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AromaOffice: Application of a Novel Linear Retention Indices Database to a Complex Hop Essential Oil

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Keywords

Hop Essential Oil, GC-MS, Linear Retention Indices, Aroma Office ^{2}D, Automated Cross Searching

Abstract

Aroma Office ^{2}D (Gerstel K.K.) is an integrated software approach for simultaneous processing of both retention index (RI) and mass spectral (MS) data for rapid and improved identification of flavor compounds. The program can be integrated into Agilent Chemstation Software and searches are performed using CAS numbers of candidate compounds after library searching and corresponding automatically generated RI values. When MS signals are too weak to be used the software allows two RI values from orthogonal columns (after GC-O organoleptic evaluation) to be cross searched in the database. This offers a very useful additional identification procedure for flavor compounds. The searchable database comprises > 10,000 compounds and offers the practicing analyst full results oriented software manipulation of RI and MS data on flavor compounds.

Introduction

The retention time of a compound on a given column phase can be expressed on a scale based on n-alkane retention times. This produces unique retention index values for compounds and serves to standardize gas chromatographic retention data. Temperature programmed GC is used widely in laboratories and values calculated using this approach are denoted as linear retention indices or programmed-temperature retention indices. Retention indices are used widely in the flavor and fragrance field and many published data bases are available [1, 2, 3]. Usually mass spectral information in addition to retention time data is available from a GC run. Either information dimension alone can often be insufficient to provide positive identification and an obvious solution here is to combine the unique attributes of both information dimensions to offer more secure identification possibilities.

Modern GC technology can offer very reproducible retention behavior and information rich mass spectral patterns are readily available from affordable benchtop instruments. In this regard data bases are available which list the retention index of compounds and their mass spectra in order of elution.

Aroma Office (Gerstel K.K.) is an integrated software approach to automatically process retention index and mass spectral data for improved identification of flavor compounds. It contains the most comprehensive data base of flavor compounds available. This is a searchable data base with retention index information on >10,000 compounds from greater than 100,000 entries from a wide range of literature references. In addition to RI values the database contains compound names, analytical conditions and literature references.

A novel odor character search is also available for flavor compounds. Various aspects of the software and database will be demonstrated with applications to a complex hop essential oil.

After creation of a suitable alkane retention index file the sample is then run under similar conditions in GC-MS mode. A library search of any compound will now display it's RI value in addition to the normal PBM hit ranking and CAS numbers. For any hit both the RI value and CAS No can be seamlessly transferred to the database for a cross search for positive identification. This cross search can be very important when the PBM hit list is composed of candidates with very similar spectral properties, and an example will be shown where this cross searching rejects a candidate with the top PBM hit score, where this combination of CAS No and RI value fail the data base search. A lower PBM hit was the correct compound. For a positive cross search result the software also displays the difference between the calculated run value and the RI average from the Aroma Office database values, together with formula, analytical conditions, literature references and compound odor character information. Cross searching can also be performed automatically on a total TIC with similar characteristics displayed for each compound.

Finally an application will be shown where automated cross searching with two RI values only from different columns are used for compound identification.

Experimental

Sample. Hop essential oil (courtesy of Robert Shellie)

Instrumentation. 7890 GC coupled with a 5977 MSD, equipped with S/SL Inlet, FPD, NPD (Agilent Technologies), ODP 3 and MultiPurpose Sampler MPS (GERSTEL).

Analysis conditions.

S/SL:	split 1:100 250°C
Columns:	30 m DB-Wax (Agilent), d_i = 0.25 mm d_f = 0.25 µm 30 m DB-5 (Agilent), d_i = 0.25 mm d_f = 0.25 µm
Pneumatics:	He, constant flow (1.0 mL/min)
Oven:	40°C (2 min), 5°C/min, 240°C, (18 min)
MSD:	Full Scan, 29-300 amu 2.68 scans/sec

Results and Discussion

Figure 1a shows the very complex TIC obtained after a split injection of hop essential oil and Figure 1b is an expanded section with a mass spectrum at 24.475 min

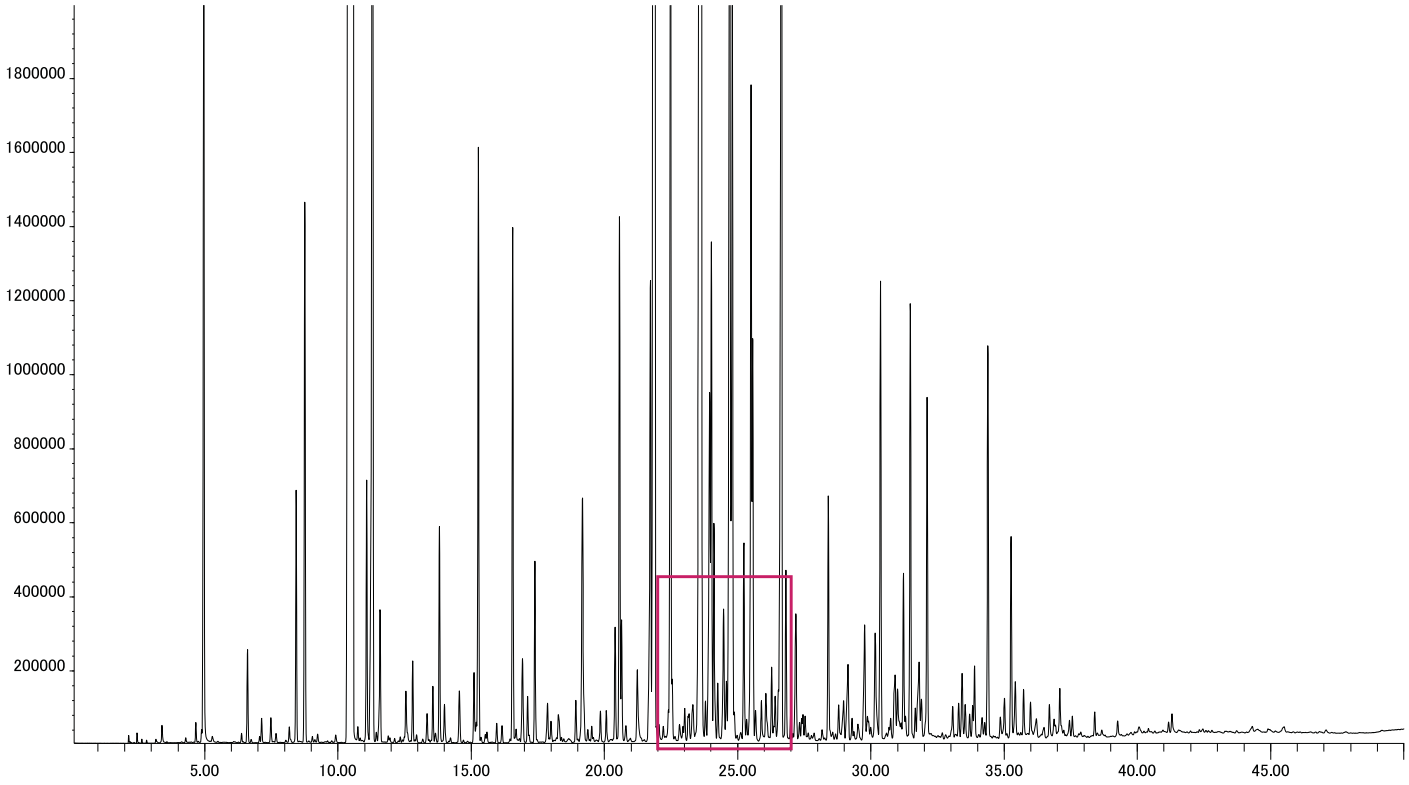


Figure 1a. TIC of a hop essential oil on DB-Wax.

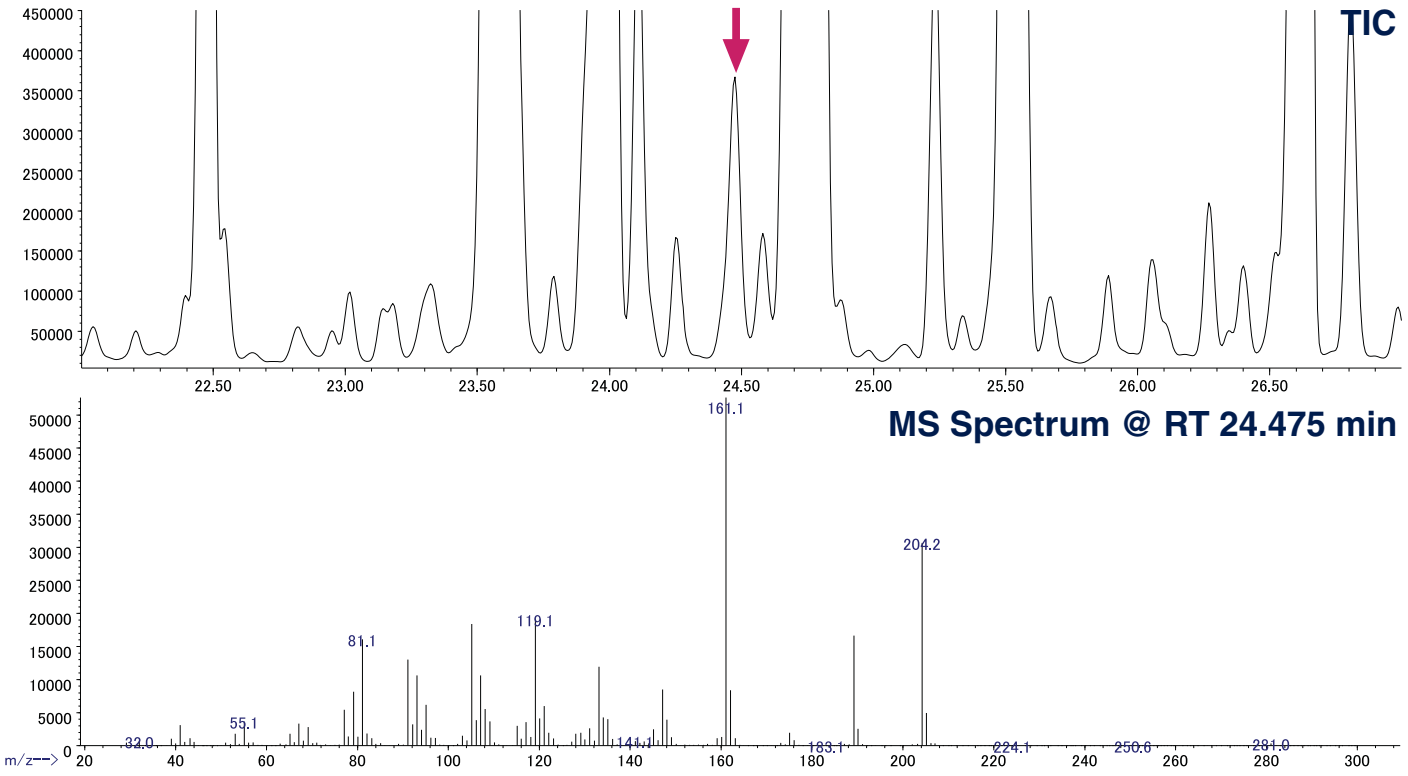
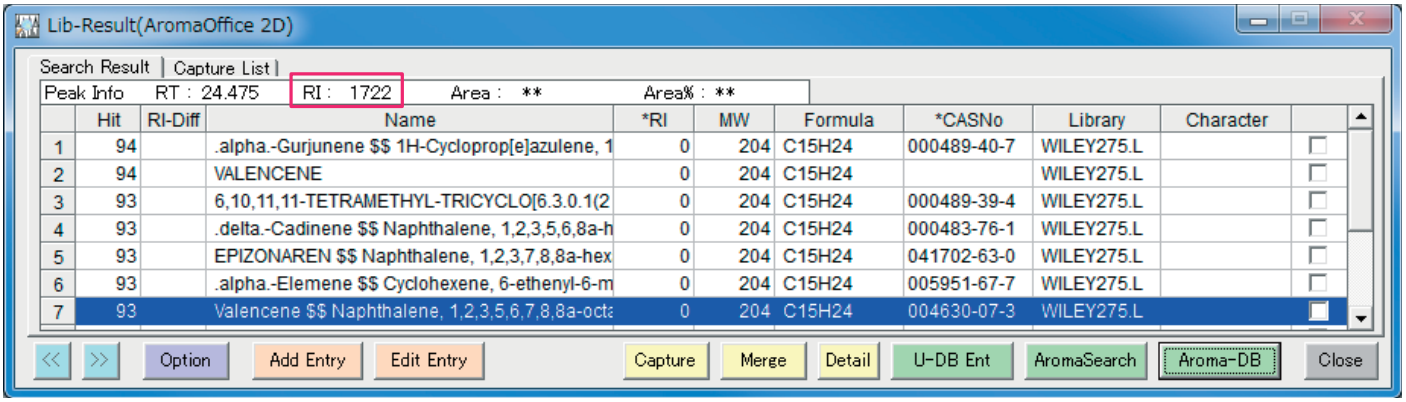


Figure 1b. Flavor compound and MS spectrum.

This spectrum is now library searched by the operator using a proprietary library supplied with the Aroma Office software and Figure 2 shows the usual PBM library search results. However a retention index of 1722 which corresponds to the retention time of 24.475 min on DB-Wax is also automatically calculated and reported on the search result screen.



	Hit	RI-Diff	Name	*RI	MW	Formula	*CASNo	Library	Character
1	94		.alpha.-Gurjunene $$ 1H-Cycloprop[e]azulene, 1	0	204	C15H24	000489-40-7	WILEY275.L	
2	94		VALENCENE	0	204	C15H24		WILEY275.L	
3	93		6,10,11,11-TETRAMETHYL-TRICYCLO[6.3.0.1(2	0	204	C15H24	000489-39-4	WILEY275.L	
4	93		.delta.-Cadinene $$ Naphthalene, 1,2,3,5,6,8a-h	0	204	C15H24	000483-76-1	WILEY275.L	
5	93		EPIZONAREN $$ Naphthalene, 1,2,3,7,8,8a-hex	0	204	C15H24	041702-63-0	WILEY275.L	
6	93		.alpha.-Elemene $$ Cyclohexene, 6-ethenyl-6-m	0	204	C15H24	005951-67-7	WILEY275.L	
7	93		Valencene $$ Naphthalene, 1,2,3,5,6,7,8,8a-octa	0	204	C15H24	004630-07-3	WILEY275.L	

Figure 2. Search result screen.

The software now allows the CAS No. of any hit together with the RI value to be sent to the Aroma Office database. The mass spectra of these compounds are very similar and positive compound identification with MS only would be very difficult. Figure 3 shows the result for alpha-Gurjunene (hit no. 1) and Valencene (hit no. 7) In the case of alpha-Gurjunene no search results are reported as the CAS No. and RI values from the database do not match. However in the case of Valencene there is such a match giving a positive identification. Notice also that the calculated RI value only differs by 2 units from the average database value. In the case of alpha-Gurjunene if the CAS No. only is used as the search condition the software now reports that this compound has an RI value of 1526 which is 196 units less than the calculated value.

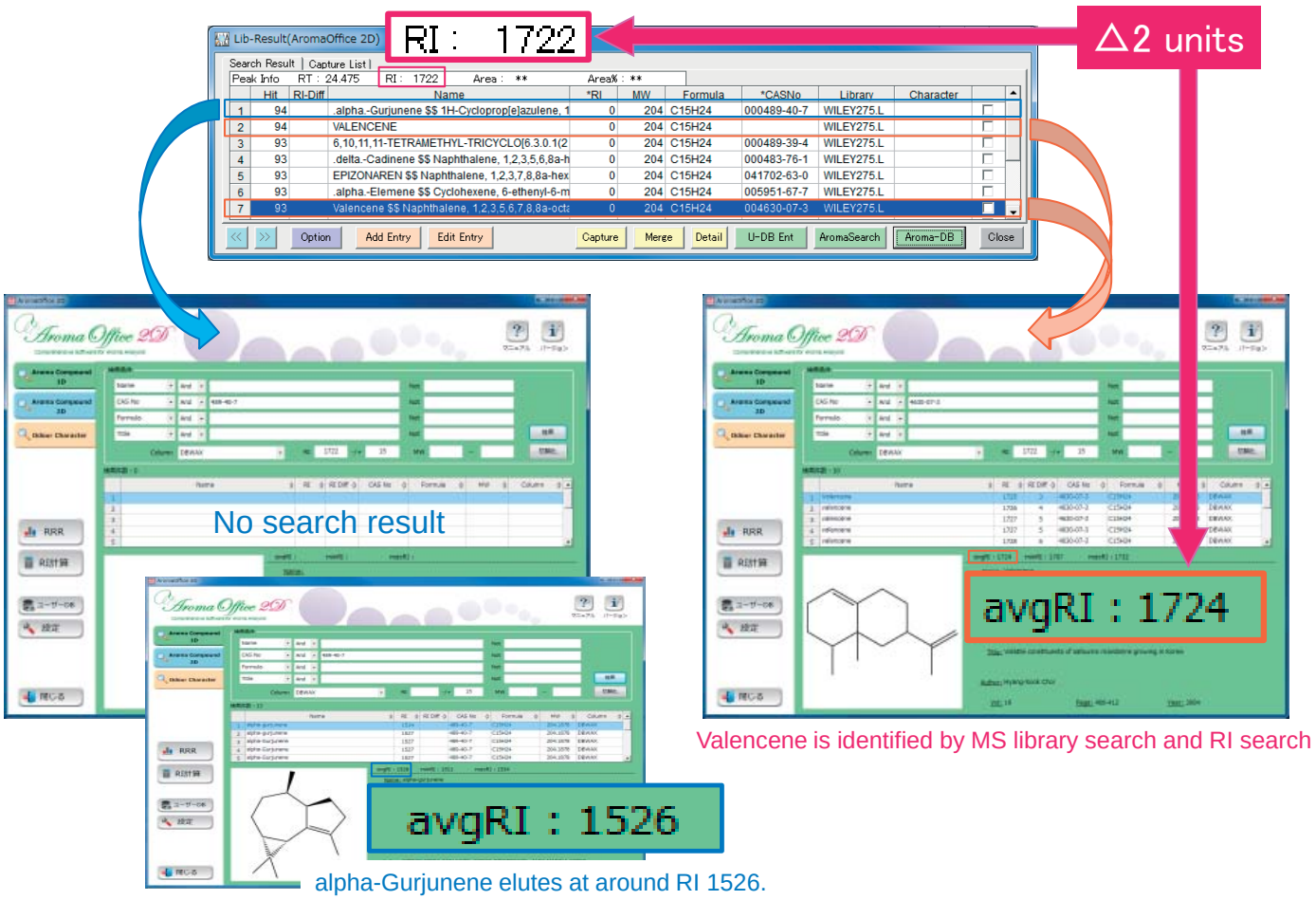


Figure 3. MS library and RI search.

The previous procedure can also be extended to the full TIC by using the Aroma Search module. In this case after TIC integration and automatic PBM library searching all CAS No's together with RI values are sent to the database. The software then reports detailed data (average RI, formula, odor character etc.) on flavor compounds that satisfy the matching CAS-RI requirement. Figure 4 shows such an output.

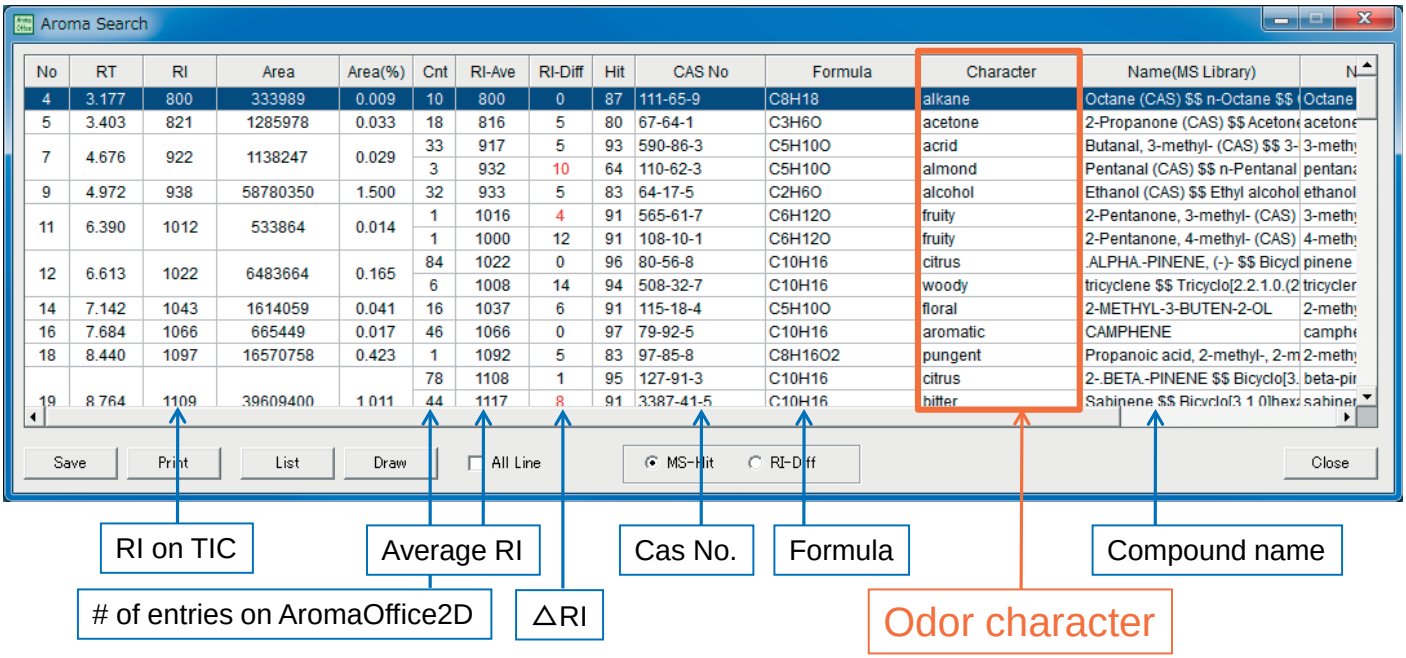


Figure 4. Aroma search result of a hop essential oil (DB-Wax). 65 odor compounds were automatically searched and confirmed by MS library and RI search.

Figure 5 now shows the TIC and an olfactory signal of the hop essential oil. An intense response with onion-like characteristics was identified at retention time 16.825 min corresponding to a retention index value of 1392 on DB-Wax. However mass spectral information at this retention time was extremely weak with no library search hits and associated CAS numbers as was previously the case.

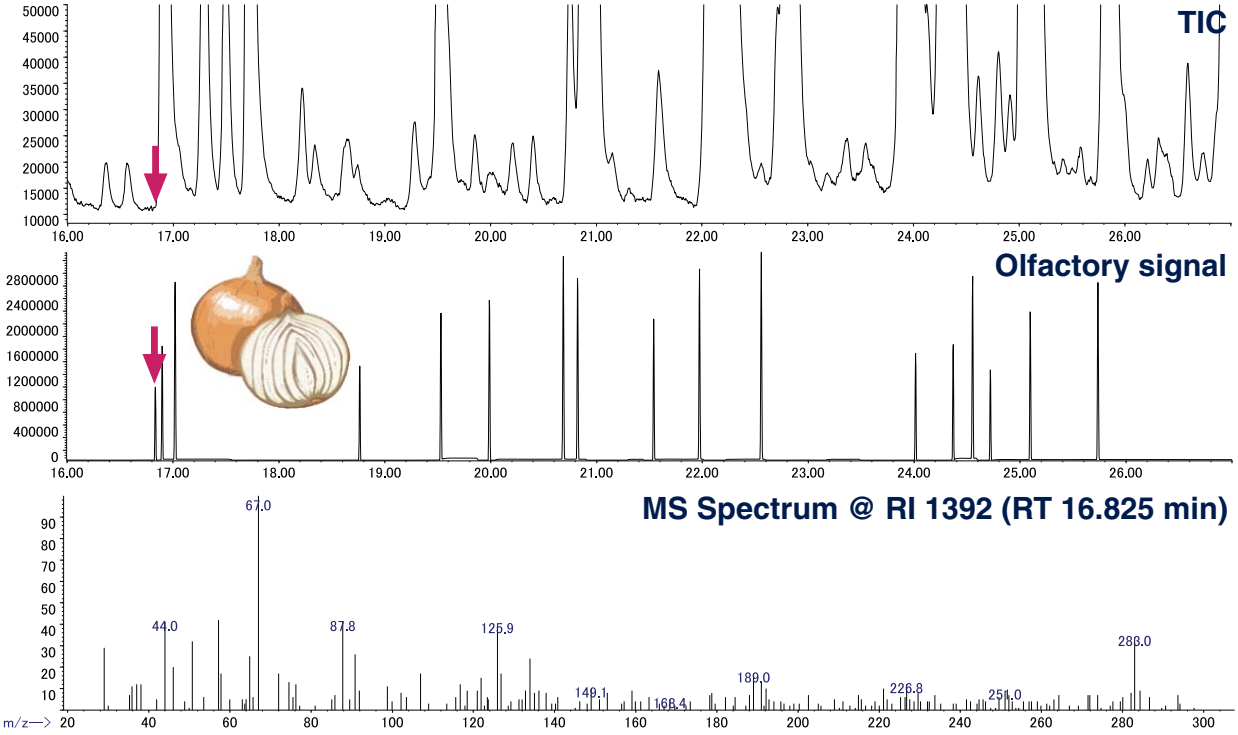


Figure 5. TIC, olfactory signal of a hop essential oil and MS spectra at RI 1392 on DB-Wax.

Figure 6 now shows the same TIC with the same olfactory signal but now run on DB-5. Again a retention index value is obtained due to the intense olfactory signal, but as before no useable library search data.

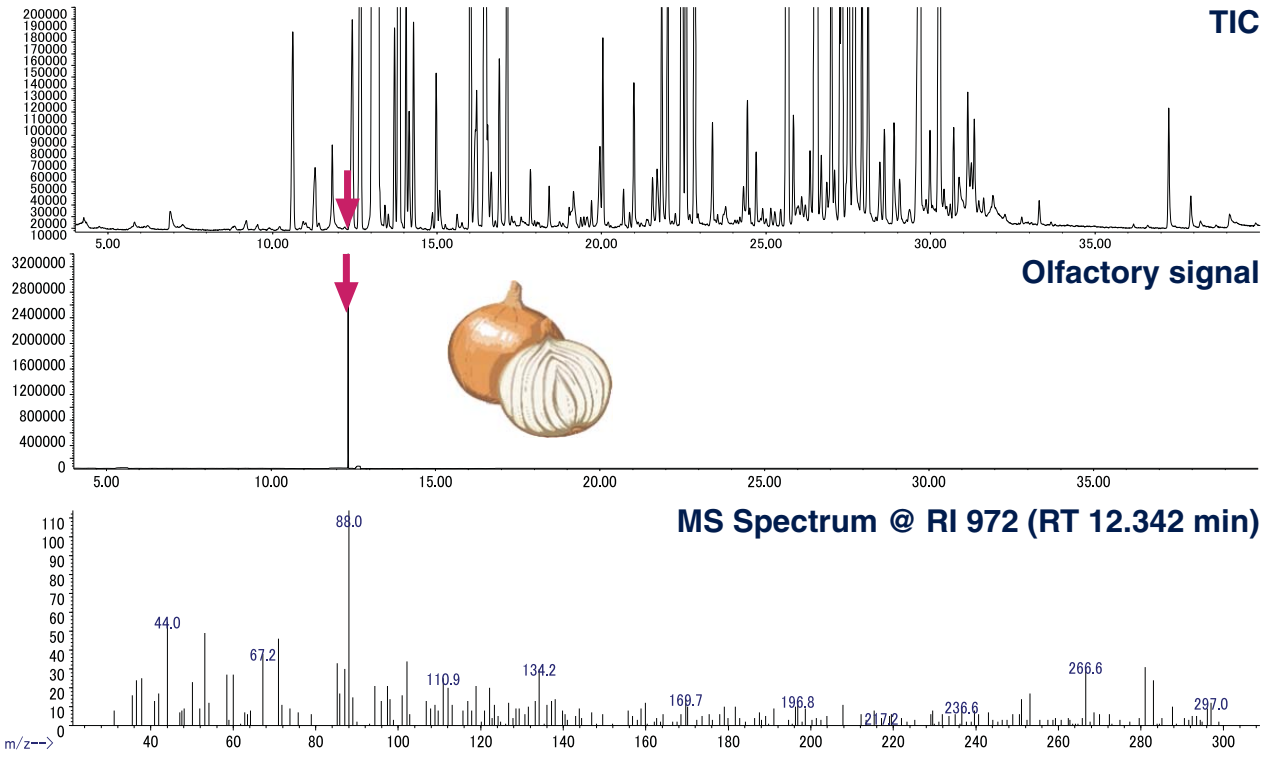


Figure 6. TIC, olfactory signal of a hop essential oil and MS spectra at RI 972 on DB-5.

Aroma Office now sends these two RI values to the database ($^1D/^2D$ option) for a cross search based on retention indices for the same compound but from different columns. The output from this procedure is shown in Figure 7.

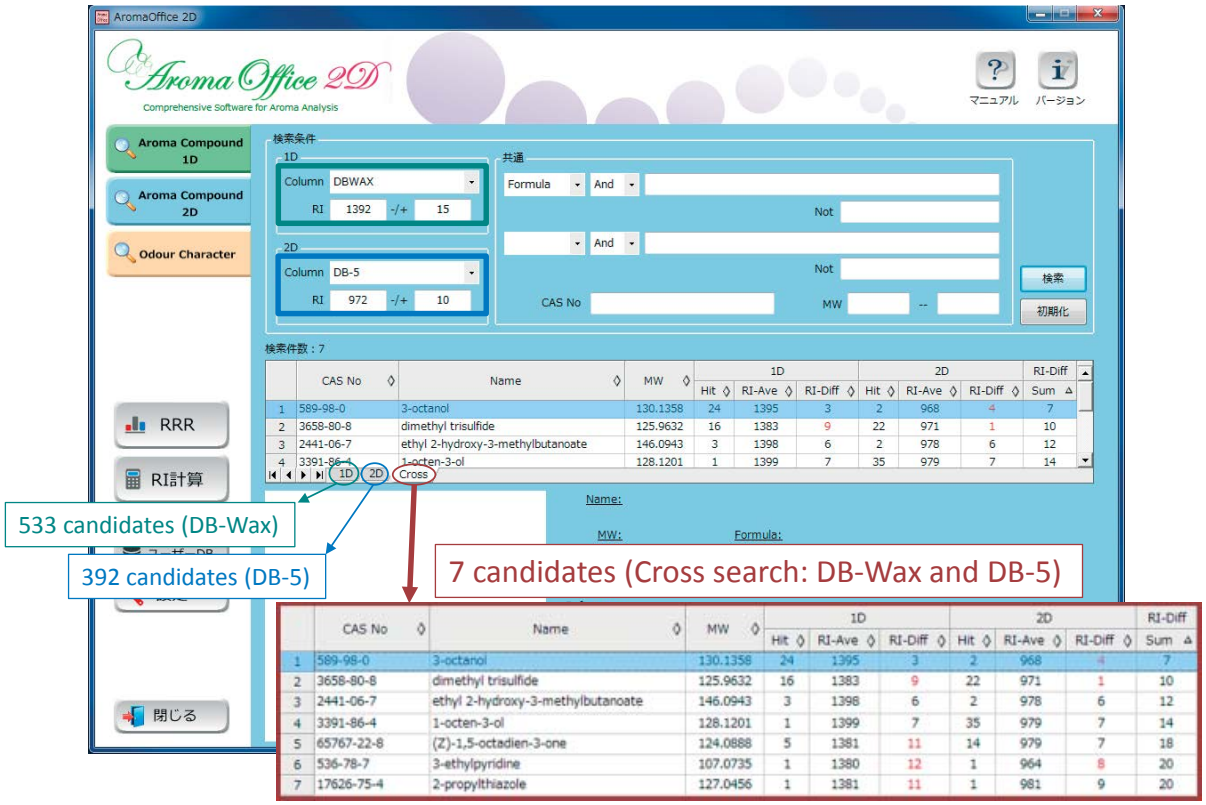


Figure 7. RI cross search result (DB-Wax and DB-5).

A search on DB-Wax alone proposed 533 possible candidates, and on DB-5 alone 392 candidates. But the constraint imposed by the cross search requirement has narrowed this down to a much more manageable 7 candidates. Two of these contain either S or N atoms and one is a Thiazole with both.

The hop oil was now run again on DB-Wax but using NPD (nitrogen) and FPD (sulfur) detectors. Figure 8 shows these elemental chromatographs and indicates only a sulfur response at RI 1392.

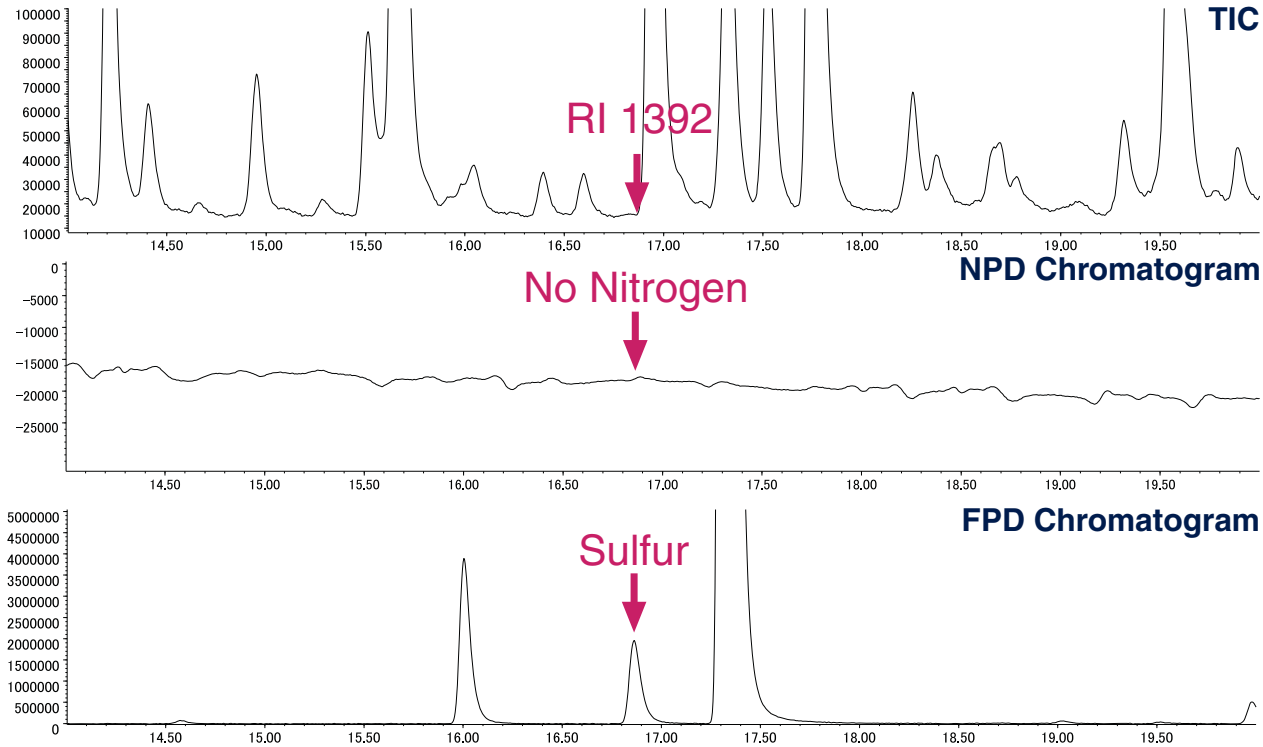


Figure 8. TIC, NPD and FPD chromatogram of a hop essential oil on DB-Wax.

This additional information can now be processed in the database and indicates that Dimethyl trisulfide is the only candidate, Figure 9a. Input of the CAS No. also generates an odor character description of the compound, Figure 9b.

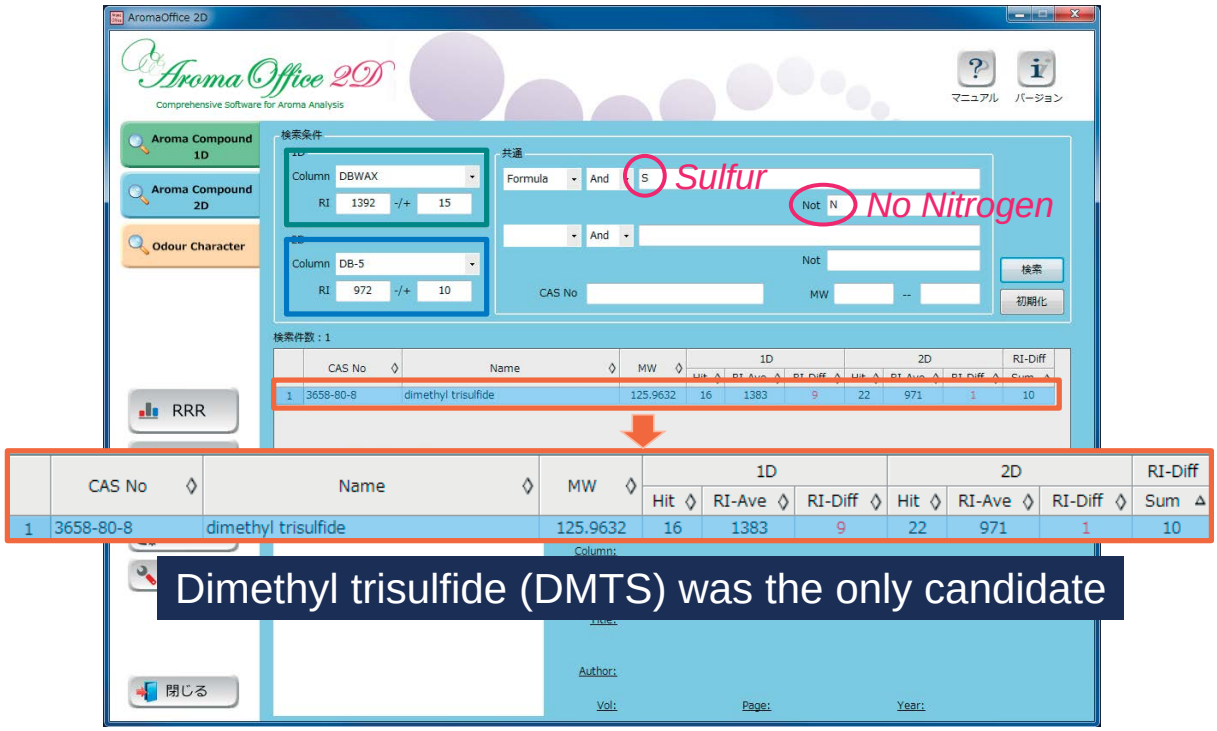


Figure 9a. RI cross search result with elemental information (DB-Wax and DB-5 with Sulfur and no Nitrogen).

Figure 9b. Odor character results for DMTS.

A DMTS standard was then injected on both DB-Wax and DB-5 columns and both retention times and retention index values matched precisely the corresponding initial olfactory values shown in Figures 5 and 6.

In more complex situations (hetero atoms not present, standards not readily available) selectable $^{1}D/^{2}D$ GC-O/MS with PFC can be used to enrich the ^{1}D active olfactory region, followed by ^{2}D GC-O/MS to obtain both the second RI value plus a confirmatory MS.

Conclusions

Previously, exploitation of retention index data in corroborative chromatographic identification of flavor compounds has involved a number of independent operations and "off-line" use of published data bases. Aroma Office simplifies this entire procedure by centralising all necessary operations in a suite of easy to access software modules. The software routines are in fact integrated into the GC-MS software for seamless transfer of both mass spectral and retention index values to the on-line database. Aroma Office is supplied with a dedicated flavor library with a searchable database from a wide range of literature references and is designed to offer new powerful tools in the field of chromatographic flavor analysis.

References

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