



GC×GC-TOFMS Quantification with Automated Calibration Tools in ChromaTOF®

LECO Corporation; Saint Joseph, Michigan USA

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1. Introduction

GC×GC-TOFMS is a powerful tool for chromatographically separating coelutions within complex samples to confidently discover more analytes. In many cases, accurate quantification is also desired and LECO's *ChromaTOF* brand software contains data processing features to help the user build calibration equations, and automatically incorporate these equations into data processing methods for calculating analyte concentrations in subsequent samples. In this example, quantification of naphthalene in a tobacco smoke extract is demonstrated. Naphthalene has been defined as one of the Harmful and Potentially Harmful Constituents (HPHCs) and is classified as a carcinogen and respiratory toxicant. As such, it must be monitored and quantified in smoke as part of federal regulations. Tobacco smoke is an inherently complex sample that benefits from analysis by GC×GC and ChromaTOF's data processing tools. The calibration feature was used to compile GC×GC peak area information for naphthalene and to determine a calibration equation, shown in Figure 1.

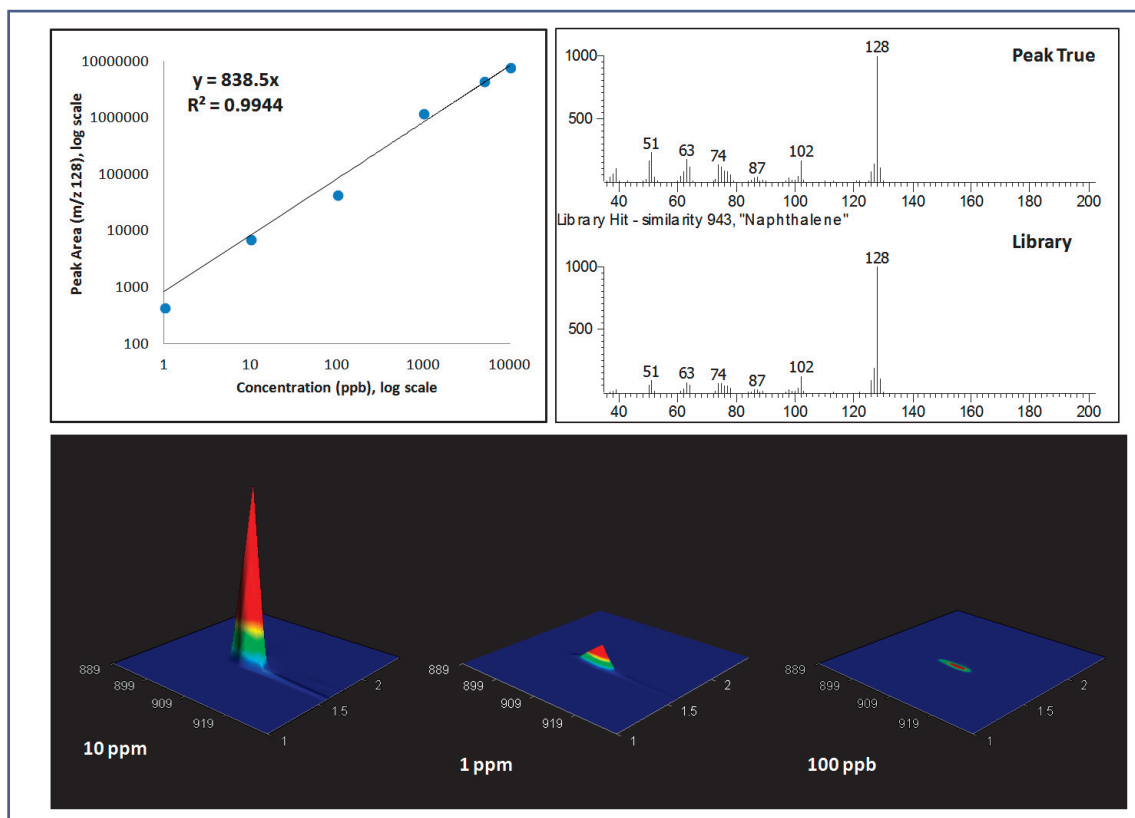


Figure 1. A dilution series for naphthalene was analyzed by GC×GC and ChromaTOF's calibration tools. The Peak True and library spectra are shown as well as peak profiles for this analyte at various concentrations.

2. Experimental

A dilution series for naphthalene (Restek, Bellefonte, PA) was prepared in methanol at concentrations ranging from 1 ppb (v/v) to 10 ppm (v/v). Smoke extracts from Kentucky 3R4F reference cigarettes (Arista Laboratories, Richmond, VA) were also analyzed. The extracts were purchased after preparation per ISO smoking conditions and constituents collected on a filter pad were extracted into methanol for analysis. GC×GC-TOFMS analyses were performed for the standards and the smoke extracts with LECO's *Pegasus 4D*, with instrument parameters and conditions listed in Table 1.

Table 1. GC×GC-TOFMS *Pegasus 4D* Instrument Conditions

Gas Chromatograph	Agilent 7890 outfitted with modulator and secondary oven
Injection	1.5 μ L injection, splitless with inlet @ 250°C
Carrier Gas	He @ 1.0 mL/min, corrected constant flow
Column One	Rxi-5Sil MS, 30 m x 0.25 mm i.d. x 0.25 μ m coating (Restek)
Column Two	Rtx-200, 1.5 m x 0.18 mm x 0.20 μ m (Restek)
Temperature Program	3 min at 45°C, ramped 8°C/min to 300°C, held 10 min Secondary oven maintained +10°C relative to primary
Modulation	3 s with temperature maintained +15°C relative to secondary oven
Transfer Line	Temperature set to 280°C
Mass Spectrometer	LECO <i>Pegasus TOFMS</i>
Ion Source Temperature	250°C
Mass Range	33-400 m/z
Acquisition Rate	200 spectra/s

3. Results and Discussion

ChromaTOF's calibration feature was used to compile the standard information and build a calibration equation for naphthalene, shown in Figure 1. The effective concentrations spanned from 1 ppb to 10 ppm with a linear equation across that range and an R^2 value of 0.9944. Naphthalene is an analyte of interest in various complex samples and is demonstrated here in a tobacco smoke extract.

A representative tobacco smoke extract is shown in Figure 2, with naphthalene highlighted. The complexity of this sample is apparent and multiple analytes that are chromatographically separated in the GC×GC data would likely coelute in a comparable GC separation. Along with the improved chromatographic resolution, *ChromaTOF*'s deconvolution algorithms further separate individual analytes in regions of remaining overlap. A zoomed view of the naphthalene peak is shown in Figure 3. The TIC is the combination of two analytes, but the chromatographic profiles were determined and XICs for unique masses for each (m/z 128 and 110) are shown. In addition to determining the pure peak profiles, the software provides pure mass spectral information for each analyte that was matched to library databases for identification. The coeluting analyte was identified as catechol, which is another HPHC with carcinogenic properties.

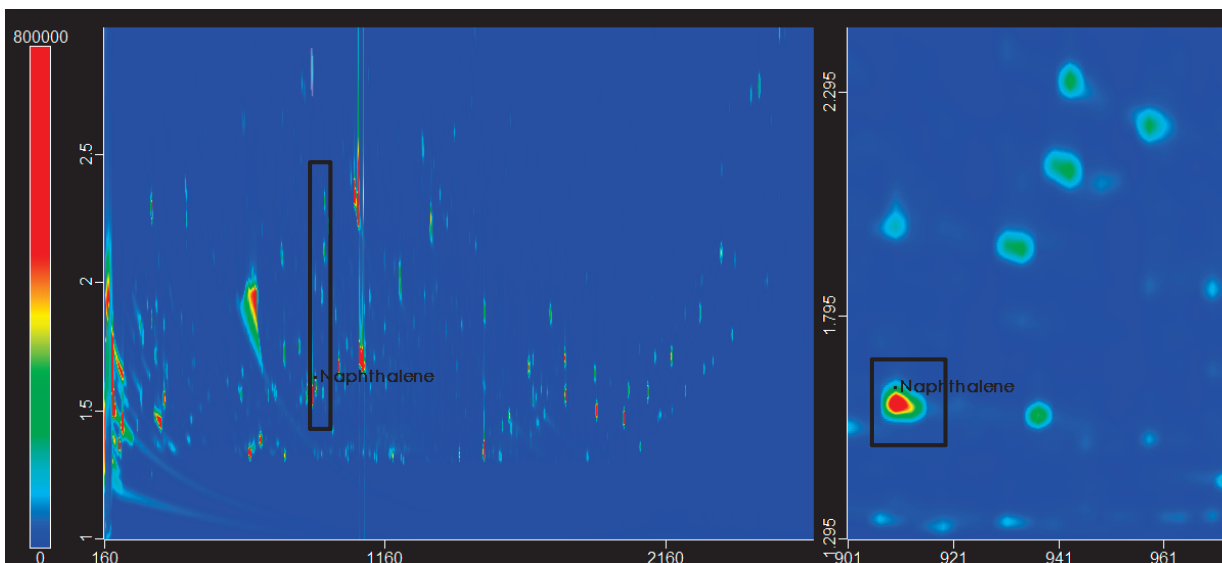


Figure 2. Naphthalene was observed in the complex tobacco smoke extracts. A zoom-in of the chromatographic region where naphthalene elutes highlights the complexity of these samples.

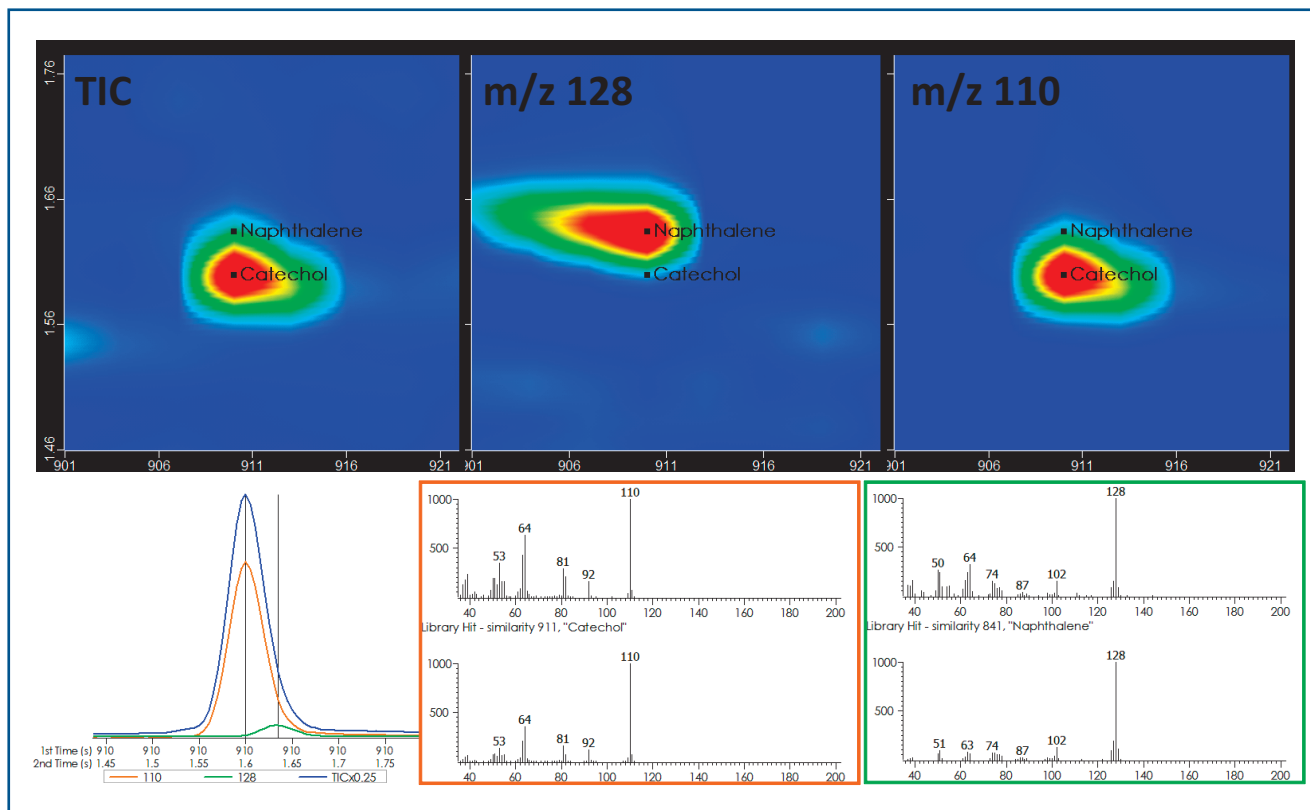


Figure 3. Deconvolution separated two coeluting analytes, naphthalene and catechol, that are both HPHCs. The pure chromatographic profiles and the pure spectra were determined for quantification and identification, respectively.

With chromatographic separation and deconvolution, the pure peak profile for naphthalene was determined in this complex sample. The calibration was applied to the naphthalene peak (m/z 128) in the tobacco extract and a concentration of 230 ppb was calculated. The naphthalene peak in the sample is shown between the 1 ppm and 100 ppb standards in Figure 4.

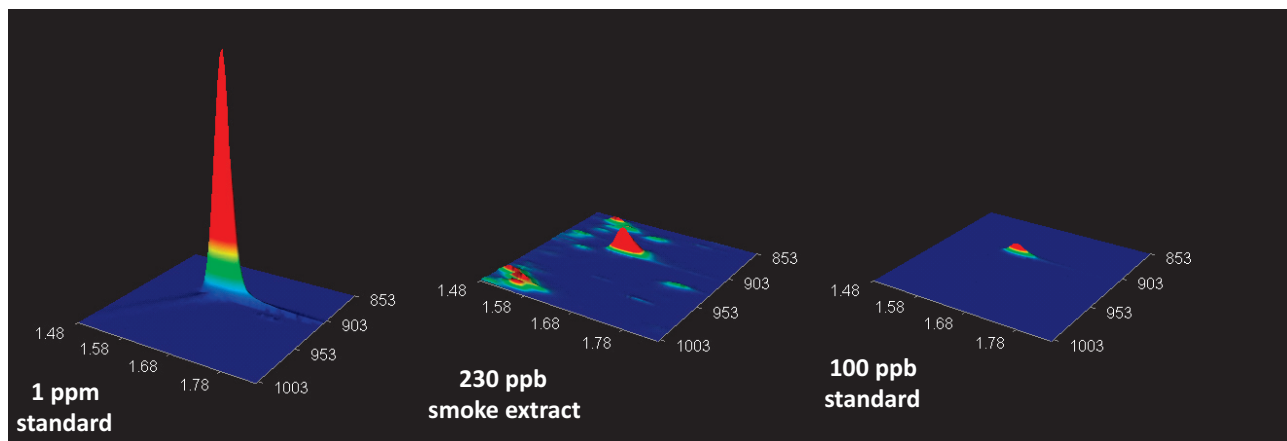


Figure 4. The concentration of naphthalene in the smoke extract sample was determined to be 230 ppb. The chromatographic peak for XIC m/z 128 is shown here for the standards and sample.

4. Conclusion

This study demonstrates the utilization of LECO's analytical tools for quantifying target analytes in GC×GC-TOFMS data. GC×GC provides excellent chromatographic separation for coelutions within complex samples. Deconvolution provides further separation for regions of remaining chromatographic overlap. Quantification of these individual analytes is possible with *ChromaTOF*'s calibration tools. A representative analyte, naphthalene, was demonstrated and calibration equations were determined from standards. The calibration equations are readily incorporated to automated data processing and were applied to an unknown tobacco smoke extract sample. These tools combine for a comprehensive analysis to confidently discover more analytes and reliably quantify them.



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