# Discovering the Scent of Celery: HS-SPME, GC-TOFMS, and Retention Indices for the Characterization of Volatiles

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1. Introduction

Determining and identifying individual analytes within a complex sample can provide important characterization information for better understanding of a sample, product, or process. This type of exploratory analysis is readily accomplished with GC-TOFMS, as it provides non-targeted data with every acquisition. The Pegasus BT adds to the chromatographic separation by taking advantage of the full mass range data with mathematical deconvolution to further separate chromatographic coelutions. These capabilities provide excellent information about a sample and the ChromaTOF® brand software contains tools to help streamline data analysis. For example, retention index methods can be created to calculate and compare retention index information for each peak in the table to support or improve library identifications. Here, we analyze a celery sample with HS-SPME coupled to GC-TOFMS. The resulting data are reviewed, with retention index information providing valuable support to analyte identifications. These instrument capabilities and software tools allow the analyst to see more analytes, have more confidence, and uncover what they might be missing.
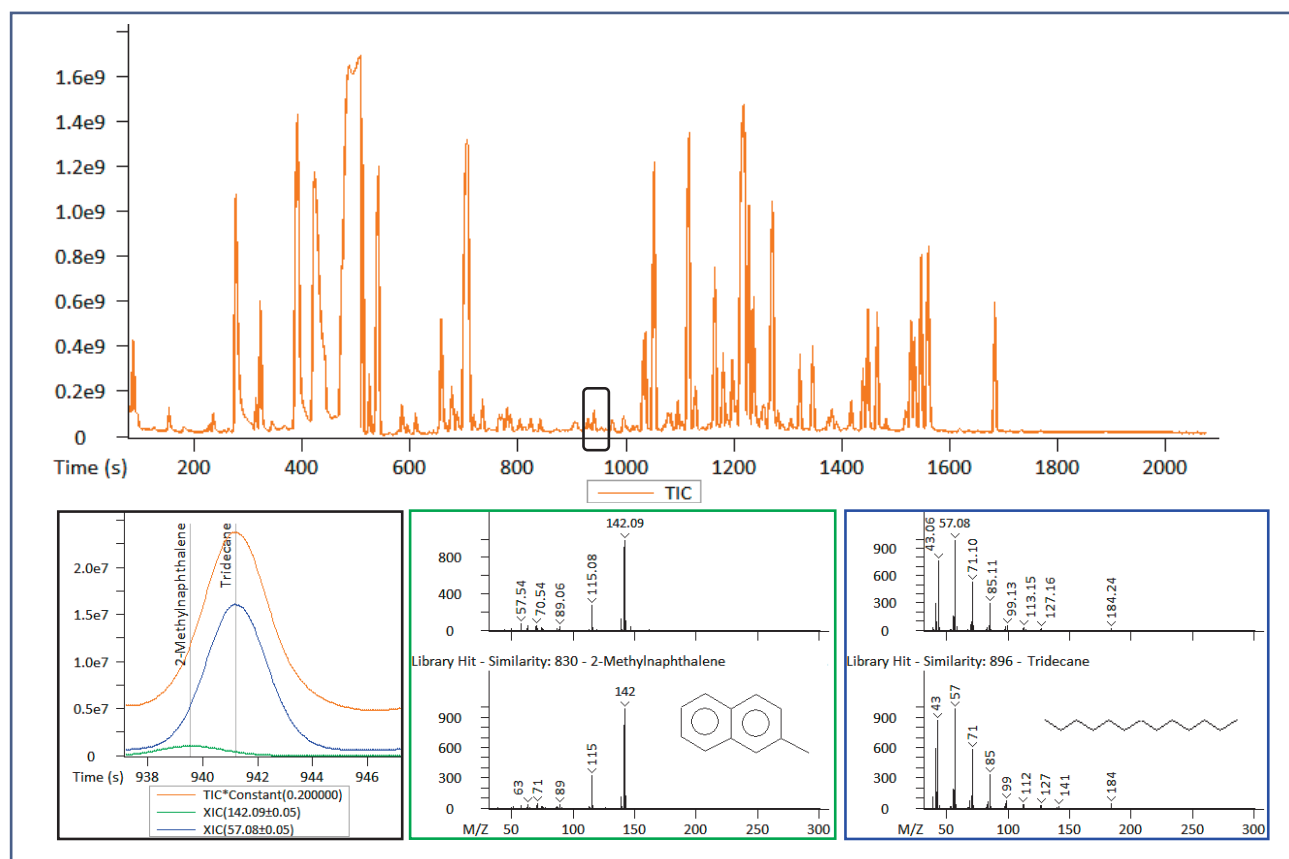
***Figure 1.** The Total ion chromatogram (TIC) for a celery sample is shown. One example of coelution is highlighted. Analytes tentatively identified as 2-methyl naphthalene and tridecane appear as a single peak in the TIC (orange trace, scaled to 20% for visualization), but are distinguishable with extracted ion chromatograms (XICs) (142.09, green and 57.08, blue). Automated processing and deconvolution determined both with their Peak True (deconvoluted) and library hit spectra shown.*
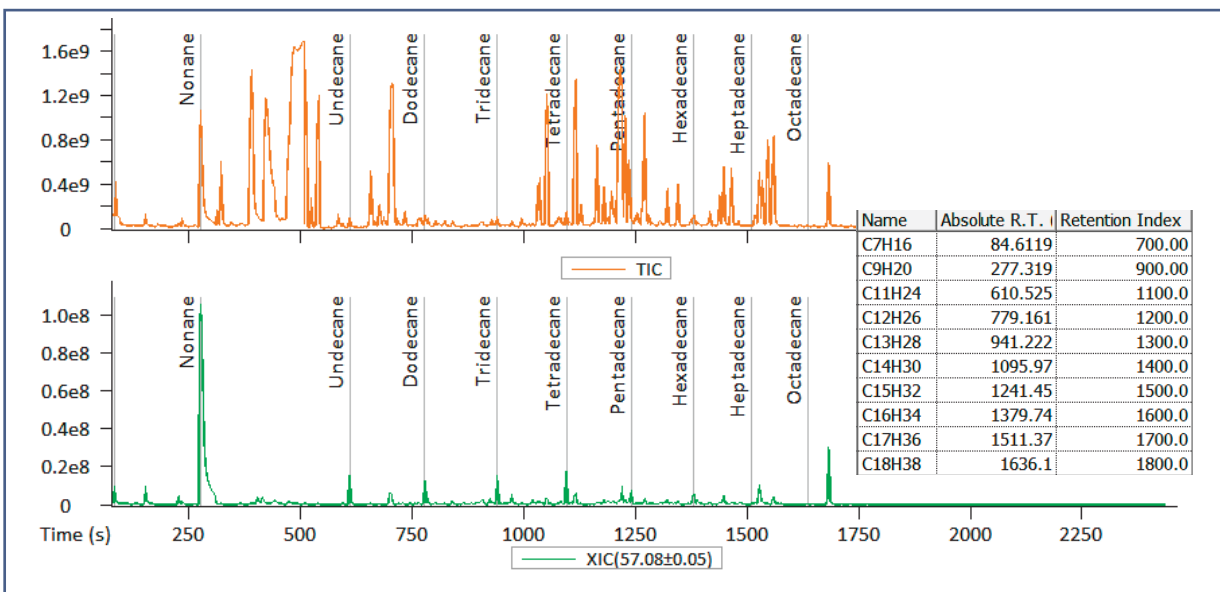
2. Experimental

A fresh celery sample was analyzed by HS-SPME and GC-TOFMS. Approximately 4 g of diced celery were placed in a 20 mL glass vial which was then incubated for 5 min at 50 °C. Extraction was performed with a DVB/CAR/PDMS fiber (Supelco) for 30 min also at 50 °C. GC-TOFMS instrument conditions are listed in Table 1.

Table 1. GC-TOFMS *(Pegasus* **BT) Conditions**

Gas Chromatograph	Agilent 7890 with LECO L-PAL3 Autosampler
Injection	2 min desorption in 250 °C inlet, splitless
Carrier Gas	He @ 1.0 mL/min
Column	Rxi-5ms, 30 m x 0.25 mm i.d. x 0.25 μm coating (Restek)
Oven Program	40 °C (2 min), ramp 5 °C/min to 200 °C, ramp 10 °C/min to 300 °C (1 min)
Transfer Line	250 °C
Mass Spectrometer	**LECO *Pegasus* BT**
Ion Source Temperature	250 °C
Mass Range	35-650 m/z
Acquisition Rate	10 spectra/s

3. Results and Discussion

A representative TIC chromatogram for the complex celery sample is shown in Figure 1. This sample contained hundreds of peaks and a specific coelution example is highlighted to show the added separation of deconvolution on top of the chromatographic separation. In this case, 2-methyl naphthalene (with sweet, floral, and woody odor properties) chromatographically overlapped with an alkane (tridecane). Automated processing successfully determined the presence of both analytes. The chromatographic profiles can be observed by plotting XICs unique to each. Deconvolution also provided pure mass spectral information for each analyte to search against library databases for tentative identifications. The known retention index information (1298 for 2-methyl naphthalene and 1300 for tridecane[1]) further supported these identifications. Retention index information is valuable in supporting or improving library identifications, and the *ChromaTOF* software can be used to calculate the experimentally observed values for all peaks in a sample. A set of known analytes are specified to connect the observed retention times to known retention index values and to build a retention index equation. Alkane standards are often run for this purpose. In the absence of this ladder, retention information can be determined with naturally present peaks. Here, enough alkanes were observed within the sample to build the retention index method, as shown in Figure 2.

Name	Absolute R.T.	Retention Index
C7H16	84.6119	700.00
C9H20	277.319	900.00
C11H24	610.525	1100.0
C12H26	779.161	1200.0
C13H28	941.222	1300.0
C14H30	1095.97	1400.0
C15H32	1241.45	1500.0
C16H34	1379.74	1600.0
C17H36	1511.37	1700.0
C18H38	1636.1	1800.0

Figure 2. Several alkanes were observed within the data and are shown with peak markers in the TIC and XIC 57.08. These were not spiked into the sample as standards, but naturally provided an alkane ladder to determine retention index information for the other peaks in the sample. A retention index method was built within the software and applied through automated processing.

The retention index method was applied to the sample to determine experimental retention index information for all peaks. These were compared to the known semi-standard non-polar retention index values from the NIST database.[1] A collection of representative analytes are shown in Table 2, including alkanes, esters, aldehdyes, ketones, terpenes, terpenoids, aromatics, alcohols, and phthalates. These analytes, along with the hundreds of others present in the data, provide good characterization information about this sample. Of note, both diethyl phthalate and dibutyl phthalate were observed in these data. And, two analytes with strong celery odor characteristics were also observed; 3-n-butylphthalide and n-butylidene phthalide.

Table 2. Representative Analytes

Peak #	Name	CAS	Similarity	R.T. (s)	Observed RI	Known RI*	% Difference	Odor Type
1	α-thujene	2867-05-2	939	314.719	922.4	929	-0.71	woody
2	α-pinene	80-56-8	941	322.867	927.3	937	-1.04	herbal
3	ß-pinene	127-91-3	929	391.591	968.6	979	-1.06	herbal
4	3-octanone	106-68-3	859	415.09	982.7	986	-0.33	herbal
5	ß-myrcene	123-35-3	852	423.595	987.8	991	-0.32	spicy
6	limonene	138-86-3	902	507.239	1038	1030	0.78	citrus
7	α-ocimene	502-99-8	935	524.87	1048.6	1047	0.15	fruity
8	γ-terpinene	99-85-4	931	542.764	1059.3	1060	-0.07	terpenic
9	acetophenone	98-86-2	910	547.236	1062	1065	-0.28	floral
10	1-octanol	111-87-5	908	562.372	1071.1	1071	0.01	waxy
11	nonanal	124-19-6	885	615.449	1102.9	1104	-0.10	aldehydic
12	naphthalene	91-20-3	947	734.675	1173.6	1182	-0.71	pungent
13	trans-carveol	1197-07-5	884	803.772	1215.2	1217	-0.15	spicy
14	n-hexyl 2-methyl butyrate	10032-15-2	846	839.642	1237.3	1236	0.11	green
15	D-carvone	2244-16-8	877	841.948	1238.7	1246	-0.59	minty
16	2-methylnaphthalene	91-57-6	830	939.554	1299	1298	0.08	floral
17	tridecane	629-50-5	896	941.222	1300	1300	0.00	
18	copaene	3856-25-5	934	1052.76	1372.1	1376	-0.28	woody
19	caryophyllene	87-44-5	904	1116.75	1414.3	1419	-0.33	spicy
20	humulene	6753-98-6	935	1164.6	1447.2	1454	-0.47	woody
21	trans-ß-farnesene	18794-84-8	934	1180.05	1457.8	1457	0.05	woody
22	α-selinene	473-13-2	932	1227.73	1490.6	1494	-0.23	amber
23	caryophyllene oxide	1139-30-6	905	1345.89	1575.5	1581	-0.35	woody
24	diethyl phthalate	84-66-2	863	1367.03	1590.8	1594	-0.20	phthalate
25	3-n-butylphthalide	6066-49-5	938	1439	1645	1656	-0.66	herbal, celery
26	n-butylidene phthalide	551-08-6	877	1465.36	1665	1675	-0.60	herbal, celery
27	cadalene	483-78-3	836	1469.98	1668.6	1674	-0.32	
28	dibutyl phthalate	84-74-2	906	1820.9	1948.2	1965	-0.85	phthalate

*Semi-standard non-polar from NIST database[1]

Retention index information supported the identification for many of these analytes and improved the identifications for others. For example, the #1 library hit for Peak #1 was for β-thujene, shown in Figure 3. Retention index information suggested that α-thujene (hit #2) was a better identification with 0.7% error instead of 4.5% error. The library spectra are very similar for these two analytes with good similarity scores for both. Changing to the second hit changed the odor properties though, and provided a better understanding of the sample.
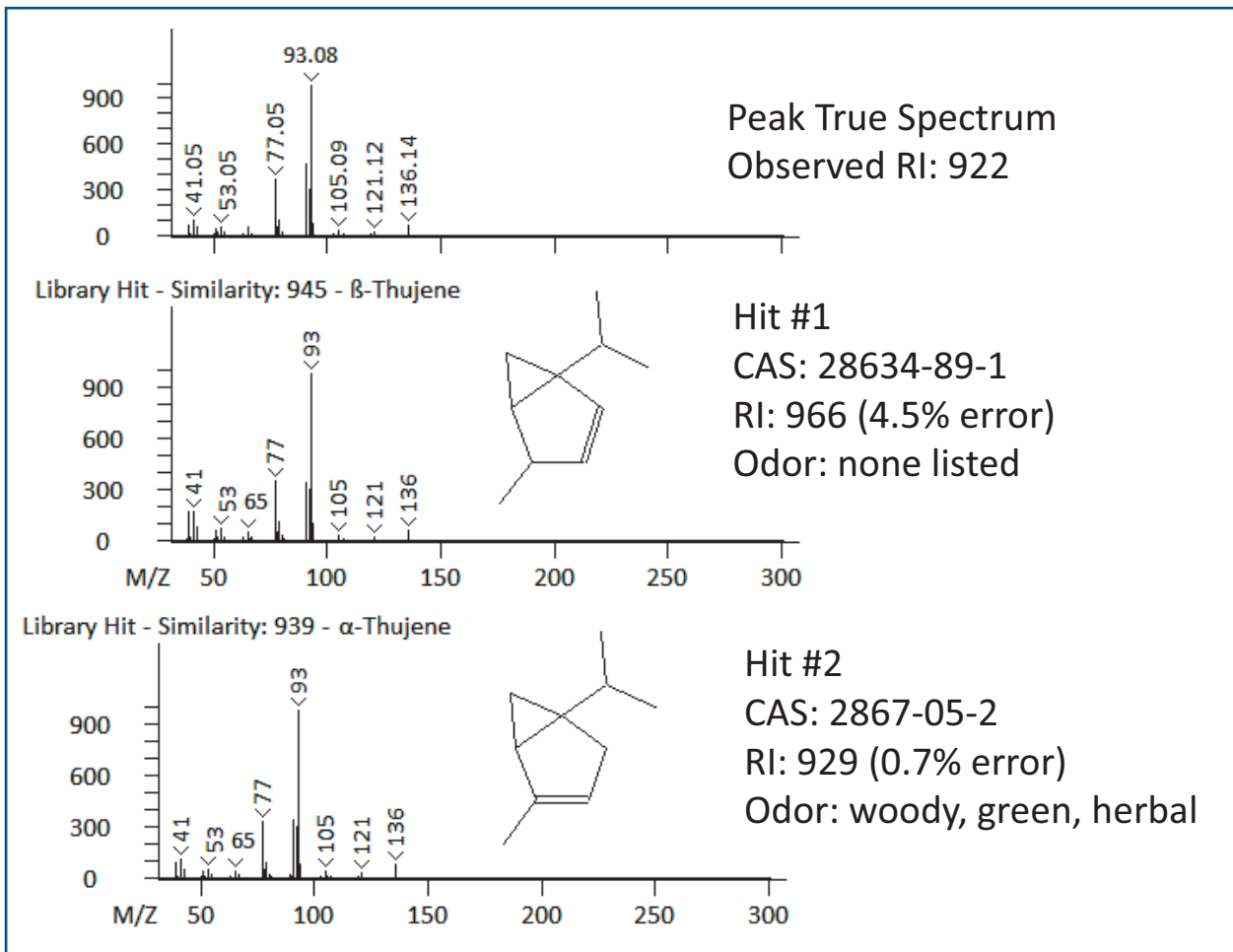
Figure 3. Retention information improved the tentative identifications of Peak #1 in Table 2.

Additional examples from Table 2 are highlighted in Figures 4 and 5 to demonstrate cases of deconvolution. Peaks #14 and 15 from Table 2 are shown in Figure 4. Deconvolution provided library searchable spectra with retention index supporting the tentative identifications. An ester and a terpenoid, with green and minty odor characteristics, respectively, were mathematically separated. Another example of closely eluting peaks is shown in Figure 5, with peaks #26 and 27 from Table 2. There is a peak buried in the tail of a large, much more concentrated peak, but the benefits of deconvolution still allowed these analytes to be tentatively identified. n-butylidene phthalide has known celery odor characteristics and is important for understanding this sample.
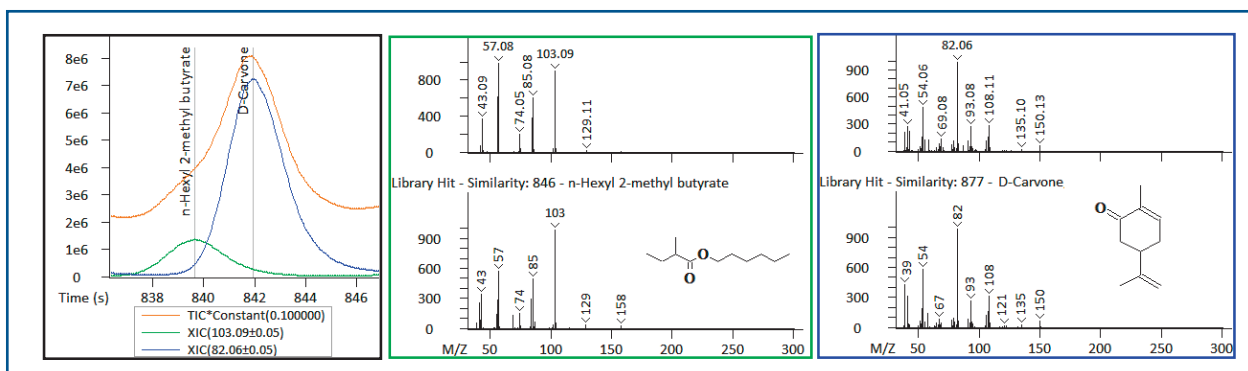

Figure 4. Peaks #14 and 15 from Table 2 are shown. Deconvolution successfully separated these two analytes.
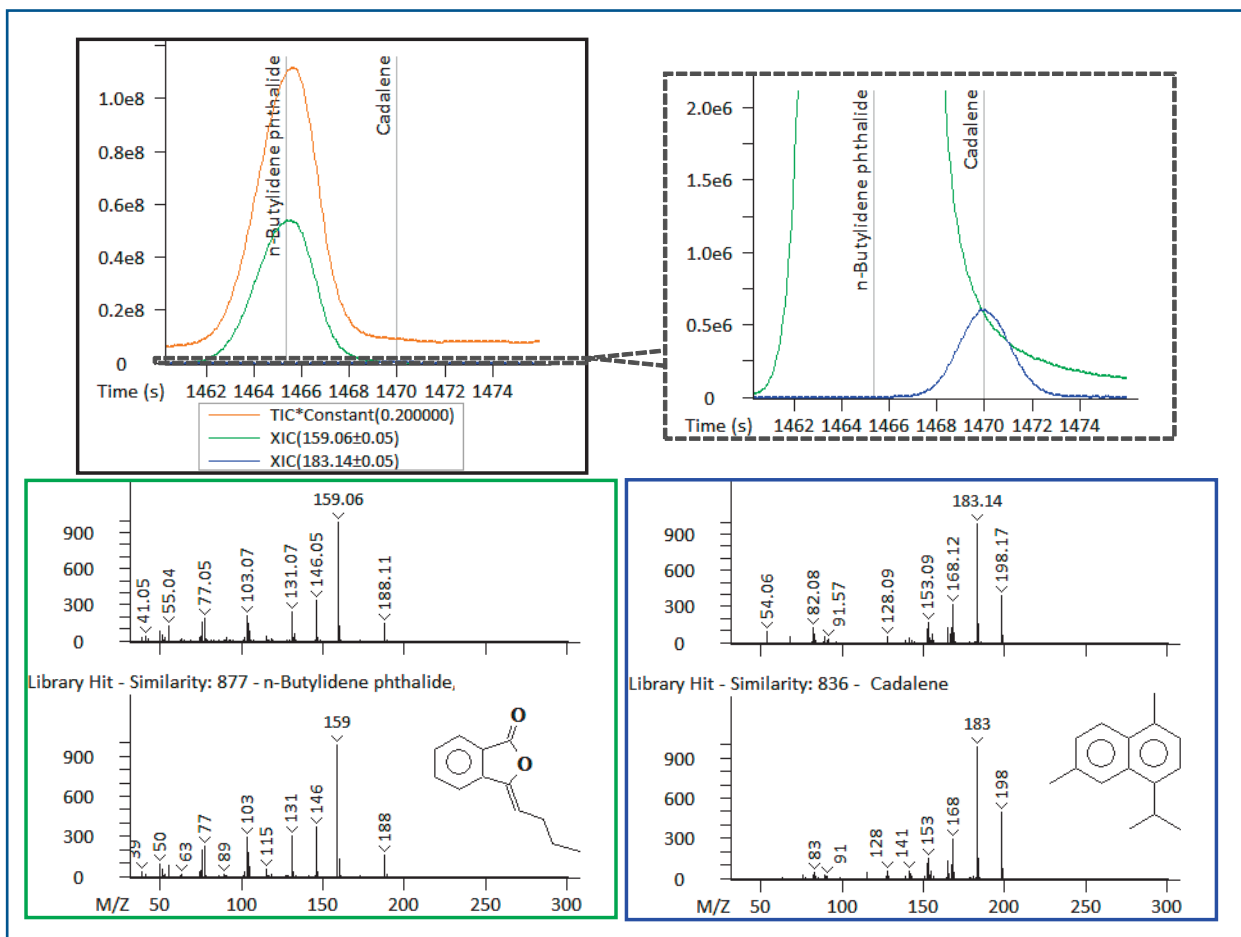
Figure 5. Peaks #26 and 27 from Table 2 are shown. Deconvolution successfully separates these analytes, even with a large concentration difference between the two.

4. Conclusion

The *Pegasus* BT was used to characterize the aroma profile of celery by determining individual analytes. Hundreds of analytes were detected in this complex sample with many contributing important odor characteristics. Deconvolution provided additional separation in instances of chromatographic coelution. Several examples were highlighted and the use of retention index and the associated *ChromaTOF* tools was demonstrated. The hardware and software capabilities allow you to see more in a standard analysis.

5. References

[1]NIST Mass Spectral Search Program for the NIST/EPA/NIH Mass Spectral Library Version 2.2, build Jun 10, 2014

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