

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

Anaïs RODRIGUES<sup>1</sup>, Jean-Marie DIMANDJA<sup>2</sup>, Djulia BENSAADA<sup>1</sup>, Pierre-Hugues STEFANUTO<sup>1</sup>, Jean-François FOCANT<sup>1</sup>

<sup>1</sup>University of Liege (ULiege) – Chemistry, Sciences, MolSys Unit

<sup>2</sup>US Food and Drug Administration's Office of Regulatory Affairs, Atlanta, USA

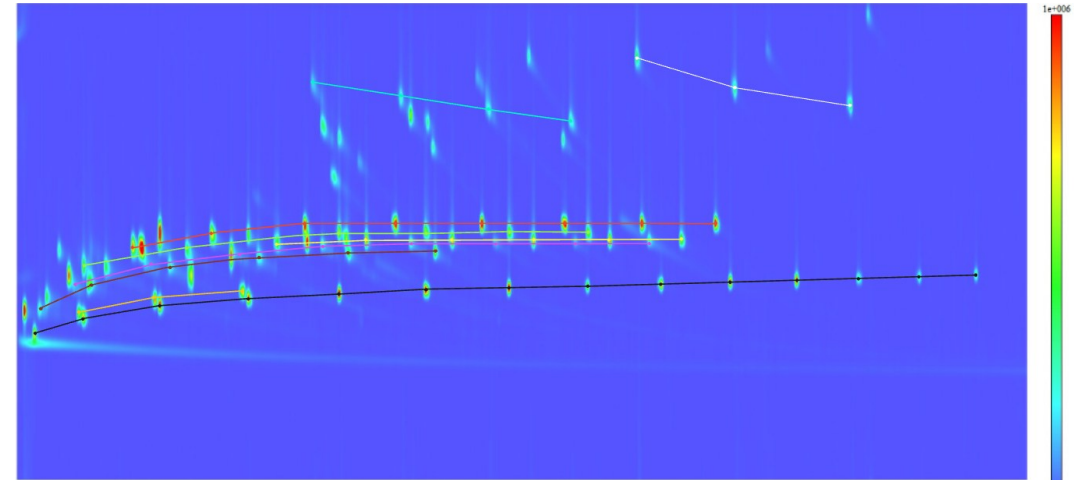
MDCW – February 4<sup>th</sup>, 2025 – Liege, Belgium

**16<sup>TH</sup> 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*



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

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CHROMATOGRAPHY A

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

Jean-Marie D. Dimandja<sup>a,\*</sup>, Garrick C. Clouden<sup>b</sup>, Ivelisse Colón<sup>c</sup>,  
Jean-François Focant<sup>d</sup>, Whitney V. Cabey<sup>a</sup>, Ritchard C. Parry<sup>e</sup>

<sup>a</sup> Department of Chemistry, Spelman College, 350 Spelman Lane, SW Box 279 Atlanta, GA 30314, USA

<sup>b</sup> Meharry Medical College, 1005 Dr. D. B. Todd Jr. Blvd., Nashville, TN 37208, USA

<sup>c</sup> Pfizer Global Research and Development, Eastern Point Road, Groton, CT 06340, USA

<sup>d</sup> Centers for Disease Control and Prevention, 4770 Buford Highway NE, Atlanta, GA 30041, USA

<sup>e</sup> LECO Corporation, 3000 Lakeview Avenue, St. Joseph, MI 49085, USA

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

Ann M. Knolhoff<sup>\*,†</sup>, Jacob H. Premo<sup>†</sup> and Christine M. Fisher



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Article

Metabolomics (2022) 18: 70

<https://doi.org/10.1007/s11306-022-01926-3>

REVIEW ARTICLE

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

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



Metabolomics (2017) 13:50

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SHORT COMMUNICATION

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

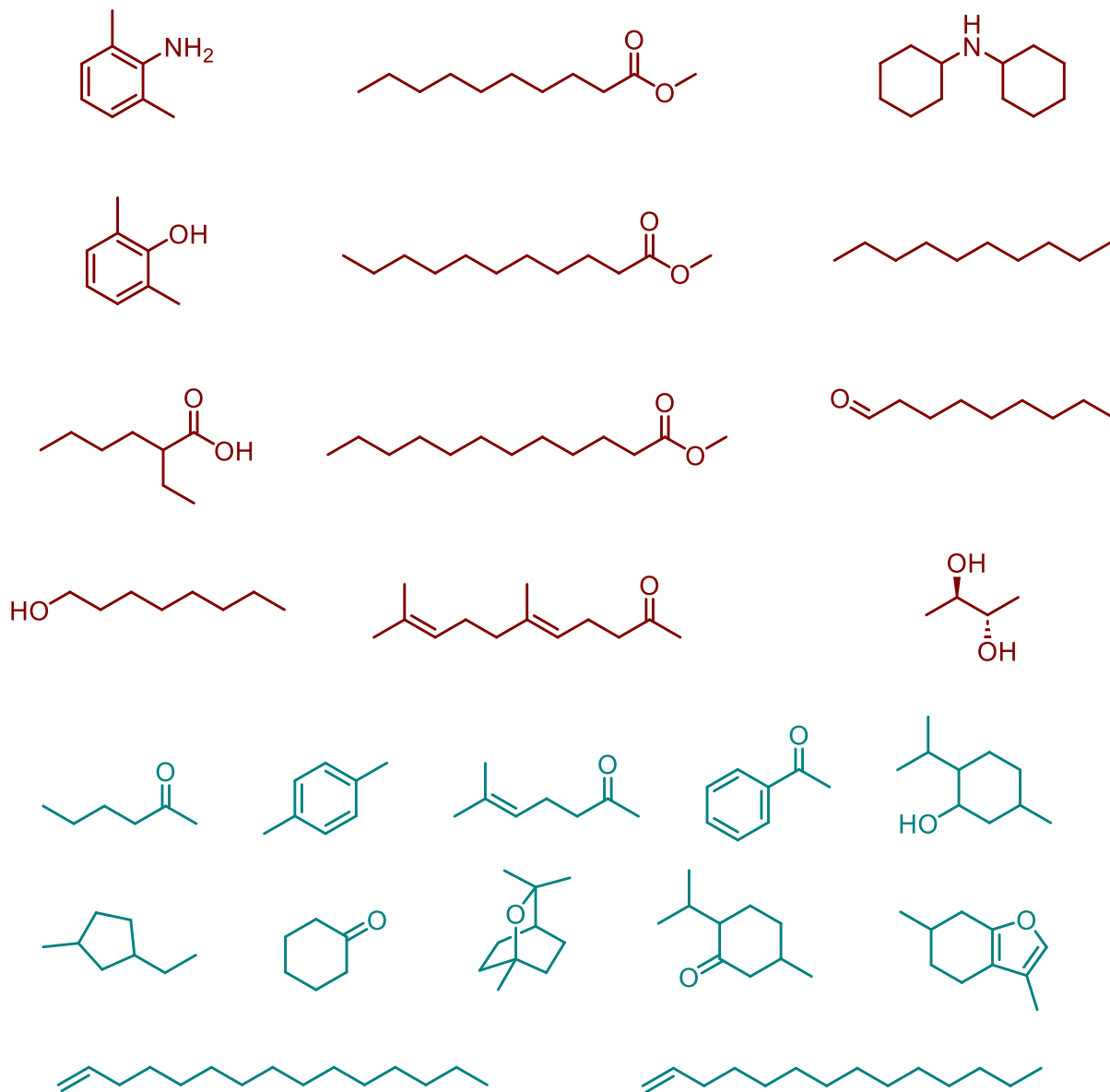
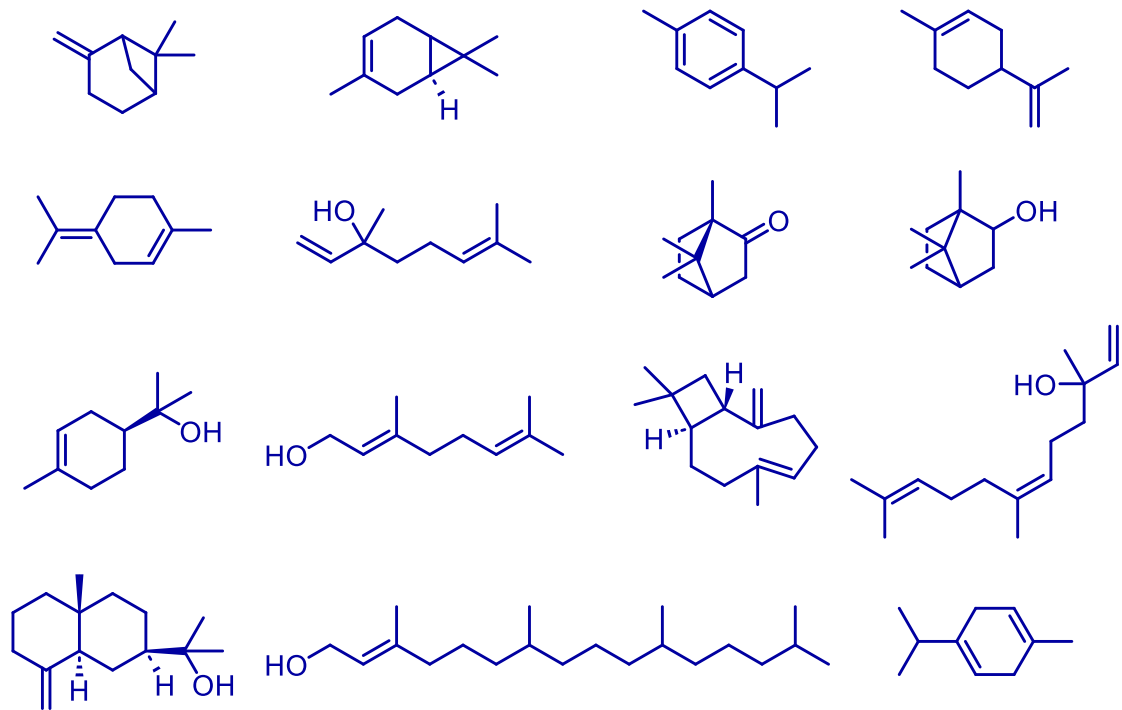
Warwick B. Dunn<sup>1</sup> · David I. Broadhurst<sup>2</sup> · Arthur Edison<sup>3</sup> · Claude Guillou<sup>4</sup> · Mark R. Viant<sup>1</sup> · Daniel W. Bearden<sup>5</sup> · Richard D. Beger<sup>6</sup>



CrossMark

# Compounds selection – QC39

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

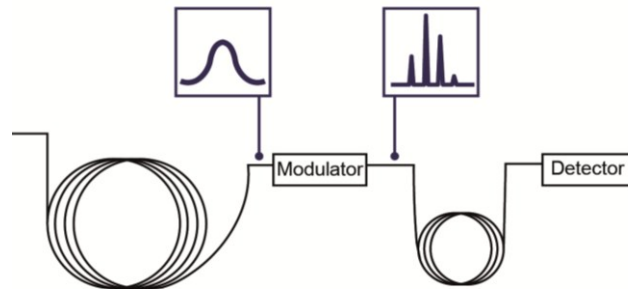


Total of 39 molecules

# GC×GC instrumentation

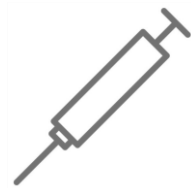
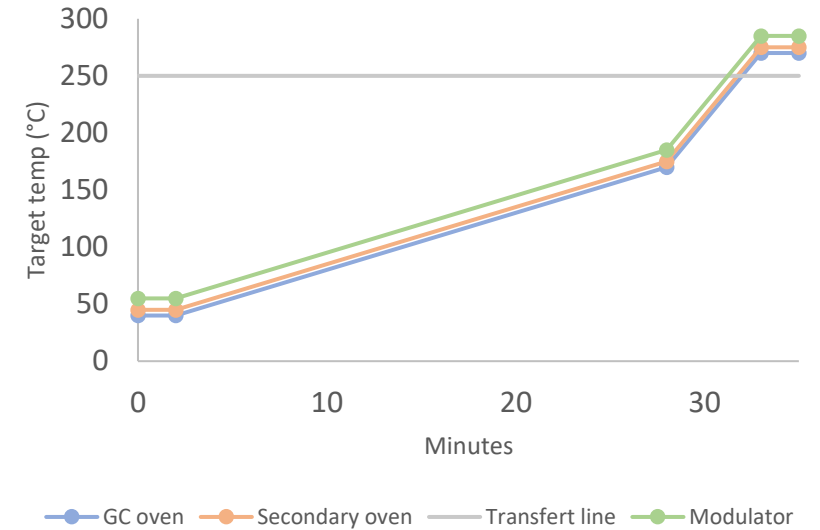


PEGASUS® BT 4D  
GC×GC-TOFMS  
LECO



“Normal set” configuration

<sup>1</sup>D: Rxi-5SilMS (30 m, 0.25 mm ID, 0.25 μm)  
<sup>2</sup>D: Rxi-17SilMS (2 m, 0.25 mm ID, 0.25 μm)

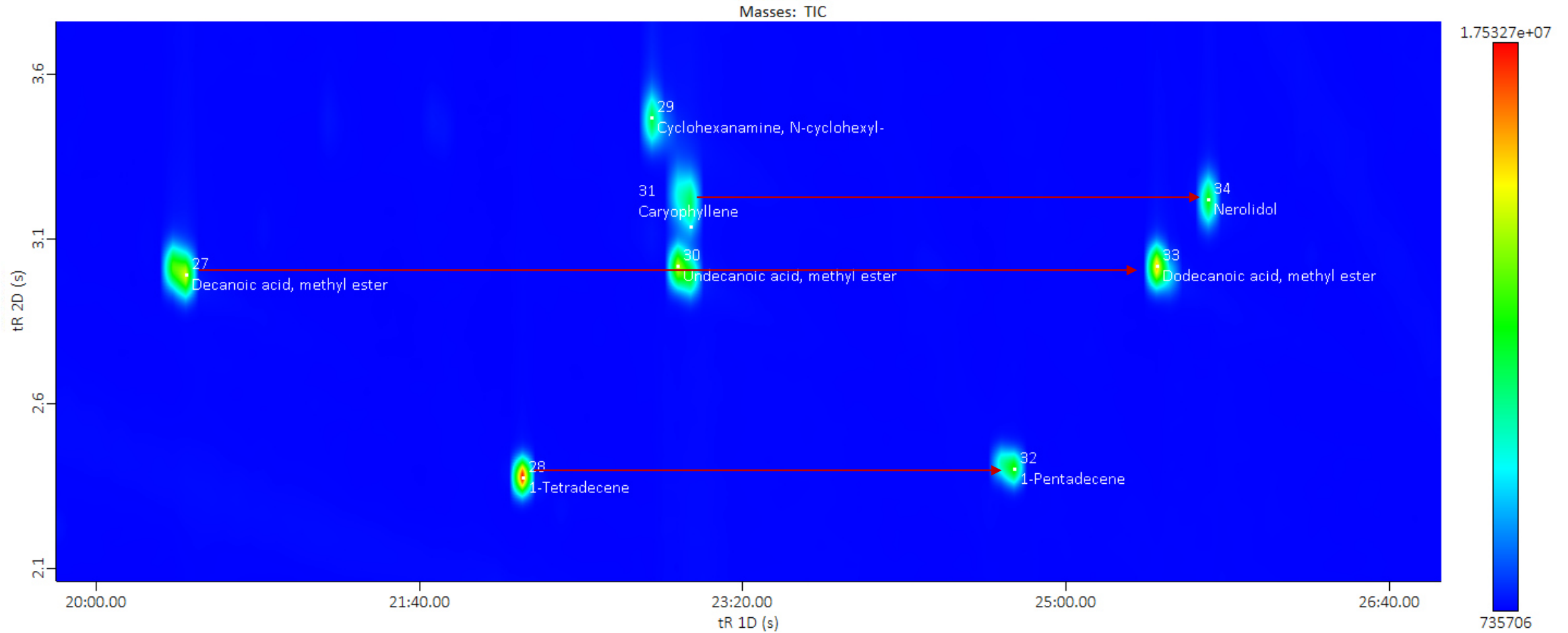


Liquid injection (1μL)



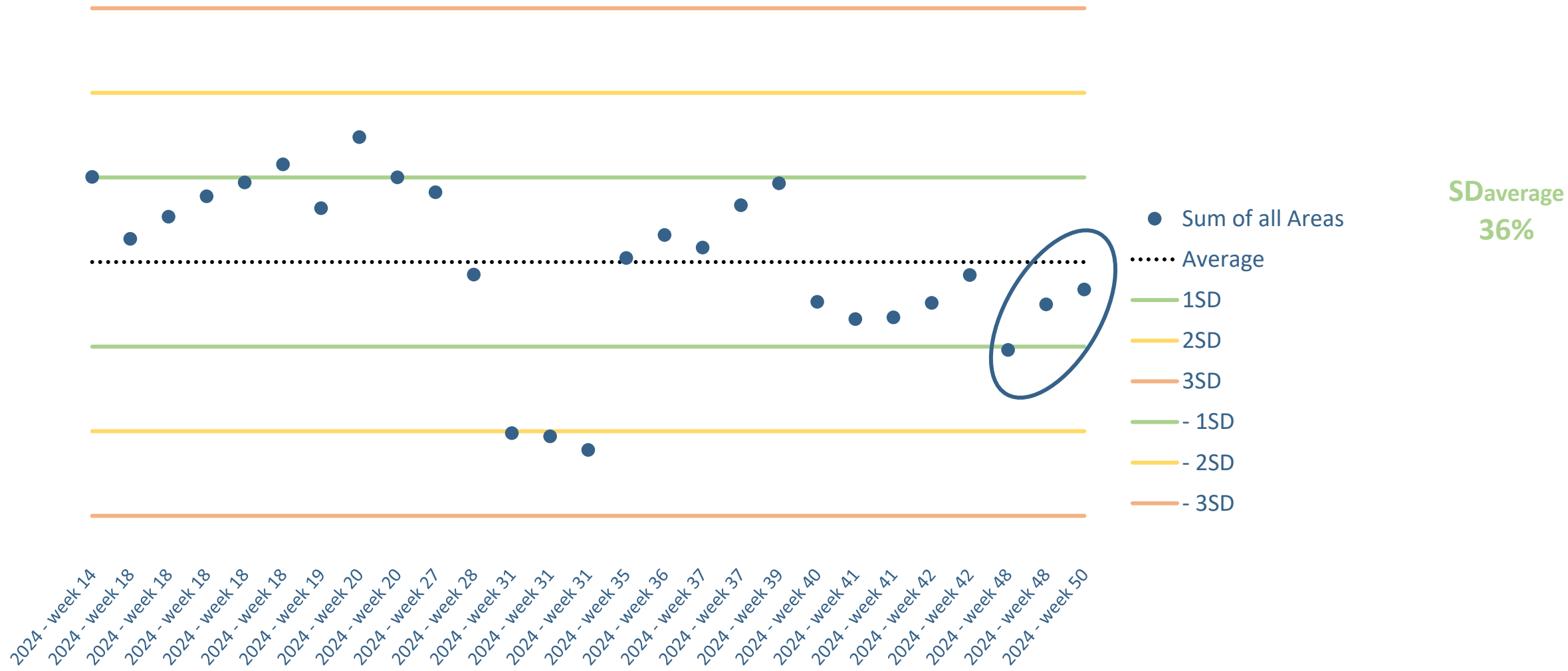
Acquisition rate 200 Hz  
Range: 35 – 450 mu

# Compounds selection – QC39

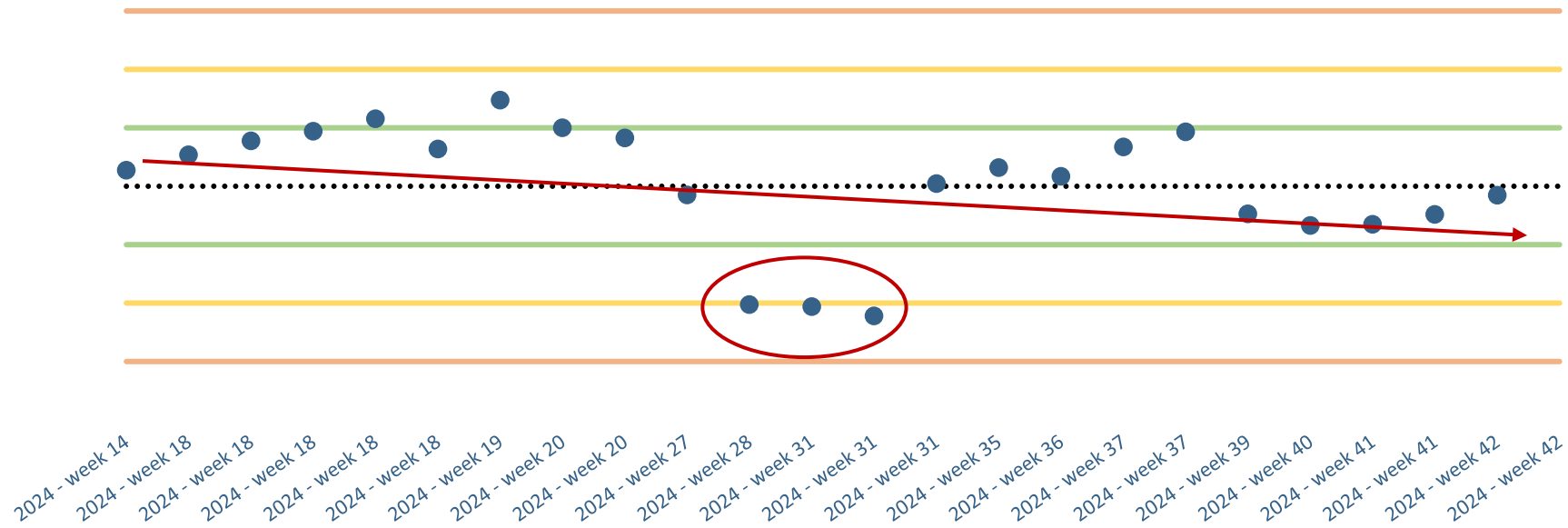


# Instrument follow-up QC chart

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

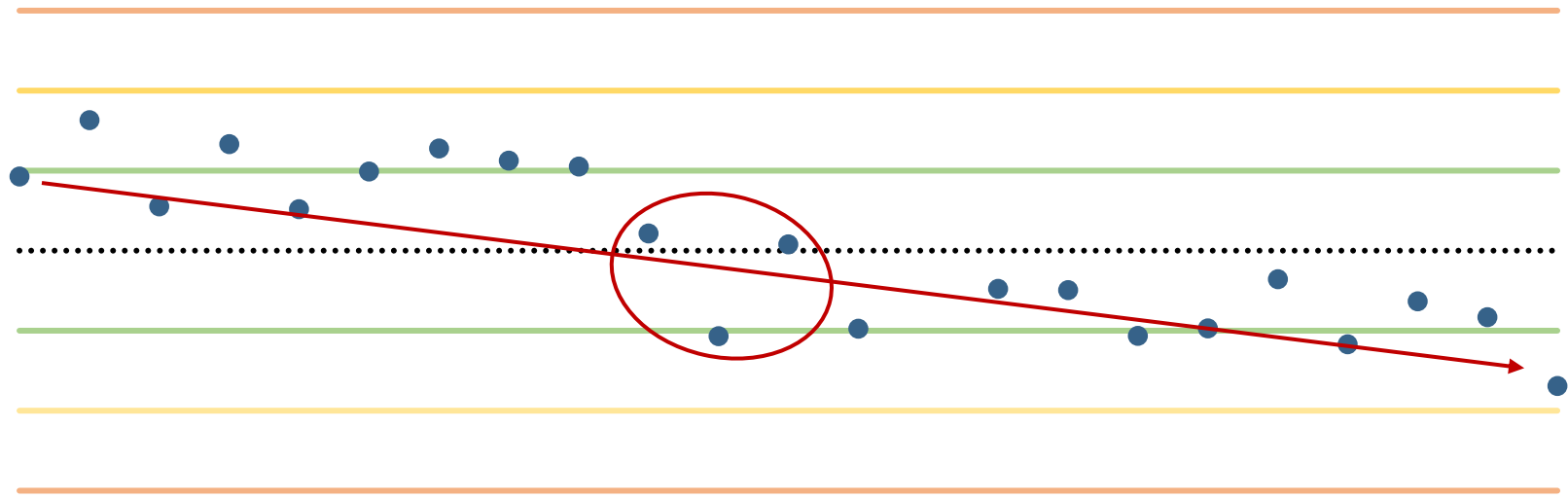


# Instrument follow-up QC chart



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

SD<sub>average</sub>  
36%



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

SD<sub>average</sub>  
15%

# Century mix project

- An interlaboratory worldwide project
- A mixture with ~100 compounds to characterize systems and column sets

- **To be used as reference**

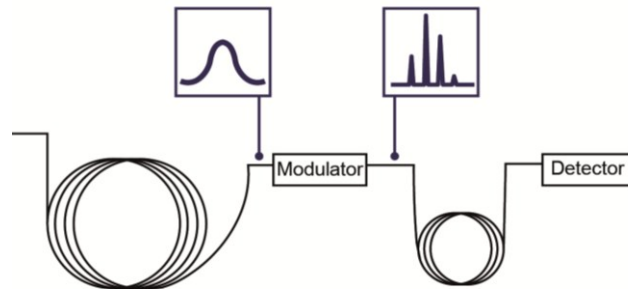
Compounds	Function
Alkanes	Mark the direction of the primary column separation gradient. Hexane(C <sub>6</sub> ) to Eicosane (C <sub>20</sub> ) range.
Aromatic hydrocarbons	Mark the direction of the secondary column separation gradient. Benzene (1-ring) to Phenanthrene (3-ring) range.
Homologous compounds	Markers of the elution order in the secondary column separation gradient. Ten series of homologous compounds include ketones (C <sub>6</sub> to C <sub>12</sub> ), primary alcohols (C <sub>5</sub> to C <sub>12</sub> ), secondary alcohols (C <sub>5</sub> to C <sub>10</sub> ), FAMES (C <sub>5</sub> to C <sub>13</sub> ), alkyl benzenes (C <sub>7</sub> to C <sub>15</sub> ), alkenes (C <sub>8</sub> to C <sub>11</sub> ), alkyl acetates (C <sub>5</sub> to C <sub>8</sub> ), carboxylic acids (C <sub>4</sub> to C <sub>8</sub> ), Lactones (C <sub>6</sub> to C <sub>9</sub> ), and phthalates (C <sub>10</sub> , C <sub>12</sub> , and C <sub>14</sub> ).
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

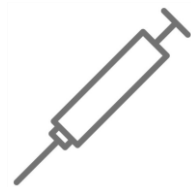
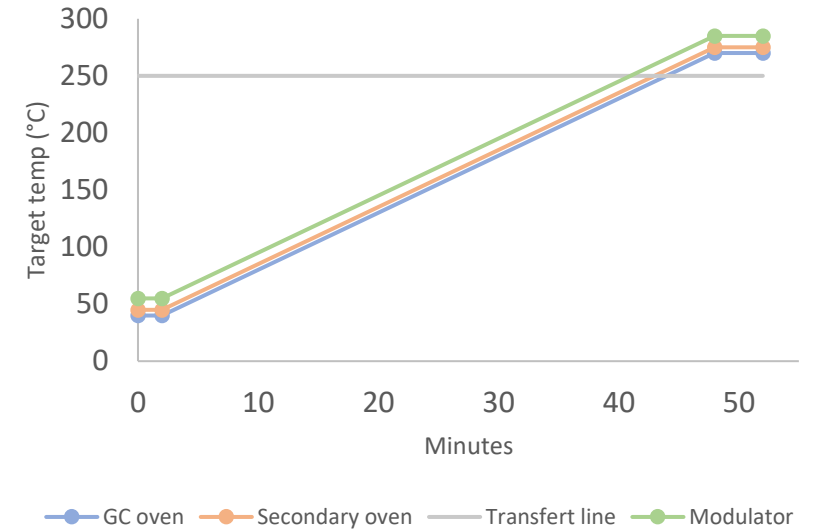


PEGASUS® BT 4D  
GC×GC-TOFMS  
LECO



“Normal set” configuration

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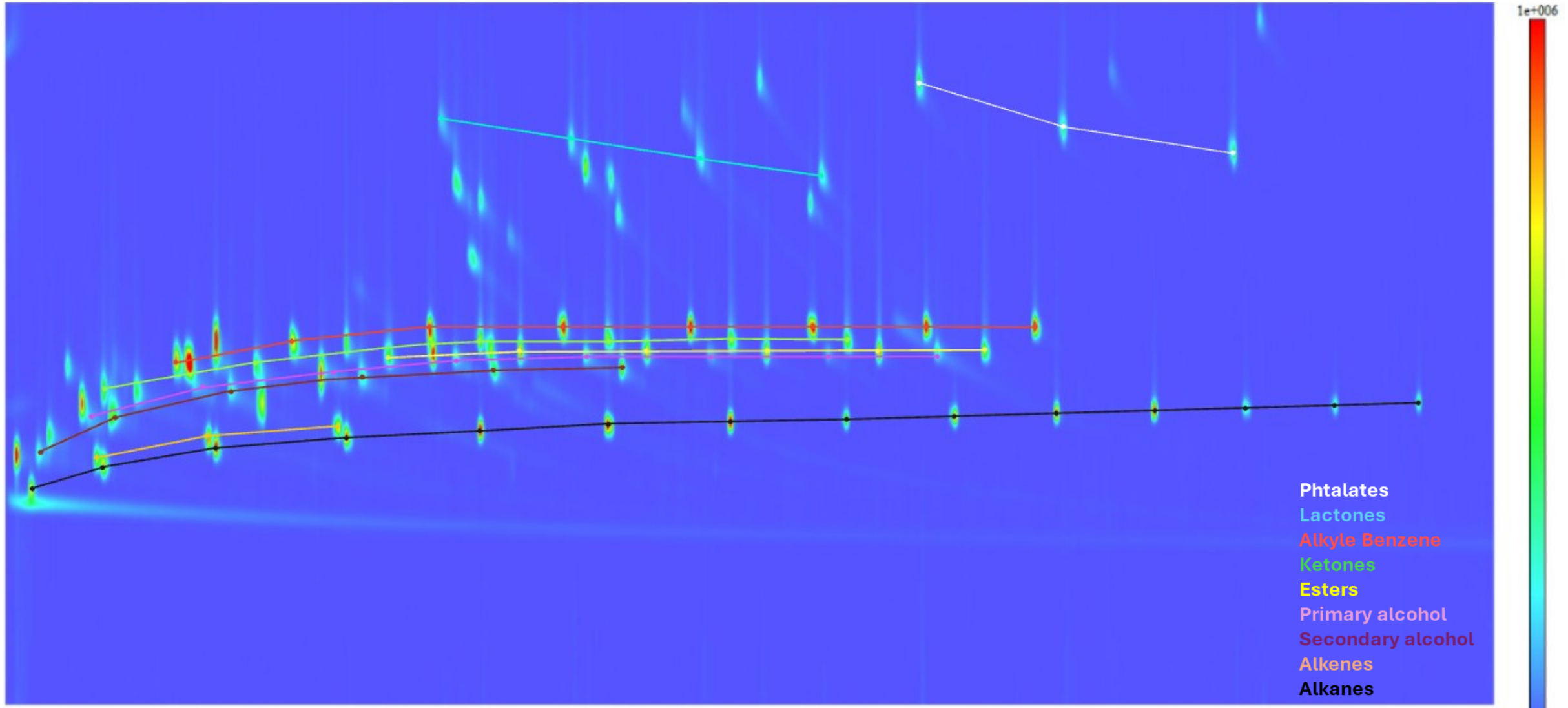
Liquid injection (1μL)



Acquisition rate 200 Hz  
Range: 35 – 450 mu

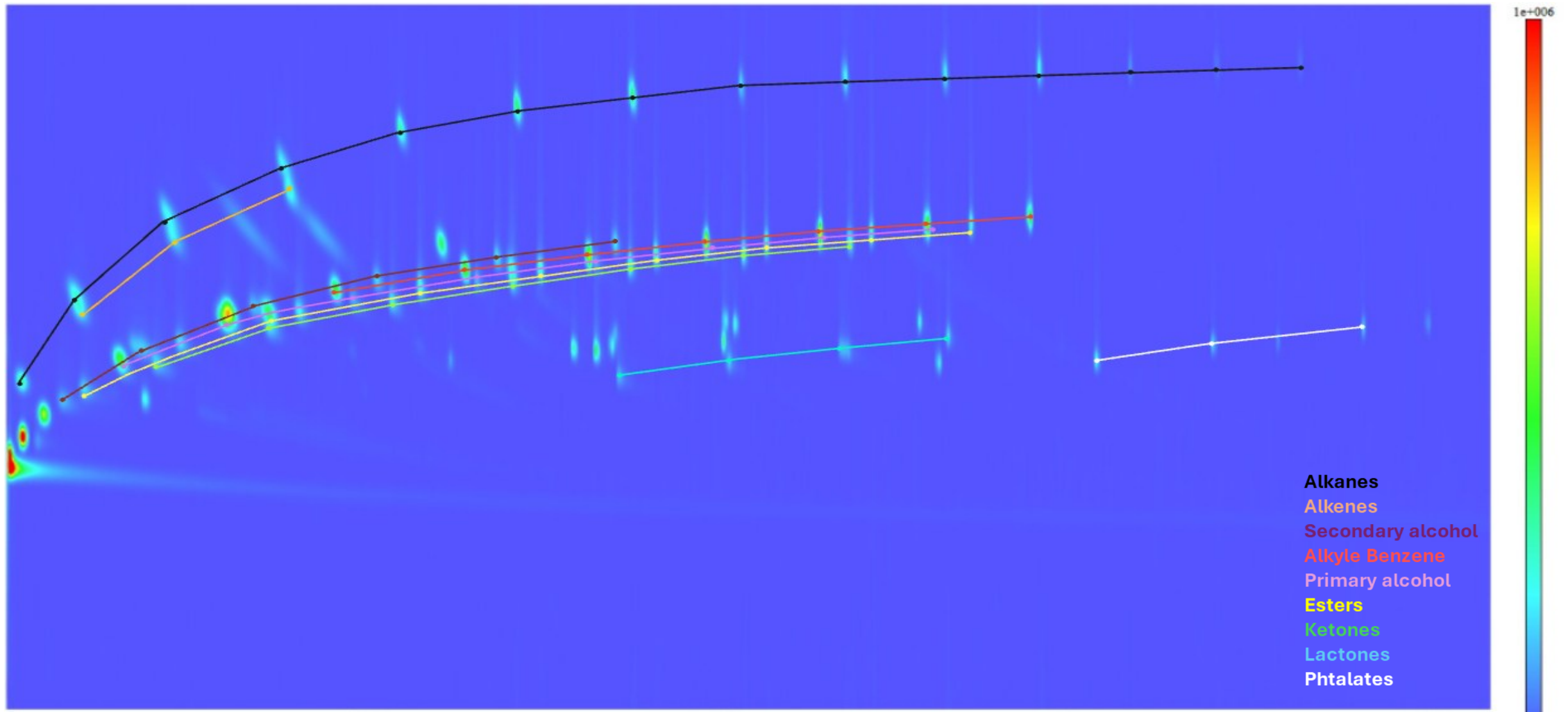
# Century mix

- Normal column set



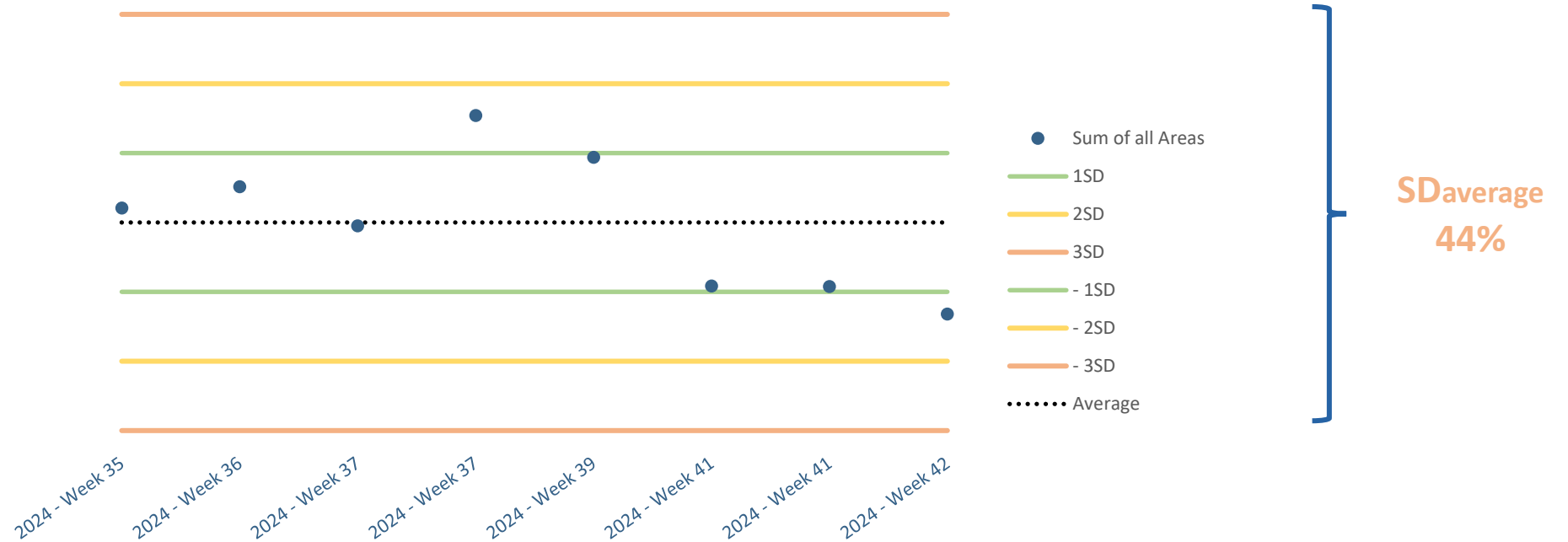
# Century mix

- 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)

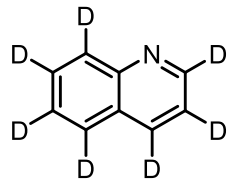


# Instrument follow-up QC chart using the CM

- Without **ISTD** correction

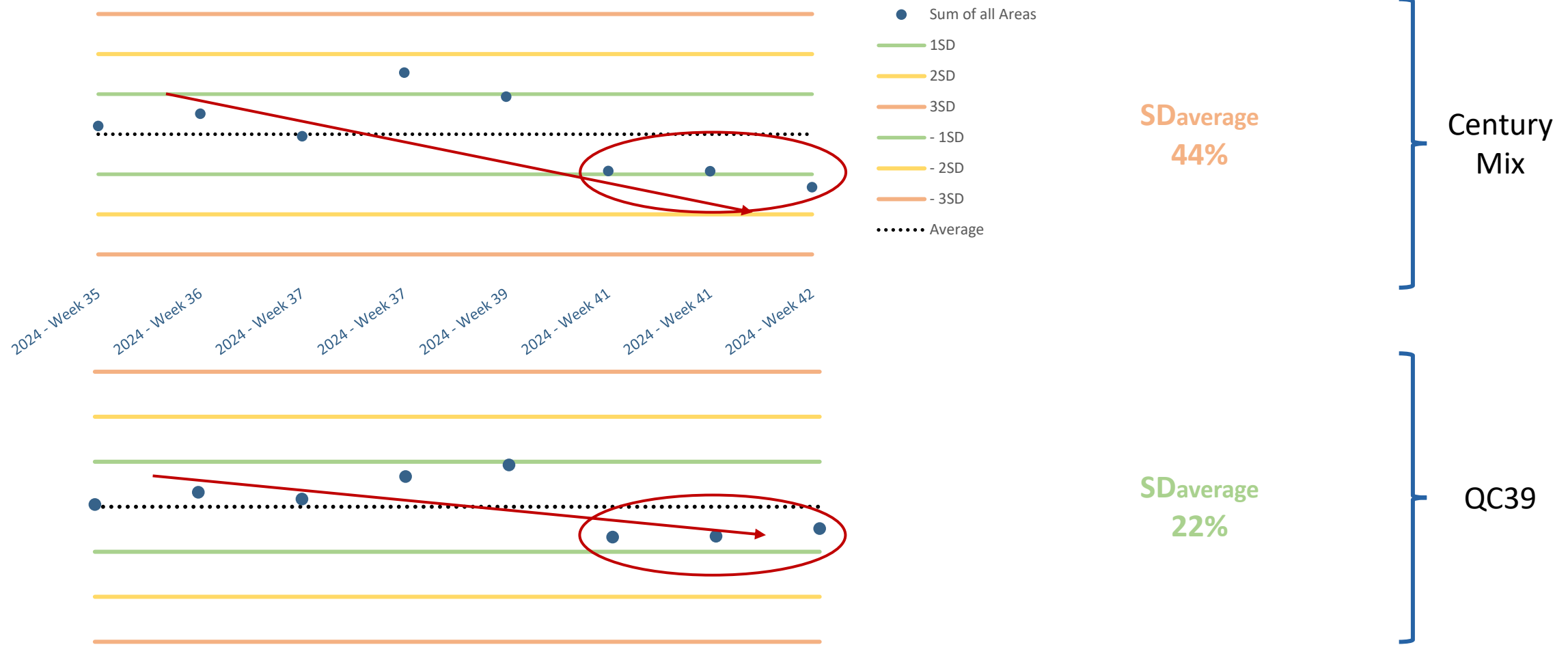


- With **ISTD** correction



# Instrument follow-up QC chart using the CM

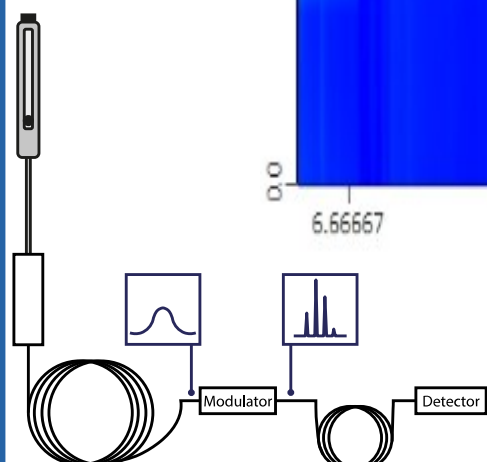
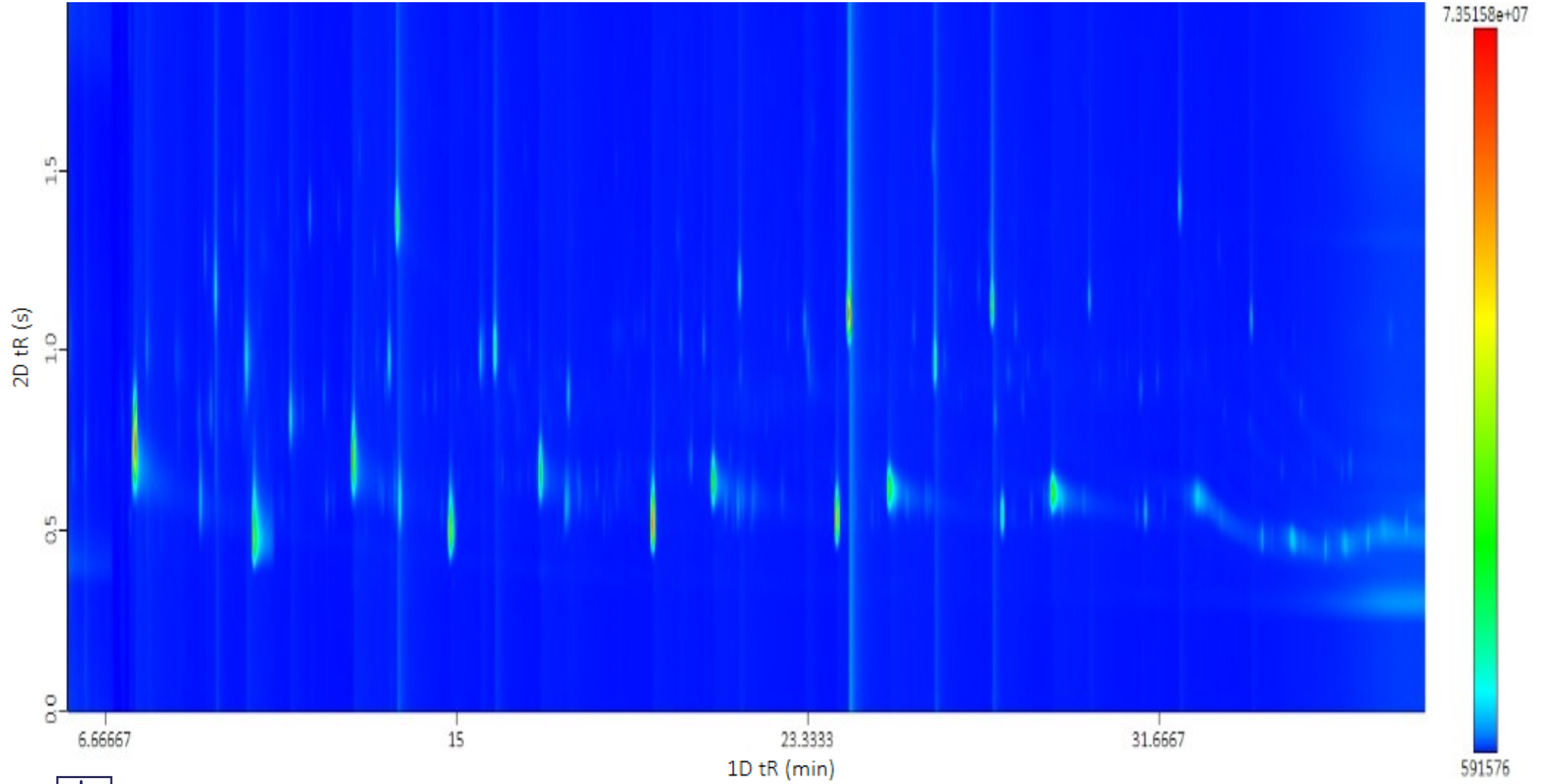
- CM vs. QC39



# QA for study follow-up



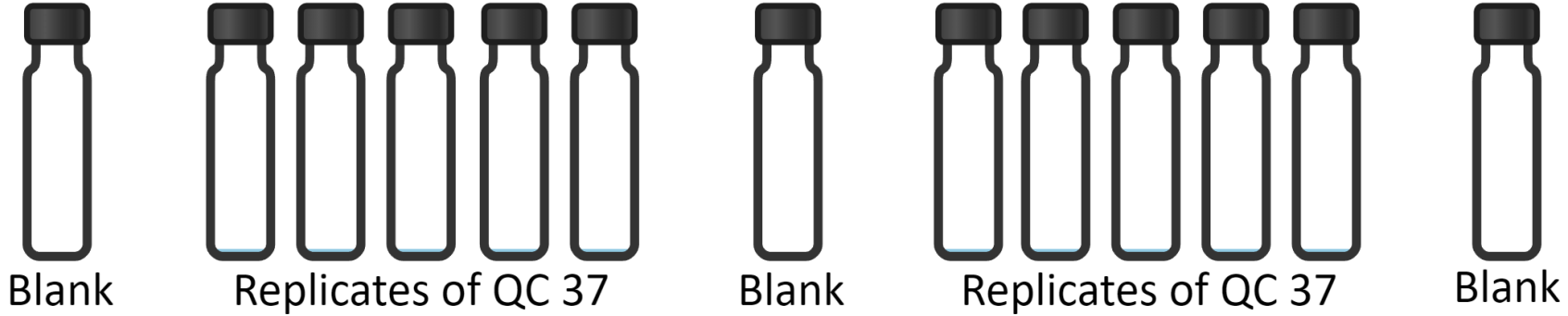
Djulia  
Bensaada



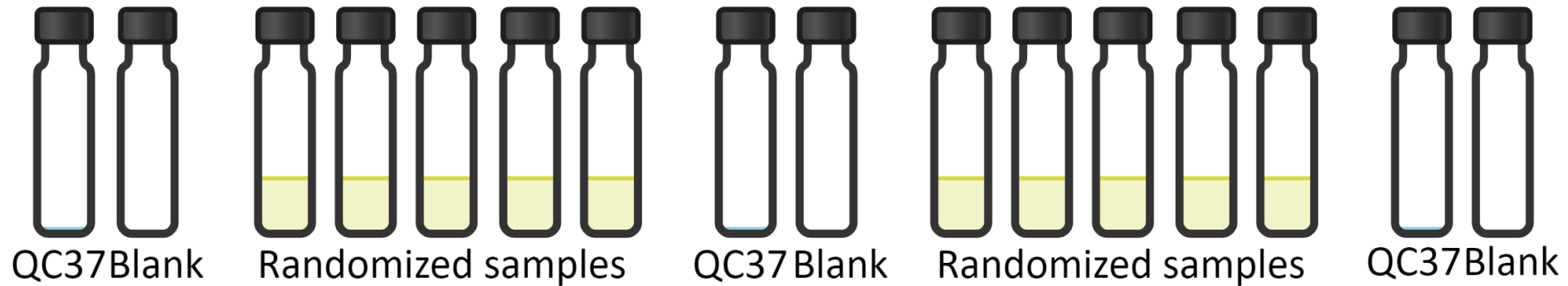


Djulia Bensaada

## Baseline for QC Chart



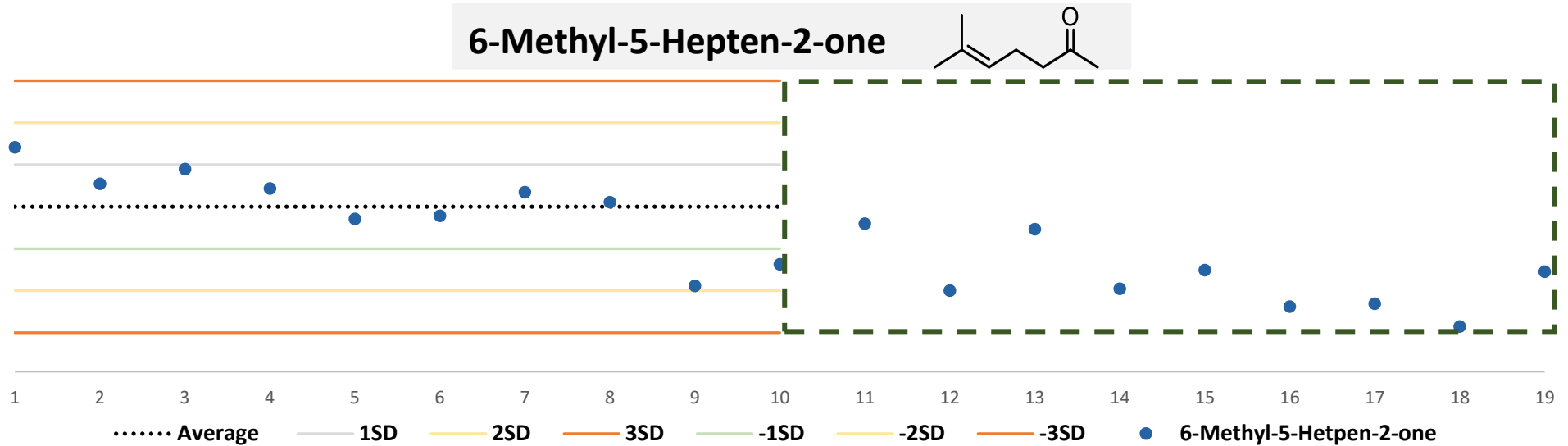
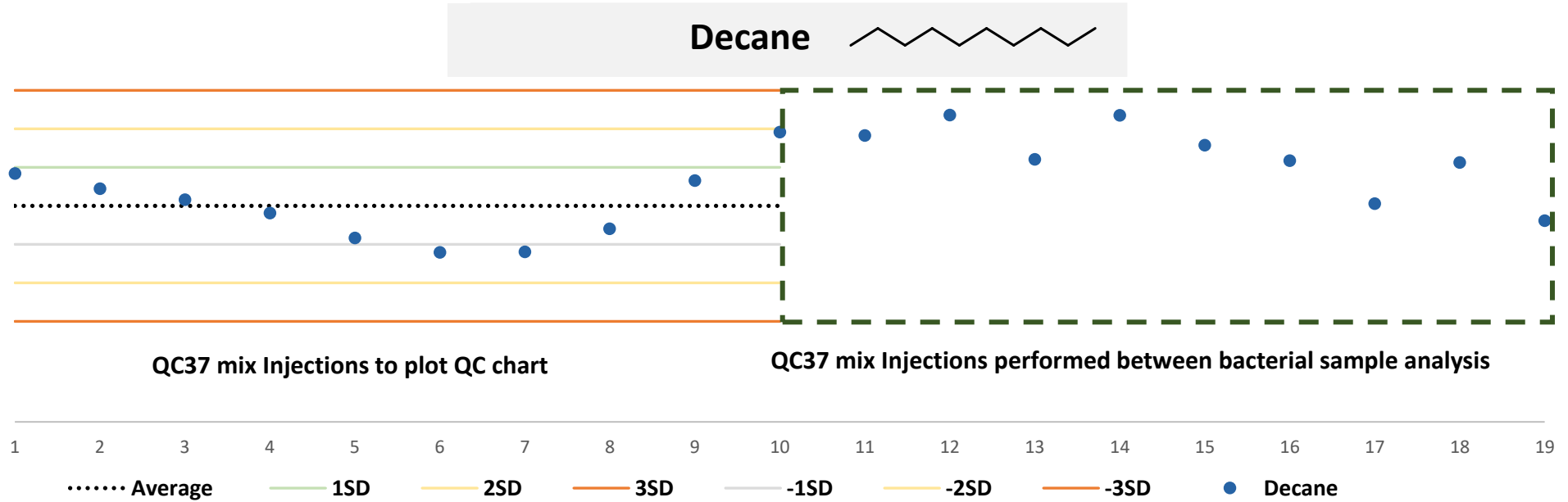
## Randomized bacterial sample analysis with QC samples



# QA for study follow-up



Djulia Bensaada



# 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

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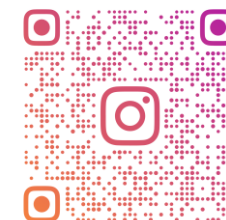
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Roberto



ROBERTO\_OBIACHEM



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