The Use of Accurate Mass, Isotope Ratios and MS/MS for the Identification of PPCPs in Water

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Introduction

During the three decades prior to the year 2000 the study of chemical pollution was confined primarily to pesticides. Following a seminal article by C. Daughton[1] this focus began to shift to the emerging environmental concern for pharmaceuticals and personal care products (PPCPs). Many of these pharmaceuticals, including estrogen, have been known as endocrine disruptors, or chemicals that disrupt the physiological function of hormones in organisms. In 2004 a report from the United States Geological Survey [2] was made as a result of discovering a high preponderance of intersex (male fish exhibiting female characteristics) in Smallmouth Bass of the Potomac River. As a result, it is important to use adequate techniques to help identify these compounds and possible metabolites.

Using accurate mass in full scan (mass range) mass spectrometry (MS) compound empirical formula can be determined for purposes of identification. Furthermore, a high degree of spectral resolution allows for selective identification among co-eluting compounds. Isotope ratios are an additional tool as they help identify compounds with high carbon numbers as well as those that contain elements like chlorine and sulfur. Although these tools do a lot to confirm chemical formula, it may still be left to the user to decide which of the possible structures of isobaric compounds apply.

To assist in the analytical need for structural elucidation, selective MS/MS by using the quadrupole time-of-flight mass spectrometer (QTOF) is implemented. Because the Agilent QTOF also has very accurate mass at the MS/MS level, it is easier to determine the structures of the product ions, which correspond as substructures of the precursor ion and thereby reduce the number of possible structures pertaining to the derived empirical formulas from several to one.

An Agilent 6510 Quadrupole Time of Flight Mass Spectrometer (QTOF) is used to analyze several surface water samples for the presence of pharmaceutical compounds. A simple gradient elution is carried out on a Rapid Resolution High Throughput Extend C18 column (particle size 1.8 µm). Of 44 potential compounds as many as 31 are proposed in one of the samples using an algorithm known as the Molecular Feature Extractor (MFE). To make comparisons among several samples another algorithm known as Mass Profiler is applied to the data processed by the MFE. Since the MFE may generate thousands of potential compounds known as features, Mass Profiler makes statistical comparisons of the features among two different samples to determine what is unique and common. All of this work is done with the full scan mass spectral data. When compounds of interest are determined, accurate mass full scan MS/MS can be invoked for structural elucidation. The results of full scan MS/MS applied to caffeine and sulfamethoxasole are included as an example and are relevant because some medications include both as ingredients.

Experimental

Sample Preparation

Prepared samples provided by the United States Geological Service National Water Quality Laboratory (USGS/NWQL) in Lakewood, Colorado. The details of the extraction procedure used are not included here, but are available upon request. Pharmaceuticals are typically extracted from surface water by using disposable polypropylene syringe cartridges that contain 0.5 g of polymeric sorbent. One liter of sample is pumped through the solid-phase extraction (SPE) cartridge. The analyte material is later eluted into 1 mL of methanol, resulting in a concentration increase of three orders of magnitude. As this is an LC/MS analysis no derivatization of the sample is required.

Following atmospheric pressure ionization, accurate mass and high resolution spectral data is acquired and then investigated by special algorithms for the determination and comparison of features which may or may not correspond to known drug compounds in a database. The database containing the chemical formula and exact neutral masses of these compounds is show in Table 1 and used to find out which compounds may be in which samples.

Targeted MS/MS is then run on the screened compounds for purposes of structural elucidation.

Table 1. List of 44 potential compounds in water samples.

Agilent 1200 series binary SL pump, degasser, wellplate sampler, and thermostatted column compartment

LC Conditions

Columns: ZORBAX RRHT Extend C18, 2.1 x 50 mm, 1.8 µm particle size (Agilent PN: 727700-902); **Column temp:** 40 °C; **Mobile phases:** A = 0.1% formic acid in water, B = 0.1% formic acid in ACN; **Flow rate:** 300 µL/min; **Linear Gradient:** 0% B at 0 min to 67% B at 10 min, and then to 100% at 11 min and held until 15 min; post run time of 10 min, **Inj. vol:** 5 µL.

MS & MS/MS Conditions

Source (pos ESI, using G3251A Dual ESI source): Nebulizer 40 psi; Drying gas flow: 9 L/min @ 350 °C; **Capillary voltage:** 3500 V;
MS > Scan range: *m/z* 100 – 1100; **Scan rate:** 1 scan/sec.
MS/MS > Collision energy = 30 V; **Scan range:** *m/z* 50 – 1000; **Scan rate:** 1 scan/sec.

Results and Discussion

Several samples from different surface water sources are analyzed, two of which are shown reported in this work. A typical injection of one of the samples is shown in Figure 1.

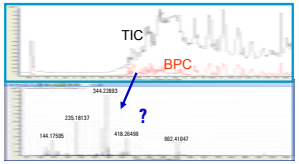

Figure 1. Manual identification of compounds involving inspection of mass spectral data can be very tedious.

With so much data to examine, it becomes very important to have an algorithm available to convert all of the data to useful information. Such an algorithm is known as Molecular Feature Extractor and is used here, employing the following steps:

- **Persistent chemical background is removed**
- **Co-eluting interferences are resolved**
- **Isotopic cluster recognized and grouped**
- **Molecular adducts are recognized**
- **2D/3D Data visualization**
- **Chemical identification (ppm, isotope matching)**

The result of analyzing Sample 1 using MFE is shown in Figure 2. Contour plots of the unprocessed versus processed data are juxtaposed to demonstrate the simplification in deriving features that may or may not be compounds of interest.

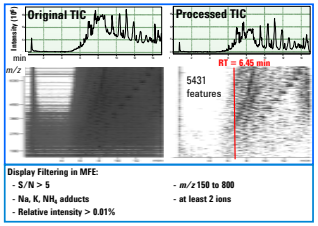

Figure 2. Processing of Sample 1 by MFE to result in features of interest. Features found at RT = 6.45 min (see Figure 3) are further explored.

If we now investigate some of the features that have been found we can begin with the apex spectrum examined in Figure 1. The retention time is 6.448 minutes as shown in Figure 3. The unprocessed spectrum at top corresponds to that shown in Figure 1. However, removing random noise and using the filtering rules of Figure 2 a processed spectrum containing 12 features is derived and shown at bottom. A subset of the features is shown at right.

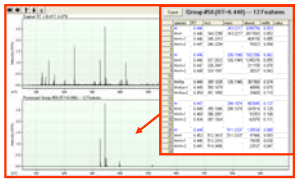

Figure 3. Twelve derived features at 6.448 minutes

Some of the features corresponding to the spectrum of Figure 2 are listed in in the table shown in Figure 3. For example, a feature that has isotopic distribution for the protonated forms reports a neutral mass of 343.2217.

By filtering the data to show features that have neutral masses within 0.005 Da of those in Table 1, there are apparently 31 of the compounds of interest in Sample 1. Figure 4 shows a match of diphenhydramine for one of the features.

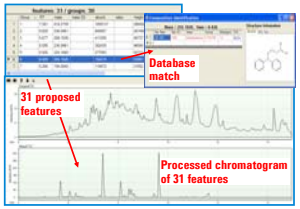

Figure 4. Thirty-one features (compounds) from database, including diphenhydramine, found in Sample 1.

Note that the compound diphenhydramine has an exact mass of 255.16231 ($C_{17}H_{21}NO$), which corresponds to the value shown in Figure 4 with an error of only -1.2 ppm. However, an actual standard of the compound should be run to verify the retention time.

Results and Discussion

An extension of determining features in a sample data file is to make comparison with features that can be found in other data files. In this work a second sample (Sample 2) is extracted, analyzed and features are also derived. Another algorithm, known as Mass Profiler, makes comparisons between the features of these two samples, which is useful for determining what features are unique or common, exist as compounds in a database like the ones shown in Table 1, or may be other compounds of interest that require further investigation.

The Mass Profiler requires that the features found in each sample are statistically significant and not random from one injection to another. Therefore, both Sample 1 and Sample 2 must be injected at least three times. The set of injections, or data files, for each sample are then combined into a group. As a result, these groupings will be called Group 1 and Group 2, respectively.

The common features among the two samples are shown in Figure 5.

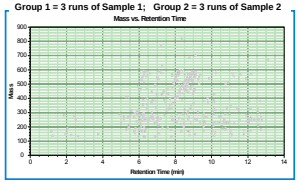

Figure 5. Features found in both Group 1 (Sample 1) and Group 2 (Sample 2), total = 346.

Of the 346 common features, how do they compare in relative abundance? One feature, possibly diphenhydramine in the database of Table 1, is common to both samples. In fact, it appears that this compound in Sample 2 is about 4 times more abundant than in Sample 1. See Figure 6.

Figure 6. Comparison of diphenhydramine in both samples

The unique capability of the QTOF is to measure the mass of product ions with a high degree of mass accuracy. After screening samples for potential compounds using full scan MS, a list of precursor ions for targeted MS/MS is generated and used in an acquisition method. A couple of examples are shown in Figure 7 in which accurate mass product ions are found for both caffeine and sulfamethoxasole. Proposed structures are generated using ACD/MS Fragmentor© (ACD, Inc., Toronto, ON, Canada).

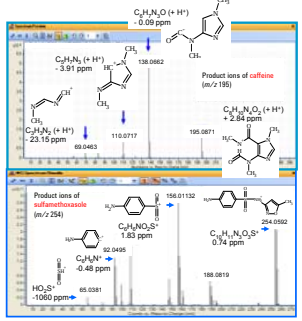

Figure 7. Calculated chemical formula given accurate mass measurement and using elements C, H, N, O, and S

Conclusions

Water samples are analyzed by LC/MS/MS using a QTOF instrument. Power of accurate mass is demonstrated in proposing compounds by empirical formula and good resolution enables the use of isotope ratios. The MFE algorithm finds a large number of possible compounds in any given sample, and Mass Profiler is used to make comparison among multiple samples. Furthermore, selective and accurate MS/MS is employed for structural elucidation.

REFERENCES

1. **"Pharmaceuticals and Personal Care Products in the Environment: Agents of Subtle Change?," C. G. Daughton and T. A Ternes, *Environmental Health Perspectives*, 107, Suppl. 6, Dec 1999.**

2. **"A Reconnaissance for Emerging Contaminants in the South Branch Potomac River, Cacapon River, and Williams River Basins, West Virginia, April-October 2004," D. B. Chambers and T. J. Leiker, Open File Report 2006-1393, United States Geological Survey, http://pubs.usgs.gov/of/2006/1393/.**

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