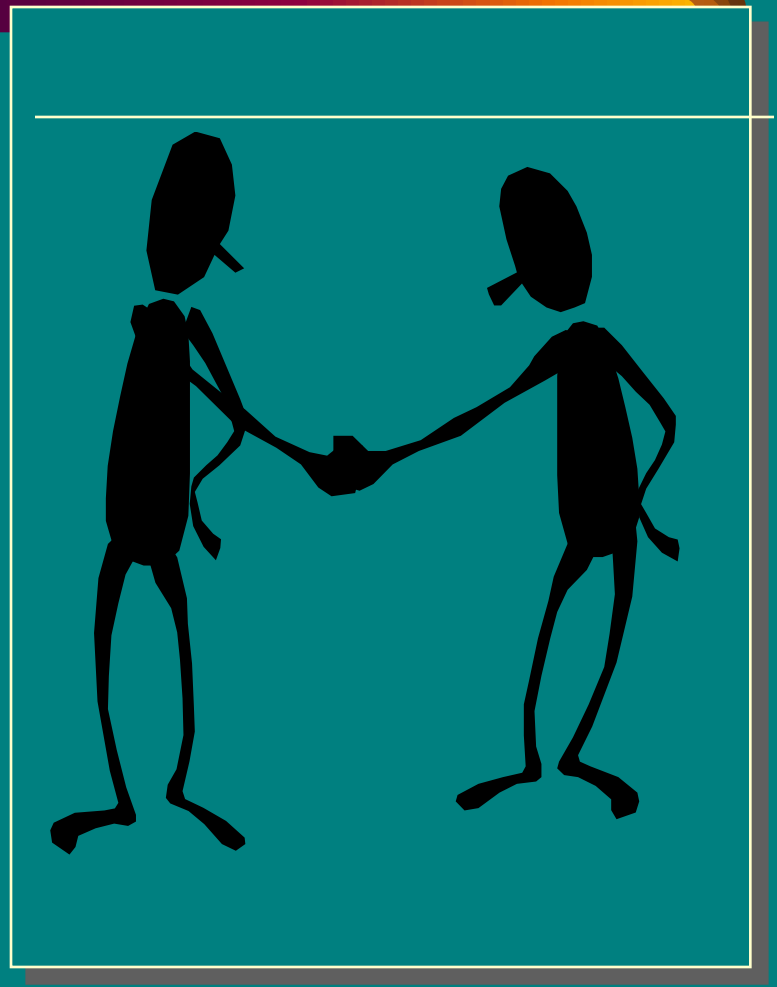
Extreme sensitivity:

Quantitative assays in the femtomolar range with use of microvolumes of sample

Admin-Lab Relationship

Compliance model

Concordance model



CONCLUSION

- Mass spectrometry coupled to adaptive and vigilant bioinformatic pattern-recognition tools will change how health & disease is detected and monitored
- Thus a transfer to newer biomarkers and biopathology signatures will open the era of “omics” diagnostics and personal management
- The result will be a nonlinear advance in our understanding of health, sports, aging, disease, prevention, risk assessment, individualization of therapy, monitoring of relapse... *(Petricoin & Liotta, Clin Chem, 2003)*

Thank you!

Questions?