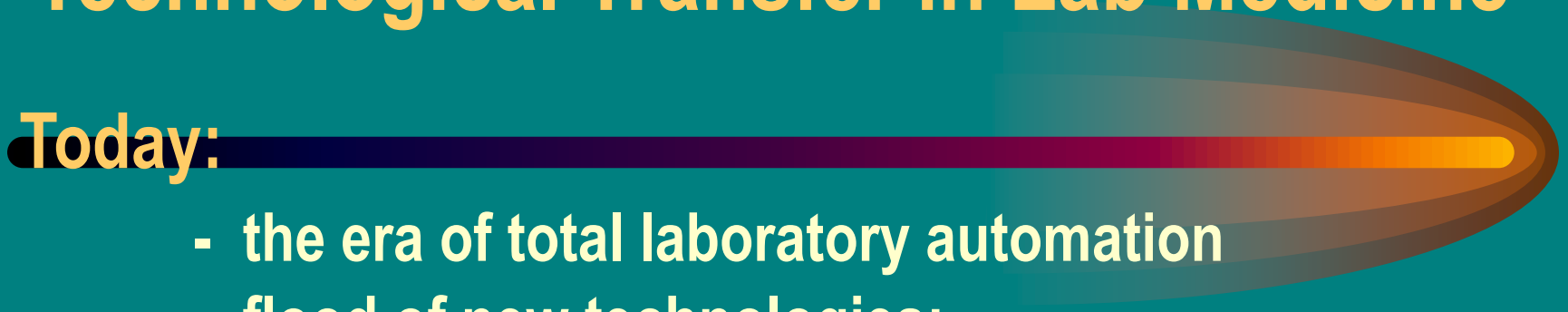
CLINICAL MASS SPECTROMETRY
ACHIEVING ROMINENCE IN MEDICINE

Dobrin Svinarov
Alexander Hospital
Medical University of Sofia, Bulgaria

SYNOPSIS

- Technological transfer in laboratory medicine
- Precision medicine is not only a NA analysis
- Principles of modern mass spectrometry
- Expanding role of MS in CLINICAL medicine
  - Chemical pathology: TDM & Tox, Endocrinology, NB screening, microbiology, Clinical chemome as a new diagnostic tool and omics'era diagnostics
  - Anatomical pathology: molecular imaging & l-knife
  - The "omics" revolution in precision 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:

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

Precision Medicine (PM)

- PM also referred as personalized medicine employs investigation of patient's genotype and phenotype to establish individually tailored disease management
  - P4 medicine:
    - Predictive
    - Proactive
    - Participatory
    - Personalized

Genotype versus Phenotype

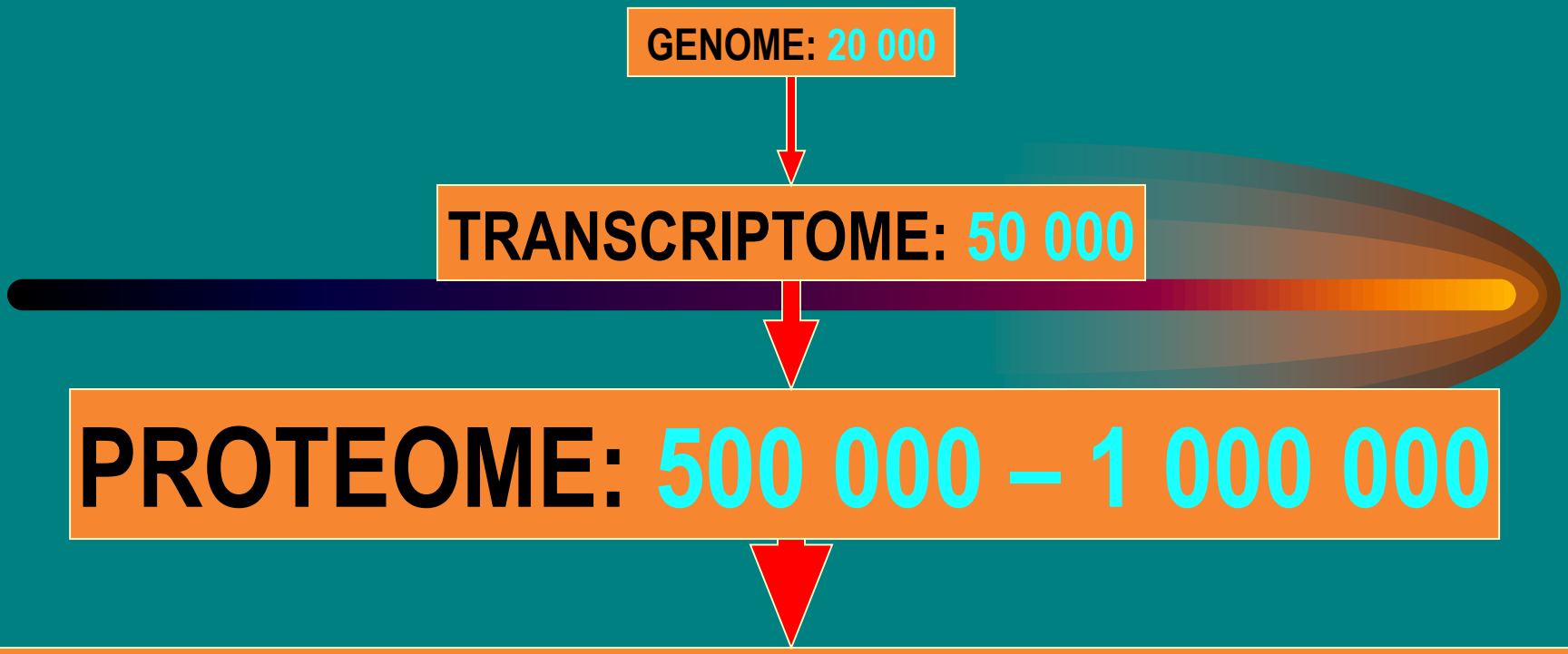
Phenotype: Variation in Organism as it Changes during Life Span

Catherpillar

Butterfly

The caterpillar and butterfly share exactly the same genome

BUT show a completely different phenotype depending on their stage of life

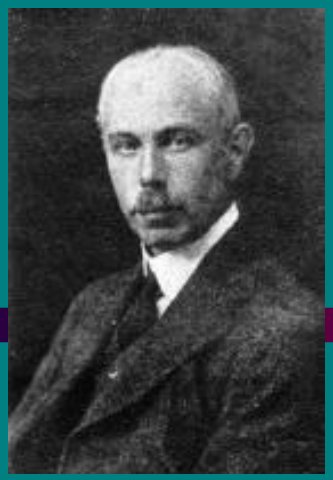

GENOME: 20 000
TRANSCRIPTOME: 50 000
PROTEOME: 500 000 – 1 000 000
METABOLOME
5 000 0000 – 10 000 000

Precision Medicine with MS?

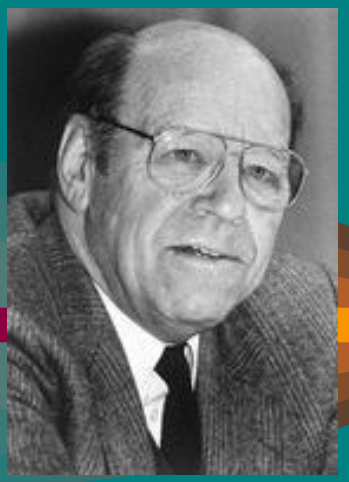
MS assays provide the actual patient's phenotype with all the environmental, pharmacological and pathological variables.

The ability to perform panel profiling with simultaneous measurement of active compounds, their precursors and metabolites in a single sample enormously amplifies informative value of results with ultimate improvement of patient care.

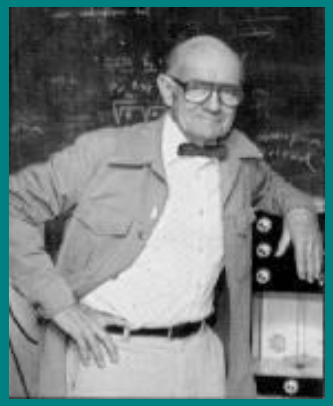
Joseph John Thomson
1865-1940, Cambridge, UK
First mass spectrometer
Nobel Prize in Physics 1906



Francis Wiliam Aston
1877-1945, Cambridge, UK
Mass spectrometry of isotopes
Nobel Prize in Chemistry 1922



Wolfgang Paul
1913-1993, Bonn, Germany
Q and Q Ion trap mass spectrometers
Nobel Prize in Physics 1989



John B Fenn
1917, Richmond, Virginia, USA
Electrospray Ionization of biomolecules
Nobel Prize in Chemistry 2002

Koichi Tanaka
1959, Shimadzu Cooperation,Japan
Matrix Assisted Lazer Desorption Ionisation
Nobel Prize in Chemistry 2002

News & Views the Clinical Chemist

Clinical Chemistry's 2016 Special Issue: Clinical Mass Spectrometry–Achieving Prominence in Laboratory Medicine

Thomas M. Annesley*

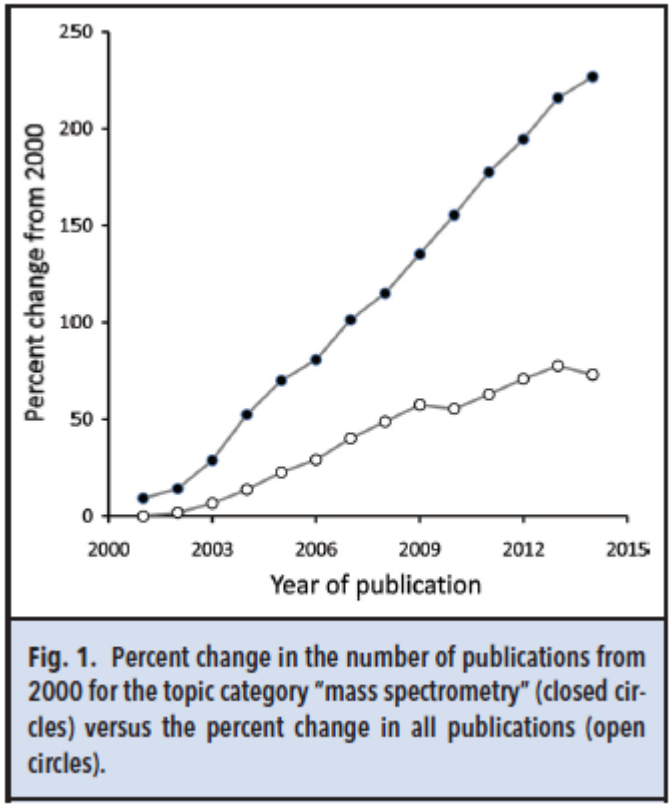
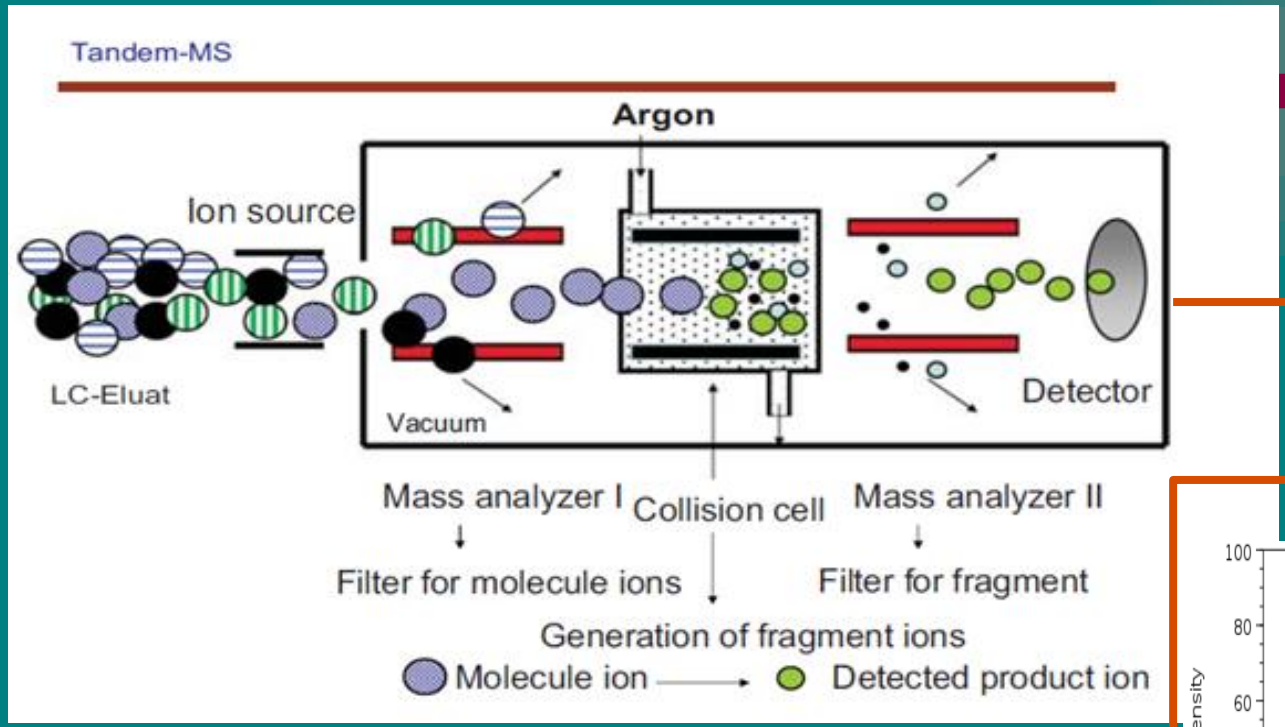
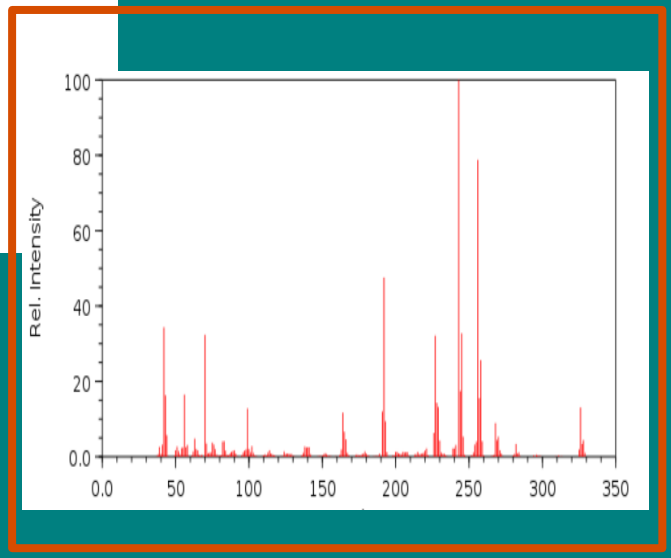


Fig. 1. Percent change in the number of publications from 2000 for the topic category "mass spectrometry" (closed circles) versus the percent change in all publications (open circles).

Principle of LC-MS/MS (QQQ)MS



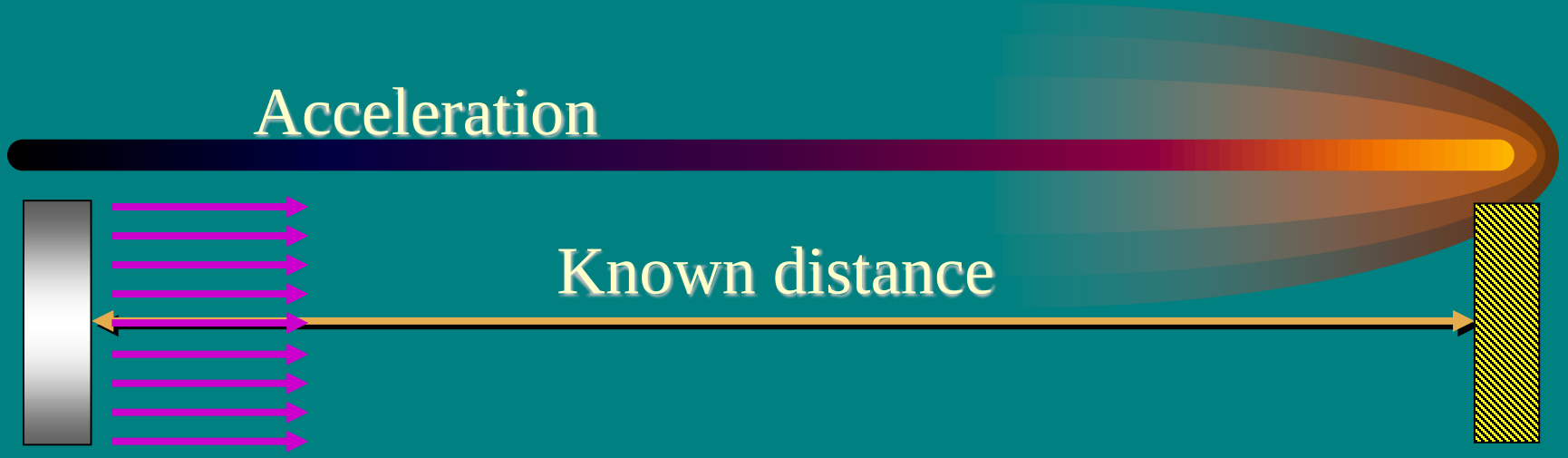


Kobold-U, Scand J Clin & Lab Invest 2012; 72 (Suppl 243):54-59, modified.

Direct sample introduction via:

- **M**atrix
- **A**ssisted
- **L**azer
- **D**esorption
- **I**onization

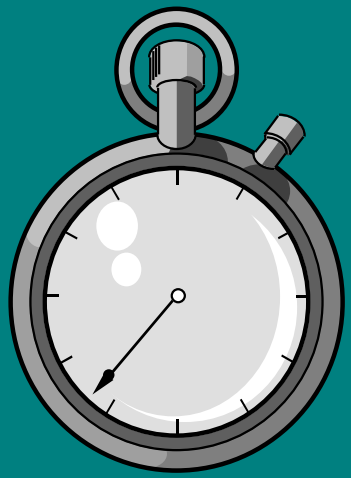
Hardware of ToF



Sample
(start point)

Detector

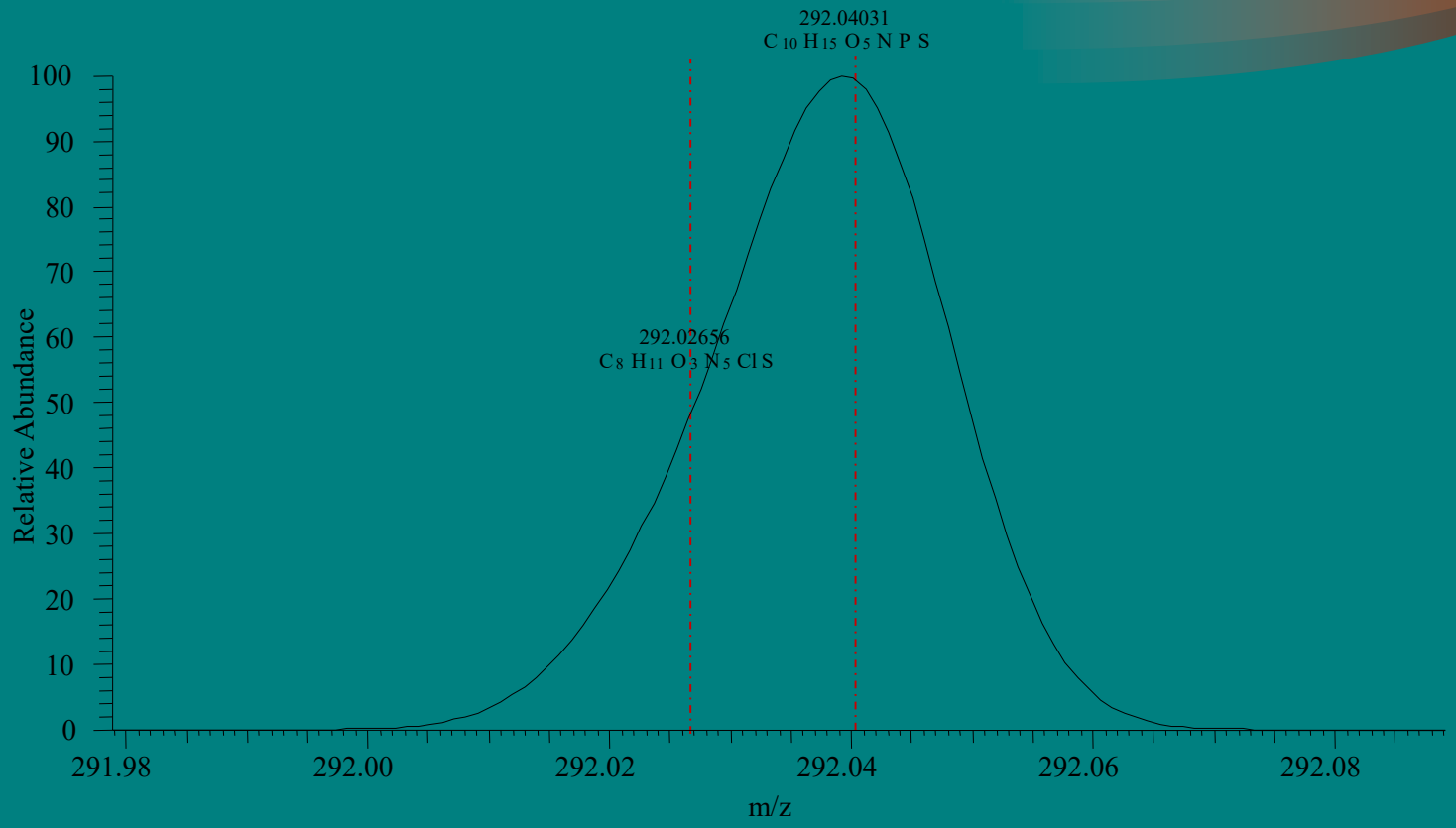
Timing
electronics



Orbitrap™ Mass Spectrometers

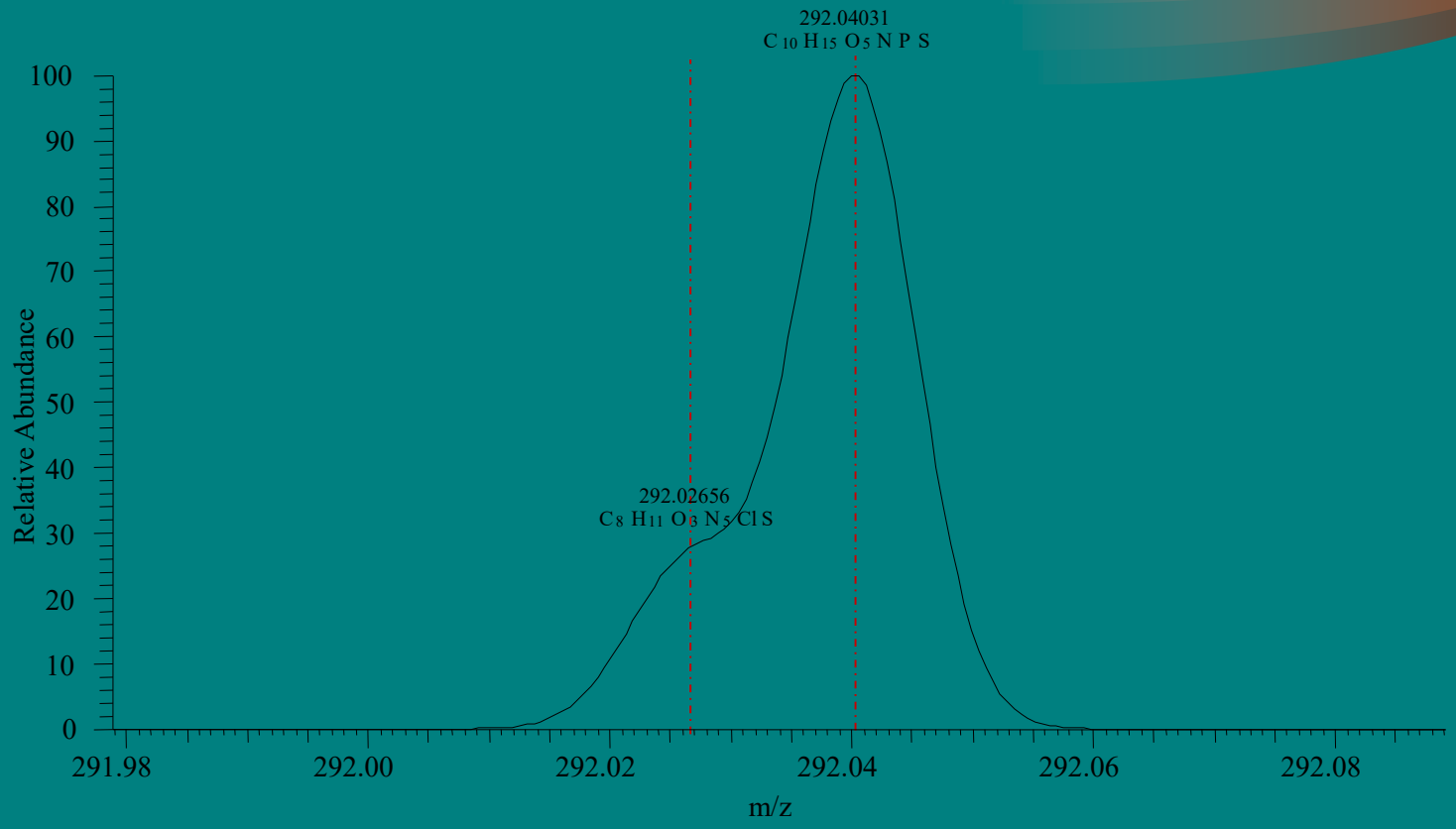
Simulated Resolution = 15,000 (Mix 1:3)

Resolution 15,000



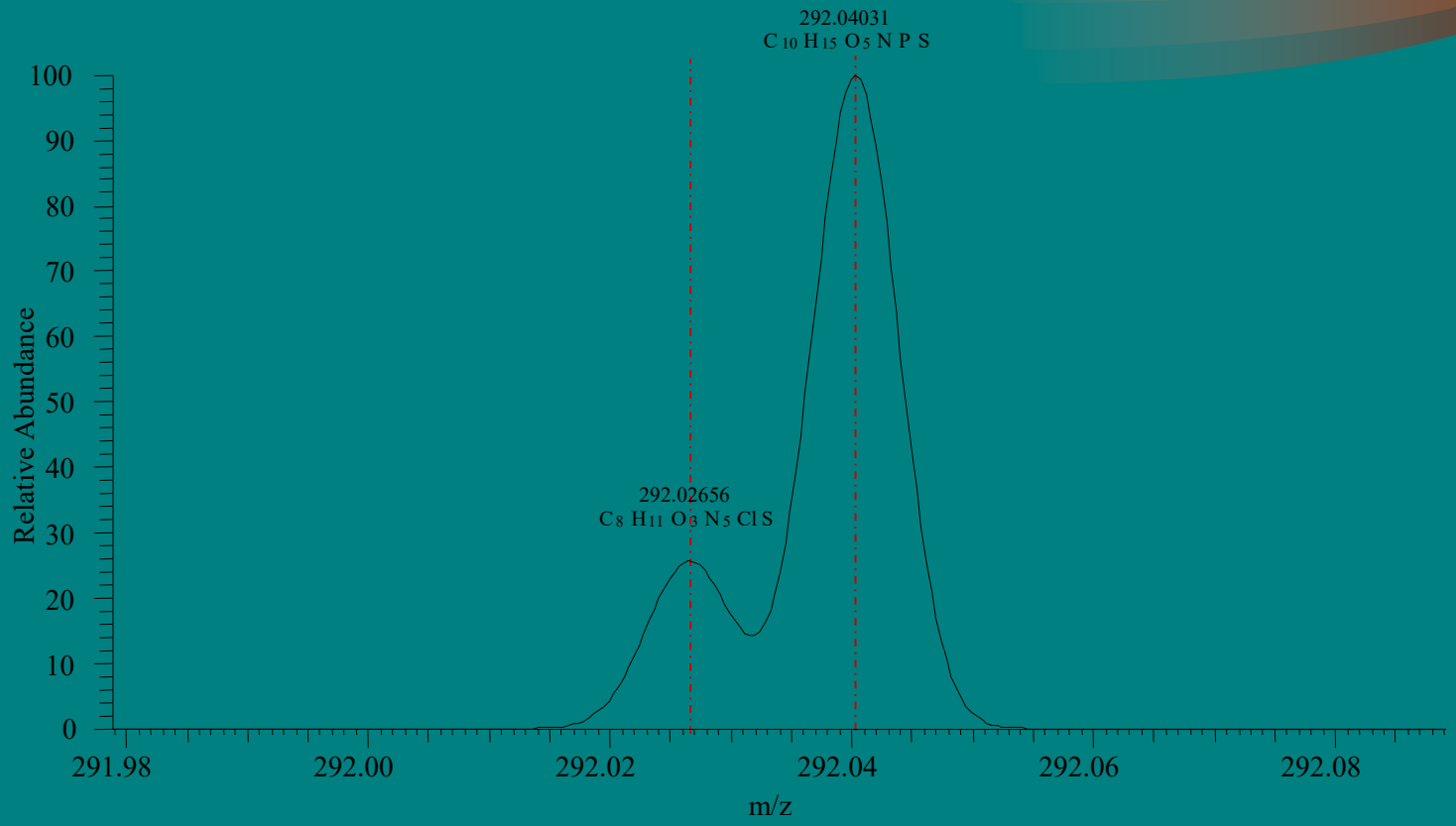
Simulated Resolution = 25,000 (Mix 1:3)

Resolution 25,000

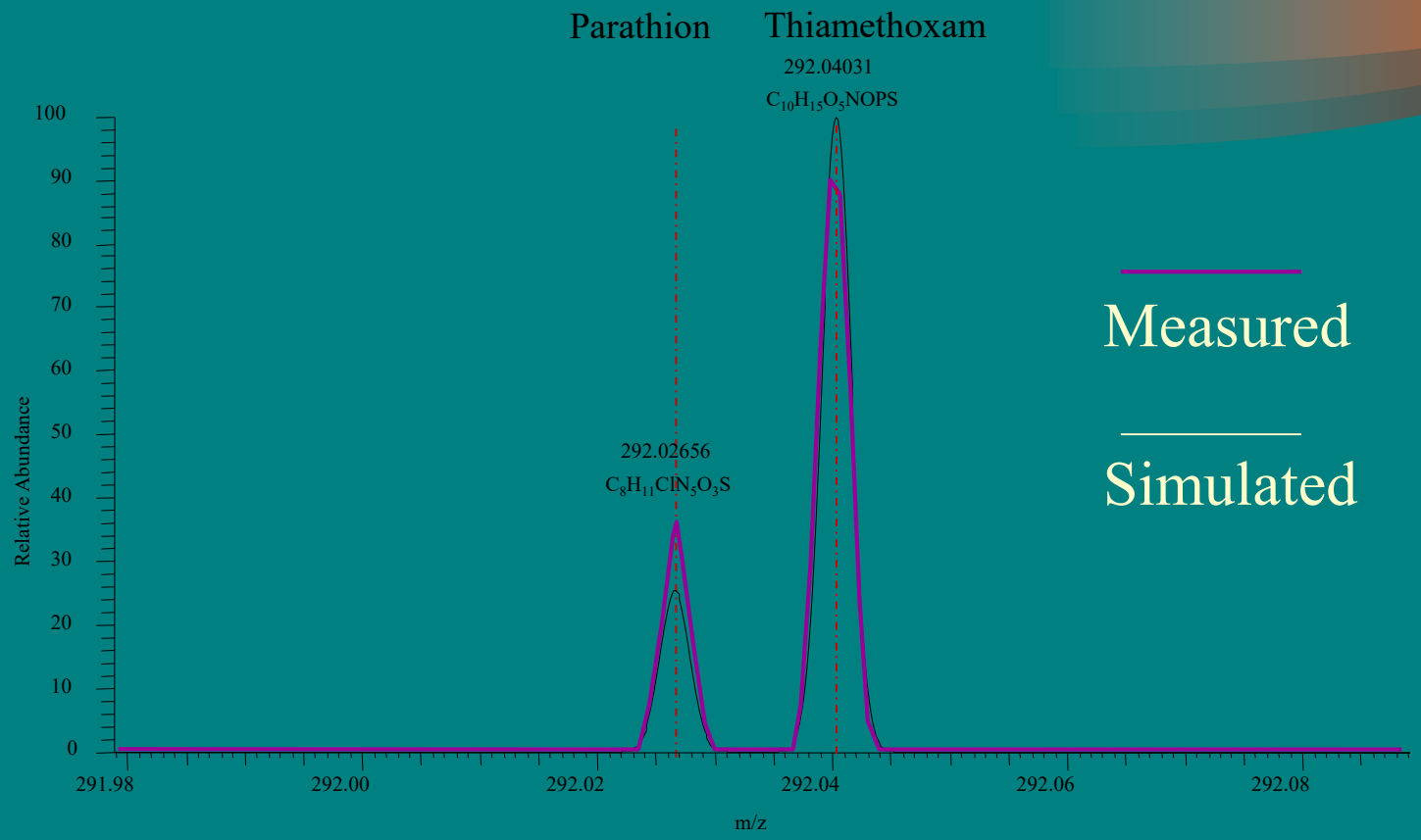


Simulated Resolution = 35,000 (Mix 1:3)

Resolution 35,000



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



Expanding role of mass spectrometry in the medical laboratory

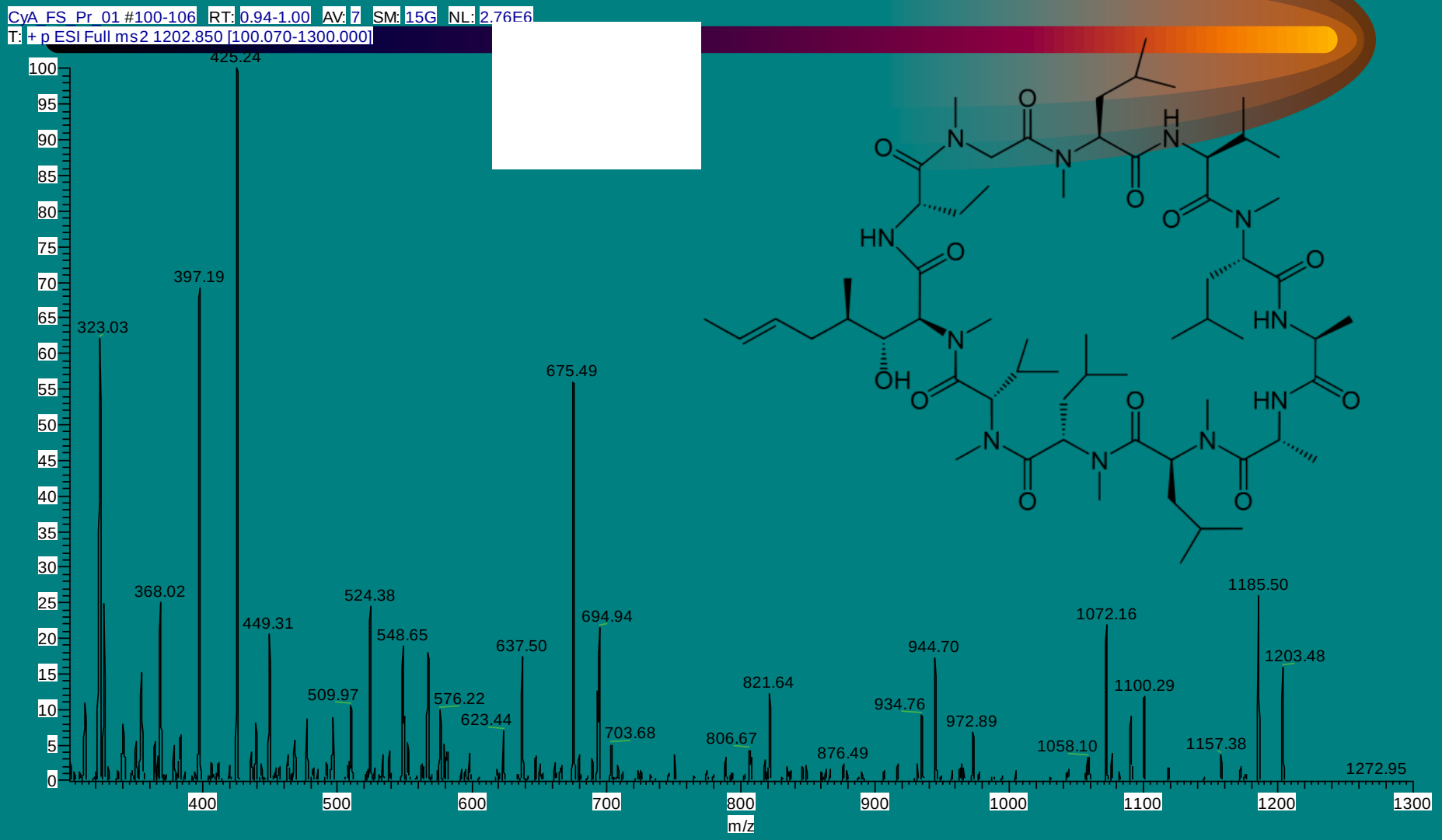
LC-MS/MS (QQQ)

- **TDM** (immunosuppressants, antiretroviral drugs, antidepressants, antipsychotics)
- **Drugs of Abuse & Clinical Toxicology**
- **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** (Angiotensins, Oxytocin, ADH, hepcidine)

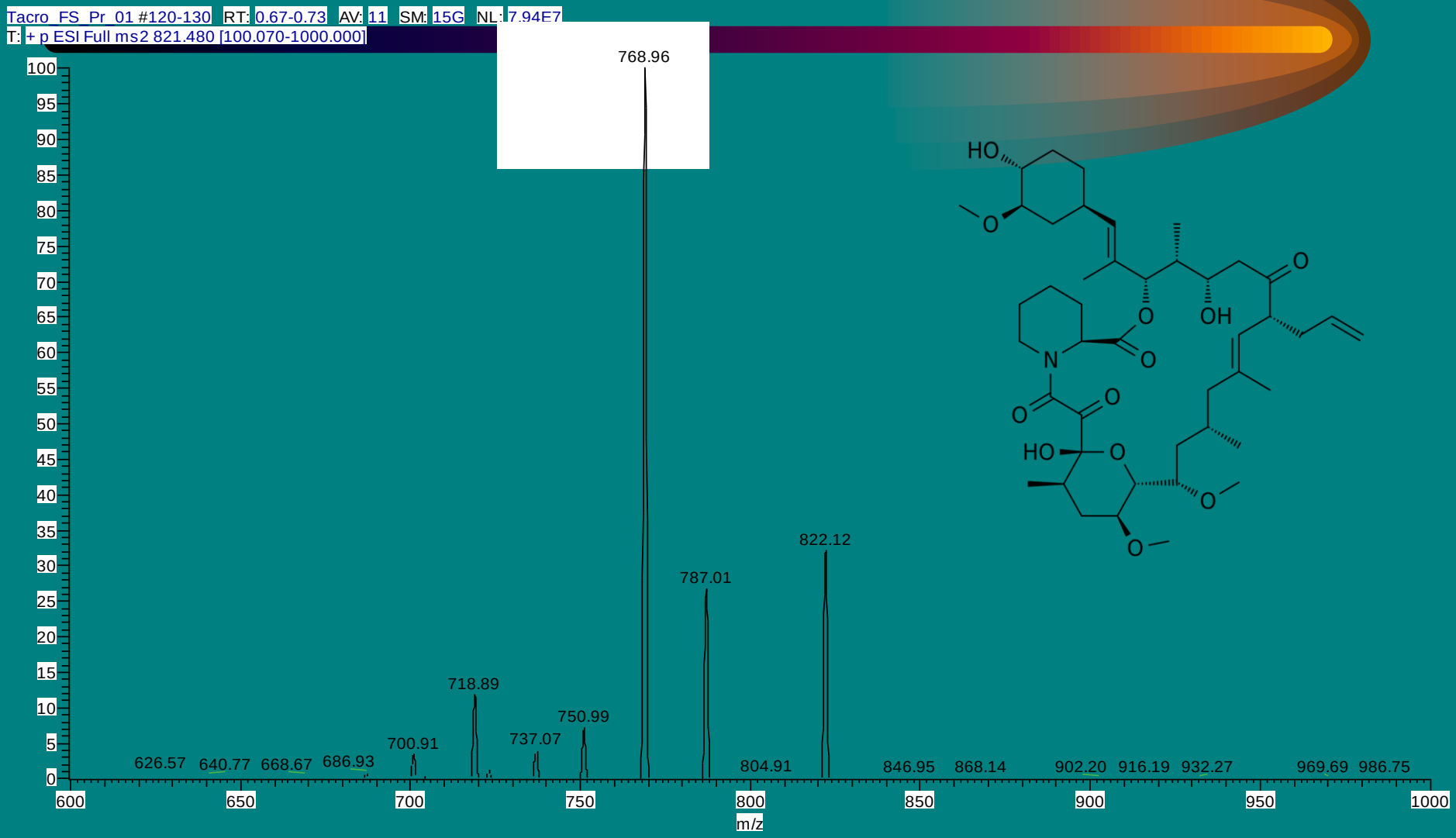
MALDI-TOF & ORBITRAP

- **Proteomics** (Research, Biomarker Discovery)
- **Medical Microbiology, Environmental & Clinical Toxicology**

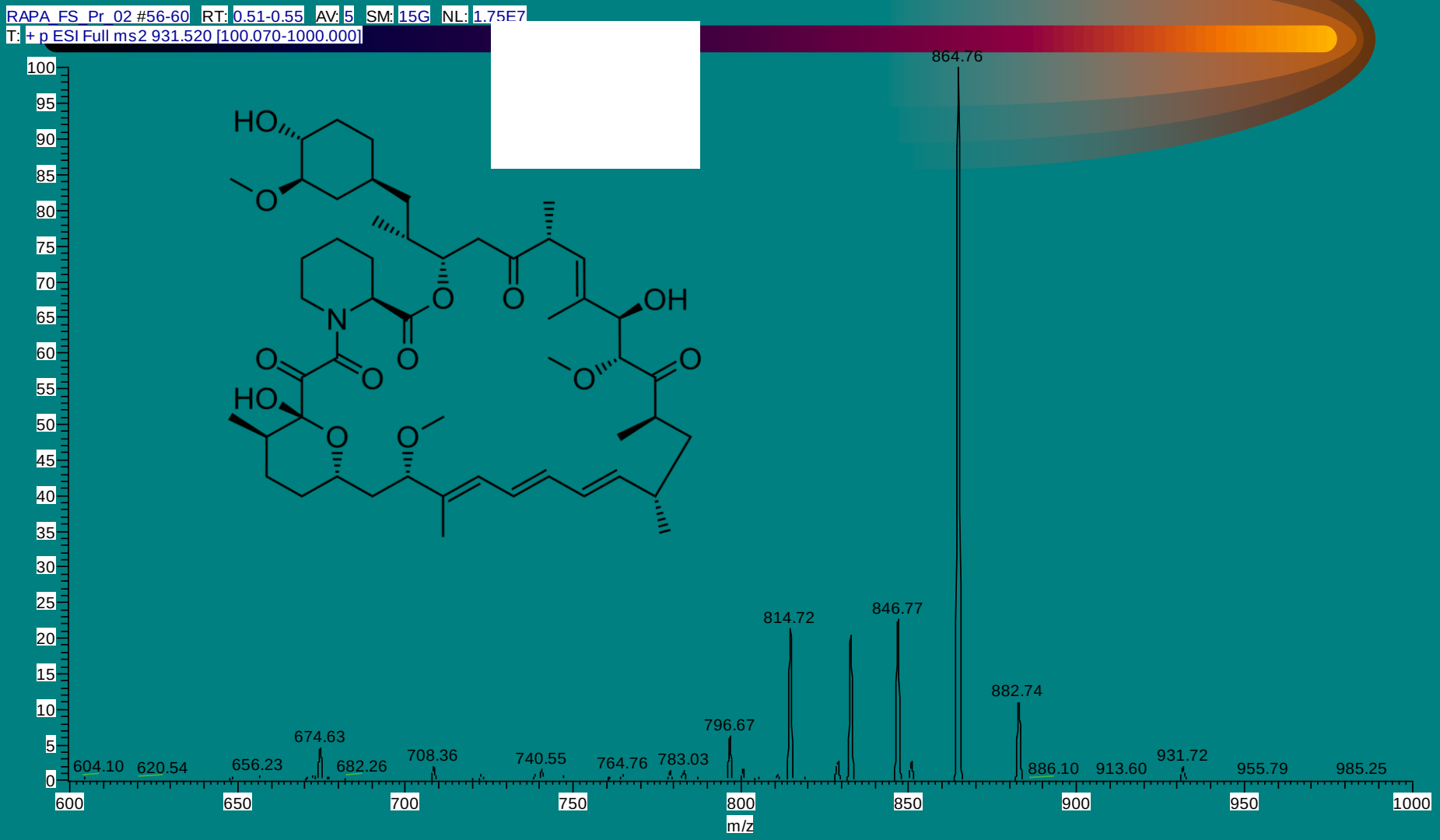
CsA - Product Spectra



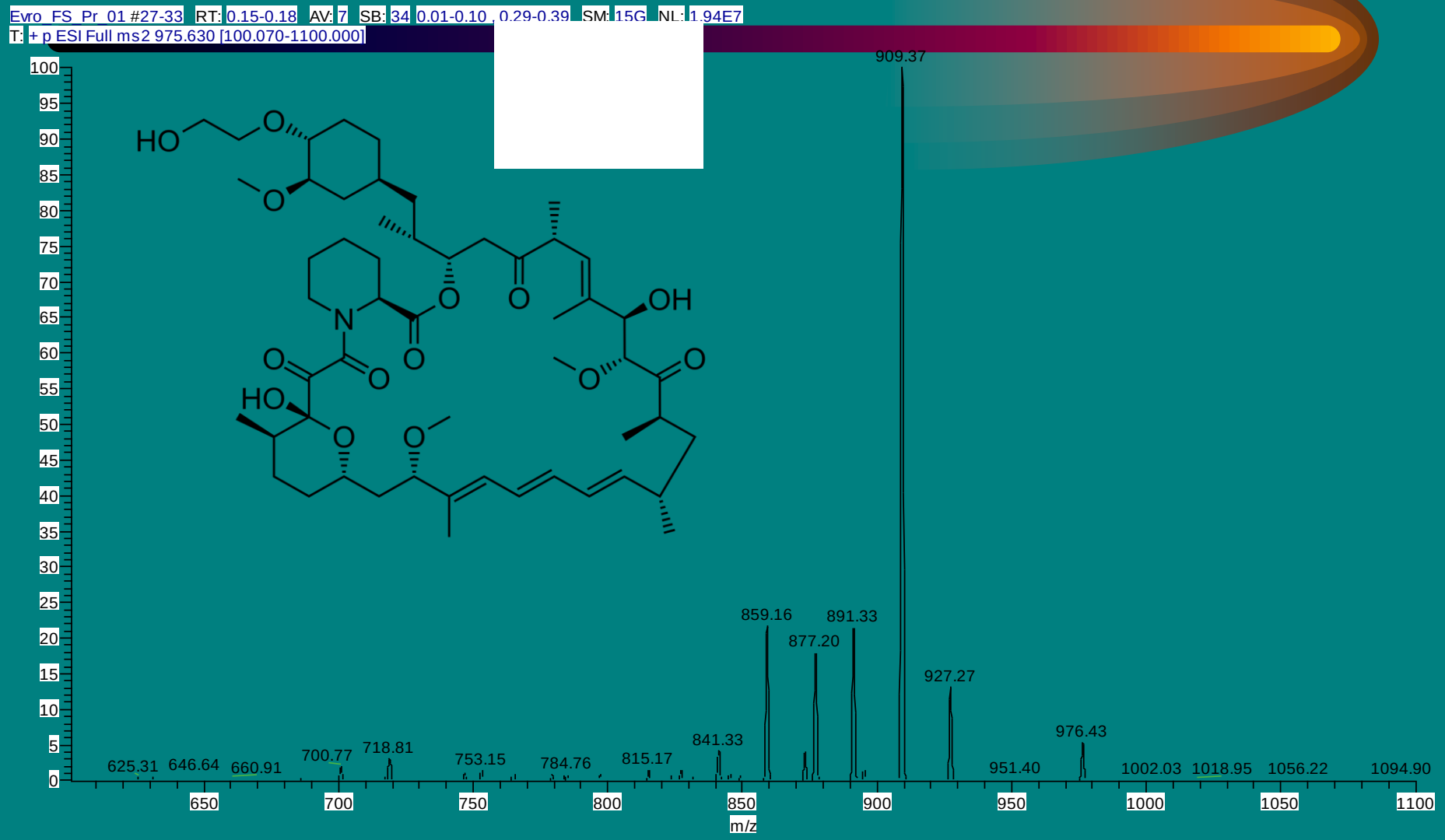
TCR- Product Spectra



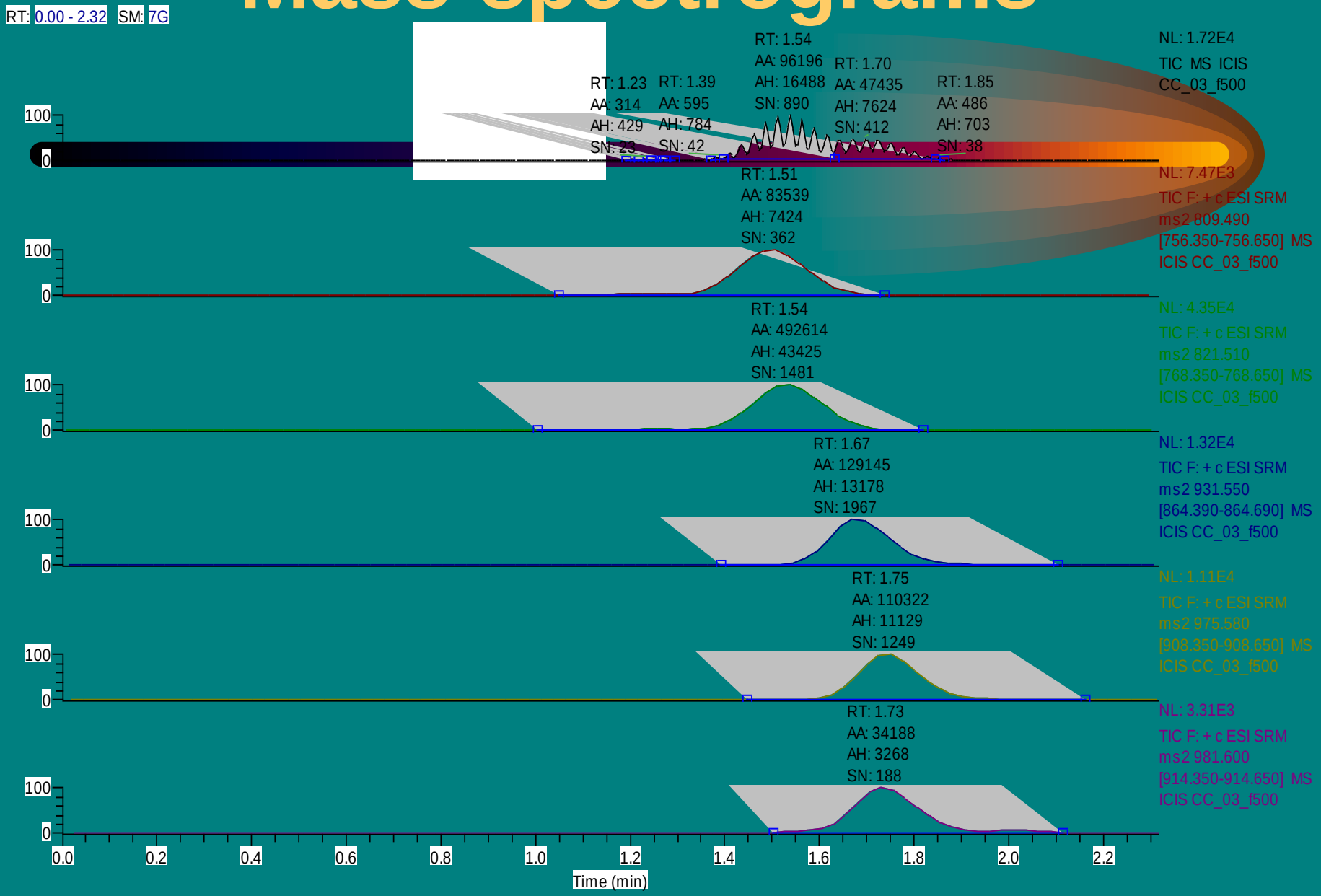
SRL- Product Spectra



ERL- Product Spectra



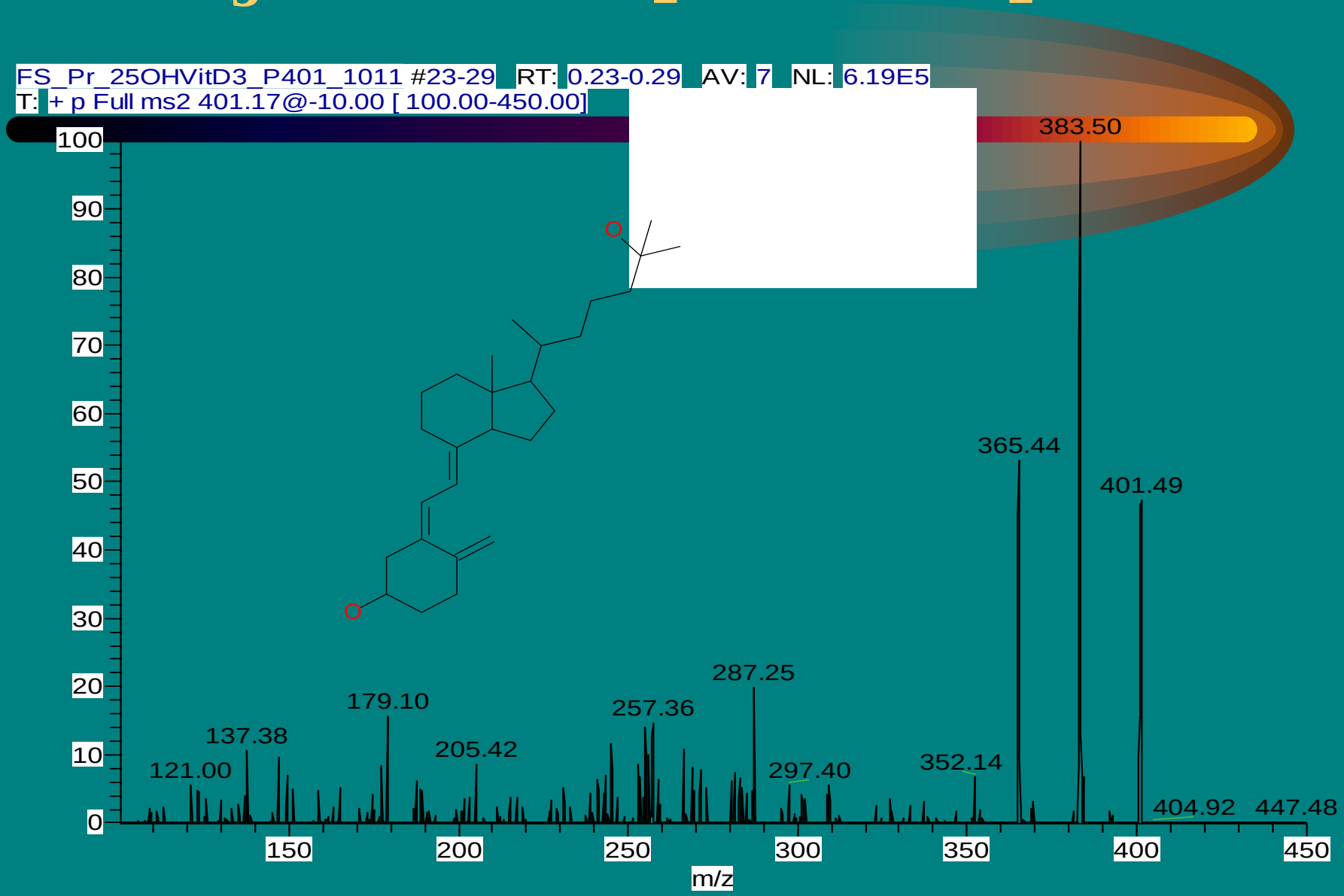
Mass-spectrograms



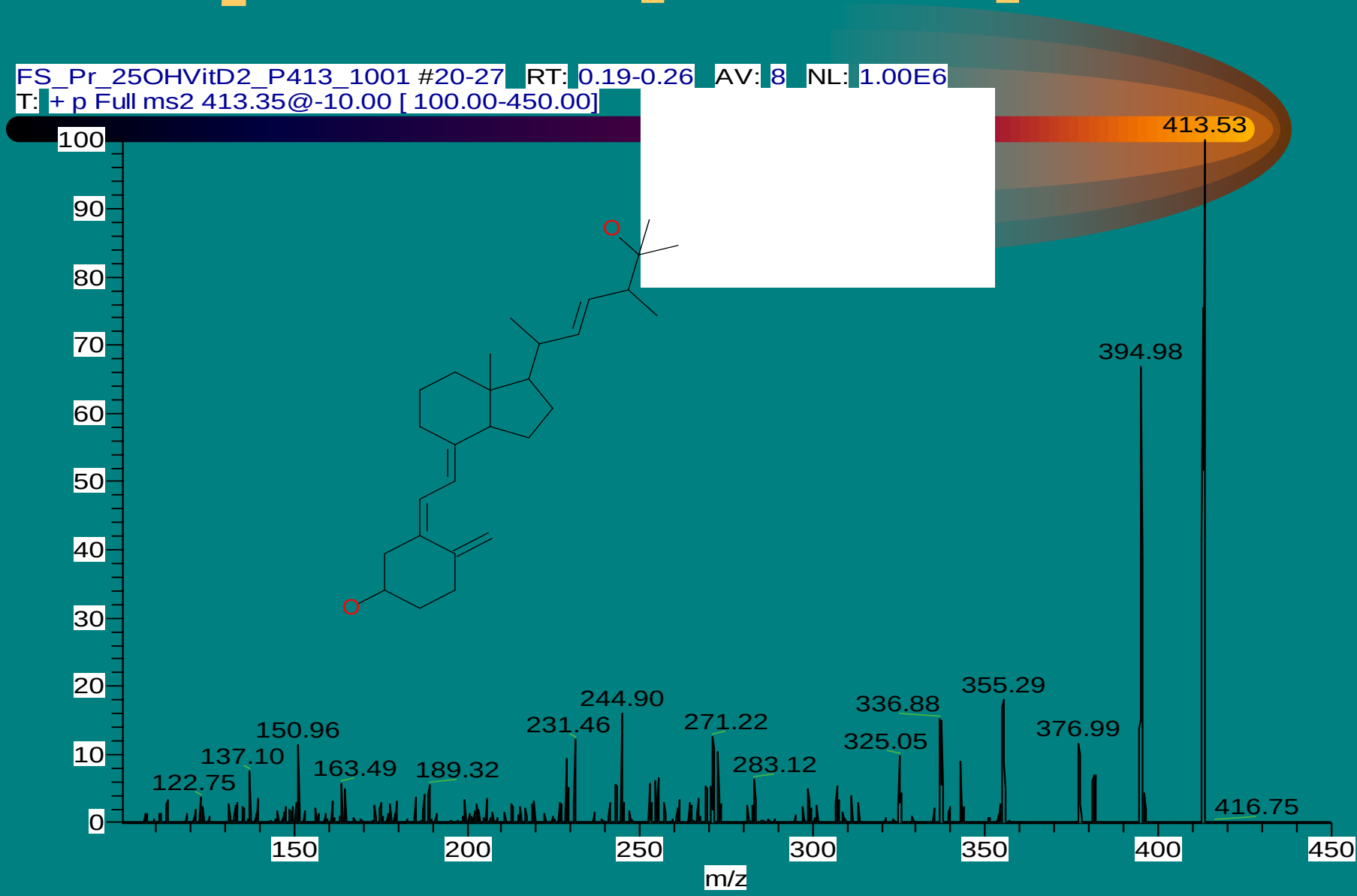
Method validation
tested at LLOQ, QC-L, QC-M, QC-H

- **Selectivity: documented by analysis of 6 different WB; ME 90-112%; RME 94-110%**
- **Accuracy: batch ± 7.3 %, between run ± 8.6%**
- **Precision: batch < 6.8 %, between run < 8.9%**
- **Recovery: 65-76%(Process efficiency)**
- **Linearity: 10 ÷ 2000 µg/L for CsA**
  1 ÷ 45 µg/L for TCR, SRL, ERL
- **Stability: freeze-thaw - 3 cycles of 24 h, post-preparative 24-96 h @ 8ºC, short-term amb. 18 h, long-term – for 7 d @ 8ºC and 120 d @ -20ºC**

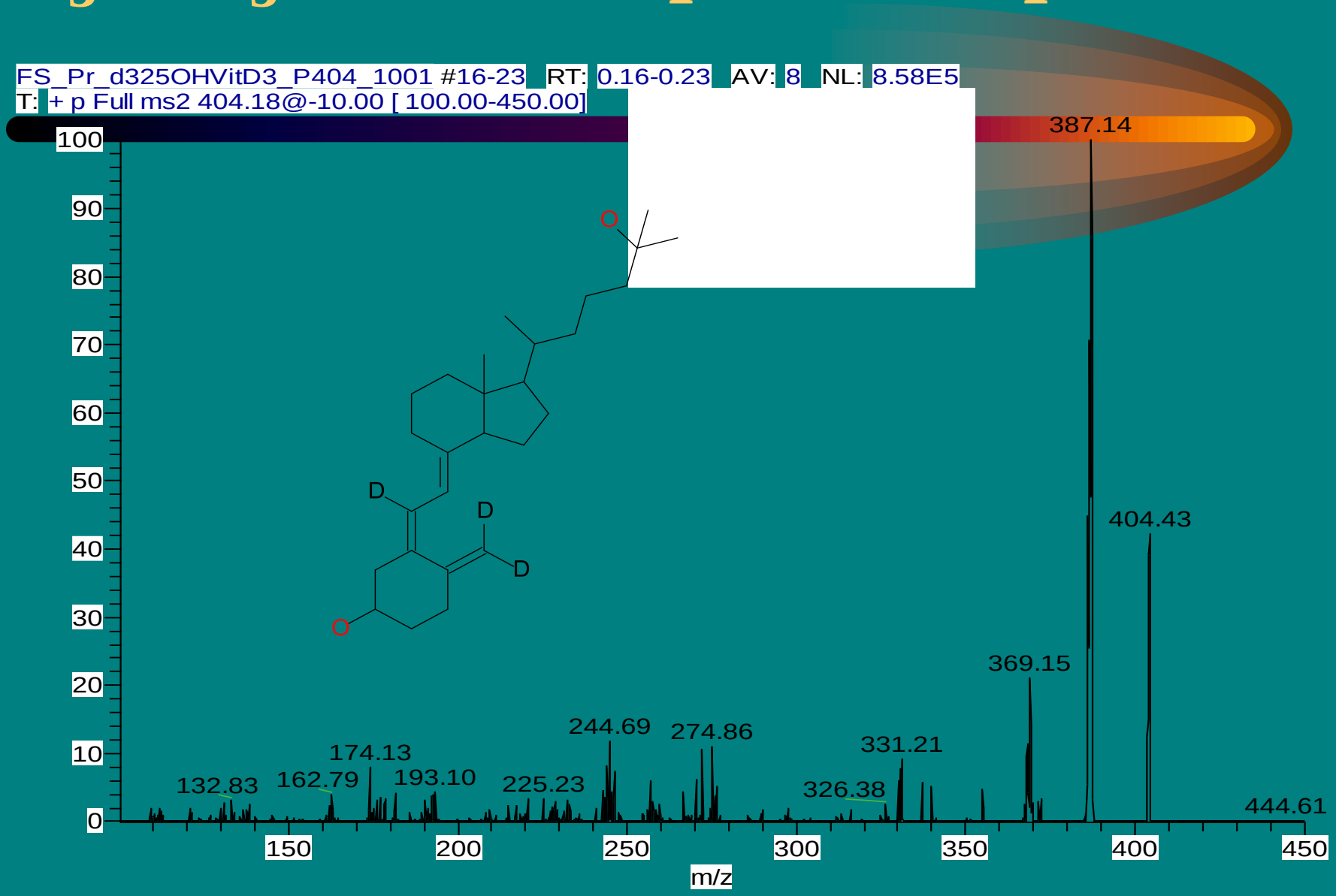
$25D_3$ Full scan-product spectra



$25D_2$ Full scan-product spectra



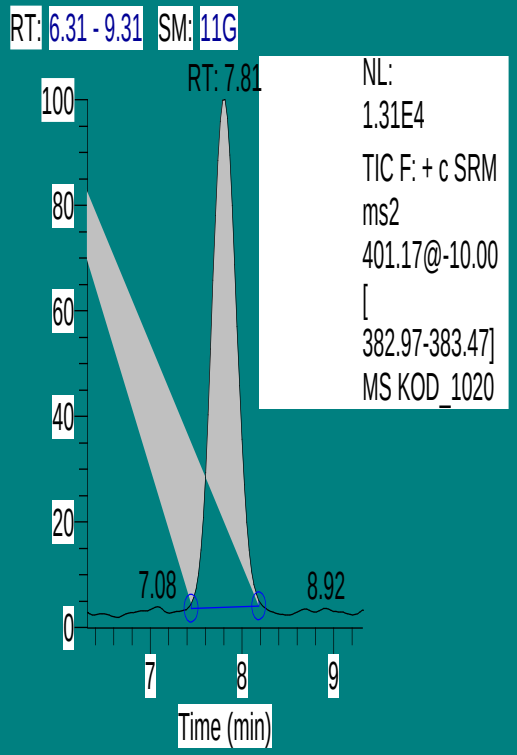
$d_3$25D$_3$ Full scan-product spectra

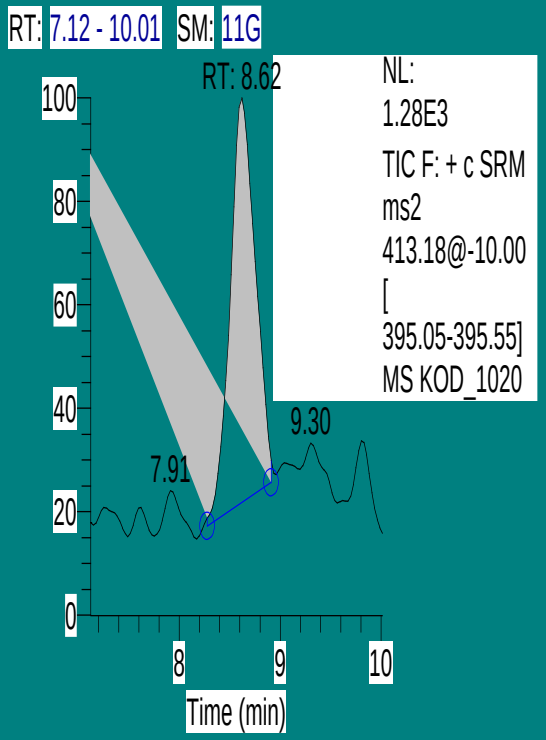


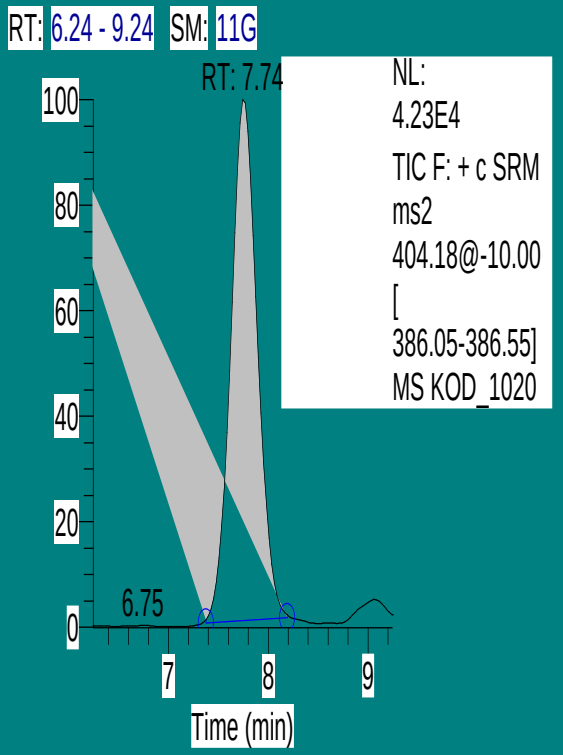
Pt Sample Mass-spectrogram

$25D_3$ $25D_2$ $d_3 25D_3$

50 nmol/L **11 nmol/L** **125 nmol/L**



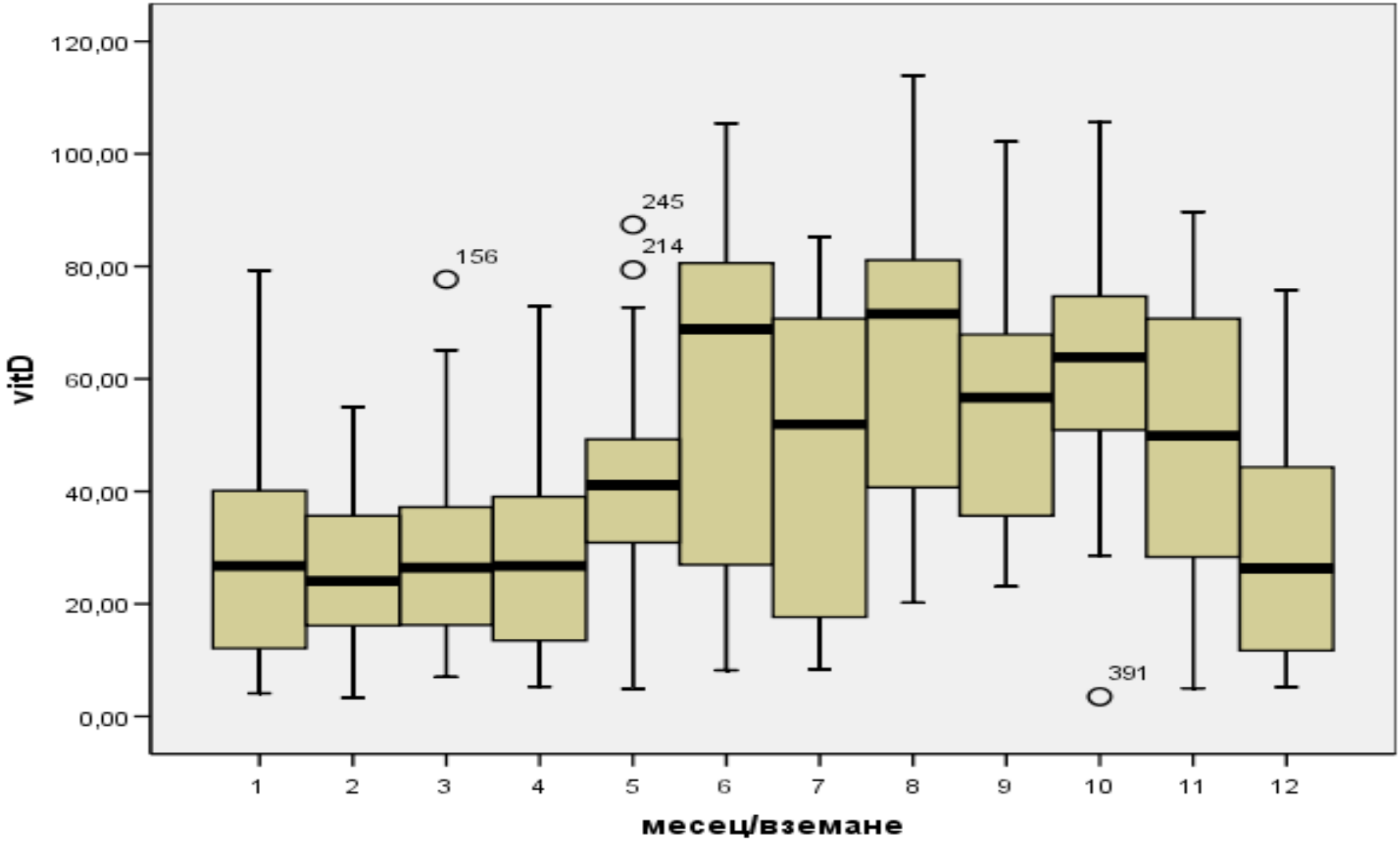




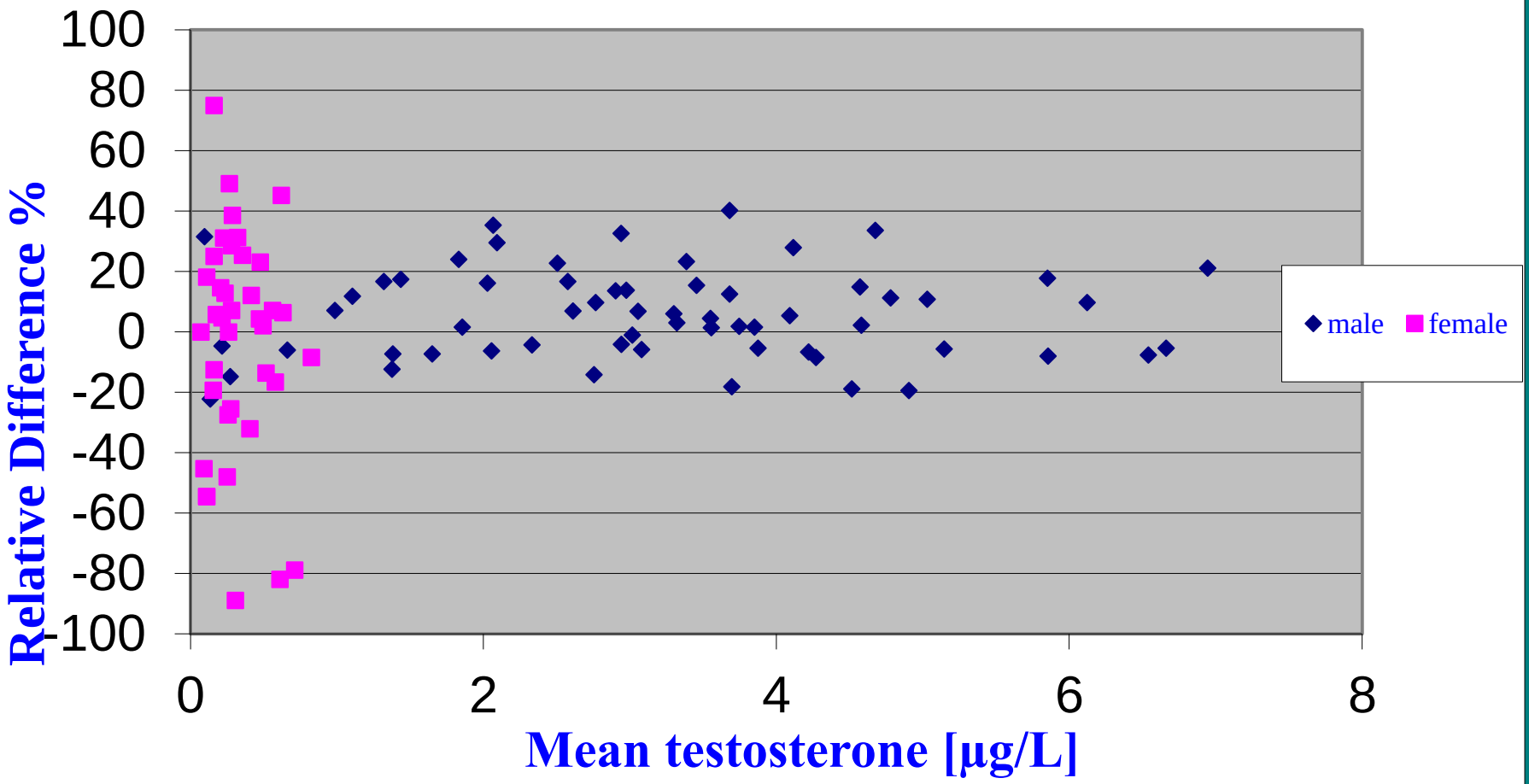
Epidemiologic study

Assessment	25D ($25D_3$+$25D_2$)	Subjects	%
Deficiency	0 ÷ 25 nmol/L	439	21.5
Insufficiency	25 ÷ 50 nmol/L	1098	54.0
Insufficiency	50 ÷ 80 nmol/L	466	23.0
Sufficiency	80 ÷ 139 nmol/L	30	1.5
Mean ± SD	39 ± 17 nmol/L	2033	100

Seasonal variations of 25OHD levels



Testosterone measured with Immunoassay & LC-MS/MS



Streit et al (unpublished)

High sensitive LC-MS/MS Analysis of Estradiol (E2) and Testosteron (T) in Plasma

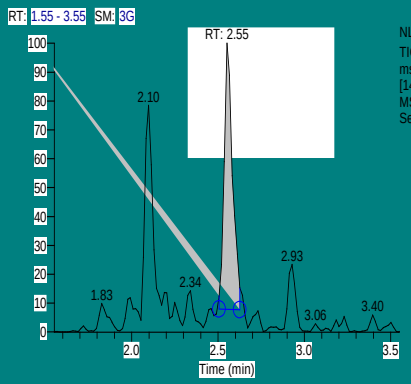
Accurate measurement of E_2

at low concentrations is required

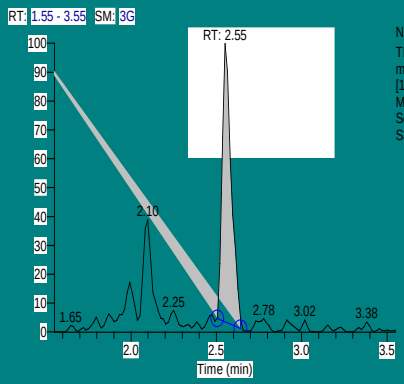
in postmenopausal women, men, children, and to assess the efficacy of anti-estrogen therapies.

The same is true for T in women, children, hypo-gonadal men and the need to control anti-androgen therapies.

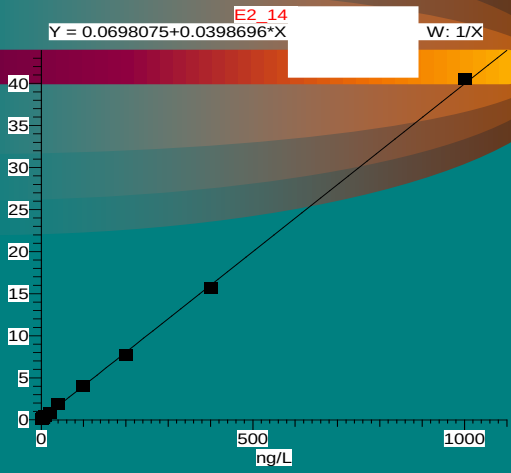
High sensitive LC-MS/MS Analysis of Estradiol (E2) in Human Plasma

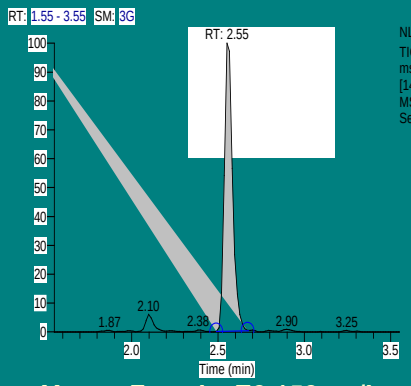


Post MP Female, E2 1.5 ng/L

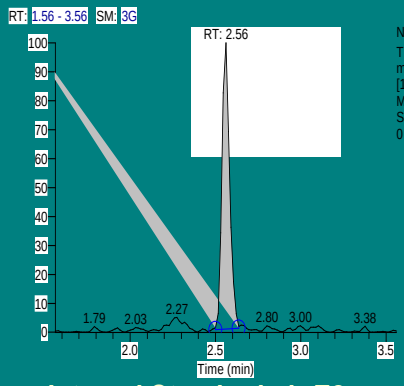


Eldery Male, E2 3.6 ng/L





Young Female, E2 152 ng/L



Internal Standard, d_3-E2

File Name	Specified Amount	Calculated Amount	% Diff
CC_00	1.000	0.934	-6.59
CC_01	2.000	2.068	3.42
CC_02	4.000	4.208	5.21
CC_03	10.000	9.857	-1.43
CC_04	20.000	18.193	-9.04
CC_05	40.000	45.714	14.28
CC_06	100.000	98.789	-1.21
CC_07	200.000	192.181	-3.91
CC_08	400.000	391.694	-2.08
CC_09	1000.000	1013.363	1.34

VALIDATION PARAMETERS

Parameter	Validation
Selectivity **Normalized ME** **Imprecision**	 **91 ÷ 111%** **< 15%**
AMI	**1.0 ÷ 1000 ng/L**
Inaccuracy **within runs** **between runs**	 **-10.0 ÷ +6.9%** **-14.7 ÷ +11.3%**
Imprecision **within runs** **between runs**	 **<12.7%** **<14.8%**

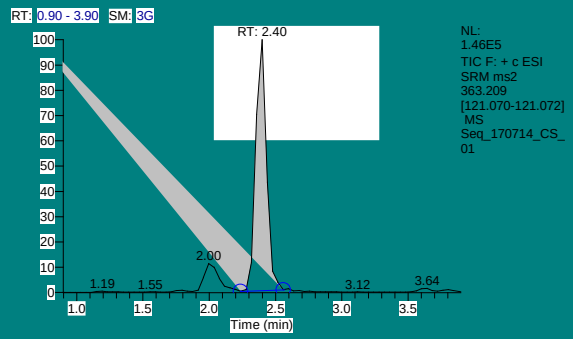
VALIDATION PARAMETERS

Preanalytical stability was proven for 2 h;
Short-term stability at room temperature for 6 h at daylight and for 4 h in the dark;
Freeze-thaw stability was confirmed for three cycles each lasting 24 h;

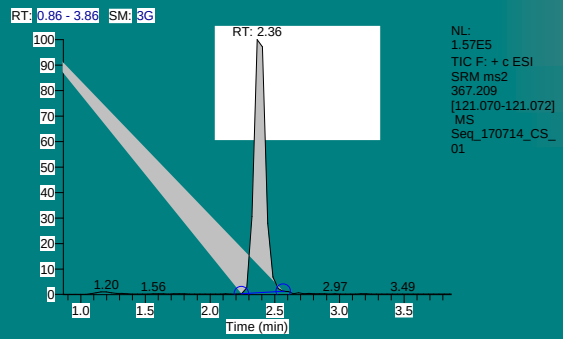
Post-preparative stability was documented for 48 h at 4^{o}C;

Stock solution stability and **long term stability in serum** were documented for 96 days at -20^{o}C.

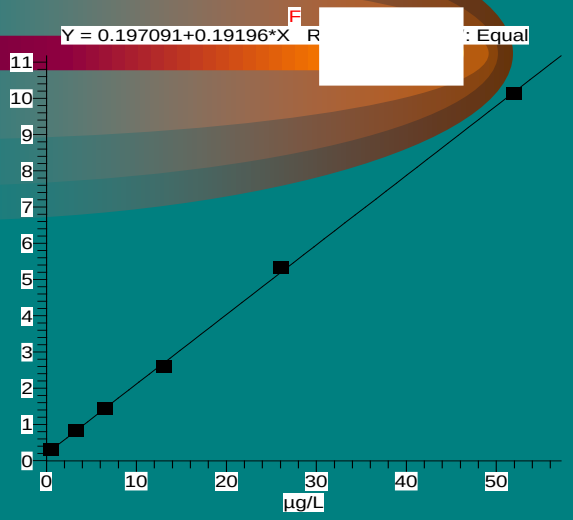
Simultaneous LC-MS/MS Analysis of Cortisol (F)and Testosteron (T) in oral fluid

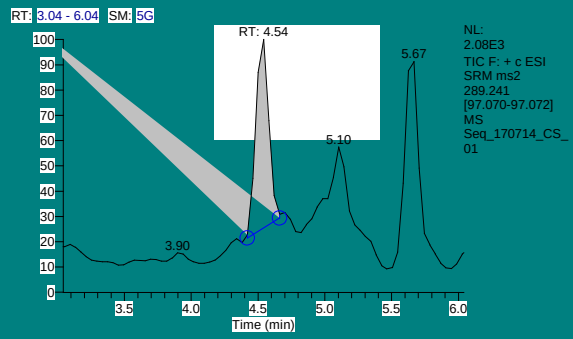


Calibration Sample01, F 2.8 µg/L

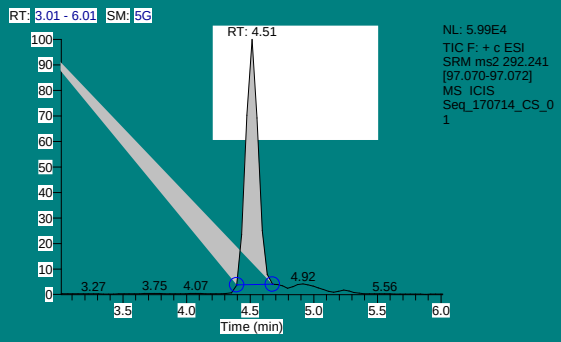


Internal Standard, d4-F

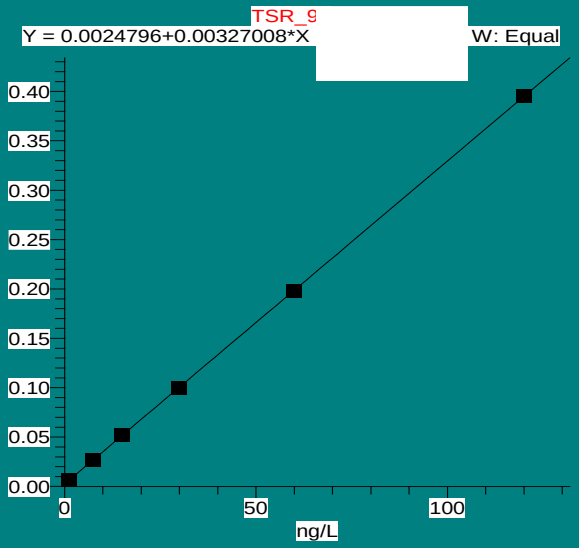




Calibration Sample01, T 6.2 ng/L



Internal Standard, d3-T



Traditional Newborn Screening

- Single Disease
- Single test
- Single Marker
- Single cut-off (N/Abn)

MS/MS Newborn Screening

- Multiple Diseases
- Single test
- Multiple Markers
- Multiple cut-offs

MS/MS Newborn Screening

> 50 ANALYTES> 30 IEM

Time < 2 min

Phenylketonuria
MSUD
Homocystinuria
Tyrosinemia type I
Argininosuccinic acidemia
Citrullinemia type I
Hyperphenylalaninemia
Tyrosinemia type II
Biopterin defects (Bios)
Tyrosinemia type III
Biopterin (Reg)
Argininemia
Hypermethioninemia
Citrullinemia type II

MCAD deficiency
VLCAD deficiency
LCHAD deficiency
TFP deficiency
Carnitine uptake defect
Glutaric acidemia type II

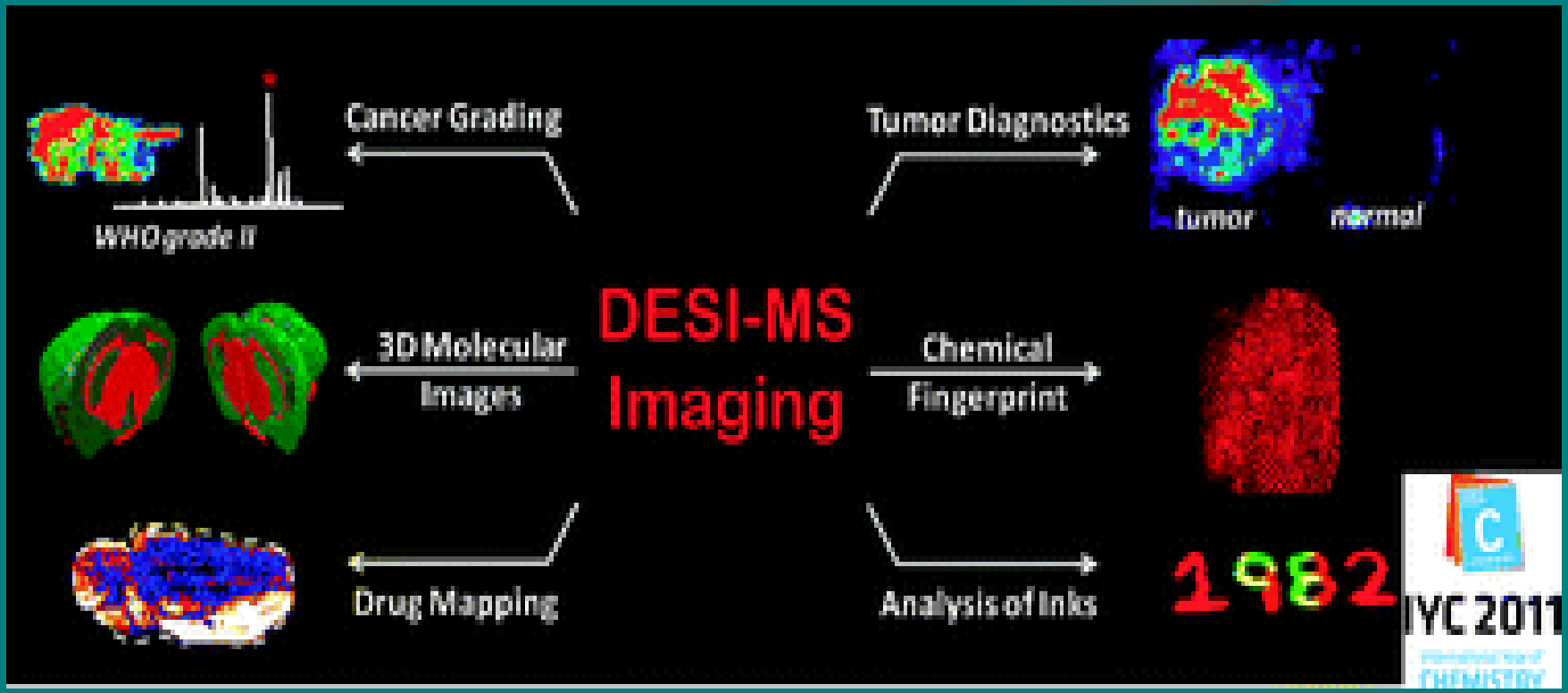
Isovaleric acidemia
Glutaric acidemia type I
Methylglutaconic acidemia
Malonic acidemia
HMG deficiency
3MCC deficiency
BKT deficiency
Multiple carboxylase deficiency
Methylmalonic acidemia (MUT)
Methylmalonic acidemia (Cbl A,B)
Propionic acidemia

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

CLINICAL MASS SPECTROMETRY MEETS CHEMICAL AND ANATOMICAL PATHOLOGY

MS IMAGING


Cancer Grading
WHO grade II
Tumor Diagnostics
tumor
normal
3D Molecular Images
DESI-MS Imaging
Chemical Fingerprint
Drug Mapping
Analysis of Inks
1982
IYC 2011

I-KNIFE MS IMAGING

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

BUT for LC-MS/MS Problems still Exist!

- High financial investment
- Limited capability for automation
- No random access
- Special expertise required
- LDTs, limited availability of IVD certified kits
- Lack of proficiency testing schemes
- Tedious method validation
- Technical support from manufacturers
- Need for harmonization and standardization
- ……

LC-MS/MS-Kits

BASIC Kit A PLUS **MasterColumn® A**

BASIC Kit A consists of:

- Mobile Phase 1
- Mobile Phase 2
- Precipitation Reagent
- Extraction Buffer
- Dilution Buffer 1
- Dilution Buffer 2
- Rinsing Solution
- Reaction vials

MasterColumn® A: Analytical column: equilibrated, with test chromatogram

PLUS

Single PARAMETER Sets

- Antiarrhythmic Drugs
- Antidepressants 2/ Psychostimulants
- Anti-HIV Drugs
- Benzodiazepines 1
- Mycophenolic Acid
- Neuroleptics 2/ *EXTENDED*
- Tricyclic Antidepressants TCA 2
- Antidepressants 1
- Antiepileptic Drugs
- Antimycotic Drugs
- Benzodiazepines 2
- Neuroleptics 1
- Tricyclic Antidepressants TCA 1

Components of each PARAMETER Set: Multilevel Plasma Calibrator Set (3PLUS1® or 6PLUS1®)
+ *MassCheck®* Plasma Control, Level I and Level II
+ Internal Standard

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™
MassTrak™

RECIPE®
ClinMass® Komplettkit

CHROMSYSTEMS

MassTox® Immunosuppressants in whole blood - ONEMinute Test

ATHENS 2017


Introducing the first fully automated
clinical analyzer with integrated
LC-MS/MS technology

CONCLUSION

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

Thank you!

Questions?