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CCS-aware wide-scope target screening utilizing LC-TIMS-HRMS and a new heated ESI source – The answer to environmental and human biomonitoring challenges

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Introduction

Thousands of chemicals are dispersed throughout the environment and may threaten ecosystems and health. Through environmental and human biomonitoring, the exposure to persistent, bioaccumulative and toxic substances can be assessed. Nowadays, various chemical classes can be reliably identified and quantified, thanks to target screening using high-resolution mass spectrometry coupled to chromatographic techniques. However, the **sample complexity** often results in **high matrix effects** and hampers the detection of compounds with low concentration (**suppressing ionization efficiency of compounds below signal-to-noise ratio**). Reliable detections are also prevented by the **complexity of MS/MS data produced by data-independent acquisition (DIA) modes**. Therefore, apart from the established target screening workflow (retention time, MS1 and MS2), **a new technology is required to identify xenobiotics with high confidence**.

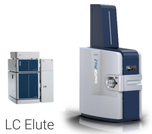
Methods

Reference standard curves & standard addition curves at a wide concentration range

Spiked samples: biota (bird eggs) & human biospecimens (urine)

Sample preparation: Generic protocols for simultaneous extraction of semi-polar to polar xenobiotics (e.g. pesticides and drugs)

Instrumentation: LC-TIMS-QTOFMS with optimized broad mass and mobility bbCID mode (DIA)



timsOFF & timsON

Data treatment: Targeted analysis using TargetScreener 4D platform for the screening of xenobiotics (precursor ion formula, RT, MS1 and MS2 qualifier ions, ion mobility-derived CCS values)

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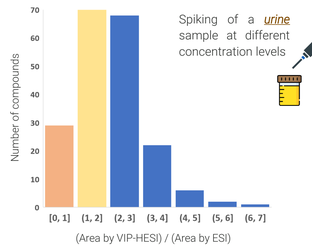
Results

Ionization efficiency: ESI vs VIP-HESI



Spiking of a urine sample at different concentration levels

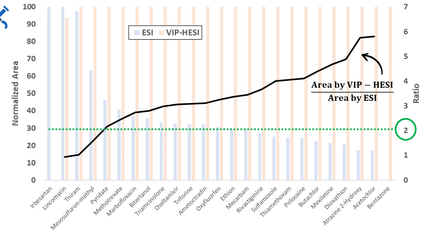


Figure 1: Distribution of 198 detected spiked compounds into groups based on the ratio of the peak area using VIP-HESI source to the peak area using ESI source at $C_{instrumental}$ = 5 µg/L

Figure 2: Presentation of a representative set of 25 compounds spiked at $C_{instrumental}$ = 25 µg/L that cover a wide range of the following chemical properties: (1) m/z, (2) logKow, (3) RT, (4) logIE (developed for ESI), (5) CCS

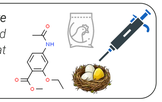
Based on the results presented in Figure 1:

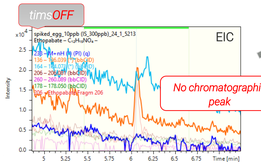
- *50% of the spiked compounds have >2 times higher response using VIP-HESI source in comparison with ESI.*
- *35% of the spiked compounds have no significant differences in response with both sources (ratio 1-2).*
- *15% of the spiked compounds show decreased response or are not detected using VIP-HESI source in comparison with ESI.*

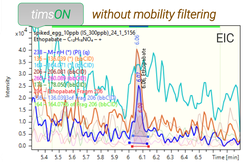
By utilizing VIP-HESI source, lower method LODs can be achieved for half of the compounds.

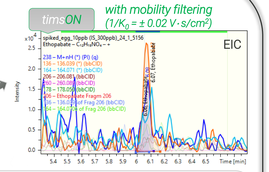
Incorporation of TIMS in LC-VIP HESI-QTOF MS

Example of ethopabate (veterinary drug) spiked in an egg sample at $C_{instrumental}$ = 10 µg/L









Addition of TIMS dimension

Peak detection

Improved signal-to-noise ratios

Data processing with ion mobility filtering

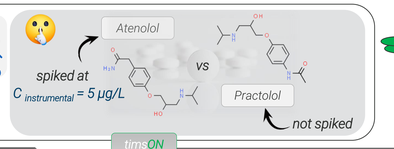
The background signal caused by the presence of co-eluting isobaric analytes and matrix components is deconvoluted.

Full-scan and bbCID EICs of even higher quality

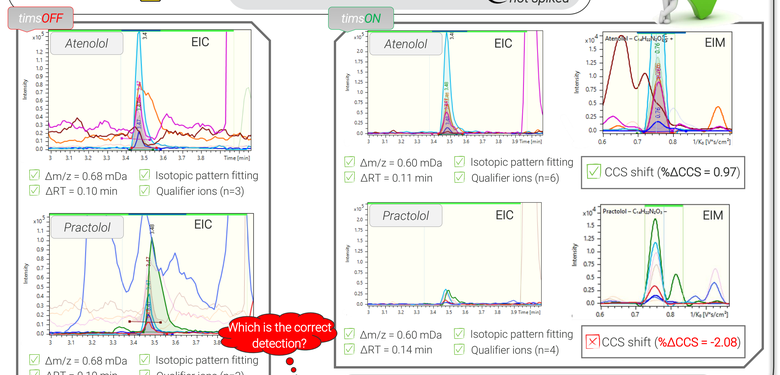
Data processing with timsOFF vs timsON

Example on the identification of co-eluting isomeric compounds in a spiked urine sample

Investigation of the presence of the isomeric compounds atenolol and practolol (pharmaceuticals)



Correct answer: Atenolol



With timsON:

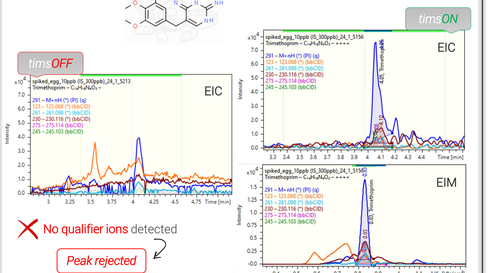
%ΔCCS as additional criterion

Reduction of false positive results

Example on the effect of ion mobility dimension in target screening using a spiked egg sample

Trimethoprim

$C_{instrumental}$ = 10 µg/L



No qualifier ions detected

Peak rejected

Detection of qualifier ions due to mobility filtering

CCS value as additional identification parameter

Peak identified

With timsON:

- *%ΔCCS as additional criterion*
- *Presence of qualifier ions due to mobility filtering*

Reduction of false negative results

Overall results of the detected compounds spiked at a urine sample at $C_{instrumental}$ = 5 µg/L

Spiked compounds detected by:

- CCS values as additional identification criterion
- TIMS-aligned full-scan MS and bbCID MS/MS spectra

...resulted in 45 additional compounds only detected with timsON

Not detected by timsOFF

Peak detected, but no qualifier ions to confirm identification by timsOFF

False negatives

With timsON, confirmation by additional identification criteria:

CCS and Qualifier ions 56% | only CCS 44%

With timsON:

Detection of additional compounds compared to timsOFF due to CCS values and qualifier ions

Conclusions

- VIP-HESI source offers enhanced sensitivity for a great number of compounds, achieving lower LODs in many cases.
- The addition of TIMS dimension in LC-HRMS improves signal-to-noise ratio.
- Ion mobility filtering during data processing provides higher quality chromatograms and full-scan MS and bbCID MS/MS spectra.
- The overall identification confidence is increased by adding CCS values.

More reliable identification of xenobiotics and lower method LODs are achieved in environmental and human biomonitoring studies.

Ion Mobility: Applications I