Identifying Disinfection Byproducts in Treated Water

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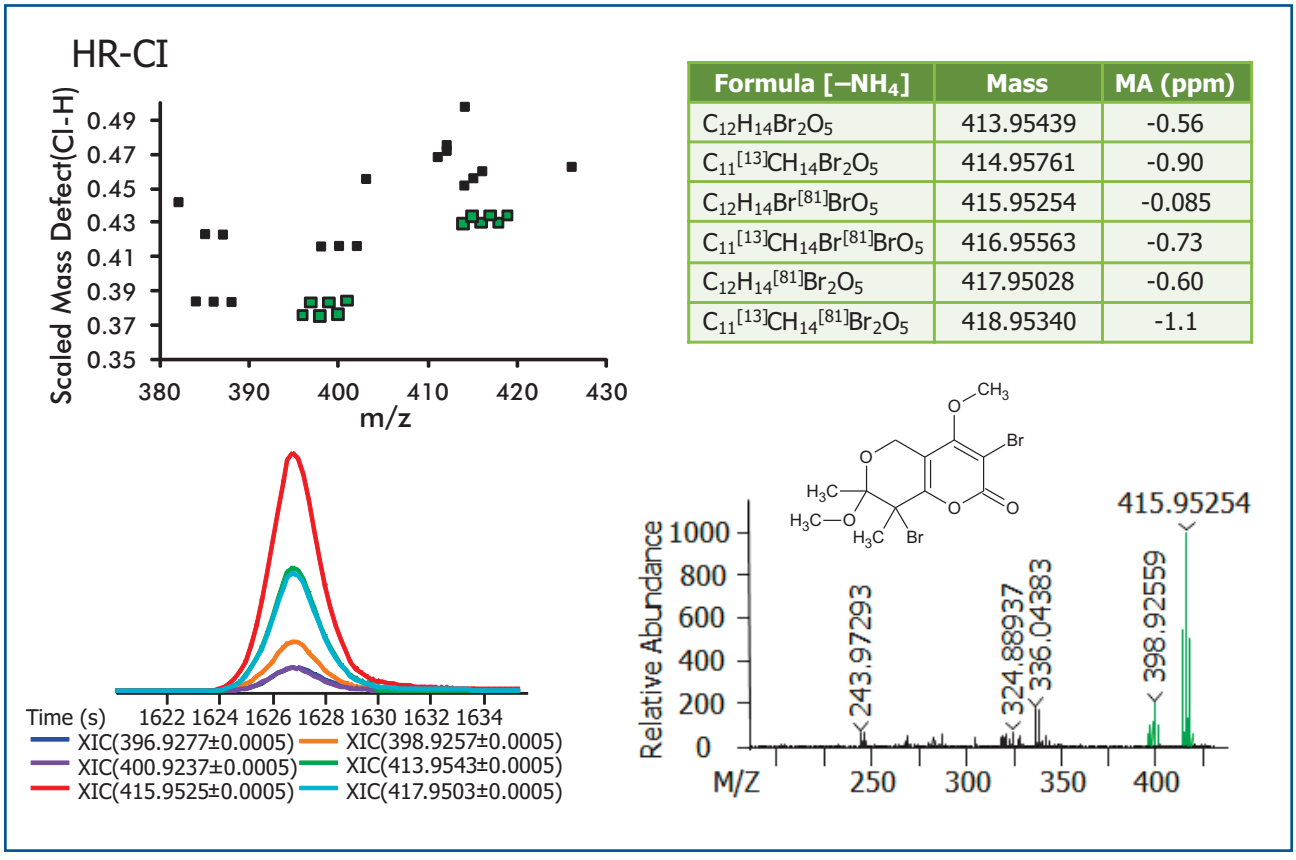
1. Introduction

LECO's Pegasus® GC-HRT was used for the identification of "known unknowns" and "unknown unknowns" in swimming pool and hot tub water. The "known unknowns" were identified by library database searching high resolution deconvoluted spectra using LECO's ChromaTOF-HRT® brand software, while the "unknown unknowns" were tentatively identified using a combination of electron ionization (EI) and chemical ionization (CI) accurate mass data for elemental composition determination. Swimming pools are treated with disinfectants to protect swimmers from pathogens and to prevent illness. Disinfectants will react with naturally occurring organic matter in water and they can also react with chemicals introduced to the water by the swimmers themselves to produce byproducts that can be potentially harmful. The chemical characterization of disinfection byproducts in swimming pools and hot tubs, very complex matrices, is one of the first steps necessary to protect swimmers. Non-target analysis is essential due to the number of contaminants that are complete unknowns. Some of the features highlighted in this note include Automated Peak Finding with High Resolution Deconvolution® (HRD®), ChemSpider search launches, mass defect plots, advanced peak filtering, and complementary EI and CI data acquisition.



Formula [–NH_4]	Mass	MA (ppm)
$C_{12}H_{14}Br_2O_5$	413.95439	-0.56
$C_{11}{}^{[13]}CH_{14}Br_2O_5$	414.95761	-0.90
$C_{12}H_{14}Br^{[81]}BrO_5$	415.95254	-0.085
$C_{11}{}^{[13]}CH_{14}Br^{[81]}BrO_5$	416.95563	-0.73
$C_{12}H_{14}{}^{[81]}Br_2O_5$	417.95028	-0.60
$C_{11}{}^{[13]}CH_{14}{}^{[81]}Br_2O_5$	418.95340	-1.1

Figure 1. Graphical representation of the workflow for the putative identification of an unknown-unknown. The user defined mass defect plot scaled by the formula Cl-H, data collected in HR-CI mode, functions as a powerful tool for the post-target analysis of halogenated compounds. The green points highlighted in the mass defect plot represent the isotope cluster of the adduct ions $[M+H]^+$ and $[M+NH_4]^+$ of an unknown compound in a hot tub sample. The deconvoluted mass spectrum, together with the accurate mass measurements with excellent mass accuracy and isotope fidelity, were used for structure elucidation and tentative compound identification.

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2. Experimental

Samples: Swimming pool and hot tub water (10 L of each) was collected and extracted through Amberlite XAD resins and concentrated to 1 mL, followed by the selective derivatization of acids with 0.5 mL of diazomethane. The extracts were then analyzed by LECO's *Pegasus* GC-HRT.

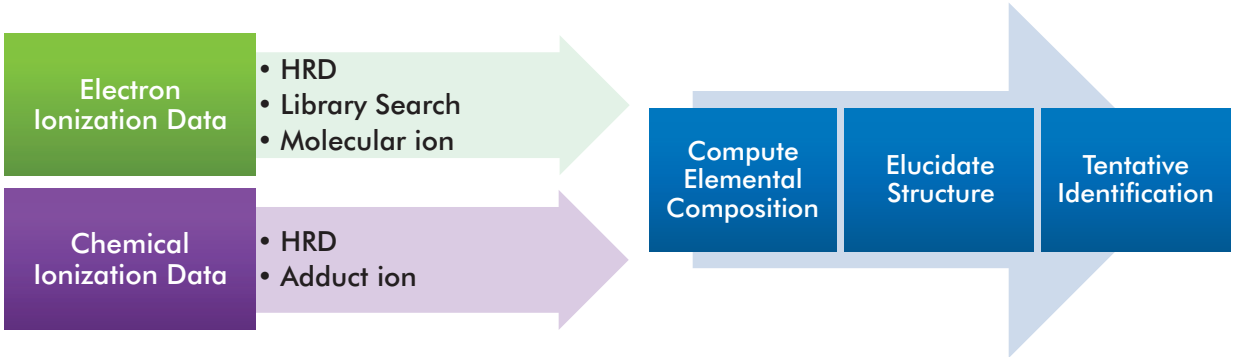
Data Analysis: Data were collected and processed with LECO's *ChromaTOF-HRT* brand software. Analyte peak finding consisted of HRD and library database searching with accurate mass confirmation. Accurate mass measurements were leveraged for confident chemical formula determination of complementary EI and CI data, which were utilized for structure elucidation and tentative compound identification. Mass defect plots with the user defined reference formula "Cl-H" were generated as a data filtering tool to target halogenated species. Furthermore, Peak Table Filter options for Cl and Br containing species, identified by their characteristic isotope patterns in their mass spectrum, were used as a post-processing option to isolate the unknown disinfection byproducts.

Gas Chromatograph	**Agilent 7890 with 7693 Autosampler**
Injection	1μL, MMI, 55°C to 250°C @ 300°C/min
Carrier Gas	He, 13 psi, Constant Pressure
Column	Rxi-5MS, 30 m x 0.25 mm i.d. x 0.25 μm coating (Restek, Bellefonte, PA, USA)
Temperature Program	35°C (4 min), to 280°C @ 9°C/min (20 min)
Transfer Line	280°C
Mass Spectrometer	**LECO *Pegasus* GC-HRT**
Acquisition Mode	High Resolution, R = 25,000 (FWHM); Ultra High Resolution, R = 50,000 (FWHM)
Ion Source Temperature	200°C (EI); 200°C (CI)
Ionization Mode	EI; CI (Reagent Gas = 5% NH_3 in CH_4 at 1.0 mL/min)
Mass Range (m/z)	HR mode = 33-650 (EI), 60-650 (CI); UHR mode = 200-650
Acquisition Rate	5 spectra/s

3. Results and Discussion

Figure 1 emphasizes a workflow which includes mass defect plots; a software tool for structure elucidation and compound identification that takes advantage of the fact that every compound, characterized by its chemical formula, has a unique mass defect resulting from the combination of its elements, measurable only with high resolution mass spectrometry. This characteristic can be leveraged to distinguish unique classes of compounds, in this case halogenated species, allowing users to confidently discover more analytes.

A typical workflow for the identification of an unknown begins with the acquisition of EI data, followed by HRD and library database searching. If the analyte(s) measured have significant fragmentation and are not identified confidently with a high library similarity score, then CI data are typically acquired, followed once again by HRD and identification of the adduct ion(s). These data are then used complementary to compute the elemental composition, elucidate a structure, and tentatively identify the compound as depicted below.



High Resolution Deconvolution is the most critical step in this workflow because it produces mass spectra that are used for all of the subsequent steps of identification. Figure 2 illustrates an example of HRD for EI data collected in UHR mode. An extracted ion chromatogram (XIC) of a characteristic m/z for each of the peaks identified during data processing is displayed in the chromatographic region from 1104 to 1112 s. The deconvoluted (Peak True) mass spectrum of a dibromo-pyridinamine demonstrates the advantage of differentiating characteristic ions from the raw data (caliper) for confident identification. Note that there were nine unique peaks deconvolved within this eight second time period.

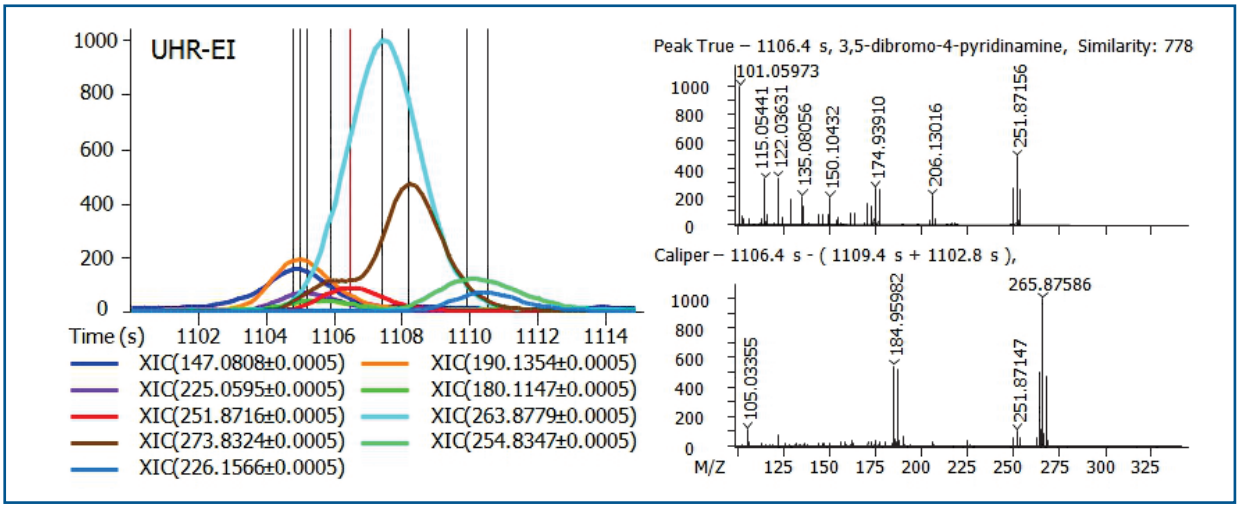


Figure 2. Extracted ion chromatograms of characteristic ions for nine peaks deconvolved using HRD. The deconvoluted (peak true) mass spectrum of the peak indicated with a red peak marker demonstrates the removal of background ions and other ions, such as masses from coeluting compounds that do not correspond to this dibromo-pyridinamine.

Electron ionization is a hard ionization technique, but it is the most widely used ionization mode for GC, in part, because of its reproducibility which has lead to the generation of vast mass spectral libraries. However, the majority of the peaks detected in the swimming pool and hot tub samples were not matched to the NIST or Wiley databases. For both samples, less than 5% of the peaks had library similarity scores greater than 750, suggesting most of the components in the samples were unknown unknowns.

True unknown analysis demands the user to acquire an accurate mass measurement of a molecular ion, which often occurs at low abundance with EI or may be absent for many thermally labile compounds. As a result, softer ionization techniques like CI have been developed to promote the detection of adduct ions. Figure 3 demonstrates the benefit of acquiring complementary EI and CI data for comprehensive structure identification. In this example, the proposed molecular ion in the EI mass spectrum is less than 2% of the base peak, creating uncertainty whether or not this ion is indeed the molecular ion. The corresponding CI mass spectrum eliminates all uncertainty as the ammonium adduct ion cluster represents the base peak. Furthermore, in the *ChromaTOF-HRT* brand software, users can compute formula for neutral losses to determine the fragments, since there is no compromise in the accurate mass measurement of fragment ions in the EI and CI spectra. Structure elucidation is feasible to propose tentative compound identification as illustrated in Figure 3.

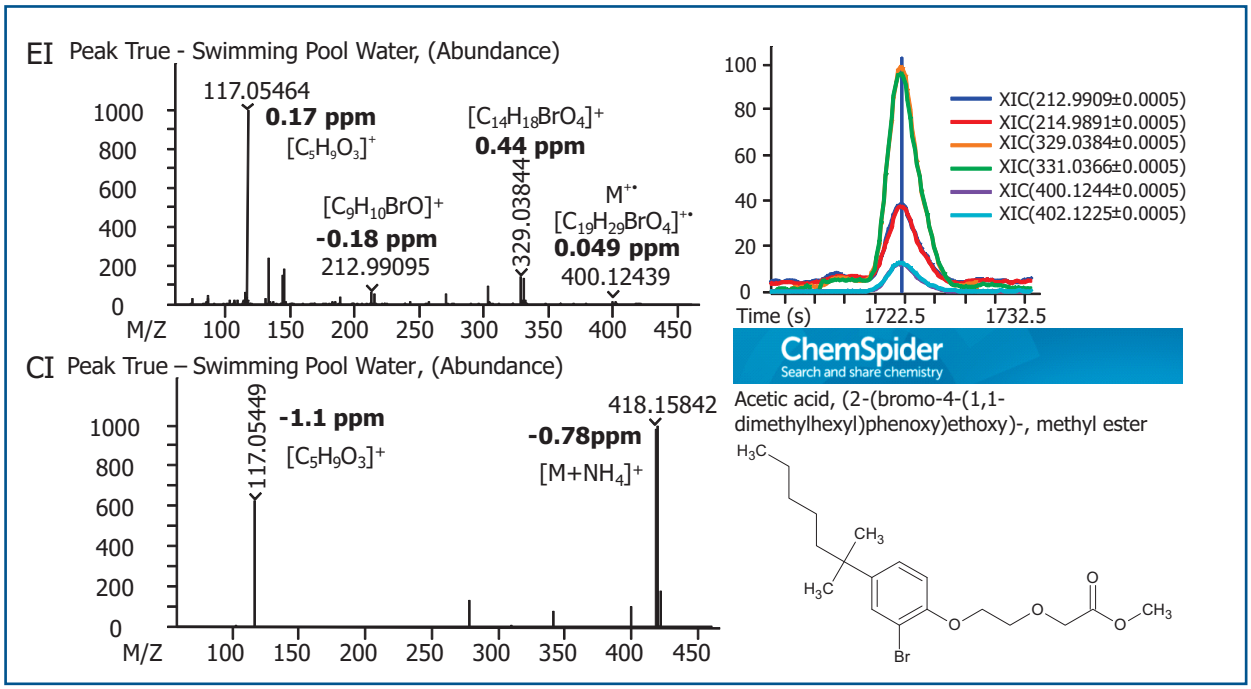


Figure 3. Complementary electron ionization and chemical ionization mass spectra enabled the proposed identification of an unknown compound in a diazomethane derivatized swimming pool sample.

Bromine was used as a disinfectant to treat the hot tub and swimming pool water, and chlorine was used to treat the tap water used to fill the pools. Therefore, in this case, compound identification could be limited to halogenated disinfection byproducts. Advanced filtering options are built into to the *ChromaTOF-HRT* brand software to manage the data analysis and visualization of very complex samples such as these, i.e. only show peaks with similarity score > 800 and/or mass accuracy ±2 ppm. The excerpt of the peak table shown in Figure 4 utilizes a Cl and Br peak filter by taking advantage of Cl and Br isotope patterns and mixed Br/Cl patterns up to 10 halogens. In the table, peaks with a library similarity score of more than 800 are assigned names, while the remaining unknown peaks are listed by peak number. Every peak in the table has a corresponding peak true (deconvoluted) mass spectrum, and chemical formula may be calculated and annotated for each m/z value. In addition, neutral differences between masses may be determined to facilitate structure elucidation. The formula assigned to the molecular ion may be searched using the web database ChemSpider, launched directly from *ChromaTOF-HRT* brand software. For the unknown peaks, the ones not assigned names by library database searching, the combination of accurate mass, fragmentation including neutral losses, elemental formula determination, and ChemSpider may be used to propose compound identification; the ultimate goal of any high resolution mass spectrometer. An example of a tentative identification reached via accurate mass formula assignment and Chemspider search is shown for 5,5'-dimethyl-3-bromocyclohex-2-enone in Figure 4.

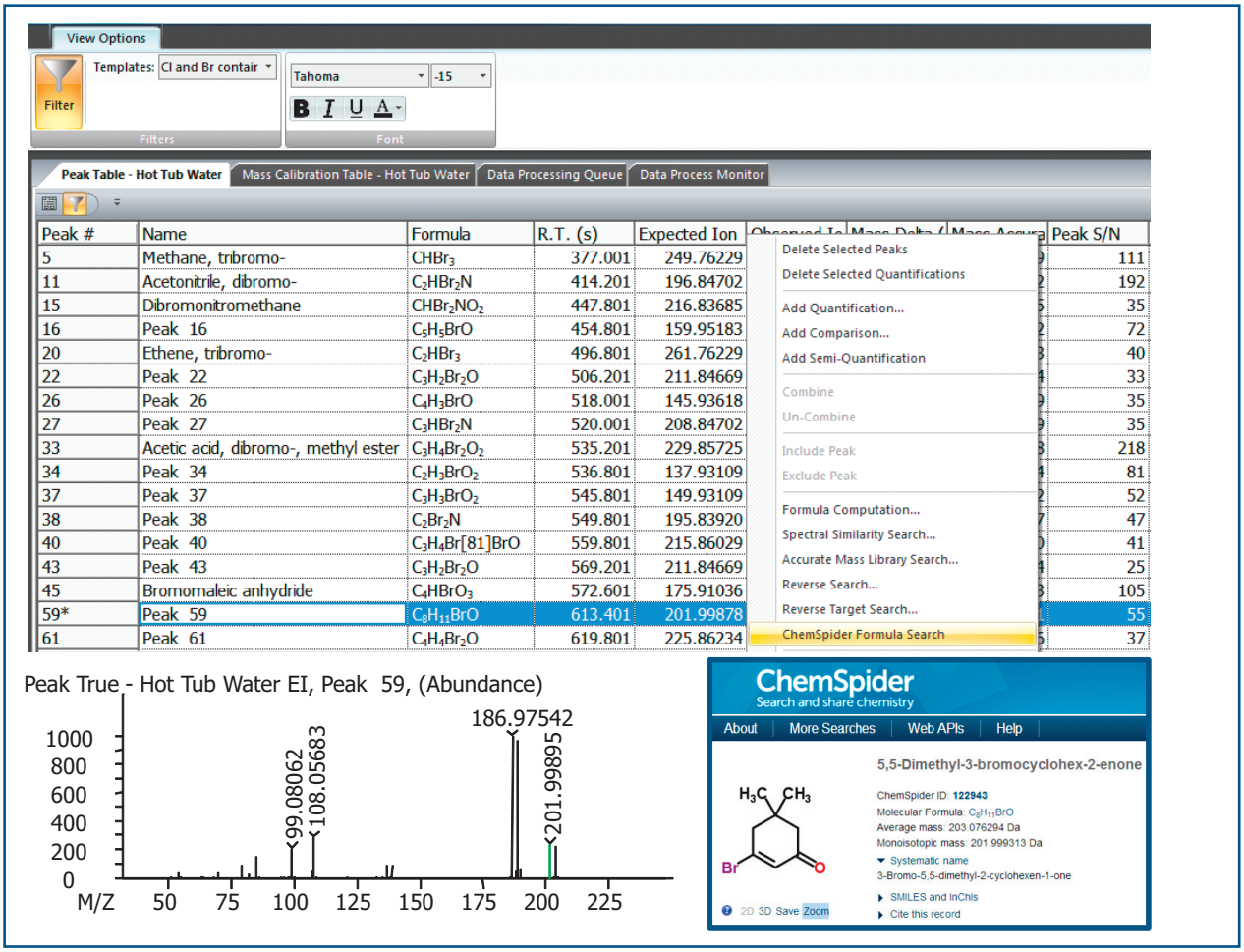


Figure 4. The Peak Table filtered for Cl and Br containing compounds facilitates the identification of halogenated disinfection byproducts. Unknown compounds for which chemical formula were generated may be searched directly from the ChromaTOF-HRT brand software by launching ChemSpider, and the appropriate structure may be proposed based on the corresponding deconvoluted (peak true) mass spectrum.

4. Conclusion

LECO's *Pegasus* GC-HRT with industry-leading mass accuracy and resolution facilitated the identification of more compounds than ever before in these samples, helping to discover what else was in the samples. The automated tools in the *ChromaTOF-HRT* bramd software made compound discovery possible with the identification of previously unknown disinfection byproducts.

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