

GLOBAL ANALYTICAL SOLUTIONS



**GERSTEL**

AppNote 7/2003

## Coupling Retention Time Locked Methods and Libraries to Automated SPME or SBSE for Analysis of Flavors and Fragrances

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### KEYWORDS

Flavors, gas chromatography, mass spectrometry, retention time locking, SPME, SBSE

### ABSTRACT

Analysis of foods, flavors and fragrances is commonly done by capillary gas chromatography coupled to a range of detectors depending on the particular needs of the analysis. Using Retention Time Locked (RTL) methods can facilitate the transfer from one instrument to another, particularly if different detectors (eg. MSD and FID) are used.

Although liquid or headspace injection methods are often used for these analyses, difficult and time-consuming sample preparation steps, or lack of sensitivity are commonly encountered. Automated Solid Phase Microextraction (SPME) or Stir Bar Sorptive Extraction (SBSE) can often provide substantial improvements to existing methods. In this contribution, we illustrate the use of RTL methods and the Agilent RTL Flavor Library when the GERSTEL MPS 2 autosampler is used to automate either SPME or SBSE as sample extraction and introduction technique. Procedures are given for calibrating the GC method using

either technique. RTL methods created for both sample introduction techniques could be moved from a GC-FID to a GC-MSD system with different column head pressure requirements.

Calibrated RTL SPME or SBSE methods were then used to analyze several sample types that otherwise would pose significant matrix interference or detection limit issues. Example chromatograms with identifications based on the RTL flavor library are given.

## INTRODUCTION

Analysis of flavor and fragrance composition can be challenging. Flavors and fragrances are usually complex mixtures that may contain many constituents that could include terpenes, diterpenes, sesquiterpenoids, etc. Common techniques used to analyze these types of samples are gas chromatography (GC) coupled to either flame ionization detector (FID) or mass spectrometer (MS).

GC/MS is widely used to separate and tentatively identify flavor constituents. There are several techniques that introduce flavor components into GC instruments. These include static headspace or enrichment techniques such as solid phase microextraction (SPME) or, more recently, stir bar sorptive extraction (SBSE). Regardless of the method used for sample introduction the identity obtained with the mass spectrometer for the flavor components is considered tentative. In order to have full confidence of the identity of a compound, standards are necessary to validate the MS findings.

Identification of flavor components with an MS detector is also difficult because usually they are mixtures of compounds with similar mass fragmentation. Since isomers give similar spectra, retention indices are commonly used to complement the information obtained with the MS. There are published libraries with retention indices for flavor and fragrances, but these libraries depend on the column type, linear velocity of the carrier gas, oven profile, etc. Due to the dependence of these experimental conditions, the retention indices can be difficult to reproduce.

A technique known as retention-time-locking (RTL) can compensate for the disadvantages mentioned before of using GC/MS. The ability to lock a GC/MS method and subsequently running samples under locked conditions has the advantage of providing complementary information to the identification obtained using a mass spectral library. When a GC/MS method is locked, the identification of the individual components is carried out with the mass spectral profile

and also by its retention time. The extra information is particularly useful when isomers with similar mass spectra are encountered.

In this study, an RTL flavor database created by David et al [1]. was used to analyze different beverage samples. The combination of the RTL capabilities along with a diversity of sample introduction into a GC creates a versatile way of analyzing flavor and fragrances samples.

## EXPERIMENTAL

*Instrumentation.* All analyses were performed on a GC (6890, Agilent Technologies) with mass selective detection (MSD). The instrument was equipped with a Thermal Desorption unit with autosampler (TDS 2 & TDS A, Gerstel), a Multipurpose sampler with SPME capability (MPS 2, Gerstel) and a PTV inlet (CIS 4, Gerstel).

### *Analysis Conditions.*

Column: 30m HP-5 (Agilent),  
 $d_i = 0.25\text{mm}$ ,  $d_f = 0.25\text{mm}$   
Pneumatics: He,  $P_i = 10.6$  psi (flavors),  
 $P_i = 20.3$  psi (pesticides)  
constant pressure  
Oven: 60°C, 3°C/min,  
240°C for flavors  
70°C (2 min), 25°C/min,  
150°C, 3°C/min,  
200°C, 8°C/min,  
300°C (5 min) for pesticides

### *Twister desorption (SBSE).*

TDS 2 splitless,  
20°C, 60°C/min, 250°C (5 min)  
PTV solvent vent (50 mL/min),  
split ratio 20:1  
-120°C (0.2 min), 12°C/s,  
280°C (3 min)

### *MPS 2 / SPME*

Fibers 100µm PDMS  
Equilibration 60°C (10 min)  
Extraction 60°C (10 min)  
PTV 0.8 min splitless  
isothermal at 250°C

### *Sample Preparation.*

*Grapefruit juice.* 0.1 ml of undiluted sample was transferred to a 20 ml headspace vial (SPME).

*Orange juice.* 10 ml of undiluted orange juice was transferred to a 10ml headspace vial. The sample was

spiked with 10 ug/l (Restek #32032 Pesticide Evaluation Mix). A Twister was added and the sample stirred at room temperature for 90 minutes. After extraction the Twister was removed, rinsed, dried and placed into a thermal desorption tube for analysis (SBSE).

*Retention Time Locking.* Identification of the flavors and pesticides was performed automatically using the Agilent Screener software in combination with the Agilent Retention Time Locking (RTL) library for flavors. For comparison purposes, mass spectral identifications were carried out using the Wiley 138 mass spectral library.

The locking compound used for the flavors was hexadecane, the inlet pressure was adjusted to give a retention time of 31.535 minutes.

The locking compound used for the pesticides was Aldrin, the inlet pressure was adjusted to give a retention time of 18.530 minutes.

## RESULTS AND DISCUSSION

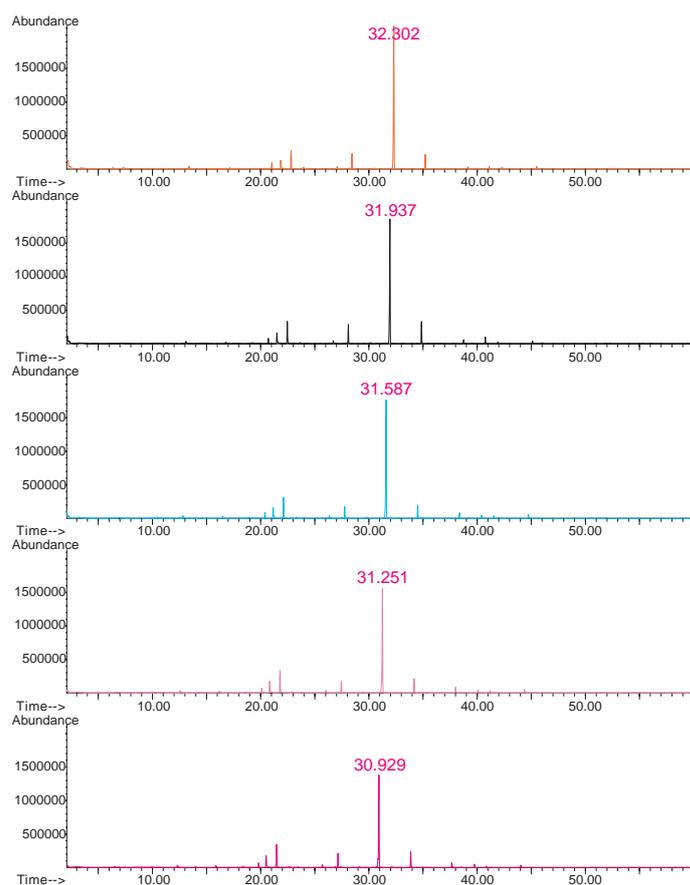
The experimental conditions used in this study are similar to the ones used by David et al [1]. In their study, they replicated the parameters used by Adams [2] in which he publishes retention indices databases useful in the flavor industry. Since all the experimental conditions are the same, the spectra and retention data are valid for all studies. In order to use the RTL flavor database, we decided to use hexadecane to lock our GC/MS method.



**Figure 1.** GC/MS system used in the study. SPME autosampler coupled with a septumless head in the front injector and Thermal Desorption Autosampler coupled with a PTV injector in the back injector.

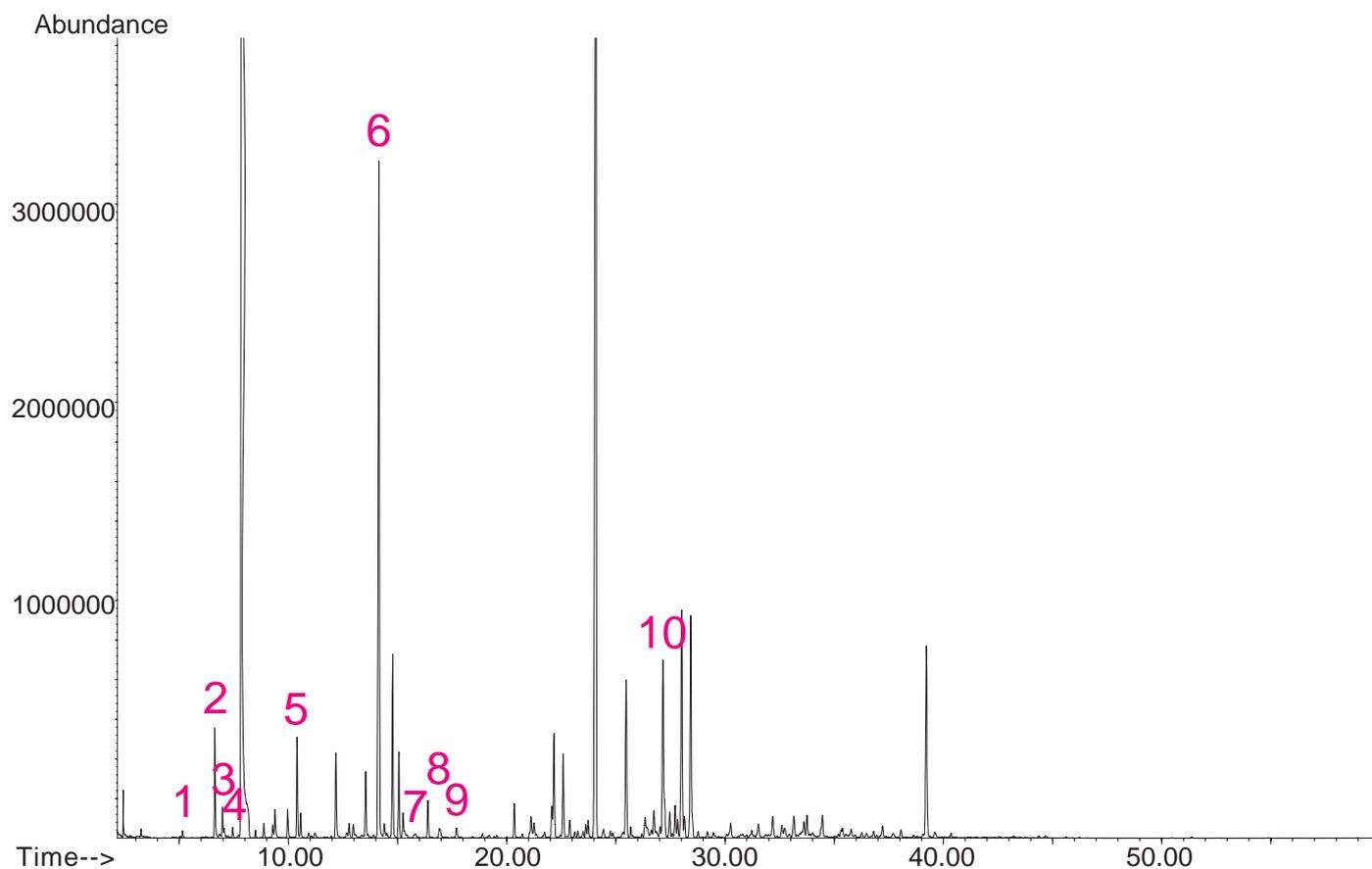
Hexadecane is part of the 400 compounds included in the flavor RTL database with an elution time of 31.535 minutes. Our system (Figure 1) included a programmable temperature vaporizer (PTV) inlet that includes a septumless head injector (CIS 4, Gerstel). The GERS-TEL septumless head (SLH) on the CIS 4 eliminates the problem of inlet septum coring common to most inlets used with the blunt-tipped SPME needles.

After some preliminary runs, the method was locked and 5 runs were automatically created using Agilent's ChemStation data acquisition software (version DA.00.01, Agilent Technologies). Figure 2 shows the 5 runs used for locking the flavor method. The RTL algorithm creates an RTL curve using the inlet pressure and the locking compound retention time. In this study, the measured retention time was 31.587 minutes and the locked retention time with a constant pressure of 10.42 psi was 31.535 minutes (obtained from the RTL database).



**Figure 2.** Retention time locking setup using hexadecane as the locking compound. The 5 individual runs were created automatically by using the Agilent's RTL method acquisition software.

We decided to examine a grapefruit juice sample (purchased at a local store) using solid phase microextraction. Figure 3 shows the TIC obtained after sampling the grapefruit juice headspace with a PDMS SMPE fiber. Table 1 shows the identities obtained for some compounds using Wiley 138 mass spectral database and the flavor RTL database.

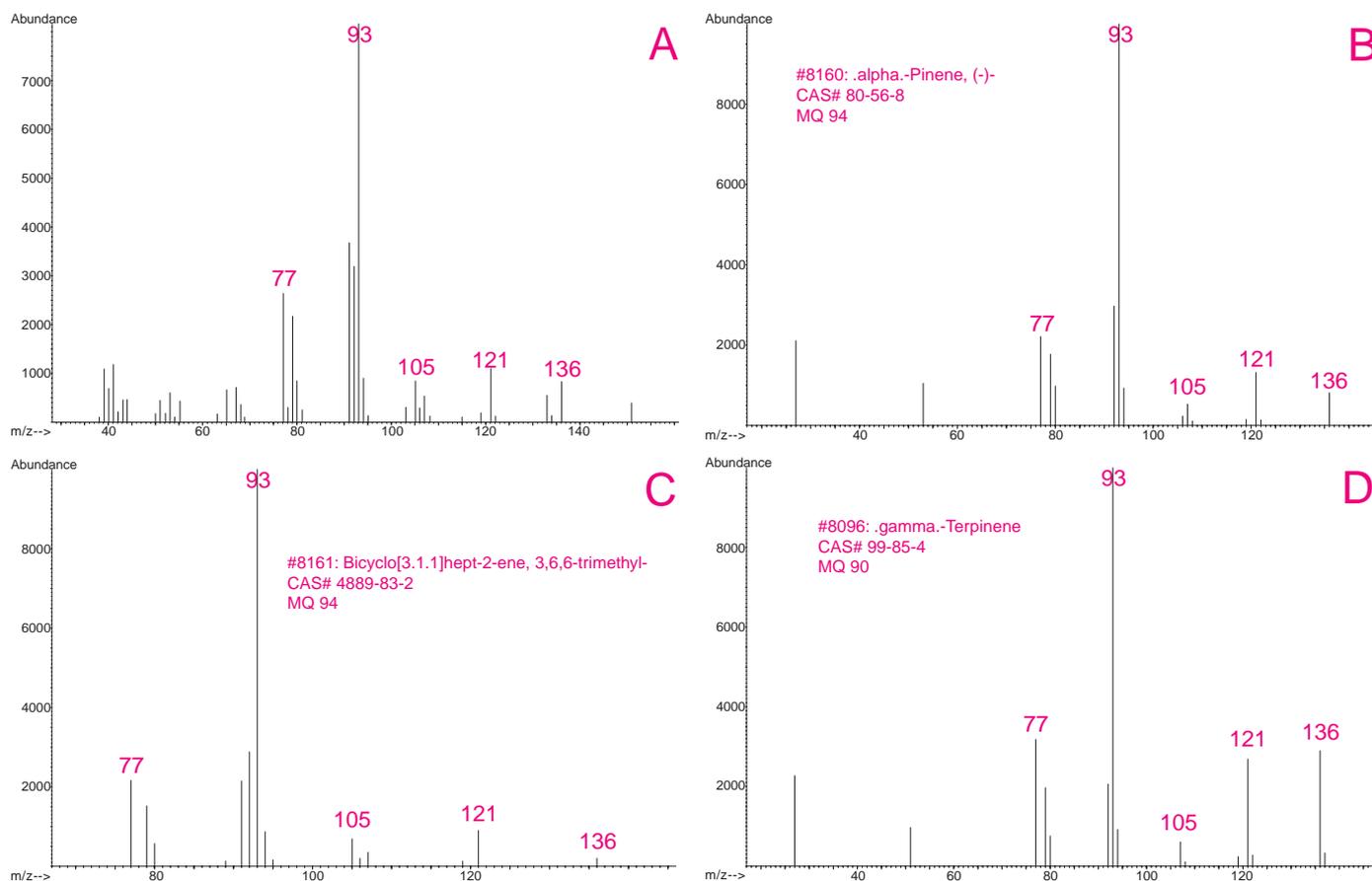


**Figure 3.** Total ion chromatogram obtained after sampling the headspace above 100  $\mu$ L of grapefruit juice with a SPME fiber using an automated sampler. Data was acquired under locked conditions using hexadecane as the locking compound.

**Table 1.** Identification of some compounds by sampling grapefruit juice with SPME fiber. If more than one match was found with the Wiley library with a quality match of over 90% an \* was placed beside the match.

Peak #	Compound	Wiley	RTL Screener
1	$\alpha$ -Pinene	X *	X
2	Myrcene	X	X
3	$\delta$ -3-Carene	X *	X
4	Cymene	X *	X
5	Linalool	X *	X
6	$\alpha$ -Terpineol	X	X
7	cis-Geraniol	X *	X
8	Carvone	X *	X
9	Geraniol	X *	X
10	Valencene	X *	X

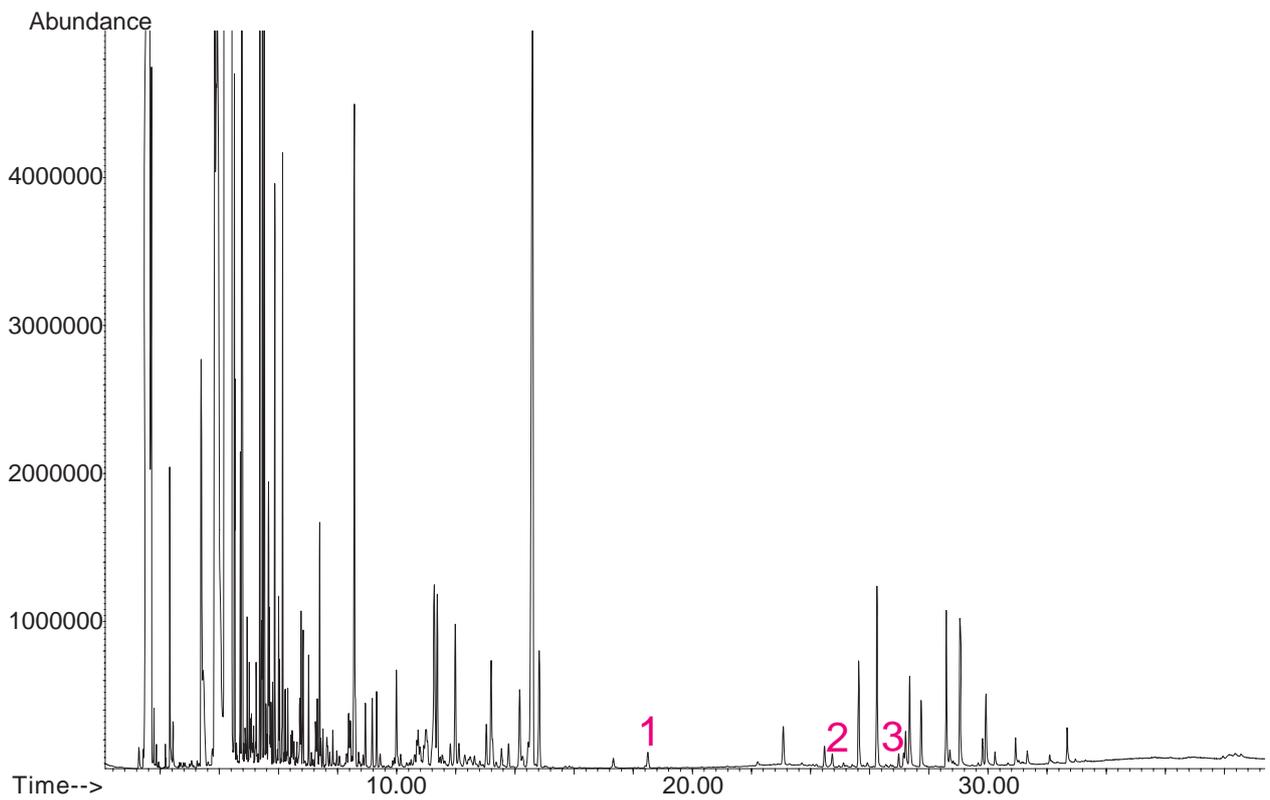
Inspection of Table 1 indicates that the additional information obtained while running the juice sample under locked conditions is complementing the mass spectral information. For example,  $\alpha$ -pinene was easily identified using the RTL database while the Wiley database gave three matches all with match qualities of over 90%. Figure 4 shows the library matches for the peak eluting at 5.14 minutes in the grapefruit juice. It can be seen in this figure that the mass spectra profiles are very similar for all the possible matches. In this particular case, the information provided by using the RTL screener clearly identifies the peak eluting at 5.14 minutes as  $\alpha$ -pinene.



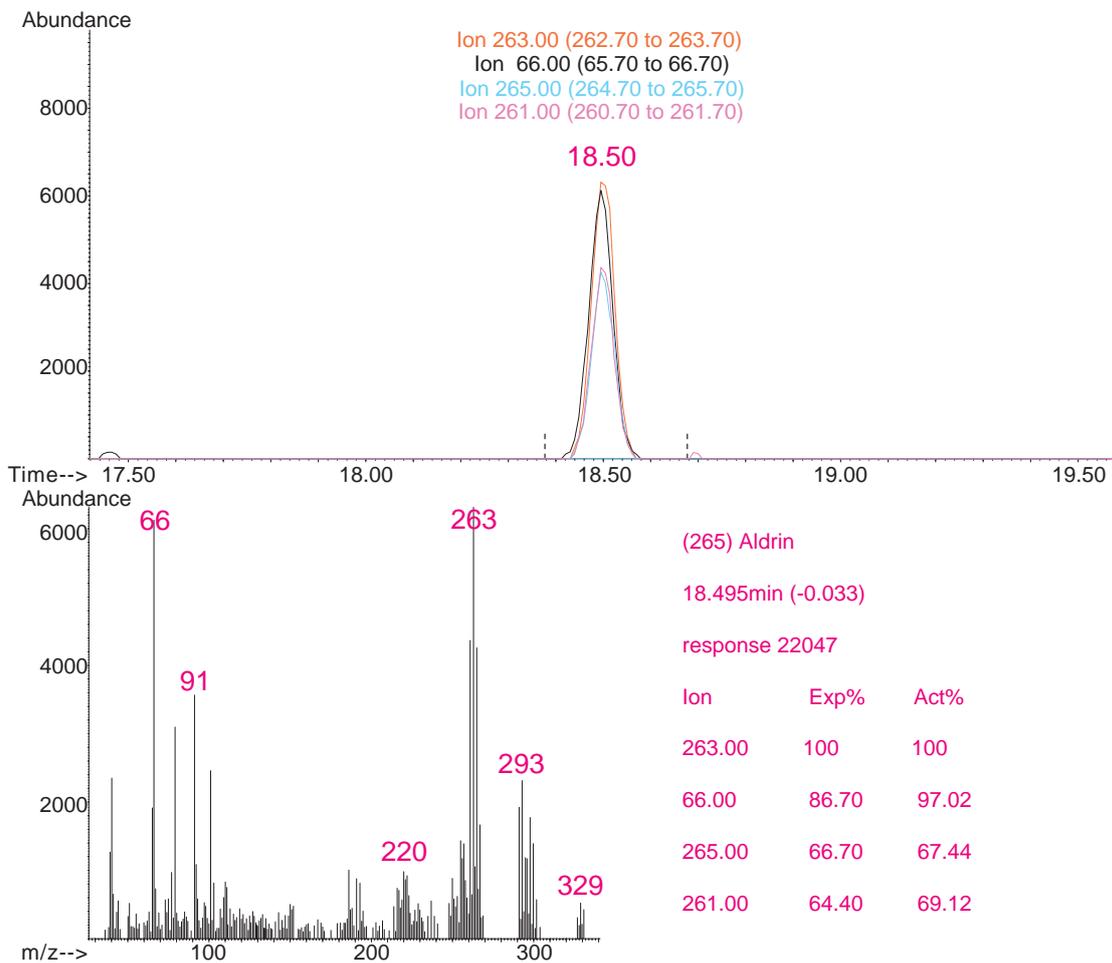
**Figure 4.** Possible library matches using Wiley 138 for peak eluting at 5.14 minutes from TIC of Figure 3. A) Scan of peak eluting at 5.14 min in grapefruit juice; B) Wiley 138 library match #1; C) Wiley 138 library match #2; D) Wiley library match #3. [Note: By using the RTL database, it was concluded that the peak eluting at 5.14 min was  $\alpha$ -pinene (spectrum displayed in B)]

We also decided to inspect the pesticide RTL database [3] using a GC/MS method. For this example, we inspected an orange juice sample spiked with pesticides. As seen in Figure 5, the combination of stir bar sorptive extraction (SBSE) coupled with a PTV and

thermal desorption system can provide a complex total-ion-chromatogram profile. It can also be seen in this figure that even at 10 ppb levels the pesticides were detected and screened with the RTL pesticide database (Figure 6).



**Figure 5.** Total ion chromatogram obtained after sampling spiked-orange juice with SBSE. The pesticides were spiked at 10 ppb levels. Peak identities are 1) Aldrin; 2) Endrin; 3) p,p' DDT.



**Figure 6.** Identification of Aldrin by the RTL screener in spiked orange juice.

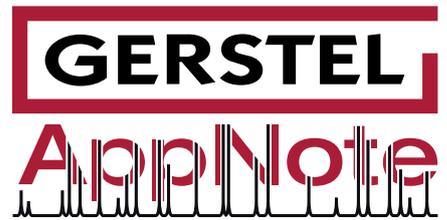
## CONCLUSIONS

By using enrichment techniques such as SPME and/or SBSE flavor constituents were easily detected using a GC/MS. Also, the use of an RTL method added meaningful retention time information supporting analytes identification.

The use of automated SPME or SBSE is compatible with RTL databases and does not adversely affect the ability to lock methods. The overall result of the combination of hardware and software created a powerful tool in screening flavor compounds quickly and accurately.

## REFERENCES

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- [3] Sandra, P et al. J. Chromatogr. A. 1000 (2003) 299-309.



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