

Analysis of gin essential oil mixtures by multidimensional and one-dimensional gas chromatography/mass spectrometry with spectral deconvolution

Kevin Mac Namara^a, Jessalin Howell^b, Yongli Huang^c, Albert Robbat Jr.^{c,*}

^a Irish Distillers - Pernod Ricard, Middleton Distillery, Middleton, Cork, Ireland

^b Seagram Lawrenceburg Distillery - Pernod Ricard, Lawrenceburg, IN, USA

^c Tufts University, Chemistry Department, 62 Talbot Avenue, Medford, MA 02155, USA

Received 2 February 2007; received in revised form 10 July 2007; accepted 12 July 2007

Available online 25 July 2007

Abstract

The composition of essential oils and their mixtures used to formulate gin is usually too complex to separate all sample components by standard capillary gas chromatography (GC). In particular, minor constituents that possess important organoleptic properties can be masked by co-elution with major sample components. A solution is provided that combines gas chromatography/mass spectrometry (GC/MS) with “interactive” spectral deconvolution software. Sequential two-dimensional (2D) GC/MS is used to produce a target compound library, with orthogonal GC–GC providing the separation power required to obtain peak retention times and the corresponding mass spectra needed for the deconvolution database. The combination of these two techniques, mass spectral deconvolution and automated sequential 2D-GC/MS, offers a very effective synergy for both identifying key constituents that determine the perception of flavor and aroma *and* the quality control needed to analyze mixtures of complex essential oils.

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Keywords: Essential oils; Gin; Flavor mixtures; Capillary GC; Mass spectrometry; Automated sequential 2D-GC/MS; Retention time locking; Mass spectrometry deconvolution; Quantitative deconvolution software

1. Introduction

In this paper we demonstrate the efficiency of using automated sequential two-dimensional (2D) gas chromatography/mass spectrometry, with heart cutting (GC–GC/MS), to make industry or sample-specific libraries, which can be used to identify components in an essential oil under typical GC/MS conditions. Our aim is to show that once the library is made, one-dimensional (1D) GC/MS with the ion fingerprint deconvolution algorithms is an effective tool to identify compounds in complex mixtures and to find differences among supposedly similar samples.

Essential oils, made through distillation, expression, or extraction of plant material, are key ingredients in a wide variety of food, beverage, and personal care products. Essen-

tial oils and their blends are extremely complex mixtures that can contain hundreds of individual compounds. Such products vary in natural product composition and can contain pesticides based on where they are sourced. Analytical methods that assess total product composition are especially important, where product quality and safeness affect brand equity. Also valuable are product profiling methods that can be used to assist in new product development. Unambiguous identification of minor compounds, which contribute significantly from a sensory perspective, is extremely difficult to accomplish when high concentration matrix constituents mask their presence in the sample. For these reasons, the analysis of essential oils creates significant challenges even when the most sophisticated high-resolution capillary gas chromatography (GC) techniques and methods are used.

Multidimensional GC–GC extends the separation power over single column GC methods [1]. As components begin to separate on the first column a portion of the sample is transferred to a second column, whose stationary phase is orthogonal to the

* Corresponding author. Tel.: +1 617 627 3474.

E-mail address: arobbat@tufts.edu (A. Robbat Jr.).

first column. Only that portion, cut to the second column and freeze-trapped, is allowed to elute with prior and post cut sample portions sent to waste. The more dissimilar the stationary phases the greater is the gain in separation space and, thus, resolution [2]. In this context, the ability to obtain a clean mass spectrum for each sample peak is the goal, which cannot be achieved by the much shorter second column and faster run-times used in comprehensive two-dimensional GC (GC \times GC) applications [3–7]. Reviews of multidimensional, heart-cut, GC for petroleum, environmental pollutants, and citrus essential oils have been published [8–10].

One of the first successful multidimensional GC–GC total product analysis applications was a tobacco essential oil in which the open/close operation of the split vent, cold trap, and heart-cut was automated as well as each oven's temperature program and data acquisition [11]. A total of 23 successive injections were used to identify 306 compounds by mass spectrometry (MS), matrix isolation FTIR, and flame ionization detection (FID). Remarkable was the number of compounds identified given the level of commercial instrument sophistication at the time. Although total product characterization was not the goal, five heart-cuts from a single injection successfully resolved on a chiral second column an enantiometric mixture of monoterpene hydrocarbons and alcohols in lemon oil [12]. More recently, the separation of cyclic, bicyclic, and non-cyclic alcohols in C12–C13 detergent alcohols was reported [13]. More than 600 peaks were found when 26, each cut 30-s in duration, heart-cuts were made. In this paper, the authors concluded that the wealth of information obtained by automating the sequence of GC–GC injections far exceeds all other multidimensional GC techniques.

Although early mass spectral and peak deconvolution routines [14–17], statistical methods of analysis [18–20], and automated library searches [21–23] made data reduction easier, extensive sample preparation and cleanup continues to limit the rate at which samples can be analyzed by GC/MS. Multistep cleanup and/or fractionation procedures are typically employed so that high levels of non-uniform background signals do not interfere with data analysis. For example, several studies [24,25] showed for pure mass spectra the most often used probability match algorithms can identify compounds against the US National Institute of Standards and Technology (NIST) and Wiley databases with good accuracy (>75%). For two-component peaks, where peak purity was 85%, accuracy dropped to 25–50%. Although deconvolution techniques have been developed and optimized for specific compound families [26–31], they cannot be easily employed for the wide range of analytes found in essential oils and where high levels of random noise or severe co-elution occurs.

AMDIS, free from NIST, is an integrated system that extracts pure spectra from complex matrices by fitting a least-square regression model to the ion chromatogram from which the spectrum is deconvolved [32]. Although AMDIS compares the extracted ion chromatogram against the library chromatogram and identifies compounds based on the spectrum similarity approach, it cannot provide quantitative identification. In contrast, LECO's deconvolution software is a fully featured program that does provide quantitative analysis. It needs extremely high

data density peaks, produced by time-of-flight mass spectrometers [33,34], since target analysis involves finding unique unaltered target ions to build a series of simultaneous equations. Both AMDIS and LECO have been used in hundreds, if not thousands, of studies. The Ion Signature software used here is also a fully featured program. The deconvolution algorithms are quantitative and have been shown in previous work to unambiguously identify low level target analytes such as explosives, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, and pesticides in the presence of high concentrations of matrix interferences such as petroleum distillates and coal tar [35–37]. In these studies, target compounds were quantitatively identified under fast GC conditions, i.e., less than 5 min.

These considerations can now be extended to the analysis of essential oils. If spectral data are available for all major and minor compounds in the sample, then this information can be incorporated into a high resolution GC/MS deconvolution method for in-depth sample characterization. In this study, the library is created by separating the natural components in the sample. Research shows that automated sequential GC–GC, although seemingly laborious, is well-suited to the assembly of a reliable mass spectral database. A further benefit is that linear retention indices and retention time locked mass spectrometry libraries can be used as additional filters [38]. This approach offers an accessible and powerful tool for characterizing complex mixtures of essential oils in a cost-efficient manner.

2. Experimental

2.1. Samples

The initial sample consisted of a mixture of 10 different proprietary amounts of botanical and citrus oils used in the formulation of gin. Two additional samples were prepared from the initial test mixture. These samples were essentially the same as the initial test sample except that the 0.7% angelica oil was replaced with 5% or 0.7% nutmeg oil. All analyses were performed on the mixtures as is without any further sample preparation.

2.2. GC/MS analysis

1D-GC/MS analysis was performed on an Agilent 6890 GC system with an Agilent 5973 MS system (Agilent Technologies, Little Falls, DE, USA). The GC system was equipped with a Gerstel CIS-4 programmed temperature vaporization (PTV) injector (Gerstel, Mülheim a.d. Ruhr, Germany). The column used was a 30 m \times 0.25 mm internal diameter (I.D.) HP5-MS fused silica with a film thickness of 0.25 μ m (Agilent Technologies). The flavor samples were injected without prior sample preparation using an MPX Autosampler (Gerstel) equipped with a 10 μ l syringe into a deactivated glass baffled liner in the PTV injector. The injection volume was 1 μ L in all cases with an initial split ratio of 20:1 and in dilution experiments at 50:1. The initial injector temperature was 60 °C immediately followed by rapid

heating to 260 °C at a rate of 10 °C/s and held for 5 min. Between injections the PTV injector was returned to its initial temperature by a Peltier cooling unit (Gerstel). The GC was operated under temperature-programmed conditions from 60 °C initial temperature and immediately to 240 °C at an oven ramp rate of 3 °C/min to give a run time of 60 min. Helium was used as carrier gas at a constant pressure of 20 kPa. The MS system was operated in scan mode with a mass range of 40–400 *m/z* at an EM voltage of 1300 V.

2.3. Retention time locking (RTL)

For the GC/MS analysis the pneumatic head pressure for the selected column and oven temperature program was set so that the retention time of *n*-pentadecane (the locking compound) is exactly 27.50 min. This is the concept of retention time locking and is an Agilent Chemstation software routine. The software prompts for five individual runs, each at a different constant head pressure, to be performed with injections of the locking compound. This is followed by an *n*-pentadecane retention time versus inlet pressure regression calibration to allow calculation of the exact required head pressure to achieve the desired retention time of the locking compound. Once the RTL calibration is established, only a single run is needed to check and relock the inlet pressure. Under these pneumatic conditions the absolute retention time can now be used as an identification tool as all other analytes in the chromatogram will also have a predictable retention time once they are run under the same locked conditions. Using this approach a locked screener data base and corresponding mass spectral library (Agilent Technologies) containing the precise retention times and spectral information on 409 flavor compounds was used in all GC/MS analyses for compound identification where applicable.

2.4. 2D-GC–GC/MS analysis

In this setup, the GC/MS configuration used in Section 2.2 is retained except that the column was disconnected from the injector and threaded through a cryotrap for connection to the outlet of a column switching device (Gerstel) mounted in an adjoining second 6890 GC system (Agilent Technologies). This GC system was equipped with a PTV injector and a flame ionization detection (FID) system. One end of a Supelcowax-10 fused silica column, 30 m × 0.25 mm I.D. with 0.25 μm film thickness (Supelco, Sigma–Aldrich Ireland) was inserted into the PTV injector in the first oven with the other end connected to the inlet of the column switching device. Additional permanent inlet and outlet capillary lines connect the column switching device to an external pneumatics module. A mass flow controller in this unit provides a constant inlet flow of 10 mL/min and a proportional valve with a pressure sensor receives the outlet flow. The countercurrent flow across the column switching device is always active until a selected heart-cut is due to be transferred to the HP5-MS GC/MS column. The outlet proportional valve acts as a pressure programmable restrictor in order to minimize pressure differences at the column switching

device before and after heart cutting. After a heart-cut the countercurrent venting flow also provides the head pressure for the second column and this can be set to allow retention time locking identification conditions for the different compounds in the heart-cuts. This system has been previously described in detail [1].

An autosampler (MPS-XL, Gerstel) was installed on the second GC system and 1 μL injections of flavor samples were made at a split ratio of 20:1. The PTV injector was rapidly heated similar to what was described in Section 2.2. The GC system was operated under temperature programmed conditions: 60 °C for 1 min, to 260 °C at 5 °C/min, then held isothermal for 10 min. Helium was used as the carrier gas at an initial pressure of 89.0 kPa for 1 min and then programmed at 1.6 kPa/min to a final pressure of 152.0 kPa, with a hold of 10 min. This pressure program mirrors the oven temperature program and further acts to minimize pressure differences during heart cutting over the entire elution temperature range on the first column. The autosampler was programmed to allow multiple sequential injections of the sample to the first column with a different unique cut being transferred per injection to the second column. Starting from a run-time of 5 min, a total of 30 sequential cuts comprising fourteen 0.5 min cuts, followed by one 2.0 min cut, ten 1.0 min cuts and five 2.0 min cuts were in turn transferred and profiled on the main column. At 1.5 min before the start of each heart-cut the cryotrap was cooled with liquid nitrogen from an initial 260 °C to –100 °C. One minute after the end of each heart-cut, the cryotrap was heated at 10 °C/s, with trapped compounds efficiently transferred to the second column. The commencement of the cold trap heating provided the start signal for the GC/MS run. The temperature program on this column was as outlined in Section 2.2.

2.5. Deconvolution software

The Ion Fingerprint Detection deconvolution algorithms were developed at Tufts University (Medford, MA, USA). The algorithms have been packaged into a fully functional data analysis software system by Ion Signature Technology (North Smithfield, RI, USA). To create the library for this software each peak in each cut was scanned to ensure that the mass spectrum for the peak was invariant. For those peaks, where only one mass spectrum was present, four ions were selected (one main and three confirmation ions) and their relative abundance calculated without regard to compound identity. For those few peaks in which multiple mass spectra were present the same information was obtained by scanning through the peak and finding at least six consecutive scans with invariant spectra. A target compound library was created for each mass spectrum that contained a compound ID No., ions, their relative abundance, a within scan relative abundance error of 20%, and an across peak scan-to-scan relative error of 10. Additionally, each unique mass spectrum was assigned a retention window, based on its corresponding compound elution as determined by the 2D retention time locking GC/MS data. This GC/MS deconvolution method was used to analyze both the test and nutmeg oil modified samples.

3. Results and discussion

Gin is made from a relatively purified spirit of known fermentation origin. It is flavored by a number of plant materials or botanicals; the most important being juniper [39,40]. The flavor and aroma of a gin can be attributed to the quality of the spirit used and the selection and composition of botanicals. Gin can be produced either by the distillation of plant materials and essential oils or by the addition of essential oils after distillation. All gins, by definition, have undergone distillation with juniper berries or have added juniper berry oil to the refined spirit. Additionally, botanicals or essential oils such as citrus oils may be added to the gin to produce unique flavors that provide distinctiveness in the marketplace. Only a few studies have appeared in the literature elucidating the composition of gin [41–43]. The most recent study found a total of 70 compounds by headspace solid-phase microextraction (HS-SPME)-GC/MS for several distilled London Dry Gins [44].

3.1. 2D-GC analysis

Fig. 1 shows a typical FID monitor chromatogram from the wax column of the essential oil mixture. Evident from peak bandwidths is the fact that many compounds coelute. Although the figure shows the first 35-min, actual run-times exceeded 45-min. No peaks of sufficient size are observable after 34-min. Also

shown are the times of each cut from the wax column to the HP-5 column; a total of 30 cuts were made. Total ion current (TIC) chromatograms at cuts 5, 15, 22, and 30 are shown below the FID chromatogram. Evident are the relatively unresolved peak profiles on the wax column as opposed to the HP-5 profiles, which are well resolved in comparison. All cuts collected prior to 15-min, when analyzed by mass spectrometry, yield, for the most part, increasingly complex but baseline separated peaks. Although difficult to discern from the figure, it is also possible to obtain a unique mass spectrum for peaks after 15-min. At least six consecutive invariant scans are obtained through these peaks, where GC separation produced peak-to-peak valleys greater than 10%.

To obtain mass spectral information for the maximum number of components in the initial test mixture, the amount injected purposely resulted in column overload for some constituents. As a consequence, 101 unique mass spectra were found. Obvious from the data, however, was the presence of additional constituents whose concentrations did not yield the minimum requirement of at least four consecutive invariant mass spectra. These compounds were not targeted for analysis; see Table 1 for the list of compounds identified using Agilent's flavor library with retention time locking, NIST's MS and retention index library, and Wiley's MS library. Despite these libraries, 14 of the 101 compounds could not be identified, see table designations 1, 2, 3, ... This mass spectral information was collected over a 30 h period by automating the entire analytical sequence.

3.2. 1D-GC/MS analysis

Fig. 2 illustrates the 1D, 60-min HP-5, total and reconstructed ion current (RIC) chromatograms. The RIC chromatogram is the result after deconvolution of the test mixture injected into the GC/MS at the same concentration used in the 2D profiling. Notably, all mass spectral patterns for all 101 targeted constituents are untangled by the deconvolution algorithms. To test whether a commercial library might also be used to identify components in the sample, Agilent's flavor library was converted into an Ion Signature deconvolution method. A total of 30 compounds was identified from the 410 compounds in the library. In general, the match between the library and sample pattern for these compounds was poorer than the match between the 2D-GC/MS library and sample. This is to be expected since the commercial library, although based on pure compounds, was not produced from the same instrument that yielded the 2D or 1D test sample mass spectra. Moreover, some commercial libraries used relative abundance acceptance criteria as high as 40% because it is conceivable that these libraries will be used with mass filters that range from very old to brand new, from linear to ion trap quadrupoles, and from time-of-flight to magnetic sector instruments. Despite the inherent error, the Ion Signature software makes inspection of compound identity easier to interpret; see discussion below. No one library source provides all the data needed to provide comprehensive characterization of a sample. Multidimensional GC-GC/MS provides a facile means to build these libraries, while the deconvolution algorithms provide the

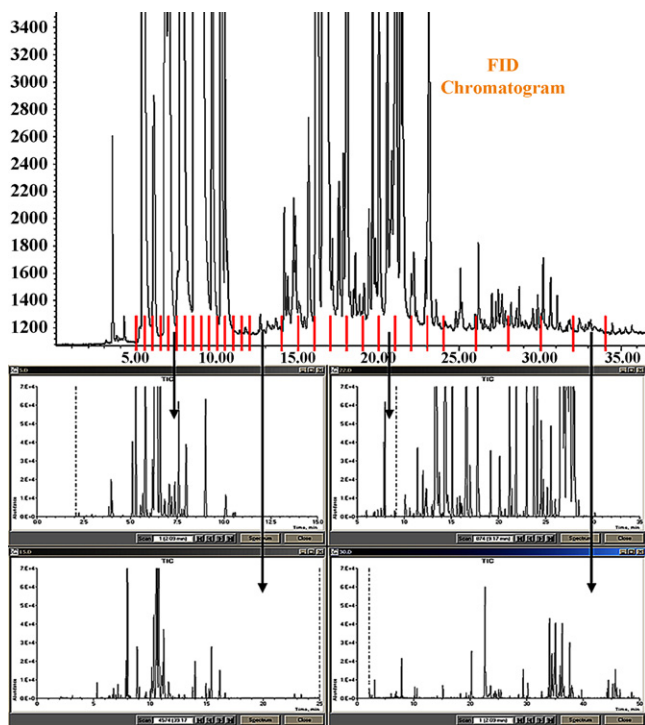


Fig. 1. FID response on the Supelcowax column of the initial test mixture comprised of 10 different proprietary amounts of botanical and citrus oils used in the formulation of gin together with the corresponding heart-cut times (red lines) for the 2D-GC/MS analysis of this mixture. Below are retention time locked total ion current (TIC) chromatograms on the HP-5 column from cuts 5, 15, 22, and 30 off of the Supelcowax column. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Table 1

The initial test sample contains a mixture of 10 different proprietary amounts of botanical and citrus oils used in the formulation of gin

No.	Compound	t_R (min)	Main ion	Ion 1 (%RA)	Ion 2 (%RA)	Ion 3 (%RA)	Ion 4 (%RA)
1	Tricyclene	5.02	93	91 (30)	79 (21)	121 (24)	
2	Thujene	5.10	93	77 (37)	79 (12)	121 (4)	
3	α -Pinene	5.23	93	91 (41)	92 (37)	77 (28)	
4	Camphene	5.68	93	121 (75)	79 (36)	107 (30)	
5	Benzaldehyde	6.09	105	106 (100)	77 (106)	51 (40)	
6	Sabinene	6.31	93	91 (45)	77 (37)	79 (26)	
7	β -Pinene	6.45	93	91 (26)	79 (23)	77 (22)	
8	Myrcene	6.78	93	69 (72)	91 (22)	79 (17)	
9	Octanal	7.20	84	57 (125)	56 (118)		
10	α -Phellandrene	7.22	93	91 (39)	77 (26)	92 (21)	
11	δ -3-Carene	7.36	93	91 (50)	77 (32)	79 (32)	
12	α -Terpinene	7.67	121	136 (53)	105 (19)		
13	<i>p</i> -Cymene	7.80	119	134 (29)	120 (10)		
14	Limonene	8.14	68	67 (75)	93 (91)	79 (42)	
15	<i>trans</i> -Ocimene	8.74	93	79 (42)	105 (20)	119 (5)	
16	2,7-Dimethyl-2,6-octadiene	8.89	69	41 (77)	82 (40)	95 (40)	
17	γ -Terpinene	9.04	93	91 (56)	136 (45)	121 (34)	
18	<i>cis</i> -Sabinene hydrate	9.34	71	93 (100)	111 (70)	121 (68)	
19	Linaloxide (<i>cis</i> , isomer B)	9.60	59	94 (60)	68 (33)	111 (44)	
20	Terpinolene	10.11	121	136 (92)	79 (40)	105 (26)	
21	Linaloxide (<i>trans</i> , isomer A)	10.23	59	94 (62)	68 (34)	81 (23)	
22	α -Pinene oxide	10.47	67	109 (62)	83 (51)	95 (30)	
23	Linalool	10.78	71	93 (86)	80 (34)	121 (29)	
24	<i>cis-p</i> -Menth-2-en-1-ol	11.28	109	79 (88)	93 (34)	137 (55)	
25	Limonene oxide	11.95	109	79 (90)	93 (95)	137 (90)	
26	<i>trans</i> -Limonene oxide epoxide	12.13	94	67 (95)	108 (70)		
27	Camphor	12.42	95	81 (72)	41 (44)	108 (42)	
28	Citronellal	12.77	41	95 (71)	67 (32)	56 (27)	
29	4-Terpineol	13.85	71	111 (50)	93 (43)	86 (27)	
30	Verbenol	14.01	100	119 (43)	137 (47)		
31	<i>p</i> -Cymen-8-ol	14.10	135	91 (24)	77 (6)	150 (14)	
32	α -Terpineol	14.40	59	93 (58)	121 (58)	136 (52)	
33	Myrtenal	14.53	94	121 (95)	105 (50)	137 (40)	
34	Ethyl octanoate	14.65	88	101 (36)	127 (28)		
35	1	14.69	121	93 (80)	105 (40)	137 (20)	
36	<i>n</i> -Decanal	14.89	57	70 (76)	68 (55)		
37	Octyl acetate	15.17	43	70 (41)	56 (37)	84 (32)	
38	2	15.24	93	71 (57)	86 (44)	111 (29)	
39	Fenchyl acetate	15.46	81	80 (43)	107 (21)	121 (26)	
40	3	16.20	112	97 (80)	83 (60)	140 (17)	
41	Citronellol	16.23	69	67 (45)	82 (46)	95 (31)	
42	Carvone	16.63	82	108 (26)	93 (31)	54 (46)	
43	Linalyl acetate	17.02	93	80 (45)	121 (40)	136 (15)	
44	Geraniol	17.06	69	41 (54)	123 (14)	53 (9)	
45	Perillaldehyde	17.79	68	79 (100)	107 (55)	135 (40)	
46	Citral	17.83	69	84 (27)	94 (16)		
47	Borneol acetate	18.36	95	93 (45)	121 (42)	80 (13)	
48	Undecanal	19.40	57	82 (78)	96 (41)	126 (32)	
49	Methyl geranate	20.08	91	43 (64)	119 (34)	92 (34)	
50	α -Cubebene	21.13	161	105 (98)	119 (93)	81 (32)	
51	Citronellyl acetate	21.20	81	95 (95)	109 (50)	123 (75)	
52	Neryl acetate	21.90	69	93 (39)	68 (40)	121 (16)	
53	α -Copaene	22.00	105	119 (98)	120 (70)		
54	4	22.29	161	119 (95)	105 (90)	81 (25)	
55	Geranyl acetate	22.71	69	93 (28)	121 (21)	136 (18)	
56	β -Elemene	23.08	93	81 (95)	68 (85)	107 (60)	
57	5	23.23	161	189 (32)	105 (62)	133 (51)	175(5)
58	Junipene	23.39	161	91 (78)	93 (52)	94 (53)	
59	α -Cedrene	23.87	119	93 (45)	105 (40)	161 (35)	
60	β -Caryophyllene	24.07	93	133 (92)	79 (76)	107 (48)	
61	6	24.43	161	105 (45)	91 (37)	119 (35)	
62	<i>cis</i> -Thujopsene	24.69	119	93 (100)	91 (40)	107 (35)	
63	γ -Elemene	24.79	121	93 (63)	107 (43)	66 (3)	
64	Aromadendrene	25.58	93	80 (29)	121 (28)	92 (16)	

Table 1 (Continued)

No.	Compound	t_R (min)	Main ion	Ion 1 (%RA)	Ion 2 (%RA)	Ion 3 (%RA)	Ion 4 (%RA)
65	2- β -Farnesene	25.77	69	93 (70)	133 (40)	161 (30)	
66	γ -Muurolene	26.36	161	93 (30)	119 (40)	204 (30)	
67	Germacrene D	26.55	161	91 (50)	119 (38)	120 (23)	
68	<i>E</i> - β -Farnesene	26.74	105	161 (80)	121 (80)	147 (70)	
69	α -Selenene	27.05	161	105 (95)	189 (98)	147 (40)	
70	α -Muurolene	27.47	161	204 (60)	107 (21)	119 (45)	
71	α -Curcumene	27.54	132	119 (26)	145 (34)	159 (6)	
72	α -Chamigrene	27.71	93	109 (35)	119 (35)	161 (35)	
73	γ -Cadinene	28.10	161	105 (41)	91 (34)	133 (22)	
74	δ -Cadinene	28.48	161	189 (20)	204 (60)	105 (43)	
75	Calamenene	28.68	119	105 (70)	161 (58)	91 (28)	
76	α -Cadinene	28.76	161	105 (53)	133 (50)	147 (17)	
77	<i>E</i> - γ -Bisabolene	28.87	105	161 (67)	91 (30)	119 (23)	
78	7	28.99	161	122 (58)	107 (50)	91 (36)	
79	α -Calacorene	29.09	157	142 (45)	141 (25)	128 (10)	
80	Elemol	29.44	93	107 (67)	121 (52)	135 (35)	
81	8	29.60	123	91 (102)	105 (96)	131 (69)	
82	Nerolidol (<i>E</i> -)	30.01	69	93 (75)	107 (44)	71 (43)	
83	<i>cis</i> -3-Hexenyl benzoate	30.20	111	95 (25)	123 (30)	161 (25)	
84	Spathulenol	30.54	91	119 (90)	79 (85)	159 (70)	
85	Caryophyllene oxide	30.72	79	93 (85)	121 (36)	161 (12)	
86	Globulol	31.50	122	107 (95)	161 (80)	189 (35)	
87	Viridifloral	31.74	109	96 (95)	81 (50)	123 (45)	
88	9	31.89	105	91 (100)	131 (100)	145 (70)	
89	Guaiol	32.09	109	93 (35)	161 (35)	179 (30)	
90	10	32.47	119	105 (50)	161 (65)	179 (25)	
91	<i>epi</i> - α -Cadinol	33.03	161	119 (36)	91 (26)	189 (11)	
92	<i>z</i> -Asarone	33.53	95	121 (95)	105 (50)	161 (60)	
93	11	33.74	157	142 (24)	175 (46)	105 (21)	
94	Cadalene	34.31	183	168 (29)	153 (17)	105 (16)	
95	12	34.75	159	91 (80)	145 (35)	220 (55)	
96	Juniper camphor	35.22	93	91 (30)	133 (30)		
97	13	35.95	162	149 (66)	147 (66)	121 (67)	
98	α -Sinesal	37.61	93	91 (50)	79 (50)	107 (50)	
99	14	41.40	149	205 (2)	150 (9)	223 (8)	
100	Cembrene	44.37	69	91 (35)	119 (25)	272 (14)	
101	Cembrene E	45.50	69	93 (53)	229 (16)	272 (9)	

Each sample component, identified by means of a 30-h 2D-GC/MS separation, is listed along with its corresponding retention time, target ions, and relative abundances.

means to identify compounds despite ion ratios that might differ from ideality.

Two examples of how “interactive” deconvolution can help the analyst ensure positive compound identification are discussed below. In each example four ions, viz., a main and three confirming ions, are used to identify each target compound. Fig. 3 shows the TIC and RIC chromatograms for terpinolene (compound 20) and the trans A isomer of linaloxide (compound 21). Despite the peak maxima for these compounds differing by less than 5 s, deconvolving their mass spectral fingerprint is straightforward when little ion fragment overlap exists. For example, only one terpinolene ion at m/z 79 will experience interference from fragments of the linaloxide, trans A isomer, see corresponding table in the figure. In contrast, all three trans-linaloxide confirmation ions at m/z 94, 68, and 81 will encounter interference from the same ions produced by electron impact ionization of terpinolene. Without deconvolution these ions will be skewed in intensity as will the peak shape adding increased uncertainty of its presence in the sample. With the ion fingerprint deconvolution algorithms, actual and expected relative abundances are well within the accepted

mass spectrometry error ranges for assigning compound identity.

Fig. 4a and b reveals the scan-to-scan variance in m/z ion signals across the chromatographic peaks of terpinolene and linaloxide, respectively. Also shown are the retention window, yellow/green dashed lines, within which each target compound is expected to elute. In these figures each ion is assigned a specific color by the software. Each set of four bars represents each compound's target ion signal at a specific scan in the peak; royal blue depicts the main ion and green, aqua blue and red the confirmation ions. As expected, contribution to m/z 79 (aqua blue bar) from linaloxide to terpinolene appears on the right-hand side of the peak in Fig. 4a. Without deconvolution the ion signal on the right-hand side of the peak will be much higher than that found with deconvolution, since the algorithms minimize signal interference from fragments of co-eluting compounds that have the same m/z as the target compound.

Visualization of an ideal deconvolved peak is shown for the trans A linaloxide isomer, where matrix interference by terpinolene at all confirming ions is easily handled by the algorithms (see Fig. 4b). After deconvolution the algorithms scale the confir-

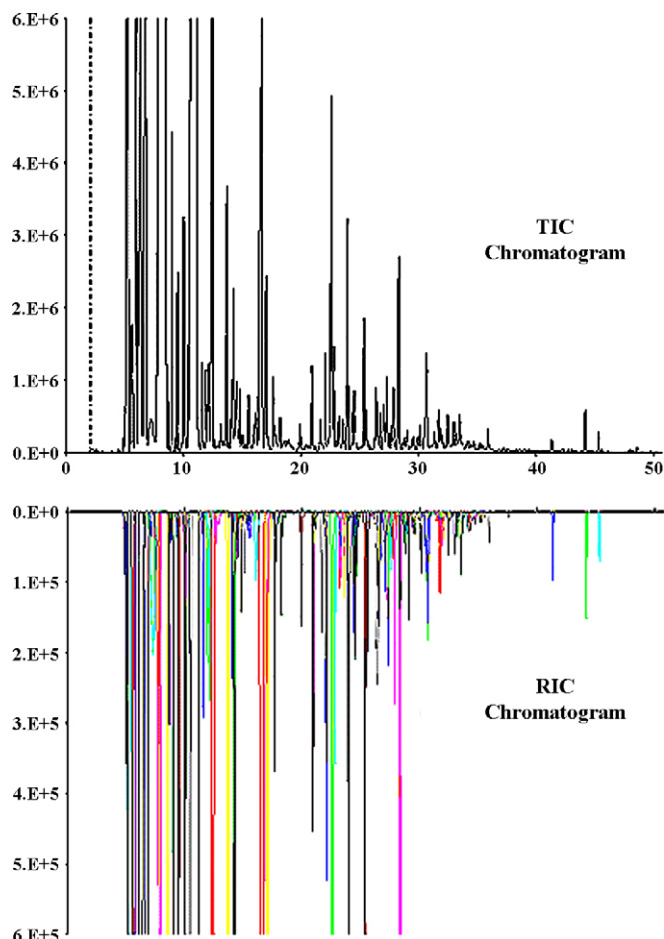


Fig. 2. GC/MS total ion current (TIC) and its reconstructed ion current (RIC) chromatogram after deconvolution of the initial test mixture under retention time locked conditions on HP-5 stationary phase. Target ions were summed so that low level analytes can be seen in the RIC trace.

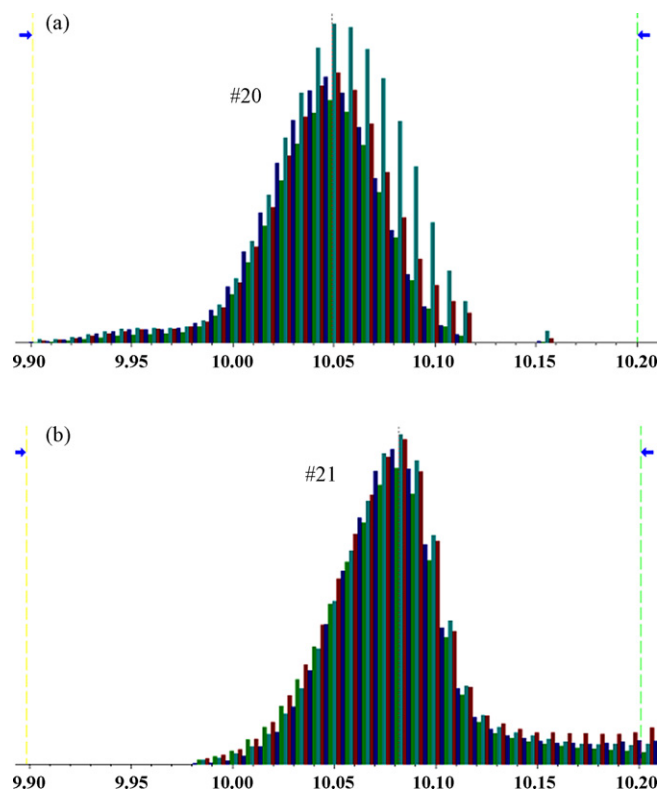


Fig. 4. (a and b) Detailed views of each RIC scan after deconvolution for terpinolene (No. 20) and linaloxide (No. 21). The blue bar depicts the signal response for each compound's main ion at each scan in the peak. Each subsequent colored bars (there are three of them) shows the signal response of each compound's confirmation ions scaled to the main ion, see table in Fig. 3.

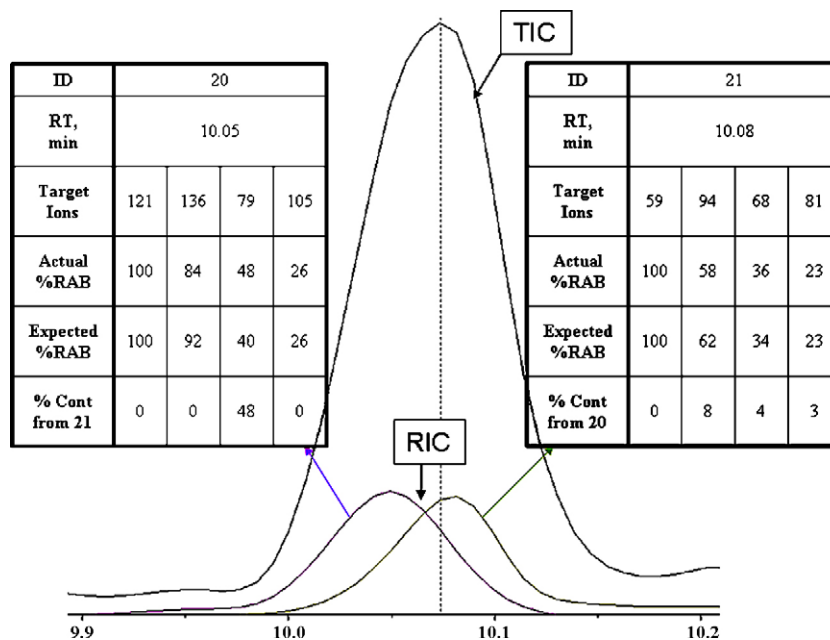


Fig. 3. Ion Signature mass spectral deconvolution of terpinolene (No. 20) and trans A isomer of linaloxide (No. 21), see the figure for retention time location. The expanded figure reveals the underlying chromatographic overlap, each compound's deconvolved retention time, the expected and actual target ion percent relative abundances (% RAB), and the amount of ion interference one compound exerts on the other.

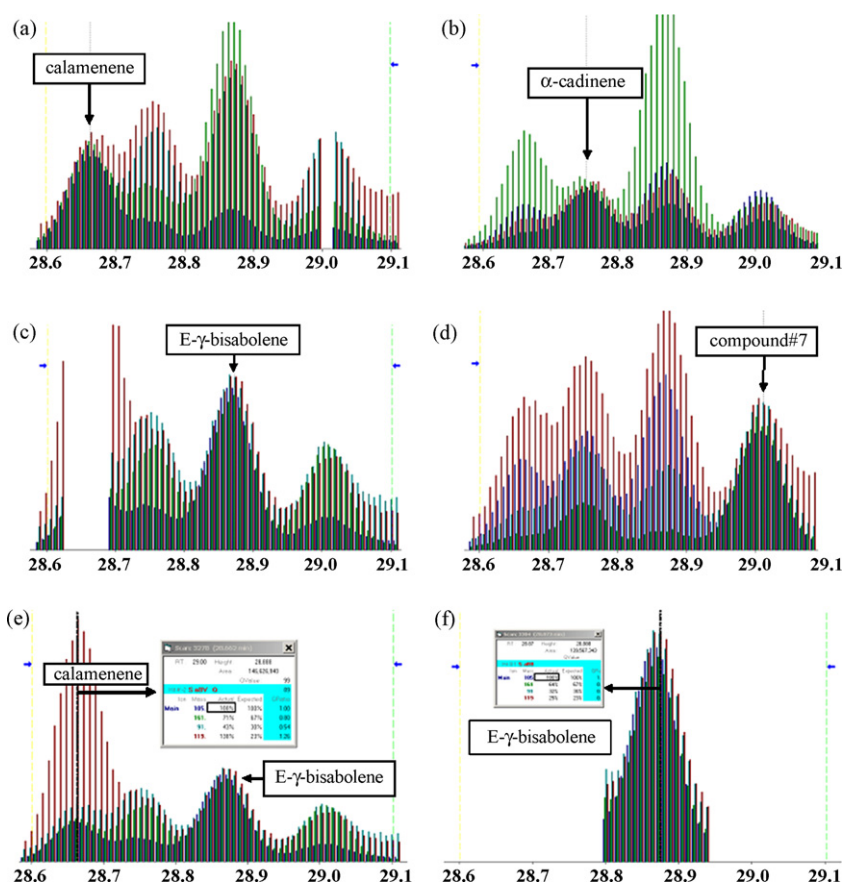


Fig. 5. (a–d) Although calamenene, α -cadinene, E- γ -bisabolene, and compound 7 have common fragment ions, their relative abundances are different. When the acceptable scan-to-scan variance for calamenene is set at 10, all target ions appear in (a) except peak 4. Similarly, when the scan-to-scan variance for E- γ -bisabolene is set to 10, all target ions appear except for peak 1, see (c). Only one compound for each peak possesses the correct target ion relative abundances as shown in the figure. (e and f) When the acceptable scan-to-scan variance for E- γ -bisabolene is widened to 15; see (e), all ions appear in the figure but as expected only peak 3 has the correct target ion relative abundances. When the acceptable scan-to-scan variance for E- γ -bisabolene is narrowed to 3; see (f), only peak 3 ions appear because they have the correct target ion relative abundances.

mation ions to the main ion, which yield an “ideal” peak having the same signal height (i.e., a flat histogram) for all target ions at a given scan making it easy to visually discern matrix effects.

Reliance on these algorithms to correctly identify target compounds is further illustrated in the next example. The detailed compound view is shown in Fig. 5 for four compounds that elute within 0.5 min of each other and have a peak-to-peak separation difference of only 0.35 min. These ranges are within expected retention time shifts due to normal instrument control instability, column aging, and/or high concentration sample matrix effects. Although relative retention time or retention index systems are used to minimize these variations, shifts in retention times, nonetheless, make it extremely difficult for an analyst to rely solely on libraries as filters to identify constituents in complex samples. Because the Ion Signature software is interactive, the analyst can select the desired scan-to-scan variance deemed acceptable. The relative error set in the deconvolution method establishes how well each scan must agree with all other scans in the peak for a given compound to be considered present in the sample.

When the four compounds shown in Fig. 5 are fragmented, the target ions for these compounds in Table 1 are produced. However, only one compound for each peak possesses the cor-

rect relative abundance at every scan. For example, only peak 2 in Fig. 5b contains the correct relative abundance for all of the α -cadinene target ions at each scan, while peaks 3 and 4 contain one or two confirming ions, respectively, with the correct relative abundances depending on which side of the peak one inspects. This example points to the inherent fallacy of relying on a single ion for confirmation in selected ion monitoring (SIM) mass spectrometry.

Most commercial libraries cannot easily change the acceptance criteria for the relative abundance match, e.g., some libraries use acceptance windows as high as 40%, nor do they have criteria for accepting the scan-to-scan variance when identifying compounds in a sample. Because of these limitations, it is easy to misidentify compound presence or absence. In contrast, the Ion Signature software allows the analyst to set both of these criteria. In the example above the relative error was set at 10 for each compound. Suppose, instead, the acceptance criteria are widened to make data acceptance more inclusive. If the relative error is set at 15 for E- γ -bisabolene, peak 3, the missing ions in Fig. 5c appear in Fig. 5e, which in the absence of deconvolution increases the potential for mistaking E- γ -bisabolene for calamenene when retention shifts occur; especially if the wrong ions are selected as confirmation ions. Confirmation ions at m/z

Table 2

Lists the 20 compounds found in nutmeg oil with their retention times, target ions and relative abundances

No.	Compound	t_R (min)	Main ion	Ion 1 (%RA)	Ion 2 (%RA)	Ion 3 (%RA)
1	15	13.71	167	81 (60)	93 (50)	139 (45)
2	Solanone	16.70	93	121 (70)	136 (65)	
3	Linalyl acetate ^a	16.89	93	80 (36)	121 (34)	
4	Safrole	18.43	162	104 (39)	131 (35)	135 (31)
5	Eugenol	21.51	164	103 (25)	77 (24)	149 (32)
6	Methyl eugenol	23.55	178	163 (27)	147 (28)	103 (23)
7	α -Bergamotene	24.65	119	93 (101)	105 (28)	77 (30)
8	(<i>E</i>)-Isoeugenol	25.50	91	77 (100)	121 (95)	
9	Methyl isoeugenol	27.41	91	79 (60)	147 (42)	
10	Elemicin	29.85	208	193 (55)	209 (13)	91 (10)
11	Spathulenol ^a	30.60	205	119 (70)	105 (59)	159 (55)
12	Caryophyllene oxide ^a	30.77	79	93 (100)	109 (50)	121 (40)
13	Ethyl laurate	31.33	88	101 (55)	157 (25)	
14	Methoxyeugenol	31.82	194	133 (22)	131 (22)	
15	B-Eudesmol	33.47	93	91 (100)	149 (100)	59 (100)
16	Myristic acid	38.20	73	129 (70)	185 (60)	228 (50)
17	Ethyl myristate	38.88	88	101 (47)	43 (20)	41 (16)
18	Isopropyl myristate	40.00	228	211 (60)	185 (60)	102 (60)
19	Palmitic acid	45.00	73	129 (60)	256 (49)	
20	Ethyl palmitate	45.76	88	101 (60)	43 (27)	41 (21)

^a Denotes compounds found in the initial test mixture.

161 and 91 are in reasonable agreement with the main ion given the wider acceptable error range. On the other hand, the signal at m/z 119 is more than 100% above the main ion threshold even after deconvolution. Again, reliance on the wrong confirming ion(s) will result in misidentifying target compounds, which is the hallmark analytical approach in SIM analysis. If the acceptance criterion is tightened (less inclusive) by setting the relative error to three for *E*- γ -bisabolene, Fig. 5f is obtained, with all other potential candidate compounds eliminated.

When the same mixture of essential oils is analyzed at a 50:1 split ratio as compared to the initial experiments at 20:1, all target compounds are still found in the sample. As less analyte is put on-column low level constituents are typically lost to instrument noise and not chemical noise. The fact that low level targets are still found in the presence of high concentration constituents allows the analyst to find key compounds that may be important to better understanding sensory science and quality control issues.

Table 2 lists the components found in nutmeg oil. Three of the 20 compounds are common to both the initial test mixture and the nutmeg oil. Mass spectral target and confirmatory ion abundance data for these twenty compounds were incorporated into a new deconvolution method. Fig. 6 shows the TIC signal after 1D-GC separation on the HP-5 column for the modified test mixture after the final solution was made to contain 0.7% nutmeg oil. Also shown is the corresponding deconvolved RIC trace of the nutmeg compounds only and after deconvolution. When compared to Fig. 2, the deconvolution software selectively picked out the nutmeg compounds only. To test this finding further the modified oil was fortified with additional nutmeg oil so that the final concentration was 5%. No new peaks were observed. The ability of the software to distinguish low levels of ingredients serves as an important tool for industries where profiling complex flavors and fragrances are important. In addition, this function of

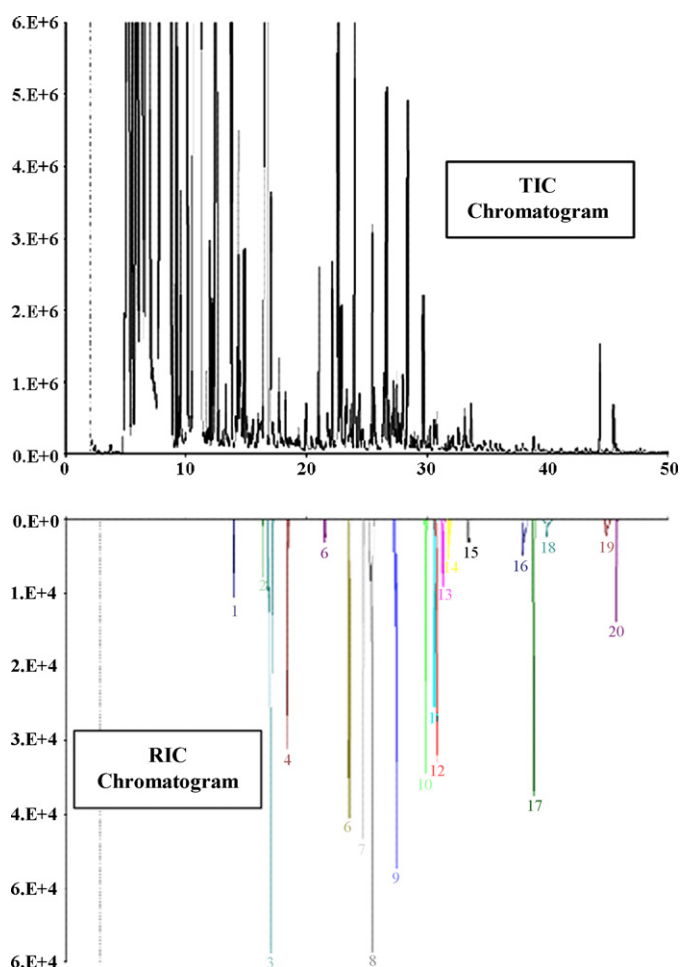


Fig. 6. GC/MS total ion current (TIC) and reconstructed ion current (RIC) chromatograms of the 0.7% nutmeg modified test mixture under retention time locked conditions on the HP5 stationary phase. The initial test mixture was modified by substituting nutmeg oil for one the 10 botanical and citrus oils used to make the initial test mixture.

the software is of practical importance for quality control by providing a screening tool for raw materials characterization.

4. Conclusion

Multidimensional GC/MS with automated sequential heart-cutting is an excellent tool to build custom libraries of compounds present in essential oils and other natural products. Once the mass spectrum, retention time window, and/or retention index for each component is known, the deconvolution method is constructed. Then, individual compounds can be identified under 1D-GC/MS operating conditions using the Ion Signature software. This means the automated sequential 2D process only needs to be done once per sample matrix. This procedure is altogether more productive than solely relying on the additional resolution provided by any 2D-GC technique or relying on long 1D-GC run-times. In fact, peak resolution becomes much less important when this software is used once the initial 2D mapping of the sample profile has been completed.

In this study, we found 101 unique mass spectra that belong to compounds from a mixture of essential oils used in making gin, whose resultant ion fingerprints under GC–GC separation produced invariant signals in at least four consecutive scans across the peak. These same fingerprints were found in the initial test mixture and a modified mixture where one of the 10 botanical and citrus oils was exchanged with nutmeg oil. In addition to identifying the constituents of the remaining initial test mixture, 17 of the 20 additional components in nutmeg were identified in the modified mixture. The peaks for the three common compounds increased in signal count as would be expected from fortifying the sample. Because the acceptable scan-to-scan variance in the relative abundance can be set in the deconvolution method, it is easy to visually inspect the peak to determine whether the target compound has been correctly identified. This makes total product characterization, shelf-life aging studies, routine quality control, and consumer flavor/aroma perception studies amenable to low level compound identification in the presence of large concentration co-eluting matrix components. The deconvolution software used in this study allows the analyst to optimize spectral search matching conditions. This approach can lead to the identification of low level analytes difficult to identify without extensive sample cleanup procedures.

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