

Automated Screening for Hazardous Components in Complex Mixtures Based on Functional Characteristics Identifiable in GCxGC–TOF-MS Data

The increased resolving power provided by comprehensive two-dimensional gas chromatography (GCxGC) extends the chromatographer's ability to rapidly detect and measure smaller components in complex mixtures beyond that which was possible previously, allowing for the identification of hazardous components in complex mixtures such as foodstuffs or emergency response samples. In target analysis, the increased numbers of peaks resulting from the sample matrix can be largely ignored during the review of data. However, when the nature of the analyte of interest is not entirely known, analysis of the samples might require screening through the entire peak table for compounds with specific chemical characteristics. For example, in the analysis of foodstuffs for pesticides (1,2), GCxGC coupled with a time-of-flight mass spectrometry (GCxGC–TOF-MS) can provide low detection limits for multiple analytes in these complex samples. Yet the question remains as to whether other toxic compounds, not included in the target list, are present in the sample. The application of automated techniques for the identification of compounds based upon characteristics detectable in mass spectral data assists greatly in answering this question.

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Methods for attempting to identify chemical compounds based upon the chromatographic properties and spectral characteristics are not new and have shown further development since the introduction of comprehensive two-dimensional gas chromatography (GCxGC). Welthagen and colleagues (3) were able to demonstrate a series of selection rules that provide discrimination between at least seven chemical classes in GCxGC chromatograms of airborne particulate matter. These rules identify compounds based upon ratios of abundances of specific masses in the spectra. Richenbach and colleagues (4) developed a computer language with its own syntax, allowing for the application of these compound class selection rules.

This work was expanded further by Vogt and colleagues (5) by applying rules based upon the knowledge-based classifiers developed by Welthagen as well as classifiers derived from chemometric analysis of spectra from the work of Varmuza and Werther (6) and the category-type classifiers of Lohninger and Varmuza (7). The work of Vogt was applied to deconvoluted spectra obtained from GCxGC–time-of-flight mass spectrometry (TOF-MS) chromatograms, and it employed the VBScript language, a dialect of BASIC language, available in the scripting option in later versions of the LECO ChromaTOF software (Leco, St. Joseph, Michigan).

Table I: Chromatographic conditions used for acquisition of GCxGC chromatograms

Injection			
Volume:	1 μ L		
Liner:	Splitless liner with wool (Restek #22401)		
Temperature:	250 $^{\circ}$ C		
Mode:	Splitless		
Column			
1	J&W Scientific DB-PONA (50 m \times 0.2 mm \times 0.5 μ m)		
2	SGE BPX-50 (2 m \times 0.1 mm \times 0.1 μ m)		
Carrier gas:	He		
Flow rate:	0.5 mL/min		
Temperature Ramp			
Primary oven			
	Initial temp: 150 $^{\circ}$ C	Duration: 3 min	
	Ramp: 3 $^{\circ}$ C/min		
	Final temp: 330 $^{\circ}$ C	Hold: 5 min	
Secondary oven			
	Initial temp: 160 $^{\circ}$ C	Duration: 3 min	
	Ramp: 3 $^{\circ}$ C/min		
	Final temp: 335 $^{\circ}$ C	Hold: 5 min	
Modulator			
	Temperature offset: 30 $^{\circ}$ C		
	Modulation time: 10 sec		
	Hot pulse time: 1.5 sec		
Mass Spectrometer (LECO Pegasus 4D)			
	Acquisition range: 29–390 u		
	Acquisition rate: 100 spectra/s		
	Source temperature: 200 $^{\circ}$ C		
	Transfer line temperature: 300 $^{\circ}$ C		

In some types of analyses, ratios of abundances of specific masses might not be particularly helpful in identifying compound classes. The relevant masses arise as isotope abundances seen with a molecular ion or as neutral losses from a molecular ion. If one can identify the molecular ion, then one can use techniques applied routinely in the interpretation of mass spectra (8) to identify compounds or classes of compounds. In a search for toxic compounds in food or environmental samples, there are no unique mass spectral identifiers for the classification “toxic.” Yet particular types of functionality are more likely to indicate a hazardous compound. For example, chlorinated and brominated compounds typically are accompanied by health risks. Frequently, these halogenated compounds can be identified by isotope ratios in molecular ions and neutral losses, indicating loss of the specific halogen atoms. In a screening method for toxic compounds, the general identification of chlorinated and brominated compounds can provide a selected list of compounds to be examined further. Similarly, sulfur-containing compounds are not as common in

the environment as compounds containing carbon, hydrogen, oxygen, and nitrogen. Sulfur is found in many pesticides. Thus, a filter for sulfur-containing compounds might be useful as well.

In this work, scripts (functions written in VBScript language) are developed to locate a parent ion and test it to determine if it matches the abundance ratio expected for compounds containing specified numbers of chlorine, bromine, or sulfur atoms.

Experimental Samples and Chromatographic Conditions

Performance of the scripts was evaluated using previously acquired data (9). In this work, undiluted samples of commercially available citrus oils were analyzed under the conditions shown in Table I.

Method of Identification of Compounds

Scripts were written to identify chlorine-, bromine-, and sulfur-containing compounds. These scripts were applied to the spectra in GCxGC chromatograms by the use of the “Classification” (with scripts) feature in the software used. This feature allows for chromatographic peaks match-

ing a mass spectral pattern described in a script function to be labeled as belonging to a specific, user-defined class.

Chlorine- and bromine-containing compounds are identified by the chlorine or bromine isotope cluster seen for the molecular ion. This identification involves three steps. First, the spectrum is evaluated from high mass to low mass to detect the highest mass signal that is clearly not background noise. Second, the signal is tested to see if it is part of the isotope cluster expected for a specific number of chlorine and bromine atoms in a molecule. Third, signals for masses that would be irrational neutral losses from a molecular ion are identified, allowing for the elimination of clusters in the spectrum that are clearly fragments — and could show signals that are from a mixture of fragments, rather than the isotope cluster being sought. A typical VBScript function for detecting dichloro compounds is shown in Figure 1.

In this function, the high and low mass limits for the spectrum are extracted to allow for general application. Starting at the upper mass limit of the spectrum, the signal for each mass is examined. Extremely weak signals, based upon signal strength, are ignored. Even if such a signal were a part of a molecular ion isotope cluster, calculations of ratios would be unreliable. If the abundance for an ion is strong enough to use in calculations, it is tested to determine if it would be the most abundant ion in an ion cluster. Signals from the next several lower masses are examined because the first signal detected might have been for a higher mass in the cluster. Once the strongest mass in an apparent ion cluster is identified, the pattern of ions is tested to determine if it matches the ion cluster being sought.

If a series of mass abundances matching the desired isotope cluster pattern has been detected, it is tested further to see if there are other ions in the spectrum that would indicate the cluster is not, in fact, the molecular ion. If the series of mass abundances being examined is not the molecular ion, it might show abundances from a mixture of fragments. The match to the desired isotope cluster becomes less certain. In Figure 1, this test is made by using a call to the function `No_Irrational_Fragments()`. In the function, the masses from 2 mass units to 10 mass units

Table II: Abundance ratio limits used to identify isotope pattern

Isotope cluster	M-35	M-2	M-1	M	M+1	M+2	M+3	M+4	M+5	M+6	M+8
Cl	>0.2		<0.8	1	<0.3	0.28–0.37					
Cl ₂				1	<0.3	0.55–0.74		0.07–0.16			
Cl ₃				1		0.7–1.1	<50% of M+2	0.23–0.38		<0.07	
Cl ₄				0.68–0.85		1	<0.5	0.45–0.59		0.05–0.16	<0.1
Cl ₅				0.05–0.7		1	<0.25	0.5–0.72		0.15–0.26	<0.35
Cl ₆				0.40–0.58		1	<0.25	0.72–0.89		0.25–0.39	<0.25
Br		<1.0	<0.67	1	>0 and <0.2	0.92–1.08	>0 and <20% of M+2	<0.2			
Br ₂				0.48–0.58		1	<0.25	0.43–0.53		<0.07	
S				1	0.02–0.18	0.04–0.07	<0.18				
S ₂				1	0.02–0.2	0.08–0.11					
S ₃				1	0.02–0.3	0.10–0.17					

lower than the low mass in the isotope cluster are examined to see if there is a signal stronger than expected for background noise. If such a signal is found, it is presumed to be a fragment, and what has been detected as a presumed isotope cluster must also be presumed to be a fragment. In this case, the spectrum cannot be considered to be an adequate match for the ion cluster being sought.

The function returns a true or false result, indicating whether the sought ion cluster was detected as the presumed molecular ion in the spectrum. In some cases, an ion cluster, that is, a fragment of the molecular ion, will be detected by one of these filters. For example, the mass spectrum of dicofol shows what might appear to be a parent ion for a dichloro compound at m/z 251. In this compound, the actual parent ion (m/z 368) is not seen. The trichloromethyl group in dicofol is easily lost from the molecular ion, leaving a relatively stable dichloro cation. While there are fragments with higher mass than the ion cluster at m/z 251, they are sufficiently weak that they are likely to be seen only as background noise. Dicofol will not be detected as a pentachloro compound, but rather as a dichloro compound — based upon the significance of the m/z 251 ion in the spectrum.

The isotope cluster for monochloro compounds includes only one abundance ratio to test. This test is easily confounded by noise. Therefore, indication of neutral loss of 35 has been included in the test used in this work.

The test for the presence of sulfur is less definitive because the strength of the M+1 and M+2 ions is considerably less

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'Purpore this function returns a value of True if the currently examined spectrum is determined
' to have a molecular ion showing the ion cluster of for two chlorine atoms.
'Assumptions made:
' 1 The molecular ion will have an intensity greater than 100
' 2 The molecular ion will be more intense than signals found at higher masses - these are
' presumed to be noise.
' 3 The abundance ratio m/m+2 for Cl2 is between 0.55 and 0.72
' 4 The abundance ratio m/m+2 for Cl2 is between 0.07 and 0.55
' 5 The abundance ratio m/m+1 is less than .3
' 6 There will be no irrational losses (loss of 2 through 10) from the molecular ion.
'Returned Value: True/False, True if compound is determined to be a dichloro compound.

Function Chlorine2()
Chlorine2 = False
Em = EndMass()
Mass = Em - 4
Finish = StartMass()
TrapMass = 0
Do While Mass>Finish
AM = intensity(Mass)
If AM > 100 Then
abm = abundance(mass)
If TrapMass = 0 and AM>100 and abm > 20 then 'if the signal is strong, it is a real ion
TrapMass = Mass
If (Finish<(Mass - 6)) then Finish = Mass - 6 'we can limit the evaluation
end if
R = Ratio(Mass+2, Mass) 'The ratio function returns the abundance ratio for masses
R1 = Ratio(Mass+1, Mass)
R2 = Ratio(Mass+4, Mass)
If R>0.55 and R<0.72 and R2<.15 and R2>.07 and R1<0.3 then 'here we test to see if this appears to be a Cl2 ion cluster
if em < mass +5 then 'if there are masses in the spectrum above the ion cluster,
threshold = 0 'we can estimate a threshold for noise detection
for i = em to (mass +5) step -1
am2 = intensity(i)
if am2 > threshold then threshold = am2
next i
if threshold > 0.1 * am then threshold = 0.1 * am
else
threshold = am * 0.1 'otherwise, we assume noise may be 10% of the molecular ion
end if
if No_Irrational_Fragments(mass,threshold) then Chlorine2 = True
exit do
end if
end if
Mass = Mass - 1 'nothing found yet? Go to the next lower ion.
Loop
End Function

```

Figure 1: Function for detecting dichloro compounds.

than that of the M+2 ions seen for chlorine and bromine. The resulting ion cluster is less distinctive. Detection of sulfur-containing compounds also requires the ability to deal with significant neutral loss of one mass unit (H⁻), as is quite notice-

able in compounds such as thiophenes.

Because the isotope cluster is less definitive, the search for the molecular ion cannot depend on simply finding a set of ions that appear in the right ratio. Small peaks, indistinguishable from background noise,

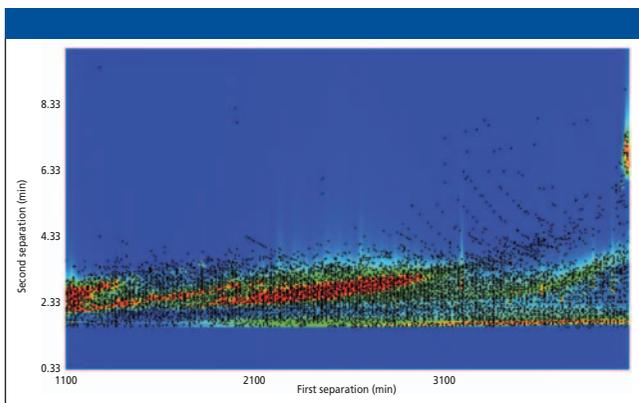


Figure 2: GCxGC chromatogram of orange oil, showing over 8000 marked peaks. The strongest signals are shown in red, with the baseline plane shown in blue. The chromatographic peaks would stand out of the plane, toward the reader. Chromatographic peaks are marked with black dots.

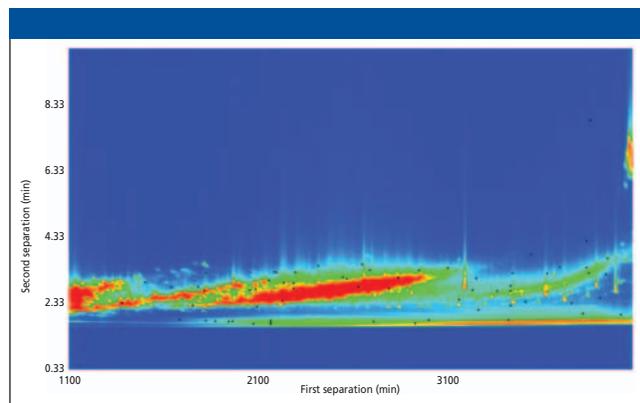


Figure 3: GCxGC chromatogram of orange oil, showing the locations of 64 peaks determined to have spectral characteristics consistent with the presence of chlorine or bromine.

can provide a fortuitous match with the ion ratios expected for sulfur-containing compounds. To avoid selecting noise for the molecular ion, the mean and standard deviation of ions showing a nonzero signal is determined as the spectrum is examined from high mass to low mass. A signal is determined to be significantly different from noise if it is greater than six times the measured standard deviation above the mean abundance determined for other detected background signals. This provides for a sufficiently strong signal that the calculation of the relative abundance of the M+1 and M+2 ions is meaningful. Once a significant signal is detected, the presence of an isotope cluster is determined in the same way it is determined for the chlorine- and bromine-containing compounds.

The constraints used for testing the various isotope clusters are shown in Table II. The ratios initially were determined from the spectra of halocarbons. The ratios for the chlorine- and bromine-containing compounds were then adjusted to allow for slight increases in abundance resulting from the presence of oxygen and sulfur in pesticides and to allow for variations resulting from background noise in weak spectra and differences in acquisition conditions. While the diagnostic ratios for chlorinated and brominated compounds are the sequence M, M+2, M+4, and so forth, the abundances of M-1 and M+1 were evaluated because strong abundances for these ions would rule out a match with the target isotope cluster. In the search for sulfur-containing compounds, the presence of a large M-1 ion requires the

expected ion ratio for the M+1 abundance to be increased to account for the presence of the M+2 abundance in the fragment that is one mass unit lower than the molecular ion. As with the tests for chlorine- and bromine-containing compounds, the reason for examining the M+1 and M+3 abundances is primarily to rule out noise. With sulfur-containing compounds, this becomes particularly important because the M+2 abundance for sulfur is quite similar to that for silicon. Silicon gives a high M+1 abundance. Therefore, limiting the acceptable M+1 abundance in the filter for sulfur-containing compounds helps to reduce the number of silicon-containing compounds detected as sulfur-containing compounds. Even so, it is helpful to construct filters for specific silanes expected to be found in column bleed and to exclude compounds matching these filters from detection as sulfur-containing compounds.

The scripts were applied to citrus oil samples that had not been spiked with pesticides to detect pesticides native to the citrus oils.

Results and Discussion

The chromatogram of Florida midseason orange oil (Figure 2) contains over 8000 marked peaks. Application of scripts designed to identify chlorinated and brominated compounds showed 64 peaks matching the criteria specified (Figure 3) and 185 peaks consistent with the presence of sulfur (Figure 4). Most of the peaks matching the criteria for peaks containing chlorine, bromine, or sulfur are coeluted with other compounds in the chromatographic plane and are detectable only with the use

of the mass spectrometer. Low-level detection of many compounds requires the combination of GCxGC with mass spectral detection. A number of these peaks were readily confirmed by examining the mass spectra and obtaining the identity of the compound, as is demonstrated with the identification of methidathion (Figure 5). In other cases, peaks were identified as compounds potentially containing chlorine or bromine with the ion cluster identifiable, but without a good library match. Figure 6 shows the spectrum for such a chromatographic peak. The ion cluster at m/z 285 appears to be that of a dichloro compound. The chromatographic peak occurs at the expected location for ronnel, the library spectrum of which is shown with the unknown in the figure. Of the 249 peaks identified by the filters, nine were determined to be known pesticides or degradation products associated with pesticides. The pesticides identified included chlorpyrifos, ronnel (fenclophos), methidathion, dicofol, chlorobenzilate, and bromopropylate.

Analysis of an unspiked sample of a California lemon oil showed over 8000 identified peaks in the chromatogram. Of these, 44 were determined to be consistent with the presence of chlorine or bromine and 153 were determined to be consistent with the presence of sulfur. Of these, six compounds were determined to be known pesticides or degradation products. The pesticides identified included chlorpyrifos, methidathion, dicofol, and bromopropylate.

Concentrations of chlorpyrifos, methidation, and bromopropylate were

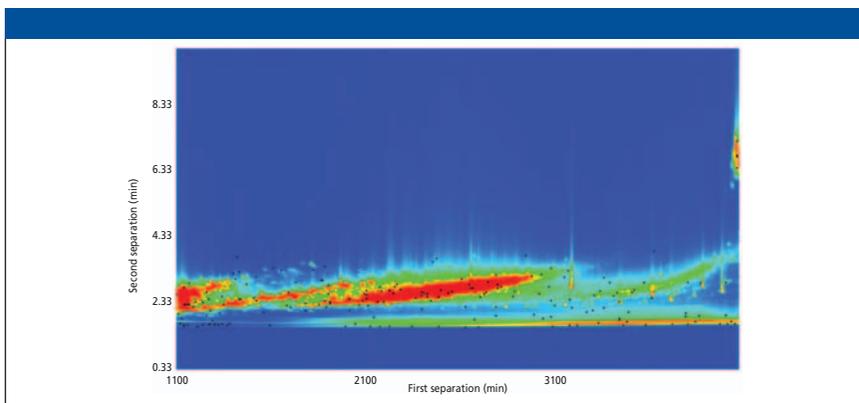


Figure 4: GCxGC chromatogram of orange oil, showing the locations of 185 peaks determined to have spectral characteristics consistent with the presence of sulfur.

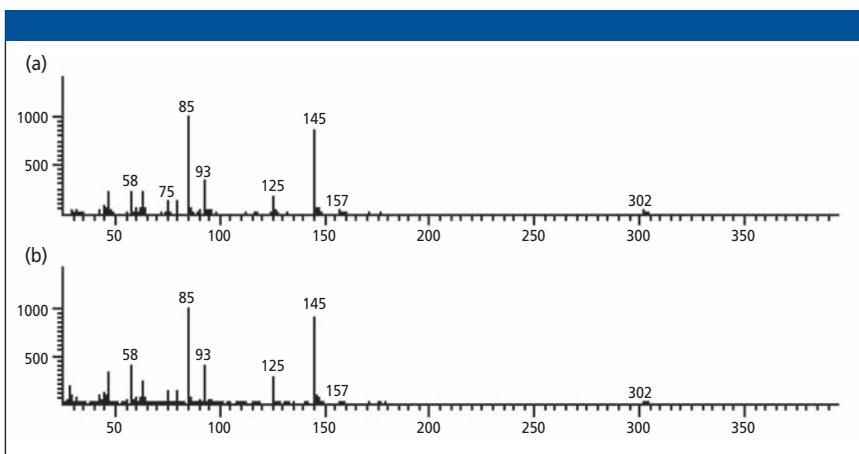


Figure 5: Methidathion was identified as matching the spectral characteristics for a sulfur-containing compound by scripting. A match of the spectrum in the chromatogram (a) to that in the library (b) and chromatographic retention time matching confirms the identification.

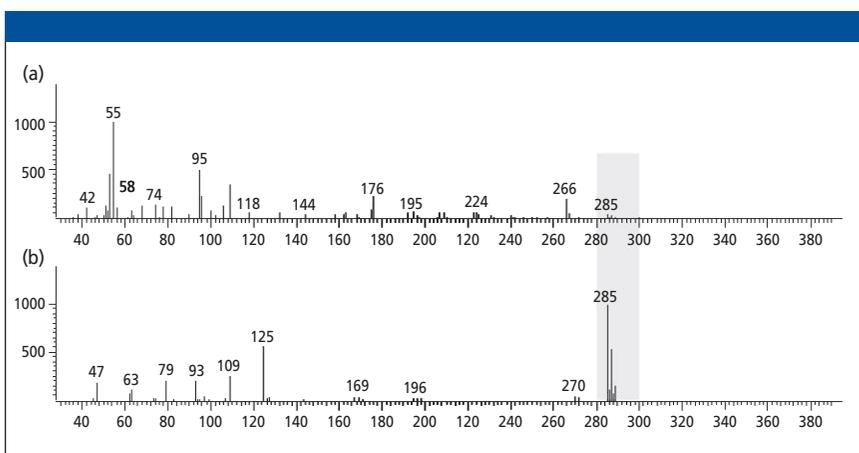


Figure 6: Ronnel (b) is identified by the presence of the chlorine isotope cluster (highlighted). While the spectrum for the chromatographic peak (a) shows coelution with at least one other compound, scripting detected the presence of a dichloro compound in the mixture. Ronnel was identified by forcing a spectral match for only the region between m/z 280 and m/z 300 (the chlorine isotope cluster) and then confirmation by location in the chromatographic plane.

below 125 ppb in the lemon oil (concentrations of pesticides were higher in the orange oil). Tetradifon was known to be present in the lemon oil at approximately

44 ppb, but it was not detected by the filters shown here. Although chlorpyrifos is detected by the filters at higher concentrations, at this low concentration, the ion

ratios for the molecular ion did not match the filter for a tetrachloro compound.

Conclusion

Automated screening of chromatographic data based upon the identification of mass spectral features offers significant assistance in the review of the GCxGC data and can identify chromatographic peaks showing spectral characteristics of compounds of interest. Although target analytical methods provide the highest degree of reliability in the identification of analytes and have the highest sensitivity, target analysis does not automatically locate compounds other than the target analytes. Peak identifications can be made, even when the spectrum is of sufficiently poor quality that the compound cannot be identified by spectral matching. This provides a complementary capability of being able to identify compounds of interest beyond those identified in a target list.

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