Addressing The Challenge of Sensitivity and Metabolite Detection in Bioanalytical Studies

European Bioanalysis Forum

1st December 2010

Gary Harland

Program Manager – Bioanalysis

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Challenges in Bioanalysis
Metabolite Monitoring and Detection

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- **[Method Development]**
  - HPLC vs UPLC
  - Resolution of Metabolites
- **[Metabolites and MIST]**
  - MS Technology
  - iMRM for metabolite detection and quantification
- **[Worked Example]**
  - MS Technology
  - iMRM for metabolite detection and quantification

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FDA MIST Guidelines

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'In general, these metabolites are of interest if they account for plasma levels >10% of total systemic exposure to drug related material, measured as area under the curve (AUC) at steady state.'

Guidance for Industry

Safety Testing of Drug Metabolites

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

February 2008
Pharmacology and Toxicology

System Solutions

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ACQUITY UPLC Xevo TQ MS

[High Sensitivity LC/MS]

ACQUITY UPLC Xevo TQ-S

[Ultra High Sensitivity LC/MS]

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[Method Development]

Metabolites

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MIST guidelines

- Metabolites with exposure over 10% of parent must be identified, characterized and quantified in studies

Isobaric metabolites

- Failure to resolve may lead to overestimation of metabolite…may trigger MIST guidelines

*(ref: Leclerq et al, Chem. Res. Toxicol. **2009**, 22, 280-293)*

Thermally Labile Metabolites

- Conversion in source back to parent can lead to overestimation of parent.
- Must resolve susceptible metabolites (e.g.……acyl glucs, N-oxides, sulphates)

Challenge: to resolve metabolites for quantitative analysis while retaining productivity

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More Samples per Hour

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- Solutions
  - Fast short column HPLC
  - Direct sample analysis with large particles
  - On-line SPE without LC

ELSEVIER

Journal of Chromatography A, 928 (2001) 41–51

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Ultra-short columns and ballistic gradients: considerations for ultra-fast chromatographic liquid chromatographic–tandem mass spectrometric analysis

Leslie A. Romanyshyn*, Philip R. Tiller

Drug Metabolism, Merck Research Laboratories, RY80-D100, P.O. Box 2000, Rahway, NJ 07065, USA

Received 19 February 2001; received in revised form 5 July 2001; accepted 11 July 2001

- These all have issues with matrix resolution, metabolite co-elution and ion suppression.

- Sub 2µm particle LC allows for rapid analysis with improved resolution and greater sensitivity

ELSEVIER

Available online at www.sciencedirect.com

SCIENCE DIRECT®

Journal of Pharmaceutical and Biomedical Analysis 40 (2006) 689–706

JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS

www.elsevier.com/locate/jpba

Orthogonal extraction/chromatography and UPLC, two powerful new techniques for bioanalytical quantitation of desloratadine and 3-hydroxydesloratadine at 25 pg/mL

Jim X. Shen, Haiping Wang[1], Samy Tadros, Roger N. Hayes*

Schering-Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, NJ 07033-1300, USA

Received 27 April 2005; received in revised form 15 June 2005; accepted 16 June 2005
Available online 10 August 2005

The Promise of Small Particles
van Deemter Curves (<500 MW)

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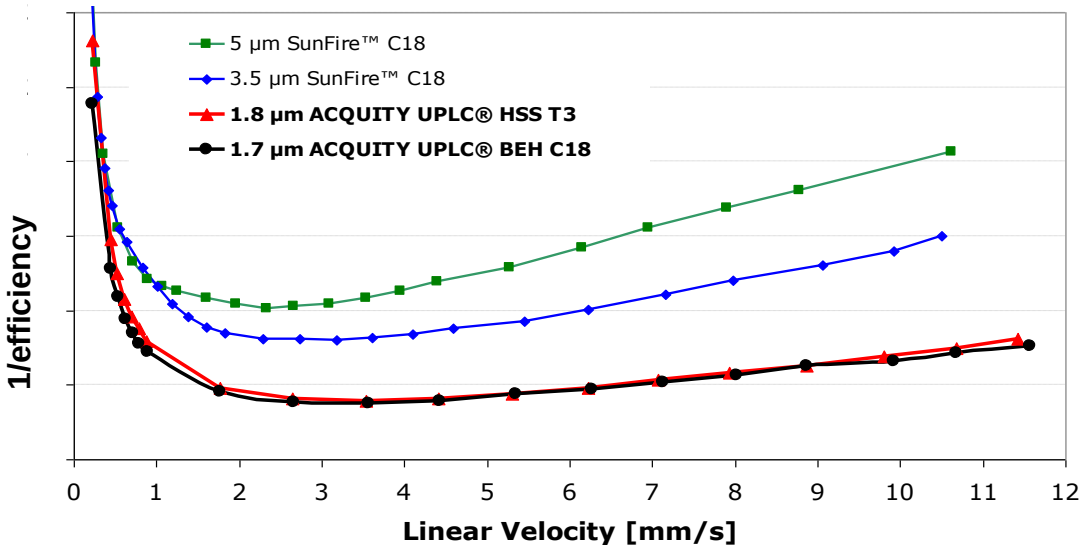

Flow rate (mL/min)												
1.0 mm ID	0.04	0.07	**0.11**	**0.14**	**0.18**	**0.21**	**0.25**	**0.28**	**0.32**	**0.35**	**0.39**	**0.42**
2.1 mm ID	0.15	0.30	**0.45**	**0.60**	**0.75**	**0.90**	**1.05**	**1.20**	**1.35**	**1.50**	**1.65**	**1.80**
4.6 mm ID	0.70	1.40	2.10	2.80	3.50	4.20	4.90	5.60	6.30	7.00	7.70	8.40

Why small particles?

Increased resolution with shorter columns

Flatter Van Deemter Curve at higher linear velocities, means less compromise for faster runs

With larger particle size like 5µm and 3.5µm, the choice must be made between speed and efficiency:

when the linear velocity increases,
the efficiency decreases,
hence the resolving power of the column decreases

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Solving Bioanalytical Challenges with UPLC® Technology:
Resolution – a customer example

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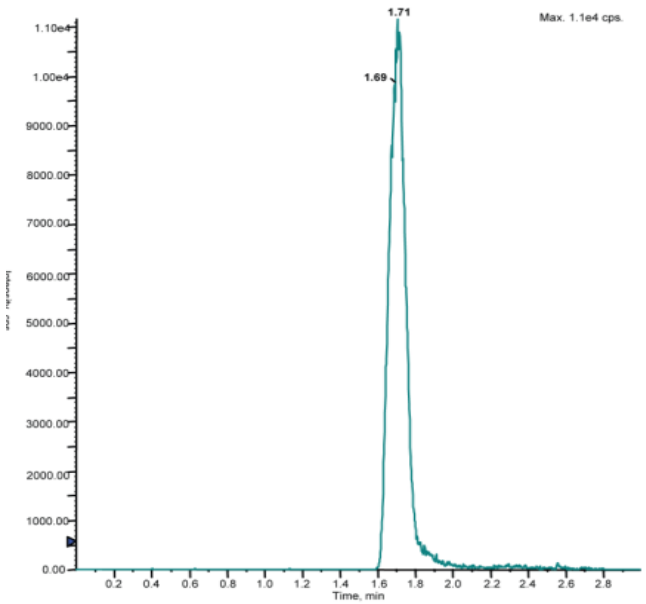
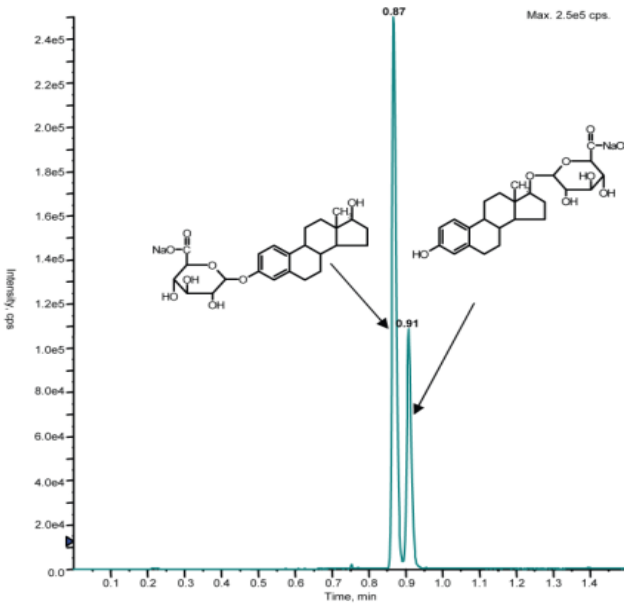
Resolution of Isobaric Compounds



HPLC resolution of Estradiol-3-Glucuronide and Estradiol-17-Glucuronide



UPLC resolution of Estradiol-3-Glucuronide and Estradiol-17-Glucuronide

Ref: Donegan M,BMS, www.cosmoscience.org/archive_2008.htm

Separation of metabolite critical pairs is essential for accurate quantitation and is becoming more important in order to satisfy regulatory expectations (MIST)

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Comparison of HPLC and UPLC sensitivity for Bioanalysis of Propranolol

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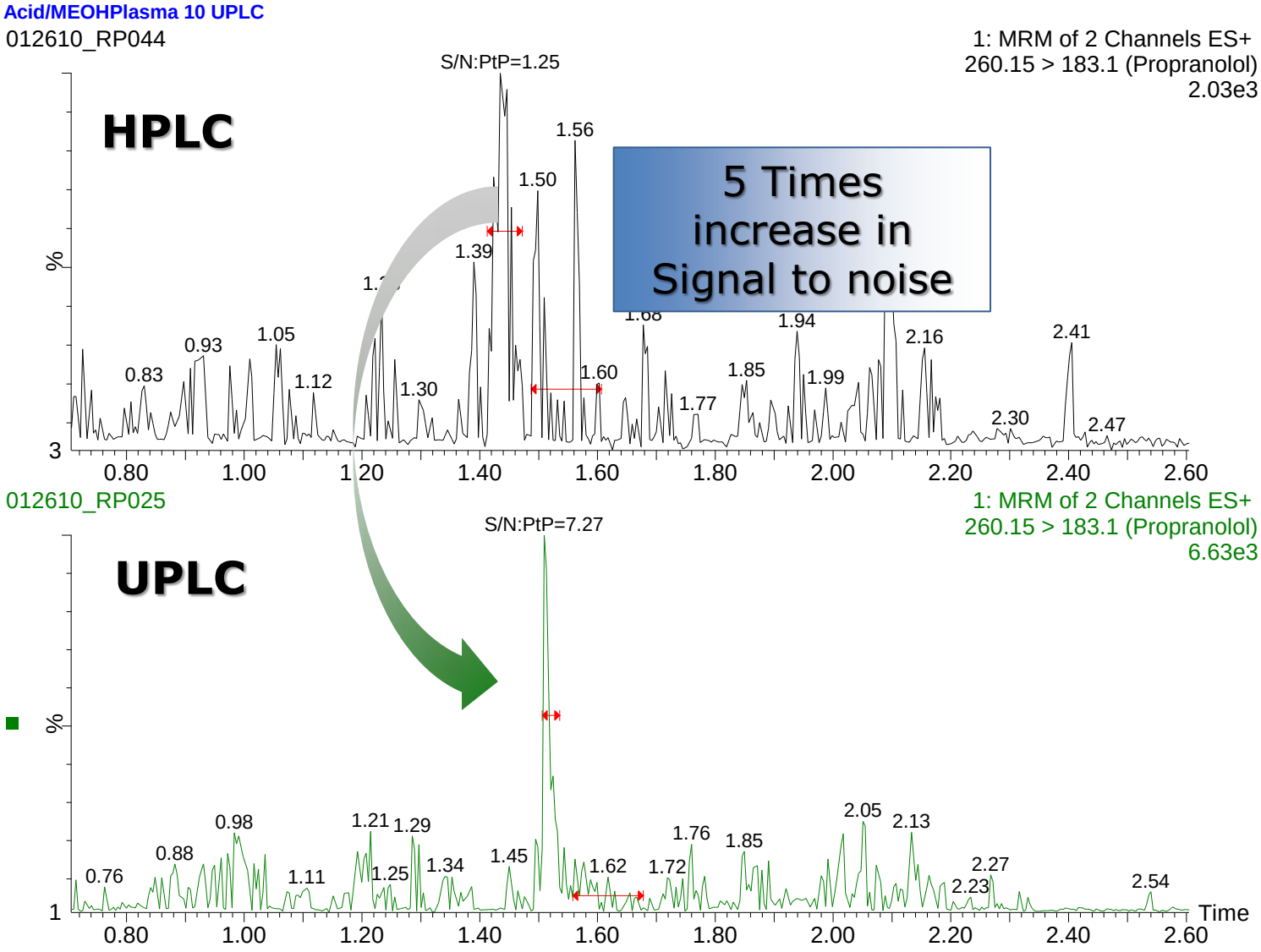

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[Metabolites Detection and Quantification]

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Challenges and Issues in Metabolite Detection

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- **[Confirmation of Metabolites]**
  - Acquiring spectra in time frame of UPLC peak
- **[Detecting metabolites]**
  - Using MRM approach to detect metabolites
  - iMRM method generator
- **[Quantification of Metabolites]**
  - Using RF factors

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Detecting Metabolites and Quantification

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- Detecting metabolites and confirming structure requires:-
  - Specificity for detection
  - Spectral data for confirmation
  - Chromatographic resolution of metabolites

- Options
  - Accurate mass full scan LC/MS(MS)
    - Best full scan data sensitivity
  - Tandem Quadrupole LC/MS/MS
    - Best detection sensitivity

 Imperial College 2010

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MS Solution for High Sensitivity Metabolite Detection and Quantification

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- MRM Detection
  - Optimal sensitivity
  - Needs method development
- Metabolite Confirmation
  - Full scan MS data required
  - MS Scan sensitivity critical
  - Ability to acquire scan data during MRM acquisition

Xevo TQ-S

Ultra Sensitive MS Detection

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New Step-Wave Optics Give Improved Ion Transfer Efficiency

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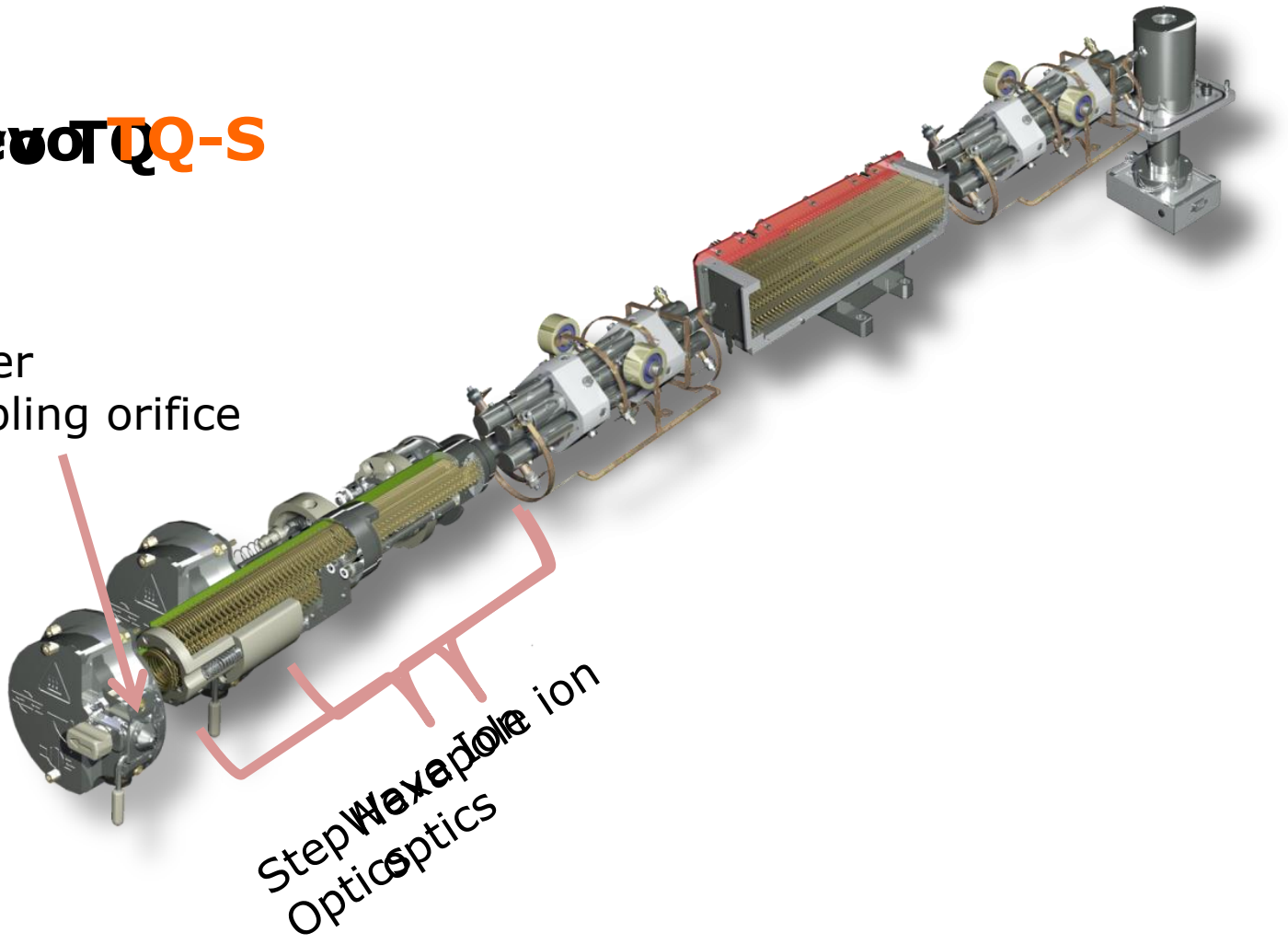
XevoTQ-S



Off Axis Design Eliminates Neutral Contamination

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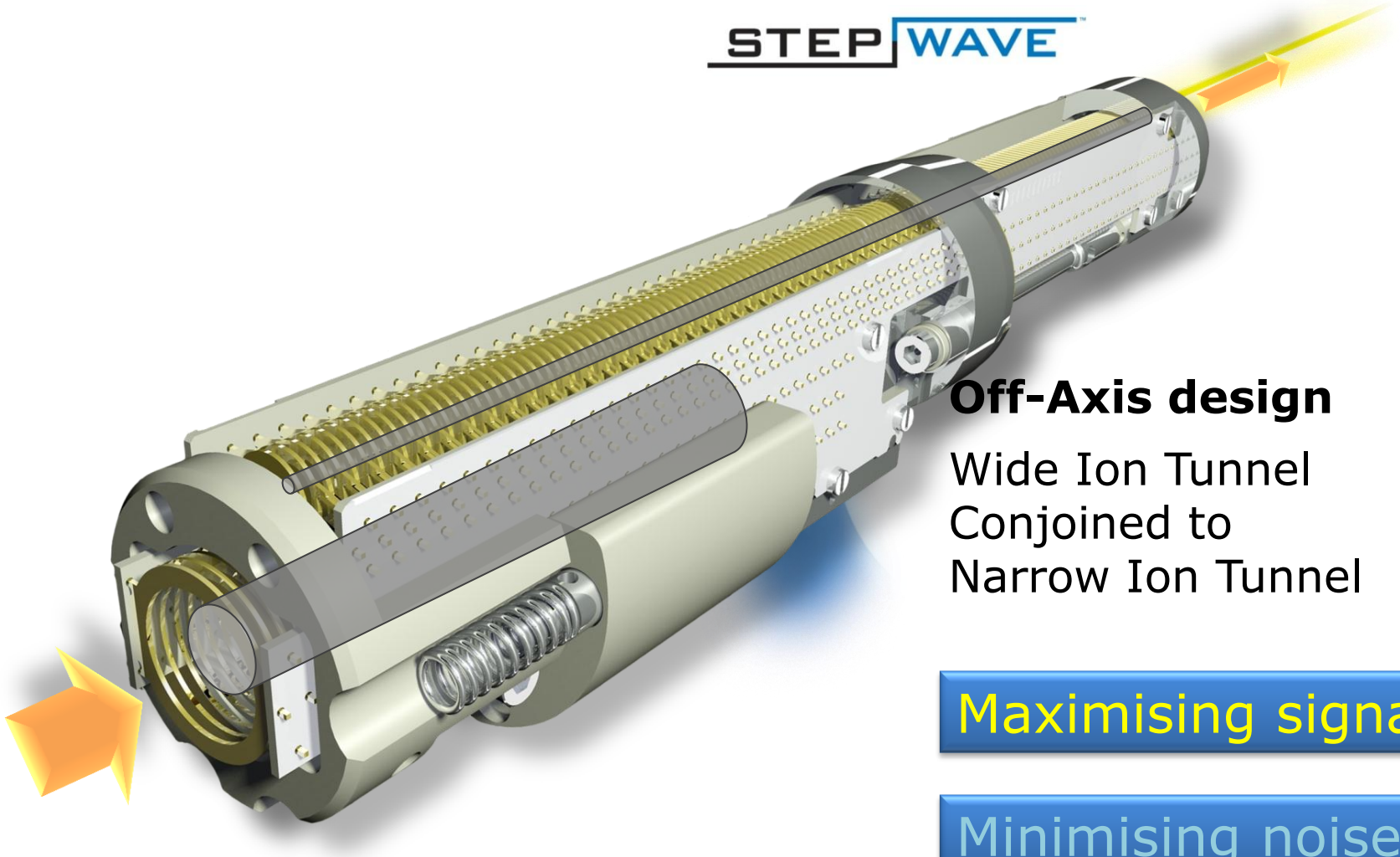

Off-Axis design

Wide Ion Tunnel
Conjoined to
Narrow Ion Tunnel

Maximising signal

Minimising noise

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Extracted Plasma Curve Desmopressin 1-10000pg/mL (Xevo TQ-S)

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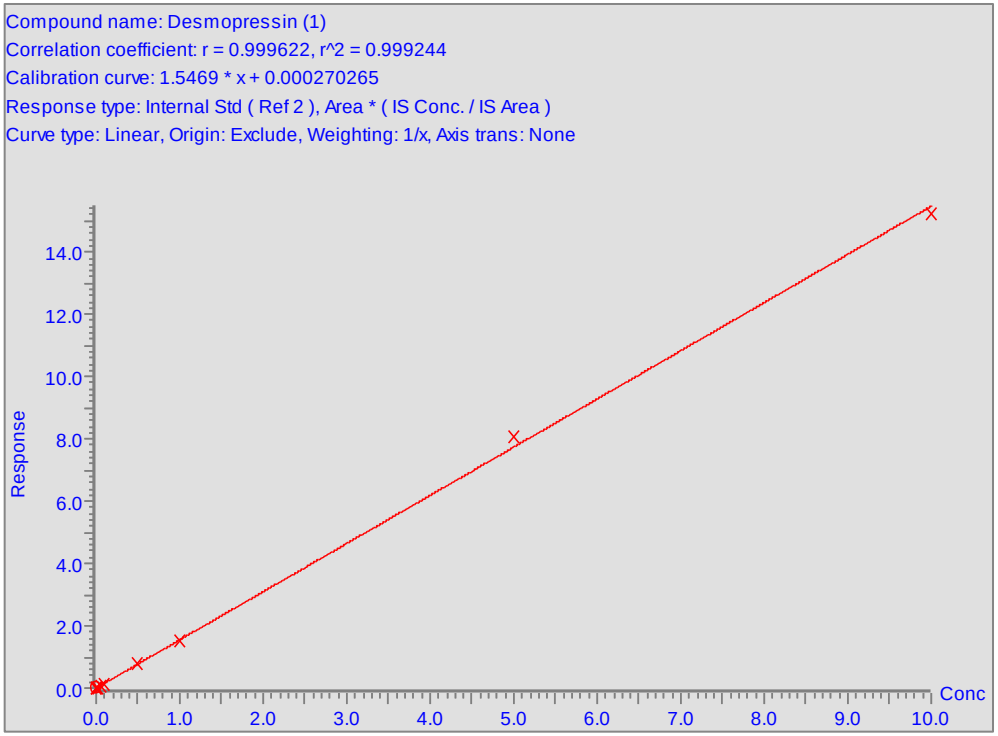

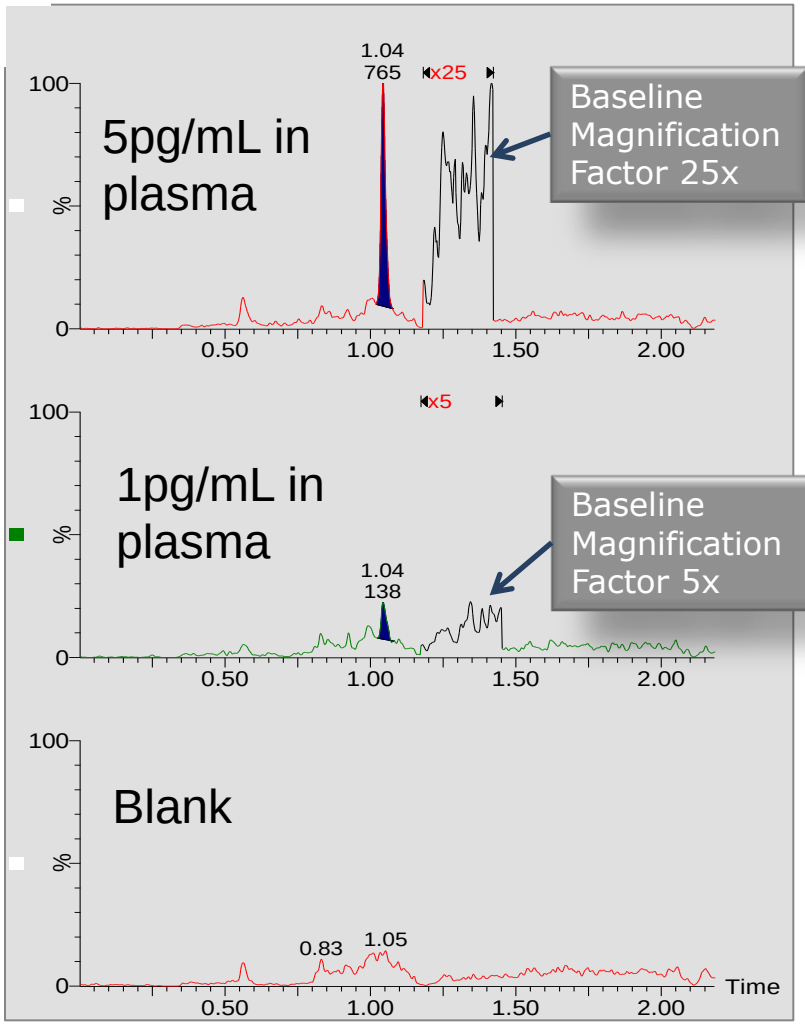

Assay Robustness

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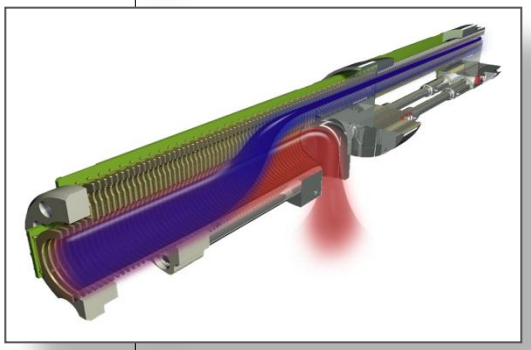
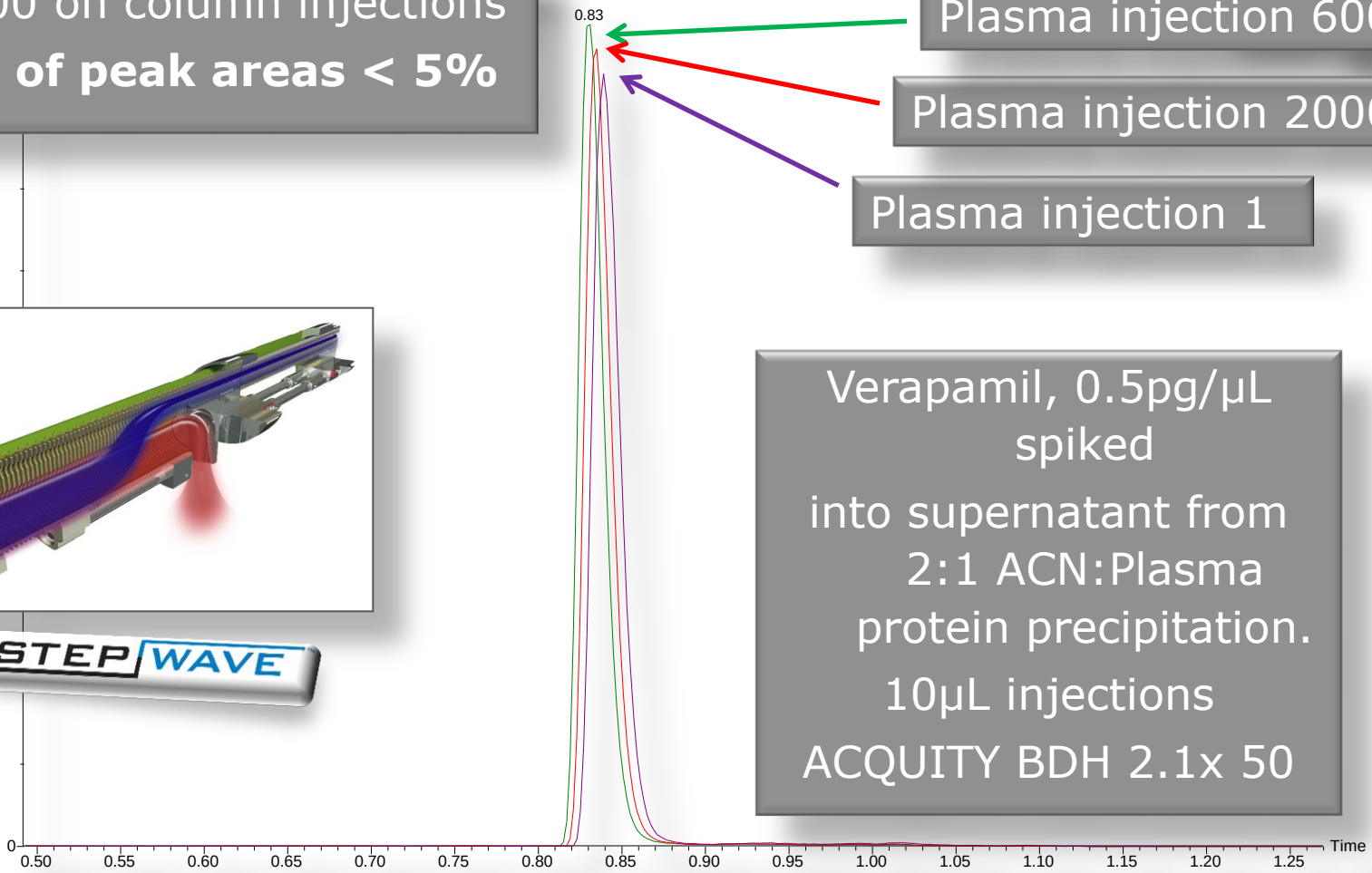
>6000 on column injections

RSD of peak areas < 5%



STEP WAVE



Verapamil, 0.5pg/µL spiked

into supernatant from 2:1 ACN:Plasma protein precipitation.

10µL injections

ACQUITY BDH 2.1x 50

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Xevo TQ-S
Xevo TQ-S
Information rich qualitative supporting data
RADAR
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Rapid MS to MRM Switching

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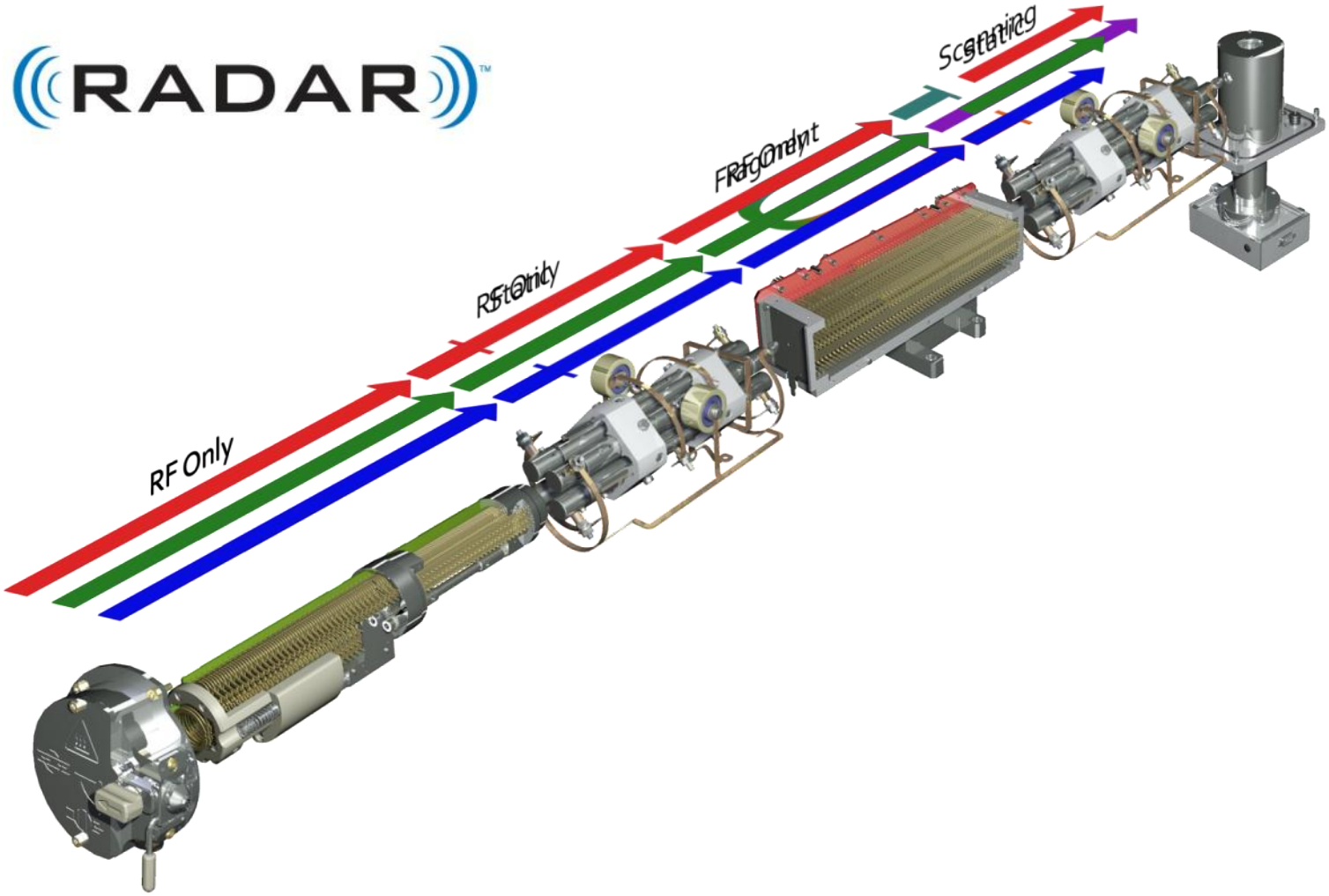

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Simultaneous MRM and Full Scan Detection

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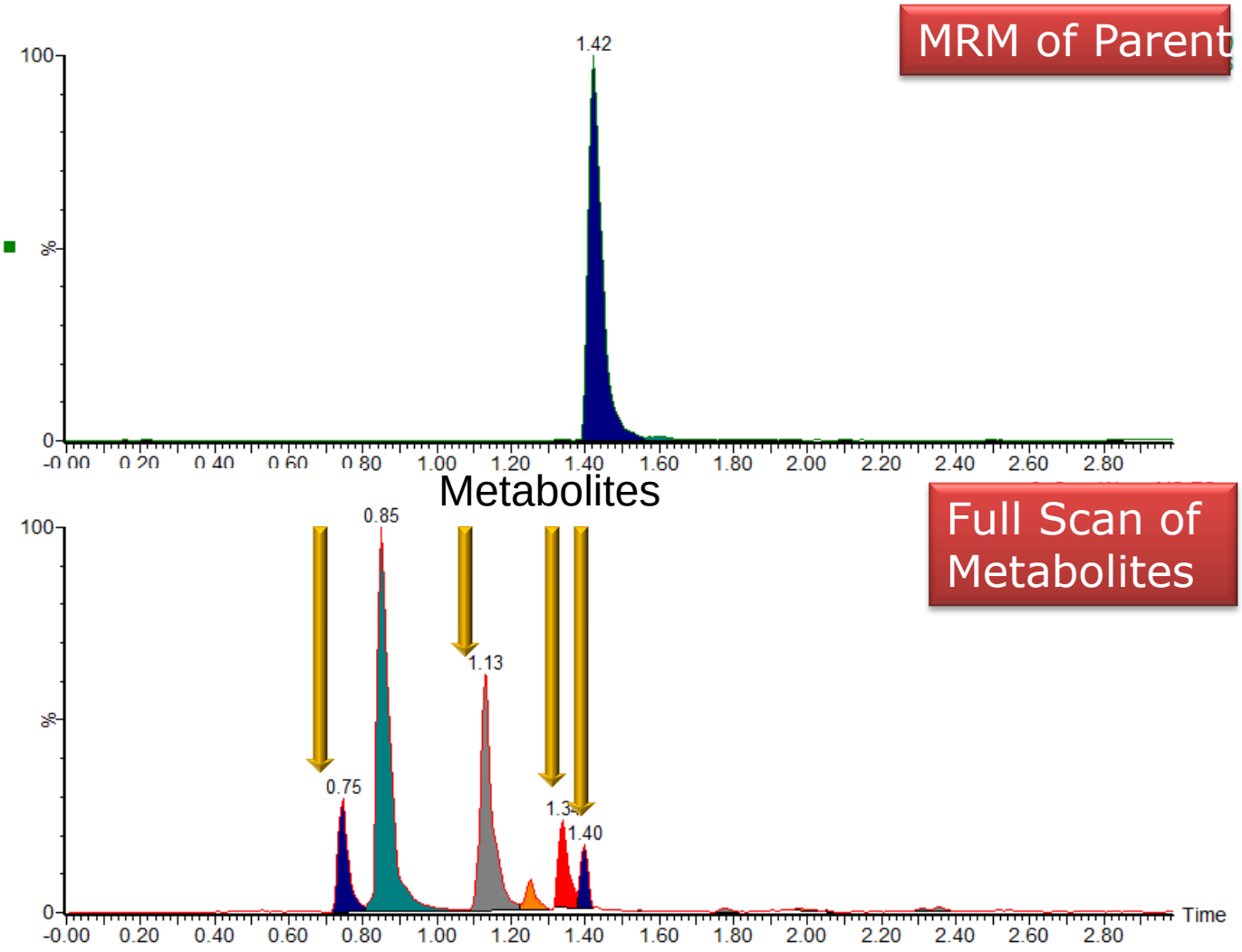

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Xevo TQ-S
Information rich qualitative supporting data
ScanWave
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Standard Product Ion Scanning

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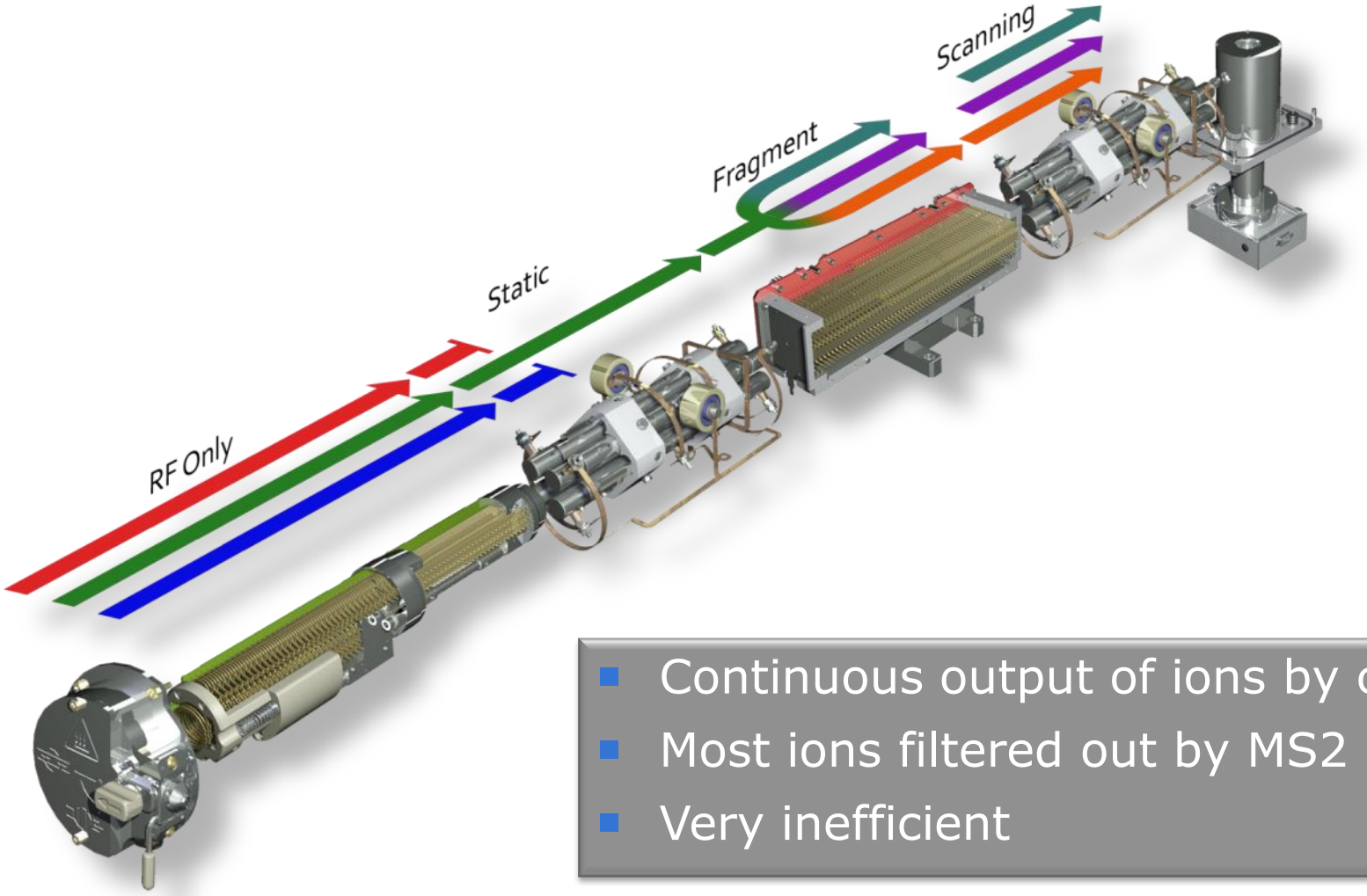

- Continuous output of ions by cell
- Most ions filtered out by MS2
- Very inefficient

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ScanWave
Enhanced Product Ion Scanning

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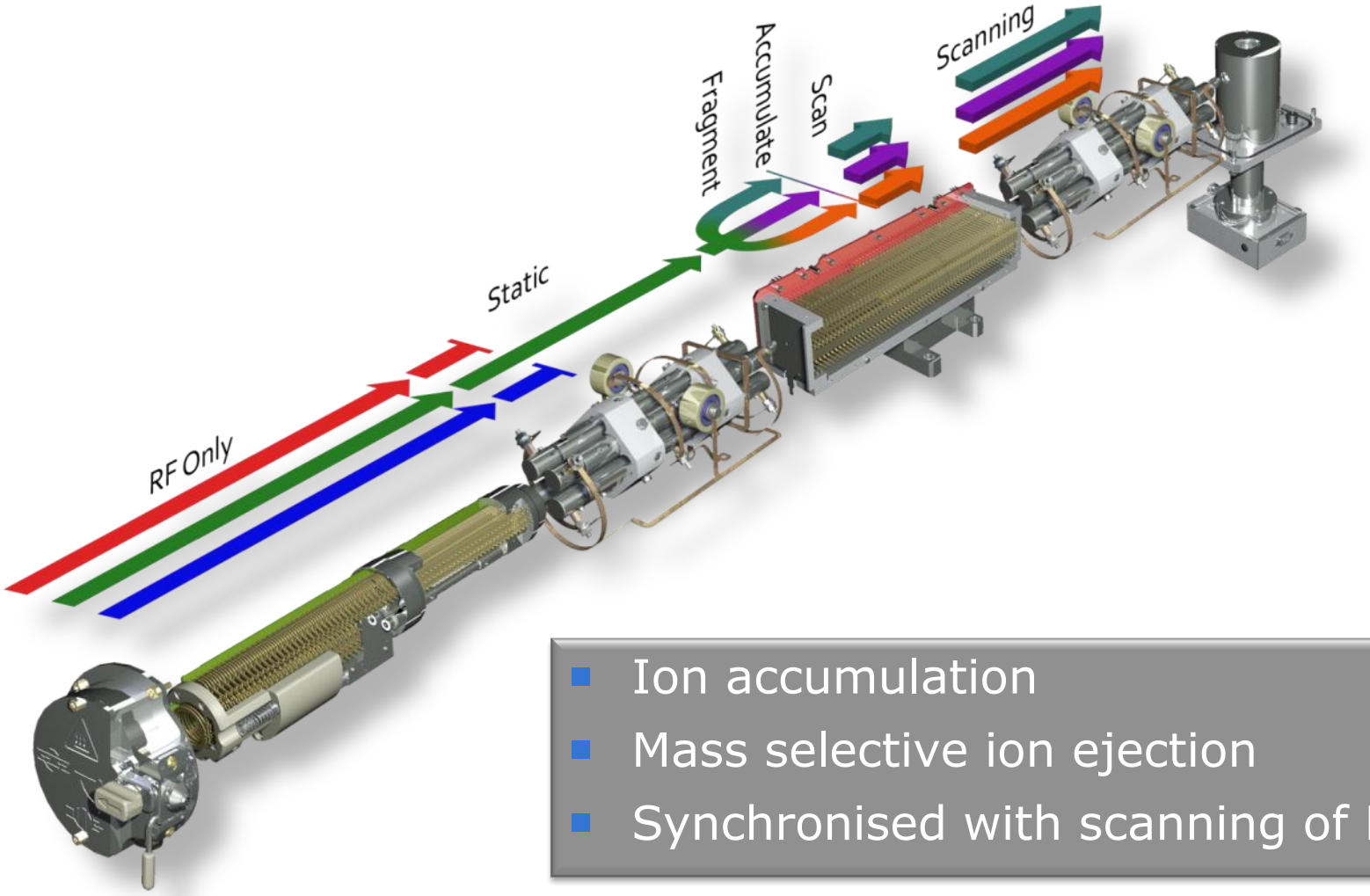

- Ion accumulation
- Mass selective ion ejection
- Synchronised with scanning of MS2

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StepWave Combined with ScanWave

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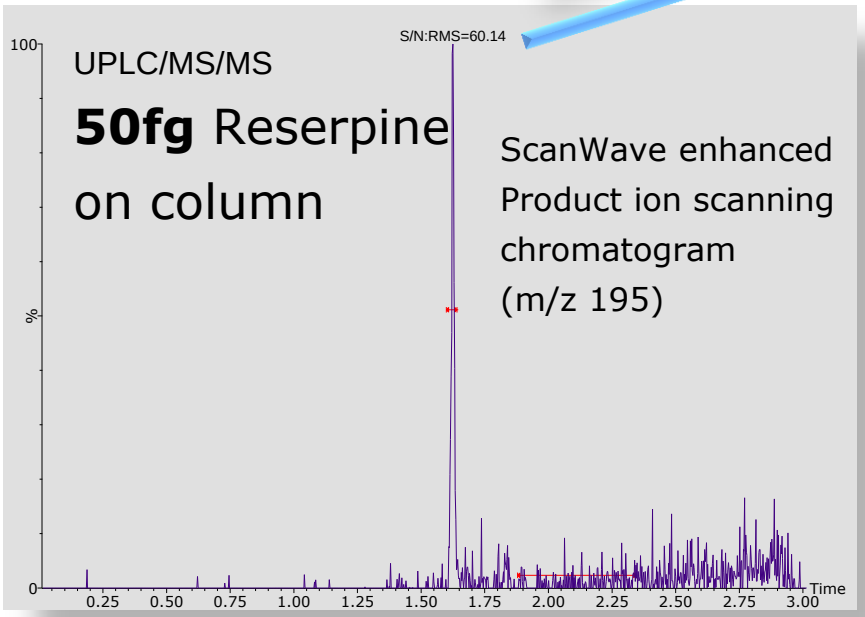

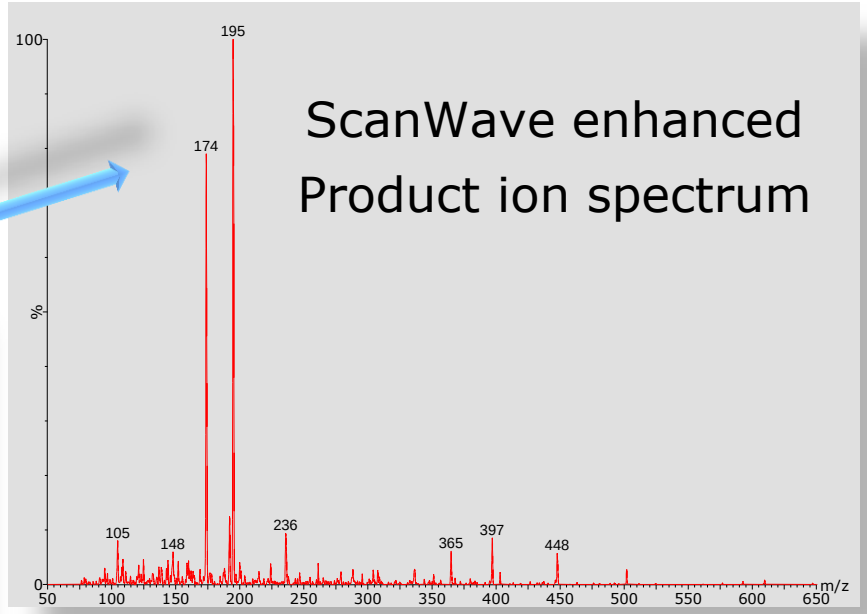

A combination providing leading-edge spectral MS/MS sensitivity

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Rapid Mode Switching
Precursor Ion Confirmation Scan

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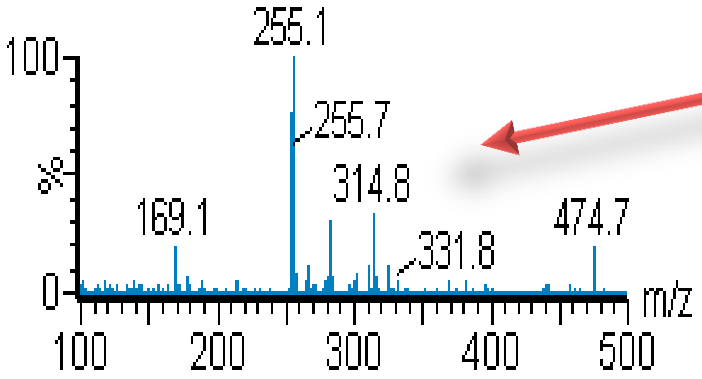
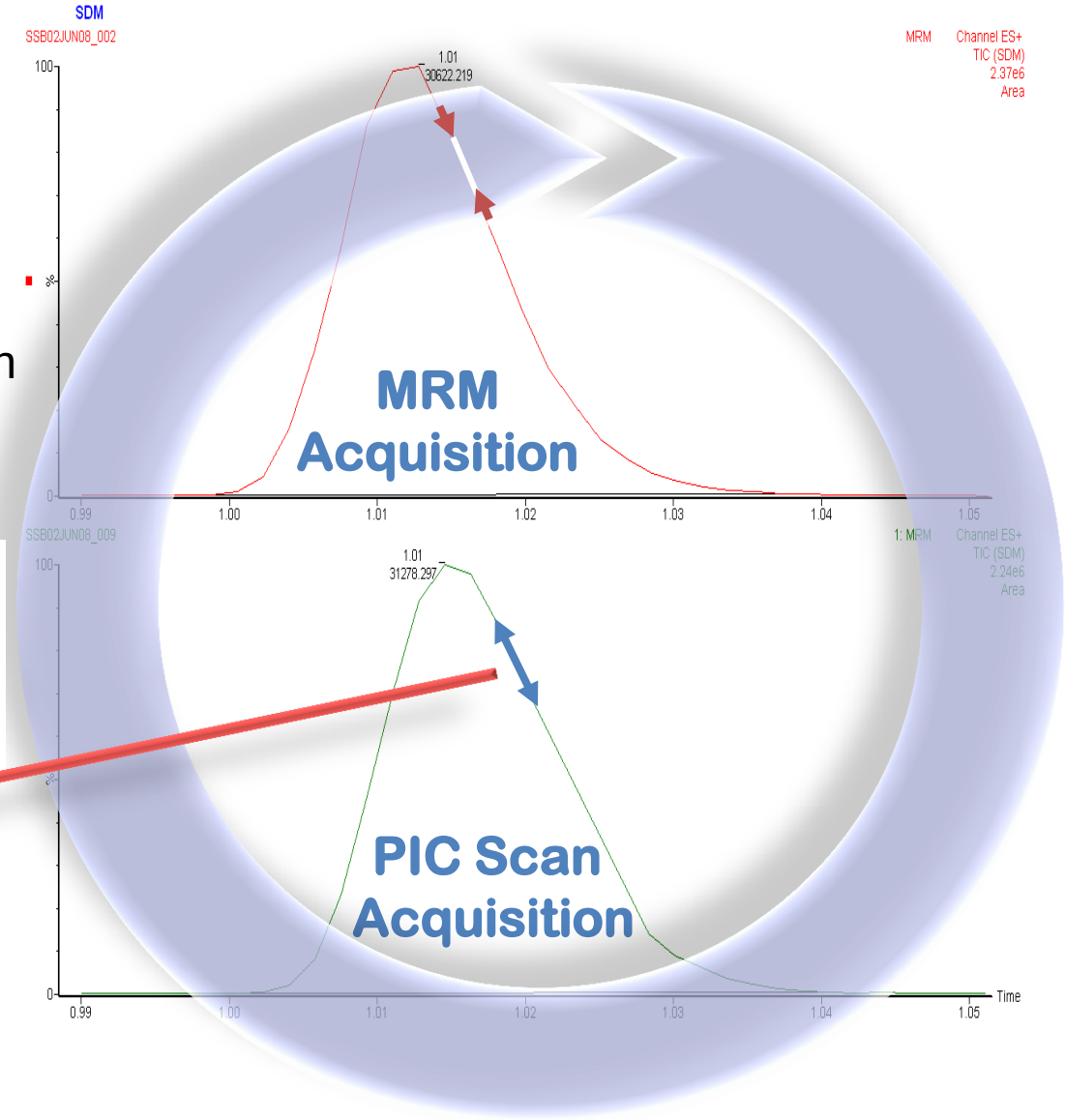
PIC SCAN:

MRM data is used as a specific trigger for the acquisition of a ScanWave enhanced product ion spectrum.





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An Alternate Approach to Full Scan Profiling of Metabolites

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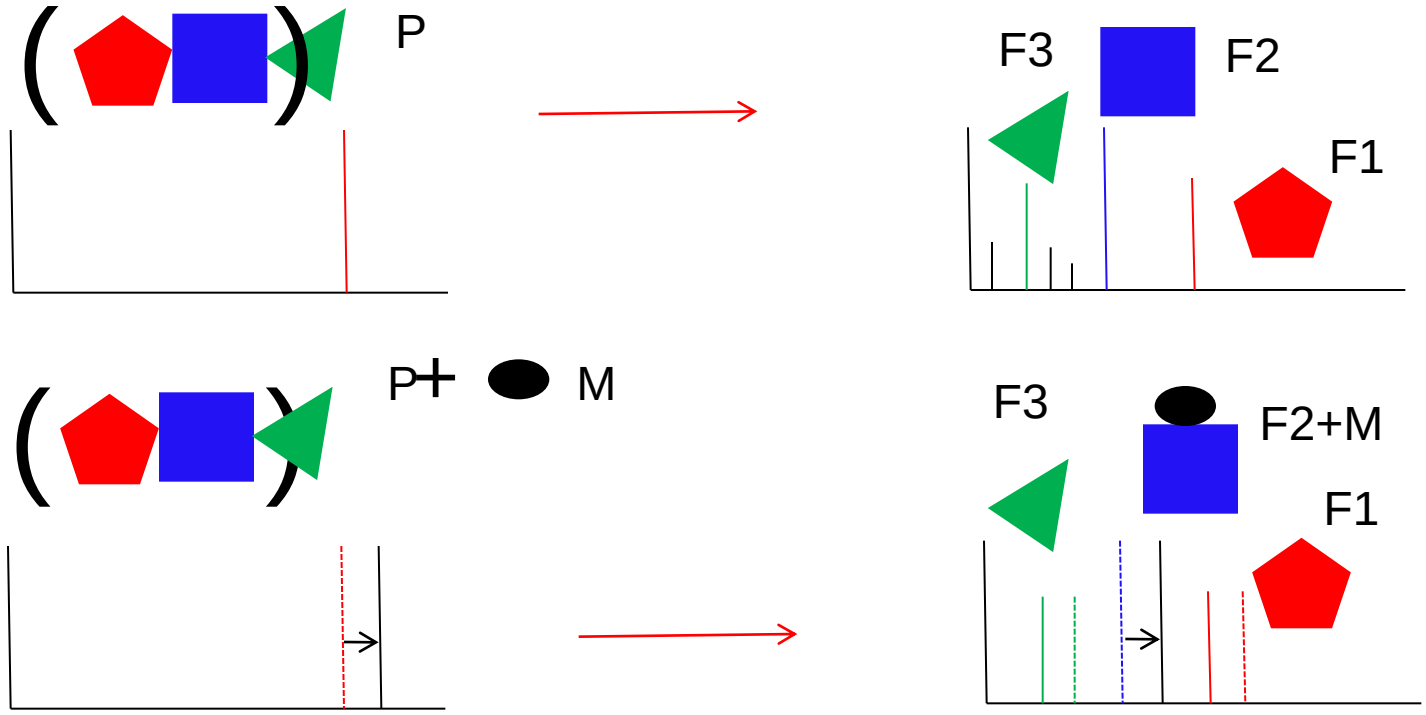
- Screen for selected panel of metabolites
  - Commonly reported metabolites
  - User definable
- Predicts metabolite MRMs based upon Parent Compound MSMS
- Generates MANY MRMs
  - 1 msec dwell
- With RADAR, full scan MS may be added to the experiment



iMRMs : P+M > F1
P+M > F1+M
P+M > F2
P+M > F2+M
P+M > F3
P+M > F3+M

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Develop Theoretical MRMs to Investigate

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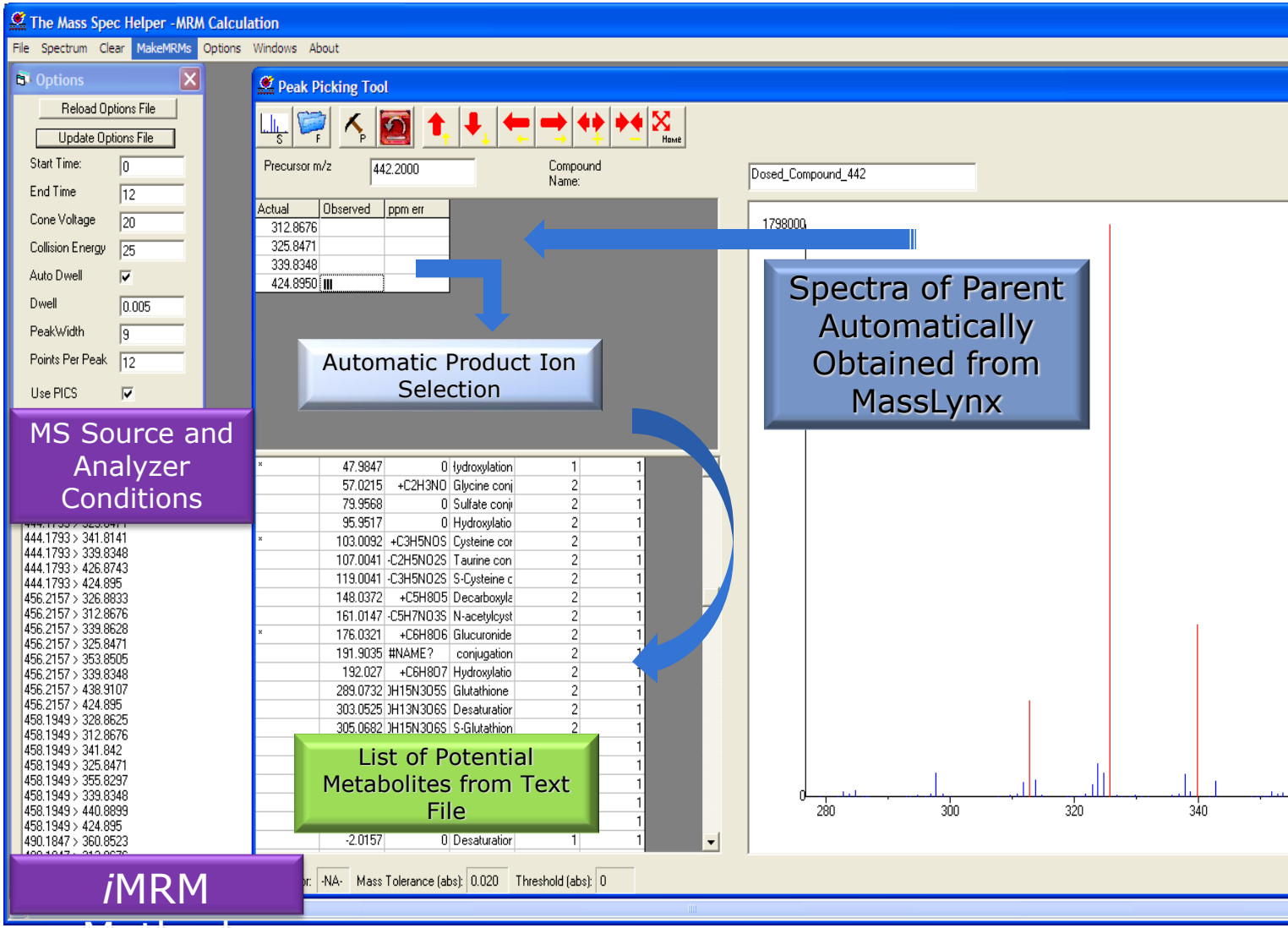
MSMS of Parent Compound

Identify Product Ions From Parent Compound

Select Metabolites from list of probable transformations

Calculate MRMs and Write MS Method File



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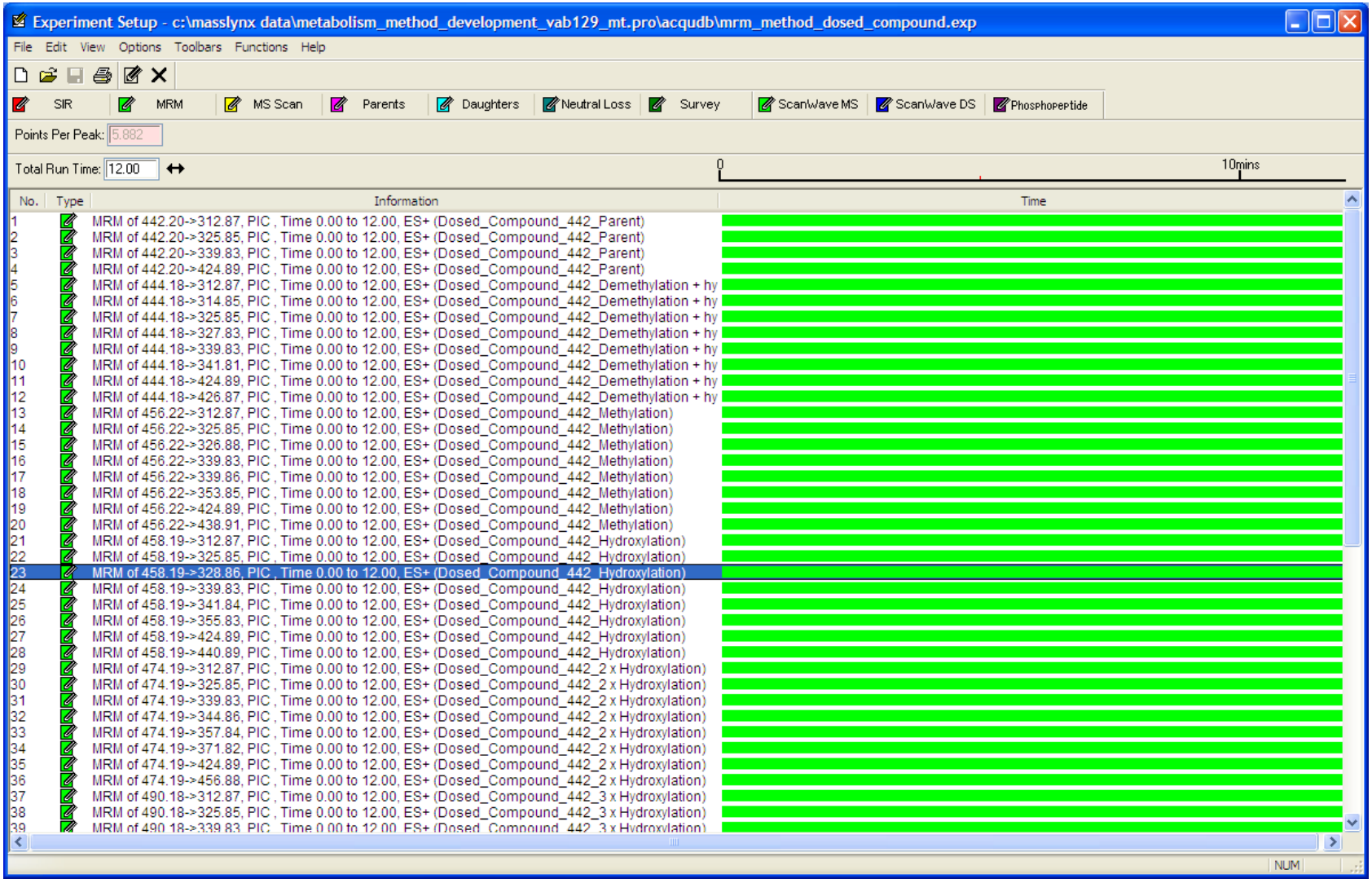
iMRM method


Experiment Setup - c:\masslynx data\metabolism_method_development_vab129_mt.pro\acqudb\mrm_method_dosed_compound.exp
File Edit View Options Toolbars Functions Help
SIR MRM MS Scan Parents Daughters Neutral Loss Survey ScanWave MS ScanWave DS Phosphopeptide
Points Per Peak: 5.882
Total Run Time: 12.00
0
10mins
No. Type Information Time
1 MRM of 442.20->312.87, PIC , Time 0.00 to 12.00, ES+ (Dosed_Compound_442_Parent)
2 MRM of 442.20->325.85, PIC , Time 0.00 to 12.00, ES+ (Dosed_Compound_442_Parent)
3 MRM of 442.20->339.83, PIC , Time 0.00 to 12.00, ES+ (Dosed_Compound_442_Parent)
4 MRM of 442.20->424.89, PIC , Time 0.00 to 12.00, ES+ (Dosed_Compound_442_Parent)
5 MRM of 444.18->312.87, PIC , Time 0.00 to 12.00, ES+ (Dosed_Compound_442_Demethylation + hy
6 MRM of 444.18->314.85, PIC , Time 0.00 to 12.00, ES+ (Dosed_Compound_442_Demethylation + hy
7 MRM of 444.18->325.85, PIC , Time 0.00 to 12.00, ES+ (Dosed_Compound_442_Demethylation + hy
8 MRM of 444.18->327.83, PIC , Time 0.00 to 12.00, ES+ (Dosed_Compound_442_Demethylation + hy
9 MRM of 444.18->339.83, PIC , Time 0.00 to 12.00, ES+ (Dosed_Compound_442_Demethylation + hy
10 MRM of 444.18->341.81, PIC , Time 0.00 to 12.00, ES+ (Dosed_Compound_442_Demethylation + hy
11 MRM of 444.18->424.89, PIC , Time 0.00 to 12.00, ES+ (Dosed_Compound_442_Demethylation + hy
12 MRM of 444.18->426.87, PIC , Time 0.00 to 12.00, ES+ (Dosed_Compound_442_Demethylation + hy
13 MRM of 456.22->312.87, PIC , Time 0.00 to 12.00, ES+ (Dosed_Compound_442_Methylation)
14 MRM of 456.22->325.85, PIC , Time 0.00 to 12.00, ES+ (Dosed_Compound_442_Methylation)
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23 MRM of 458.19->328.86, PIC , Time 0.00 to 12.00, ES+ (Dosed_Compound_442_Hydroxylation)
24 MRM of 458.19->339.83, PIC , Time 0.00 to 12.00, ES+ (Dosed_Compound_442_Hydroxylation)
25 MRM of 458.19->341.84, PIC , Time 0.00 to 12.00, ES+ (Dosed_Compound_442_Hydroxylation)
26 MRM of 458.19->355.83, PIC , Time 0.00 to 12.00, ES+ (Dosed_Compound_442_Hydroxylation)
27 MRM of 458.19->424.89, PIC , Time 0.00 to 12.00, ES+ (Dosed_Compound_442_Hydroxylation)
28 MRM of 458.19->440.89, PIC , Time 0.00 to 12.00, ES+ (Dosed_Compound_442_Hydroxylation)
29 MRM of 474.19->312.87, PIC , Time 0.00 to 12.00, ES+ (Dosed_Compound_442_2 x Hydroxylation)
30 MRM of 474.19->325.85, PIC , Time 0.00 to 12.00, ES+ (Dosed_Compound_442_2 x Hydroxylation)
31 MRM of 474.19->339.83, PIC , Time 0.00 to 12.00, ES+ (Dosed_Compound_442_2 x Hydroxylation)
32 MRM of 474.19->344.86, PIC , Time 0.00 to 12.00, ES+ (Dosed_Compound_442_2 x Hydroxylation)
33 MRM of 474.19->357.84, PIC , Time 0.00 to 12.00, ES+ (Dosed_Compound_442_2 x Hydroxylation)
34 MRM of 474.19->371.82, PIC , Time 0.00 to 12.00, ES+ (Dosed_Compound_442_2 x Hydroxylation)
35 MRM of 474.19->424.89, PIC , Time 0.00 to 12.00, ES+ (Dosed_Compound_442_2 x Hydroxylation)
36 MRM of 474.19->456.88, PIC , Time 0.00 to 12.00, ES+ (Dosed_Compound_442_2 x Hydroxylation)
37 MRM of 490.18->312.87, PIC , Time 0.00 to 12.00, ES+ (Dosed_Compound_442_3 x Hydroxylation)
38 MRM of 490.18->325.85, PIC , Time 0.00 to 12.00, ES+ (Dosed_Compound_442_3 x Hydroxylation)
39 MRM of 490.18->339.83, PIC , Time 0.00 to 12.00, ES+ (Dosed_Compound_442_3 x Hydroxylation)
NUM

Comparison to Full Scan Analysis

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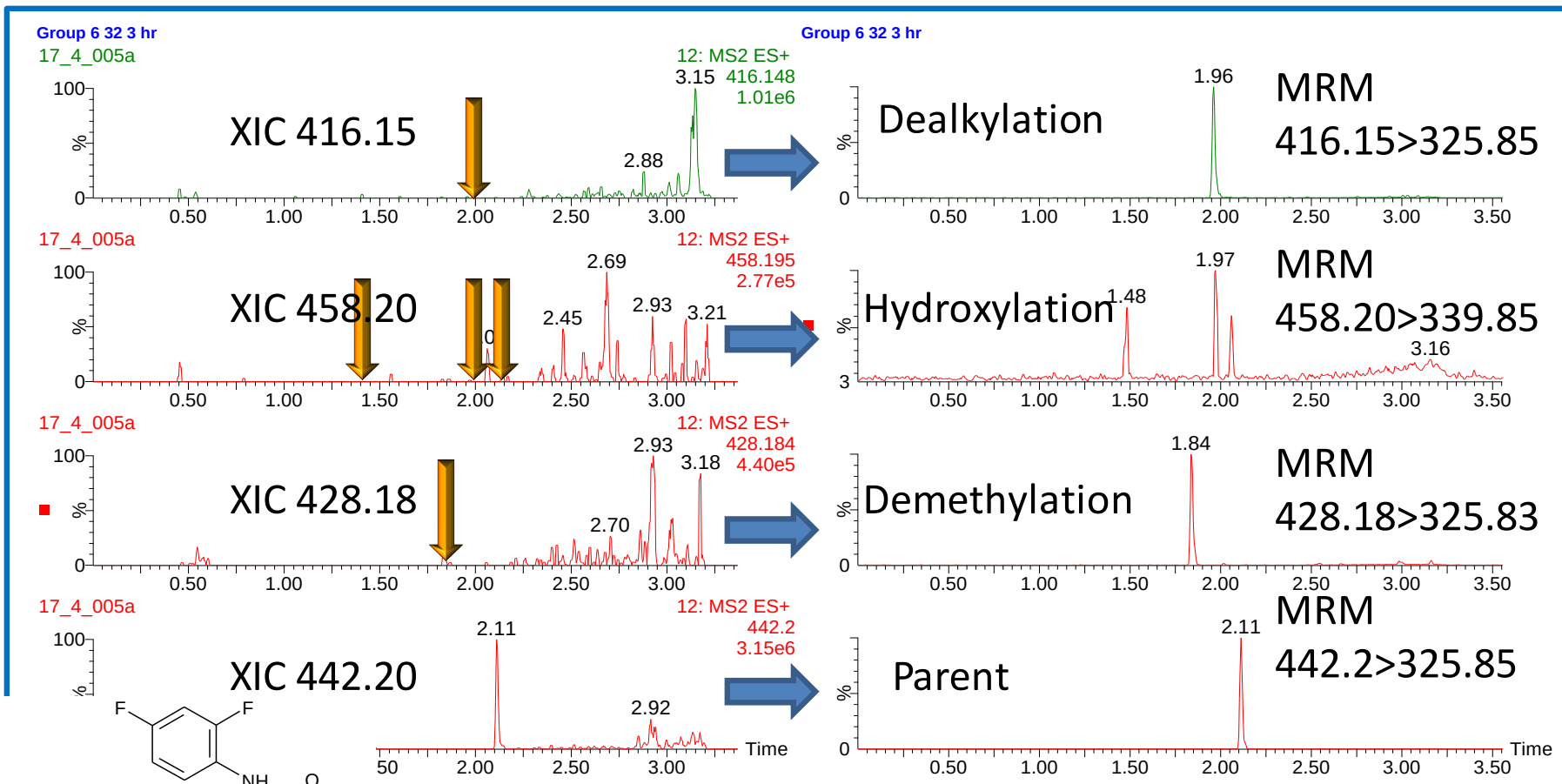
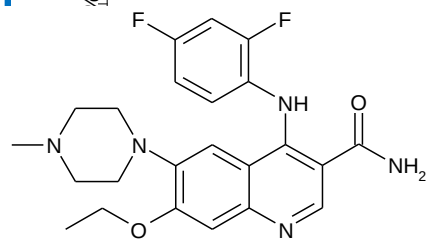
Full Scan Mode Data

MRM Mode Data





Courtesy of Prof Ian Wilson

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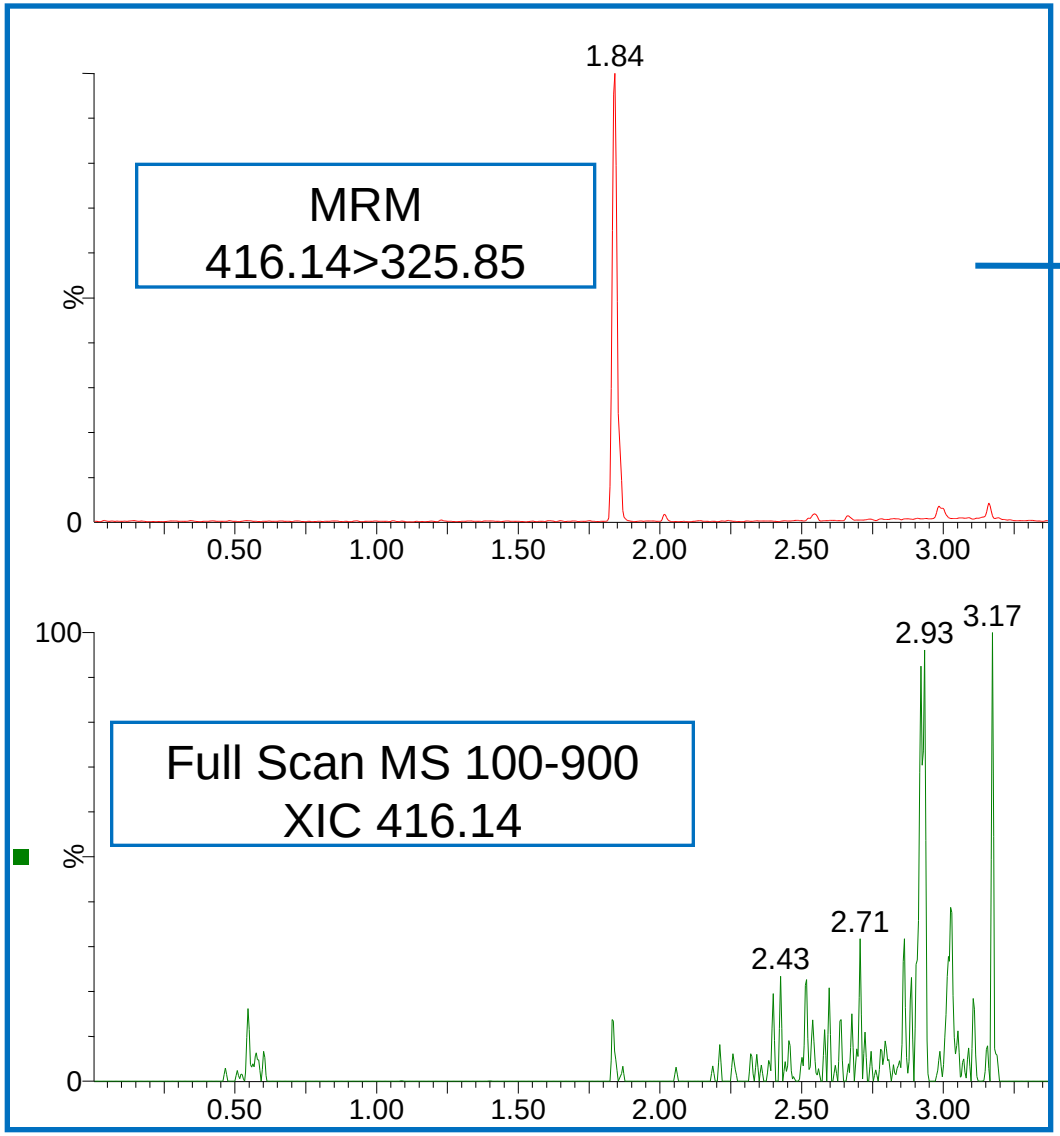
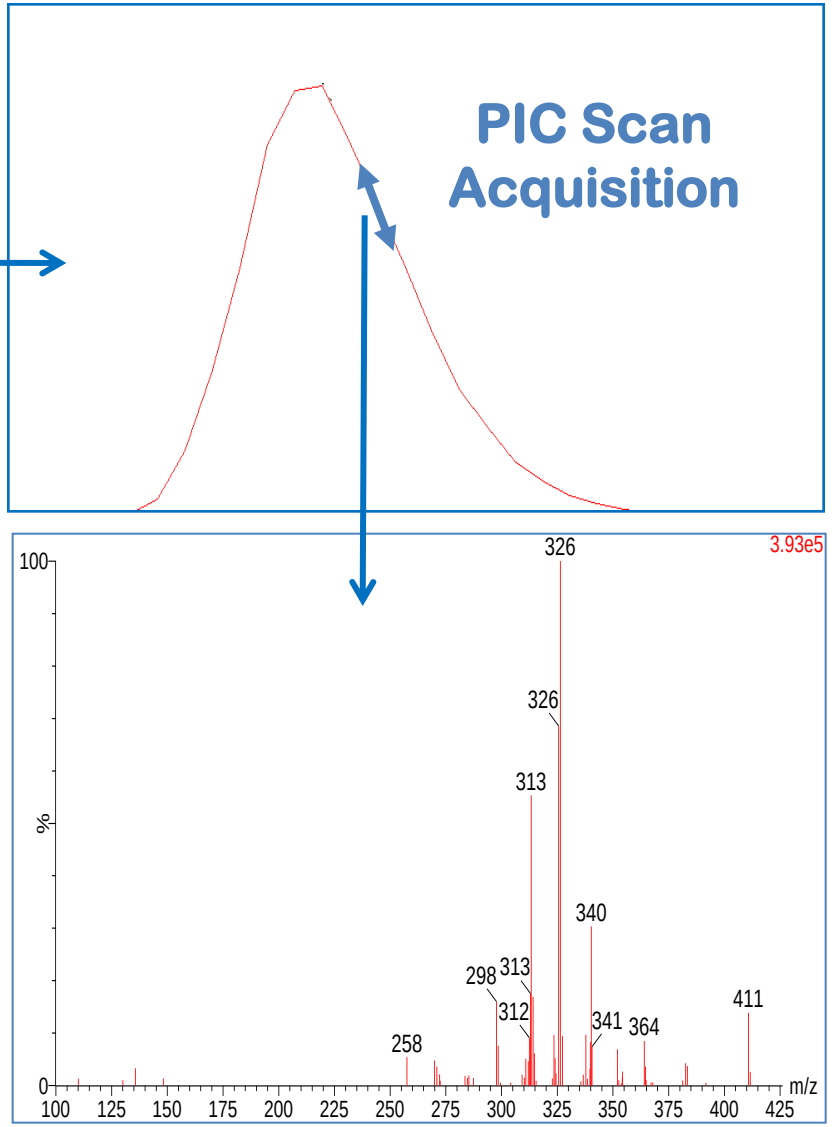
m/z 428 -Dealkylation





Simple Metabolite Detection and Confirmation

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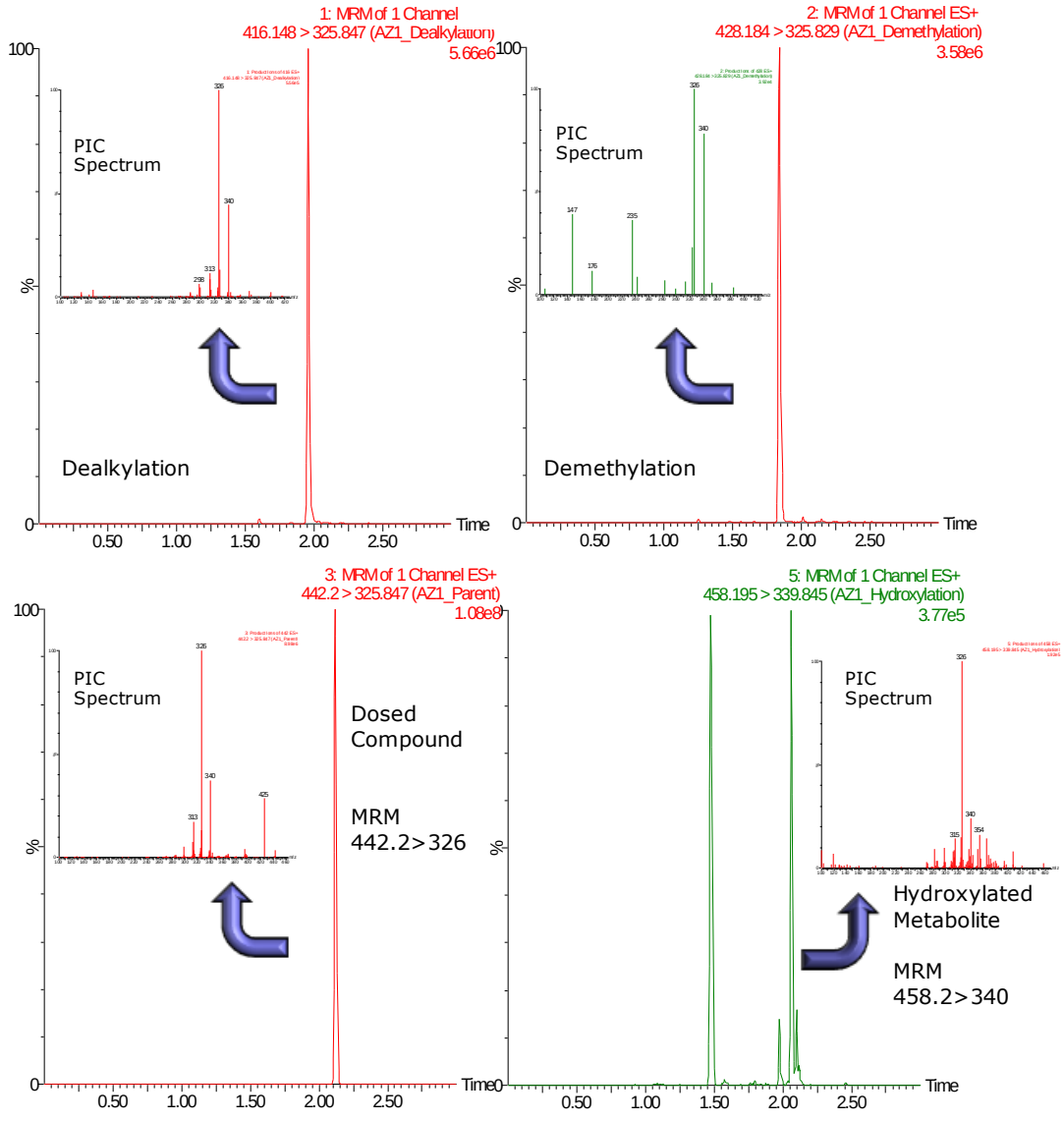

- [Benefits]
  - Metabolites detected with maximum sensitivity
  - Spectra acquired in the time scale of UPLC peak
  - Data acquired in one analytical run, no need for confirmatory experiment

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Quantification of Metabolites

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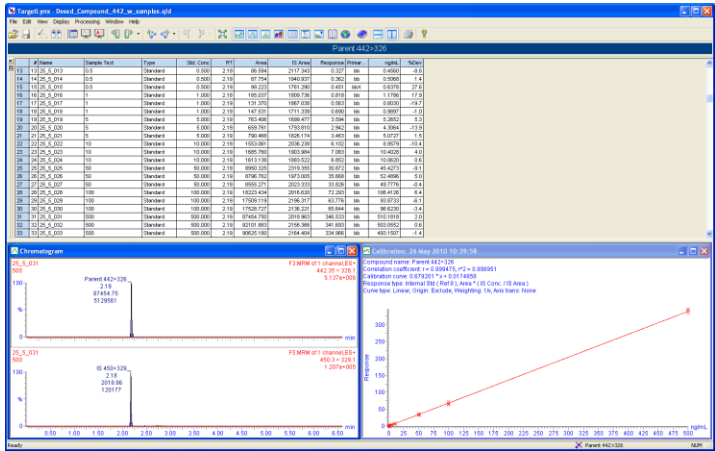
- Response factor can be created using a radio label dose and a comparison of the MS response

$$RF = \frac{MSStd\ \mathrm{Re}sponse}{MSMetabolite\ \mathrm{Re}sponse} \times \frac{Radiolabel\ _Metabolite}{RadioLabel\ _S\tan dard}$$

- Standard calibration line can be used to quantify metabolites.

- TargetLynx can do this automatically



Metabolic Stability Plots with TargetLynx data from MRMs

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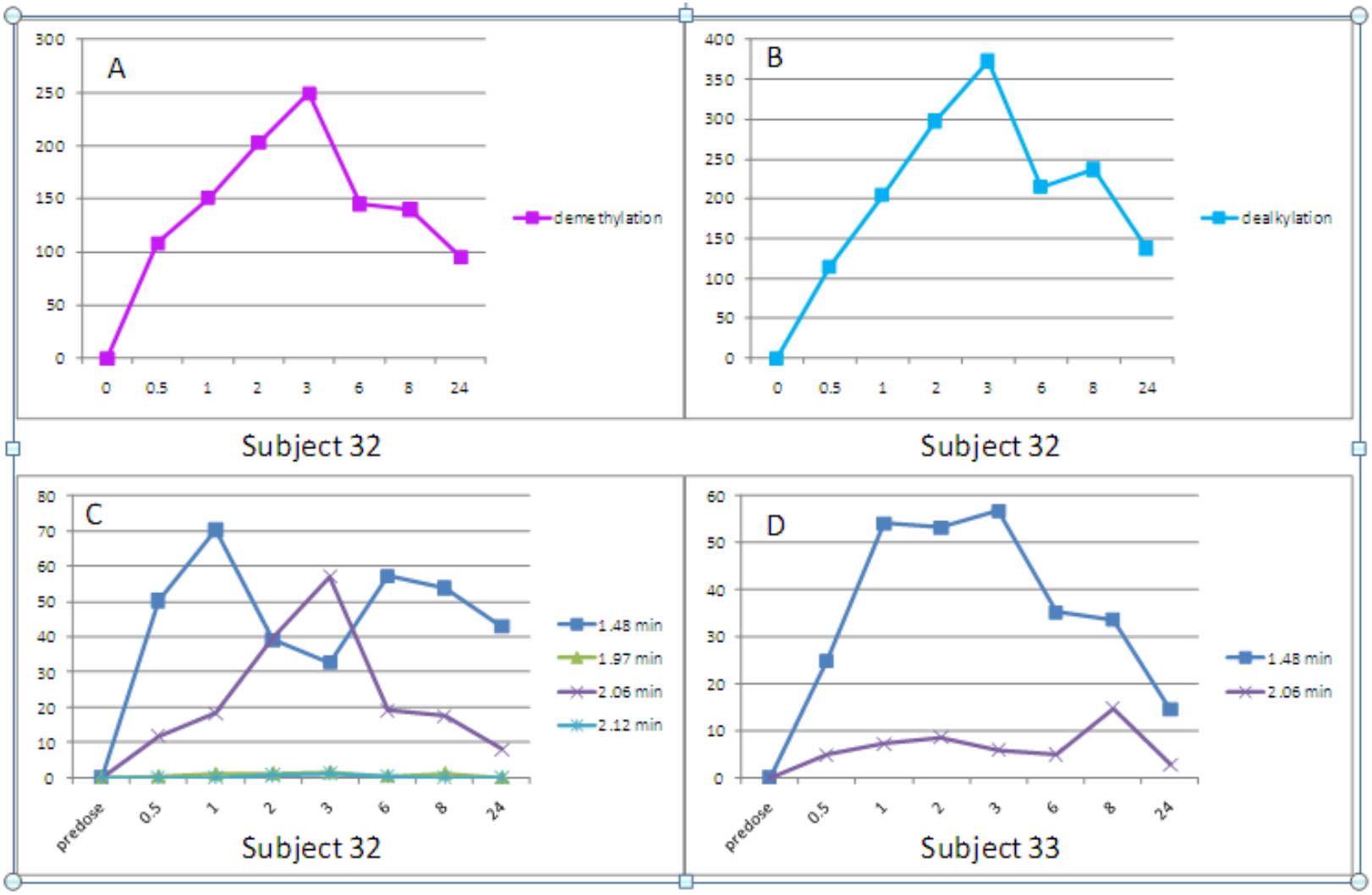

Figure Concentration versus time curves for [A] Demethylated metabolite; [B] Dealkylated metabolite; [C] Hydroxylated metabolites at various Rt in subject 32 and 33 [D]

Conclusions

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- Combination of Xevo TQ-S, OASIS SPE and UPLC provide unprecedented levels of productivity sensitivity and resolution.
- Software compliance, data management, processing and reporting are simplified with MassLynx and NuGenisis SDMS.
- Spectral sensitivity and PIC functionality of Xevo TQ(S) of allows simple metabolite detection and quantification.
- Peptide analysis simplified with Xevo TQ-S, OASIS SPE and ACQUITY PST columns.
- Sensitivity challenge of dried blood spots addressed with nanoACQUITY and Xevo TQ-S

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