



The Use of High Resolution Accurate Mass GC/Q-TOF and Chemometrics in the Identification of Environmental Pollutants in Wastewater Effluents

Application Note

Environmental

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Abstract

A GC/Q-TOF method employing the Agilent 7200 series GC/Q-TOF system and chemometric analysis tools in Agilent Mass Profiler Professional software has been used to effectively identify environmental pollutants in complex effluent samples from multiple wastewater treatment sites and track their transformation during the treatment process.



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Introduction

Efforts to characterize the fate of environmental pollutants during wastewater treatment are hampered by the large number of compounds present in various wastewater streams [1]. Untargeted analysis of pesticides and other environmental pollutants in wastewaters using gas chromatography/mass spectrometry (GC/MS) can benefit from comprehensive EI libraries available for this technique, such as NIST 14 (containing over 200,000 EI spectra with retention index (RI) values for over 80,000 compounds). However, the highly complex chromatograms and very large data sets characteristic for this workflow represent a substantial analytical challenge [2,3].

While deconvolution of unit mass electron ionization (EI) data followed by a mass spectral library search is the most typical workflow used for the identification of environmental pollutants, this approach does not provide enough confidence in compound identification, especially in case of poor library matching. Using the high resolution, accurate mass capability of GC/Q-TOF provides analysts the required tools for reliable compound identification.

This application note presents a novel combined untargeted and targeted approach that uses high resolution accurate mass quadrupole-time-of-flight mass spectrometry (Q-TOF MS) to increase the efficacy of identification of large numbers of unknown compounds in wastewater. Furthermore, chemometric techniques using Agilent Mass Profiler Professional (MPP) software are then used for statistical analysis and data interpretation to ascertain the fate of environmental pollutants during wastewater treatment.

Experimental

Instruments

This study was performed on an Agilent 7890B GC system coupled to an Agilent 7200 series GC/Q-TOF system. The instrument conditions are listed in Table 1.

Table 1. GC and Mass Spectrometer Conditions

GC run conditions	
Column	Agilent DB-5 MS Ultra Inert, 30 m × 0.25 mm, 0.25 µm film (p/n 122-5532U1)
Injection volume	1 µL
Split ratio	10:1
Split/Splitless inlet temperature	300 °C
Oven temperature program	70 °C for 2 minutes 25 °C/min to 160 °C 3 °C/min to 205 °C 8 °C/min to 280 °C, 7.5 minutes hold 40 °C/min to 325 °C, 2 minutes hold
Carrier gas	Helium at 1.5 mL/min constant flow
Transfer line temperature	300 °C
MS conditions	
Ionization mode	EI
Source temperature	280 °C
Quadrupole temperature	150 °C
Mass range	50 to 600 <i>m/z</i>

Sample preparation

Settled primary and final effluent samples from three wastewater treatment works in South Wales, UK, were collected over a period of a few days. Five replicates of final effluent and primary effluent samples from each of the sites, as well as blanks, were extracted with dichloromethane, and concentrated to low volume. Internal standard deuterated phenanthrene (D₁₀) was added to each sample prior to extraction.

Data processing and statistical analysis

The data were processed by chromatographic deconvolution using the Unknowns Analysis tool in Agilent MassHunter Quantitative Analysis software (version B.07), followed by tentative compound identification by comparison to the NIST 14 mass spectral library. The identification of environmental contaminants was further confirmed using the accurate mass tools available in the MassHunter Qualitative Analysis software (version B.07). A set of approximately 200 putative contaminants of potential interest was then selected from the list of identified components, and semiquantitation was performed using MassHunter Quantitative Analysis.

The results from quantitation analysis were subsequently imported and processed in the multivariate statistical package Mass Profiler Professional (MPP, version 13) to evaluate the transformation of environmental pollutants in the wastewater treatment works. Figure 1 outlines the data analysis workflow.

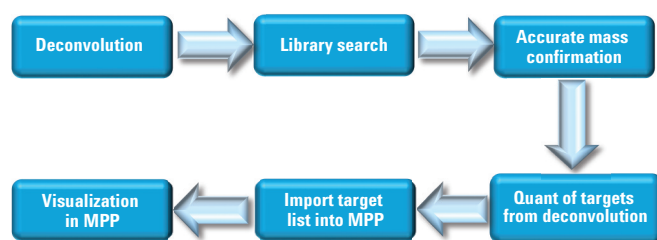


Figure 1. Data analysis workflow.

Results and Discussion

Chromatographic deconvolution, library search and accurate mass confirmation

Using chromatographic deconvolution and NIST library search, approximately 600 components were tentatively identified in each sample (Figure 2). Compound identity was further confirmed using accurate mass information, relative isotope abundance information, and MassHunter accurate mass tools, including Molecular Formula Generator (MFG) (Figure 3). Excellent mass accuracy and small isotope abundance error facilitated confirmation of tentative hits (Table 2). The data were further processed in MassHunter Quantitative Analysis software using peak areas normalized to the internal standard.

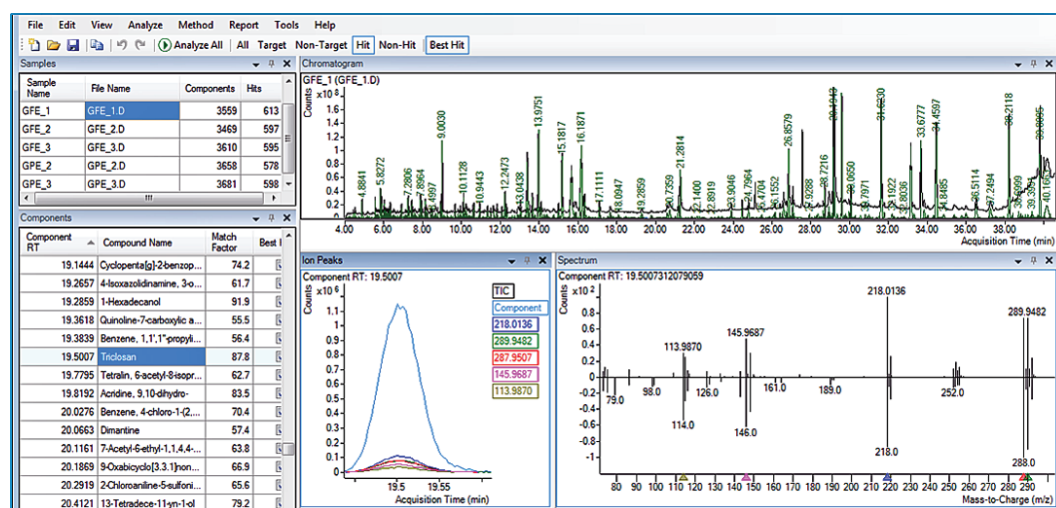


Figure 2. The Unknowns Analysis tool was used to perform deconvolution and the NIST library search. The lower middle panel shows deconvoluted ions selected for the component. They all have the same peak shape, confirming that they all belong to the same component, and thus aiding in its identification (triclosan in this case).

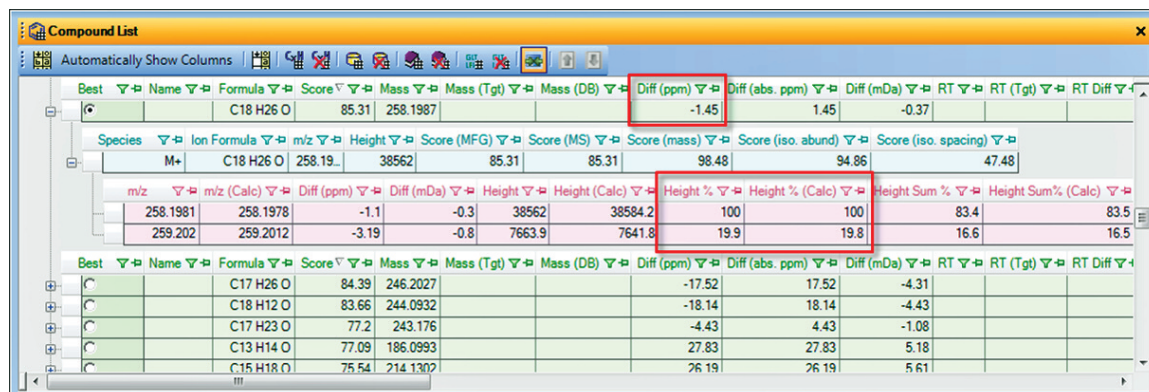


Figure 3. Molecular Formula Generator results.

Table 2. Mass and M+1 Molecular Ion Isotope Abundance Error for Some of the Compounds in the Final Effluent of Site 3

Compound	Formula	Absolute m/z	Calculated m/z	Mass error for MI* (ppm)	M+1 Abundance error (%)
p-Dichlorobenzene	C ₆ H ₄ Cl ₂	145.9684	145.9685	-0.69	0.3
1,2,4-Trithiolane	C ₂ H ₄ S ₃	123.9469	123.947	-0.81	-0.7
2,6-Dichlorophenol	C ₆ H ₄ Cl ₂ O	161.963	161.9634	-2.47	-1.2
Benzothiazole	C ₇ H ₅ NS	135.0142	135.0137	3.70	-0.1
3,5-Dichloroaniline	C ₆ H ₅ Cl ₂ N	160.9795	160.9794	0.62	0.8
Chloroxylenol	C ₈ H ₉ ClO	156.0336	156.0336	-0.30	0.7
5-Methylbenzotriazole	C ₇ H ₇ N ₃	133.0636	133.0634	1.00	0.9
2,3,4-Trichloroaniline	C ₆ H ₄ Cl ₃ N	194.9404	194.9404	0.02	-1.5
4-Methyl-1H-benzotriazole	C ₇ H ₇ N ₃	133.0634	133.0634	-0.20	-0.5
Crotamiton	C ₁₃ H ₁₇ NO	203.131	203.1305	2.46	2.2
Benzophenone	C ₁₃ H ₁₀ O	182.0733	182.0726	3.84	0.1
Tonalide (ANTH)	C ₁₈ H ₂₆ O	258.1981	258.1978	1.16	0.1
Average				1.44	0.76

*MI = Molecular ion

Statistical analysis

A target list of putative contaminants of potential interest was chosen, and the quantitation results were subsequently imported into and processed in the multivariate statistical package MPP to evaluate the transformation of pollutants in the wastewater treatment plants. The data analysis workflow is outlined in Figure 1.

Principal component analysis

Principal Component Analysis (PCA) is a frequently employed unsupervised multivariate statistical analysis technique for data dimensionality reduction. PCA analysis revealed distinct data clusters that represented differences in composition and abundance between all three wastewater treatment plant (WWTW) sites, as well as differences between the primary and final effluents of each site (Figure 4). The grouping of data points along the Z-axis suggests chemical similarity in the primary effluents for all three sites.

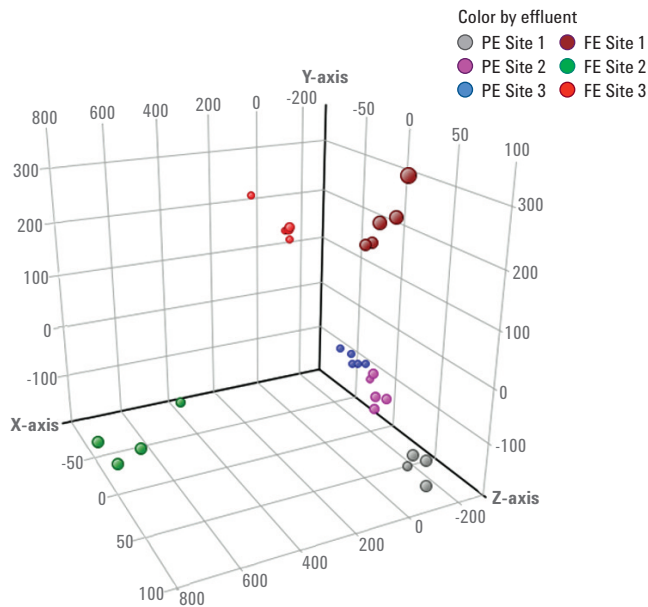


Figure 4. PCA plots confirmed the existence of distinct clusters of compounds for the replicate samples of each type of effluent. Primary Effluent (PE); Final Effluent (FE).

Heatmap analysis

A heatmap was created in MPP to display the transformation of the environmental pollutants between the primary and final effluents of the three WWTW plants (Figure 5). A few of these compounds are shown in the detailed view on the right. The abundance of some compounds decreased in the final effluent relative to the primary effluent. For example, caffeine and tonalide (AHTN) were significantly decreased in the final effluents of all three sites while cashmeran (DPMI) did not significantly change in relative abundance for Sites 1 and 2. Other compounds actually increased in abundance in the final effluent versus the primary effluent, such as 2,3,4-trichlorophenol at Site 3.

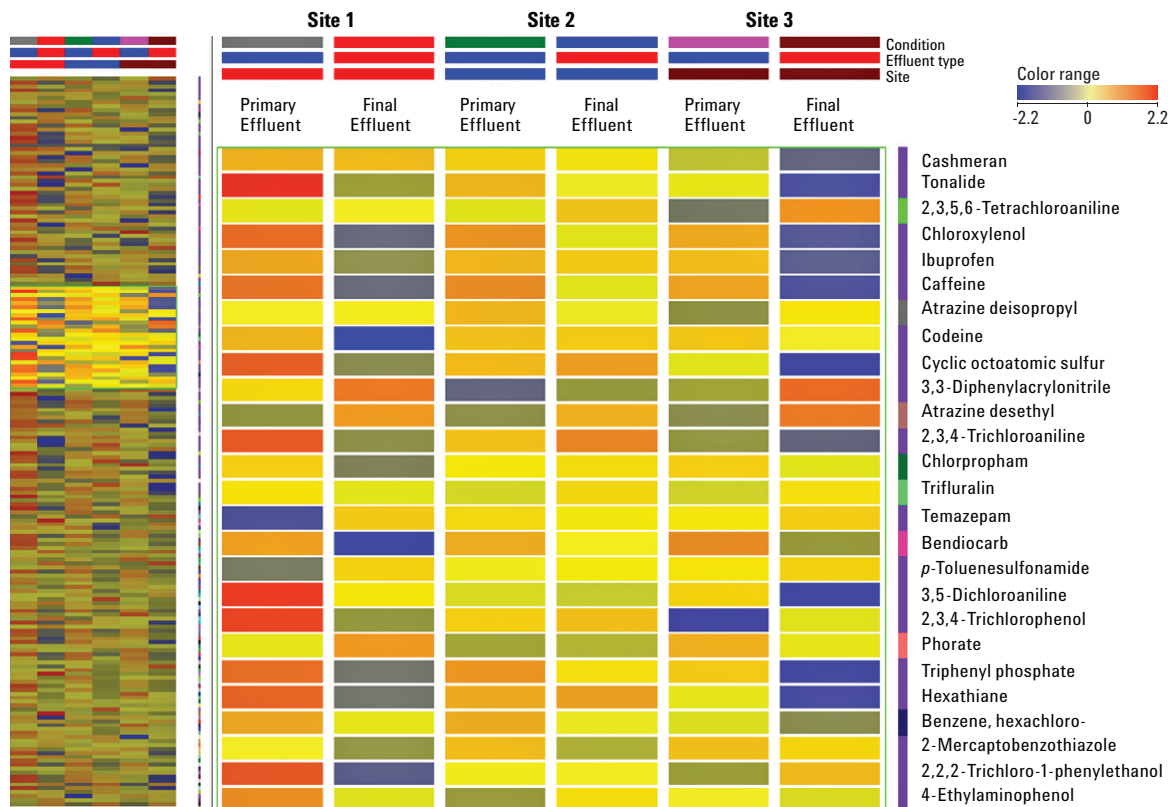


Figure 5. MPP Heatmap summary (left) and detailed view (right). Columns represent effluent type and treatment site, and the rows represent compounds.

K-means clustering analysis

The K-means clustering tool in MPP divides entities (compounds in this case), into groups (clusters) based on similarity of their behavior under different conditions (primary and secondary effluents in this case). K-means clusters are constructed so that the average behavior (increase or decrease in abundance measured by degree of fold change) in each group is distinct from any of the other groups. Figure 6

illustrates this analysis for two groups of compounds analyzed at Site 3. In the left panel, all members of the group decrease in abundance in a similar manner in the final effluent with respect to that of the primary effluent. Conversely, all members of the group in the right panel increase in abundance in a similar manner in the final effluent. In this way, several groups of compounds with similar changes in abundance were identified.

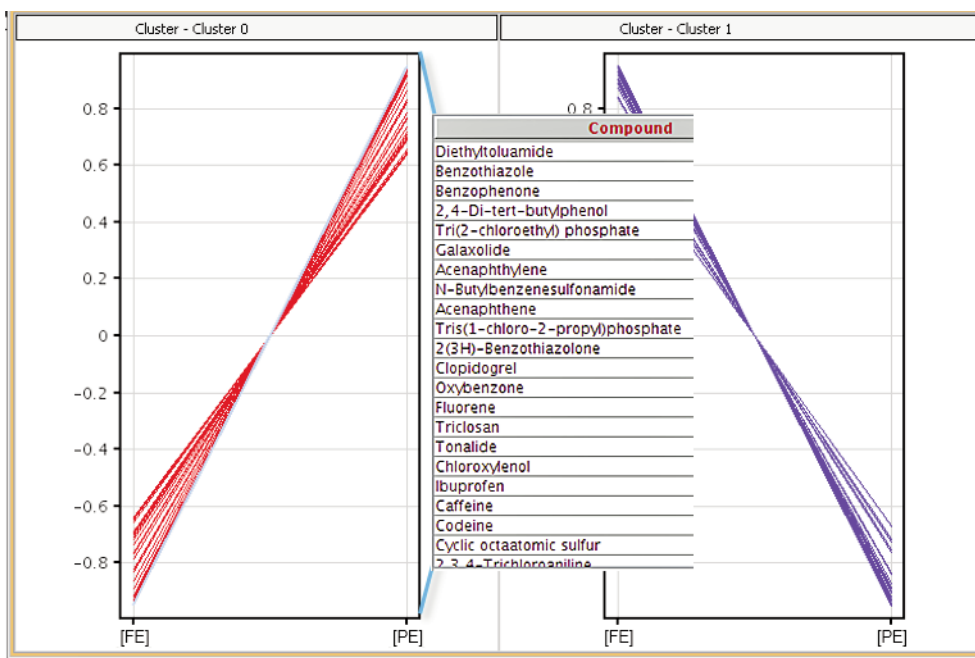


Figure 6. K-means clustering of two representative groups of compounds from Site 3 that display similar degree of fold change in abundance between primary effluent (right side of each graph) and final effluent (left side of each graph). Some of the compounds present in the left hand cluster are shown in the text box.

Venn diagram

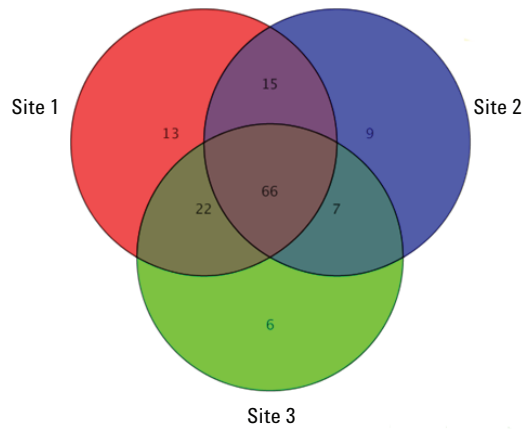
To visually compare treatment sites with respect to the number of shared and unique compounds removed or accumulated during the course of the treatment, Venn diagrams were used (Figure 7). For all the treatment sites, the number of compounds removed from primary effluents (left side of Figure 7) was larger than the number accumulated.

Conclusions

The combination of gas chromatography, high resolution Q-TOF mass spectrometry, and chemometrics techniques was successfully used to characterize and identify environmental pollutants in complex effluent samples from multiple wastewater treatment sites. Data analysis, using both targeted and untargeted approaches, revealed a number of compounds, including pharmaceuticals, benzothiazole-based corrosion inhibitors and polycyclic musks that were specifically present in the primary effluents, but were reduced to significantly lower concentrations in final effluents.

The statistical analysis tools in Agilent Mass Profiler Professional enabled easy and rapid visualization of the results using multiple statistical approaches that revealed similarities and differences in treatment modalities between treatment sites as well as between groups of compounds. This approach can thus facilitate our understanding of the effectiveness of wastewater treatment for the removal of trace organic pollutants.

A Compounds reduced in abundance in final effluent



B Compounds increased in abundance in final effluent

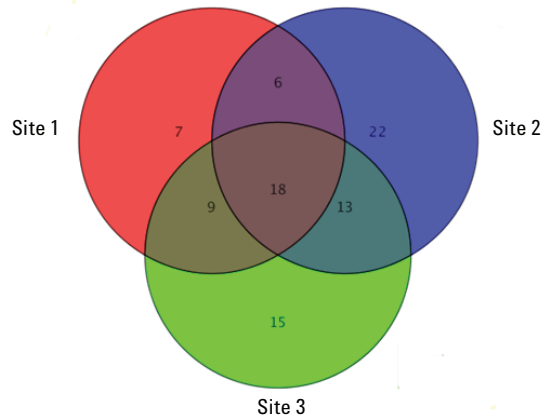


Figure 7. Venn diagrams showing compounds present at significantly higher levels in primary effluents as compared to the final effluents (A), and those accumulated in final effluents as compared to the primary effluents (B). The numbers of compounds shared by multiple sites are depicted by the overlap in the diagrams.

References

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2. T. Portoles, E. Pitarch, F. J. Lopez, F. Hernandez. "Development and validation of a rapid and wide-scope qualitative screening method for detection and identification of organic pollutants in natural water and wastewater by gas chromatography time-of-flight mass spectrometry." *J. Chromatogr. A* **1218(2)**, 303-15 (2011).
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