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Multidimensional GC Analysis of Complex Samples

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ABSTRACT

To perform chromatographic analysis of real-world samples, analysts often must deal with either complex sample types such as essential oils and petroleum fractions, or complex matrices like biological fluids, foods, sludge, or polymers. Once the sample has been prepared for analysis, resolution of all the individual compounds present by means of a single chromatographic separation can be challenging due to the different ranges of polarity, boiling point, solubility, MW, and concentration of the components. It is therefore desirable to use innovative yet robust techniques that allow resolution of as many types of compounds as possible.

Coupling columns having different polarities can significantly enhance the resolution of complex samples. This approach is commonly known as multidimensional gas chromatography. In this study, we coupled two low thermal mass (LTM) GC column modules (GERSTEL-MACH) with dissimilar column phases using a valveless, software-controlled column switching device for heartcutting GC-GC. The GERSTEL-MACH has a resistive heating system rather than a convection oven which allows for rapid heating and

cooling rates. In addition, the column modules can be independently programmed for optimal separation and minimum analysis time.

Examples of complex flavor and fragrance samples separated using the above system show that heartcutting fractions from the first column and transferring them to the second column provided better identification of more compounds than conventional single-dimension GC analysis.

This system was also used to identify trace components responsible for off-odors in headspace samples from polymers by coupling an olfactometry detector to the powerful heartcutting technique. Two main advantages of the instrumental configuration used were the simple, robust design and short analysis times.

INTRODUCTION

Gas chromatography (GC) is widely used to analyze samples having complex volatile profiles; however it is often difficult to fully resolve all components on a single chromatographic column. When coupled to a mass selective detector (MSD) the goal is often tentative identification of specific compounds present in the sample. Issues arise when complex samples are analyzed using GC/MS and co-elution interferes with the mass spectral identification of individual compounds.

In many cases, it is only necessary to further resolve sections of a chromatogram, or groups of peaks in a region of interest. This can be readily accomplished adapting standard GC equipment and data analysis software by connecting two columns with different phases and selectively transferring compounds from one column into the other using a flow-control column switching device. This approach is commonly known as classic heartcut GC-GC. This technique is generally more economical and effective for these analyses than comprehensive GCxGC, which requires specialized fast modulation devices, fast mass spectrometers (TOF) and software capable of displaying data in three dimensions. Heartcut GC-GC is also better suited to managing samples with components spanning a wide concentration range.

In this study, we coupled two low thermal mass (LTM) GC columns using a multi-column switching system that also includes a cryogenic trap at the head of the second column. Two different sample types - a liquid spearmint oil fraction and a solid vinyl shower curtain - were used to illustrate the resolving power of the heartcut GC-GC system.

EXPERIMENTAL

Instrumentation. Analyses were performed on a 6890 GC equipped with a 5973 mass selective detector and flame ionization detector (Agilent Technologies), PTV inlet (CIS 4) and thermal desorption unit with autosampler (TDS 2 & TDS A), a multi-dimensional column switching system with cryotrap (MCS 2/CTS 2) and olfactory detection port (ODP 2) and dual Modular Accelerated Column Heaters (GERSTEL-MACH).

Analysis conditions.

TDS 2	splitless, 20°C, 60°C/min, 250°C (5 min)
PTV	solvent vent (50 mL/min), splitless -120°C (0.2 min), 12°C/s, 280°C (3 min)
Columns	30m DB-5MS (Agilent), LTM $d_i = 0.32\text{mm}$, $d_f = 1.0\mu\text{m}$ for pre-column 30m INNOWax (Agilent), LTM $d_i = 0.25\text{mm}$, $d_f = 0.25\mu\text{m}$ for main column
Initial flows: (spearmint)	1.8 mL/min for pre-column 1.2 mL/min for main-column
Initial flows: (curtain)	1.89 mL/min for pre-column 1.64 mL/min for main-column
Heaters: (spearmint)	60°C (1 min); 10°C/min; 280°C (6 min) for pre-column 50°C (13.5 min); 10°C/min; 220°C (4 min) for main-column
Heaters: (curtain)	40°C (2 min); 10°C/min; 280°C (5 min) for pre-column 40°C (37 min); 10°C/min; 220°C (5 min) for main-column

Sample preparation. 5 μL of neat spearmint oil, terpeneless (Sigma Aldrich, part # W30321-6) was transferred into a 20 mL headspace vial. A Twister stir bar was suspended above the sample and the vial was crimp capped. The sample was extracted for 1 hour at room temperature. Approximately 0.5 g of vinyl shower curtain was weighed into a 20 mL headspace vial. A Twister stir bar was suspended above the sample and the vial was crimp capped. The sample was extracted at least 16 hrs at room temperature.

Following extractions the stir bars were removed and placed into conditioned thermal desorption tubes for analysis.

RESULTS AND DISCUSSION

The instrumentation we used for this analysis is shown in Figure 1. This system includes a thermal desorber for sample introduction by either direct thermal extraction or preconcentration onto adsorbents or other adsorptive phases. Separations are performed on independently controlled low thermal mass GERSTEL-MACH modules allowing optimization of the conditions for pre-

column and main column separations. The precolumn separation is monitored by a flame ionization detector (FID) and the main column cuts are detected with either an MSD or MSD/ODP. The pneumatic flow diagram for this system with the countercurrent flow “On” (no heartcut) is shown in Figure 2.

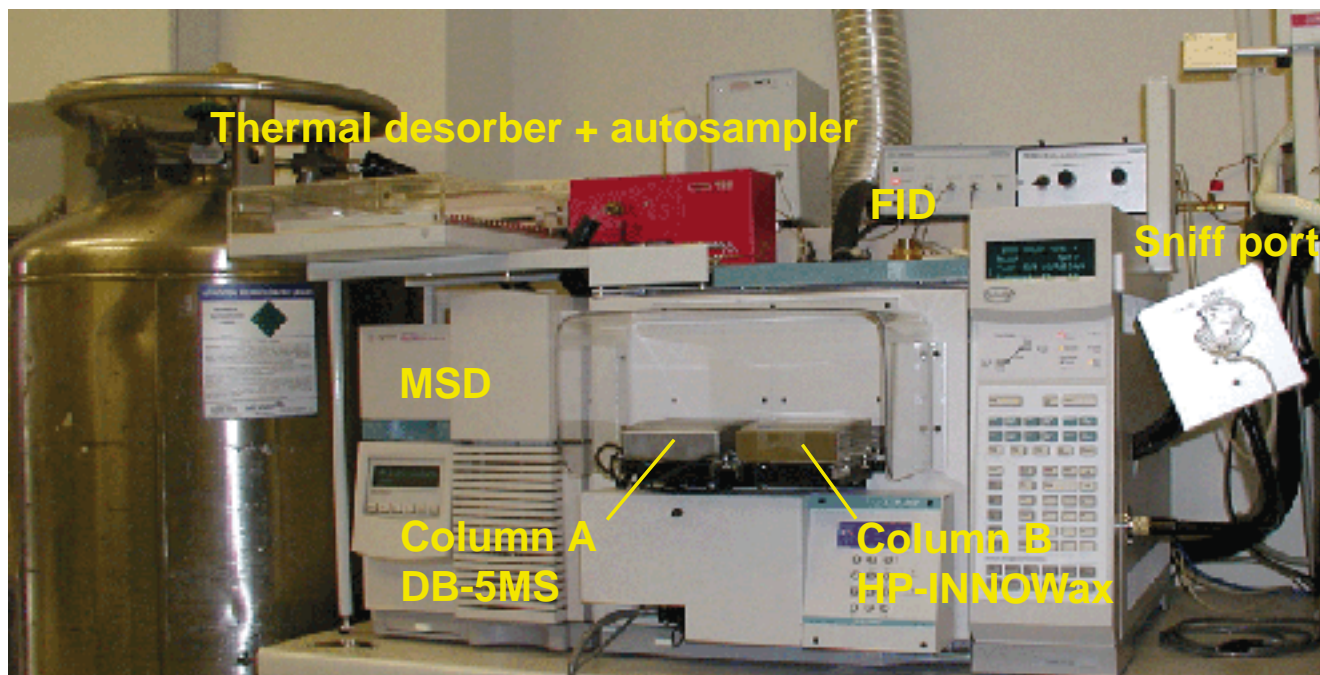


Figure 1. Multidimensional GC-GC instrument used for the study.

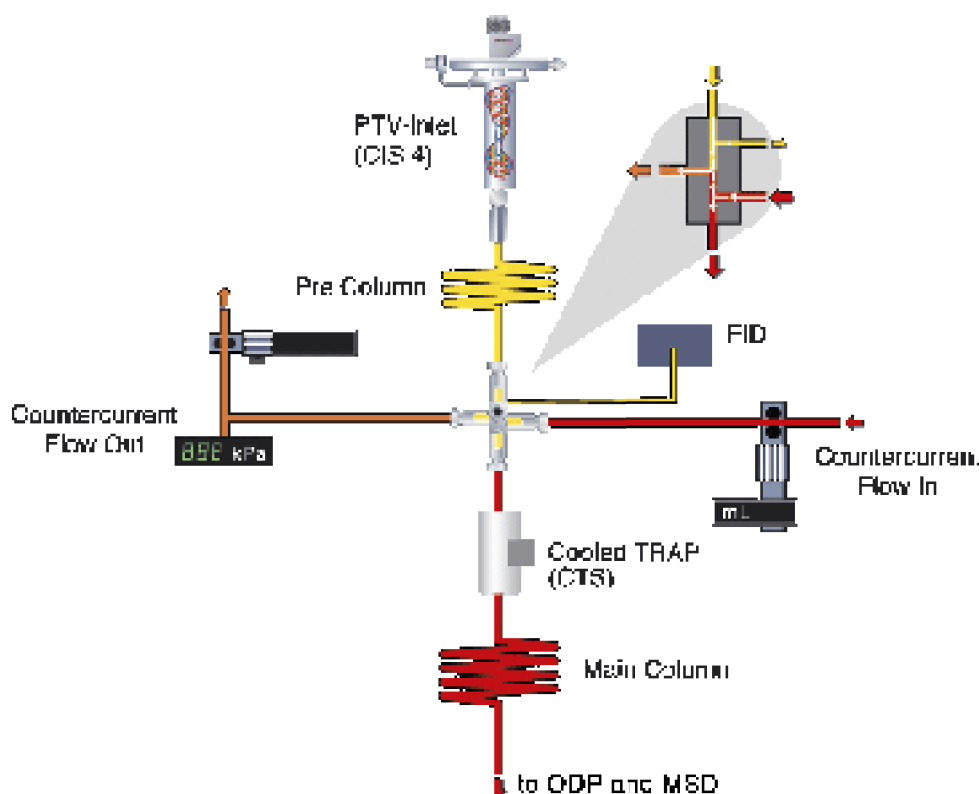


Figure 2. Pneumatic flow diagram for GERSTEL multidimensional system in venting condition, with countercurrent flow “On”.

The GERSTEL software that controls every module of the instrument is fully integrated into Agilent ChemStation software which facilitates the creation, optimization, and storage of methods.

The critical steps necessary to successfully identify trace odor components are:

1. Introduction of sufficient mass of the compounds of interest into the column to obtain adequate response at the detector (MSD or ODP).
2. Identification of the regions of interest in the pre-column chromatogram.
3. Transfer (heartcut) regions containing compounds of interest and interfering matrix components onto a second, orthogonal GC column to improve separation.
4. Identify the resolved components using an MSD and mass spectral library.

To illustrate the challenges associated with identification of trace volatile components in a complex mixture, we selected two different sample types: a spearmint oil fraction and a vinyl shower curtain with a distinctive “plastic” odor.

Spearmint oil separation

Step 1 - Sample introduction. The choice of an appropriate sample introduction technique is critical to all subsequent steps. In trace analysis, it is often necessary to heavily overload the GC column with the major sample components to insure sufficient mass of the trace components is available for detection. For simplicity we chose to concentrate headspace volatiles onto the PDMS phase on a Twister stir bar, although dynamically purging the sample headspace onto a packed adsorbent tube is another option.

Step 2 - Identification of co-elution regions in spearmint oil.

Figure 3a shows the chromatogram obtained without any cuts from a single Twister desorption. We focused on two regions of the chromatogram where peaks were not completely resolved. In this figure, the TIC of the second dimension without cuts shows acceptable low background (Figure 3b).

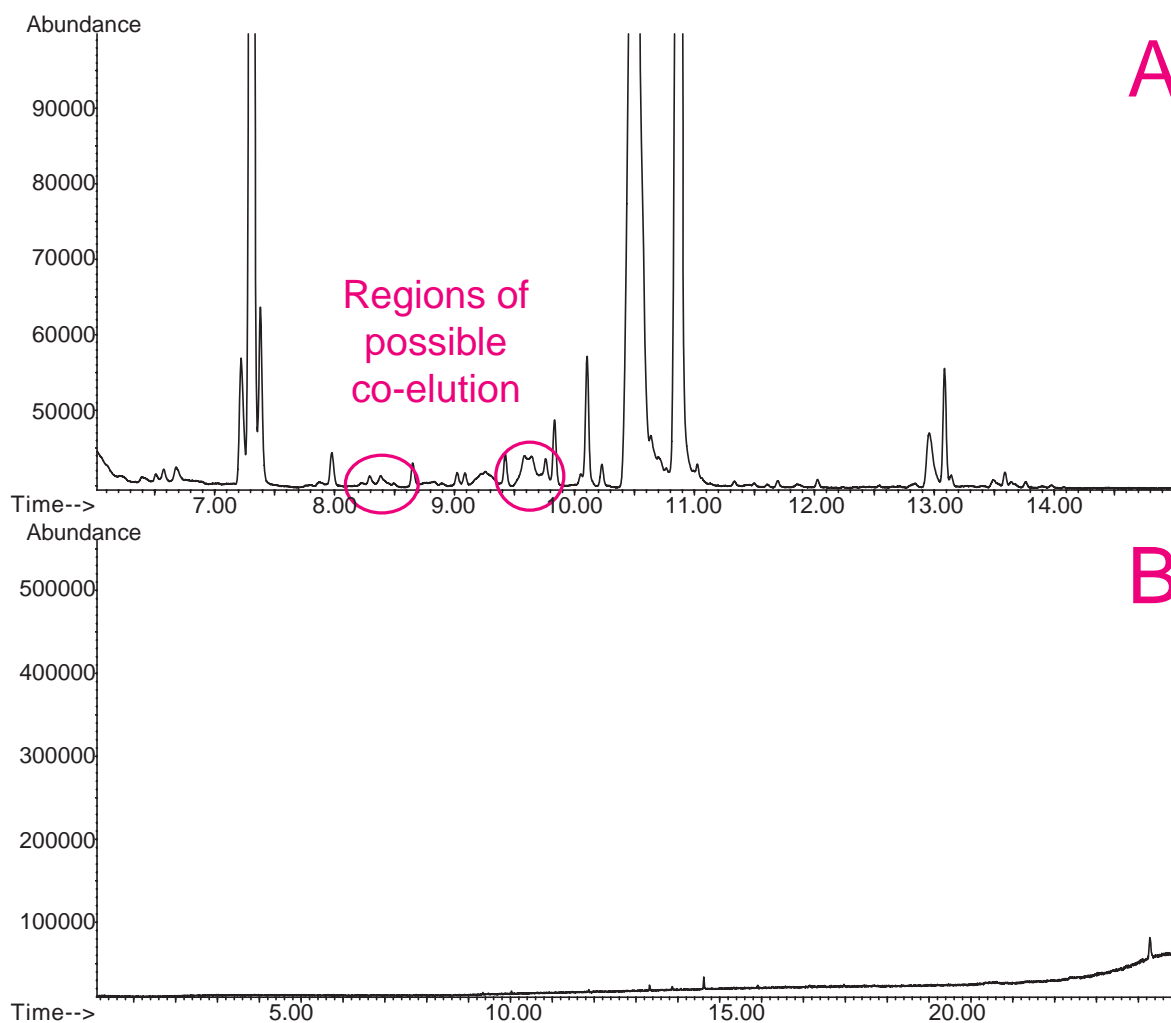


Figure 3. (A) FID trace from GC-GC precolumn separation of spearmint oil; (B) TIC from main column without any cuts.

Step 3 - Heartcut regions onto second column. The multidimensional GC-GC system was configured with a DB5-MS precolumn and an HP-Innowax column for the main separation. Figure 4a shows the precolumn separation in duplicate illustrating excellent run-to-run reproducibility. The region containing the group of co-eluting compounds (9.36-9.90 min) was cut into the main column with cold trapping. The chromatogram from the precolumn suggested at least five components were present in this region.

Step 4 - Identification of components transferred to main column. Figure 4b shows the total ion chromatogram from duplicate injections on the main column. Note that the 0.54 minute heartcut from the precolumn transferred more than 15 components to the main column. This figure also illustrates excellent reproducibility between duplicate cuts.

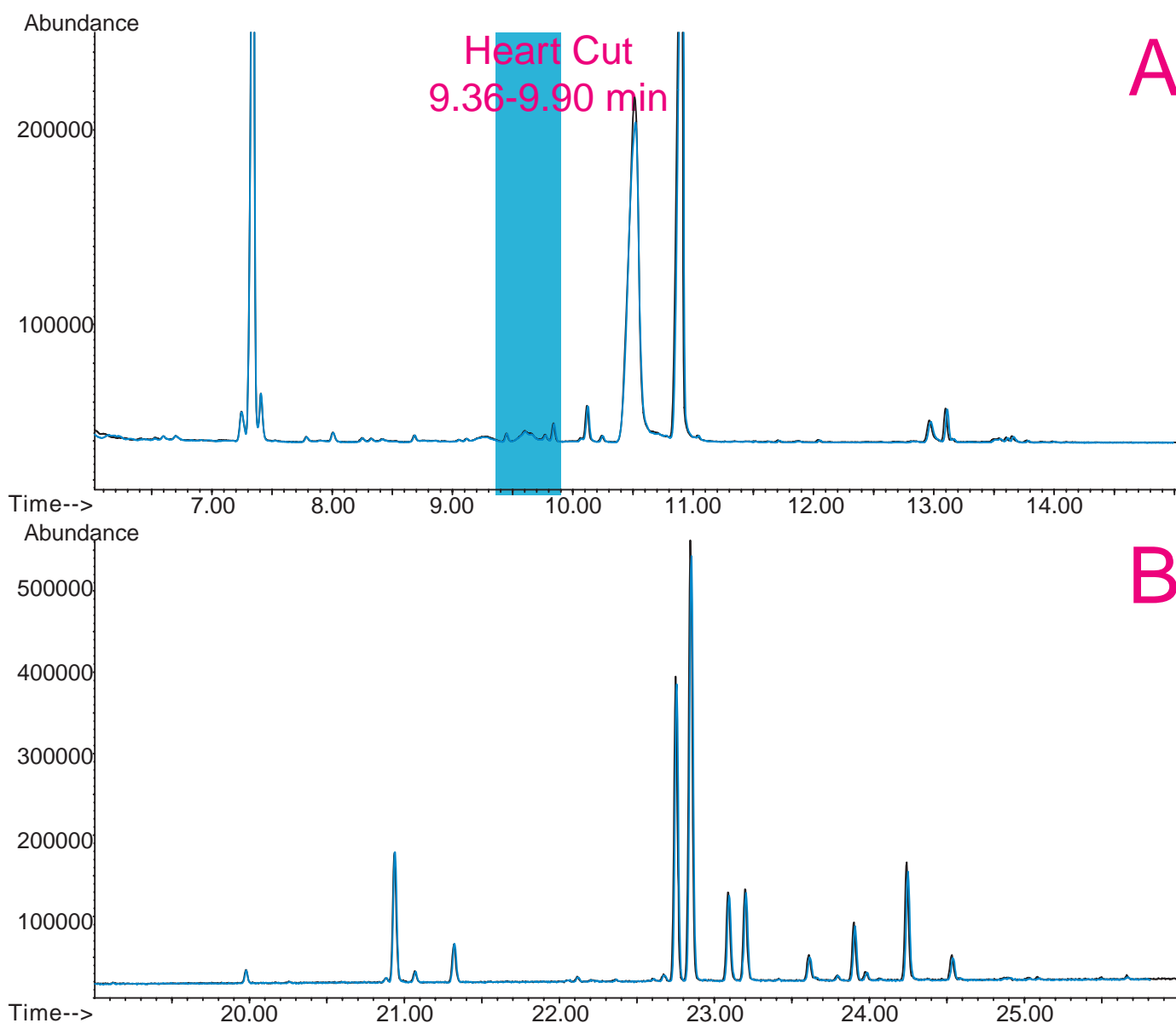


Figure 4. (A) Overlay of FID trace replicas from GC-GC precolumn separation of spearmint oil; (B) TIC overlay of spearmint oil replicas from GC-GC main column (9.36-9.9 min heartcut).

The multidimensional system includes cryogenic trapping and therefore different regions can be trapped and analyzed together in the second dimension. To illustrate this capability we created a method with two cuts: 8.15-8.6 min and 9.36-9.90 min. Figure 5a shows

the precolumn FID trace and main column TIC (Figure 5b) for the analysis with two cuts. The components resulting from the additional cut at 8.15-8.6 min are highlighted in the figure.

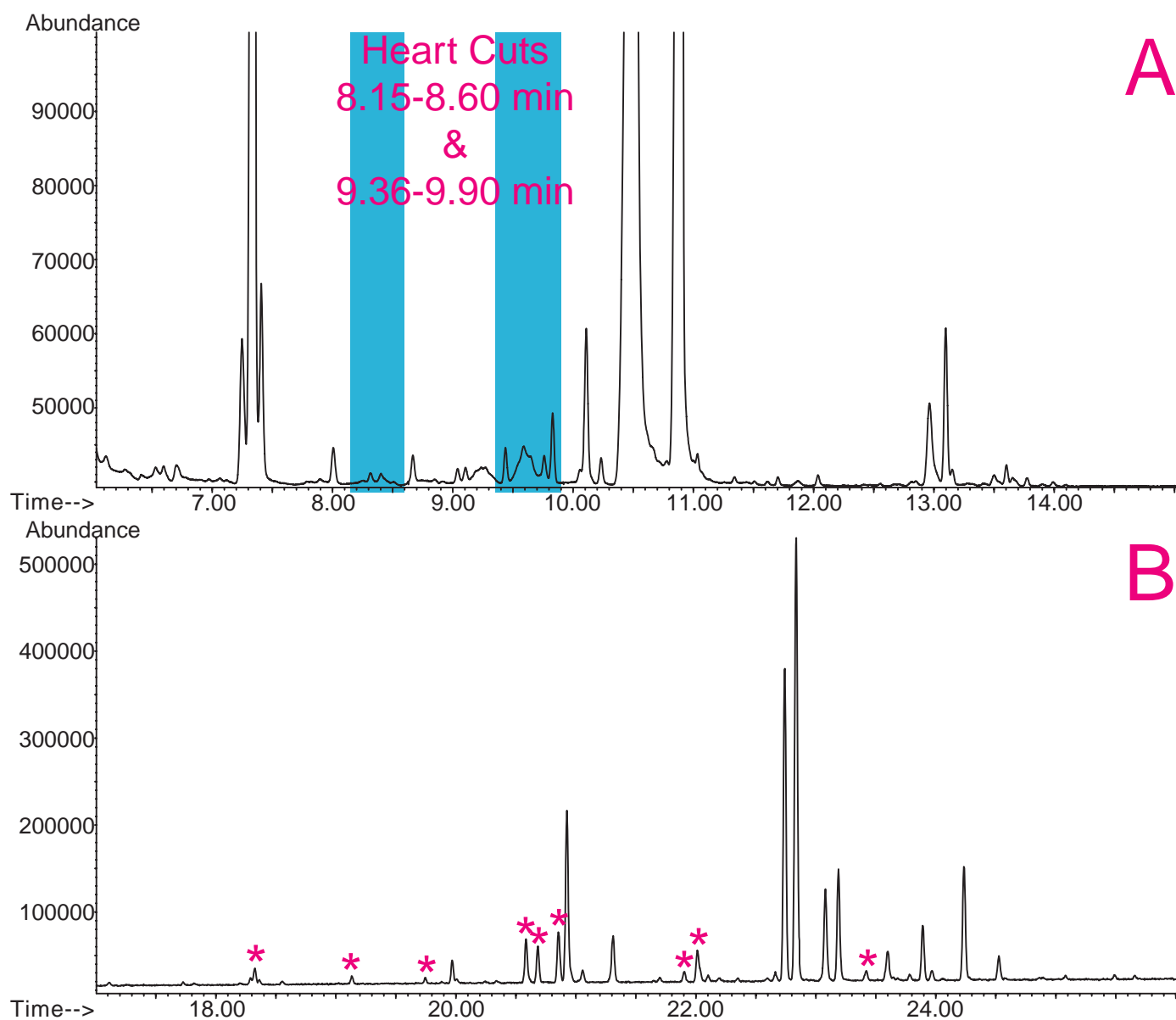


Figure 5. (A) FID trace from GC-GC precolumn separation of spearmint oil with two cuts; (B) TIC from GC-GC main column separation of 8.15-8.6 min and 9.36-9.9 min heartcut regions.

The compounds eluting from the main column were tentatively identified using the NIST 02 mass spectral library and included a match for trans-limonene oxide (RT=20.86 min) which had not been identified in our single dimension separations.

Vinyl shower curtain odor

Step 1 - Sample introduction. Prior work had shown that it was necessary to concentrate the headspace volatiles from several hundred milligrams of shower

curtain sample prior to introduction into the GC in order to detect the characteristic “shower curtain” odor at the sniff port. In trace odor analysis, it is often necessary to heavily overload the GC column with the major matrix components to insure sufficient mass of the odor active components is available for detection. For simplicity we chose to concentrate headspace volatiles into the PDMS phase on a Twister stir bar, although dynamically purging the sample headspace onto a packed adsorbent tube is another option.

Step 2 - Identification of odor regions. At least six distinct regions containing odors were identified in the separation on an HP5-MS column. We focused on the 19-21 minute region of the chromatogram where the strongest odor identified as “shower curtain” was located. Direct inspection of the data from the single dimension separation showed a large hydrocarbon background, and phenol and 2-ethyl-1-hexanol were tentatively identified eluting in this region by matches with the NIST02 MS library. It was impossible to identify any other possible odor components due to the hydrocarbon background.

Step 3 - Heart cut odor regions onto second column. The multidimensional GC-GC system was configured with a 1 μ m DB5-MS precolumn and an HP-Innowax column for the main separation.

Figure 6a shows the precolumn separation in which the region containing the distinctive odor causing compounds (19-21min) was cut into the main column without cold trapping. This allowed the hydrocarbon interference to move through the main column while the more polar components naturally refocused on the wax phase.

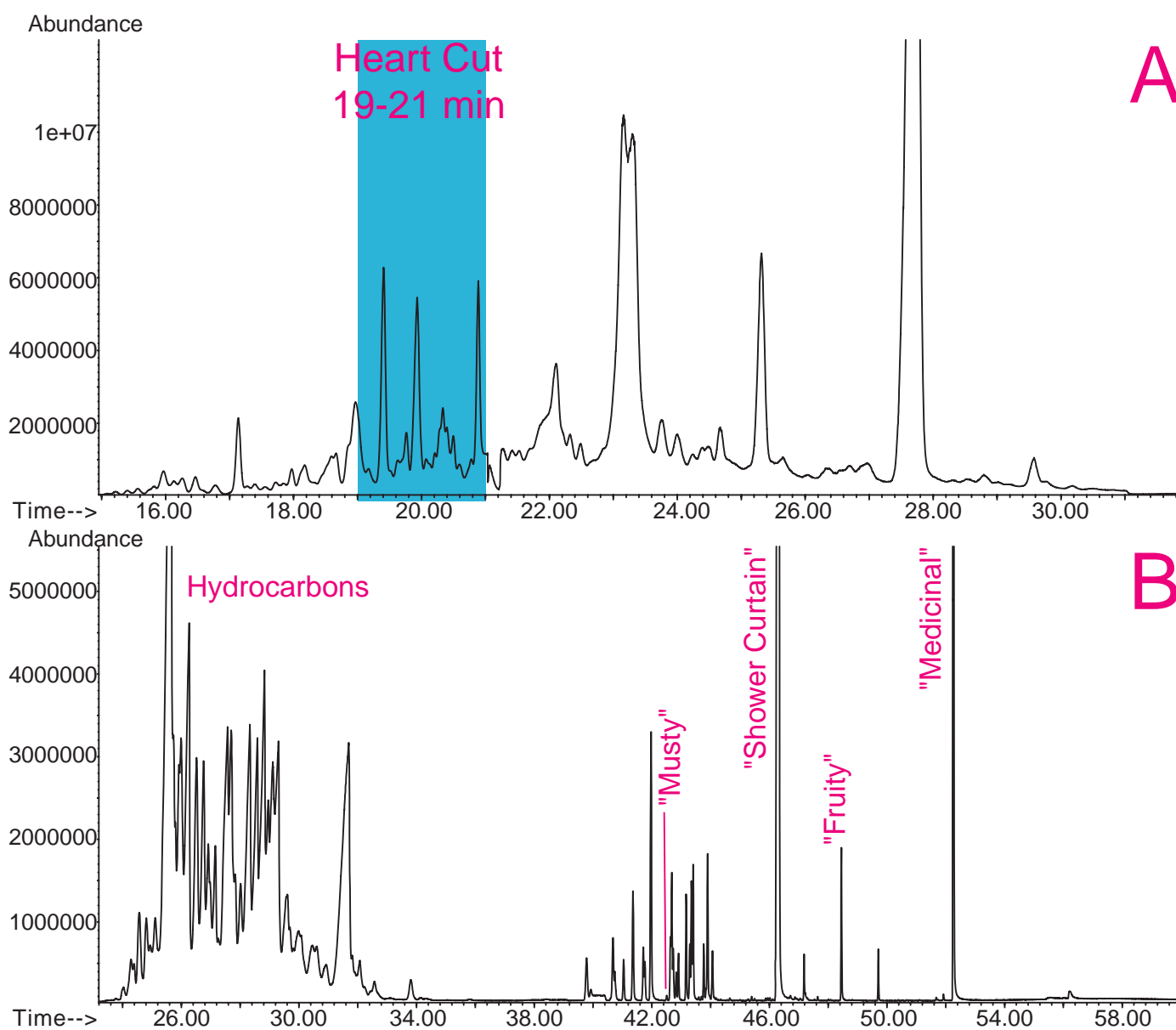


Figure 6. (A) FID trace from GC-GC precolumn separation of vinyl shower curtain liner volatiles. (B) TIC from GC-GC main column separation of 19-21min heart cut from precolumn.

Step 4 - Identification of odor causing components. Figure 6b shows the chromatogram from the main column with four distinctly different odor descriptors identified. Note that the two minute heart cut from

the precolumn transferred over 100 components to the main column. The components responsible for these odors are now nearly baseline resolved from any interference. Figure 7 shows the mass spectrum of the

component identified as the most similar to the “shower curtain” smell and the NIST02 library match obtained with 2-ethyl-1-hexanol. A flavor database identified this component as having a „rose“ or „green“ odor.

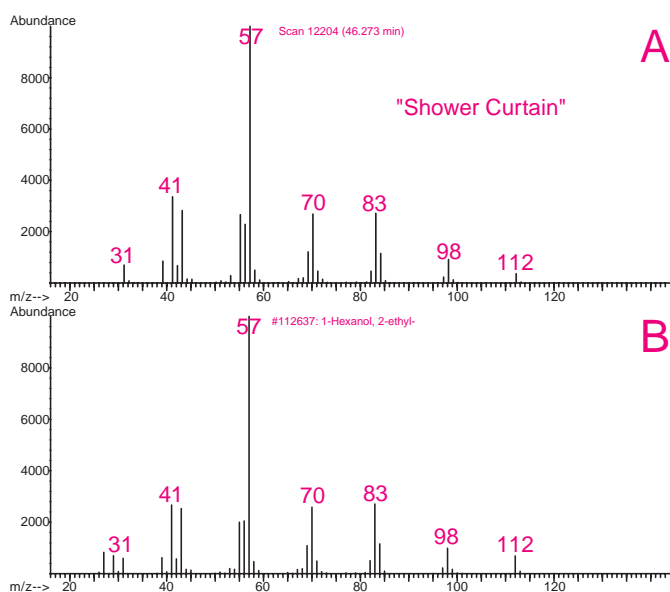


Figure 7. (A) MSD spectrum from “shower curtain” odor peak. (B) NIST98 MS library match with 2-ethyl-1-hexanol.

Figure 8 shows the mass spectrum of a small peak identified as having a “musty” odor, and the library match with 2-ethyl-1-hexenal. This peak is less than 0.2% of the size of the 2-ethyl-1-hexanol peak, yet it contributes a distinctly musty odor to the sample. A peak this size would clearly be impossible to identify in a single dimensional separation from this matrix. The remaining components and descriptors from this cut are listed in Table 1. To fully characterize the odor from the sample, each odor region from the precolumn could be cut individually into the main column for further separation and identification.

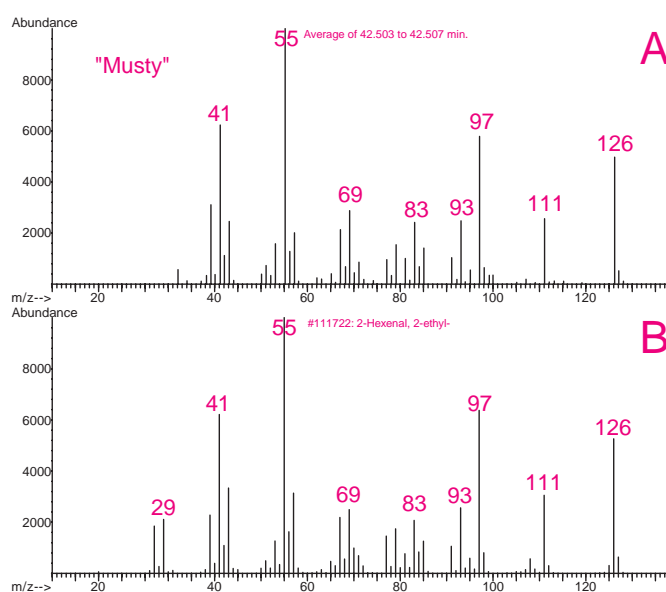


Figure 8. (A) MSD spectrum from “musty” odor peak. (B) NIST98 MS library match with 2-ethyl-1-hexenal.

Table 1. Odor descriptors, tentative assignments and NIST98 library match quality for the four odor compounds from a single cut from shower curtain sample, Figure 4b.

Peak	Descriptor	Compound	MQ
1	Musty	2-Ethyl 2-hexenal	94
2	Shower curtain	2-Ethyl 1-hexanol	86
3	Plastic fruity	Acetophenone	94
4	Medicinal	Phenol	95

CONCLUSIONS

Resolution and identification of trace compounds in complex samples can be effectively accomplished by multidimensional GC-GC/MS with heartcutting. The four steps used to identify trace components by multidimensional GC-GC are:

1. Introduce sufficient mass of the compounds of interest into the precolumn to obtain adequate response at the main column detector (MSD or ODP).
2. Identify the regions of interest in the precolumn chromatogram.
3. Transfer (heartcut) regions containing compounds of interest and interfering matrix components onto a second, orthogonal GC column to improve separation.
4. Identify the resolved components using an MSD and mass spectral library.

The GERSTEL multidimensional MCS system is a powerful tool for heartcut GC-GC separations of complex samples. When coupled to the GERSTEL MACH modules it provides a flexible system for independently optimizing precolumn and main column separations for resolution or speed on a single GC instrument platform.

Using this approach, trans-limonene oxide was identified in a complex region of a chromatogram from a liquid spearmint oil fraction. Likewise, 2-ethyl-1-hexanol and 2-ethyl-1-hexenal were identified as compounds that contribute to the characteristic odor from a vinyl shower curtain.



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