Analysis of Essential Oil Compounds Using Retention Time Locked Methods and Retention Time Databases

Application

Food and Flavors

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Abstract

Two retention time locked methods for the analysis of essential oil compounds are described. The first method is a gas chromatography/flame ionization detector method using a 50 m × 320 µm id × 1.05 µm HP-5 column. The second method is a gas chromatography/mass spectrometry method using a 30 m × 250 µm id × 0.25 µm HP-5MS column. Retention times of approximately 400 essential compounds were measured using both methods, and two retention time databases were created. Flavor compounds in food extracts or essential oil constituents can be automatically searched based on retention times and/or mass spectra. Finally, transformation of existing retention index libraries into locked retention time databases is discussed.

Introduction

Capillary gas chromatography (GC) has been for many years the method of choice for the analysis of essential oils [1]. The constituents of essential oils are identified using a combination of different GC techniques, including GC with flame ionization detection (FID) and determination of retention indices, GC with olfactometric detection (sniffing), GC in combination with mass spectrometry (GC/MS) and GC with element-selective detection (flame photometric detection, nitrogen phosphorous detection, atomic emission detection, etc). Although GC/MS is probably the most powerful technique, and extended mass spectral libraries are available, it does not allow complete identification. Essential oils are complex mixtures of monoterpenes, monoterpenoids, sesquiterpenes, sesquiterpenoids, diterpenes, and diterpenoids. No single capillary column can resolve all possible compounds, and spectral data are not always conclusive because isomers give similar spectra. In flavor and fragrance quality control, retention indices are still frequently used as a complementary technique to GC/MS. Several libraries are

available with retention indices for many flavor and fragrance compounds [2–4]. Retention indices are less dependent on operational parameters than absolute retention times, but they still depend significantly on the column type (stationary phase and supplier), on the temperature program, and to a lesser extent, on the carrier gas velocity. Therefore, it is sometimes difficult to reproduce published retention indices in different laboratories. Moreover, most companies in the flavor and fragrance industry are still using in-house methods based on historical choices of columns and conditions. Finally, the use of retention indices (with n-alkanes as reference compounds) is not possible with element-selective detectors. A peak detected at a certain retention time using a sulphur selective detector, for instance, might be difficult to locate in an FID or MS chromatogram.

Recent developments in GC have led to the ability of locking and matching retention times for a given application [5-6]. 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. Moreover, retention time locking can also be used in combination with different detectors, and exact scaling of GC/FID, GC/sniffing, GC/MS, and GC/AED chromatograms is possible [6]. Retention time locking and retention time databases are, therefore, excellent tools in essential oil and in flavor QA/QC analysis.

In this paper, two retention time locked methods are presented. For each method, a retention time database is available containing approximately 400 flavor compounds and essential oil constituents. The first method is a GC/FID method. A long, thick film column is used in combination with a slow temperature program. These conditions are frequently used in QA/QC analysis in the flavor and essential oil industry because a high sample capacity is combined with a high resolving power, resulting in a detailed picture of the samples. The second method is a GC/MS method. For this method, a 30 m × 0.25 mm id × 0.25 µm HP-5MS column was selected because this is the most frequently used column in GC/MS analysis. While the resolution and sample capacity are lower on this column, the GC/MS analysis mainly focuses on identification of analytes. For this method, a combined retention time and mass spectral library Screener Database is available.

Because a lot of retention data are already available as retention indices, it was also evaluated if these data could be transferred into absolute retention times that match with locked retention times. It was shown that retention indices from existing retention index libraries can be recalculated as absolute retention times that match with experimental data.

Experimental

GC/FID analyses were performed on an Agilent 6980 gas chromatograph equipped with a split/splitless inlet. Separation was done on a 50 m × 0.32 mm id × 1.05 µm HP-5 column (β=72) (Agilent part number 19091J-215). The analytical conditions are summarized in Table 1. Helium at approximately 85 kPa (12.33 psi) constant pressure was used as carrier gas. The inlet pressure was adjusted to give a retention time of 70.000 min for n-pentadecane. This is done by retention time locking (RTL), using five runs at different pressures (respectively 70, 80, 90, 100, and 110 kPa), and plotting the retention time of n-pentadecane as a function of the inlet pressure [5]. From this curve, the inlet pressure can be calculated to adjust the retention time of n-pentadecane to exactly 70.000 min. The analytical conditions in this GC/FID method are typical conditions used in quality control of essential oils. The column choice and the slow temperature program offer high resolution and a detailed sample profiling. The column also offers high sample capacity, which is also important in essential oil profiling, because important trace constituents can be present and elute close to major constituents. On columns with restricted sample capacity, overloading and, consequently, peak leading is frequently observed for the main constituents.

Table 1. GC/FID Conditions

Column	50 m × 0.32 mm id × 1.05 µm HP-5 (β=72) (Agilent part number 19091J-215)
Injection	Split, split ratio 25:1, 250 °C, 1 µL injection volume
Carrier	Helium (approximately 85 kPa) (12.33 psi), constant pressure
RTL	The inlet pressure is adjusted to give a retention time of 70.000 min for n-pentadecane
Oven program	50 °C to 280 °C at 2 °C/min (110 min analysis time)
Detection	FID, 300 °C

Table 2. GC/MS Conditions

Column	30 m × 0.25 mm id × 0.25 µm HP-5MS (β=72) (Agilent part number 19091S-433)
Injection	Split, split ratio 25:1, 250 °C, 1 µL injection volume
Carrier	Helium (approximately 65 kPa) (9.43 psi), constant pressure
RTL	The inlet pressure is adjusted to give a retention time of 27.500 min for n-pentadecane
Oven program	60 °C to 240 °C at 3 °C/min (60 min analysis time)
Detection	MS in scan mode (40–400 amu); solvent delay: 2 min; transfer line: 300 °C

The second method is a GC/MS method. GC/MS analyses were performed on an Agilent 6980 gas chromatograph equipped with a split/splitless inlet in combination with an Agilent 5973N MSD. Separation was done on a 30 m × 0.25 mm id × 0.25 µm HP-5MS column (β=250) (Agilent part number 19091S-433). The analytical conditions are summarized in Table 2. Helium at approximately 65 kPa (9.43 psi) constant pressure was used as carrier gas. The inlet pressure was adjusted to give a retention time of 27.500 min for n-pentadecane. This is done by retention time locking, using five runs at different pressures, and plotting the retention time of n-pentadecane as a function of the inlet pressure. This is automatically done by starting the "acquire RTL calibration runs" command in the GC/MS instrument control. From this curve the inlet pressure can be calculated to adjust the retention time of n-pentadecane to exactly 27.500 min. These analytical conditions can be used to screen essential oils using GC/MS. Essential oil constituents can be identified based on the mass spectral data, and on retention times, using a screener library. The operational conditions are identical to the conditions used by Adams [4]. Spectra and retention data published in this reference are also valid for this method.

Test mixtures of flavor compounds and n-alkanes were prepared from pure chemicals at 0.1% concentration in carbon tetrachloride or chloroform. Essential oil mixtures are diluted in carbon tetrachloride or chloroform at a 5% level (50 mg/mL).

Results and Discussion

GC/FID Method

The described GC/FID method is used for quality control of essential oil mixtures. The long, thick film column results in high resolution and high sample capacity. Traces of important compounds can be detected besides the main constituents. A typical separation obtained by this method appears in Figure 1, showing the analysis of a Spanish orange oil. The chromatogram shows a detailed picture of the main compounds and of minor constituents. This type of analysis, giving both qualitative and quantitative information, is used for quality control of essential oils. This GC/FID method was locked to n-pentadecane (t_R = 70.000 min). Under these locked conditions, n-decane elutes at 31.640 min and n-eicosane at 99.557 min. Using these conditions, a retention time locked database was created containing approximately 400 compounds that are important in quality control of essential oil mixtures. Using this database and the GC ChemStation RTL option, peaks in the GC chromatogram can be identified based on a retention time search in a given retention time window (for instance ±0.2 min). For the 10 main peaks of the Spanish orange oil, the results of such a retention time search are given in Table 3. It is clear, that in some cases, several compounds elute in the 0.4-min window and further identification is needed. However, this tool already allows an excellent profiling of samples.

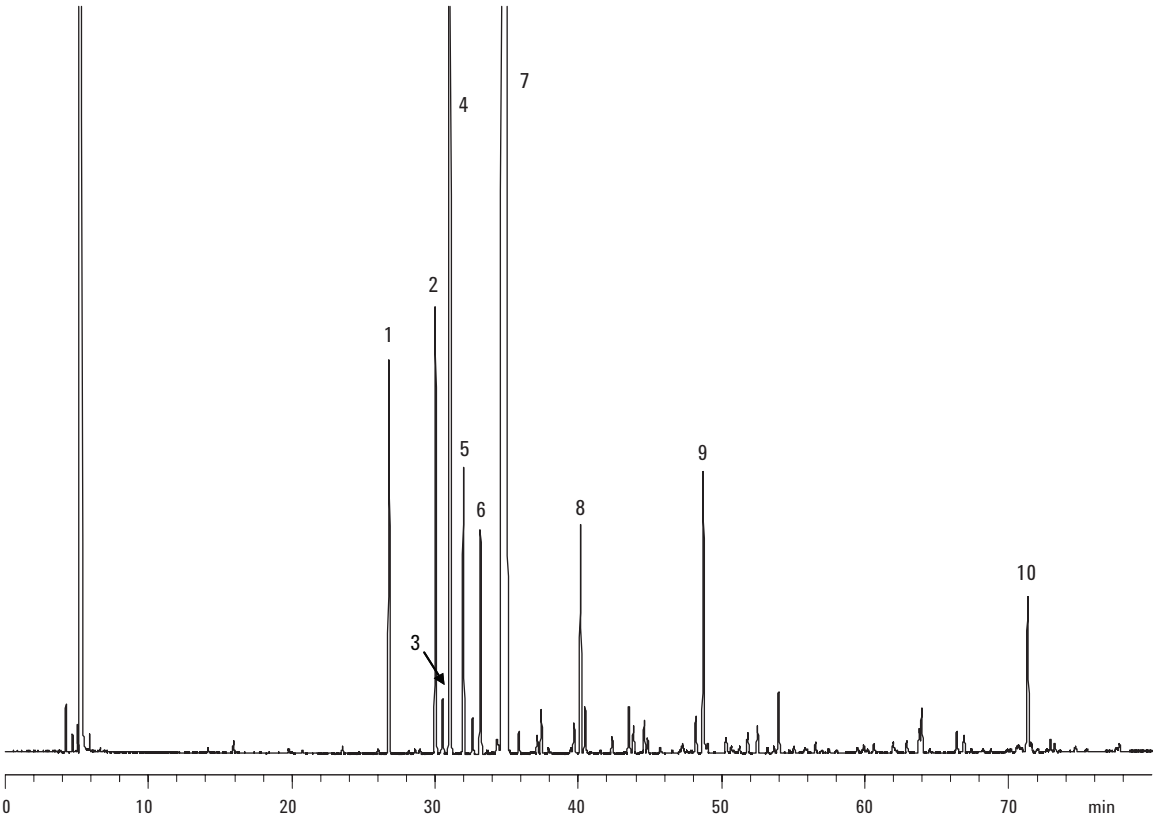

Figure 1. GC/FID chromatogram of Spanish orange oil. (Conditions: Table 1, peak identification: Table 3)

Table 3 Identification of main compounds in Spanish orange oil using a retention time database and a combined mass spectral and retention time identification.

Peak number	GC/FID* t_R (min)	t_R identification	GC/MS t_R (min)	MS + t_R identification
1	26.793	α-pinene	5.172	α-pinene
2	30.042	1-octen-3-ol 3-(methylthio)-1-propanol sabinene	6.181	sabinene
3	30.539	hexanoic acid β-pinene 6-methyl-5-hepten-2-one	6.282	β-pinene
4	31.053	2-octanone myrcene furfuryl acetate	6.658	myrcene
5	31.987	octanal	6.987	octanal
6	33.190	*trans*-2-hexenoic acid Δ-3-carene	7.267	Δ-3-carene
7	35.001	limonene benzylalcohol ocimene	8.130	limonene

Table 3 Continued

Peak number	GC/FID* t_R (min)	t_R identification	GC/MS t_R (min)	MS + t_R identification
8	40.162	n-undecane *cis*-3-hexenylpropionate δ-hexalactone 1-methyl-2,3-cyclohexadione linalool methyl benzoate	10.391	linalool
9	48.728	dihydrocarveol methyl salicylate estragole n-decanal octylacetate	14.750	n-decanal
10	71.366	anisylproprionate valencene piperonyl acetate	27.134	valencene

* For the GC/FID retention time identification, a 0.4-min window was used (±0.2 min)

Another example of the GC/FID method appears in Figure 2, showing the analysis of an Indonesian nutmeg oil. Again a very detailed chromatogram is obtained. Using the retention time locked database, most constituents are identified. The small peak eluting at 67.468 min is, for instance, identified as isoeugenol. This is an important flavor compound.

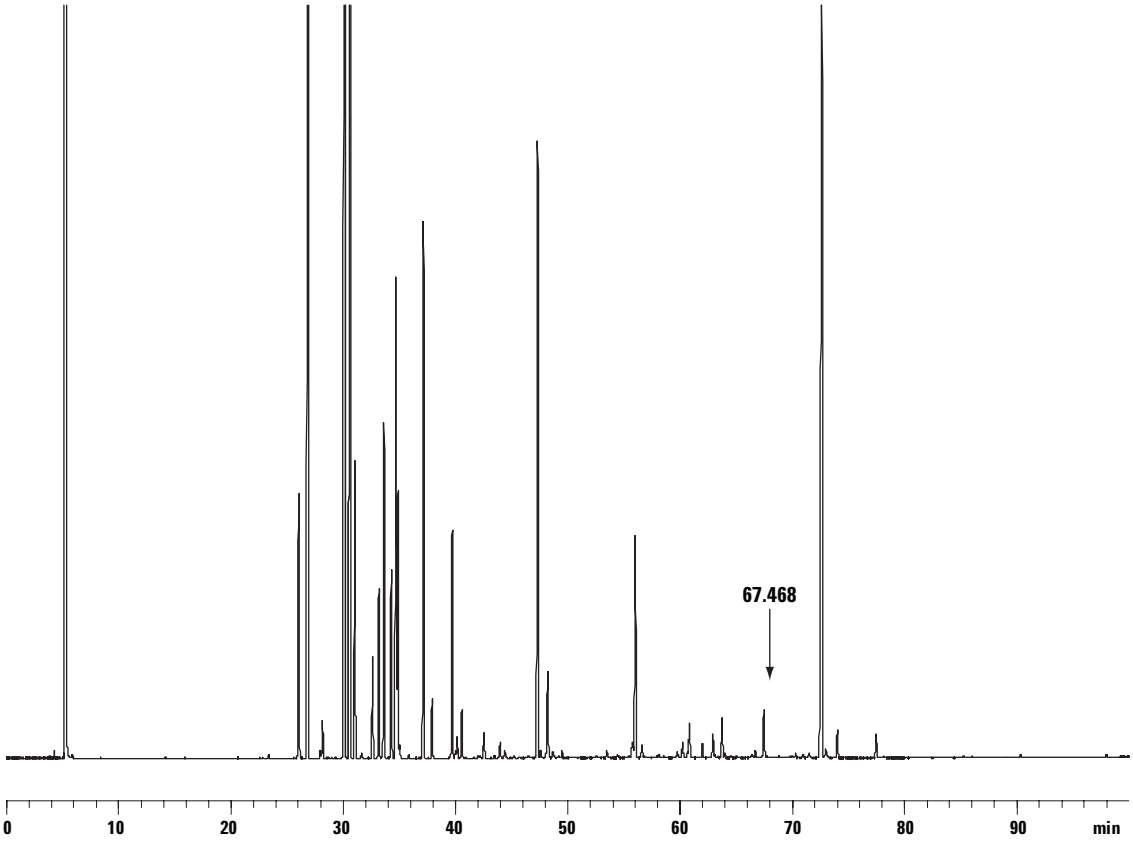

Figure 2. GC/FID chromatogram of Indonesian nutmeg oil. (Conditions: Table 1)

GC/MS Method

For confirmation of solute identities and for the identification of unknown peaks, the essential oils are analyzed by GC/MS. The described method uses a standard column and a faster temperature program. These conditions are similar to the method published by Adams [4]. The chromatogram obtained for the Spanish orange oil is given in Figure 3. A similar separation is obtained as in Figure 1, but the resolution is lower due to the lower column plate number. Moreover, some peak overloading can be observed. Due to the fact that a different temperature program is used, the compounds also elute at different temperatures (method not translated) and, therefore, also some differences in relative elution profiles are observed (see, for instance, relative elution of peaks 2, 3, and 4). Nevertheless, this method can be used for detailed identification of the essential oil constituents. For this GC/MS method, a mass spectral library was created containing approximately 400 compounds, together with the retention times. With this library, identification is possible based on mass spectra AND on retention time. The identification of the 10 main compounds (same as labelled in Figure 1) using the Results Screener is included in Table 3. The combination of mass spectral and retention time data allows complete identification. It is also important to notice that the correct compound was, in all cases, already selected in the GC/FID retention time window.

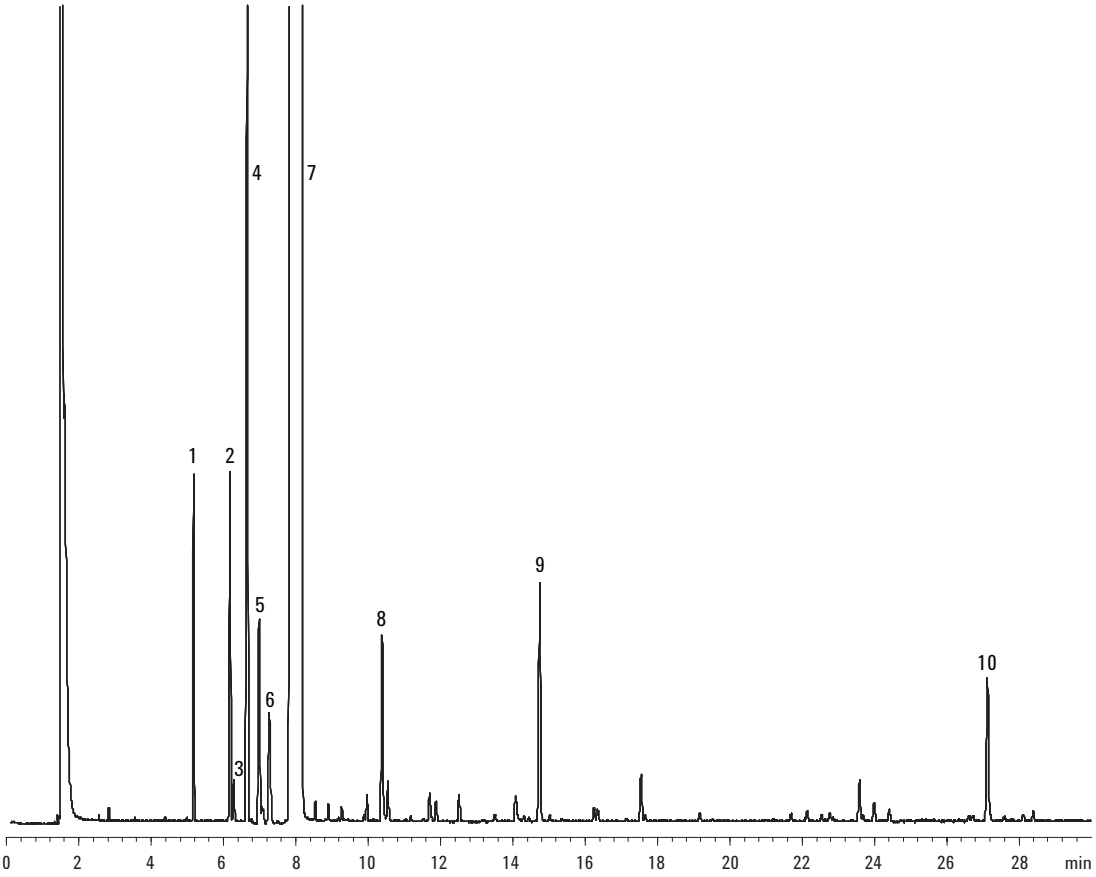

Figure 3. GC/MS chromatogram of Spanish orange oil. (Conditions: Table 2, peak identification: Table 3)

The Indonesian nutmeg oil was also analyzed by this GC/MS method. The chromatogram is given in Figure 4. A classical library search using the standard NIST mass spectral library of the peak at 25.304 min gave isoeugenol as the first hit (probability 96%) and eugenol as the second hit (probability 94%). The spectra of both compounds are very similar (Figure 5). Using the Results Screener, the compound was identified unequivocally as isoeugenol (retention time plus mass spectral match). This example clearly demonstrates the power of combined retention time and mass spectral search.

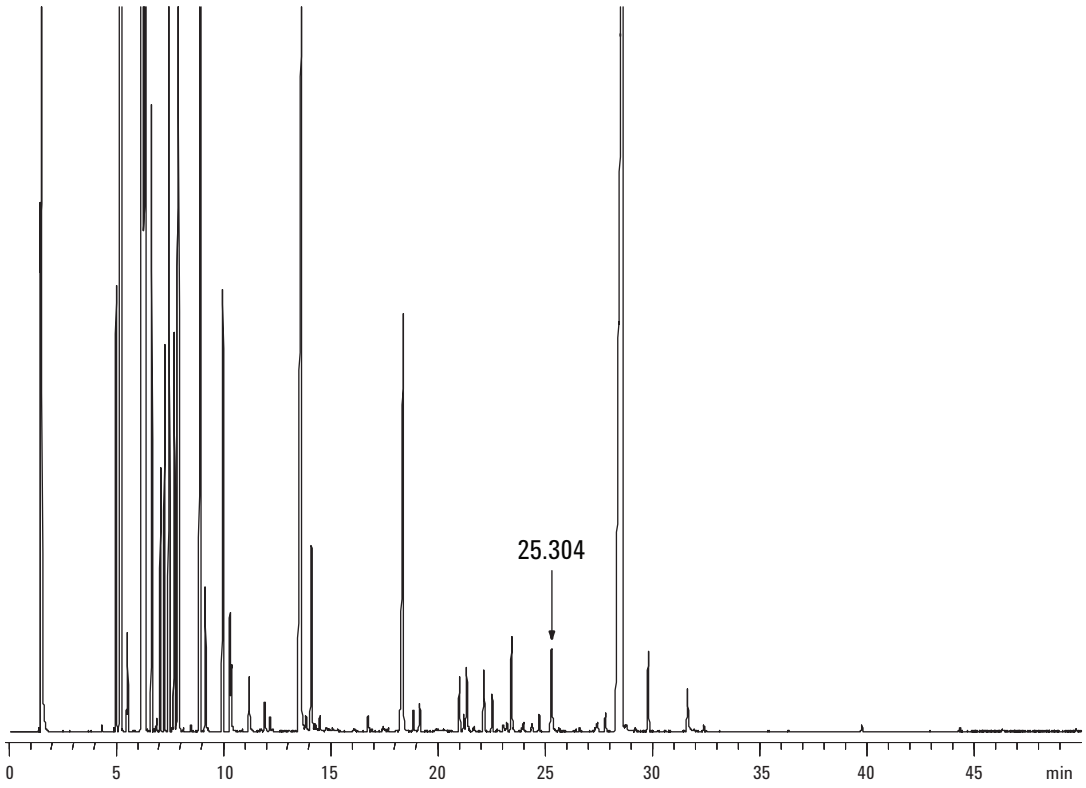

Figure 4. GC/MS chromatogram of Indonesian nutmeg oil. (Conditions: Table 2)

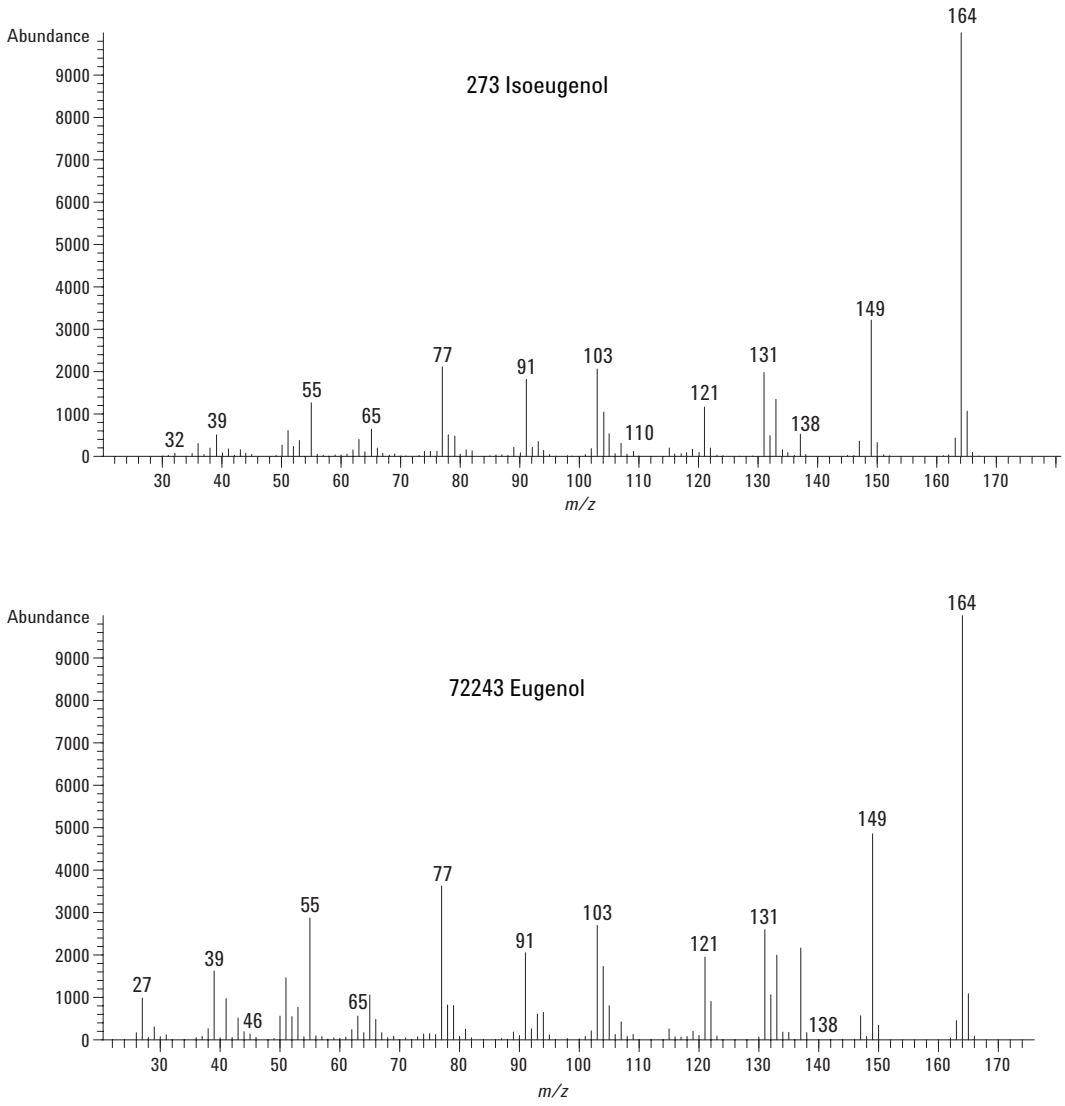

Figure 5. Comparison of mass spectra of eugenol and isoeugenol.

Transformation of Retention Indices

Further it was evaluated if published retention indices could be transferred into retention times and if these calculated retention times match with experimental data. A total of 34 test solutes were analyzed. The compounds are listed in Table 4, together with the FEMA code, the retention index in an existing database (RI) [7], and the measured retention time ($t_{R\,exp}$) under retention time locked conditions. From the retention index, the absolute retention time was calculated using the retention times of n-alkanes as reference compounds. The calculated values are also listed in Table 4 ($t_{R\,calc}$). Thus, these retention times are not the original retention times used for the retention index calculation, but calculated values. In the last column, the difference between calculated and experimental retention times are also given. From these data, it is clear that the calculated and experimental retention times match very well (within ±0.2 min). This means that retention times can be calculated from the retention indices present in an existing database using the locked retention times for n-alkanes if the column dimensions and the temperature program are the same. This is also valid for the GC/MS method as shown in the following example. Isoeugenol is present in the database of

Table 4. FEMA names, FEMA codes, retention indices, calculated retention times, experimental retention times, and retention time differences for test solutes.

Compound	FEMA name	FEMA code	RI	$t_{R\ calc}$ (min)	$t_{R\ exp}$ (min)	$t_{r\ diff}$ (min)
1	Acetal	2002	725.1	11.836	11.836	0.000
2	Amyl alcohol	2056	760.0	13.844	13.833	-0.011
3	Hexyl alcohol	2567	865.8	21.068	20.941	-0.127
4	Anisole	2097	923.0	25.529	25.403	-0.126
5	Ethyl acetoacetate	2415	944.4	27.300	27.319	0.019
6	Heptyl alcohol	2548	968.4	29.285	29.180	-0.105
7	Octanal	2797	1003.8	32.218	32.215	-0.003
8	Methyl 3-(methylthio)propionate	2720	1026.8	34.140	34.121	-0.019
9	Benzyl alcohol	2137	1036.9	34.984	35.011	0.027
10	Isoamyl butyrate	2060	1055.5	36.539	36.524	-0.015
11	Octyl alcohol	2800	1069.7	37.726	37.663	-0.063
12	Acetophenone	2009	1072.4	37.952	38.119	0.167
13	Benzylformate	2145	1081.2	38.688	38.851	0.163
14	Benzyl acetate	2135	1169.1	45.848	45,915	0.067
15	Allylheptanoate	2031	1180.0	46.729	46.664	-0.065
16	Decanal	2362	1207.4	48.917	48.970	0.053
17	Benzyl propionate	2150	1266.2	53.444	53.378	-0.066
18	1-Decanol	2365	1272.1	53.899	53.904	0.005
19	Anisyl alcohol	2099	1295.0	55.662	55.799	0.137
20	Isobornyl acetate	2160	1301.7	56.171	56.252	0.081
21	Benzyl isobutyrate	2141	1305.0	56.411	56.452	0.041
22	Undecanal	3092	1310.6	56.819	56.798	-0.021
23	Triacetin	2007	1344.4	59.279	59.192	-0.087
24	Benzyl butyrate	2140	1354.5	60.014	60.049	0.035
25	Acetanisole	2005	1369.0	61.070	60.960	-0.110
26	gamma-Nonalactone	2781	1373.4	61.390	61.273	-0.117
27	Anisyl acetate	2098	1426.0	65.116	65.203	0.087
28	Allyl cyclohexylpropionate	2026	1435.0	65.735	65.747	0.012
29	Lauryl alcohol	2617	1475.0	68.489	68.531	0.042
30	Isoamyl octanoate	2080	1487.0	69.315	69.230	-0.085
31	Isoamyl phenylacetate	2081	1503.0	70.405	70.387	-0.018
32	Ethyl-methylphenylglycidate	2444	1517.0	71.317	71.447	0.130
33	Ethyl-3-phenylglycidate	2454	1529.0	72.099	72.063	-0.036
34	gamma-Undecalactone	3091	1589.0	76.007	75.955	-0.052

Adams [4] with a retention index of 1447. This retention index can be transferred into an absolute retention time using the following formula:

$$\left[\frac{[RI - (Z \times 100)]}{100} \times \left(t_{R_{Z+1}} - t_{R_Z}\right)\right] + t_{R_Z} = t_{R_X}$$

whereby: RI = retention index (from existing data base),
Z = carbon number of preceding n-alkane,
t_{RZ+1} and t_{RZ} = retention times of following and preceding n-alkanes (in RTL method) and
t_{RX} = retention time of solute in RTL method

For isoeugenol, with an RI = 1447, and the preceding tetradecane (Z=14) eluting at 23.26 min (t_{RZ}), and the following n-pentadecane (Z+1) eluting at 27.50 min (t_{RZ+1}) using the retention time locked GC/MS method, the calculated retention time is 25.25 min. This corresponds well (within ±0.2 min) with the measured retention time (25.30 min). Using these calculations, compounds can also be added to the RTL databases.

Conclusion

Two methods were developed for the analysis of essential oils. The first method is used for quality control analysis. The method is locked using n-pentadecane as locking standard. A retention time locked database, containing approximately

400 compounds was created. This database can be used to identify constituents based on their absolute retention time under the locked conditions. The locked method also guarantees retention time stability in function of time, between columns and between instruments.

Secondly, a GC/MS method was developed. This method can be used for identification of essential oil constituents. Identification is done based on the combination of retention time and mass spectral matching.

Finally, it is shown that retention indices for flavor compounds measured under specific operational conditions can be transferred into locked retention times using the locked retention times of n-alkanes. Thus, existing retention index databases can be translated into locked retention time databases. Moreover, a retention time locked database is not restricted to the use of one (FID) detector, but compounds detected by any GC detector can be searched if the locked method is used.

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