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The Century Mix as QC for untargeted metabolomics using two-dimensional gas chromatography

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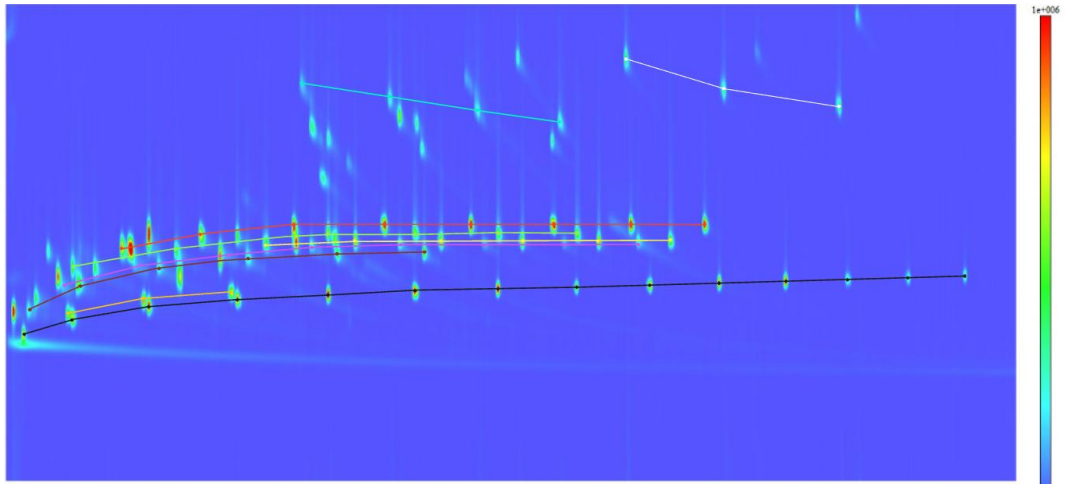
MDCW – February 4th, 2025 – Liege, Belgium

16TH Multidimensional Chromatography Workshop

A Novel QC Standard for Untargeted Metabolomics

- **Untargeted metabolomics** presents a **challenge** of navigating complex chemical data
- The **Century Mix** leverages the precision of **two-dimensional gas chromatography** to bring **clarity** and **reproducibility** to these analyses
- Discover how this innovation could be implementing in **quality control in metabolomics**



Introduction: QA/QC

- Quality assurance (QA) and quality control (QC) are two aspects of quality management
- QA: “part of quality management focused on providing confidence that quality requirements will be fulfilled”
- QC: “part of quality management focused on fulfilling quality requirements”
- Well known in industry and for targeted analysis
- **What about untargeted analysis and metabolomics?**

Quality System, Quality Assurance, and Quality Control Relationships
ISO 9000 – Quality management

Imler, K., 2006, *Quality Progress*, 39(6), 57.

Introduction: QA/QC

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Journal of Chromatography A, 1019 (2003) 261–272

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Standardized test mixture for the characterization of comprehensive two-dimensional gas chromatography columns: the Phillips mix

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[d] *Centers for Disease Control and Prevention, 4770 Buford Highway NE, Atlanta, GA 30041, USA*
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Metabolomics (2022) 18: 70
https://doi.org/10.1007/s11306-022-01926-3

REVIEW ARTICLE

Check for updates

Quality assurance and quality control reporting in untargeted metabolic phenotyping: mQACC recommendations for analytical quality management

Jennifer A. Kirwan[1,2,3] · Helen Gika[4,5] · Richard D. Beger[6] · Dan Bearden[7] · Warwick B. Dunn[8] · Royston Goodacre[8] · Georgios Theodoridis[9,5] · Michael Witting[10] · Li-Rong Yu[6] · Ian D. Wilson[8,11] on behalf of the metabolomics Quality Assurance and Quality Control Consortium (mQACC)

analytical chemistry

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Article

A Proposed Quality Control Standard Mixture and Its Uses for Evaluating Nontargeted and Suspect Screening LC/HR-MS Method Performance

Ann M. Knolhoff,*[,†] Jacob H. Premo,[†] and Christine M. Fisher

Cite This: *Anal. Chem.* 2021, 93, 1596–1603

Read Online

Metabolomics (2017) 13:50
DOI 10.1007/s11306-017-1188-9

CrossMark

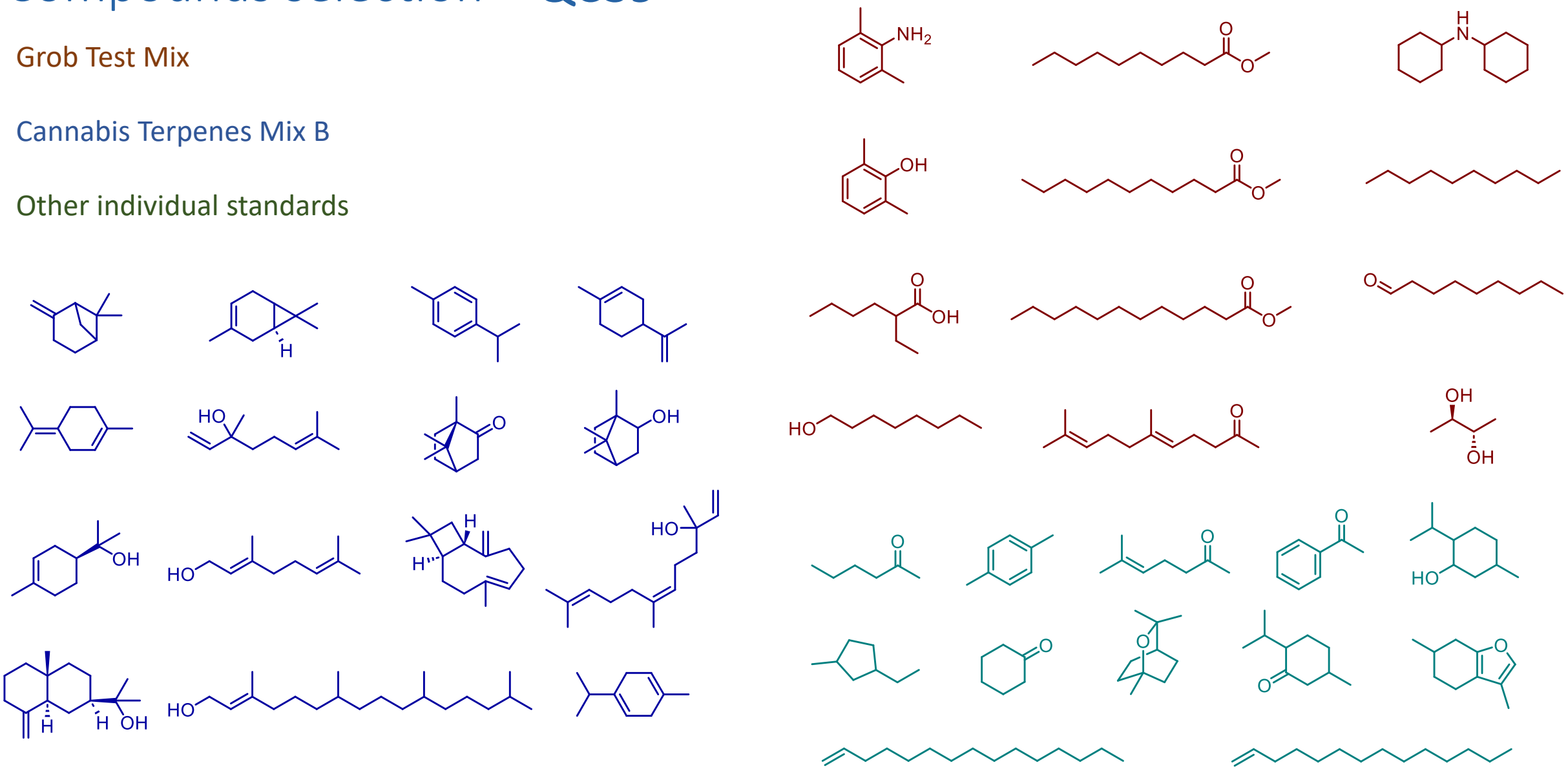
SHORT COMMUNICATION

Quality assurance and quality control processes: summary of a metabolomics community questionnaire

Warwick B. Dunn[1] · David I. Broadhurst[2] · Arthur Edison[3] · Claude Guillou[4] · Mark R. Viant[1] · Daniel W. Bearden[5] · Richard D. Beger[6]

Compounds selection – QC39

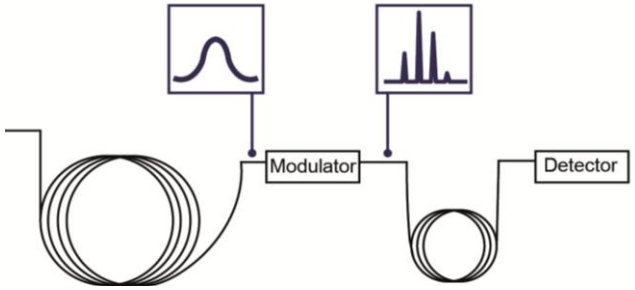
- Grob Test Mix
- Cannabis Terpenes Mix B
- Other individual standards



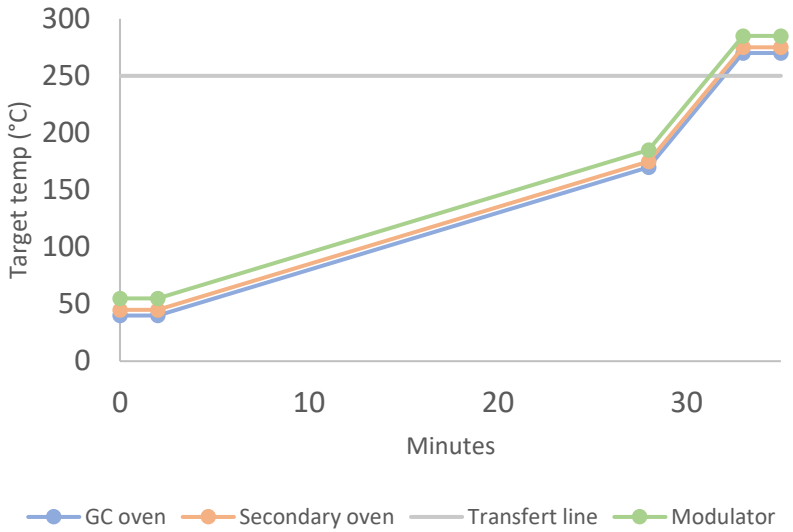
Total of 39 molecules

GC×GC instrumentation

PEGASUS® BT 4D
GC×GC-TOFMS
LECO



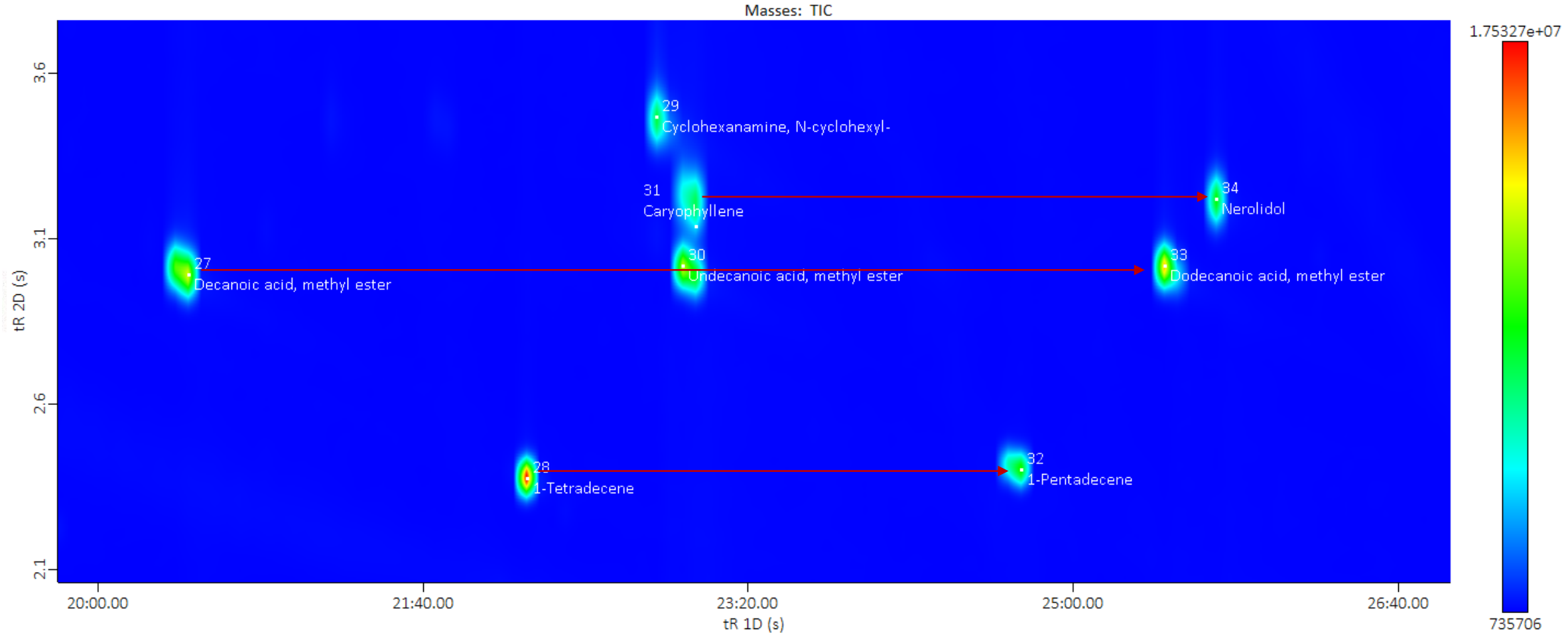
"Normal set" configuration
[1]D: Rxi-5SilMS (30 m, 0.25 mm ID, 0.25 µm)
[2]D: Rxi-17SilMS (2 m, 0.25 mm ID, 0.25 µm)



Liquid injection (1µL)

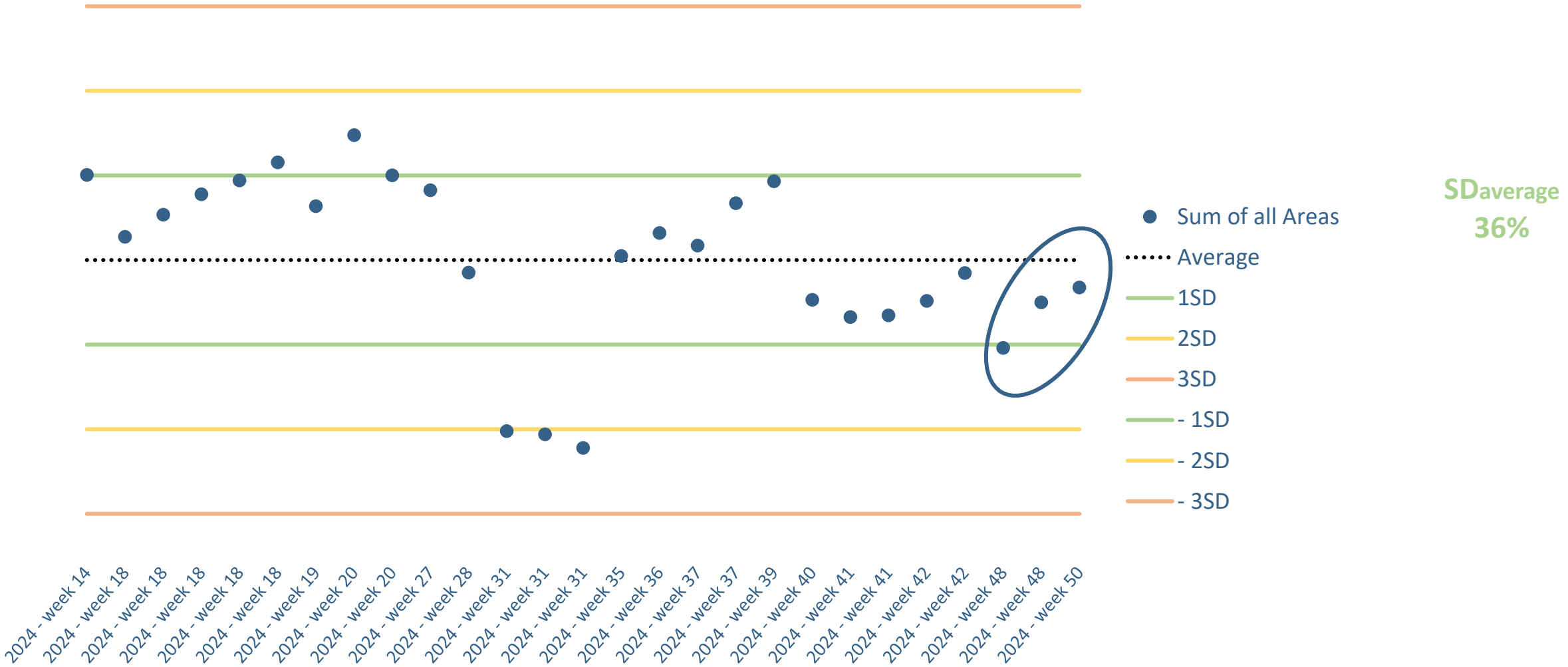
Acquisition rate 200 Hz
Range: 35 – 450 mu

Compounds selection – QC39



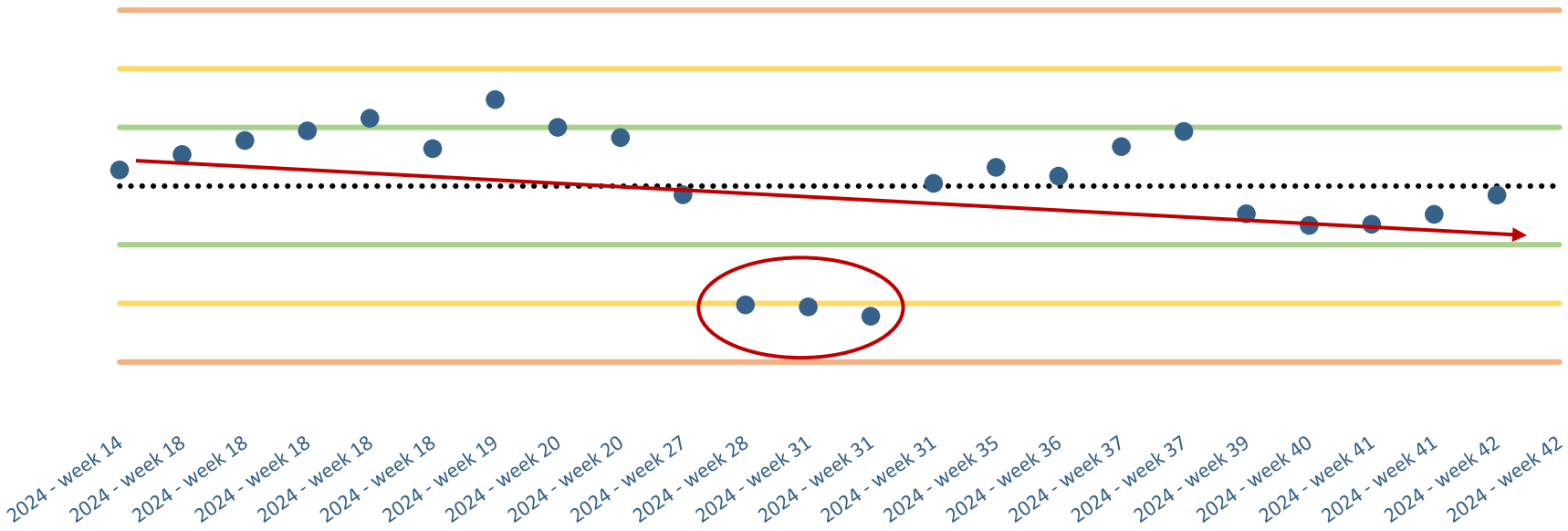
Instrument follow-up QC chart

- Building of a **follow-up** QC chart **within a year** with the **score** of all areas from the 39 compounds of our QC39



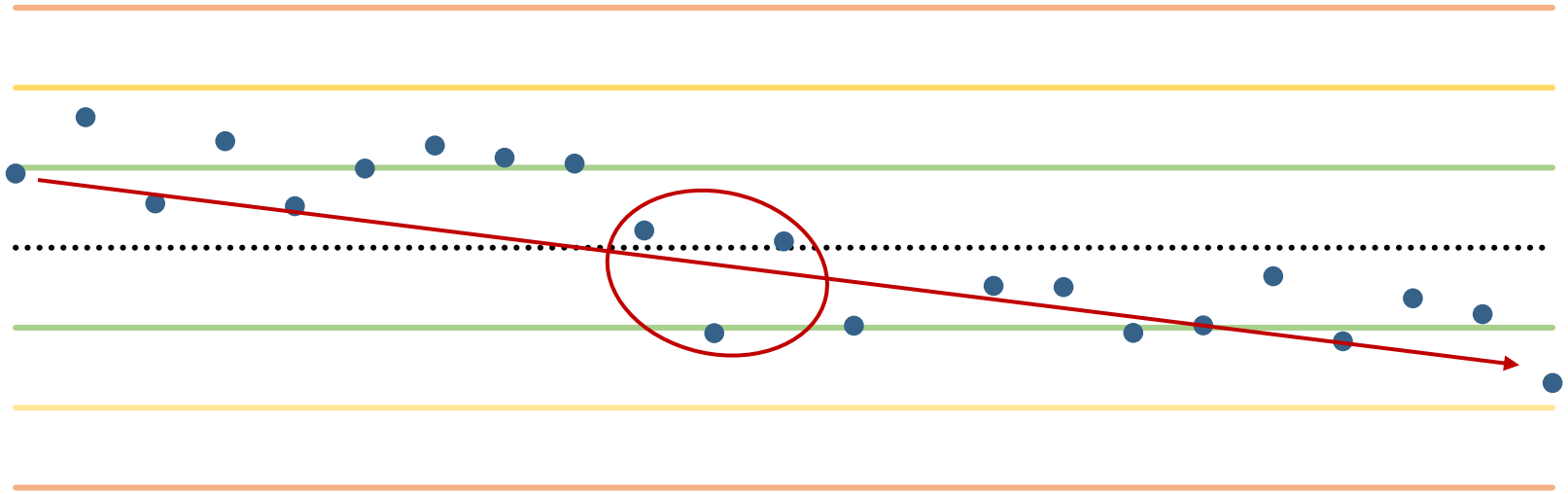
SDaverage
36%

Instrument follow-up QC chart



- With PQN normalization and without internal standard (ISTD) correction

SDaverage
36%



- With ISTD correction (6-Methyl-5-heptene-2-one)

SDaverage
15%

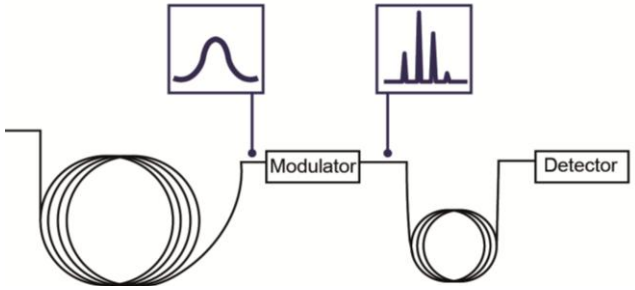
Century mix project

- An interlaboratory worldwide project
- A mixture with ~100 compounds to characterize systems and column sets
- **To be used as refe**

Compounds	Function
Alkanes	Mark the direction of the primary column separation gradient. Hexane(C_6) to Eicosane (C_{20}) range.
Aromatic hydrocarbons	Mark the direction of the secondary column separation gradient. Benzene (1-ring) to Phenanthrene (3-ring) range.
Homologous compounds	Markers of the elution order in the secondary column separation gradient. Ten series of homologous compounds include ketones (C_6 to C_{12}), primary alcohols (C_5 to C_{12}), secondary alcohols (C_5 to C_{10}), FAMES (C_5 to C_{13}), alkyl benzenes (C_7 to C_{15}), alkenes (C_8 to C_{11}), alkyl acetates (C_5 to C_8), carboxylic acids (C_4 to C_8), Lactones (C_6 to C_9), and phthalates (C_{10},C_{12}, and C_{14}).
Grob mix and McReynolds compounds	Markers of selectivity metrics connected to 1D GC.
Miscellaneous compounds	Selected marker compounds connected to a variety of application areas (bioanalytical, environmental, foods and flavors)

GC×GC instrumentation

PEGASUS® BT 4D
GC×GC-TOFMS
LECO



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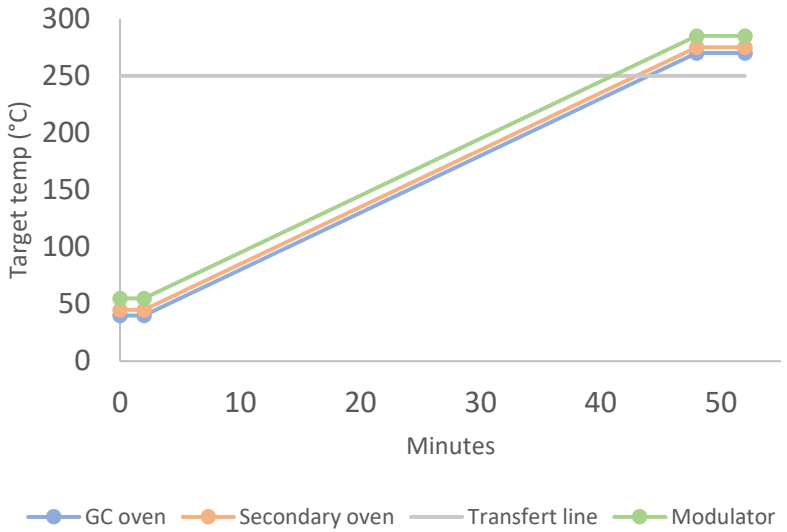
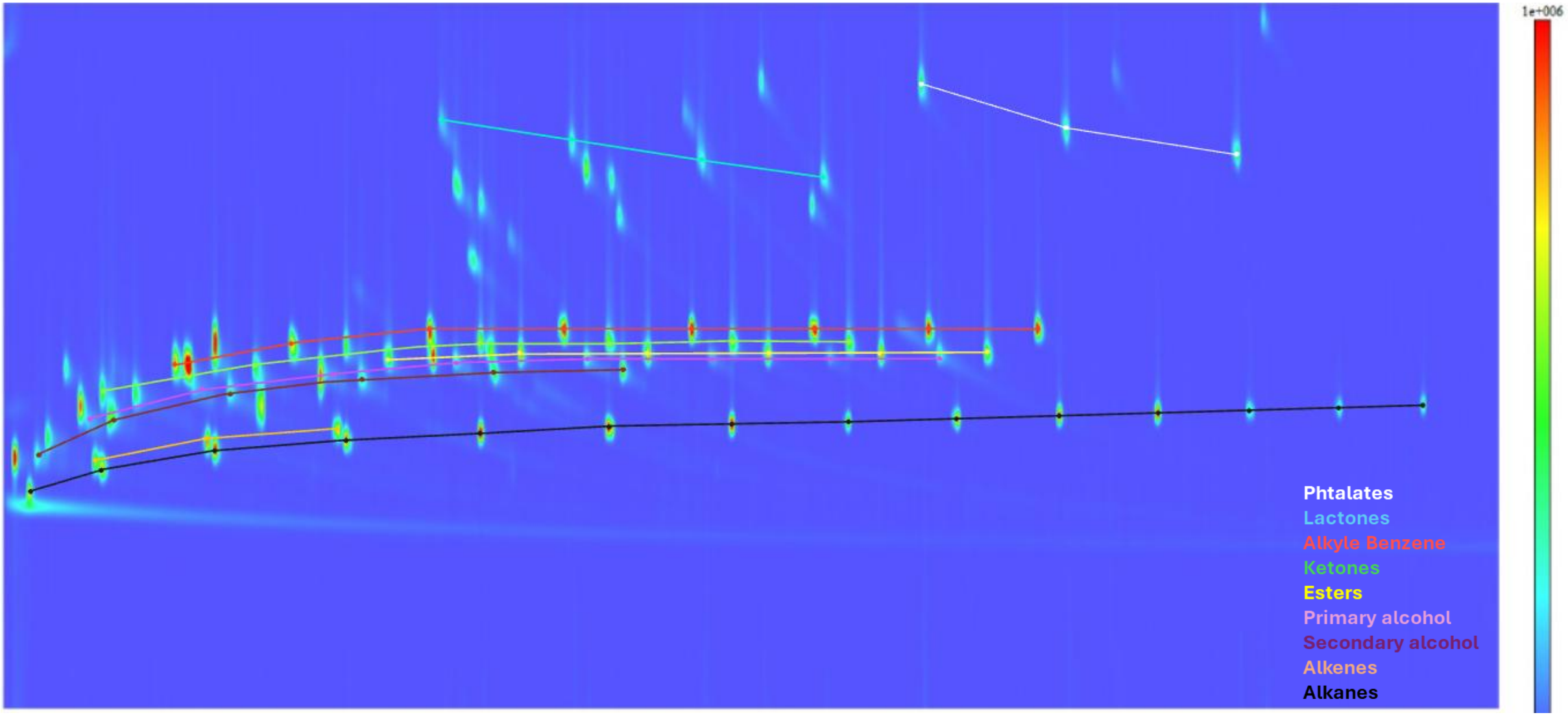


Liquid injection (1µL)

Acquisition rate 200 Hz
Range: 35 – 450 mu

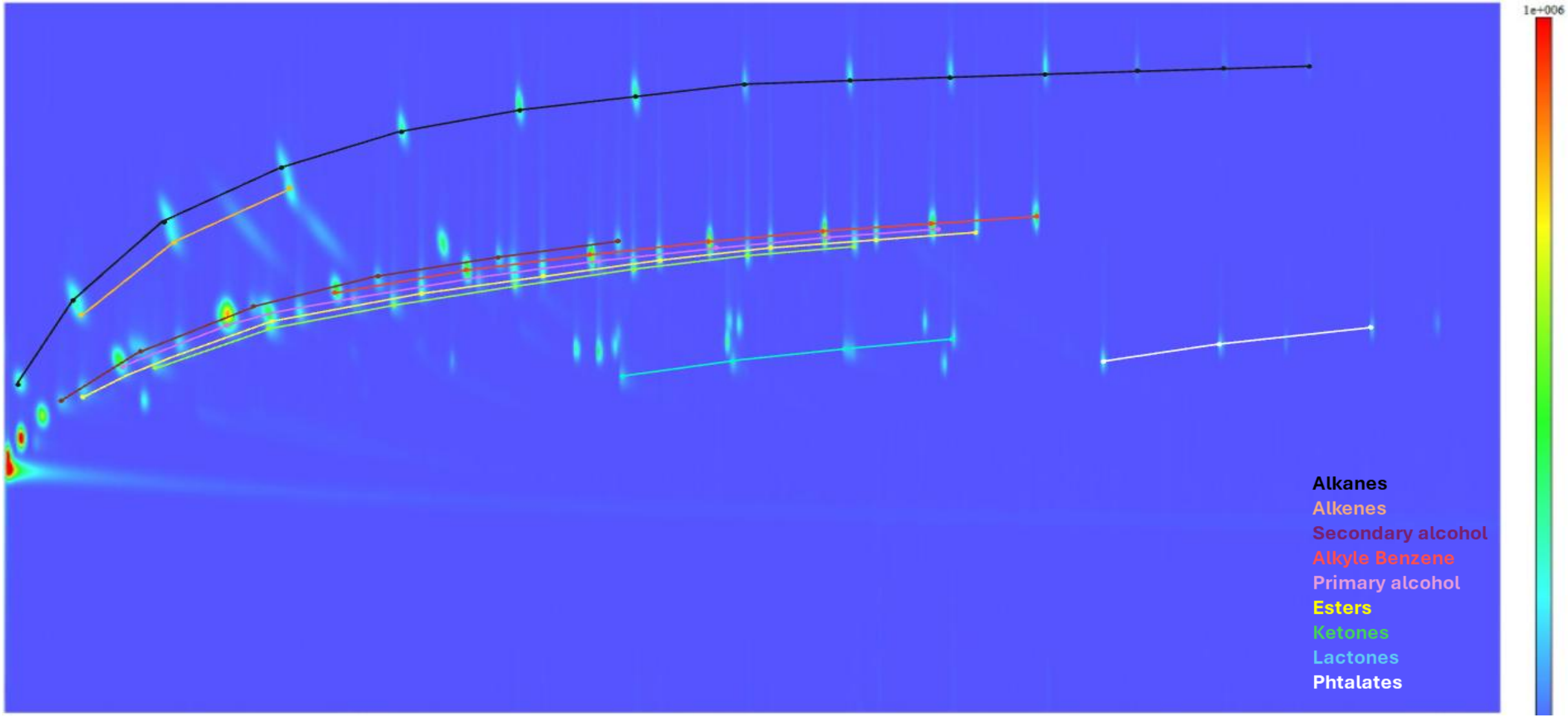
Century mix

- Normal column set



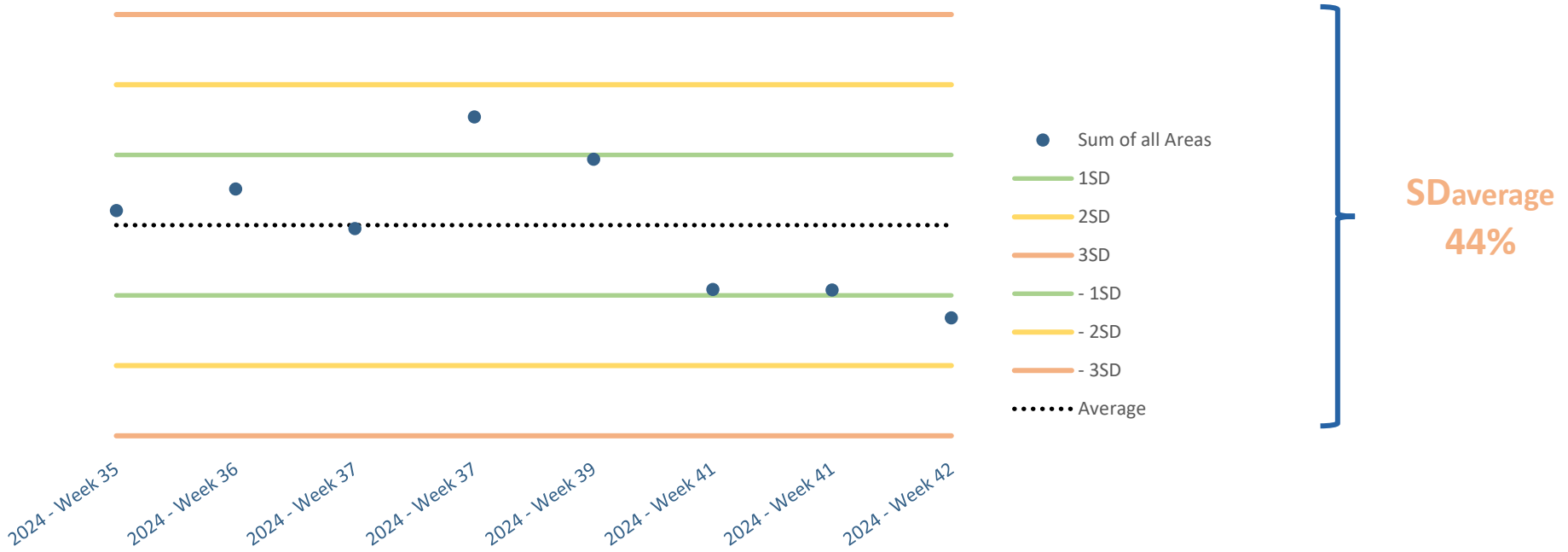
Century mix

- Reverse column set



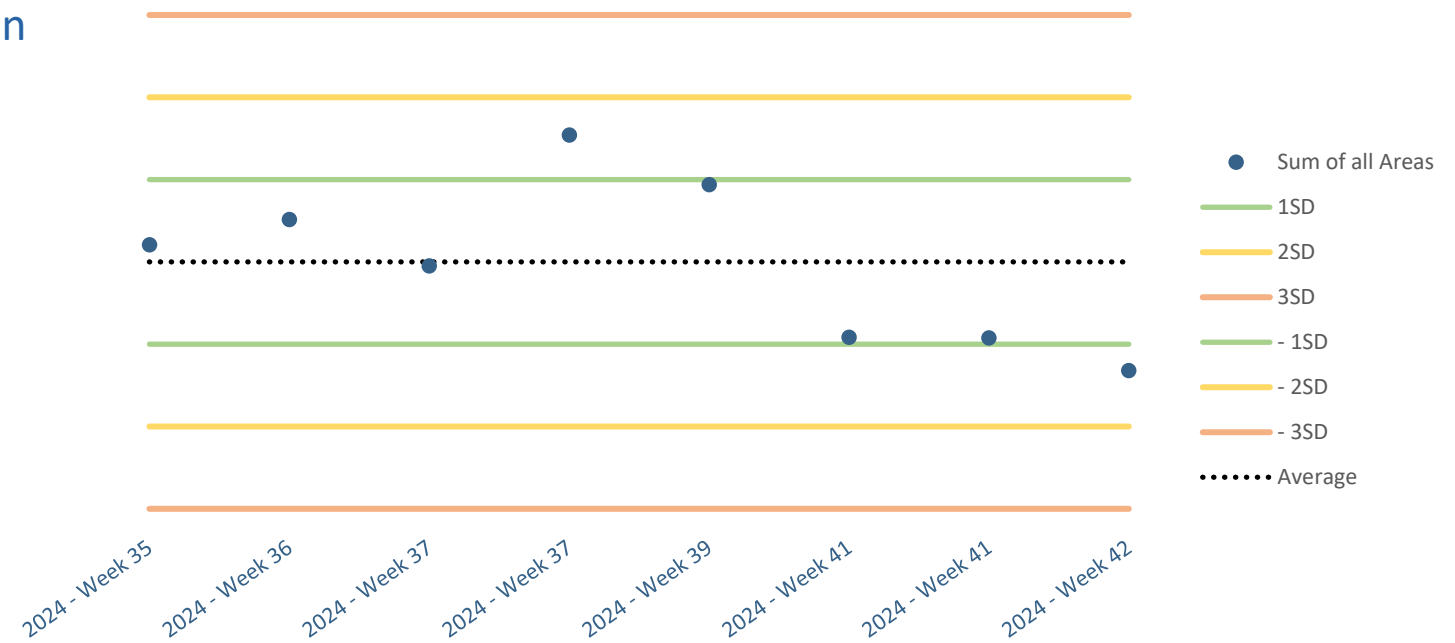
Instrument follow-up QC chart using the CM

- Building of a **sum of all areas** QC chart with the **score** of all areas from **60 compounds** of the **Century Mix** (homologous series)



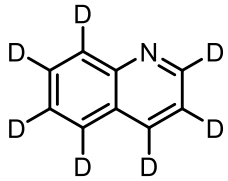
Instrument follow-up QC chart using the CM

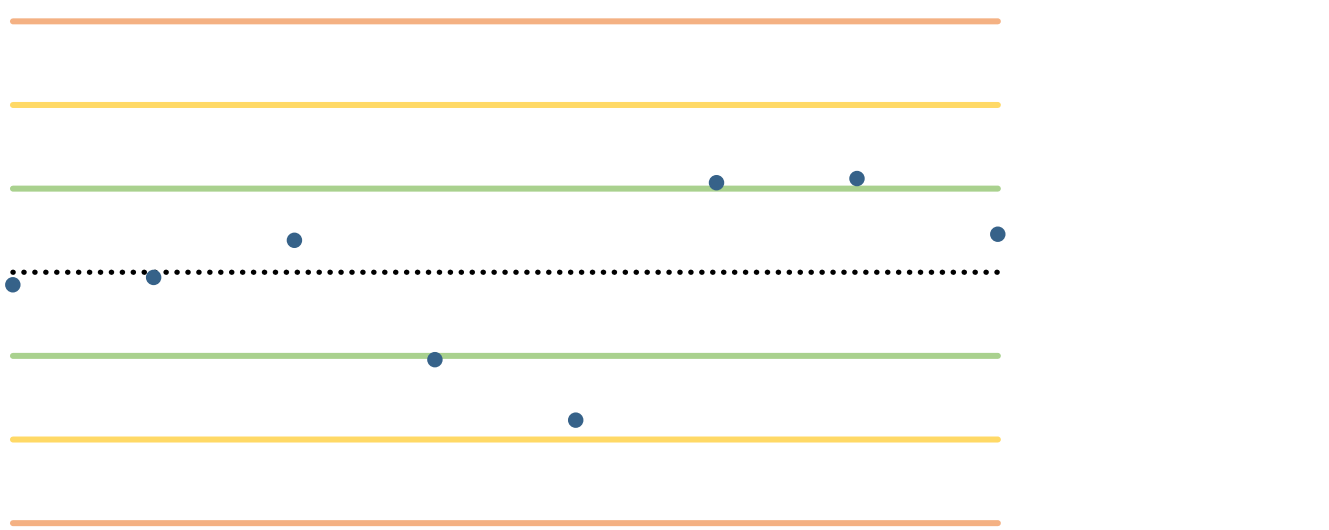
- Without **ISTD** correction



SDaverage
44%

- With **ISTD** correction

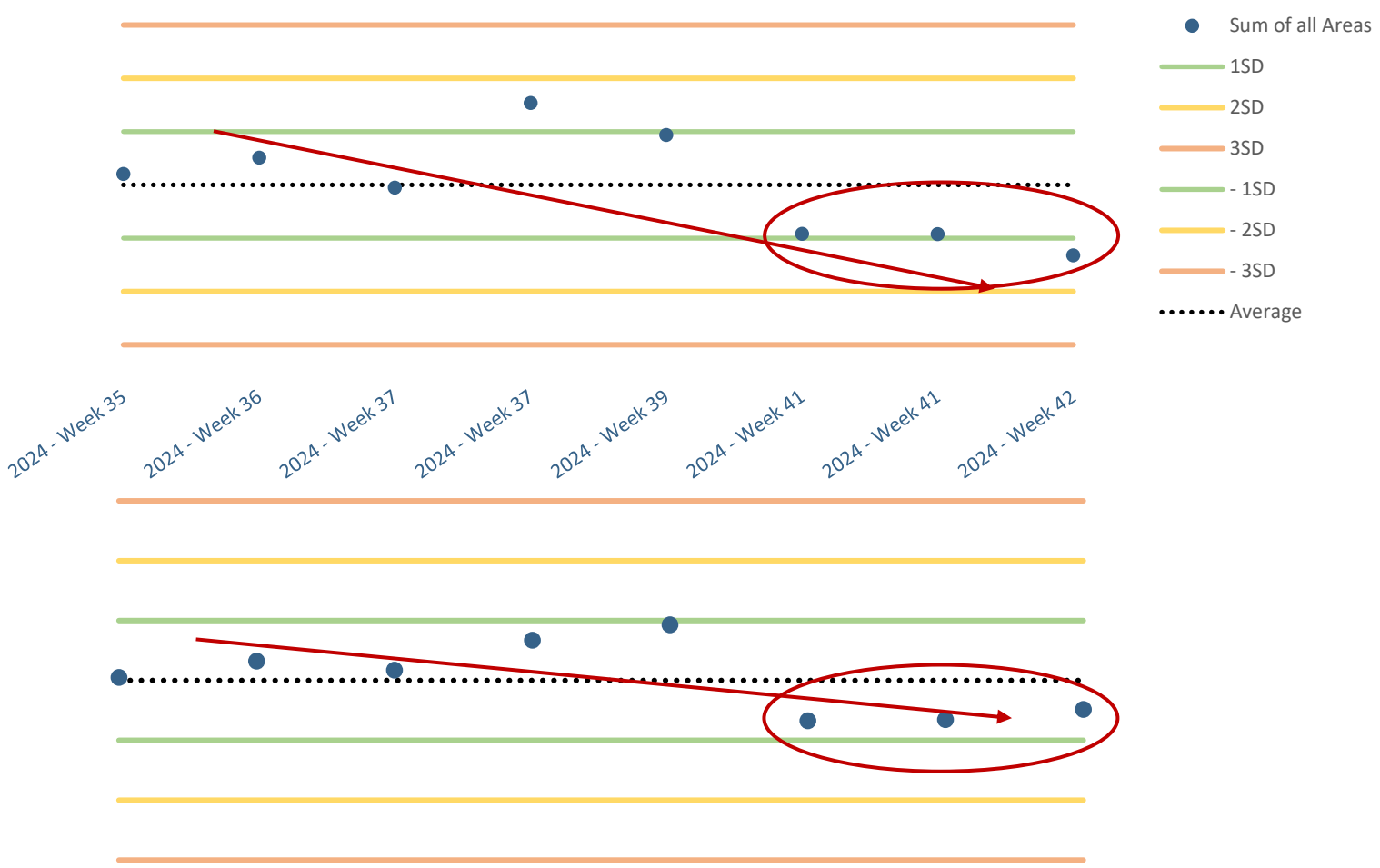




SDaverage
28%

Instrument follow-up QC chart using the CM

- CM *vs.* QC39



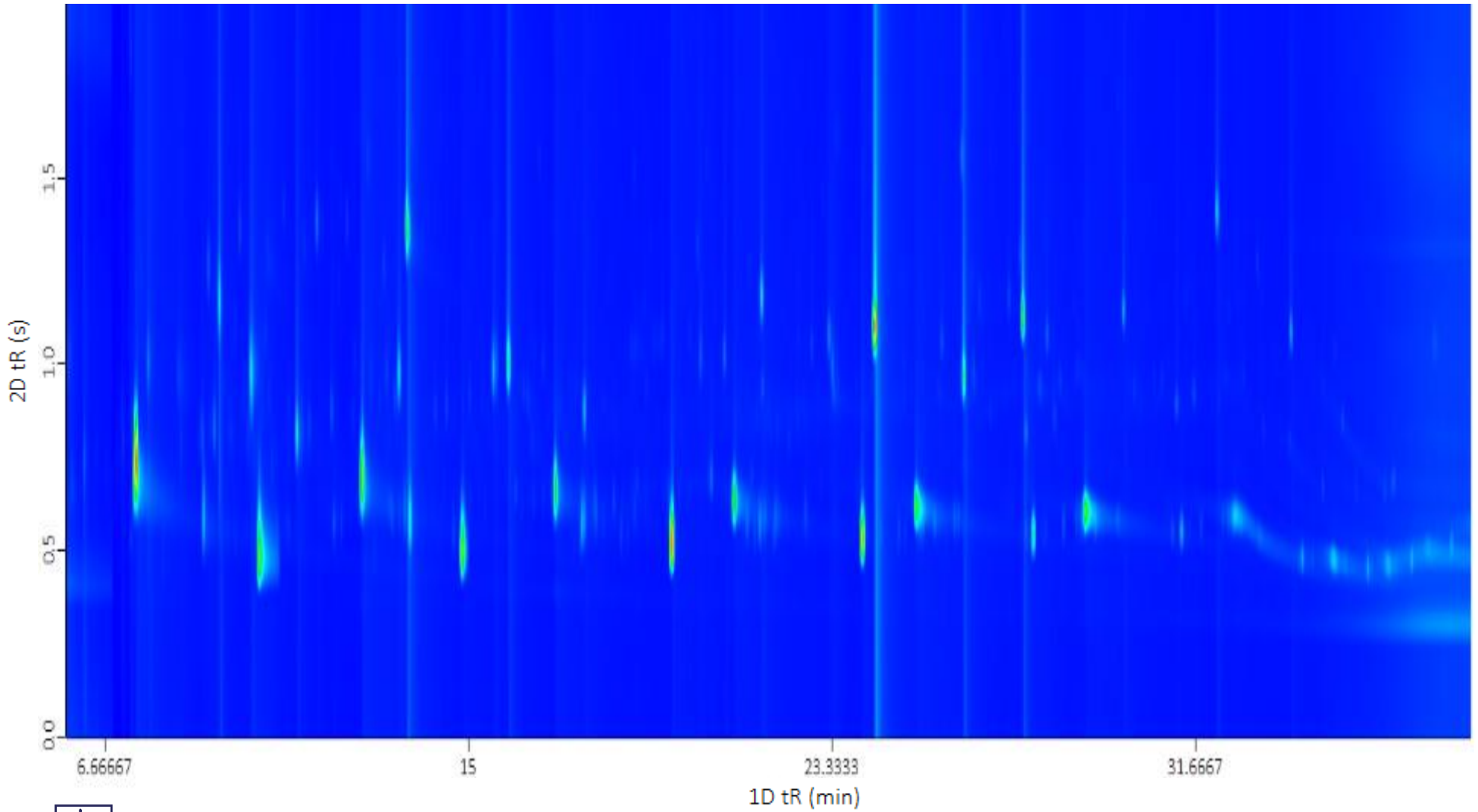
SDaverage 44%

Century Mix

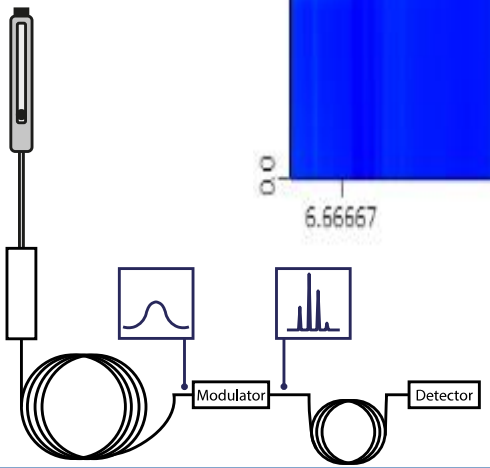
SDaverage 22%

QC39

QA for study follow-up



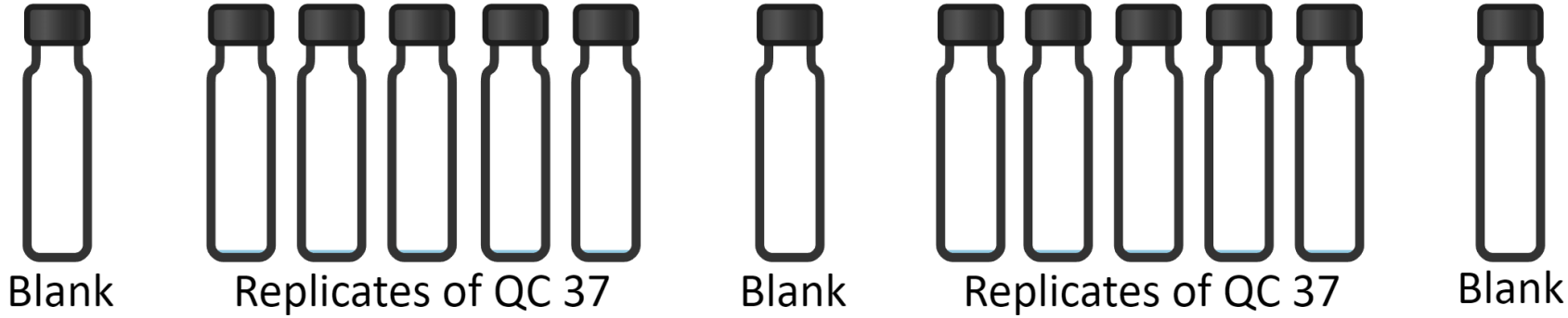
Djulia Bensaada



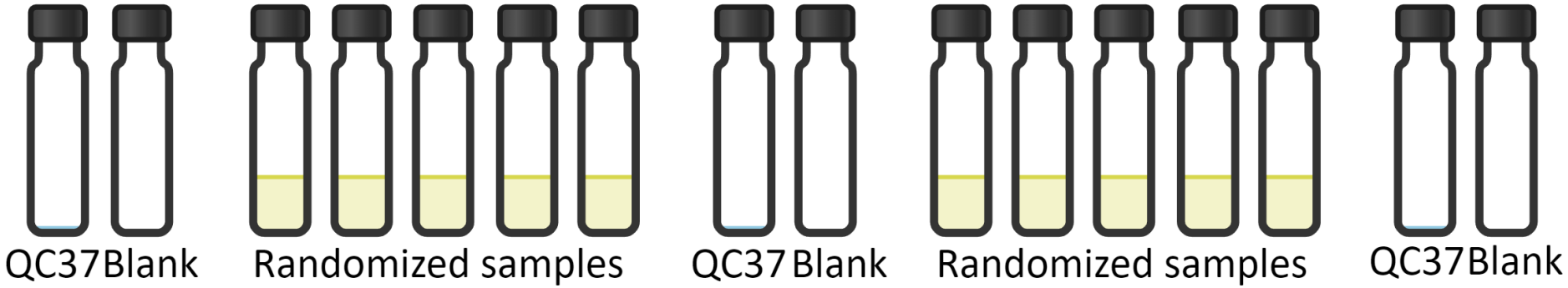
QA for study follow-up

Djulia Bensaada

Baseline for QC Chart



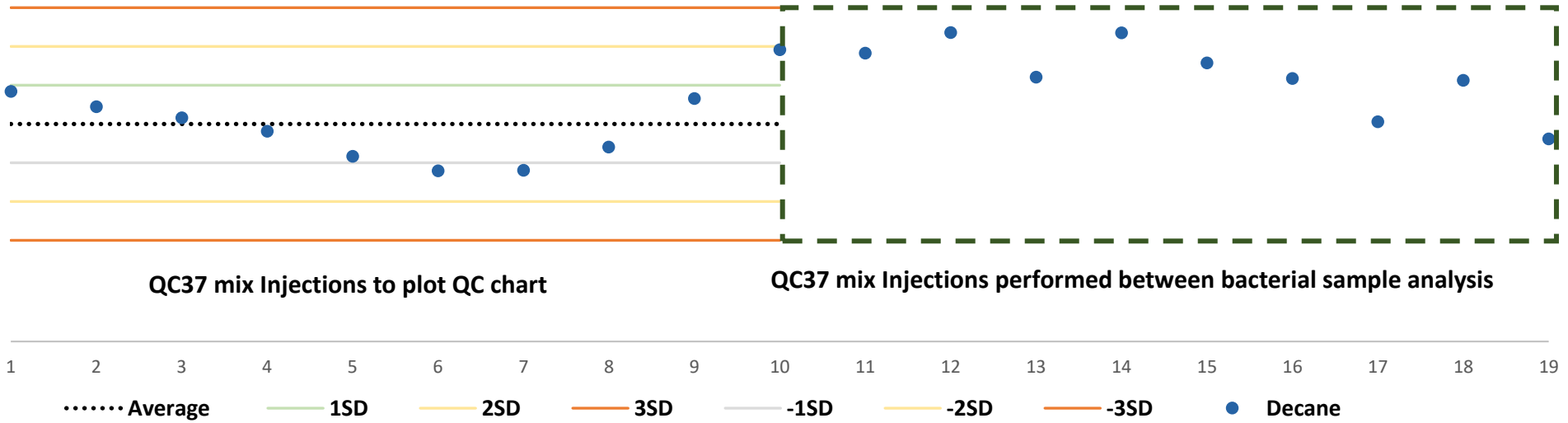
Randomized bacterial sample analysis with QC samples



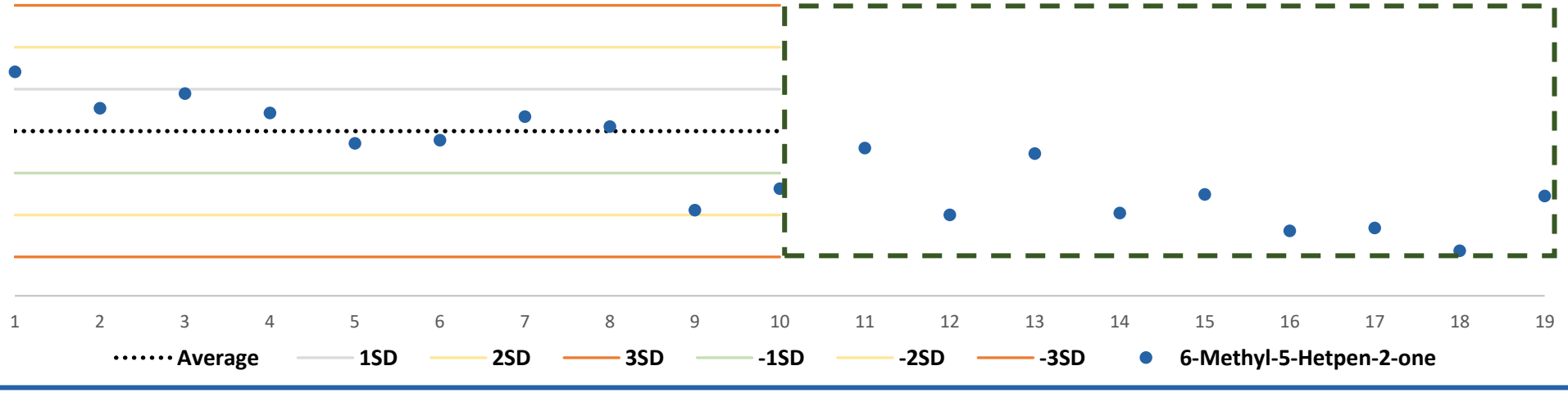
QA for study follow-up

Djulia Bensaada

Decane



6-Methyl-5-Hepten-2-one



Take home messages

- **Following more compounds helps monitoring instruments: CM to be use as QC**
- Building instrument follow-up QC charts **+** include QCs for study follow-up
- **Base** for your QA/QC system in metabolomics

- **Continue follow-up** on Peltier instrument and **implement QA/QC system** on our other instruments using both **QC39** and **CM**
- Dedicated **QC mixtures** derivate from the CM
- Work on **multivariate chart control** based on **PCA** to evaluate reproducibility

Organic and Biological Analytical Chemistry Group

Acknowledgements

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MolSys

Prof. Jef Focant

Dr. PH Stefanuto

Dr. Kinjal Bhatt

Thibaut Dejong

Thibault Massenet

Anika Lokker

Géraldine Dumont

Djulia Bensaada

Dr. Armélinda Agnello

Stéphanie Hendrick

Grégory Gridelet

Vicky Borguet

Alex Haway

Océane Tirsel

Thibault Rudnik

Roberto

ROBERTO_OBIACHEM

Prof. John Dimandja
(FDA)

TRAJAN

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FDA

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This research was supported by the University of Liege under Special Funds for Research, **IPD-STEMA Program**