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Accurate Mass Retention Time Locked Flavor Database by GC/Q-TOF

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Introduction

Companies in the flavor and fragrance industry often use proprietary GC/MS databases based on historical methods for research and quality control. Fortunately, many of their MS based libraries are built upon common non-polar columns and so their retention index information can be converted into an absolute retention time.

Although quadrupole based GC/MS is a powerful technique in the flavor and fragrance industry, it is not optimal for identifying new flavorants. The use of an accurate mass instrument with MS/MS capabilities allows for structure elucidation of novel flavorants in the part per million concentration range without the need for isolating compounds.

Using retention time locking, it is no longer necessary to calculate the retention index, but the absolute retention time can be used as an identification tool. Of course, retention times are still dependent on operating conditions, but small differences in carrier gas velocity and column length are compensated by re-locking the GC method by adjustment of the column head pressure. After re-locking, elution temperatures of the solutes are also constant. Retention time locking and retention time databases are excellent tools in essential oil and in flavor QA/QC analysis. Incorporating both accurate mass information and absolute retention times are useful in the identification of isomeric flavorants.

Methods

Sample Preparation

This database was developed on analytical standards run either in split mode or using Solid Phase Micro Extraction (SPME). The standards were originally made in either ethanol or hexanes at 1 mg/mL concentration dependent on solubility. The standards were diluted down to 10 ppm concentration in hexanes. Further dilutions were also made dependent on ionization efficiency of the specific flavorant.

Volatile samples were prepared in 20-mL headspace vials. HS-SPME was performed using a conditioned 50/30 µm DVB/Carboxen/PDMS StableFlex SPME fiber. Semi-volatile compounds were injected using a split/splitless inlet run in split mode. The Retention Time Locked (RTL) GC method was utilized for separation of flavor compounds to construct a targeted flavor database. The spectra were acquired in full spectrum acquisition mode.

Methods

Analytical Conditions

This study was performed using an Agilent 7890 GC coupled to an Agilent 7200 series Quadrupole-Time-of-Flight (Figure 1). GC and MS conditions are described in Table 1. A CTC-PAL was installed for the headspace SPME samples.

The SPME samples were equilibrated at 40 °C for 3 min with 500 rpm agitation. The fiber was then lowered into the headspace and the samples were extracted for 3 min before being desorbed in the inlet for 3 min. After 1 minute the purge flow flushed out the inlet. The fiber was left in the inlet for 3 additional minutes until the gas saver was turned on and the fiber was retracted.

The split injections were done using the same inlet and oven conditions to minimize the impact of the different injection techniques.

Figure 1. 7200 series GC/Q-TOF system.

GC and MS Conditions:	
Column	HP-5 MS, 30 meter, 0.25 mm ID, 0.25 µm film
Injection volume	1:10 Split or SPME
Purge to Split Vent	60 mL/min at 1 min
Split/Splitless inlet temperature	300 °C
Oven temperature program	40 °C for 10 min 4 °C/min to 140 °C, 0 min hold 10 °C/min to 200 °C, 0 min hold 50 °C/min to 300 °C, 2 min hold 50 °C/min to 325 °C, 0.5 min hold
Carrier gas	Helium at 1.2 mL/min constant flow
Transfer line temperature	300 °C
Ionization mode	EI
Source temperature	230°C
Quadrupole temperature	150°C
Scan range	40 to 800 m/z
Spectral acquisition rate	5 Hz, collecting both in centroid and profile modes

Table 1. GC-MS conditions used in the study.

Results and Discussion

Chromatogram Deconvolution

The data was processed by chromatogram deconvolution using the Find by Chromatographic Deconvolution tool of MassHunter Qualitative software. Chromatogram deconvolution was able to extract clean spectra from background noise based on both retention time and peak shape. The compounds could then be searched against a unit mass database, generating a Match Factor score comparable to the score generated by NIST.

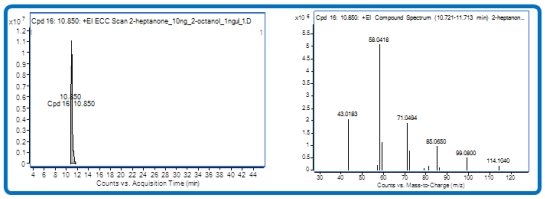

Figure 2. Find by Chromatographic Deconvolution tool was used to extract spectra.

Find by Formula and Fragment Formula Annotation

The Find by Formula and Fragment Formula Annotation tools were then used to automatically identify fragments of the compound identified by library search. In Find by Formula, a match score was generated based on abundance ratios, isotope spacing and isotope m/z match.

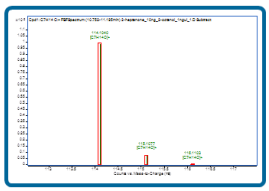

Figure 3. Find By Formula results for 2-heptanone. The match score is 99.59; it is based on abundance ratios, isotope spacing and isotope m/z match. These ions are compatible with the formula $C_7H_{14}O$, Moreover, the mass difference is only 0.73 ppm.

Library Editor

The Library Editor can read commercial EI spectral libraries. Currently the editor considers the standard *.L format databases as compressed and cannot write to that format. However it is possible to import custom library data in JCAMP formats. GC/MS ChemStation can create the JCAMP format files and LIB2NIST program can create the HPJ format. There is also a script to automatically import structures (.MOL files) from the ChemSpider web site.

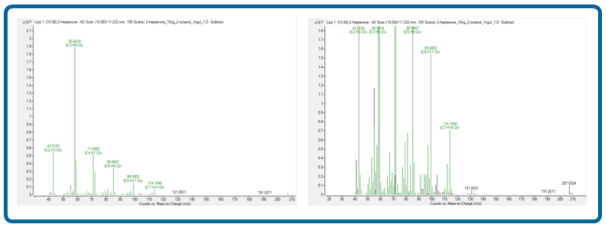

Figure 4. A fragment is annotated and colored green when the formula of the fragment is a subset of the molecular formula identified by a library search. This is useful for rapid confirmation of the compound. Both spectra in this figure are the same, the one on the right is expanded in order to show low-abundance fragments that are not compatible with the $C_7H_{14}O$ structure. These ions can be manually filtered out in the Library Editor.

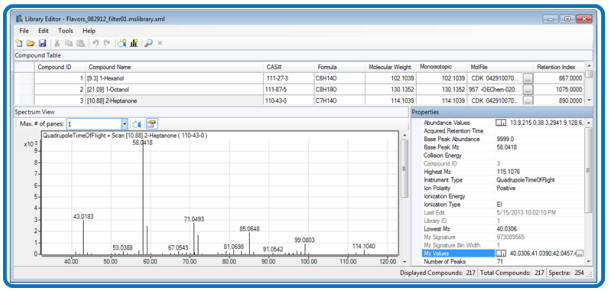

Figure 5. Library Editor saves data in either binary or XML formats. It has a full featured search function and can include alternative names, boiling point, melting point, and user defined features. The spectra shown above has not been filtered and still contains matrix ions.

It is also possible to import spectra from the Find by Chromatographic Deconvolution and the Find by Integration algorithms. It would be useful to include spectra generated by these algorithms in the database if these techniques are frequently used for library searching.

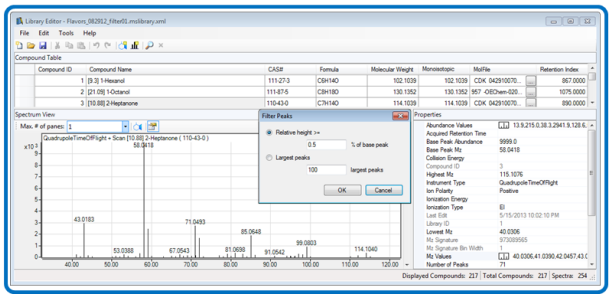

Figure 6. Library curation with relative height and peak filters.

Results and Discussion

Clearly one of the first steps in creating a high quality accurate mass database is to know which ions are not compatible with the structure. Most of the outliers identified by Fragment Formula Annotation are small and can be readily filtered out with judicious relative height and peak filters. Especially with background subtracted spectra, manual curation should be considered if there are significant amounts of noise present even when a filter as large as 1 % of the base peak has already been applied.

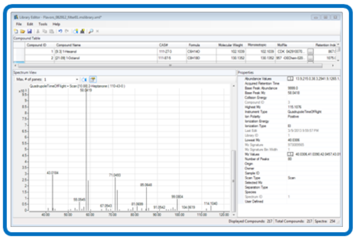

Figure 7. The spectra can also be manually curated. This is done by clicking on the m/z value portion of the properties window which opens up a spectrum edit window.

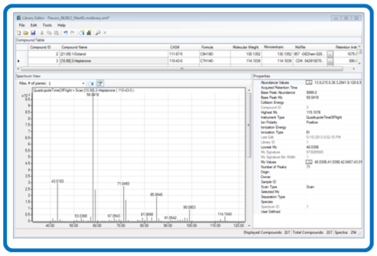

Figure 8. The ions that the Molecular Formula Generator and Fragment Formula Annotation identified as not compatible with the identified compound formula can be easily removed. In the future there will be a Personal Compound Database Library option that automatically filters the spectra and convert the accurate mass results into exact mass.

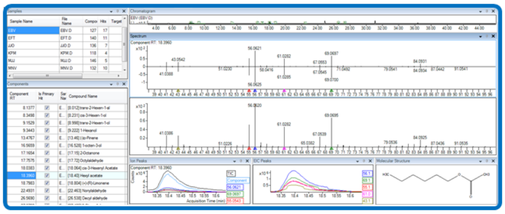

Figure 9. The constructed accurate mass flavor library containing over 200 compounds was tested against an extra virgin olive oil samples. A number of flavor compound library hits were found in each sample. Example shows hexyl acetate hit, a compound responsible for fruity notes in olive oil.

Absolute Retention Time

Now that we have demonstrated that it is straight forward to add accurate mass spectra libraries to existing flavor databases we want to demonstrate that Retention Time Locking can be used to generate Absolute Retention Times from existing RI libraries.

$$[RI - (Z \times 100)]/100 \times (RT_{z+1} - RT_Z)] + RT_z = RT_x$$

RI = retention index

Z = carbon number of preceding n-alkane

RT_{z+1} = Retention time of following n-alkane

RT_z = Retention time of preceding n-alkane

RT_x = Calculated retention time

Figure 10. With modern pneumatics and precision GC oven temperature control, it is straight forward to automatically convert Retention Index methods to Retention Time Locked methods. We can then apply this formula to generate Absolute Retention Times for compounds that are no longer available.

Name	RI	RT Cal	Min	Sec
[11.601] Heptaldehyde	902	11.6216	0.0206	1.236
[11.608] 2-Heptanol	902	11.6216	0.0136	0.816
[11.663] Ethyl valerate	904	11.7432	0.0802	4.812
[11.923] Furfuryl mercaptan	909	12.0472	0.1242	7.452
[12.045] 2,5-Dimethylpyrazine	909	12.0472	0.0022	0.132
[12.11] 2-Furyl methyl ketone	910	12.108	0.002	0.12
[12.123] Oxolan-2-one	912	12.2296	0.1066	6.396
[12.129] 2,6-Dimethylpyrazine	912	12.2296	0.1006	6.036
[12.385] Anisole	916	12.4728	0.0878	5.268
[12.505] 2,3-Dimethylpyrazine	918	12.5944	0.0894	5.364
[12.618] Pentyl acetate	920	12.716	0.098	5.88
[13.263] Methyl hexanoate	929	13.2632	0.0002	0.012
[13.328] 4,5-Dimethylthiazole	930	13.324	0.004	0.24
[13.46] (-)a-Pinene	932	13.4436	0.0144	0.864
[13.375] 2-Vinylpyrazine	932	13.4436	0.0706	4.236
[13.823] Ethy 3-hydroxybutyrate	938	13.8104	0.0126	0.756
[14.233] Camphene	946	14.2968	0.0638	3.828

Name	RI	RT Cal	Min	Sec
[14.92] 2-Ethoxythiazole	956	14.9048	0.0152	0.912
[14.907] γ-Valerolactone	956	14.9048	0.0022	0.132
[15.0875] trans-2-Heptenal	959	15.0872	0.0002	0.012
[15.102] Benzaldehyde	960	15.148	0.046	2.76
[15.96] ß-Pinene	973	15.9384	0.0216	1.296
[15.883] 2-Methoxy-3-methylpyrazine	973	15.9384	0.0584	3.504
[16.08] 1-Heptanol	976	16.1208	0.0408	2.448
[16.397] 1-Octen-3-one	981	16.4248	0.0278	1.668
[16.528] 1-octen-3-ol	983	16.5464	0.0184	1.104
[16.785] Phenol	988	16.8504	0.0654	3.924
[16.8] 2-Methoxy-5-methylpyrazine	988	16.8504	0.0504	3.024
[16.915] 3-Octanone	989	16.9112	0.0038	0.228
[17.15] 2-Octanone	993	17.1544	0.0044	0.264
[17.146] 2,4,5-Trimethylthiazole	993	17.1544	0.0084	0.504
[17.332] 2-Ethyl-6-methylpyrazine	996	17.3368	0.0048	0.288
[17.40] 3-Octanol	997	17.3976	0.0024	0.144

Table 2. Deviations are small (~ 2 s) between calculated and experimentally determined retention times for flavorants. The differences would be larger if the RI values were calculated with a method that differed substantially from the experimental method.

Conclusions

We have constructed a GC/Q-TOF library that currently contains about 200 flavor compounds with accurate mass information for all the major fragments. The core of the library is comprised of the wine volatiles with many heterocycles such as pyrazines, furanones, and lactones added to provide nut, roasted, and dairy flavors. We used tools such as the Find By Formula and Fragment Formula Annotation in combination with library editing functions to create clean, accurate mass EI spectral library entries that can be incorporated in proprietary EI based GC/MS databases. This demonstrates the use of combining accurate mass EI database and retention time locking for the identification of isobaric compounds.