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Identification of Unknown Contaminants in Water Samples from ISS Employing Liquid Chromatography/Mass Spectrometry/Mass Spectrometry

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ABSTRACT

Mass Spectrometry/Mass Spectrometry (MS/MS) is a powerful technique for identifying unknown organic compounds. For non-volatile or thermally unstable unknowns dissolved in liquids, liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) is often the variety of MS/MS used for the identification. One type of LC/MS/MS that is rapidly becoming popular is time-of-flight (TOF) mass spectrometry. This technique is now in use at the Johnson Space Center for identification of unknown nonvolatile organics in water samples from the space program. An example of the successful identification of one unknown is reviewed in detail in this paper. The advantages of time-of-flight instrumentation are demonstrated through this example as well as the strategy employed in using time-of-flight data to identify unknowns.

INTRODUCTION

Mass spectrometry generates more information from less sample than almost all other laboratory analysis techniques. This makes it essential for qualitative and quantitative analysis of the rare water samples from various aspects of the space program. For nonvolatile or thermally-labile analytes, liquid chromatography/mass spectrometry (LC/MS) is the mass spectrometric technique of choice [1]. However, data processing and interpretation from this technique are more difficult than gas chromatography/mass spectrometry (GC/MS) techniques. A result of this difficulty is that more unknown organic compounds remain unidentified by this technique. The reason for the advantage that GC/MS has over LC/MS in qualitative analysis is the availability of numerous probability-based-matching libraries that can locate the closest fingerprint matches to a mass spectrum of an unknown, from 200000 or more user-contributed mass spectra [2,3]. These spectral libraries are available for GC/MS, because GC/MS analysis conditions are much more similar from instrument to instrument than are LC/MS. Databases are not yet available for LC/MS. So, when analysts employ LC/MS to generate a mass spectrum of an unknown contaminant, they must interpret the spectrum themselves employing knowledge of structure and fragmentation. Another disadvantage to LC/MS is, because it ionizes the analytes less harshly than GC/MS, fewer peaks (clues) exist in LC/MS spectra. (While the scarcity of peaks in LC/MS make the spectra more difficult to interpret than those of GC/MS, the LC/MS peaks generally contain more ions, as only a few peaks dominate the spectrum, and this can be viewed as an advantage in the sensitivity of the method for quantitation.) Thus, when a laboratory characterizes a water sample as completely as possible, the majority of contaminants that remain unidentified are the nonvolatile organics, the ones only detectable by LC/MS.

Time-of-flight based mass spectrometers have been reborn in the past 15 years [4] because of their ability to produce easier-to-interpret mass spectra, and are increasingly being incorporated into LC/MS instrumentation. TOF instruments are able to do this by determining mass much more accurately than most other mass spectrometers and are classified as “accurate mass” instruments [5].

The Water and Food Analytical Laboratory (WAFAL) at the Johnson Space Center analyzes water samples from the space program, and now uses time-of-flight instrumentation to identify nonvolatile organics. This paper describes the use of this instrument to identify one unknown recently detected in a water sample analyzed by the WAFAL lab. Other such identifications have been done, and many more are anticipated, but this one analysis is used as an illustration of the power of the instrument and the detective work that is needed given the lack of mass spectral databases for LC/MS.

The goal of the laboratory is to attain 90% "TOC Accountability". This means that if a water sample is found to contain 10 mg/L of total organic carbon (TOC) by a TOC Analyzer, then 9 mg/L of that carbon should be attributable to organic compounds already identified and quantified in it. Low TOC accountability means that the organic compounds in the sample are largely uncharacterized and could include toxic chemicals. The WAFAL lab averages approximately 60% accountability in humidity condensate samples [6] but much less in potable water samples, where it is more important.

TOC Accountability is the reason that the largest peak observed in this water sample (using this instrument configuration) was selected for identification first. The unknown could do the most harm if toxic and could increase accountability the most if successfully identified. A more detailed list of organics found in humidity condensate is provided in the Schultz paper referenced above.

INSTRUMENT OVERVIEW

HIGH PERFORMANCE LIQUID CHROMATOGRAPH (HPLC) – The HPLC employed is a "Surveyor" Model from Thermo Electron. It injects 50 uL of a water sample onto a column of solid-phase packing material. For this work a "Restek Allure" organic acid column, Model 9165585-700, 300x4.6x5uM, was used. The mobil phase was 0.3 mL/minute of 12% acetonitrile in water. The concentration gradient was 12% to 100% over 20 minutes, holding for 10 minutes, then back to 12%. Ammonium acetate was added at 0.005 molar concentration to the mobil phase as a buffer/ionization aid. Analytes separate in the column and flow one-by-one into the interface and are sprayed into the mass spectrometer in ionic form.

MASS SPECTROMETER – An Applied Biosystems QStar Elite Mass Spectrometer was used. It involves a quadrupole in series with a collision cell and a time-of-flight tube. This type of mass spectrometer is referred to in the industry as a "QTOF" for "quadrupole-time-of-flight.

HIGH RESOLUTION INSTRUMENTS - QTOF instruments are considered "high resolution", sharing this classification with double-focusing and Fourier transform ion cyclotron resonance mass spectrometers [7]. Most mass spectrometers, e.g. ion traps or quadrupoles, are "unit mass" instruments. Unit mass LC-MS instruments often require further fragmentation, or more degrees of MS, to identify an unknown compound. For example, if a unit mass LC/MS analysis detects an ion of mass 75 in positive ion mode, one assumes this is accurate to within one m/z, where m/z means the molecular (ionic) weight in daltons divided by the number of charges. This means that the ionic weight, if it contains one positive charge as most analytes do in positive mode, ranges from 74.7 to 75.7 daltons. In this case, the unknown ion could be H2O-C-COOH+, which has mass 75.0076, or it could be NH2-CH2CH2CH2-NH3+, which has mass 75.0916. Further fragmentation of this ion (called MS/MS) would be necessary to identify it, and even then knowledge of the mass of the fragment may not narrow down the number of candidate identities to one.

High Resolution mass spectrometers are called accurate mass instruments. These instruments can easily distinguish ions differing in mass by 50 millidaltons, or 0.05 m/z. In the mass 75 example, these ions differed in mass by 0.084 m/z, which exceeds 0.05. Therefore, if a QTOF mass spectrometer had detected the unknown ion of mass 75, and it had been mass-calibrated prior to this analysis, the operator could reject one candidate or the other without doubt.

The "Analyst" [8] software of the QStar has a calculator function which will list all possible formulas within a given number of millidaltons of the mass of the detected ion (peak), and these are clues to the identity of the unknown. The theoretical (exact) masses of most all ionic combinations of atoms have been tabulated for years. This software contains this information in a searchable database. The search can be narrowed using various properties the user suspects about the unknown ion and enters as search criteria.

Because QTOF instruments can employ MS/MS techniques to break an unknown ion ("precursor ion") into many "product ion" fragments, and because the calculator will suggest formulas for these, many of the candidate formulas that the QStar's Calculator has already suggested for the precursor ion can be discarded, because the sum of the pieces must contain the same atoms as does the unknown molecule. Therefore, by employing this powerful technique of "exact mass" or "accurate mass" determination repeatedly within the same unknown compound, one can often successfully identify it.

LC/MS ANALYSIS OF THE SAMPLE

The problem-solving involved in unknown compound identification by LC/MS/MS is easier to describe by the use of an example. In July of 2007, a sample of U.S. Lab Humidity Condensate, from the International Space Station (ISS) , returned on the Space Shuttle (Mission ISS13A/STS117), was analyzed on the QStar for unknowns. With our instrument, four different modes of ionization are possible between the HPLC column and the mass spectrometer. Their description is beyond the scope of this paper, but the first mode we employed was electrospray positive [9]. (The other three modes are yet to be applied to this sample and could produce and identify other peaks.)

The water sample was injected into the HPLC and the HPLC effluent was continually sprayed into the QStar, which was operating as an LC-MS rather than an LC-MS-MS at this point. This means that all ions were

allowed to pass through the first mass spectrometer (the quadrupole) and into the second mass spectrometer(the time-of-flight). The time-of-flight mass spectrometer was generating a mass spectrum every 100 microseconds for 50 minutes. These data are all stored in one datafile and can be analyzed and replotted in a number of ways.

LC/MS DATA PROCESSING

After chromatographing the sample, the first graph an analyst normally plots is the "Total Ion Current" or "TIC", an HPLC chromatogram in which total MS signal (regardless of mass) is plotted vs. elution time. These plots are normally not visually appealing because so much noise is added into every minute of the plot- peaks can be hidden throughout. But the TIC serves to show that you actually acquired data and, if peaks are visible in the TIC, they are probably major peaks. Figure 1 shows the TIC; only a few peaks are visible and one peak stands out as being much larger than the rest, the one at 32.26 minutes. Identification of that peak is the subject of this paper.

The next plots an analyst employs are mass chromatograms. A software program searches through the entire dataset for peaks not visible in the TIC, by sequentially searching all individual mass signals and generating a list of masses of eluting (often hidden) peaks. Plots are then generated of only that mass as a function of time. This eliminates most of the instrument noise that comes from monitoring many masses and concentrates on a mass that does have one or more peaks eluting. Figure 2 contains three mass chromatograms and shows how peaks not visible in the TIC can appear once the operator plots the appropriate mass chromatograms. The top pane is a replot of the TIC. The next plot down is a mass chromatogram of mass 112.9-113.1 as a function of time. A sharp peak, later identified as uracil, was found eluting at 35.58 minutes. It is barely visible, if at all, in the TIC. In the third pane, a peak was found and identified as the amino acid histidine. The fourth pane contains the unknown, to be identified later in this paper, with a mass in the range of 207.1 to 207.3. It is much taller than the first two peaks as indicated by the y-axis labels of detector counts (the software auto-scales each graph).

The next step in this research was to view a mass spectrum of the peak at mass 207. One purpose of plotting the mass spectrum is to verify that the ion about to be identified is the protonated molecule. In positive mode, an LC/MS generally adds a proton, in the electrospray ion source, to a molecule giving a peak in the mass spectrum at one dalton above it's molecular weight. This ion is called (M + H)+. However, other ions can be formed from the same molecule. Two protons can be added to give (M + 2H)2+. Other cations, present in the sample, can be added to the molecule by the same mechanism, to form ion-molecule adducts such as (M + Na)+, (M + NH4)+, etc. (Humidity condensate from crew cabin air always contains some sodium and ammonium.) Therefore, one must look carefully at the mass spectrum of a molecule to learn its true molecular weight, which peaks are cation adducts, and which peaks, if any, are fragments of the molecule (because some fragments form even in the LC/MS run, before fragmentation is being actively encouraged). Figure 3 is a plot of the mass spectrum after subtracting out ions present in the background (from ionized solvent clusters, buffer, etc.). The mass spectrum provides much information. The peak just to the right of mass 207, the "X+1 isotope peak", is m/z 208, as expected. If it were 207.5, we would be looking at a doubly charged ion of mass 414 and our unknown would be 412 grams per mole, even though the observed peak is 207. This isotope peak tells us that the mass 207 ion is singly charged. Because mass 207 is exactly 17 m/z less than another major peak and 22 m/z less than another major peak, we can surmise that 207 is the protonated molecule, 224 is the ammonia adduct (the molecular weight of the uncharged unknown molecule, or 206, plus the molecular weight of ammonium ion, 18), and mass 229 is the sodium adduct. It was difficult to explain mass 339 until it was realized that a cesium salt had been used for mass calibration, and residual cesium remained in the LC/MS interface. Mass 339 is the cesium adduct. Now that we know that mass 207 is the ion to identify, the "nitrogen rule" [10] is applied: "An even-electron ion containing an even number of nitrogen atoms will appear at an odd mass number." LC/MS produces virtually all even-electron ions. Therefore, our mass 207 ion contains 0,2,4, etc. nitrogen atoms in its formula.

Besides pinpointing the protonated molecule (to make identification easier) and verifying that the ion under scrutiny is singly-charged, the mass spectrum tells whether any sulfur, chlorine, bromine, or silicon atoms are present in the unknown. The "X+2 ion" tells this, or mass 209. If mass 209 were >3.4 % of the height of mass 207, then the presence of these atoms would be possible. But mass 209 is 18 intensity counts, while mass 207 is 1300. That percentage is 1.3, ruling out sulfur, bromine, chlorine, and silicon, based on their isotopic abundances in nature.

RE-ANALYZING THE SAMPLE IN LC/MS/MS MODE

We now have the accurate mass of the protonated unknown: 207.1620 daltons. We could put the instrument software to work to suggest possible molecular formulas for it right now. However, with a mass as high as 207, numerous possible candidate formulas for this unknown would probably be suggested by the instrument software. We would most likely need MS-MS fragmentation data to select which formula is correct. Also, the preliminary analysis had been done with a mass calibration of less than ideal quality because the mass range was too wide; when one doesn't know what masses to expect, the calibration is performed over a wide mass range s o that no peaks lie outside of the range. We had originally run a 2 point calibration from

133Da to 829Da. We decided to recalibrate at a nerrower range (46-376) to improve mass accuracy, and to re-analyze the sample in LC/MS/MS mode. In this mode, we set the first MS (the quadrupole) to only allow mass 207 through, set the collision cell to bombard this ion with nitrogen, and measured the fragment masses (and some unfragmented mass 207) in the more-accurately-calibrated time-of-flight mass spectrometer. The following are the accurate masses of the eight ions detected in this experiment in daltons: 207.1541, 151.0972, 145.1233, 133.0856, 101.0952, 89.0627, 57.0713, and 45.0343.

CALCULATING POTENTIAL MOLECULAR FORMULAS

The instrument software can suggest possible molecular formulas for any ion if its mass is known accurately. When one searches the software for possible formulas of the protonated unknown (mass 207.1541) as well as for each of the seven fragments, a wealth of information is available. In addition, each fragment ion above implies the accurate mass of a neutral molecule, lost during fragmentation, but not detected as a peak because it is uncharged. This is because, for a charged ion to spit into two pieces, one will still carry the charge (and is called a “fragment ion”) while the other will be called a “neutral”. Such neutrals are eliminated by the vacuum pumps and go undetected. For example, for mass 207 to fragment into mass 151, it had to have lost a neutral molecule weighing 207.1541-151.0972=56.0569. Mass 56.0569 was not detected, but its existence was implied and it can also be searched for possible formulas, doubling the information available.

Figures 4 and 5 are screenprints from the Analyst Software showing the “Calculator” function in use identifying possible formulas for the protonated molecule (mass 207). First, the user must input the criteria the Calculator is to use for the search. Before searching, the Calculator wants to know the allowable range of carbon atoms, hydrogen atoms, etc. in any given fragment (“Elements and Limits” window in Figure 4). It also requires data as to how much mass inaccuracy is allowed (“Tolerance”). For example, if the measured mass is 45.0343 Da, and one possible formula it calculates to produce this mass is C2H5O+, weighing 45.0335 Da, is this mass so far away that the operator wishes to reject it from consideration? The Calculator also asks if the operator wishes to consider radical formulas, not normally present in LC/MS, but still possible. In Figure 4, we set the “Electronic State” as “Even”, excluding radicals from consideration. Finally, the Calculator can determine the number of “rings plus double bonds” in its proposed formulas, so the operator can specify the ranges of this parameter to accept and reject. These are the “DBE” parameters, for Double Bond Equivalents in Figure 4. If the DBE parameter is wisely specified, the software will automatically reject some absurd formulas.

The criteria we used for the Calculator are as follows: mass accuracy must be within 10mDa (since mass accuracy upon calibration was 1 mDa, and accuracy gets worse once analysis conditions change). Only carbon, hydrogen, nitrogen, and oxygen are present. (We had not yet ruled out the presence of F, I, or P, but these increase dramatically the number of proposed formulas requiring consideration, so we decided to revisit these possibilities if an identification was not obtained without them.) Allowable ranges are 30%-98% carbon, 5-20% hydrogen, 0-40% nitrogen and oxygen. Cations are allowed to have -0.5 to +8.0 double bond equivalents, and neutrals are allowed to have -2.0 to +8.0.

Figure 5 shows the results of a search of Mass 207.1541 (the unknown protonated molecule) using the above criteria. While 10 formulas are listed, in order of closeness to the experimental mass, only five formulas are within 10 mDa of the experimental mass. Because we listed 10 mDA as our tolerance, it is unclear why the second five were listed, but they were ignored. The first five formulas are very important, because one of them (it is hoped) is the correct formula for the unknown ion.

The Calculator’s results for each fragment ion and neutral are listed in Table 1. These will serve to determine which of the five candidates for the unknown molecular formula is correct (if any) and what its structure might be. In general, the smallest fragments in Table 1 have the fewest candidate formulas. They also have the fewest possible structures that have a given formula and still make sense chemically. So the first fragments one would consider are usually the lowest weight fragments. However, in the case of this unknown molecule, line 13 of Table 1 gives us the biggest clue. The fragment of mass 145.1233 is only given one possible formula, C8H17O2+. Further over on Line 13 is the formula for the neutral molecule that split out to leave that fragment: C2H6O2. Adding these two formulas together gives us the molecular formula for our unknown protonated molecule: C10H23O4+. It is the third choice down on the list in Figure 5. The remaining parts of Table 1 are now only needed to learn the structure of the unknown, not the formula.

The next part of Table I that got our attention was Line 1, the fragment of mass 45.0343. It can only be C2H5O+. This had to have come from one of three groups: ethoxy CH3CH2O−, hydroxyethyl HOCH2CH2−, or a methyl ether CH3OCH2−. With any fragments coming from LC/MS/MS, such as those in in Table 1, we assume that they came from an “end” of the unknown molecule, rather than being buried somewhere in the middle, where multiple carbon-carbon bonds would have to break for the fragment to form. If fragments emerged from the centers of molecules, then protein sequencing by mass spectrometry would not be possible. Therefore, one of these three groups makes up the very end (terminus) of the unknown molecule.

The next most useful piece of the puzzle is Line #4 of Table 1, where we get a C4H9+ fragment. This could only come from a normal butyl group −CH2CH2CH2CH3, a sec-butyl group −CH(CH3)CH2CH3, an isobutyl group −CH2CH(CH3)2, or a tert-butyl group −C(CH3)3. This group has to be at the other end of the molecule, because we already established that an oxygen atom is 1-2 carbons in from one end, and this fragment has no oxygen. (It is still possible that our unknown molecule has 3 or 4 "ends", because one of its carbon atoms could be branched.)

We now have a general knowledge of the structure of the unprotonated unknown. It contains C2H5O− at one end and −C4H9 at the other. From subtraction we infer that the center of the molecule is of the formula C4H8O3.

To add more detail to the structure, we need another piece of the puzzle. Line 7 of Table 1 contains an ion of mass 89.0627 with two candidate formulas. One candidate formula contains nitrogen. We already know that our unknown has no nitrogen, so the only possible makeup of this fragment is C4H9O2+. Our current molecular structure, with as much detail as we can give it, is [C2H5O]-[C4H8O3]-[C4H9]. Which end of the molecule was the mass 89 fragment cleaved from? If it came from the side with the butyl group, it would have to have been present as −OOC4H9. This would make our unknown molecule an unstable organic peroxide-not likely in a humidity condensate sample. C4H9O2+ probably came from the other end where the C2H5O is. Since C4H9O2+ contains two oxygen atoms somewhere in the span of four carbon units, and the left-most [C2H5O]- portion of the unknown only contains one oxygen, this tells us more about the center [C4H8O2] section of the unknown. The first two carbon units of the center [C4H8O2] have one oxygen attached. Therefore, our unknown molecule looks like this: [C2H5O]−[C2H4O]−[C2H4O2]−[C4H9] with the mass 89 fragment making up the first two pieces.

Again, to add more detail to the structure, more information from Table 1 is needed. Line #9 of the table shows a fragmentation in which a mass 101 ion, C6H13O+, breaks off. This fragment had to have come from the right side of the unknown, as drawn above, because it contains only one oxygen, and the unknown contains (from the left) two oxygens within the first four carbon units. Therefore, the right side of the unknown is −[C2H4O]−[C4H9]. The unknown's structure now contains a bit more detail: [C2H5O]-[C2H4O]-O-[C2H4O]-[C4H9]. The first two subunits, [C2H5O]-C2H4O]- can be arranged in one of two structures: HOCH2CH2CH2OCH2O− or HOCH2CH2OCH2CH2O−. The structures differ in whether the oxygen atoms are evenly-spaced or unevenly-spaced.

Does Table 1 contain any evidence as to which of the two structures make up the left half of the unknown molecule? It does: Line 13 shows a neutral loss of C2H6O2. This had to have come from the very left end of the molecule because it contains one or more oxygen atoms. The fact that it contains two oxygens over the span of two carbons shows that the second structure is the correct one, in which the oxygens are evenly-spaced. In the first structure, counting from the left, three methylene groups, −CH2−, occur before two oxygen atoms do, so this cannot be it.

We now know the entire structure of the unknown molecule, except for the arrangement of the butyl group on the end. It is HOCH2CH2OCH2CH2OCH2CH2OC4H9. This is triethylene glycol mono-n-butyl ether (or sec-butyl, tert-butyl, or isobutyl). We saw no evidence of fragmentation of the butyl group, so we can't distinguish. At this point, we consulted the chemical supplier catalogs in order to find which, if any, of these compounds are available for purchase. Searching by the molecular formula, C10H22O4, we found 1,1,6,6-tetramethoxyethane, tripropylene glycol monomethyl ether and triethylene glycol mono butyl ether (this is the common name, referring to the n-butyl derivative). The third compound in the catalog could be the unknown; the first two could not be. It was worth the time and money to purchase the compound and analyze it under the same conditions.

Figure 6 displays the spectra resulting from LC/MS/MS analysis of a 3 ppm solution of the suspected compound, followed immediately by analysis of the water sample under the same conditions. All of the fragments listed in Table 1 occur in both spectra, in the same intensity ratios, and with virtually the same accurate masses. The fine print above each spectrum shows that the peaks eluted from the HPLC column with the same retention time: 31.9 minutes. This confirms the identity of the unknown: triethylene glycol mono n-butyl ether (TGBE).

QUANTITATIVE ANALYSIS

To compare levels of TGBE in various types of water samples from the space program, a quantitative method is needed. Quantitation can be done with QTOF type mass spectrometers. They do not always have the wide linear ranges that GC/MS instruments do, but they are more specific (interference-free) if run in LC/MS/MS mode. An LC/MS/MS method was developed that isolates the protonated molecule, mass 207, in the quadrupole, rejecting all other masses. It fragments the ion and quantitates using mass 151. The QStar's response to TGBE proved to be very sensitive.It is linear from 20ppb to 300 ppb, becoming quadratic at higher levels. Once the method was established and verified, it was used to quantify TGBE in seven water samples from ISS and the Space Shuttle. Some samples required dilution if they exceeded 300 ppb. Results are listed in Table 2.

Table 2: Concentrations of TGBE in Shuttle and ISS Water Samples

Sample Name	Sample Description	[TGBE], ppm
2006-0921-007	U.S. Lab Condensate	2.130
2007-0625-008	U.S. Lab Condensate	1.120
2007-0823-001	ISS Potable Water	<0.02
2007-1109-005	U.S. Lab Condensate	1.540
2008-0108-009	ISS Potable Water	<0.02
2008-0108-012	ISS Potable Water	<0.02
2008-0108-014	Raw Condensate	0.039

CONCLUSION

In the example described above, the major unknown peak in the water sample was identified as triethylene glycol mono-n-butyl ether. As the instrument was found to be very sensitive to TGBE, and because the instrument's response factor varies greatly from compound to compound, TGBE may not be the most abundant analyte in this sample that is detectable by LC/MS/MS. Three other ionization modes are yet to be employed on these samples and may identify more abundant contaminants.

High resolution LC/MS/MS is a powerful technique for qualitative analysis of nonvolatile organics in water samples. It can then serve to quantify these contaminants, but several variables associated with the analytical method must be optimized.

For qualitative analysis, some detective work is needed, and in many cases not all questions are answered by the technique. The chemist must first identify the protonated (or deprotonated in negative mode) molecule and fragment it under properly-chosen conditions. The chemist must use specialized software to search for candidate formulas of ions as well as of neutral losses. Most importantly, the chemist must mentally assemble the suspected molecular parts into an intact molecule which proves to be the unknown.

This work is relevant to the goal of identifying major specific contributors to the total organic carbon (as measured by TOC—see ref 6) in samples of water and wastewater from ISS and Shuttle. This is a significant part of a larger program of water quality monitoring. Shuttle and Space Station environments are exhaustively monitored for contaminants in the air and water to include any recurring or emerging major unknowns. Humidity condensate reflects the quality of the cabin atmosphere to a great extent and is a major source of drinking water via the regenerative systems.

Because the instrument is proving to be highly sensitive, it should be possible to identify contaminants in ISS potable water, where the current % TOC accountability is lower and the importance is greater. That is the planned direction for further work.

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DEFINITIONS, ACRONYMS, ABBREVIATIONS

Here is the Definitions section. This is an optional section.

DBE: "Double bond equivalents" – the sum of the number of rings plus double bonds in a molecule or ion. This parameter can be calculated if the formula is known.

HPLC: High performance liquid chromatography, an instrumental technique that separates analytes using a solid stationary phase and a liquid mobil phase.

ISS: International Space Station

JSC: Johnson Space Center

LC/MS: Separation of analytes by high performance liquid chromatography, followed by mass spectrometry. "Liquid chromatography/mass spectrometry."

LC/MS/MS: Separation of analytes by high performance liquid chromatography, followed by ionization, fragmentation, and detection by tandem mass spectrometry.

QTOF: Quadrupole time-of-flight. A transmission-quadrupolem/z analyzer, followed by a quadrupole collision cell, followed by a time-of-flight m/z analyzer.

MS/MS: Mass spectrometry/mass spectrometry or tandem mass spectrometry

m/z: Mass per unit charge

(M+H)+: The protonated molecule. This is the derivative of the unknown that is normally studied in a positive ion mass spectrum.

TGBE: Triethylene glycol mono-n-butyl ether, the identity of the unknown. An acronym developed by this laboratory to significantly decrease the number of pages in this paper.

TIC: "Total ion current" – the sum of all intensities in a mass spectrum.

TOF: Time-of-flight mass spectrometry. This type of instrument uses no external force to separate ions of different mass to charge ratios.

WAFAL: Water and Food Analytical Laboratory, NASA Johnson Space Center.

X+2 Ion: A derivative of the ion of interest (the X ion) which contains a naturally occurring isotope with mass 2 daltons greater than that in the X ion, and causing a MS peak 2 daltons higher.

APPENDIX