A Strategy for an Unknown Screening Approach on Environmental Samples Using HRAM Mass Spectrometry

Olaf Scheibner,[1] Patrizia van Baar,[2] Florian Wode,[2] Uwe Dünnbier,[2] Kristi Akervik,[3] Jamie Humphrie,[3] Maciej Bromirski[1]
[1]Thermo Fisher Scientific, Bremen, Germany; [2]Berliner Wasserbetriebe, Berlin, Germany;
 [3]Thermo Fisher Scientific, Austin, TX, USA

Overview

Purpose: Run a general unknown screening approach in an automated fashion.

Methods: Surface water samples form the city of Berlin, Germany, were analyzed in full scan/AIF mode with a Thermo Scientific™ Exactive™ Plus mass spectrometer and analyzed in a widely automated workflow using Thermo Scientific™ TraceFinder™ 3.1 and Thermo Scientific™ SIEVE™ 2.1 software.

Results: Differences in the load of contaminants could be easily determined in the different samples. Easy detection and identification of a significant number of contaminants was achieved.

Introduction

The analysis of food and environmental samples for contaminants by LC-MS has become a quick and cost-effective routine application when run in a targeted fashion, but this method disregards events or circumstances not taken into account beforehand. Run in a non-targeted fashion, this method is known to be laborious and time-consuming, making it everything but a routine application. New-generation software now links in quantitative and (unknown) screening approaches to one smoothly integrated workflow, tying together component detection capabilities of unknown screening workflows with the identification capabilities of targeted screening and quantification software. Here we show how one data set can serve for routine high throughput quantitative analysis and for versatile non-targeted investigations in a highly automated manner.

Methods

Sample Preparation

Four samples of surface water from different sources were taken and analyzed without any further treatment. In addition, one neat standard as a control sample and one tap-water sample as a reference sample were run in the same sequence.

Liquid Chromatography (or more generically, separations)

For online sample concentration and chromatographic separation, a Thermo Scientific™ EQuan™ LC-MS system was used (Figure 1). A sample volume of 1000 μL was injected onto a Thermo Scientific™ Hypersil GOLD™ 20×2.1 mm trapping column with subsequent elution onto a Thermo Scientific™ Accucore™ RP-MS 18 analytic column. A 6.7-minute solvent gradient was applied as shown in Figure 2. This resulted in a total cycle time of 15 minutes for sample injection, online concentration, and chromatographic separation.

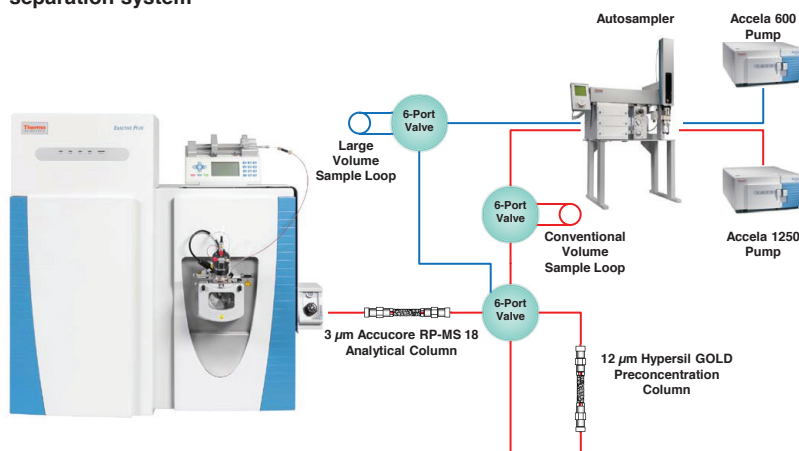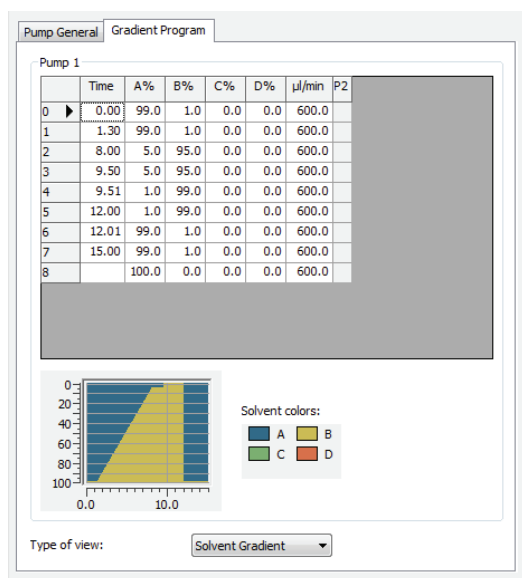**FIGURE 1. Schematics of the EQuan online solid phase extraction and separation system**
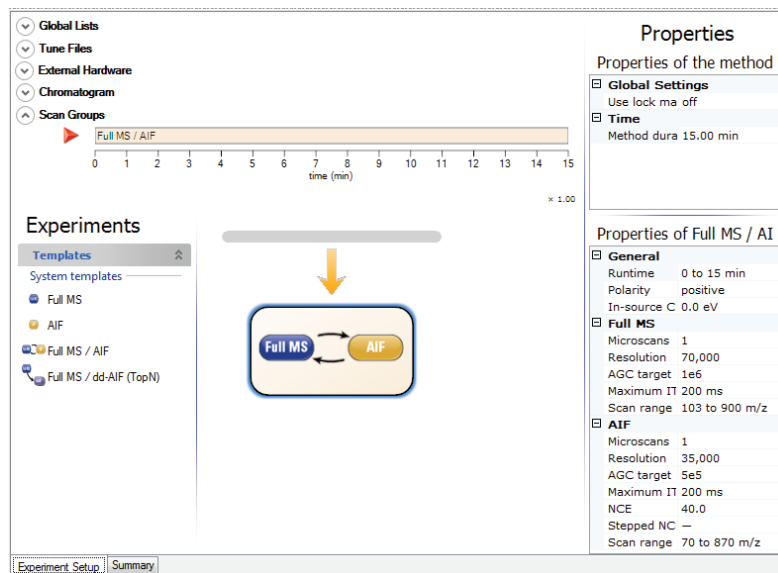
FIGURE 2. Analytical gradient of the LC method

Mass Spectrometry

For mass spectrometric detection, an Exactive Plus mass spectrometer was used and run in full scan/all ion fragmentation (AIF) mode. In this mode, full scans are permanently alternated with AIF fragmentation scans. A resolution setting of 70,000 (FWHM @ *m/z* 200) was used (Figure 3). A mass range of *m/z* 103 to 900 was applied (resp. *m/z* 70 to 870 and resolution setting 35,000 FWHM for the AIF scans) to be prepared for all possible contaminants. The mass axis of the system was calibrated with the standard calibration mix once prior to measurement. Further optimization of the instrument (compound tuning) was not required.

FIGURE 3. Exactive Plus method setup

Data Analysis

For data analysis, SIEVE 2.1 and TraceFinder 3.1 software were used. All data acquired with TraceFinder software was transferred automatically to SIEVE software, and then retransferred into TraceFinder after processing in SIEVE. Final processing, reporting, and archiving took place in TraceFinder software.

Results

Suspect Screening

The more simple way of screening is the suspect screen, using a large list of components possibly present in a sample (Figure 4). It avoids the critical step of condition-free component detection, but works already without analytical standards which could serve for confirmation by providing valid retention time, ion ratios, and more. In this case, a built-in database with about 1,000 components was used, containing name, elemental composition, and fragment information. Additionally, a matching spectral library containing roughly 4,000 HR/AM MS[2] spectra is available inside the application. As a result, isotopic pattern match, fragment search, and MS[2] library search were used for result confirmation.
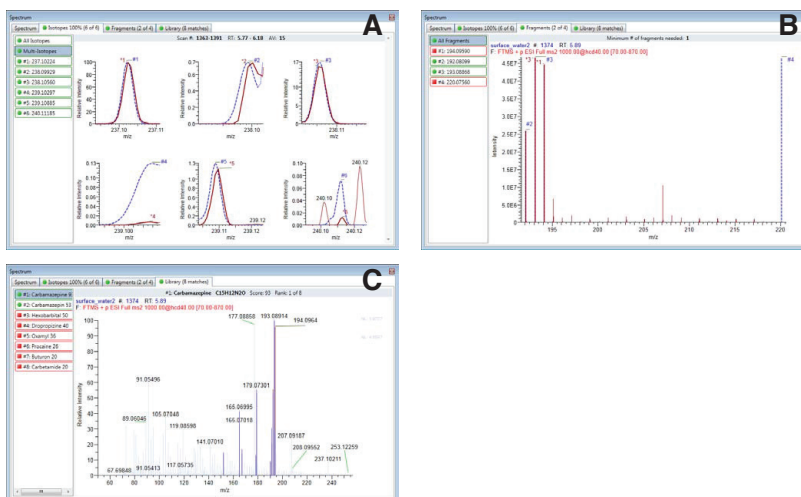
FIGURE 4. Suspect screening result view

As to be expected, it was possible to identify a good number of contaminants, yielding a match on all three confirming points. On the other hand, it was clear that this method of screening did not cover all possible compounds, as was visible from the neat standard (as used normally for target screening on these samples) measured in the same batch. A clear benefit could be seen in the fact that fragment information and library spectra were present for additional confirmation (Figure 5).

In addition, a larger database with 2,900 components was applied, but still left open the question of contaminants not found because they may not be members of this list.
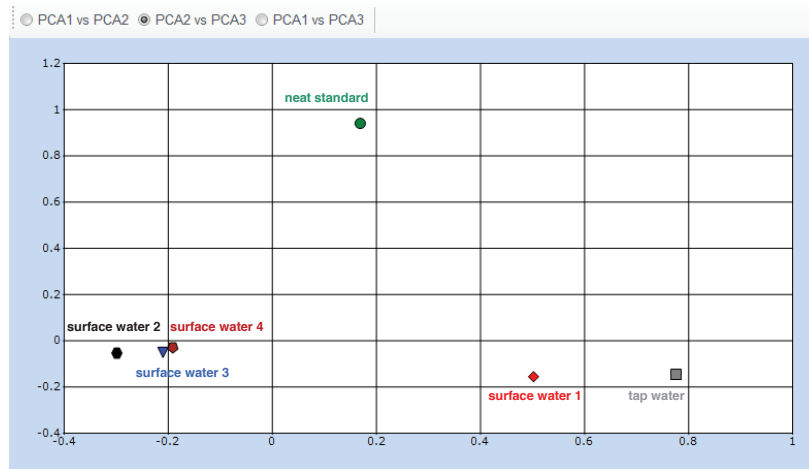
FIGURE 5. Three stages of confirmation in suspect screen: isotope pattern match, fragment search, and library search; A: Isotopic pattern overlay; B: Fragment overlay; C: Library comparison

Unknown Screening

As a consequence of the limitations of a suspect screen, an unknown screening workflow was run. For this, the measured sequence was transferred to the screening application, SIEVE, for unconditioned component detection. Since all necessary settings and parameters were transferred from TraceFinder software to SIEVE software automatically, the component detection process could be started immediately. As a result, 5,000 components were detected. Since such a list contains all components regardless of their significance, refinement of this list was clearly needed. As part of the process, all samples were referenced against the tap-water sample, so a simple filter could be applied to remove matrix and background signals from the result list, leaving 1,829 components in the list. Application of a principal component analysis to this result revealed that three water samples were closely related, while one water sample (surface water 1, see Figure 6) seemed to be rather different in its content, so the filter for significant components could be further refined.

FIGURE 6. PCA result after filtering for significant differences

This time the filter was set to look only for significant changes in the samples surface water 1 and surface water 2 (Figure 7). This reduced the list of components to 1,671, which were sent to the ChemSpider database for identification. This search returned 1,529 identifications. Closing the SIEVE application automatically transferred this result list back to the TraceFinder software, where it was imported as a new compound database.
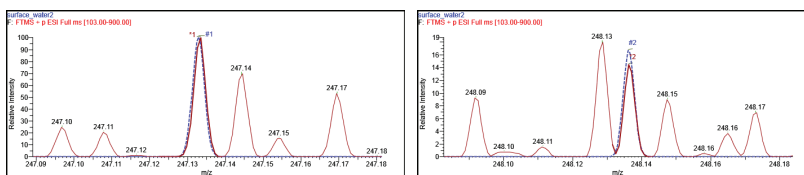
FIGURE 7. Confirmation of the unknown screening results from SIEVE software, propiconazole taken as an example: the extracted ion chromatogram at the determined retention time gives a clear signal free from interferences, the isotope pattern match shows close to perfect overlay

For confirmation and reporting of the results, this compound database was used for a normal suspect screening. The advantage of looping the results back to the TraceFinder software was to be able to handle all data – target, suspect, and unknown screen – in one application and to be able to use the same data review and report templates.

It became visible that some components were coeluting with higher amounts of matrix, but it was still possible to extract significant signals from the surrounding matrix, maintaining full mass accuracy despite the low signal intensity. Figure 8 shows the example of the component Loxoprofen, where the surrounding matrix signals have roughly the same intensity as the first and second isotope signal of the compound. Still, the analyte signals are clearly resolved from the background and matrix signals, so the compound can easily be detected and confirmed. Key to this clear separation of analyte and matrix signals is the high resolving power of R = 70000 @ *m/z* 200 used in this analysis.

FIGURE 8. Importance of sufficient resolution for unambiguous identification of components: the monoisotopic signal (A) and the first isotope signal (B) are surrounded by matrix signals of similar intensity, which are only separated by means of the high resolving power used

Since all final processing was done in one application, the results of target, suspect, and unknown screening could easily be combined into one result, making result reporting and archiving one single step. Since all data transfer between the two applications is fully automated, Table 1 shows a short selection of compounds that had not been part of the initial target screening, but were found in the unknown screening process.

Table 1. Selection of additional contaminants not present in previous target and suspect screen

Compound Name	Formula	m/z (Apex)	m/z (Delta (ppm))	RT (Measured)	Isotopic Pattern Score (%)
Bisoprolol	C18H31NO4	326.2330	0.57	5.12	100
Candesartan	C24H20N6O3	441.1671	-0.50	6.56	100
Carbofuran	C12H15NO3	222.1127	-0.19	5.18	98
Dibenzylamine	C14H15N	198.1277	-0.66	7.31	98
Irbesartan	C25H28N6O	429.2401	-0.03	6.45	100
Loxoprofen	C15H18O3	247.1332	0.45	5.52	85
Mexacarbate	C12H18N2O2	223.1443	-0.06	5.53	96
Oxazepam	C15H11ClN2O2	287.0584	0.48	6.29	96
Propiconazole	C15H17Cl2N3O2	342.0774	0.21	7.43	89
Tramadol	C16H25NO2	264.1961	0.10	4.35	100

Conclusion

In this example of environmental analysis. we could show that it is possible to enhance the capabilities of target and suspect screening with its limitations by a streamlined general unknown screening with a high degree of automation from within one application. The resolving power of the Exactive Plus benchtop Orbitrap MS system is the driving force behind the selectivity and reliability of the obtained results because this serves for the separation of the analyte peaks from background and matrix signals.

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