TargetScreener 4D workflow: Providing solutions in environmental monitoring studies

Abstract

This Application note highlights the importance of advanced analytical methodologies for comprehensive environmental monitoring to control threats from thousands of daily-released organic chemicals. Trapped Ion Mobility Spectrometry (TIMS) coupled to High Resolution Mass Spectrometry (HRMS) enhances the reliable identification of various contaminants, including several with persistent bioaccumulative and toxic characteristics (PBT).
TIMS offers significant advantages by improving the data quality. Moreover, the TargetScreener 4D database includes ion mobility-derived collision cross section (CCS) information for thousands of target compounds, ensuring comprehensive and confident compound identification.

Keywords:
TargetScreener 4D, timsTOF, CCS values, environmental monitoring

Introduction

Thousands of chemicals originating from anthropogenic sources are released daily into the environment. There is an increasing concern to humans and animals health as well the environment in general. Therefore a comprehensive and systematic environmental monitoring is necessary for the establishment of effective mitigation measures for contaminants with PBT properties. This study investigates the unique contribution of TIMS to HRMS to achieve the confident identification of thousands of contaminants. TIMS adds highly significant benefits which have an extreme importance when dealing with a large number of targets.
Aside of simply applying the CCS values as an added criterion for compound identification, TIMS generates an additional, truly orthogonal separation to HPLC and MS and efficiently cleans up background noise in both MS and MS/MS for increased ID scoring in different environmental matrices.
Therefore standard solution mixes containing more than 1,000 environmental contaminants in total were used for the TargetScreener 4D database setup. CCS values were registered for each ion species, making it an efficient solution for comprehensive compound identification with highest confidence.
The included compounds belonged to several classes, such as pharmaceuticals, personal care products, drugs of abuse, pesticides, as well as their transformation products. As test samples, different environmental matrices were utilized, representing the challenge and needed performance for a comprehensive screening approach.

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Experimental

The typical concentration levels for the standard solution mixes containing > 1,000 environmental contaminants were C=100 ug/L. The solutions were analyzed in triplicate to ensure repeatability. General sample preparation protocols for the extraction of a broad range of contaminants from different environmental matrices were applied to reduce differences in the results by preparation artefacts. All analyses were performed using the Elute UHPLC, the ESI source (Apollo II) and the timsTOF Pro 2 HRMS (Bruker, Bremen, Germany).
Data was acquired in broadband Collision Induced Dissociation (bbCID) mode with either ion mobility switched on or off (timsON or timsOFF). This acquisition mode is a data independent method providing possibilities of retrospective analysis. TargetScreener 4D methods and database were applied for sample screening and confident identification and validation of all targets.

Database setup and data quality check

As a starting point, a CCS-aware database with a high number of environmental contaminants was established. The CCS values were determined experimentally for the analytes for which standards were available. For the remaining analytes that were included in the previous version of the 3-dimensional TargetScreener, CCS prediction was implemented from their elemental composition and structure with a machine learning approach. CCS prediction was also implemented for analytes with experimental CCS values, and both matched typically within 2% error. Figure 1 shows a part of the library containing compound information for:

- Exact *m/z* values representing the most abundant ion in full scan MS
- Retention time (RT) for the applied standard UHPLC separation method
- $1/K_0$ and the derived CCS values for each ion species corresponding to a compound
- Qualifier bbCID MS/MS ions & full scan MS ions (ions with >50% relative intensity are set as mandatory ions)

m/z	RT	Formula	Name	CCS	1/K0	Qual1	Qual2	Qual3	Qual4	Qual4 Name	Qual4 1/K0	Qual1 Spectrum Type	Qual2 Spectrum Type	Qual3 Spectrum Type	Qual4 Spectrum Type
303.2319	9.47	C20H30O2	17-alpha-Methyltestosterone	177.63	0.8487	97.0648	109.0648	285.2213				bbCID	bbCID	bbCID	fullscan
313.0795	7.96	C14H11F3N2O3	5-Hydroxyflunixin	168.58	0.8065	295.0689						bbCID	bbCID	bbCID	fullscan
328.1543	3.87	C19H21NO4	6-O-Monoacetylmorphine (MAM)	176.31	0.8451	211.0754	268.1332	193.0648				bbCID	bbCID	bbCID	fullscan
284.1194	5.05	C16H14N3O1F1	7-Aminoflunitrazepam	164.48	0.7836	135.0917	227.0979	256.1245				bbCID	bbCID	bbCID	fullscan
337.2122	4.44	C18H28N2O4	Acebutolol	190.11	0.9122	116.1070	72.0808	74.0600				bbCID	bbCID	bbCID	fullscan
170.1176	2.93	C9H15NO2	Aceclidine	138.31	0.6400	82.0651	128.1070	110.0964				bbCID	bbCID	bbCID	fullscan
238.0993	9.81	C13H17ClNO^1+	Alachlor Fragm 238	149.85	0.7079	162.1277	147.1043	132.0808	270.12553	Alachlor (M+H)	0.7513	bbCID	bbCID	bbCID	fullscan
198.1349	4.91	C8H15N5O	Atrazine 2-Hydroxy	147.94	0.6915	156.0880	86.0349	114.0662	395.2626	Atrazine 2-Hydroxy (2M+H)	0.9994	bbCID	bbCID	bbCID	fullscan
404.1241	8.45	C22H17N3O5	Azoxystrobin	195.72	0.9452	372.0979	344.1030	329.0795				bbCID	bbCID	bbCID	fullscan
309.1822	3.29	C17H20N6	Baquiloprim	179.10	0.8564	294.1587	277.1322	171.0917				bbCID	bbCID	bbCID	fullscan
316.0080	6.71	C14H10N3O1Br1	Bromazepam	162.29	0.7767	182.0839	209.0947	288.0131				bbCID	bbCID	bbCID	fullscan
319.0804	5.72	C16N2H19Br	Brompheniramine	167.19	0.8005	274.0226	167.0730					bbCID	bbCID	bbCID	fullscan
195.0877	4.27	C8H10N4O2	Caffeine	140.46	0.6559	138.0662	83.0604	110.0713				bbCID	bbCID	bbCID	fullscan
441.1670	6.94	C24H20N6O3	Candesartan	196.75	0.9528	207.0917	263.1291	235.0978				bbCID	bbCID	bbCID	fullscan
341.2111	8.81	C22H28O3	Canrenone	185.87	0.8923	107.0855	97.0648	187.1117				bbCID	bbCID	bbCID	fullscan
306.2064	9.38	C18H27NO3	Capsaicin	183.16	0.8755	137.0597	122.0362	69.0699				bbCID	bbCID	bbCID	fullscan

Figure 1.
Establishment of a library containing compound information.

Ethirimol

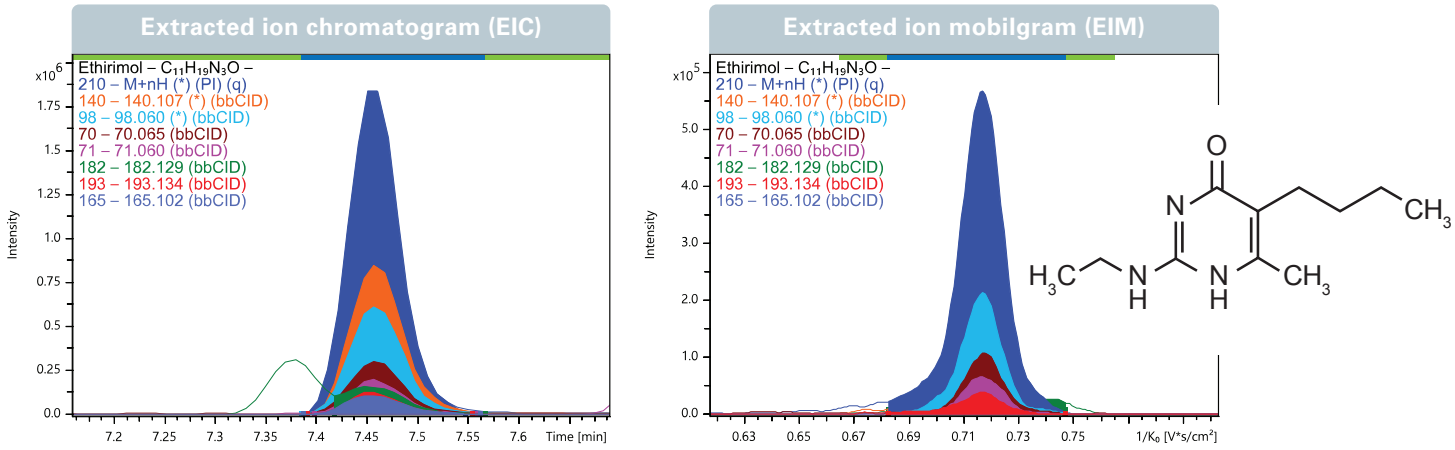


Figure 2. Extracted ion chromatogram and ion mobilogram for Ethirimol at a reference standard solution of 100 ppb or µg/L.

Figure 2 shows the extracted ion chromatogram (EIC) and the extracted ion mobilogram (EIM) of the fungicide Ethirimol in the TASQ® (Target Analysis for Screening and Quantitation) software as one example.

Quality assurance for CCS measurements

To ensure the quality of the CCS measurements, a quality control protocol was implemented. Triplicate analyses of a standard biomolecule mixture were performed. These biomolecules were part of the Unified CCS Compendium [1] with validated CCS values based on DTIMS-HRMS.
These measurements were carried out before, during, and after analyzing the standard solution mixes. The protocol aimed to evaluate CCS accuracy by calculating %ΔCCS between the experimental and the reference CCS values. Additionally, CCS precision was assessed in terms of %RSD (n=3).

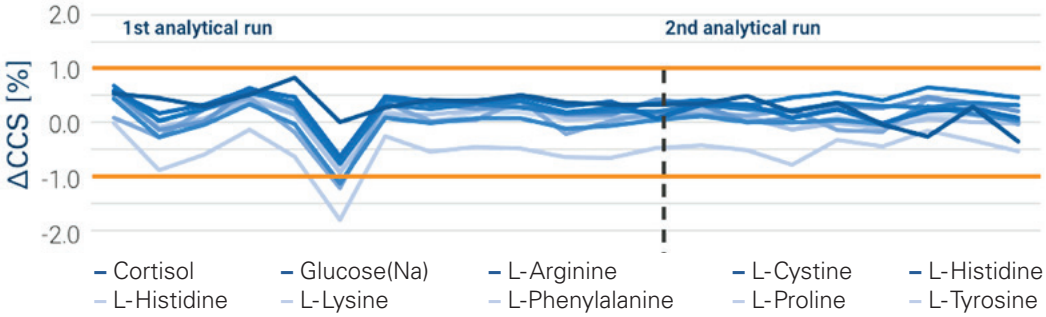


Figure 3. Evaluation of injections before, during and after each analysis.

[1] J. A. Picache et al., Collision cross section compendium to annotate and predict multi-omic compound identities, Chem. Sci., 2019, 10, 983–993, DOI: 10.1039/C8SC04396E.

Criteria for acquiring CCS measurements:

- Individual compound RSD ≤ 0.7% [1]
- Maximum individual ΔCCS ≤ 1% [1]
- %ΔCCS of experimental CCS values in comparison with the corresponding literature values measured by drift tube ion mobility <2%

Separation of isomeric compounds based on varied ion mobilities

In Figure 4, the extracted ion chromatogram of *m/z* 264.1958 is presented, illustrating the presence of two isomeric compounds, namely O-Desmethylvenlafaxine, a primary metabolite of the antidepressant Venlafaxine, and the opioid analgesic Tramadol, in hospital wastewater. Due to their structural similarity (isomeric compounds), these compounds exhibit significant overlap when separated solely using UHPLC. However, by combining the known RT of each compound with ion mobility, distinct and well-separated mobility peaks for the two compounds (represented by green and blue colors) can be observed. Furthermore, utilizing the known $1/K_0$ values (ion mobility values K_0) not only achieves fully resolved EIM, but also enables confident identification of each isomeric compound due to mobility filtering of the EICs.

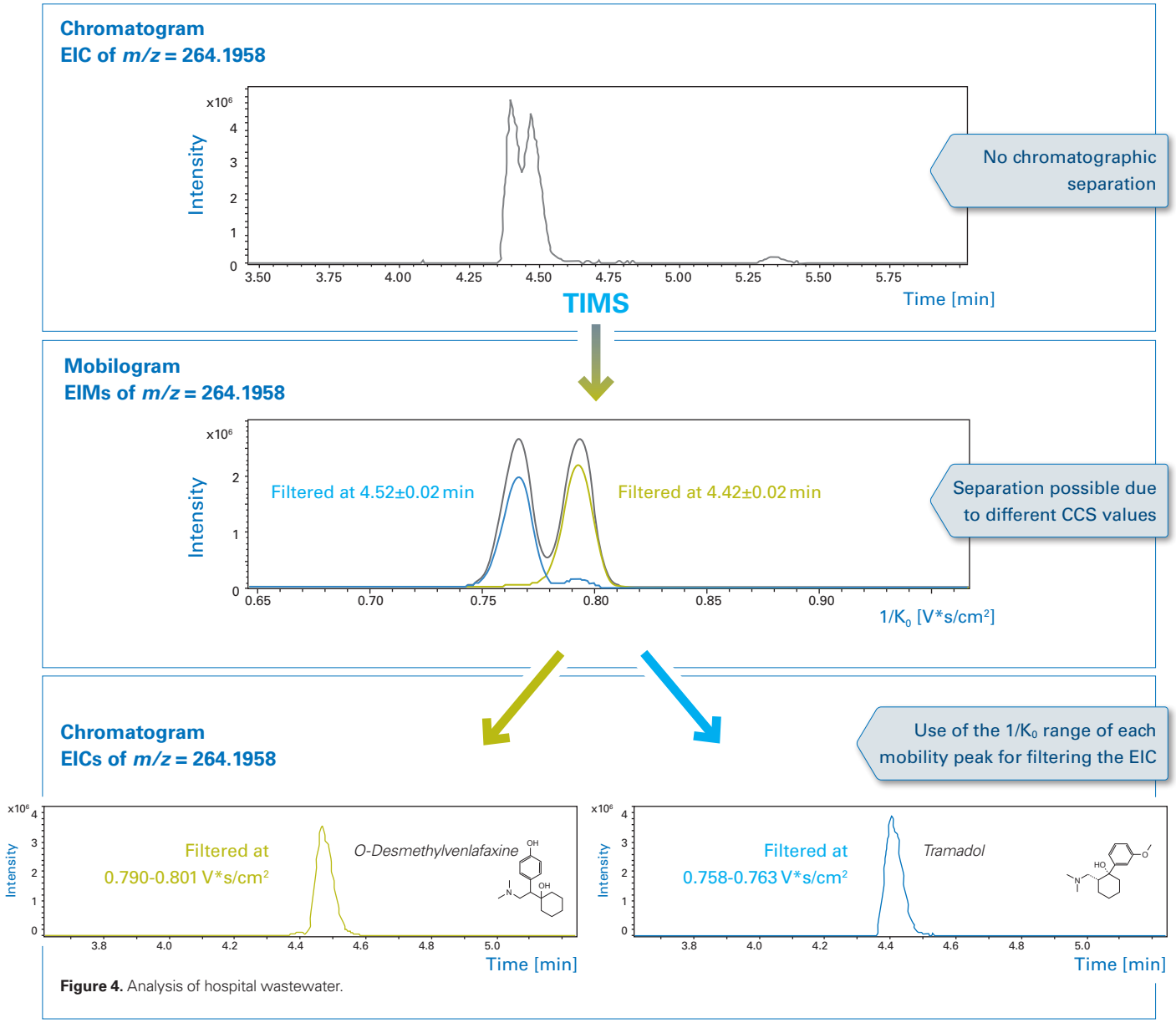
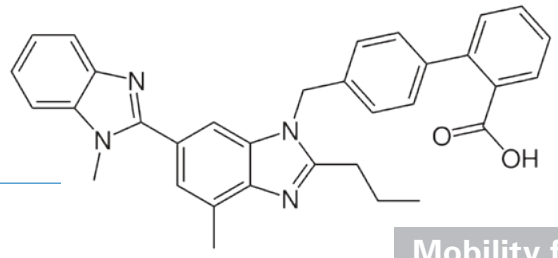


Figure 4. Analysis of hospital wastewater.

Achieving lower method detection limit

In addition to facilitating the separation of isomeric compounds and introducing a new criterion for compound identification, ion mobility significantly enhances the quality of MS and MS/MS data, leading to a remarkable improvement in analysis sensitivity. Figure 5 showcases two EICs of the drug Telmisartan (*m/z* 515.2442 ± 0.005 Da) in seawater, one with mobility filtering OFF and the other with mobility filtering ON using a filter of $1/K_0$ width ± 0.01 V*s/cm^2. The signal-to-noise ratio (S/N) of the EIC increases by nearly one order of magnitude, resulting in a much cleaner peak. This enhancement has the potential to significantly improve the method limit of detection (LOD) for Telmisartan.



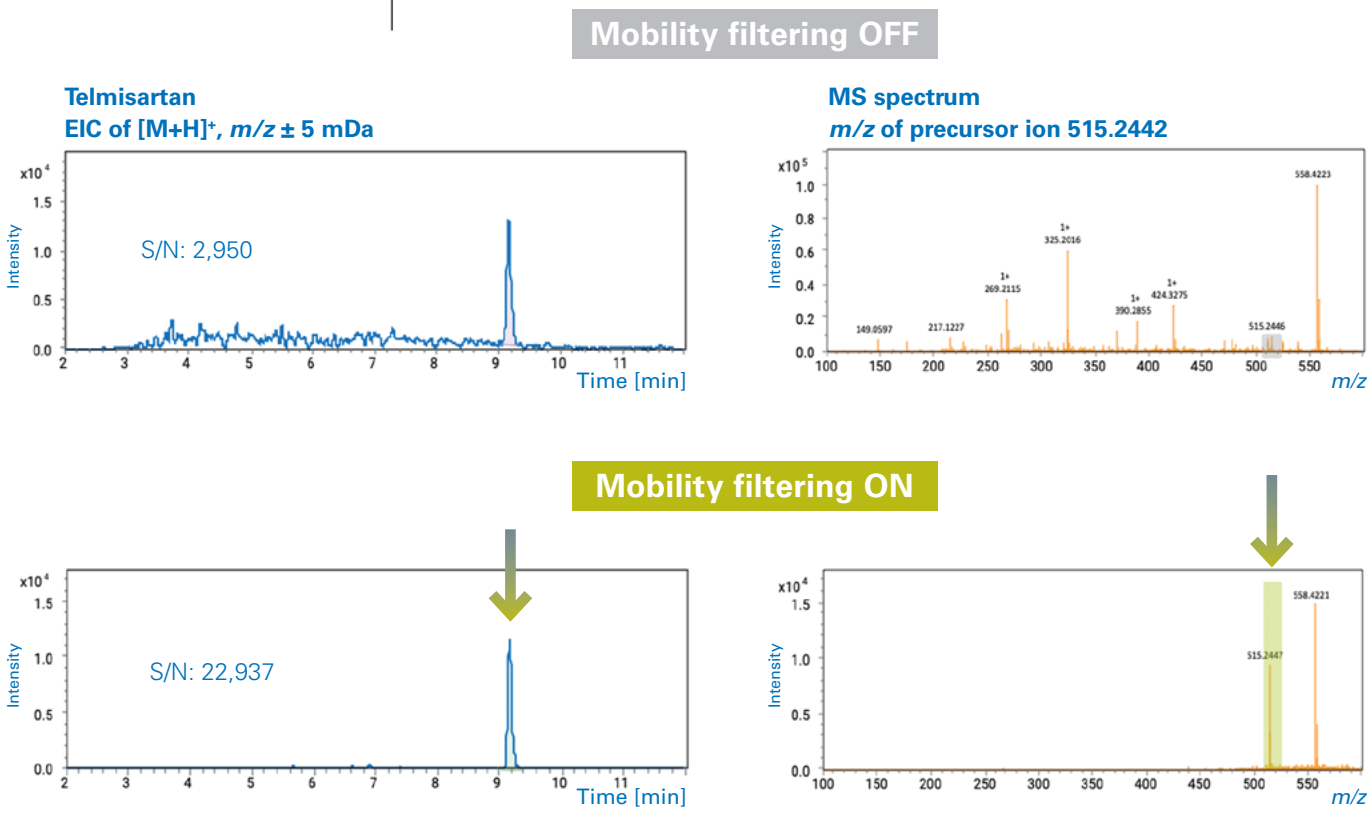


Figure 5. EICs and MS spectra of Telmisartan with Mobility filtering OFF and ON ($1/K_0$ width ± 0.01 V*s/cm^2).

This spectral clean-up from background noise is as well observed in the MS spectrum of Telmisartan itself. Applying the ion mobility filter on the acquired raw data eliminates background noise and highly cleans up the MS spectrum for the component of interest.

This demonstrates even more clearly the advantage of TIMS for achieving lower detection limits and a more definite and confident identification of each component of interest.

Comprehensive overview of analysis results in TASQ

A standard representation of the graphical user interface (GUI) and the information provided by the software TASQ regarding the investigated contaminants in a river water sample are presented in Figure 6. The GUI provides a comprehensive view of the compound identification, facilitated by the multi-dimensional MRSQC score, which considers identification parameters such as *m/z* accuracy (mass-to-charge ratio, M), retention time shift (R), isotope pattern fitting (mSigma, S), qualifier ions (Q) and CCS error values (C). This score allows for a quick visual assessment of the compound's quality.

Overview of the analysis in TASQ

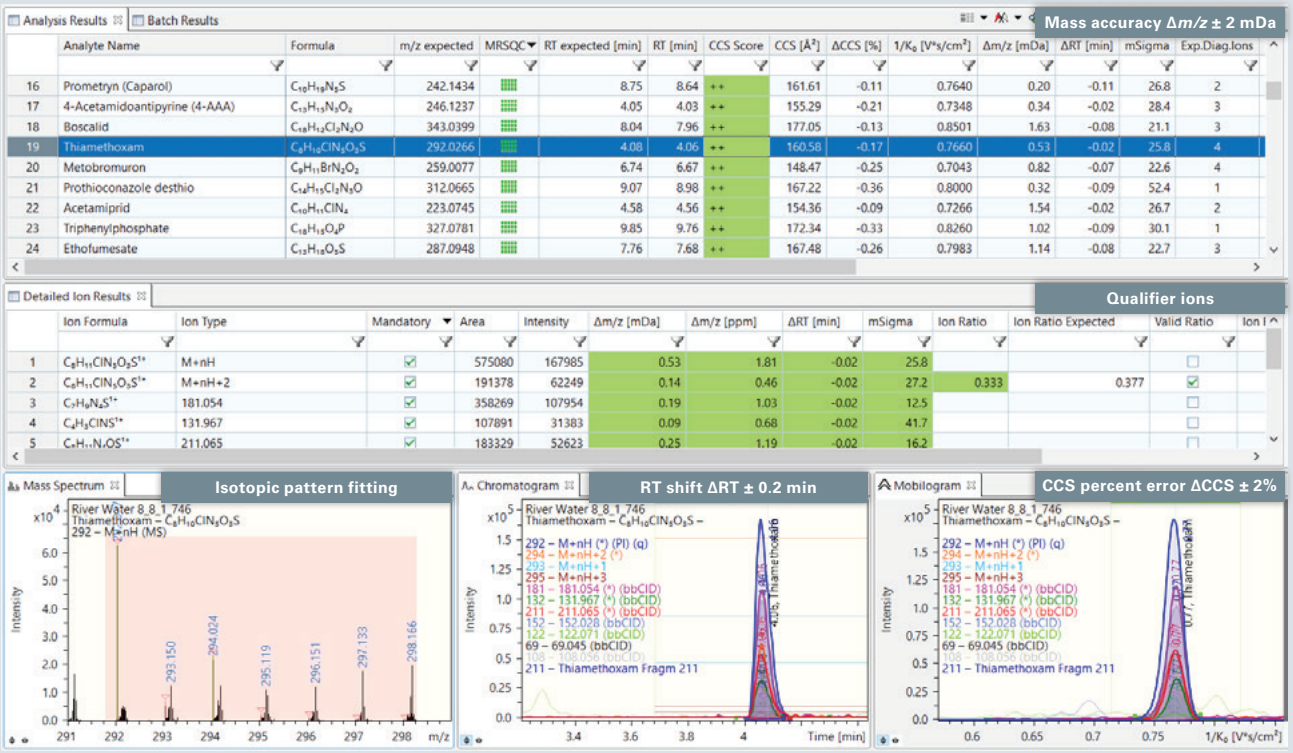
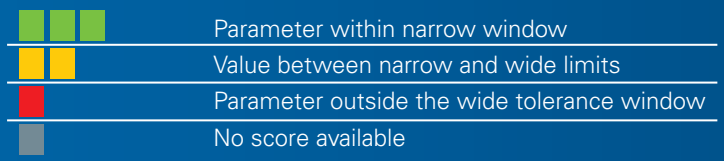


Figure 6. Overview in the software TASQ to show the overall analytical performance of the TargetScreener 4D workflow. Shown is the example of the insecticide Thiamethoxam in river water.

Mass accuracy
Retention time
m**S**igma value (Isotopic pattern)
Qualifier ions (Diagnostic ions)
CCS value

Color-coded screening results

Straightforward reviewing of either a sample or an analyte as desired by the analyst. Once reviewed, qualitative and quantitative sample reports are then easily generated.



4D-Identification: Strengthening wide-scope target screening with ion mobility-derived CCS values

This workflow typically achieves mass accuracies within the range of ± 2 mDa. By simply clicking on a specific compound of interest, users can easily access an intuitive overview of the qualifier ions' detection and matches, as well as the isotope patterns, RT, and CCS values, which can be manually inspected for further verification.

CCS scoring demonstrates the CCS robustness in standard and complex samples. Moreover, the data quality is positively influenced by using ion mobility filtering. This will result in less reviewing and more confidence in the result.

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"TargetScreener 4D is an exceptional solution for comprehensive xenobiotic screening, transforming analytical capabilities across various fields including environmental, food, and toxicology. By integrating ion mobility-derived CCS values with TIMS technology into LC-QTOFMS, confidence in compound identification is enhanced.
Ion mobility filtering during data processing improves sensitivity, enabling detection of trace-level xenobiotics. When traditional two-dimensional separation techniques may fall short, TargetScreener 4D offers exceptional performance for research and routine projects."

Summary

In this study, a CCS-aware database was etablished, containing LC-ESI-TIMS-HRMS data of more than 1,000 environmental contaminants, typically found in environmental matrices. A highly curated quality assurance of the CCS measurements was performed to validate the application and usefulness of this criterion for target identification. This results in a 4D wide-scope target screening approach for identifying contaminants in environmental samples and demonstrates the benefits of LC-TIMS-HRMS in environmental monitoring studies.

The hyphenation of TIMS with HRMS benefits environmental monitoring and improves both data quality as well as information retrieval dramatically, in particular if thousands of compounds have to be screened in a single sample simultaneously. The ion mobility filtering suppresses matrix signal and provides higher quality mass spectra even in highly complex matrices. This in turn highly improves sensitivity and limits of detection for any analyte. Ion mobility derived CCS values are an additional identification criterion and reinforce the established identification point system for wide-scope target screening, enhancing the identification confidence in environmental monitoring.

Finally, the added 4th dimension of TIMS assists in the separation of various isomeric/isobaric co-eluting compounds. Isomeric analytes like Tramadol and *O*-Desmethylvenlafaxine pose an analytical challenge. TIMS provides an additional dimension for separation of such compounds that permits quantitative analysis

TargetScreener 4D workflow including TIMS provides:

1) Clean-up of chromatograms and spectra

2) Additional ID criterion

3) Separation and quantitation of isomers

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