Identification of Compounds in South African Stream Samples Using Ion Composition Elucidation (ICE)

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Introduction

Many target compounds have been identified and quantified in surface waters from industrialized countries. A major study by the USGS targeted 95 compounds in streams throughout the U.S.[1] Analytical methods for target compounds usually employ cleanup procedures to remove potential mass interferences and utilize selected ion recording (SIR) to provide low detection limits. Such an approach, however, could overlook non-target compounds that might be present and that could pose risks to ecosystems or to humans. In an ideal world, all compounds present would be identified, quantified, and evaluated for toxicity.

Ion Composition Elucidation (ICE)

The U.S. EPA's Environmental Chemistry Branch is identifying as many compounds as possible in combined acid and base/neutral extracts (1 mL total) of six 4-L stream samples collected near Johannesburg, South Africa, using Ion Composition Elucidation (ICE), a high resolution mass spectrometric technique developed in-house for a Finnigan MAT 900S double focusing mass spectrometer. This Selected Ion Recording (SIR)-based technique measures the exact masses of an ion and its +1 and +2 isotopic mass peak profiles that arise from heavier isotopes such as ^{13}C, ^{2}H, ^{15}N, ^{17}O, ^{18}O, ^{33}S, and ^{34}S. The abundances of the +1 and +2 profiles relative to the monoisotopic ion's profile are also measured for compounds by the technique illustrated in Figure 1.[2]

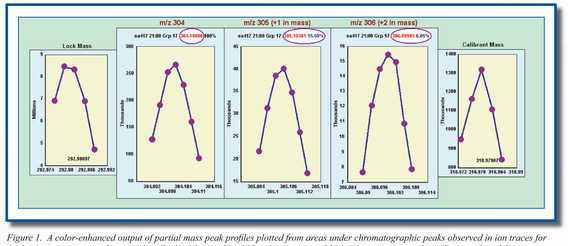


Figure 1. A color-enhanced output of partial mass peak profiles plotted from areas under chromatographic peaks observed in ion traces for each m/z ratio corresponding to a dot. Each of the m/z 304, 305 (+1 in mass), and 306 (+2 in mass) partial profiles was plotted from seven ion traces, and the lock and calibration mass profiles were each plotted using five ion traces. Three exact masses (red) and two relative abundances (blue) were calculated from these profiles. The corresponding theoretical values for partial profiles from $C_{13}H_{21}N_2O_3PS^+$, the molecular ion of diazinon (dimpylate), are: **304.10105, 305.10396, 306.09944, 15.18%,** *and* **6.11%.**

Automated comparison of measured and calculated values of these three exact masses and two relative abundances generally provides a unique composition for the apparent molecular ion.[3] Determining the exact masses of fragment ions then provides their compositions. This methodology realizes the full potential of high resolving power for separating components by exact mass on the time scale of a chromatographic peak. Mass spectral interpretation based on ion compositions often provides tentative identifications when there are:

- multiple library matches,
- convoluted mass spectra,
- poor quality mass spectra, or
- no library matches.

Notice: The U.S. Environmental Protection Agency (EPA), through its Office of Research and Development (ORD), funded and performed the research described. This poster has been subjected to the EPA's peer and administrative review and has been approved for presentation.

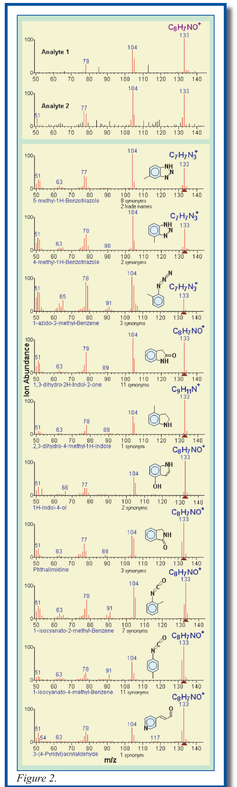


Figure 2.

Multiple Library Matches

Two background-subtracted mass spectra are shown in the top part of Figure 2. M/z 133, 104, and 78 were entered in the NIST library program and 10 plausible matches with three different molecular ion compositions were found. Thus far, ICE was used to determine the molecular ion composition of the first analyte, C_7H_7NO, which limited its possible identity to six compounds (assuming the analyte mass spectrum was in the NIST library). The two isocyanatobenzenes are unstable in water, but could form from decomposition of precursor compounds in the injection port. The azido compound is probably unstable or explosive. These three compounds are not likely to persist in a stream. For the remaining 4 isomers, the NIST library provided 11 synonyms for 1H-indol-4-ol, while the others had 3 or less. This compound was purchased and provided the same retention time as the analyte. The second analyte is most likely 5-methylbenzotriazole, since the eight library synonyms included two trade names. This compound has been quantified by USGS in U.S. waters.

Convoluted Mass Spectra

When hundreds of compounds are present in an extract, coelution of multiple components is common. The background-subtracted (middle) mass spectrum in Figure 3 did not correspond to a single compound and no NIST library matches resembled the mass spectrum. A loss of 4 from the m/z 139 ion to produce the m/z 135 ion was unlikely, but both ions could have been fragment ions of an unobserved molecular ion. ICE was used to determine the compositions of the m/z 135 and 139 ions. Each had an integer number of rings and double bonds suggesting each was a molecular ion and that the mass spectrum resulted from at least two compounds. Entering C_7H_5NS and $C_7H_9NO_2$ into the NIST library provided five good matches and one good match with multiple ions in the convoluted mass spectrum, respectively. Benzothiazole had three commercial names, while the other four compounds had none. Benzothiazole was purchased and it provided the same retention time as the analyte. The compound providing the single library match for the m/z 139 ion is not readily available. Determination of the molecular ion's composition provides support for its tentative identification in addition to the library match. The library matches are the top and bottom mass spectra in Figure 3. Most of the ions in the convoluted mass spectrum are accounted for, but m/z 121 was produced from a third unidentified compound.

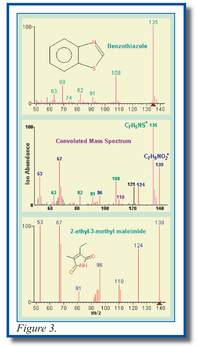


Figure 3.

Poor-Quality Mass Spectra

The negative mass defects for ions in the poor-quality, background-subtracted mass spectrum displayed in Figure 4 and the presence of m/z 249, 251, and 253 ions suggested an ultra-trace level of a halogenated compound was contributing to the mass spectrum. ICE determined the m/z 249 ion's composition was $C_6H_{12}Cl_3PO_4^{+\cdot}$. M/z 249, 251, and 223 were entered into the NIST library program and two compounds were found that contained a P atom and at least four O atoms. One provided ions at m/z 279 and 281, which were absent from the analyte mass spectrum. The remaining compound was a flame retardant, tris(2-chloroethyl)phosphate ($C_6H_{12}Cl_3PO_4$) for which the $[M-Cl]^+$ isotopic cluster yielded the highest-mass ions observed. It was purchased and found to have the same retention time as the analyte.

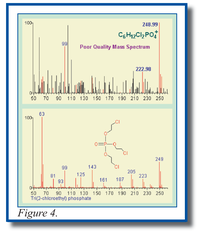
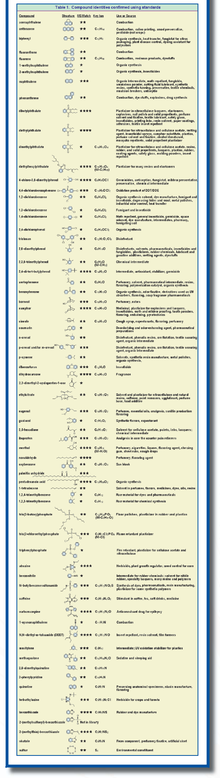


Figure 4.

Table 1. Compound Identities Confirmed Using Standards



No Library Matches

The background-subtracted mass spectrum in Figure 5 was not found in the NIST library. The molecular ion was determined to be $C_7H_5NO_2S_2^+$ using ICE. Ordinarily, the compositions of the fragment ions would also be determined and candidate molecules consistent with all ions would be hypothesized. However, in this fortuitous example, a chemical company's catalog contained only one compound with this composition. In addition, the fragment ions observed were plausible based on the compound's structure. It was purchased and its retention time was the same as that of the analyte.

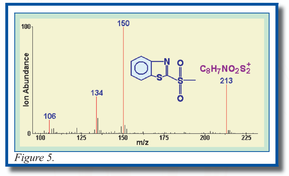


Figure 5.

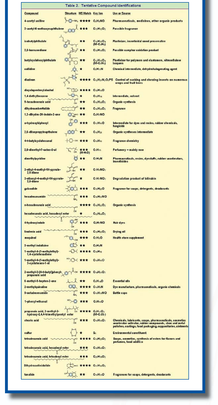
Compounds Identified and Tentatively Identified

The analytes listed in Table 1 and their standards had the same retention times. The analyte mass spectra when compared to those of the standards ranged from nearly identical to containing only a few ions in common. The quality of the background-subtracted mass spectra and library matches were visually judged to be poor, fair, good, or excellent as indicated by one to four stars. Often for poor quality mass spectra, the composition of the molecular ion or other high-mass ion (key ion in the table) was determined and entered into the NIST library before candidate compounds became apparent. These ion compositions provided support for many tentative identifications before the standards were purchased. For low-level analytes and for instances where significant mass interferences were present, the ion compositions provided important additional confirmation of each compound's identity. Additional compounds will be added and blanks in the table will be filled as this work progresses.

In Table 2 are listed analytes for which library matches were found and for which the composition of the molecular ion or a high-mass ion (key ion) was consistent with the library match. For analytes with standards available for purchase, most of these tentative identifications will be proven correct. Where standards are not available, the ion composition strengthens the tentative identification. Compounds of anthropogenic origin could be traced upstream to their sources to confirm their identities based on their industrial, commercial, or household usage. Some analytes could be byproducts or degradation products.

At least 40 additional analytes not listed in either table provided plausible library matches and will be investigated further.

Table 2. Tentative Compound Identifications



Importance of Software Versatility

The versatility of the Finnigan ICL® and ICIS® software made ICE studies of the hundreds of analytes in these complex extracts possible. Using resolving powers of 3,000 and 10,000, up to 32 ions were studied for each injection; each ion was from a different analyte. Template files in Lotus® 9.1 based on the center masses of ions, start and end times for each SIR group, and the mass resolution, all entered by the user, provided analyte specific ASCII files. These files were run as procedures by the data system to prepare SIR menus and to tune the instrument (ICL), and after data acquisition, to display ion chromatograms, to integrate peak areas, and to write an ASCII report file of m/z ratios and areas (ICIS). This ability to execute ICL and ICIS procedures provided to the data system as ASCII files of instructions is essential for applying ICE to such complex extracts. Grange and Sovocool suggest that all research-grade instruments should provide software with this degree of versatility to enable operators to explore and automate new ways of using the instruments. ICE is a product of such exploration.

Conclusion

- Streams in South Africa contain many of the same compounds found in streams in North America and Europe.
- Ion Composition Elucidation (ICE) is a valuable tool for identifying non-target compounds in complex environmental samples.

References

[1]**"Pharmaceuticals, Hormones, and Other Organic Wastewater Contaminants in U.S. Streams, 1999-2000: A National Reconnaissance,"** DW Kolpin, ET Furlong, MT Meyer, EM Thurman, SD Zaugg, LB Barber, and HT Buxton, *Environ. Sci. Technol.*, **2002**, *36*, 1202-1211.

Final drafts of references 2, 3, and other detailed information about ICE and its applications are available at: **http://www.epa.gov/nerlesd1/chemistry/ice/default.htm**

[2]**"Determination of Elemental Compositions from Mass Peak Profiles of the Molecular (M), M+1 and M+2 Ions,"** Grange AH, Donnelly JR, Sovocool GW, Brumley WC, *Anal. Chem.*, **1996**, *68*, 553-560.

[3]**"A Mass Peak Profile Generation Model to Facilitate Determination of Elemental Compositions of Ions Based on Exact Masses and Isotopic Abundances,"** Grange AH, Brumley WC, *J. Amer. Soc. Mass Spectrom.*, **1997**, *8*, 170-182.

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