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A Selectable Single or Multi-dimensional GC System with Heart-Cut Fraction Collection and Dual Detection for Trace Analysis of Complex Samples

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Abstract
Identification of important trace components in complex samples like fragrances, natural products, petroleum fractions or polymers can be challenging. Achieving the mass on column and resolution necessary to locate and identify trace components using a single chromatographic separation can be difficult if not impossible. Parallel detection with a selective detector and MSD can help locate the region of interest within the complex chromatogram, but lack of sufficient resolution may still preclude reliable identifications. Heartcutting and 2D GC using columns with different polarities can significantly improve the resolution of complex regions and is the most powerful approach for trace component identification. This configuration requires a selective detector after both the pre-column and analytical column to track the components of interest through both separations, adding complexity.

We used Stir Bar Sorptive Extraction as a solventless means to introduce sufficient mass of sample onto the pre-column. When additional mass is necessary, a cryotrap after the pre-column can function as a fraction collector to accumulate fractions from many replicates of the sample. We coupled low thermal mass (LTM) GC column modules with dissimilar column phases using a software-controlled column switching device to perform heartcutting 2D-GC on two sample types. This system was configured with dual detectors to track and identify trace components of interest and a 6-port high temperature valve to allow selecting single column or multidimensional operation with dual detection in either configuration.

Separation and identification of selected trace flavor components from alcoholic beverages and sulfur compounds from food products demonstrated the effectiveness of this system. The main advantages of this configuration were the simple selection of single or two dimensional operation, the ability to collect multiple fractions to maximize signal from trace components in the second dimension, and parallel MSD and selective detection to assure correct identification of components of interest.

Introduction

Gas chromatography (GC) is widely used to analyze samples with complex volatiles profiles which are difficult to resolve 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 coelution interferes with the mass spectral identification of individual compounds.

In many cases, it is only necessary to further resolve groups of peaks in a region of interest. Regions of interest may be known from prior studies, or may be identified by using selective detection techniques such as olfactometry for odors or element-specific detectors like the Pulsed Flame Photometric Detector (PFPD) for sulfur. Resolving these regions can be readily accomplished using standard GC equipment and data analysis software by connecting two columns with different phases and selectively transferring compounds from one column into the other. This approach is commonly known as classic heartcut GC-GC.

In this study, we coupled two GC columns with dissimilar phases in independently programmable low thermal mass (LTM) heating modules using a software-controlled GC-GC heartcut system with a cryogenic trap at the head of the second column. A manual 6-port valve in the system allows the user to select whether the main detectors (MSD, Olfactory or PFPD) are in-line after the pre-column or main column simplifying switching between single and multidimensional operation. The performance of the system was illustrated by identifying aroma components in Amaretto, and organosulfur species in sauerkraut and soy sauce.

Experimental

Instrumentation. Analyses were performed on a 6890 GC equipped with a 5973 mass selective detector, a flame ionization detector (Agilent Technologies), a 5380 PFPD pulsed flame photometric detector (OI Analytical), PTV inlet (CIS 4, GERSTEL) and thermal desorption unit with autosampler (TDS 2 & TDS A, GERSTEL), a multi-dimensional column switching system with cryotrap (MCS 2/CTS 2, GERSTEL), an olfactory detection port (ODP, GERSTEL), a six-port high temperature valve (Valco), and dual Modular Accelerated Column Heaters (MACH, GERSTEL).

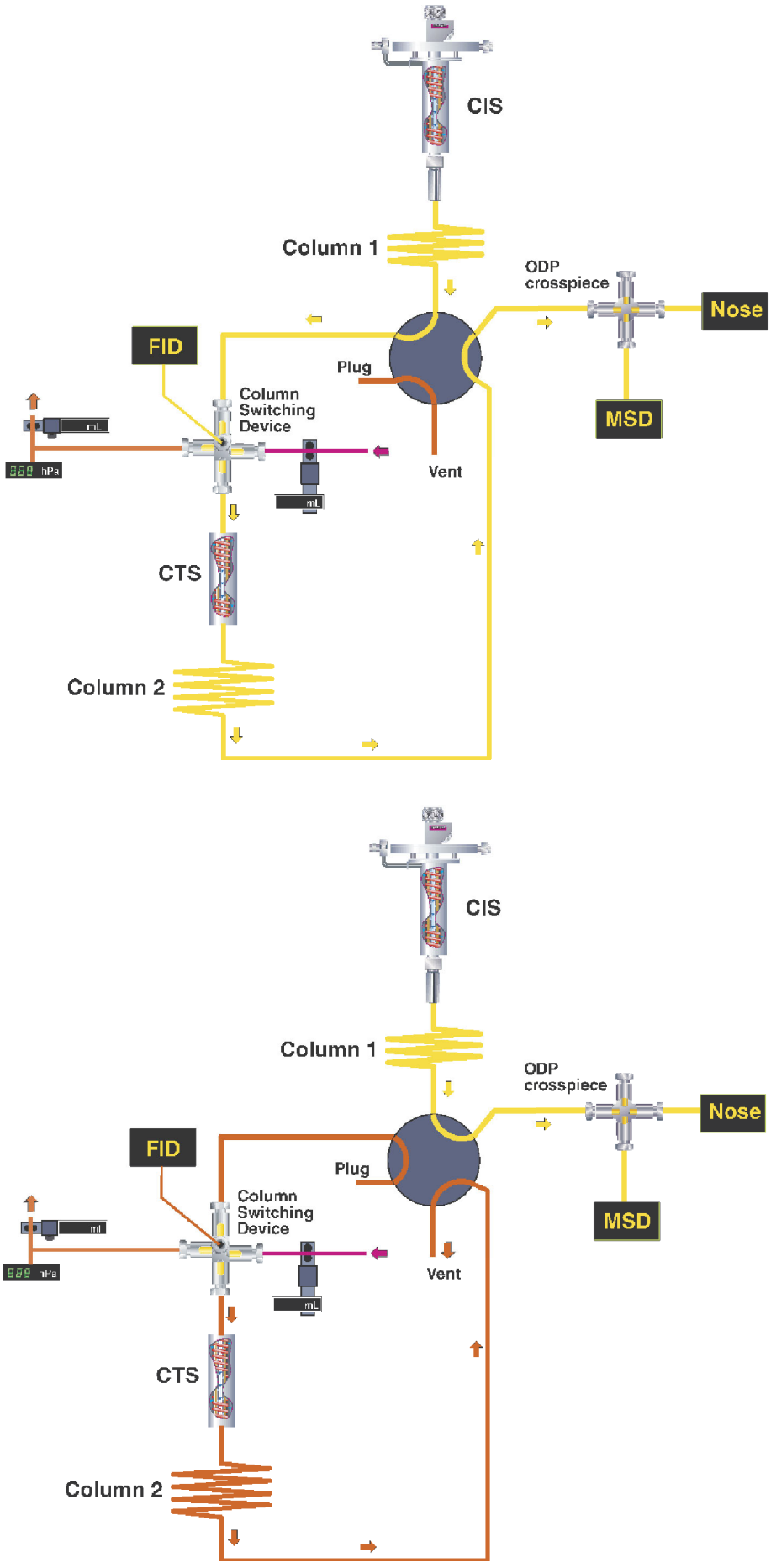

Figure 1. Schematic diagram of GC-GC system with 6-port valve for selecting single or multidimensional operation.

Analysis conditions.

TDS 2	splitless, 20°C, 60°C/min, 250°C (5 min)
PTV	0.2 min solvent vent (50 mL/min), splitless -120°C, 12°C/s, 280°C (3 min)
Pre-Column	15 m RTX-5 (Restek), LTM d_i= 0.25 mm, d_f= 0.25 µm
Main Column	30 m INNOWax (Agilent), LTM d_i= 0.25 mm, d_f= 0.25 µm
Initial flows:	1.6 mL/min for pre-column 1.5 mL/min for main-column
MACH: (Amaretto)	50°C; 20°C/min; 280°C (5 min) for pre-column 40°C (10 min); 10°C/min; 220°C (5 min) for main-column
MACH: (Soy sauce)	40°C (1 min); 25°C/min; 280°C (5 min) for pre-column 60°C (8 min); 60°C/min; 120°C (2 min); 7°C/min; 220°C (5 min) for main-column
MACH: (Sauerkraut- juice)	40°C (1 min); 15°C/min; 280°C (5 min) for pre-column 40°C (12 min); 10°C/min; 220°C (5 min) for main-column

Sample preparation. 10 mLs of sample diluted 1:10 in bottled H_2O (Amaretto) or prepared neat (soy sauce & sauerkraut juice) were pipetted into a 10 mL headspace vial. Stir Bar Sorptive Extraction was performed by adding a Twister to the sample, capping the vial, and extracting for 1 hr at room temperature with 1200 rpm stirring. The stir bars were removed, rinsed with bottled H_2O, blotted dry and placed into conditioned thermal desorption tubes for analysis.

Results and Discussion

Identification of aromas in Amaretto liquor. We configured the selectable single/multidimensional system with dual detection (Olfactory Detection Port/MSD) to identify aroma compounds in Amaretto liquor. A Twister stir bar used to extract the 1:10 diluted Amaretto was first analyzed with the single dimension system to identify regions in the chromatogram containing characteristic aroma compounds (Figure 2). The group of peaks eluting near 7 minutes contained a spicy citrus aroma.

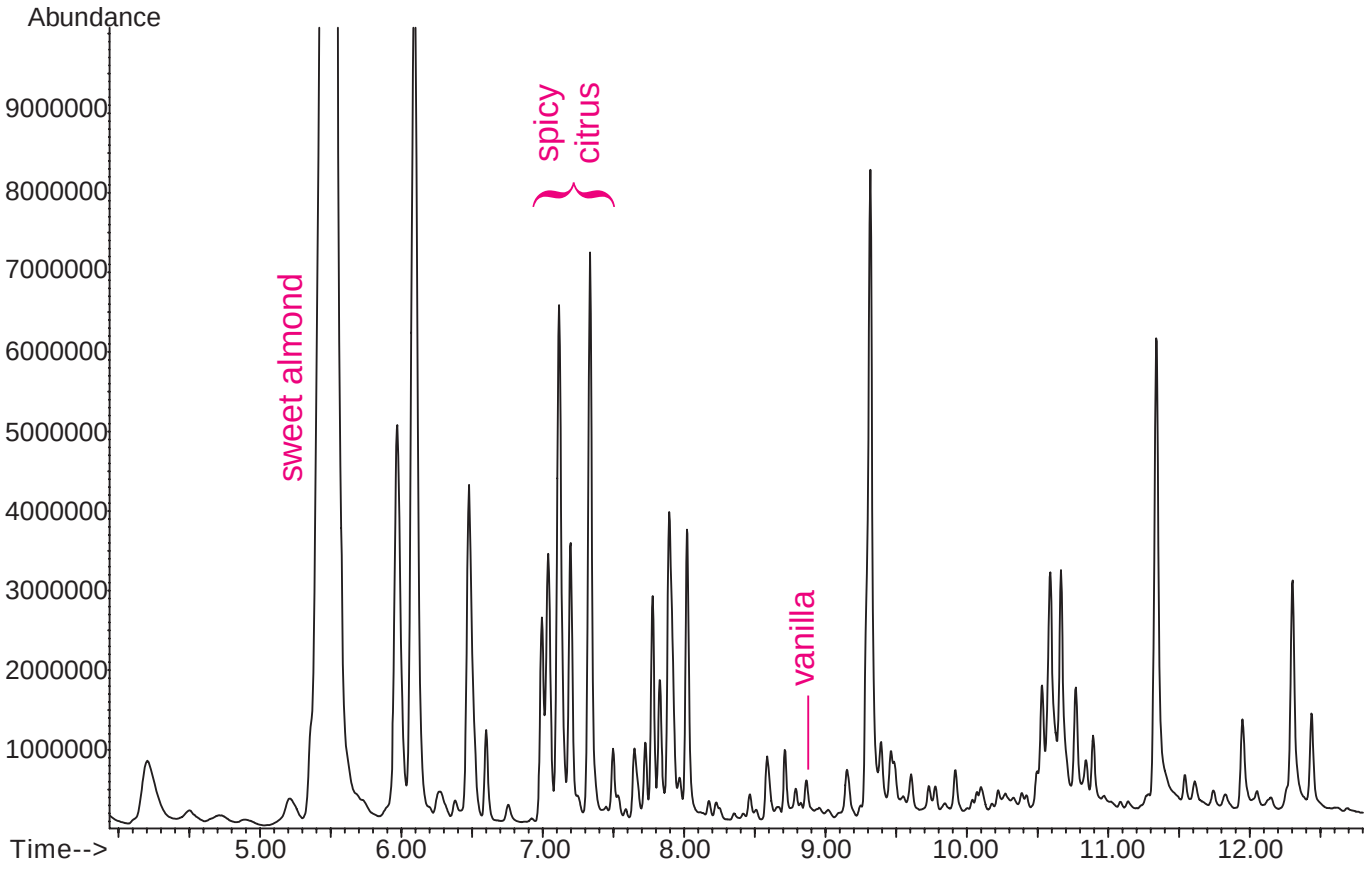

Figure 2. Single dimension separation of Amaretto flavor extract on a 30m x 0.25mm x 0.25µm DB-5MS column with main fragrance regions identified.

The 6-port valve was then turned to the multidimensional configuration and a second Twister stir bar was analyzed with a heart cut in the 7.9-8.5 minute region to transfer the group of peaks to the cold trap at the head of the second (main) column. Heating the cold trap to start the second dimension separation (Figure 3) gave a good separation of peaks, allowing identification of decanal as the compound responsible for the spicy citrus aroma. In addition, seven more trace aroma compounds could be detected in this same heart cut fraction (Table 1).

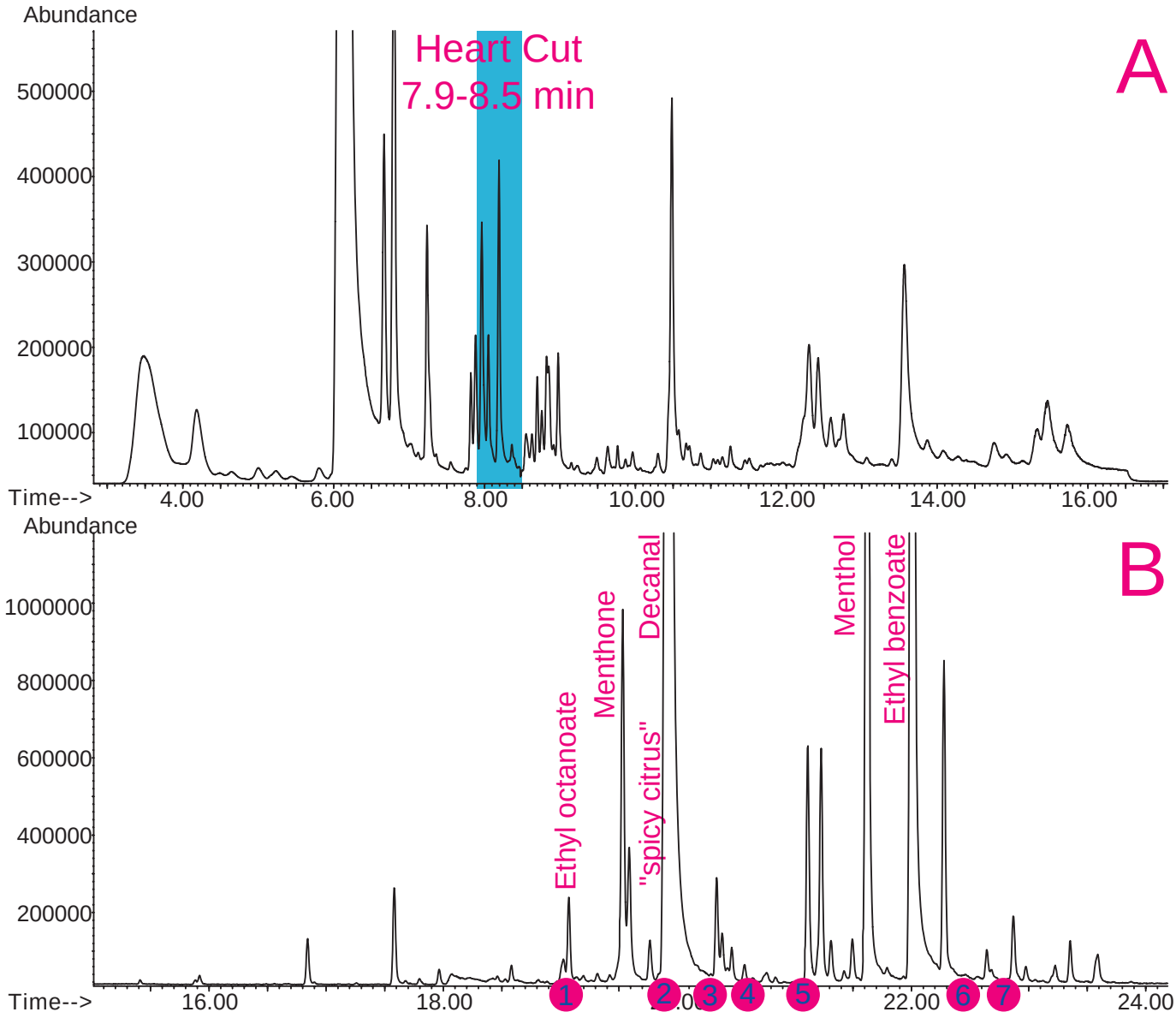

Figure 3. (A) Pre-column separation of Amaretto extract on a 30m x 0.25mm x 0.25µm DB-5MS column with heartcut region (7.9-8.5 minutes) highlighted. (B) Main column separation of heartcut region with major "spicy citrus" aroma identified as decanal. Descriptors for additional aroma regions are listed in Table 1.

Table 1. Amaretto – 1 cut (3 stir bars).

Peak No.	Retention Time	Descriptor	Compound I.D.	Match Quality
1	19.04	Floral	Ethyloctanoate	94
2	19.98	Spicy citrus	Decanal	91
3	20.27	Green	Unidentified	
4	20.45	Peppery	Unidentified	
5	21.03	Rubbery	Unidentified	
6	22.44	Oily	Unidentified	
7	22.72	Musty	Unidentified	

Identification of sulfur compounds in sauerkraut juice. We then configured the selectable single/multidimensional system with dual detection (PFPD/MSD) to identify sulfur compounds in foods. Figure 4 shows the first dimension separation of a Twister extract of sauerkraut juice. Over 65 sulfur compounds were detected in this extract.

The 6-port valve was then turned to the multidimensional configuration and a second Twister stir bar was analyzed with a heart cut in the 8.3-9.3 minute region to transfer a group of peaks containing sulfur species to the cold trap at the head of the second (main) column. Heating the cold trap to start the second dimension separation (Figure 5) gave a good separation of peaks, allowing identification of trithiolane (Figure 6) and several other compounds in this fraction.

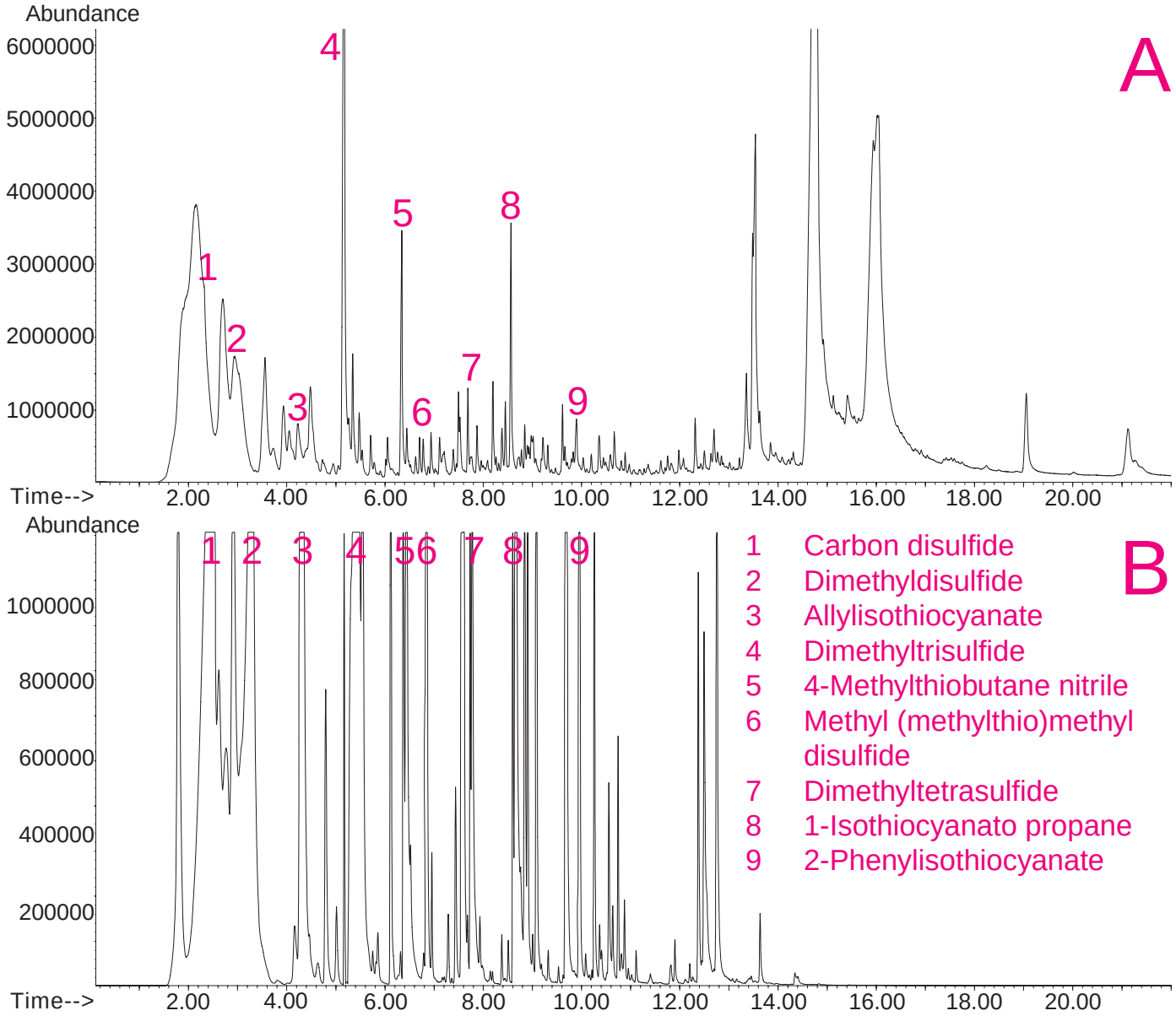

Figure 4. Single dimension separation of commercial sauerkraut juice extract with TIC (A) and PFPD (B) response. Peaks corresponding to main organosulfur compounds are numbered.

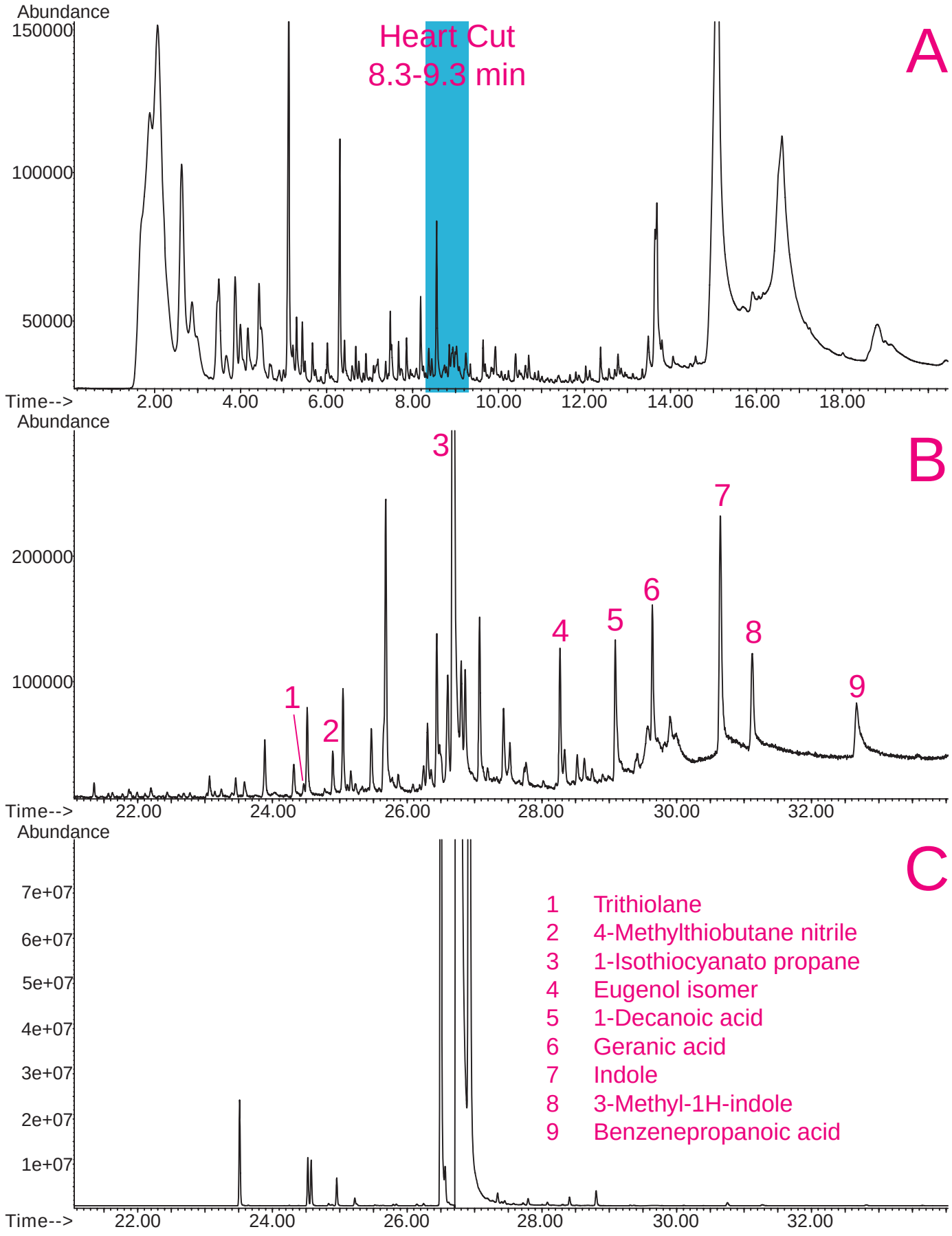

Figure 5. Pre-column separation of sauerkraut juice extract with heartcut region (8.3-9.3 minutes) highlighted. Main column separation TIC (B) and PFPD response (C) of heartcut region with three sulfur and six additional compounds identified.

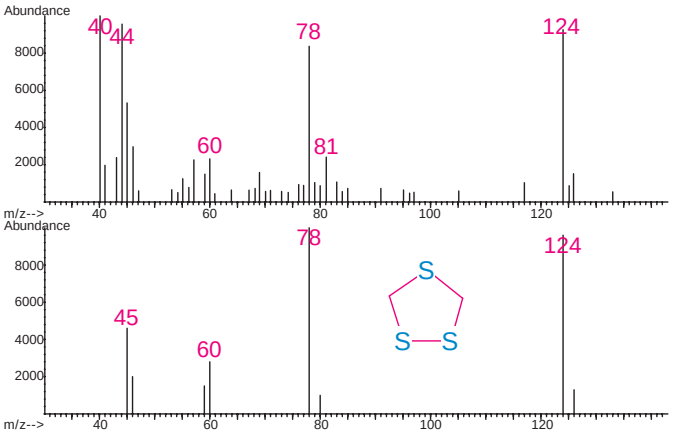

Figure 6. Wiley138 library match for trithiolane detected in sauerkraut juice.

Fast multidimensional GC with fraction collection in soy sauce. We used the same selectable single/multidimensional system with dual detection (PFPD/MSD) to identify sulfur compounds in soy sauce. Figure 7 shows the first dimension separation of a Twister extract of soy sauce run with fast heating (50C/min). Over 30 sulfur compounds were detected in this extract.

The 6-port valve was then turned to the multidimensional configuration and a second Twister stir bar was analyzed with a heart cut in the 5.51-5.92 minute region containing several sulfur compounds. This region was chosen because of the major interference from a large benzoic acid peak. Heating the cold trap at the head of the main column to start the second dimension separation (Figure 8) gave a good separation of peaks, all well resolved from the benzoic acid interference. Several compounds were identified in this fraction, but despite obtaining better mass spectra of the sulfur species no convincing mass spectral library matches were obtained.

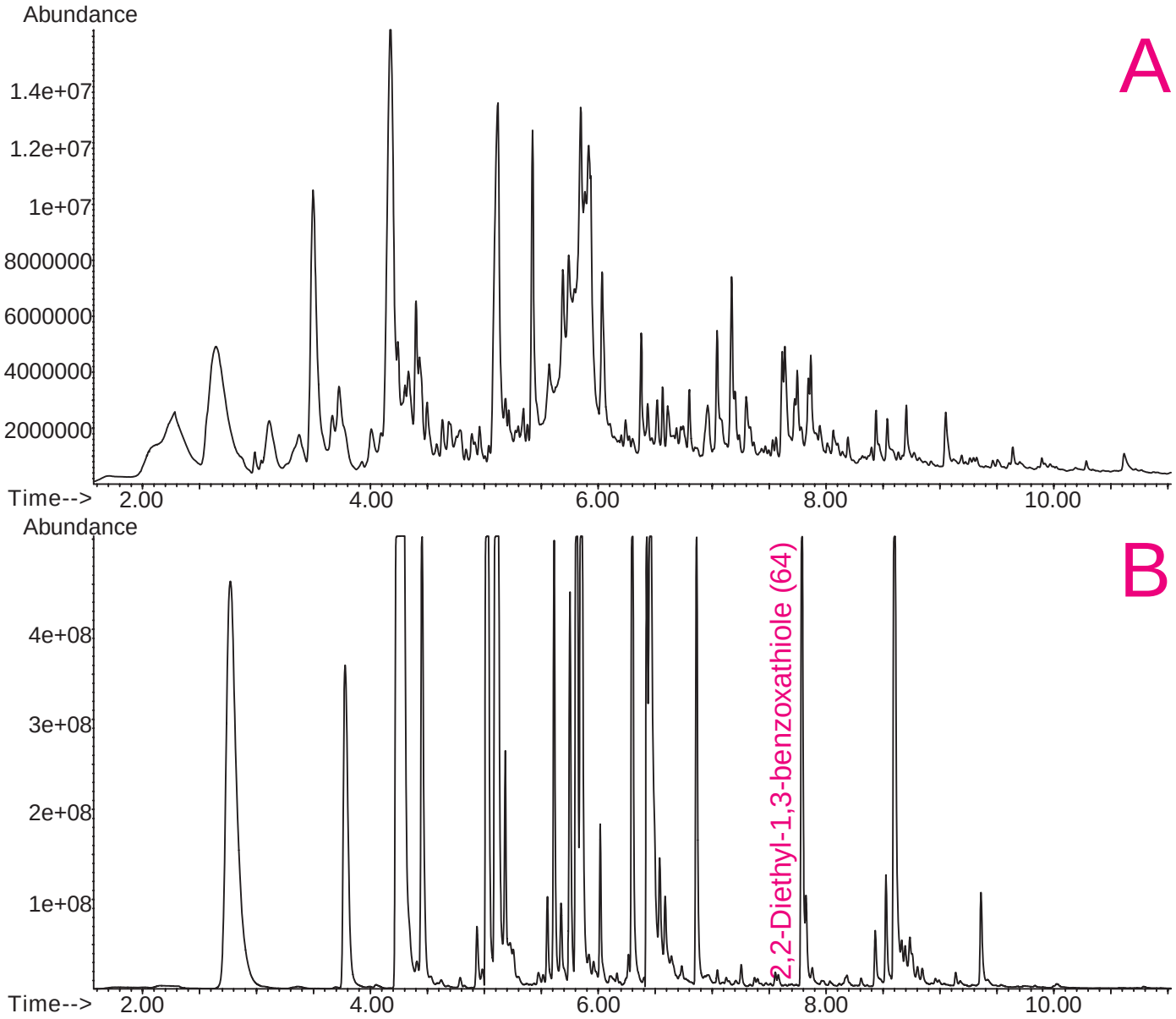

Figure 7. Single dimension separation of soy sauce extract with TIC (A) and PFPD (B) response. Only one organosulfur compound could be tentatively identified.

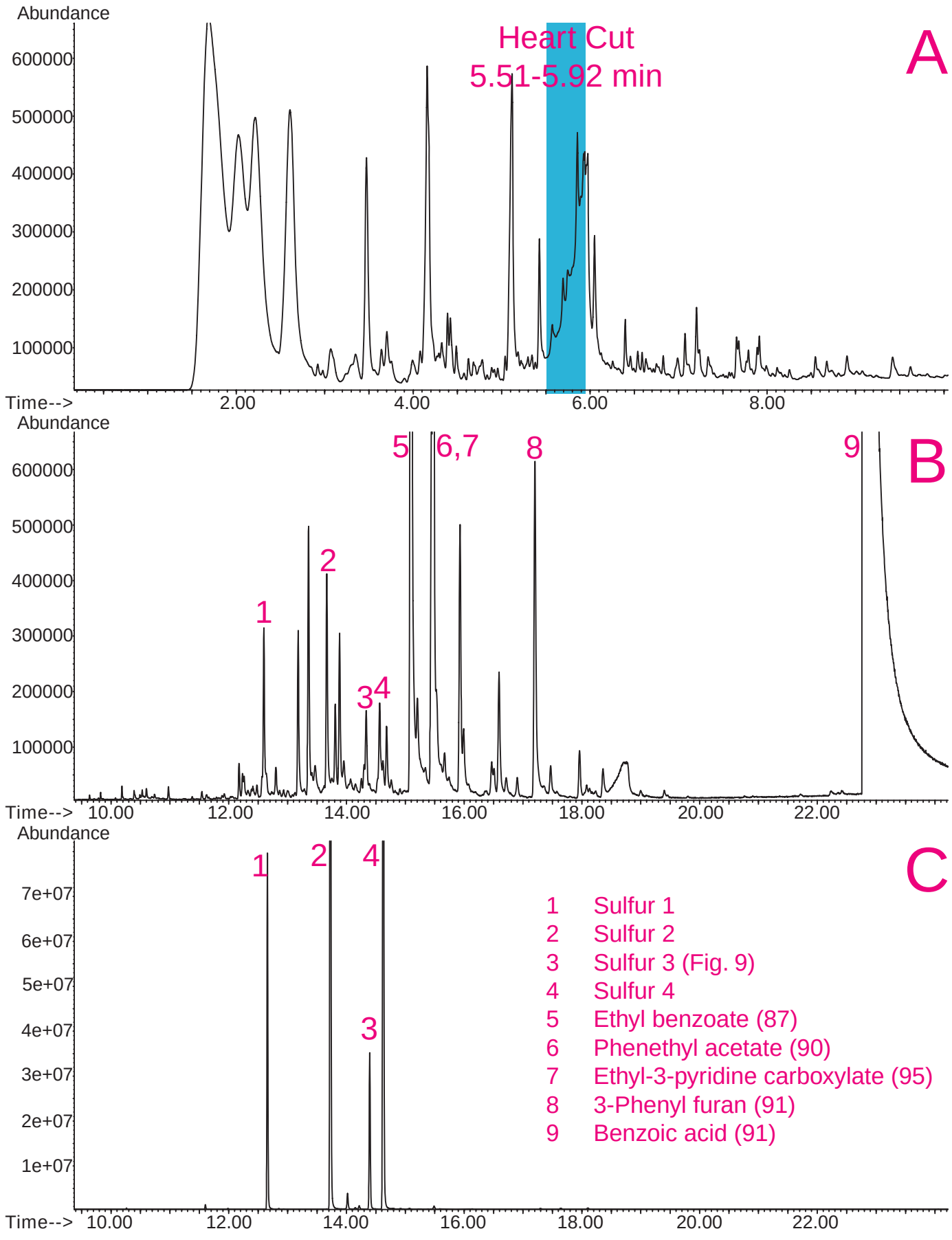

Figure 8. Fast pre-column separation of soy sauce extract with heartcut region (5.51-5.92 minutes) highlighted. Main column separation TIC (B) and PFPD response (C) of heartcut region with three sulfur and six additional compounds identified.

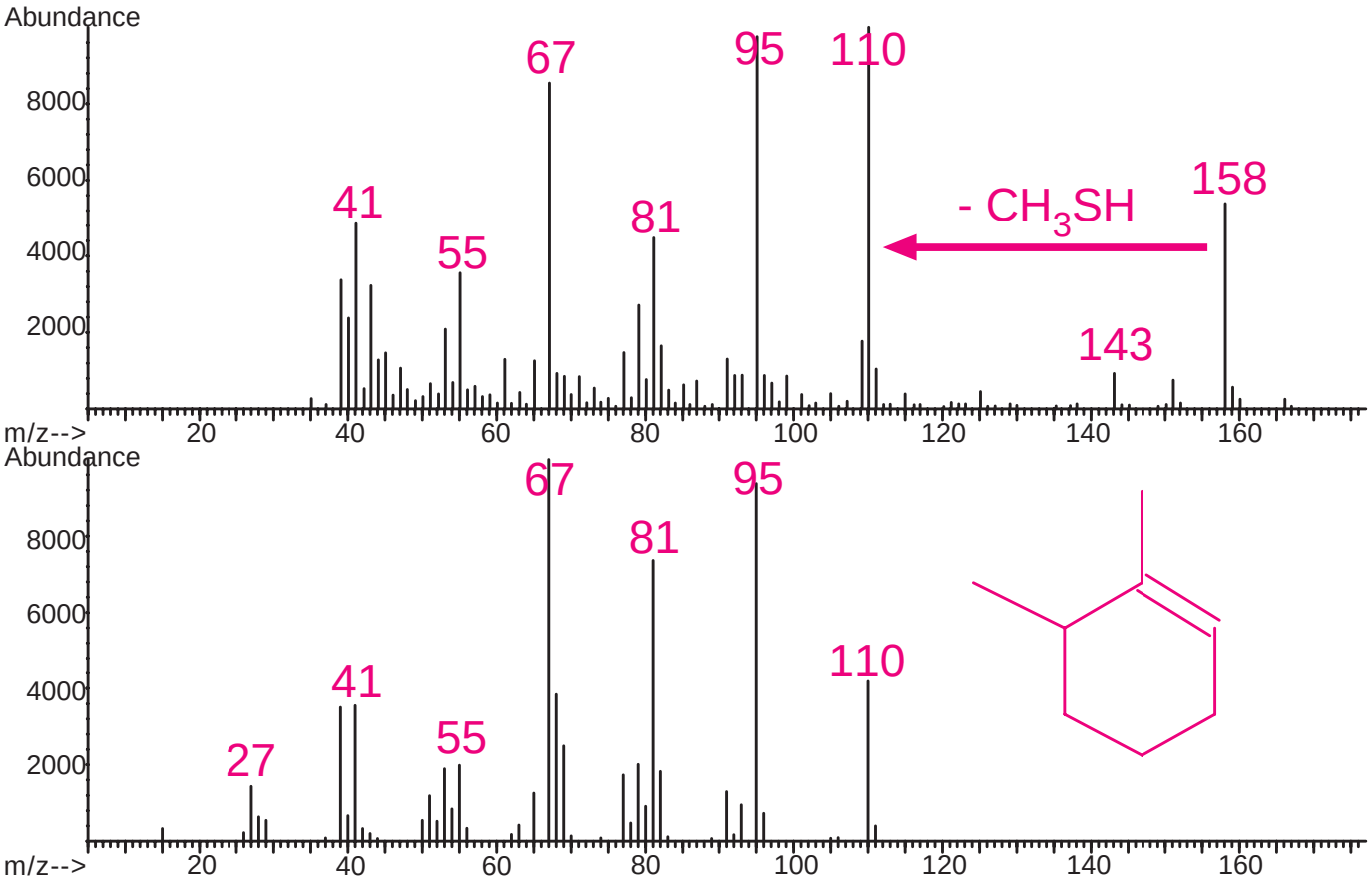

Figure 9. NIST98 library match for trace sulfur compound detected in soy sauce. Several sulfur compounds exhibited loss of 48 AMU, perhaps consistent with loss of CH3SH fragment as shown.

To illustrate the fraction collection capability on the cold trap, three Twister stir bars were desorbed simultaneously in a single desorption tube and the heart cut fraction was trapped in the cold trap. Two more tubes containing three Twisters each were subsequently desorbed with the same heart cut region. Figure 10 shows the additional signal available in the second dimension separation after accumulating the fractions from nine Twisters.

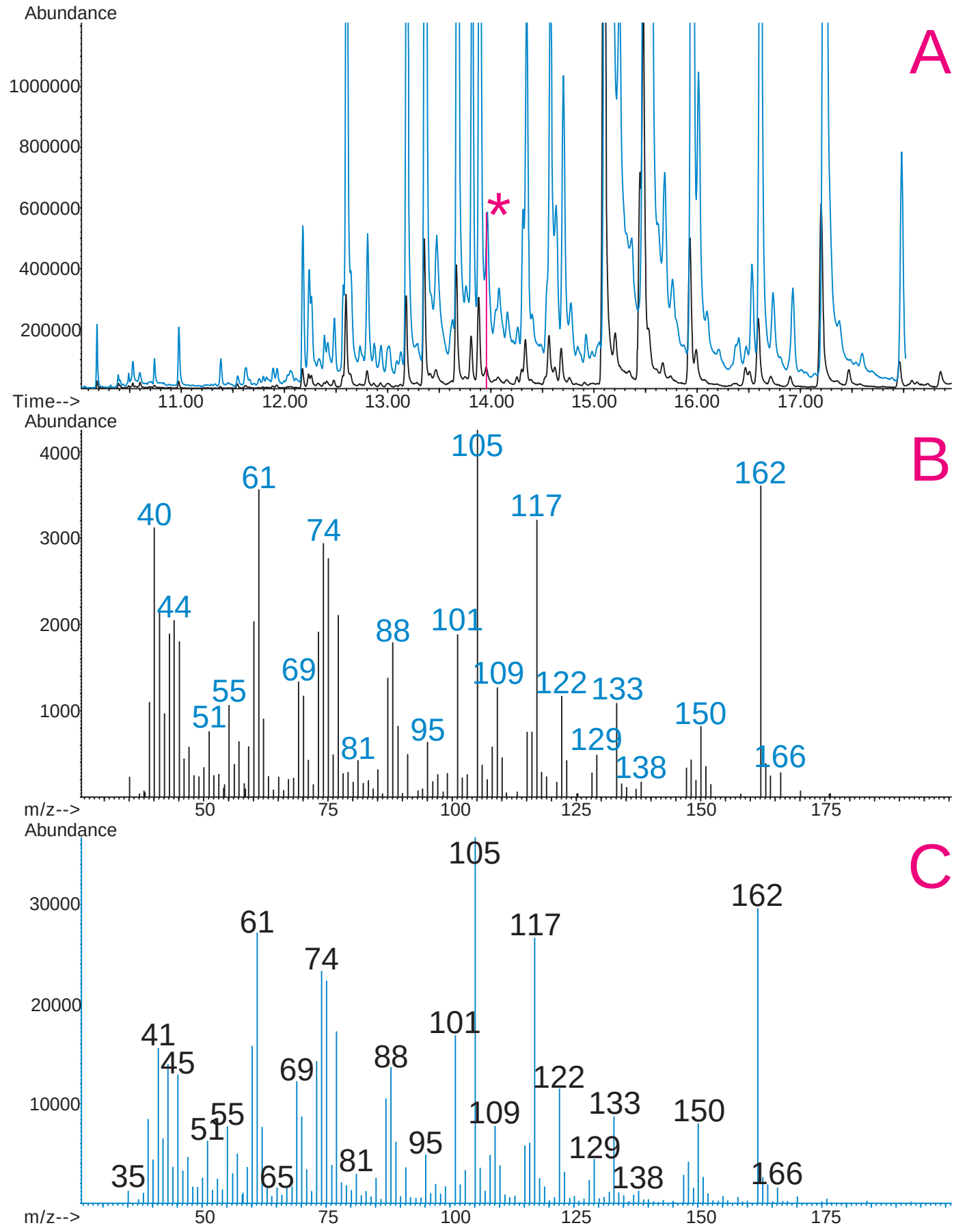

Figure 10. Overlay of heartcut region (5.51-5.92) TIC from single Twister and 3x3 Twisters (with cold fraction collection) illustrating the additional MS response possible with fraction collection. Mass spectra from single Twister (B) and nine Twisters (C) also show the increased abundance across the spectrum.

Conclusions

The major steps to trace compound identification by heartcut GC-GC are:

- Introduce sufficient mass of the compounds of interest into the column to obtain adequate response at the selective detector (ODP or PFPD) and the MSD.
- Identify the regions of the chromatogram in which target compounds elute using a selective detector.
- Reconfigure the GC for heartcut GC-GC.
- Heart cut regions containing target compounds and interfering matrix components onto a second, orthogonal GC column to separate matrix interferences from the compounds of interest.
- Identify the resolved components using selective detection and MSD in parallel.

Identification of odors in complex samples can be most effectively accomplished by multidimensional GC-GC/MS if an olfactometry detector using the human nose is used to help pinpoint the compounds responsible for the odor on both the pre-column and main column. Using this approach, we identified decanal and ethyl octanoate as contributors to the characteristic odor associated with Amaretto, in addition to the expected benzaldehyde and vanillin.

Identification of organosulfur compounds in foods can be most effectively accomplished by multidimensional GC-GC/MS if a PFPD configured for sulfur response is used to help pinpoint the compounds on both the pre-column and main column. Using this approach, over 65 sulfur species were detected in sauerkraut juice extract, and eleven could be tentatively identified by MS library matching. Over 30 sulfur species were detected in soy sauce extract with only one tentatively identified based on MS library matching. Mass spectra were obtained for most other sulfur compounds, and structure elucidation is ongoing.

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