Essential Oils—Area Percent Calculations Using Deconvoluted Total Ion Chromatogram

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Key Words: GC-TOFMS

1. Introduction

When using a mass spectrometer as a detector for gas chromatography to obtain what is called a Total Ion Chromatogram (TIC), it is common to sum all the acquired masses over the time range collected. When the chromatography is simple or all peaks are resolved, the TIC gives a good representation of the chromatography. However, when the chromatography is complex and coelutions occur, the TIC may not be a good indicator of all the peaks present in the sample because some peaks may appear as one. In this instance, one must have previous knowledge about the sample to know which components are coeluting, or resort to special deconvolution programs in order to identify the coeluting peaks.

To further complicate things, many analysts like to correlate the signals obtained by the mass spectrometer to those obtained using other detectors such as an FID. When using detectors such as an FID, it is common to do quantifications based on area percent calculations. This methodology does not translate well when using mass spectrometers as detectors because the response factors for the analytes are not the same. In addition, when coelutions occur the TIC only shows an overall profile rather than individual peaks. It may not be possible to know how much area belongs to which peak in the coelution. Time-of-Flight mass spectrometers have an advantage over scanning instruments when it comes to coelutions because in a TOFMS system the ion ratios across a peak remain constant. This is known as "spectral continuity". Because of this spectral continuity, the LECO ChromaTOF® software is capable of deconvoluting coeluting peaks and computing more precise areas for coelutions; thus area percent calculations are possible even when the TIC is not defined for coeluting peaks.

In this application note, an example is shown on the analysis of an Essential Oil imported from France called Bourdonol which is a Geranium Bourbon supplement. It will be demonstrated that accurate area percents can be calculated even for closely coeluting peaks.

2. Experimental Conditions

GC-Parameters:
Agilent 6890 (EPC Mode)
Column:
DB-5; 10 m x 0.18 mm x 0.18 µm film
Injector Temperature: 200°C
Split Ratio: 100:1
Oven Program:
40°C for 1 minute to 320°C at 40°C/minute, hold 1 minute
Flow Rate:
Constant Flow at 1.5 ml/minute

MS-Parameters: Pegasus II GC-TOFMS (EI Mode)
Mass Range: 45 to 450 amu
Acquisition Rate: 50 spectra/second
Ion Source Temp: 200°C
Total Acquisition Time: 9 minutes

3. Results and Discussion

Figure 1 shows the chromatogram for the Bourdonol Essential Oil. The chromatogram was processed automatically using a signal to noise threshold of 50:1 and a peak width of 2 seconds. This means that the Processing algorithm ignores peaks that have a signal-to-noise (S/N) ratio of less than 50:1. The Peak Find algorithm identified 150 analytes with a large number of peaks coeluting. Automatic spectral deconvolution was done and a peak table was generated followed by a library search. It is possible that more than one peak may have the same name since the Library Search algorithm cannot tell the eluting order of isomers. In addition, only the first hit for each analyte is displayed in the peak table, which may not be the correct one. Table 1 shows the peak table for this chromatogram.

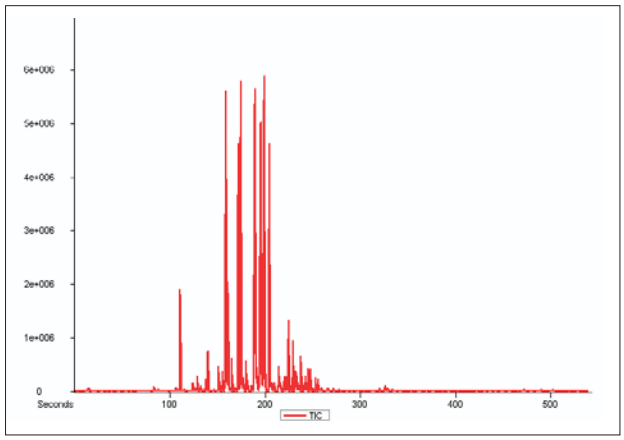

Figure 1. Chromatogram of Bourdonol Essential Oil.

Another dilemma demonstrated by this sample is the calculation of area percent of coeluting peaks since some peaks coelute so closely that the software does not detect inflection points. A few examples are shown in the following figures.

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Table 1. Peak Table for Bourdonol Essential Oil

Peak #	Name	R.T.	Quant Masses	Area	Area %
1	Dimethyl ether	13.44	DT	494,820	0.021
2	Ethene, methoxy-	14.26	DT	840,820	0.036
3	Formic acid	14.74	DT	2,264,200	0.097
4	Formic acid, ethyl ester	15.02	DT	138,180	0.006
5	Acetic acid, anhydride with formic acid	17.94	DT	515,470	0.022
6	3-Buten-2-ol, 2-methyl-	19.94	DT	278,430	0.012
7	Propanoic acid	28.94	DT	497,010	0.021
8	1-Butanol, 3-methyl-	38.30	DT	559,540	0.024
9	1-Butanol, 2-methyl-	39.24	DT	376,290	0.016
10	2-Butenal, 3-methyl-	53.26	DT	326,550	0.014
11	Pentanoic acid	53.66	DT	508,680	0.022
12	1-Butanol, 3-methyl-, formate	56.24	DT	824,960	0.035
13	1-Pentanol, 3-methyl-	79.68	DT	173,340	0.007
14	3-Hexen-1-ol, (Z)-	83.44	DT	3,065,200	0.131
15	2-Hexen-1-ol, (E)-	87.82	DT	815,640	0.035
16	Acetic acid, 2-ethylbutyl ester	99.22	DT	152,410	0.007
17	4-Hexen-1-ol, acetate	106.64	DT	1,487,500	0.064
18	Formic acid, cyclohexyl ester	108.76	DT	224,110	0.010
19	3-Thujene	108.90	DT	318,920	0.014
20	1R-á-Pinene	110.92	DT	64,975,000	2.776
21	Camphene	115.42	DT	351,530	0.015
22	(-)-á-Pinene	124.22	DT	3,554,100	0.152
23	Bicyclo[4.1.0]heptane, 7-(1-methylethylidene)-	126.46	DT	681,710	0.029
24	Menth-1(8)-ene	126.66	DT	1,023,400	0.044
25	á-Myrcene	129.06	DT	7,771,100	0.332
26	3-Heptanol, 6-methyl-	130.22	DT	197,000	0.008
27	á-Pinene	132.40	DT	1,881,100	0.080
28	à-Phellandrene	132.56	DT	1,264,900	0.054
29	p-Menth-8-ene, cis-	132.76	DT	728,790	0.031
30	2-Hexen-1-ol, acetate, (Z)-	136.06	DT	442,560	0.019
31	2-Menthene	137.54	DT	98,416	0.004
32	o-Cymene	138.20	DT	5,960,500	0.255
33	D-Limonene	139.50	DT	4,956,600	0.212
34	Menthyl acetate	139.74	DT	607,600	0.026
35	Cineole	140.08	DT	18,371,000	0.785
36	3-Methyl-apopinene	142.14	DT	129,020	0.006
37	á-cis-Ocimene	144.98	DT	92,548	0.004
38	Benzene, butyl-	146.78	DT	484,350	0.021
39	4-Carene, (1S,3R,6R)-(-)-	147.72	DT	108,290	0.005
40	3-Octyl methylphosphonofluoridate	149.66	DT	86,085	0.004
41	cis-Linalool Oxide	151.36	DT	13,149,000	0.562
42	trans-Linaloloxide	155.44	DT	10,437,000	0.446
43	1,6-Octadien-3-ol, 3,7-dimethyl-	159.30	DT	274,890,000	11.744
44	Rose oxide	161.44	DT	46,809,000	2.000
45	Phenylethyl Alcohol	161.70	DT	2,455,600	0.105
46	cis-2-Pinanol	164.12	DT	1,088,000	0.046
47	2H-Pyran, tetrahydro-4-methyl-2-(2-methyl-1-propenyl)-	165.32	DT	15,947,000	0.681
48	Cyclopropane, trimethyl (2-methyl-1-propenylidene)-	165.56	DT	2,056,600	0.088
49	1,3-Cyclohexadiene, 1,2,6,6-tetramethyl-	168.54	DT	1,728,500	0.074
50	3,8,12-Tri-O-acetoxy-7-desoxyingol-7-one	169.58	DT	193,400	0.008
51	Cyclohexanol, 5-methyl-2-(1-methylethenyl)-, [1R-(1à,2á,5à)]-	169.78	DT	1,098,700	0.047
52	Cyclohexanone, 5-methyl-2-(1-methylethyl)-, cis-	171.98	DT	148,420,000	6.341
53	Cyclohexanone, 5-methyl-2-(1-methylethyl)-, trans-	174.90	DT	262,220,000	11.202
54	Isomenthylamine	176.18	DT	760,960	0.033
55	Formic acid, 2-phenylethyl ester	176.38	DT	1,495,900	0.064
56	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-	177.32	DT	5,975,900	0.255
57	Pyrazine, 2-methoxy-3-(2-methylpropyl)-	177.72	DT	4,412,000	0.188
58	Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1à,2â,5à)-	178.74	DT	475,590	0.020
59	(+)-à-Terpineol (p-menth-1-en-8-ol)	180.34	DT	15,324,000	0.655
60	1-Octanol, 3,7-dimethyl-	181.16	DT	2,639,900	0.113
61	Bicyclo[3.1.0]hexane, 4-(1-methylethylidene)-	181.88	DT	4,592,300	0.196
62	O-Ethyl S-2-diethylaminoethyl ethylphosphonothiolate	185.80	DT	1,853,000	0.079
63	Tetrahydroionol	189.74	DT	426,950,000	18.240
64	Cyclohexane, 1-methyl-3-(1-methylethylidene)-	191.32	DT	3,167,400	0.135
65	á-Citral	191.50	DT	3,254,300	0.139
66	2-Octene, 2,6-dimethyl-	191.96	DT	3,489,100	0.149
67	3,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-	192.16	DT	3,844,900	0.164
68	1,6-Octadien-3-ol, 3,7-dimethyl-, (ñ)-	195.58	DT	314,840,000	13.450
69	7-Hexadecyne	196.82	DT	3,788,300	0.162
70	trans-Citral	197.90	DT	5,887,900	0.252
71	6-Octen-1-ol, 3,7-dimethyl-, formate	199.30	DT	242,020,000	10.339
72	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z) -	200.14	DT	12,538,000	0.536
73	1,6-Octadien-3-ol, 3,7-dimethyl-, formate	204.70	DT	113,070,000	4.831
74	6-Octenoic acid, 3,7-dimethyl-, methyl ester	207.20	DT	9,147,000	0.391
75	4-Chloro-3-n-hexyltetrahydropyrane	208.02	DT	45,109	0.002
76	Neric acid	209.64	DT	4,046,200	0.173
77	2-Butenoic acid, 2-methyl-, 2-methylpropyl ester, (E)-	209.90	DT	2,936,300	0.125
78	1,2-Cyclohexanediol, 3-methyl-6-(1-methylethyl)-, (1à,2á,3à,6à)-	210.56	DT	740,620	0.032
79	Cyclohexanol, 2-methyl-3-(1-methylethenyl)- , acetate, (1à,2à,3à)-	210.66	DT	1,075,000	0.046
80	Z-11,12-Epoxytetradecan-1-ol	211.22	DT	253,040	0.011
81	cis-2,6-Dimethyl-2,6-octadiene	214.44	DT	12,643,000	0.540
82	à-Cubebene	215.00	DT	1,063,200	0.045
83	Phenol, 2-methoxy-3-(2-propenyl)-	215.66	DT	1,866,500	0.080
84	Neric acid	215.96	DT	6,547,800	0.280
85	Nerol acetate	216.64	DT	2,759,800	0.118
86	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z) -	220.38	DT	5,651,600	0.241
87	Copaene	220.56	DT	2,173,000	0.093
88	3,9-Epoxypregn-16-ene-14-18-diol-20-one, 7,11-diacetoxy-3-methoxy-	221.44	DT	102,440	0.004
89	Cyclohexene, 6-ethenyl-6-methyl-1-(1-methylethyl)-3-(1-methylethylidene)-, (S)-	222.08	DT	6,905,100	0.295
90	Cyclobuta[1,2:3,4]dicyclopentene, decahydro-3a-methyl-6-methylene-1-(1-methylethyl)-, [1S-(1à,3aà,3bá,6aá,6bà)]	222.50	DT	2,793,500	0.119
91	[5-(3-Methoxymethoxy-10,13-dimethyl-2,3,4,9,10,11,12,13,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-17-yl)-hex-1-ynyl]-trime	223.16	DT	32,428	0.001
92	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-	223.44	DT	2,041,300	0.087
93	Butane, 1,1'-sulfinylbis-	223.58	DT	1,127,600	0.048
94	Diphenyl ether	224.76	DT	35,224,000	1.505
95	2-Methyl-1-undecanol	225.60	DT	78,978	0.003
96	1,4-Methanoazulene, decahydro-4,8,8-trimethyl-9-methylene-, [1S-(1à,3aá,4à,8aá)]-	226.88	DT	601,500	0.026
97	Isoledene	228.00	DT	2,731,100	0.117
98	Caryophyllene	229.40	DT	26,807,000	1.145
99	6-Octen-1-ol, 3,7-dimethyl-, propanoate	230.96	DT	520,370	0.022
100	á-Cubebene	231.02	DT	196,010	0.008
101	2,4,6-Octatriene, 2,6-dimethyl-, (E,Z)-	231.26	DT	22,424	0.001
102	Citronellyl propionate	231.90	DT	7,112,000	0.304
103	à-Guaiene	232.48	DT	9,834,000	0.420
104	Isoledene	233.44	DT	7,680,600	0.328
105	Butanoic acid, 3-methyl-, 1-ethenyl-1,5-dimethyl-4-hexenyl ester	233.82	DT	1,327,100	0.057
106	Isoledene	234.80	DT	4,434,000	0.189
107	à-Caryophyllene	235.82	DT	2,852,200	0.122
108	(+)-Cyclosativene	237.30	DT	2,349,200	0.100
109	Geranyl propionate	237.72	DT	17,622,000	0.753
110	(-)-Calamenene	238.44	DT	1,280,600	0.055
111	2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalene	239.58	DT	3,110,700	0.133
112	Germacrene D	240.78	DT	626,070	0.027
113	Isolongifolan-8-ol	241.58	DT	3,260,100	0.139
114	(ñ)-Cadinene	242.48	DT	7,077,300	0.302
115	3,7-Cyclodecadien-1-one, 10-(1-methylethenyl)-, (E,E)-	243.46	DT	852,410	0.036
116	Azulene, 1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1à,4à,7á)]-	243.94	DT	1,620,200	0.069
117	Isoledene	244.12	DT	1,066,100	0.046
118	à-Bulnesene	245.22	DT	11,539,000	0.493
119	Azulene, 1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethylidene)-, (1S-cis)-	245.70	DT	615,200	0.026
120	2-Methyl-2,3-pentanediol	246.26	DT	257,550	0.011
121	ç-Cadinene	246.78	DT	170,350	0.007
122	à-Guaiene	247.04	DT	504,040	0.022
123	Phenol, 2-methoxy-4-(2-propenyl)-, acetate	247.40	DT	6,365,200	0.272
124	Butanoic acid, 3,7-dimethyl-6-octenyl ester	247.66	DT	8,572,800	0.366
125	á-Cadinene, (-)-	248.16	DT	2,065,400	0.088
126	Propanoic acid, 2-methyl-, 3,7-dimethyl-2,6-octadienyl ester, (E)-	249.30	DT	384,520	0.016
127	5-(4,4,3-Trimethylpentyl)-2-pyridinecarboxylic acid	250.28	DT	24,983	0.001
128	1H-Cycloprop[e]azulene, 1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1aà,4à,4aá,7bà)]-	250.84	DT	1,287,500	0.055
129	Acetic acid, cyano-, methyl ester	251.58	DT	36,356	0.002
130	6-(3-Isopropenylcyclopropyl)-1-enyl)-6-methylhept-3-en-2-one	251.88	DT	207,890	0.009
131	Eudesma-3,7(11)-diene	252.00	DT	399,980	0.017
132	Nerol acetate	253.36	DT	7,039,700	0.301
133	Guanidine, monothiocyanate	254.56	DT	65,114	0.003
134	à-Guaiene	255.80	DT	5,723,800	0.245
135	3,4-Hexanedione, 2,2,5-trimethyl-	256.72	DT	122,800	0.005
136	2-Butenoic acid, 2-methyl-, 2-phenylethyl ester, (E)-	258.34	DT	732,570	0.031
137	4-(3,3-Dimethyl-but-1-ynyl)-4-hydroxy-2,6,6-trimethylcyclohex-2-enone	259.32	DT	340,100	0.015
138	Caryophyllene oxide	259.52	DT	1,346,400	0.058
139	Benzophenone	266.14	DT	1,116,800	0.048
140	cis-3-Hexenyl phenyl acetate	266.44	DT	522,230	0.022
141	Citronellyl tiglate	269.90	DT	123,000	0.005
142	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-	271.70	DT	1,348,300	0.058
143	Geranyl tiglate	277.50	DT	507,260	0.022
144	cis-Linalool Oxide	285.04	DT	114,300	0.005
145	(-)-cis-Carane	320.44	DT	1,136,900	0.049
146	Neryl phenylacetate	326.10	DT	2,857,800	0.122
147	6-Octen-1-ol, 3,7-dimethyl-, acetate	329.16	DT	1,512,800	0.065
148	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-, (E,E)-	334.04	DT	933,200	0.040
149	2-Naphthuric acid	472.38	DT	1,024,900	0.044
150	Capric acid triglyceride	490.00	DT	1,024,400	0.044
	Total			2,340,800,000	100.000

The following examples (from the sample above) illustrate the problems associated with area percent calculations using a mass spectrometer. In the first example, the peaks are calculated using the natural inflection points and valleys in the TIC as detected by most software packages. Normally a perpendicular is drawn between valleys of coeluting compounds, but this is only an approximation. The problem is compounded when peaks coelute so closely that there are no defined valleys between them. In this instance, most software packages can only guess at the location to draw perpendiculars from the peak apexes.

Figure 2 shows the TIC section of the Bourdonol Essential Oil between 213.5 and 217.5 seconds. Inspection of the TIC only reveals the presence of four peaks (in actuality there are five peaks). It is difficult to assign integration limits when the peaks are not well defined or there are no defined valleys between the peaks. This is the main reason why long chromatographic runs are needed by most chromatographic platforms to ensure accurate peak areas. Table 2 shows a section of the peak table as calculated using the area percent from the TIC. These numbers are incorrect for the reasons noted above.

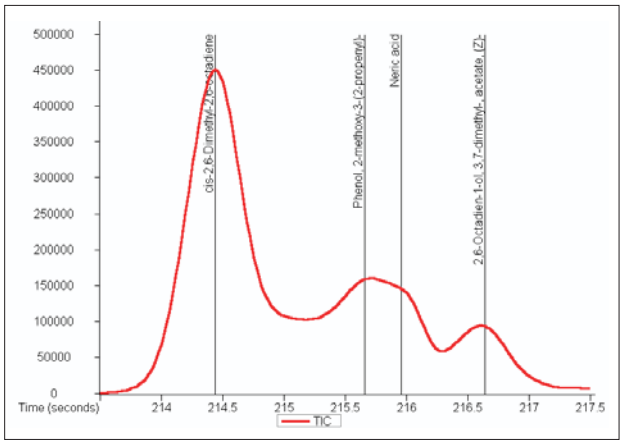

Figure 2. Chromatogram Section of Bourdonol Essential Oil (Coeluting Peaks).

Table 2. Erroneous Area Percent Calculation Based on the TIC

Peak #	Name	R.T.	Quant Masses	AREA	AREA %
81	cis-2,6-Dimethyl-2,6-octadiene	214.44	TIC	14422000	0.549
82	Phenol, 2-methoxy-3-(2-propenyl)-	215.66	TIC	4357900	0.166
83	Neric acid	215.96	TIC	2800200	0.106
84	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)-	216.64	TIC	3069800	0.117

The areas calculated from the TIC are incorrect because the software cannot assign good integration limits. First, the area for peak 81 (cis-2,6-Dimethyl-2,6-octadiene) is only approximate because there is not enough valley between the adjacent peak. Second, the area for peak 82 (2-methoxy-3-(2-propenyl)-phenol is also not correct because it also has added contribution from peak 83 (Neric acid). The best the software can do is to draw a perpendicular between peaks 82 and 83. Peak 83 has the same problem as peak 82 where the coelution is so close that good integration areas are difficult to calculate. The area for peak 84 is underestimated because there is not enough valley between the adjacent peak.

The ChromaTOF software can actually calculate accurate areas for each of the peaks shown in the example based on the spectral continuity of the peaks. By specifying in the QUANT MASSES section of the peak table a deconvoluted TIC (DTIC) the software is instructed to calculate how much contribution each peak makes to the TIC so that good integration limits can be calculated. Table 3 shows the area percent calculation based on the deconvoluted TIC. Note that the ChromaTOF software detects the presence of five peaks. Peak 82 (Cubebene) is not obvious from the TIC. Now the integration limits are set correctly and areas that are more accurate can be calculated for more reliable area percent results.

Table 3. Area Percent Calculation Based on the DTIC as Computed by Software

Peak #	Name	R.T.	Quant Masses	Area	Area %
81	cis-2,6-Dimethyl-2,6-octadiene	$214.44	DT	12,150,000	0.516
82	à-Cubebene	$215.00	DT	1,058,700	0.045
83	Phenol, 2-methoxy-3-(2-propenyl)-	$215.66	DT	1,860,200	0.079
84	Neric acid	$215.96	DT	6,133,700	0.260
85	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)-	$216.64	DT	2,034,200	0.086

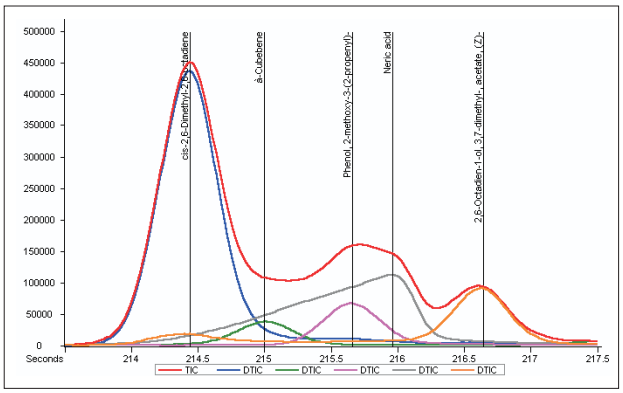

Figure 3. Chromatogram Section of Bourdonol Essential Oil (Coeluting Peaks with DTIC).

Figure 3 shows the same chromatographic section as Figure 2 with the deconvoluted TIC sections for each peak. The software can easily calculate integration limits without trying to fit gaussian shapes in the coelution (to calculate areas) as some other deconvolution packages do. The areas are calculated on the actual shape of the peak. Note that the Neric acid peak is fronting and does not have a gaussian shape, but the software gives a good area percent calculation.

A more extreme case is shown in Figure 4 for a pair of peaks that coelute very closely in the region of 160.5 and 162.5 seconds. In addition to the close coelution, the concentration difference between the peaks is substantial. In this case, most chromatographic platforms would not detect a coelution since the TIC shows a well-defined peak. Using the TIC as the quantification mass, the software calculates the areas for both peaks the same since it cannot define inflection points or valleys for close coeluting peaks. Table 4 shows the area percent calculation using the TIC while Table 5 shows the peak areas based on the DTIC.

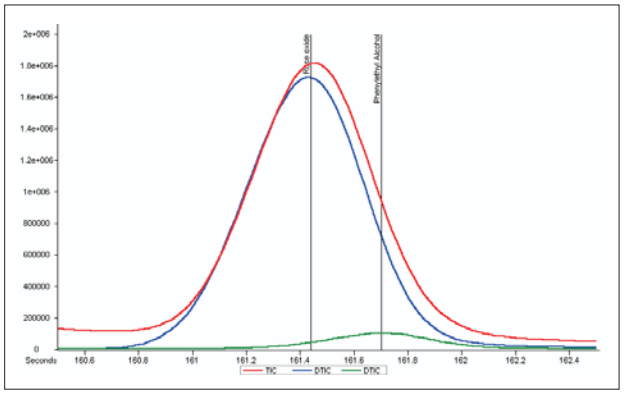

Figure 4. Chromatogram Section of Bourdonol Essential Oil (Closely Coeluting Peaks).

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Table 4. Area Percent Calculation Based on the TIC as Computed by Software for Above Peaks

Peak #	Name	R.T.	Quant Masses	Area	Area %
44	Rose oxide	161.44	T	49,124,000	2.0366
45	Phenylethyl Alcohol	161.70	T	49,138,000	2.0372

Table 5. Area Percent Calculation Based on the DTIC as Computed by Software for Above Peaks

Peak #	Name	R.T.	Quant Masses	Area	Area %
44	Rose oxide	161.44	DT	46,809,000	1.981
45	Phenylethyl Alcohol	161.70	DT	2,455,600	0.104

4. Conclusions

The analysis of Bourdonol Essential Oil demonstrates that accurate area percent calculations can be easily done even when chromatographic coelutions take place. By instructing the processing software, it is possible to obtain accurate area contributions from coelutions even when peaks have a non-gaussian shape. This allows the chromatography to be compressed in time so that more samples can be analyzed.

The strength of the Pegasus GC-TOFMS for the analysis of these complex mixtures lies in its automated data handling capabilities. Peak finding and spectral determination is possible due to the uniqueness of the data acquisition system, which allows up to 500 spectra/second to be collected while simultaneously obtaining non-skewed spectra (spectral continuity). Library searching and area percent determinations can be accomplished very rapidly, improving analytical results and productivity.

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