Using Multi-Dimensional GC with a GC-Orbitrap to Separate Isomers for Identification of Unknowns

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ABSTRACT

Heart-cutting multi-dimensional gas chromatography (MDGC) is an efficient approach for the separation of co-eluting compounds in complex matrices, particularly for isomers. Deans' Switch is a device that can do heart-cutting on a gas chromatography mass spectrometry (GC-MS) system. Coupling Deans' Switch with high resolution accurate mass (HRAM) GC-MS could not only separate isomers or co-eluting compounds from one dimension to the second, but also offers superior resolution and excellent mass accuracy for fragment annotations, which can significantly increase confident identification. In this study, a Thermo Scientific™ Deans' Switch device with a Thermo Scientific™ Q Exactive™ GC Orbitrap™ GC-MS/MS system was used to transfer co-eluted lemon oil chromatography bands to the second dimension. Complete separation was then achieved on the polar secondary column. Compound identification was performed by library searching against an HRAM GC-Orbitrap library and confirmed by accurate mass determination of the molecular formula. Furthermore, multiple peaks can be cut and sent to the second dimension without retention time drifts. Excellent mass accuracies were achieved throughout the whole process.

INTRODUCTION

HRAM GC-MS has become a popular tool for comprehensive sample characterization because of its high selectivity full-scan acquisition. Co-eluting peaks with the same nominal mass, which interfere at nominal resolution, can be spectrally separated at high resolution, allowing for the detection and identification of more compounds in matrix. However, co-eluting isomers can still be problematic as they have exactly the same masses for both their molecular and fragment ions. Furthermore, the retention indices of these isomers are quite close to each other, which makes for harder identification. In this case, HRAM GC-MS alone is ineffective for separation.

Heart-cutting MDGC is a comprehensive technique to separate and identify isomers in complex matrices that was first reported 60 years ago.[1,2] A major breakthrough in heart-cutting MDGC was achieved by Deans at the end of the 1960s. The invention of Deans' Switch allowed flow switching from a primary column to a secondary column, which allows the transfer of an analyte from the primary column to a secondary column that typically has similar dimensions but substantially different phase polarity compared with the primary column. In this study, a lemon oil sample was analyzed on the Deans' Switch coupled with a Q Exactive GC Orbitrap GC-MS/MS system to fully separate co-eluted monoterpenes. The advantages of using the Deans' Switch device are listed below:

- Fully deactivated auxiliary gas channels, which enhances quantitative accuracy and provides high reproducibility without peak tailing
- Zero dead volume port connections, which eliminates peak broadening
- Low thermal mass without cold spots, which avoids sample condensation

MATERIALS AND METHODS

Sample Preparation

Terpene standards were purchased from Sigma-Aldrich™, including amino acids. Lemon oil was purchased from Sigma-Aldrich and then diluted in a solvent mixture of hexane and acetone (9:1).

Data Analysis

Data was acquired and processed using Thermo Scientific™ TraceFinder™ 4.1 software. In addition, compound identification was made by searching an HRAM GC-Orbitrap library.

Deans' Switch

The Thermo Scientific microfluidic Deans' Switch system provides the hardware and the calculation software that allows for precise heart-cutting in a chromatogram. Figure 1 shows a five port microfluidics device and two capillary columns connected to two different detectors: Thermo Scientific™ Instant Connect Electron Flame Ionization Detector (FID) and the Q Exactive GC Orbitrap mass spectrometer (MS). The eluent from one column is diverted to a second column at specific times and for specific durations in the chromatographic run. Because the second column has different polarity, the analytes of interest that are unable to be separated on the first column can often be better separated on the second. The detector types used can be FID, MS, and other Thermo Scientific detectors.

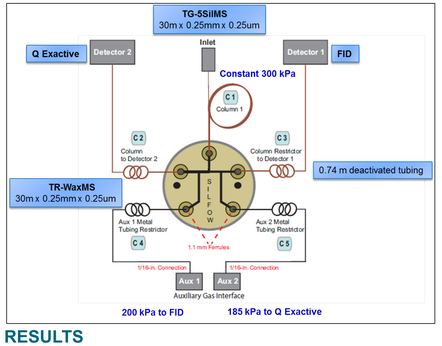
Inlet flow and two auxiliary gas module channels are controlled by constant pressures. Different oven temperatures could significantly change the flow rate into the two columns. Hence, it is necessary to calculate the pressures in the calculation software as shown in Figure 2. Checking the auxiliary channel 1 will divert the flow directly to the first detector, therefore all the analytes will be detected on detector 1. On the other hand, checking the auxiliary channel 2 will do heart-cutting of peaks that would elute at that time directly to the secondary column. The oven temperature that the analyte of interest elutes at needs to be applied in the calculator to properly adjust pressures from auxiliary channels. The arrows on the software show the direction of flow in the device. If the current settings are reasonable, the software will show "cutting is Ok". Then the corresponding parameters can be loaded into the TraceFinder instrument editor for data acquisition.

Table 1. Gas chromatograph and mass spectrometer analytical parameters and the microfluidic Deans' Switch five-port plate.

TRACE 1310 GC System Parameters			
Primary Column	Thermo Scientific™ TRACEGOLD™ TG-5SilMS 30mx0.25mmx0.25um	Injection Volume:	1.0 µL
Secondary Column	Thermo Scientific™ TRACE™ TR-WaxMS GC 30mx0.25mmx0.25um	Liner:	Single taper without glass wool
Primary Column Pressure, kPa	He, 300 kPa	Inlet:	250 °C
Aux 1 Gas Pressure, kPa	He, 200 kPa	Inlet Module and Mode:	Split 10:1 (EI)
Aux 2 Gas Pressure, kPa	He, 185 kPa		
Oven Temperature Program:			
Temperature 1:	40 °C	Hold Time:	0.5 min.
Rate:	5 °C/min.	Temperature 2:	200 °C

Q Exactive GC Orbitrap GC-MS/MS Parameters	
Transfer Line:	250 °C
Ionization Type:	Electron Ion (EI)
Ion Source:	250 °C
Electron Energy:	70 eV
Acquisition Mode:	Full-scan
Mass Range (*m/z*):	50-500 (EI)
Lock masses (*m/z*):	73.04680; 133.01356; 207.03235; 281.05114; 355.06990

Figure 1. The set up of Deans' Switch on a Q Exactive GC Orbitrap GC-MS/MS system.



RESULTS

Terpene Analysis in Lemon Oil

Terpene hydrocarbons such as monoterpenes and sesquiterpenes are widely found in citrus oils. The determination of the essential oil volatile profile is especially important for an evaluation of quality and authenticity. The separation of very complex citrus oil matrices through uni-dimensional GC-MS is relatively difficult as co-elutions are inevitable, and even sophisticated deconvolution software packages are sometimes not able to fully deconvolute them. Co-eluted isomers have exactly the same molecular ions such for classes such as the monoterpene ($C_{10}H_{16}$), and almost the same fragment ions (Figure 3). This means that HRAM MS won't help to differentiate them. Therefore, two-dimensional GC-MS system is a promising tool to resolve the peaks that coelute on a primary column but can be separated on a secondary column. As we know, lemon oil is a common citrus oil that contains high concentrations of limonene, a lemon-like odor monoterpene. It is has been widely used as an additive in industrial cleaning solvents and in cosmetics.

Figure 2. Deans' Switch calculator for diverting flow to primary and secondary columns.

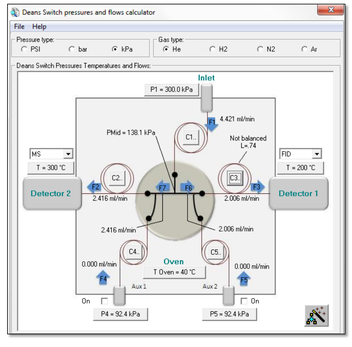


Figure 3. Comparison of library reference spectra between limonene and ocimene.

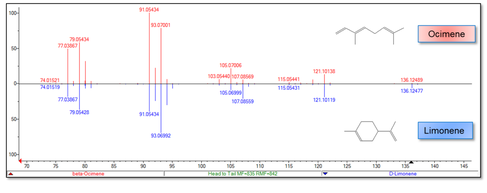


Table 2. Retention indices of limonene and ocimene on different phase polarities.

Retention Index	Semi Non-polar	Polar
Limonene	1030±2	1200±7
Ocimene	1037±7	1250±4

Detecting trace amounts of monoterpenes in lemon oil is essential for quality control. Ocimene, a monoterpene with a pleasant odor appreciated in perfumery, is most frequently found in essential oils. It has almost the same fragment ions as limonene. Also, their retention indices (RI) on semi non-polar columns are nearly identical (Table 1) so it makes it difficult to separate ocimene from limonene, usually found in high concentration, on a uni-dimensional GC column setup. However, their RI difference is larger on a polar phase column as shown in Table 1. So the Deans' Switch can help to cut them onto a polar secondary column for separation.

In the beginning, lemon oil samples were acquired solely on an FID detector to get the retention time of limonene (10.99 min), as shown in Figure 4. On the subsequent run, the large limonene peak was cut at 10.99 min from the primary column to the secondary column. The cut peak was immediately sent to a secondary column, where a complete separation was made (Figure 5). A tiny peak was found at 13.23 min on the secondary column, which was identified as ocimene by library searching through the HRAM GC-Orbitrap library. It's worth noting that these two monoterpenes can be baseline separated on a wax column. In order to test the multiple heart-cutting capability of the Deans' Switch device, limonene and linalool were both cut from the primary column onto the secondary column, as shown in Figure 6. On the secondary column, it shows a large limonene peak, a separated ocimene peak, a linalool peak, and an unknown interference peak separated from linalool (Figure 7).

Figure 4. (Top) Limonene peak at 10.99 min on the primary column chromatogram detected by FID before heart-cutting; (Bottom) limonene peak was removed from the primary column after heart-cutting.

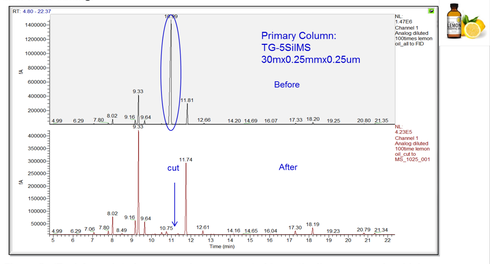


Figure 5. (Top) A co-eluted compound was separated from the limonene peak which was diverted from the primary column by heart-cutting. (Bottom) Identification was performed through the HRAM GC-Orbitrap library which demonstrated it was ocimene.

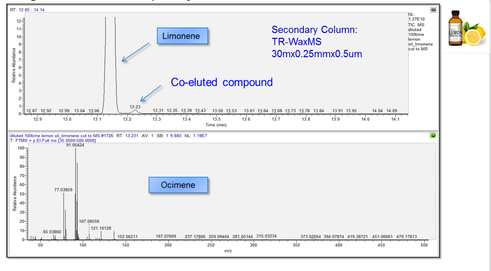


Figure 6. (Top) Before multiple peaks were cut from the primary column by using Deans' Switch. (Bottom) after heart-cutting, limonene and linalool peaks were removed from the primary column chromatogram.

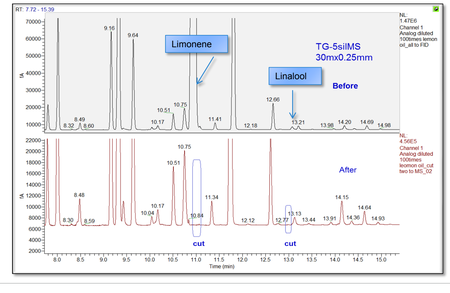
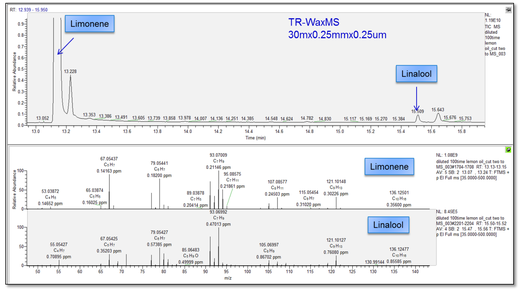


Figure 7. (Top) limonene and linalool were sent to the secondary column. Their co-eluted interferences were fully separated on the secondary column; (Bottom) Identifications were performed through the HRAM GC-Orbitrap library and mass accuracies were maintained of less than 1 ppm for all fragment ions and molecular ions.



CONCLUSIONS

Heart-cutting Deans' Switch is an alternative way to separate co-eluted isomers on an HRAM GC-MS system. The key benefits of using Deans' Switch coupled with a HRAM Q Exactive GC Orbitrap system are listed below:

- Easy to operate: column connections are simple; flow calculation software is intuitive.
- Reliable system: heart-cutting occurs immediately with flow switch, so retention times are highly reproducible. Multiple peaks can be cut from the first dimension without affecting the retention times of other peaks.
- Fully deactivated hardware: Inert microfluidic plate, nuts, ferrules and gas channels.
- Excellent mass accuracy of less than 1 ppm is consistently maintained during the whole process which increases the confidence of identification.

REFERENCES

1. Tranchida, P. Q.; Sciarrone, D.; Dugo, P.; Mondello, L. Heart-cutting multidimensional gas chromatography: A review of recent evolution, applications, and future prospects. *Anal. Chim. Acta.* 2012, 716, 66-75.
2. Seeley, J. V.; Seeley, S. K., Multidimensional Gas Chromatography: Fundamental Advances and New Applications, *Anal. Chem.* 2013, 85, 557−578.

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