# High Performance Comprehensive Two-Dimensional Gas Chromatography Coupled with a High Resolution Multi-Reflecting TOFMS for Confident Non-Target Analyte Identification

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## Introduction

Performing non-target analyte identification on most environmental sample matrices can be an extremely difficult task to accomplish with confidence. Being able to chromatographically separate all the possible pollutants from a complex environmental matrix is one of the major challenges for labs. High performance comprehensive two-dimensional gas chromatography is an effective tool for the separation of compounds within a complex matrix. Confident identification of non-target analytes is best accomplished with the use of high resolution mass spectrometry. Combining the separation power of two-dimensional gas chromatography, with resolving power greater than 25,000, and sub ppm mass accuracies of a high resolution multi-reflecting TOFMS is the ideal solution for confident compound identification within a complex sample matrix.

## Case

Soil and wastewater effluent samples were analyzed from the treatment facility at the Penn State Office of Physical Plant, and some curious sample identifications were generated. The samples were first analyzed on a Pegasus® 4D GCxGC-TOFMS. Some major peaks of interest were identified in the wastewater effluent sample as 2-Chloro-6-methylphenyl isocyanate with good library similarity values in the mid 800's. Since the sample being analyzed was a waste water sample, it is very unlikely that an isocyanate would be present due to its reactivity to water. With this lack of confidence in the library identification of these peaks, it was decided to analyze these samples on a *Pegasus* GC-HRT 4D to complement the benefits of GCxGC with the mass accuracy of a high resolution TOFMS. The initial peak find of the data produced the same NIST Library identifications for the isocyanate compounds, but further investigation of the accurate mass information told a different story.

When the samples were first analyzed, the mass accuracy of the instrument was checked against the surrogates that were injected with the soil extracts. Table 1 shows a list of the surrogates analyzed along with their respective formulas and mass errors.

**Table 1. List of surrogates injected in the soil extract used to check the mass accuracy of the instrument.**

| Surrogate | Formula | HT/4D Similarity | HRT Similarity | Molecular Ion | Mass Error (ppm) |
|---|---|---|---|---|---|
| 2-Fluorophenol | $C_6H_5FO$ | 895 | 807 | 112.0319 | -0.69 |
| Nitrobenzene-D5 | $C_6D_5NO_2$ | 898 | 817 | 128.0629 | 0.15 |
| 2-Fluorobiphenyl | $C_{12}H_9F$ | 953 | 891 | 172.0683 | 0.68 |
| PCB 18 Trichlorobiphenyl | $C_{12}H_7Cl_3$ | 937 | 877 | 255.9608 | -1.08 |
| PCB 28 Trichlorobiphenyl | $C_{12}H_7Cl_3$ | 943 | 899 | 255.9608 | 0.03 |
| PCB 52 Tetrachlorobiphenyl | $C_{12}H_6Cl_4$ | 925 | 884 | 289.9218 | 0.69 |
| Triphenylmethane | $C_{19}H_{16}$ | 866 | 525 | 244.1247 | 0.43 |
| p-Terphenyl-D14 | $C_{18}D_{14}$ | 875 | 844 | 244.1969 | 0.78 |
| Tris(1,3-dichloroisopropyl)phosphate | $C_9H_{15}Cl_6O_4P$ | 907 | 836 | 427.8834 | N/A |
| Triphenyl phosphate | $C_{18}H_{15}O_4P$ | 828 | 811 | 326.0702 | -2.68 |

Figure 1 shows the chromatogram view of Triphenylmethane as it relates to the surrounding compounds. The low similarity match value for this compound is very likely due to poor chromatographic resolution and a complex sample deconvolution.

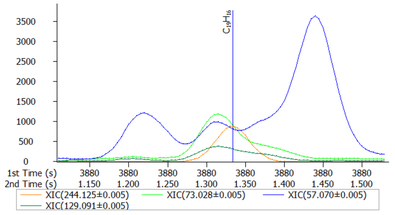



**Figure 1. Chromatogram of the surrogate Triphenylmethane displaying the complex deconvolution necessary for a good library match.**

Figure 2 shows the zoomed deconvoluted spectrum of the molecular ion $[C_{18}H_{15}O_4P]^+$ for the surrogate Triphenyl phosphate along with the $(M\text{-}H)^+$ ion $[C_{18}H_{14}O_4P]^+$. One of the isotopes of the $(M\text{-}H)^+$ ion is $[^{13}CC_{17}H_{14}O_4P]^+$, which is displayed as m/z 326.06581. Since the ion $[C_{18}H_{15}O_4P]^+$ and the isotope $[^{13}CC_{17}H_{14}O_4P]^+$ require a resolving power of 104,000 to properly resolve them, the mass error of -2.68 is due to the contribution of the $[^{13}CC_{17}H_{14}O_4P]^+$ isotope from the $(M\text{-}H)^+$ ion.

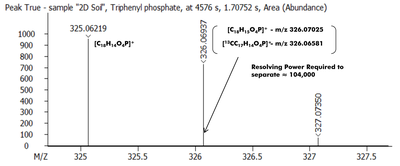



**Figure 2 The zoomed deconvoluted spectrum of the molecular ion $[C_{18}H_{15}O_4P]^+$ for the surrogate Triphenyl phosphate along with the $(M\text{-}H)^+$ ion $[^{13}CC_{17}H_{14}O_4P]^+$.**

Now that the mass accuracy of the instrument is confirmed, confident use of the accurate mass data can be implemented for analyte identification. Figure 3 shows a zoomed in contour plot highlighting one of the isocyanate peaks in question. The peak table in Table 2 shows a good library similarity match value for the spectrum generated, but the accurate mass information associated with the formula for 2-Chloro-6-methylphenyl isocyanate indicates this is likely not the correct compound name. LECO's Formula Calculator was used next to generate the best possible formulas that correspond with the observed ion m/z 167.02453. A 2 ppm mass window was employed, and various halogens selected to be included as potential elements for the formula. Table 3 shows the results generated by the Formula Calculator.

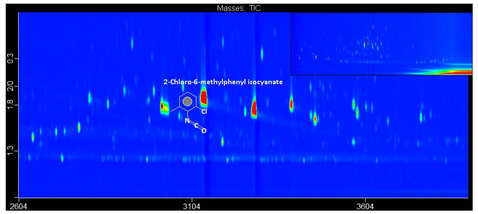



**Figure 3. Contour plot of Penn State waste water effluent sample collected on a *Pegasus* GC-HRT 4D**

**Table 2. Peak Table.**

| Analyte Name | Formula | Similarity | Base Mass | Expected Ion m/z | Mass Accuracy (ppm) | Peak Quality | Observed Ion m/z |
|---|---|---|---|---|---|---|---|
| 2-Chloro-6-methylphenyl isocyanate | $C_9H_6ClNO$ | 832 | 104.04958 | 167.01324 | 67.57 | 0.99 | 16702453 |

**Table 3. Possible formula calculations.**

| Formula | Expected Ion m/z | Observed Ion m/z | Mass Delta (mDa) | Mass Accuracy (ppm) |
|---|---|---|---|---|
| $C_7H_6ClN_3$ | 167.02448 | 167.02453 | 0.054 | 0.32 |
| $C_8H_5F_3NOS_2$ | 167.02446 | 167.02453 | 0.066 | 0.40 |
| $C_9H_5NO_3S$ | 167.02468 | 167.02453 | -0.150 | -0.90 |
| $C_{10}H_5N_4OP$ | 167.02430 | 167.02453 | 0.230 | 1.38 |

The next step in determining the proper formula was to compare the theoretical isotope abundances for each of the possible formulas with the experimental isotope abundances. Figure 4 shows a zoomed in portion of the deconvoluted spectrum around the molecular ion, along with a screen shot of the Mass Calculator for the formula $C_7H_6ClN_3$. The isotope abundances for each of the potential formulas were tabulated in Table 4. Close inspection of the isotope abundances for each of the formulas as compared to the experimental data shows a very close match to the formula $C_7H_6ClN_3$.

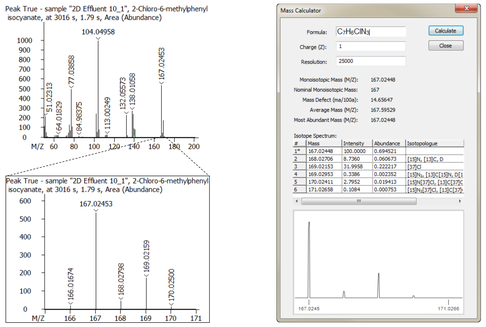



**Figure 4. Deconvoluted spectrum of the unknown compound with a zoomed in portion displaying the molecular ion and its isotopes next to a screenshot of LECO's Mass Calculator for theoretical isotope abundance values.**

**Table 4. Isotope Abundances for each proposed formula.**

| | | Formula Isotope Abundances | | | |
|---|---|---|---|---|---|
| Observed Ion m/z | Observed Abundance | $C_7H_6ClN_3$ | $C_8H_5F_3NOS_2$ | $C_9H_5NO_3S$ | $C_{10}H_5N_4OP$ |
| 167.02453 | 100 | 100 | 100 | 100 | 100 |
| 168.02798 | 8.55 | 8.74 | 4.10 | 5.74 | 7.69 |
| 169.02159 | 32.34 | 32.00 | 0.48 | 4.51 | 0.46 |
| 170.02500 | 2.79 | 2.80 | 0.02 | 0.23 | 0.02 |

To further confirm the postulate for the formula $C_7H_6ClN_3$, a closer look of the $(M\text{-}H)^+$ ion $[C_7H_5ClN_3]^+$ and its isotopes was performed. Figure 5 displays a screen shot of the Mass Calculator, which is displaying the molecular ion for the formula $C_7H_5ClN_3$ and its corresponding isotopes. Figure 6 is a screen shot of the Mass Resolution Calculator, which calculates the resolving power necessary for proper separation of two specified ions. The ions selected in this figure are of the $[C_7H_5{}^{37}ClN_3]^+$ and the $[^{13}CC_6H_6ClN_3]^+$ ions.

Figure 7 shows the zoomed in spectrum plot of the molecular ion with a further zoomed in plot displaying the separation of the $[C_7H_5{}^{37}ClN_3]^+$ ion and the $[^{13}CC_6H_6ClN_3]^+$ ion. With the full mass range resolving power greater than 25,000 in High Resolution mode, the instrument was able to resolve the 13mDa difference between the $[C_7H_5{}^{37}ClN_3]^+$ ion (m/z 168.01449) and the $[^{13}CC_6H_6ClN_3]^+$ ion (m/z 168.02798). The presence of the $[C_7H_5{}^{37}ClN_3]^+$ ion further confirms the presence of a chlorinated isotope.

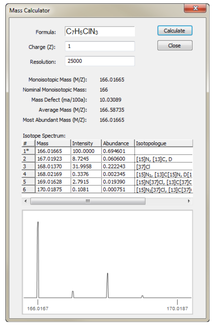



**Figure 5. Screen shot of the Mass Calculator displaying the $(M\text{-}H)^+$ ion and its associated isotopes.**

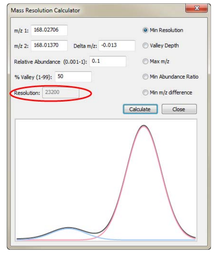
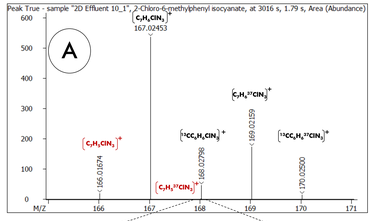



**Figure 6. Screen shot of the Mass Resolution Calculator displaying the resolving power required to separate the two specified ions.**



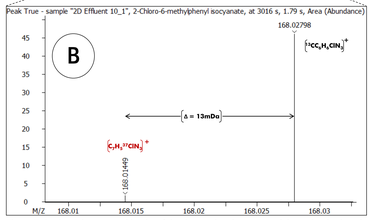




**Figure 7. Section A is a zoomed in plot of the deconvoluted spectrum of the molecular ion of the unknown compound and its associated isotopes. The ions in red are of the $(M\text{-}H)^+$ ion and one of its isotopes. Section B is a further zoomed in plot of the $C_7H_5{}^{37}ClN_3$ isotope of the $(M\text{-}H)^+$ ion and the $^{13}CC_6H_6ClN_3$ isotope of the molecular ion.**

With a confident formula determined from the high resolution accurate mass data, the next step focused on some of the benefits of using GCxGC for chromatographic separation of the sample components. One of the advantages of using GCxGC for chromatographic separation is the high degree of organization based on chemical structure that can be seen in the resulting chromatogram. Components from the same class are aligned in bands based on the two separation mechanisms used. In this case, the chromatogram is organized by carbon number in the first dimension due to the use of a non-polar primary column, and by polarity in the second dimension due to the use of a polar secondary column.

Further investigation of the sample peaks within the same chromatographic band as the unknown peak show a possible similarity to a class of Methylated Benzotriazoles. Figure 8 shows the expanded contour plot of the sample highlighting the $C_7H_6ClN_3$ peak along with a peak identified as 4-Methyl-1H-benzotriazole. With the idea that the $C_7H_6ClN_3$ peak might be a chlorinated methylbenzotriazole, the next step would be to determine if this is a logical identification. Further investigation of users upstream to the treatment facility showed the use of halogenated benzotriazoles as corrosion inhibitors. It is therefore concluded that the $C_7H_6ClN_3$ peak in question is very likely a chlorinated methylbenzotriazole.

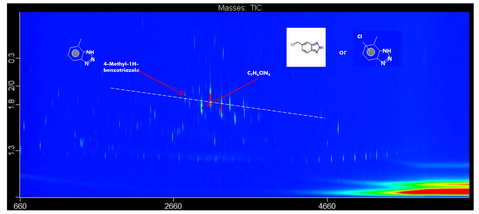



**Figure 8. Contour plot of the waste water effluent sample showing the unknown peak in the same band as a methylbenzotriazole.**

## Conclusion

Confident analyte identification can be a challenging task when analyzing complex sample matrices. The use of comprehensive two-dimensional gas chromatography to help increase chromatographic resolution is a major step in tackling the problem of confident peak identification in a complex sample matrix. The use of high resolution accurate mass data is another important tool to be used for proper identification. Combining the separation power of two-dimensional gas chromatography, with resolving power greater than 25,000, and sub ppm mass accuracies of a high resolution multi-reflecting TOFMS is the ideal solution to confident compound identification within a complex sample matrix.

## Acknowledgements

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