Food & Environmental

Metabolomic Profiling of Accurate Mass LC-MS/MS Data to Identify Unexpected Environmental Pollutants

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Overview

There is growing concern that commonly used Pharmaceuticals and Personal Care Products (PPCP) are entering and contaminating the drinking water supply. The use of targeted quantitation of PPCP has been well established but there is an emerging trend to also screen for and identify unexpected environmental pollutants. The new AB SCIEX TripleTOF™ 5600 LC/MS/MS was used to profile environmental samples for unexpected pollutants, to identify and characterize the chemical composition and structure of the pollutants, and to quantify the concentration in collected water samples.

Introduction

Pharmaceuticals and Personal Care Products (PPCP) are environmental contaminants of growing concern. In order to properly assess the effects of such compounds on our environment it is necessary to accurately monitor their presence.[1] The diversity of chemical properties of these compounds makes method development challenging. Liquid Chromatography coupled to tandem Mass Spectrometry (LC-MS/MS) is able to analyze polar, semi-volatile, and thermally labile compounds covering a wide molecular weight range, such as pesticides, antibiotics, drugs of abuse, x-ray contrast agents, drinking water disinfection by-products etc. In addition, state-of-the-art LC-MS/MS instruments operated in selective Multiple Reaction Monitoring (MRM) mode, offer unmatched selectivity and sensitivity to quantify PPCP reproducibly at trace levels without time consuming and extensive sample preparation.[2]

More recently there is a growing interest from environmental researchers to also screen for and identify non-targeted compounds in environmental samples, including metabolites and degradates, but also completely unexpected pollutants. The new AB SCIEX TripleTOF™ 5600 LC/MS/MS system is capable of performing highly sensitive and fast MS scanning experiments to search for unknown molecular ions while also performing selective and characteristic MS/MS scanning for further compound identification and, therefore, is the instrument of choice for this challenging task. General unknown screening workflows do not use a target analyte list and compound detection is not based on any a priori knowledge, including retention times and information on possible molecular and fragment ions. Therefore, acquired chromatograms are very rich in information and can easily contain thousands of ions from both any compounds present in the sample as well as from the sample matrix itself. Thus, powerful software tools are needed to explore such data to identify the unexpected compound.

In metabolomic investigation the change (greek: ‘meta’) of the fingerprint of a system is systematically studied. Originally developed to study cellular processes, such as small-molecule metabolite profiles, statistical data analysis tools are suitable to also study changes in environmental systems and, thus, to identify unexpected environmental pollutants.

This article describes the use of the new AB SCIEX TripleTOF™ 5600 system for the screening for unexpected environmental pollutants applying statistical data analysis using MarkerView™ Software. The detected compounds were identified based on empirical calculation of the molecular formula, interpretation of MS/MS fragments and mass spectral library searching. The acquired high resolution and accurate mass spectra were further used to quantify the amounts of detected and identified environmental pollutants in a large set of environmental water samples.

Method Details

- More than 70 water samples in different cities and countries from different type of waters, including drinking water, creeks, rivers, lakes, sea etc were collected by different scientist
- LC-MS/MS analysis in triplicates and in a randomized order
- Ultra High Pressure Liquid Chromatography (UHPLC) using a Shimadzu UFLCXR system with a Phenomenex Kinetex 2.6 µm (100x2.1 mm) column and a gradient of water and acetonitrile with 0.1% formic acid
- Flow rate of 0.5 mL/min and injection volume of 50 µL
- Total run time of 15 min
- AB SCIEX TripleTOF™ 5600 System with Accelerator TOF™ Analyzer and Electrospray Ionization source
- Continuous recalibration for EasyMass™ Accuracy
- IDA experiment with a TOF-MS survey (accumulation time of 100 ms) and up to 10 dependent MS/MS scans (accumulation time of 50 ms)
- Standardized collision energy (CE) = 35 V with collision energy spread (CES) = ±15 V
- Statistical data processing using MarkerView™ Software
- Compound identification and quantitation using PeakView™ Software

Results and Discussion

The new AB SCIEX TripleTOF™ 5600 system with Accelerator TOF™ Analyzer and continuous recalibration for EasyMass™ Accuracy delivers high resolution mass information and stable mass accuracy of ~1 ppm at fast acquisition speed in MS and MS/MS mode. Figure 1 shows an example of MS and MS/MS spectra of the tricyclic antidepressant Clomipramine highlighting the excellent resolution and mass accuracy over the entire mass range.

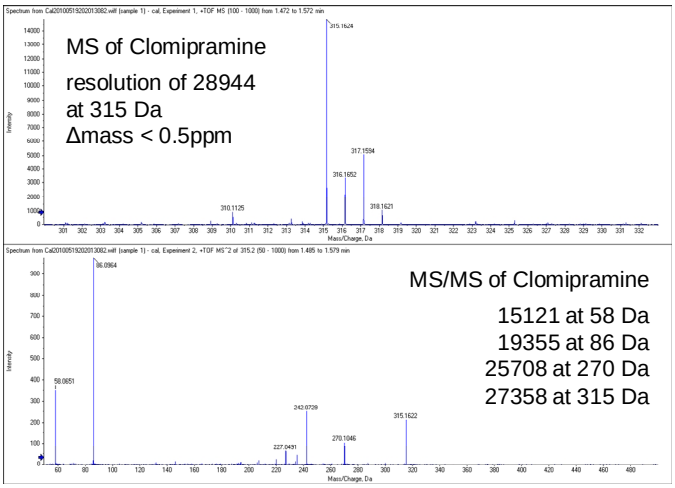

Figure 1. Resolution and mass accuracy of Clomipramine in MS and MS/MS mode using the AB SCIEX TripleTOF™ 5600 System

The metabolomic data profiling tool is Principal Components Analysis (PCA). LC-MS/MS data was processed using PCA in MarkerView™ Software. PCA finds combinations of variables, in this case based on retention times, mass signals and intensities, that explain most of the variance present in a data set. For each principal component every sample has a score and every variable has a loading that represents its contribution to the combination. It is common to plot the scores and loadings of two principal components to visualize analytical results. Figure 2 shows the scores plot of all >70 water samples. Water samples are color coded based on sample type (i.e. green – samples from wilderness areas, blue – drinking water, and red – samples from urban areas). The scores plot clearly differentiates samples originating from different water types.

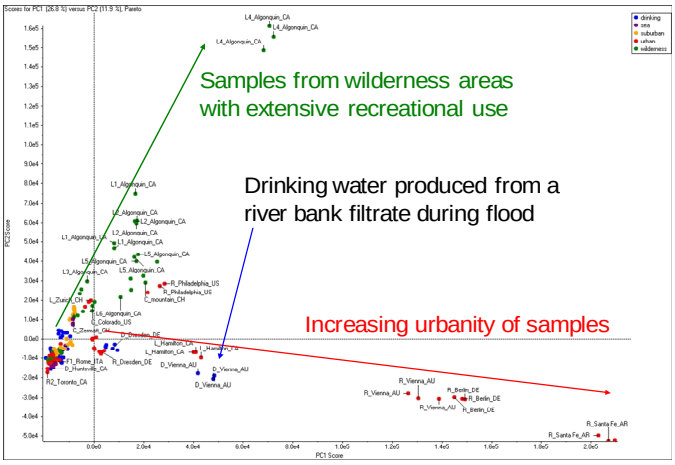

Figure 2. Scores plot of PCA of more than 70 water samples indicating trends by sample comparison

For example, river water samples arranged depending on urbanity at the bottom of the plot. One drinking water sample was collected during flood season. The position of this sample in the scores plot suggests higher pollution of that drinking water produced from river bank filtrate and asks for further investigation.

Samples collected in remote and wilderness areas arrange in the top part of the scores plot. The corresponding loading plot assists to find characteristic marker ions for this grouping. The loading plot in Figure 3 indicates mass 192 Da at 7.7 min.

The information of the unexpected ion characteristic for all water samples from wilderness areas was used to further explore a samples collected from Lake Zürich in Switzerland. The high resolution and accurate mass spectra of the detected molecular ion and the automatically collected MS/MS spectrum is shown in Figure 4. Both spectra were further processed using the Formula Finder of PeakView™ Software to calculate empirical formulas for the molecular ion and detected fragment ions. The Formula Finder software shown in Figure 5 uses accurate mass information, the isotopic pattern and logic on distribution of chemical elements for this task.

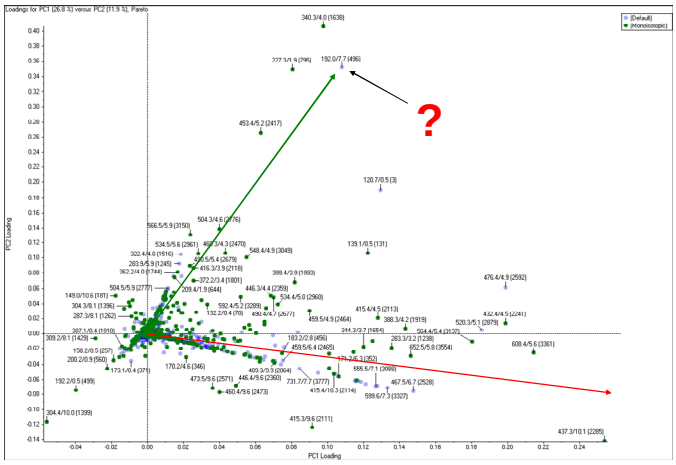

Figure 3. Loadings plot of PCA of characteristic mass-retention time-pairs to separate studied water samples

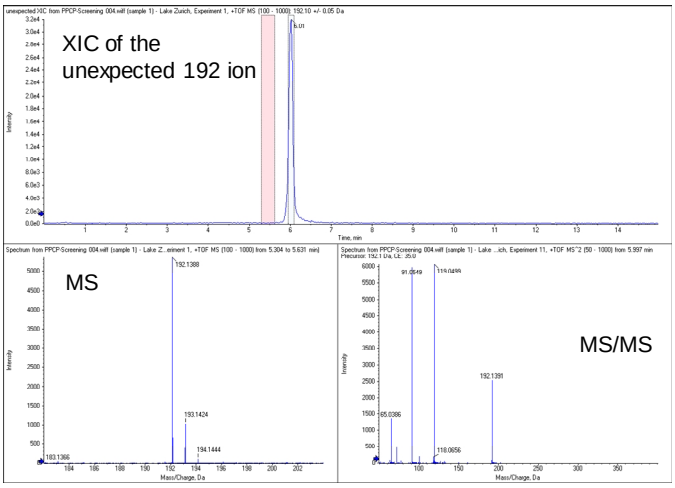

Figure 4. XIC of 192.1 Da +/- 50 mDa with high resolution accurate mass MS (left) and MS/MS spectra (right)

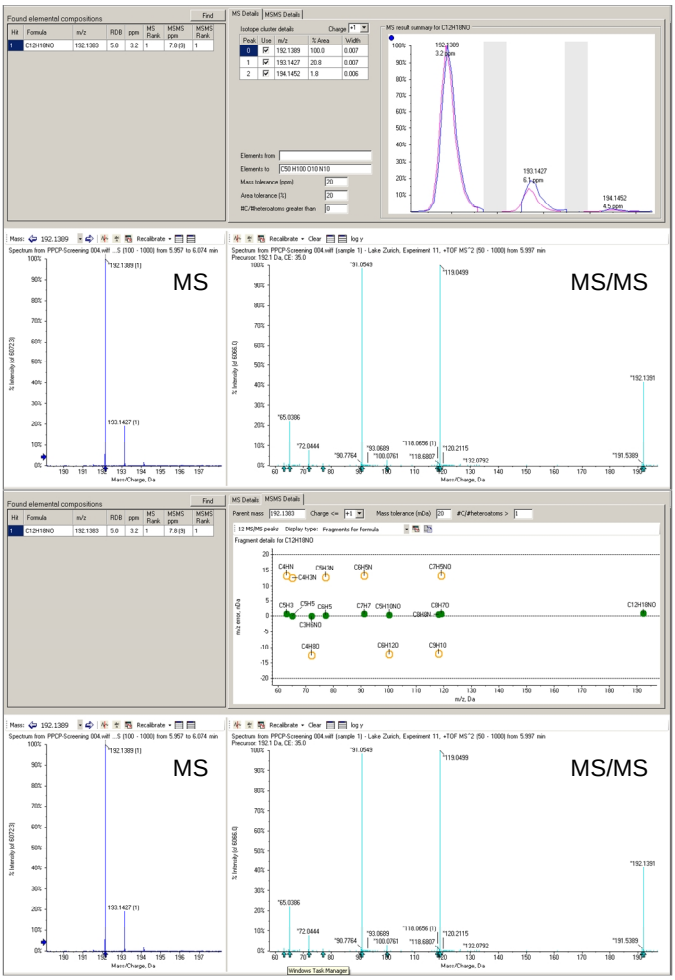

Figure 5. Formula Finder software to empirically calculate the formula of the molecular ion and fragment ions of the unexpected environmental pollutant (192 Da at 7.7 min)

The information gained from data processing was used to characterize the structure of the identified environmental pollutant (Figure 6) and identify the molecule as DEET (N,N-Diethyl-m-toluamide).

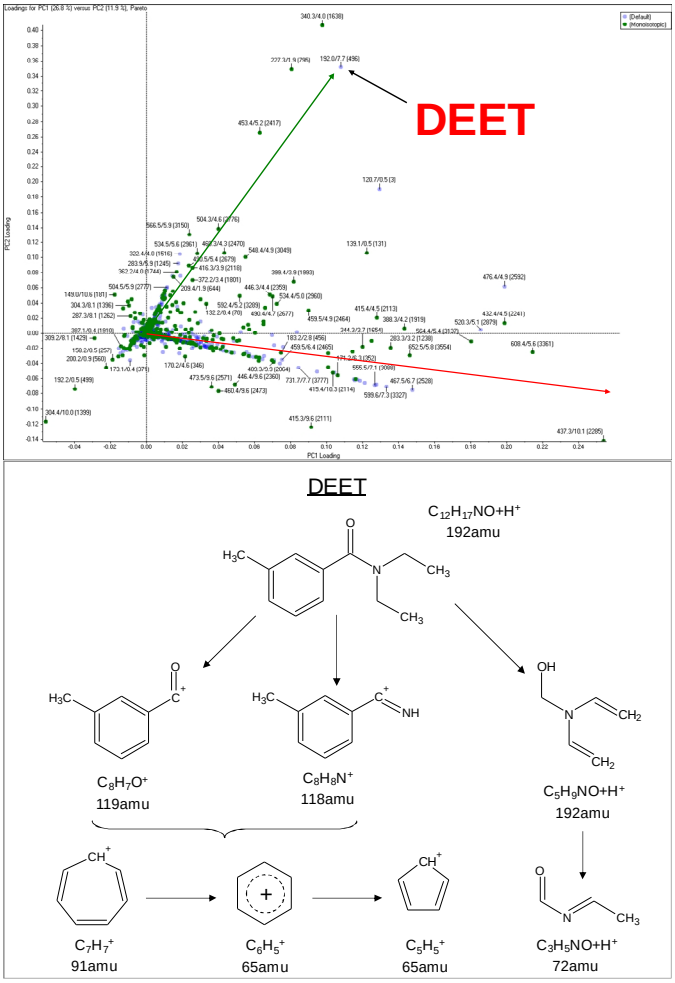

Figure 6. Structural characterization of MS/MS fragments of the unexpected environmental pollutant

After purchase of an analytical standard the identified environmental pollutant was quantified in the collected environmental samples using the XIC Manager in PeakView™ Software (Figure 7).

The profile plot of DEET (Figure 8) shows that this compound is widespread in the environment and can even be found in remote areas with high recreational use, such as Algonquin Provincial Park, a popular canoeing destination in Ontario (Canada), in the Rocky Mountains of Colorado (US), and in the Alps (Switzerland).

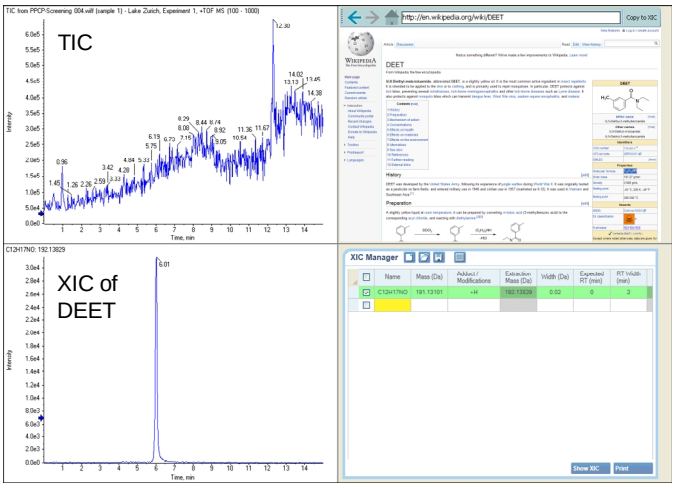

Figure 7. Quantitation of DEET in environmental water samples using the web browser of the XIC Manager of PeakView™ Software

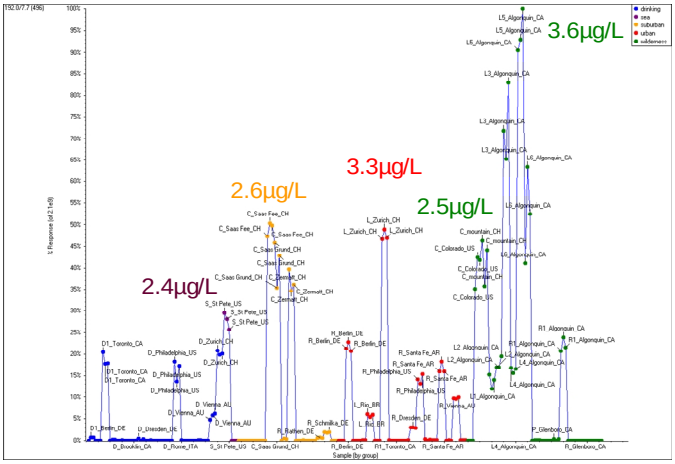

Figure 8. Quantitation of DEET in environmental water sample

Conclusion

The new AB SCIEX TripleTOF™ 5600 LC/MS/MS System and powerful software tools were used to screen for non-targeted environmental pollutants.

More than 70 samples collected all over the world were processed in MarkerView™ Software using metabolomic profiling and statistical analysis (Principal Components Analysis PCA) to detect unexpected compounds. High resolution and accurate mass information was used to empirically calculate the formula of molecular ions and MS/MS fragment ions using Formula Finder of PeakView™ Software. After compound identification the unexpected pollutant was quantified in all collected water samples. Finally, the collected full scan data can be further processed retrospectively to identify more unexpected environmental pollutants.

Acknowledgements

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References

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