Using a Chlorine Filter for Accurate-Mass Data Analysis of Environmental Samples

Application Note

Environmental

Authors

Imma Ferrer and E. Michael Thurman
Center for Environmental Mass Spectrometry
University of Colorado
Boulder, Colorado
USA

Abstract

A chlorine mass-filter is used to screen both LC/TOF-MS and LC/QTOF-MS data files in order to discover compounds that contain chlorine. The chlorine filter uses Mass Hunter software to generate formulae of chlorine containing compounds. Examples are shown in the analysis of an environmental water sample (the South Platte River after wastewater discharge) for pharmaceuticals. The chlorine filter is a useful data analysis tool for complex sample analysis in the field of environmental chemistry.

Introduction

Data files generated by LC/TOF-MS and LC/QTOF-MS contain literally thousands of individual ions that are difficult to evaluate by manual techniques. It is important to have software that makes data analysis rapid and effective. One such data analysis tool that we report in this application note is the chlorine mass filter. Chlorine appears in many pesticides and pharmaceutical products that are important to environmental analysis. Because chlorine contains two isotopes, Cl^{35} and Cl^{37}, there is a distinctive A+2 isotope pattern that is generated by a single chlorine atom in a molecule. Furthermore, there is a isotopic mass defect that occurs with chlorine-37 that makes the identification of chlorine in a molecule relatively easy [1]. More than one chlorine atom in a molecule generates an A+2 and A+4 isotopic pattern, which is characteristic and commonly shown in all mass spectrometry books as a key to compound identification of chlorinated compounds [2].

In this application note, we have automated the MassHunter software to generate formulae that contain chlorine from a data file of a water sample from surface water that is contaminated with wastewater. Furthermore, the automated report includes a database search using either a forensic or pesticide database for compound identification.

Agilent Technologies

At this point the user needs only to check the data report for quality control and quality assurance purposes. The chlorine mass filter will work on any .d data file from environmental samples, such as food for pesticides or water samples for pharmaceuticals.

Experimental Conditions

Any of the Agilent accurate mass analysis instruments, the Model 6200 or 6500 series, may be used to generate data files for this analysis procedure. We applied the 6220 to generate data files of pharmaceuticals in surface water, which is from the South Platte River and is affected by the Denver Metropolitan area and wastewater from Denver. The data files, as .d files, are ready for software analysis using the MassHunter software package with Qualitative Analysis. The same Qualitative Analysis software is available on all of the accurate mass instruments, the Model 6200 or 6500 series.

Results and Discussion

Applying the Chlorine Mass Filter

The chlorine filter is applied by an eight-step procedure that begins with first opening the Qualitative Analysis software package and opening the .d file of interest.

Step 1. Open the .d file of interest with the Qualitative Analysis software package, the green icon. Go to the Find Compounds Menu (see Figure 1) and open the Find by Molecular Feature tab. Figure 1 shows the blue arrow that activates this program. The tab open is called compound filters and is set for a relative height of 1.5% and absolute height of 10,000 counts. To obtain detection of even lower abundance compounds, the relative height might also be left unchecked. However, these settings may need to be adjusted for the noise levels of both individual instruments and data sets. These three settings allow for a filter of a complicated sample and take full advantage of the software's ability to find small peaks that contain chlorine.

The other settings needed are *ion species* at H+ and Na+ for the two positive ion species. In negative ion, the setting would be H-. *Extraction* is for small molecules (chromatographic) and use peaks with height greater than 1000 counts. Again, this setting may need to be adjusted for individual instrument background and sample set noise, *Charge State* is set for peak spacing of 0.0025 plus 7 ppm, isotope model is common organic molecules, and limit assigned charge state to a maximum of one. The *Mass Filter* is blank and the *Mass Defect* is blank. The *Results* is to extract the EIC, highlight all compounds, and delete previous results. These settings should result in a clean run of the program at this point, that is, the Molecular Feature.

Step 2. Run the Find by Molecular Feature program by clicking the **arrow** shown in Figure 1. This program identifies all of the ions in the .d file and groups ions together that are related, such as isotopic clusters and sodium adducts. It does not group fragment ions, but considers them as different compounds.

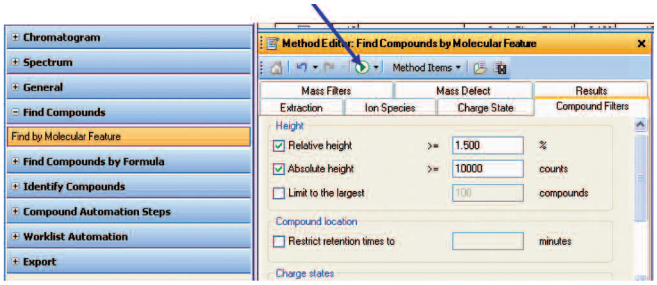


Figure 1. Find by Molecular Feature.

Step 3. Open the Identify Compounds tab (Figure 2) and click the **Generate Formulas** tab. This opens the window shown in Figure 2, and here, one specifies two chlorine atoms and at least one chlorine atom in the table (see Figure 2 with arrows). Note that this example filters the compounds for only 1–2 chlorines but one could select as many chlorines as desired. *Limits* tab and *Charge State* tabs are shown in Figure 3 with a setting that will give good results. Copy these values.

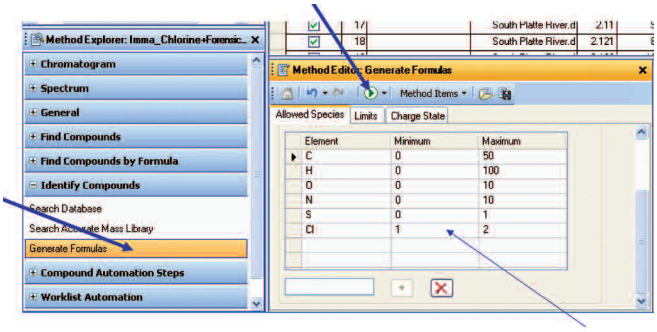
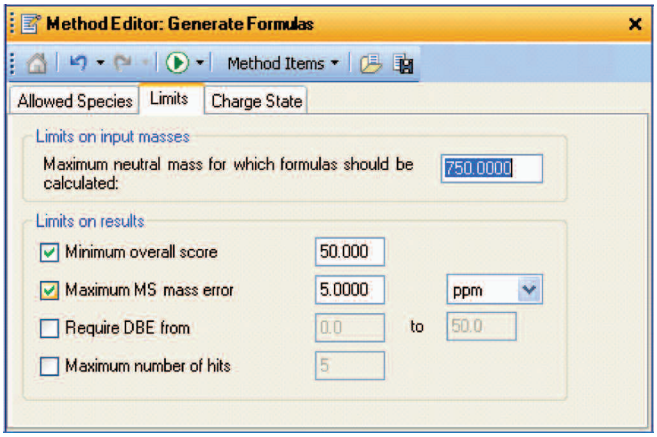


Figure 2. Identify Compounds and Generate Formulas.



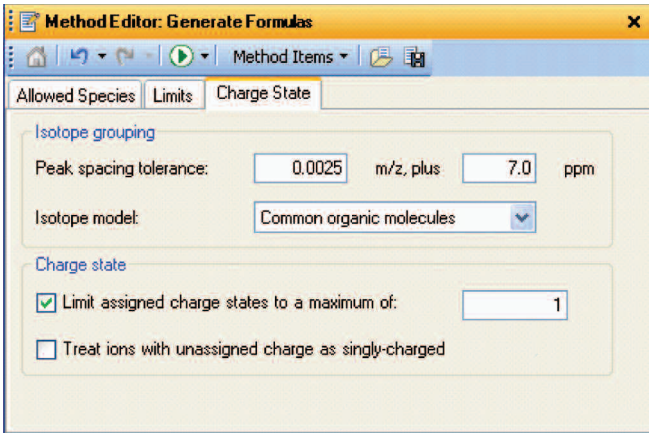


Figure 3. Other tab settings.

Step 4. Run the Generate Formulas program with the forced chlorine atoms. This generates formula for all of the features that were identified in Step 2 and will include only formulae that contain 1 to 2 chlorines.

Step 5. Open the **Search Database** pane and open the tab called Search Criteria. Select **Molecular Formula** as shown in Figure 4. This will force the search to report only those compounds that match the found formulae (and will only contain 1 to 2 chlorines). Next select the **Database**. Here specify the **Forensic** or **Pesticide Database** (Figure 5). The tab settings are: + Ions set at +H, *-Ions* set at –H, *Search Results* set at Blank, *Peak Limits* set at 5, and *Search Criteria* set at Mass and 5 ppm. These settings will work well to identify compounds. The Forensic database contains over 7,500 compounds including pharmaceuticals and pesticides. The chlorinated compounds are grouped under a column labeled *MFG Formula* or molecular formula generator.

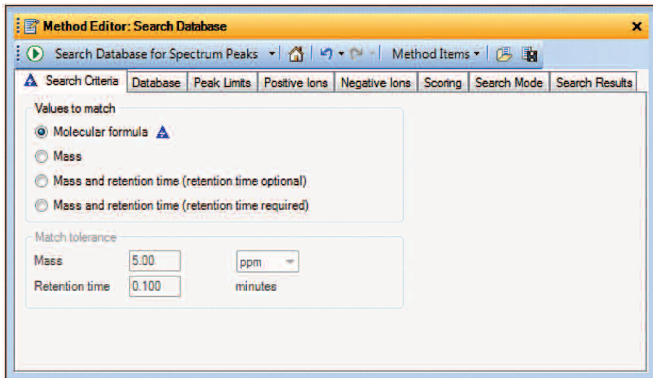


Figure 4. Select search criteria by Molecular formula.

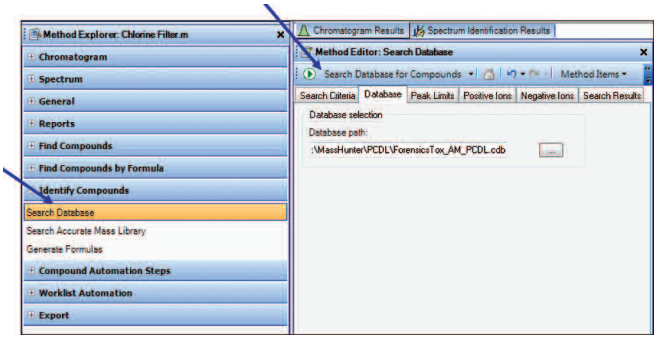


Figure 5. Search Database program using forensics database of 7500 compounds.

Step 6. Run the Search Database Program. This program now goes in and checks all of the formulas that were generated in Step 4 and assigns a name to the compound if it appears in the database. If the formula is not in the database, the Name is left blank in the final report. In this case, the best fit formula is printed in the column labeled Molecular Formula Generator for all chlorinated compounds.

Step 7. Print the Report. The report contains the following columns: Compound label, Retention Time, Mass of Neutral compound, Name (if available in the database), Molecular Formula Generator (MFG) elemental formula, MFG difference in mass accuracy, Database Formula (DB), and DB difference in mass accuracy. The report for the river sample is shown in Figure 6.

Qualitative Compound Report

Data File	South Platte River.d	**Sample Name**	
Sample Type	Sample	**Position**	P1-C6
Instrument Name	Instrument 1	**User Name**	
Acq Method	Multiresidue_Pestticides.m	**Acquired Time**	8/10/2009 7:09:22 PM
IRM Calibration Status	Success	**DA Method**	Chlorine Filter Automated.m
Comment			

Compound Table

Compound Label	RT	Mass	Name	Formula	MFG Formula	MFG Diff (ppm)	DB Formula	DB Diff (ppm)
Cpd 464: Lamotrigine	13.641	255.0077	Lamotrigine	C9 H7 Cl2 N5	C9 H7 Cl2 N5	0.54	C9 H7 Cl2 N5	0.54
Cpd 499: Ketamine	13.858	237.0923	Ketamine	C13 H16 Cl N O	C13 H16 Cl N O	-0.89	C13 H16 Cl N O	-0.89
Cpd 637: C9 H10 Cl N	15.265	167.0502		C9 H10 Cl N	C9 H10 Cl N	-0.39	C9 H10 Cl N	
Cpd 640: C13 H20 Cl N O	15.266	241.1233		C13 H20 Cl N O	C13 H20 Cl N O	0.33	C13 H20 Cl N O	
Cpd 735: C19 H27 Cl N6 O2 S	16.37	438.1611		C19 H27 Cl N6 O2 S	C19 H27 Cl N6 O2 S	-1.36	C19 H27 Cl N6 O2 S	
Cpd 775: C14 H34 Cl2 N10 O2	16.768	444.2245		C14 H34 Cl2 N10 O2	C14 H34 Cl2 N10 O2	-0.5	C14 H34 Cl2 N10 O2	
Cpd 889: C18 H29 Cl N2 O3 S	18.264	388.1583		C18 H29 Cl N2 O3 S	C18 H29 Cl N2 O3 S	1.18	C18 H29 Cl N2 O3 S	
Cpd 1015: Losartan	19.635	422.1629	Losartan	C22 H23 Cl N6 O	C22 H23 Cl N6 O	-1.7	C22 H23 Cl N6 O	-1.7
Cpd 1137: C18 H29 Cl N2 O6 S	21.105	436.1439		C18 H29 Cl N2 O6 S	C18 H29 Cl N2 O6 S	-0.9	C18 H29 Cl N2 O6 S	
Cpd 1159: C28 H41 Cl N4 O2	21.311	500.2929		C28 H41 Cl N4 O2	C28 H41 Cl N4 O2	-2.1	C28 H41 Cl N4 O2	
Cpd 1194: C18 H33 Cl N2 O4	21.64	376.2118		C18 H33 Cl N2 O4	C18 H33 Cl N2 O4	2.91	C18 H33 Cl N2 O4	

Figure 6. Printout of Report for database search of forensics to determine for the first time an antidepressant in riverwater [3].

Step 8. QA/QC Manual Report. Finally, it is necessary for the operator to manually check the assigned chlorinated compounds. This is done by manually calling up the ion at the correct retention time and checking the isotopic signature of the ion. Figure 7 shows an example of this for lamotrigine found in the South Platte River that received wastewater from a nearby effluent. This is an antidepressant drug that was detected in the sample at a retention time of 13.4 minutes [3]. The measured mass of the ion was *m/z* 256.0150 and shows an isotopic signature for both an A+2 and an A+4 isotope at masses of *m/z* 258.0121 and *m/z* 260.0092. It is important to note the relative isotopic mass defect of both the A+2 and the A+4 isotope for this compound. The relative mass defects are -0.0029 and -0.0029 for both peaks. These values are quite close to the theoretical value of -0.0030 and are excellent evidence for the presence of two chlorine atoms in the molecule [1]. This step is the critical step of the chlorine filter and is a manual step at this time. Because the molecular formula generator looks at mass only, it is possible for some formulae to be generated that

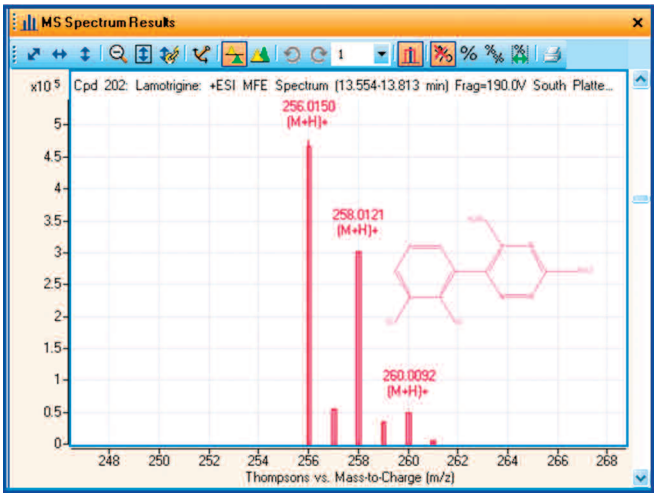


Figure 7. Mass spectrum for lamotrigine.

do not fit the isotopic signature. In this example from the South Platte River, we had approximately 100 possible chlorinated peaks of which 20 were identified to contain chlorine based on the relative mass defect and the isotopic signature.

Of these 20 different ions, there were 15 different components and the remaining five ions were fragment ions based on the retention time matching of the ions. For example, Figure 8 shows the structure of cetirizine, which was matched by the forensics database at 18.3 minutes with a protonated molecule at *m/z* 389.1626 and a fragment ion at the same retention time with a *m/z* 201.0466. The combination of mass accuracy, database matching, and identifying a fragment ion shows the power of using the chlorine mass-filter to find and identify trace chlorinated substituents in water samples impacted by wastewater. The application shown here of the chlorine mass-filter will work equally well for food samples contaminated with pesticides or other similar environmental samples.

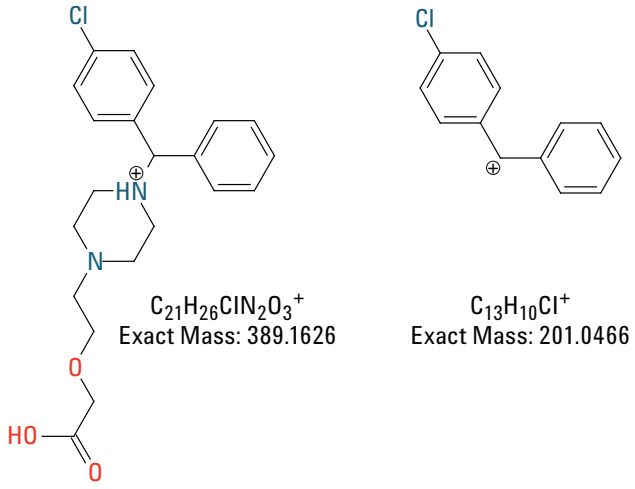


Figure 8. Cetirizine example showing the two ions that were found at the same retention time of 18.3 minutes.

Conclusions

Using accurate mass LC/MS with the Agilent 6200 or 6500 series TOF or Q-TOF and MassHunter Qualitative analysis version 5.0, a chlorine filter is described that effectively mines single MS data for all compounds that contain 1 to 2 chlorines. The process can be modified to contain any specified number of halogens or for that matter any specific elements, for example a fluorine filter or iron. The process can be automated using Worklist Automation and then manually QC'ed as described.

References

1. E.M. Thurman, I. Ferrer, 2010, The isotopic mass defect: A tool for limiting molecular formula by accurate mass: *Analytical and Bioanalytical Chemistry*, v. 397: 2807-2816.
2. R.M. Smith, 2004, Understanding Mass Spectra, John Wiley & Sons, Inc., New York, 290p.
3. I. Ferrer, E.M. Thurman, 2010, Identification of a new antidepressant and its glucuronide metabolite in water samples using liquid chromatography/quadrupole time-of-flight mass spectrometry: *Analytical Chemistry*, v. 82: 8161-8168.

For More Information

For more information on our products and services, visit our Web site at www.agilent.com/chem.

www.agilent.com/chem

Printed in the USA
December 5, 2011
5990-9431EN

Agilent Technologies