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Electrospray Ionization and samples complexity in Meta-metabolomics: a biomarker or a suppressed ion?

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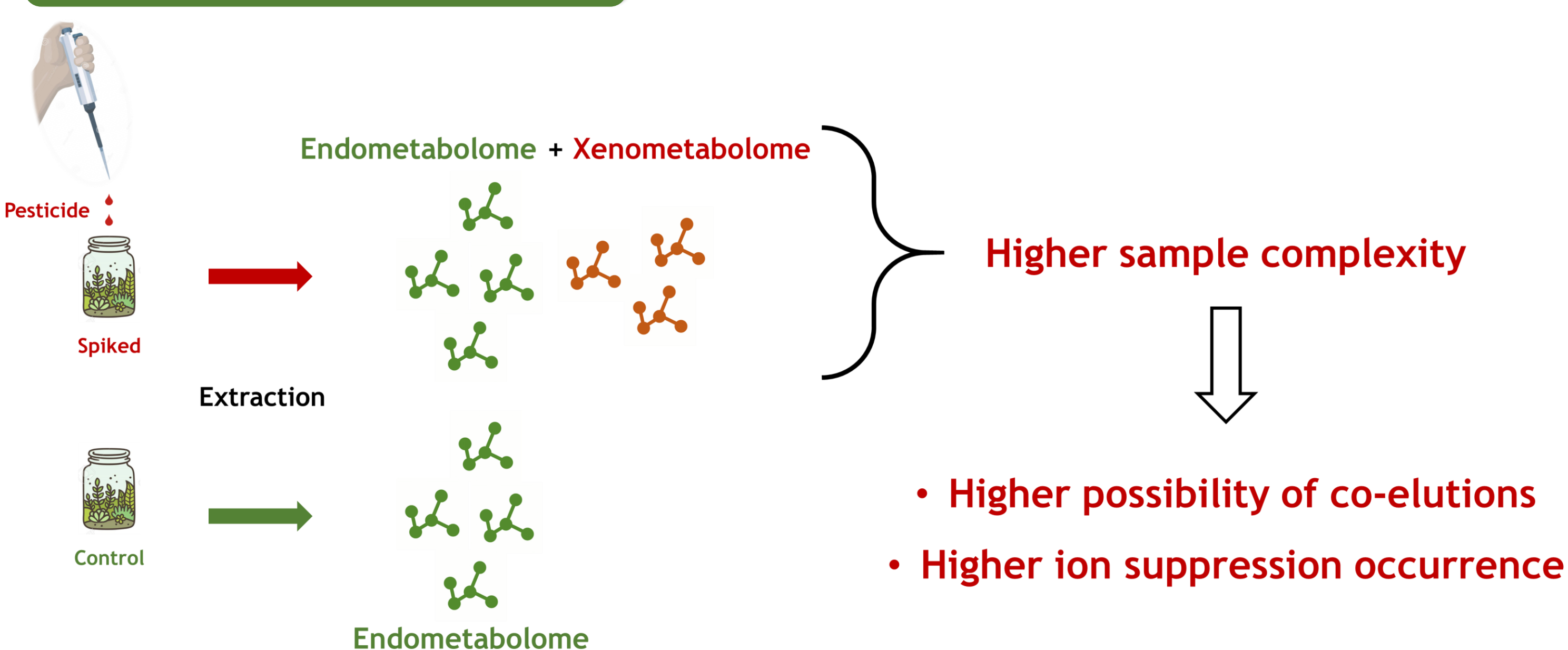
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Introduction

Electrospray Ionization is one of the most used ionization techniques for LC-MS-based metabolomics. However, it presents several drawbacks, e.g. the ion suppression phenomenon, causing ion intensity decrease. More the sample is complex, higher is the occurrence of the phenomenon. Thus, studying samples with different complexities may lead to consider some biologically non-significant molecular traces as markers of discrimination. This is due to ion suppression occurring in complex samples.

1. Problematic



In case of co-elution of an endometabolite and a xenometabolite:

- » An ion suppression for the endometabolite may occur
- » This provokes a decrease of its signal in the spiked sample
- » The signal of this endometabolite will not be impacted in the control sample
- » The endometabolite will be considered as a marker of discrimination
- » The discrimination is biologically insignificant: potential false positives

2. Experiment

2.1. Sample preparation

- ✓ Environmental sample: lagoon sediments (5 replicates/group)
 - Control (Ctr): non-spiked samples (5 µL of H₂O)
 - Spiked (Bti): 5 µL of 10× field dose of *Bacillus thuringiensis israelensis*
- ✓ Extraction performed after 15 days of incubation in microcosms
- ✓ Meta-metabolome extraction: ACN-based QuEChERS (Lehotay 2006)

2.2. Chemical and statistical analyses

- LC-HRMS**
- Thermo Vanquish UHPLC system
 - Phenomenex Kinetex 2.6 µm Polar C18 - mobile phases: H₂O/ACN + 0.1 % FA
 - Bruker maXis ESI-QToF High Resolution Mass Spectrometry
 - ESI+ FullMS scan range: m/z 80-1600

- Data Analysis**
- Data preprocessing: Workflow4metabolomics platform
 - Statistical analysis: MetaboAnalyst platform
 - Manual processing: Compass DataAnalysis 4.3 software
 - Manual mathematical analysis: Microsoft Excel 2016

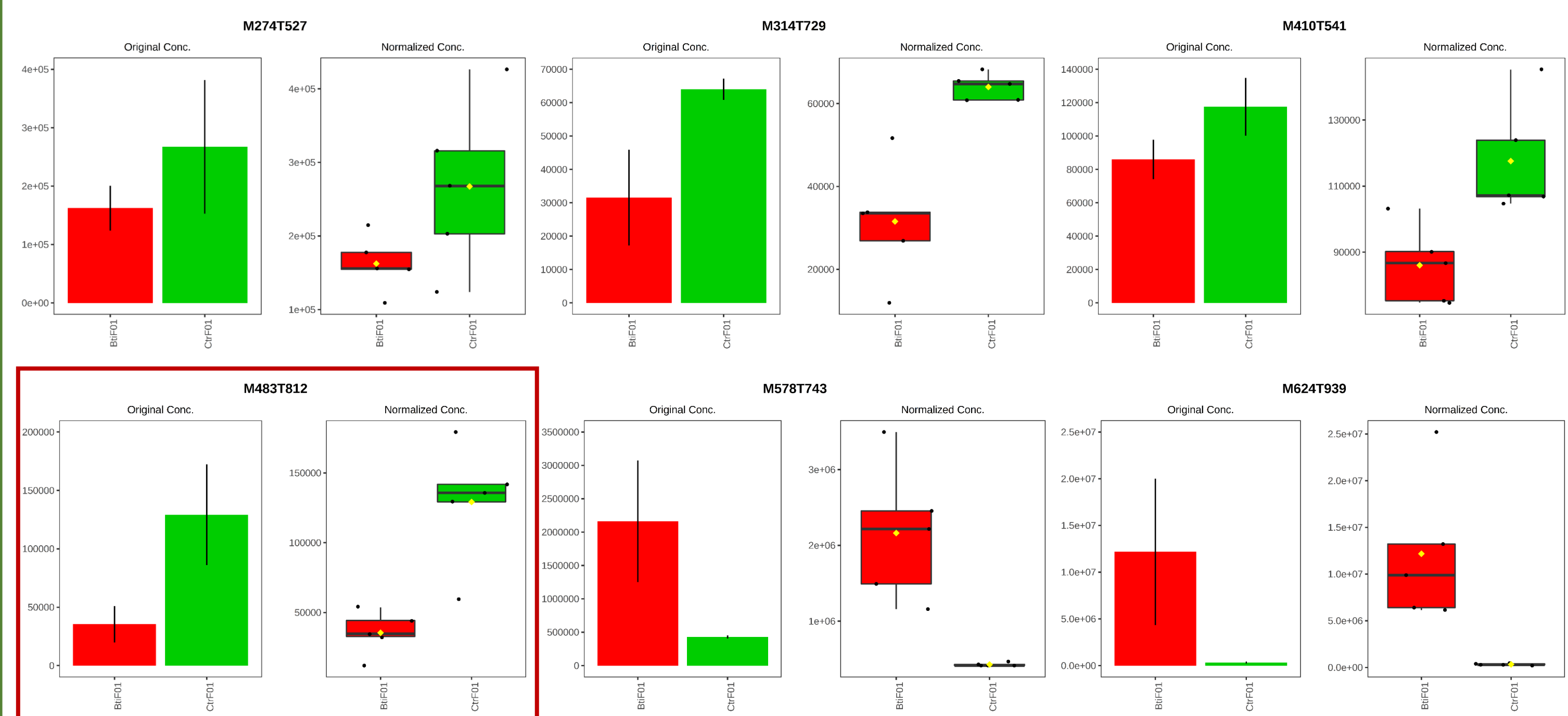


2.3. Suppressed ions/biomarkers verification

- ✓ Application of a series of dilutions on the different sample extracts
- ✓ 5 concentration levels: 1, 1/2, 1/4, 1/6, 1/10
- ✓ 3 replicates/group for each concentration level
- ✓ The matrix effect/ion suppression occurrence decreases by dilution

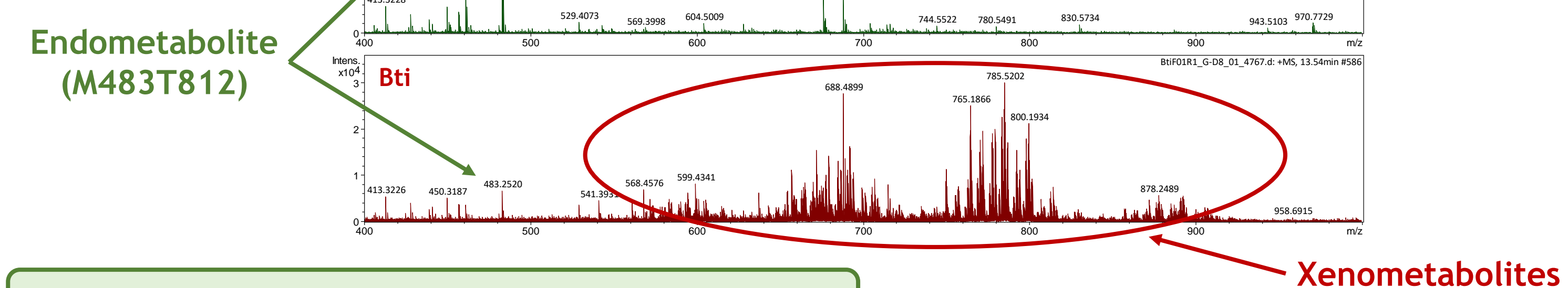
3. Results

3.1. Endogenous markers of discrimination



Raw data check:

ex: M483T812

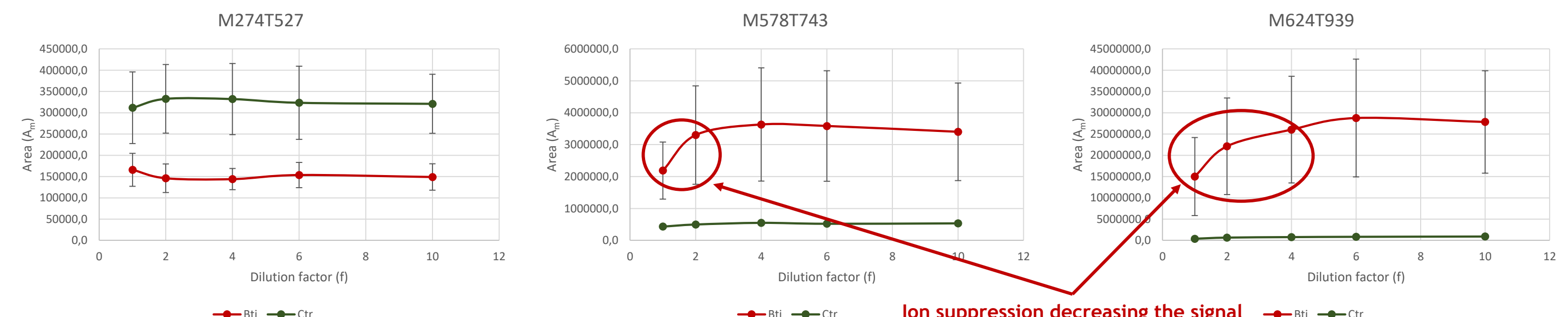


3.2. Confirmation of the ion suppression hypothesis

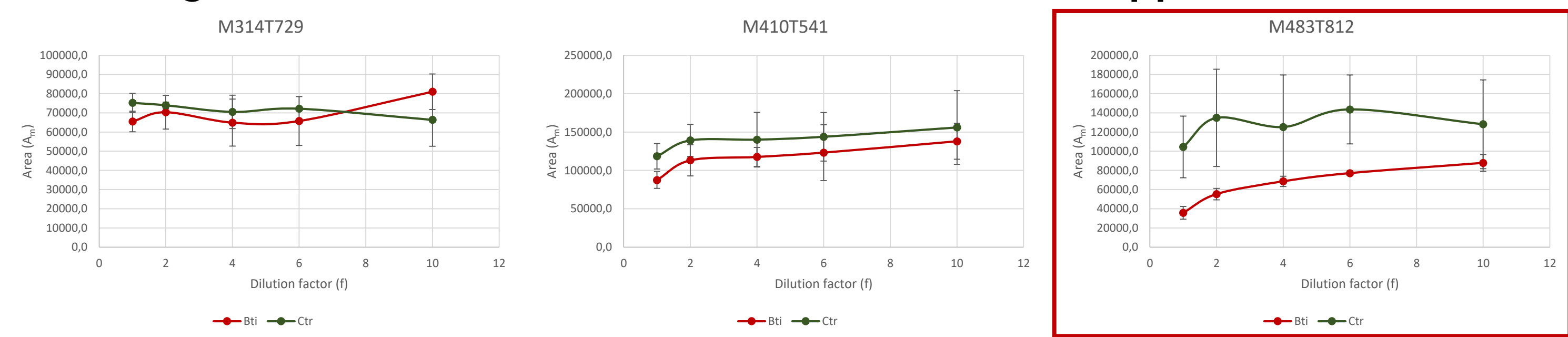
$$A_m = A_r \times f$$

A_m : Multiplied peak area; A_r : Real peak area; f : Dilution factor

➤ The significant difference was maintained after dilution: biomarker ✓



➤ The significant difference was lost after dilution: suppressed ion ✗



4. Conclusions

Critical analysis of the analytical data is essential for reliable conclusions

In case of co-elution between endometabolites and xenometabolites:

- ✓ The significance of the difference in relative-concentration should be revised
- ✓ A series of dilutions should be applied and tested
- ✓ If the significant difference is maintained after dilution: biomarker is validated
- ✓ If the significant difference is lost after dilution: the marker is a suppressed ion

Acknowledgments



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RGate Profile

