Tracking juniper berry content in oils and distillates by spectral deconvolution of gas chromatography/mass spectrometry data

Albert Robbat Jr.*, Amanda Kowalsick, Jessalin Howell[1]

Tufts University, 62 Talbot Ave., Medford, MA 02155, USA

A B S T R A C T

The complex nature of botanicals and essential oils makes it difficult to identify all of the constituents by gas chromatography/mass spectrometry (GC/MS) alone. In this paper, automated sequential, multidimensional gas chromatography/mass spectrometry (GC–GC/MS) was used to obtain a matrix-specific, retention time/mass spectrometry library of 190 juniper berry oil compounds. GC/MS analysis on stationary phases with different polarities confirmed the identities of each compound when spectral deconvolution software was used to analyze the oil. Also analyzed were distillates of juniper berry and its oil as well as gin from four different manufacturers. Findings showed the chemical content of juniper berry can be traced from starting material to final product and can be used to authenticate and differentiate brands.

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1. Introduction

Botanicals and their essential oils are complex natural products, which contain hundreds of organic compounds often added to flavor foods and beverages and provide aroma. Essential oils are also promoted aggressively as anticancer, antidiabetic, antibacterial, antiviral, and antioxidants as well as potential agents to prevent and treat cardiovascular diseases [1–7]. Nonetheless, even when clinical trials aimed at determining efficacy are performed disputes exist over outcomes. For example, lavender oil is thought to reduce stress and tension. In a recent study, the authors claimed the oil did not act as a mood enhancer nor did it reduce the pain or stress of the participants [8]. Based on this finding, an argument ensued in the literature questioning why the authors did not analyze the oils by gas chromatography/mass spectrometry (GC/MS) [9]. The assumption being that the oils used were somehow different from lavender oils used in other studies that demonstrated efficacy. The authors countered with the following argument: GC/MS is not commonly used in trials nor can it provide sufficient clarity as suggested by the critics [10]. Both authors are correct. GC/MS cannot provide the separation needed to quantitatively identify all components

in a complex essential oil nor can it delineate subtle compositional differences from the same or different suppliers. On the other hand, without this information how can one draw definitive conclusions or inferences of potential mechanisms if the sample itself is unknown? How can outcomes be correlated when an essential oil is compared against individual components to assess synergistic effects, potential mechanisms of action, or therapeutic value?

In 2002, the U.S. passed into law the requirement that all food, beverage, and pharmaceutical companies compile and track the genealogy of their products, which the government defined as the chemical characterization of raw materials to final products [11]. The law also requires the government to develop instrumentation and methods to detect adulterants no matter the complexity of the sample. The aim is to ensure product quality, authenticity, and safety. Although the International Organization for Standardization (ISO) offers some guidance, no regulatory mandate exists to use specific procedures. Our aim is to develop the knowledge to meet these challenges.

Toward this end, the combination of automated sequential, multidimensional gas chromatography/mass spectrometry (GC–GC/MS) and spectral deconvolution is explored for building comprehensive retention time and mass spectrometry libraries of essential oil compounds. Multidimensional GC employing two dissimilar stationary phases provides far more efficient separation than a single column, where resolution increases only when column length increases significantly. For example, doubling the column length increases resolution by 1.4 and leads to loss of low concentration analytes if band broadening occurs. This is a problem for low

* Corresponding author. Tel.: +1 617 627 3474; fax: +1 617 627 3443.
  E-mail address: albert.robbat@tufts.edu (A. Robbat Jr.).
  [1] Formerly of Pernod Ricard-Americas, for additional information contact David Doyle, VP – Quality and Research & Development, david.doyle@pernod-ricard-americas.com.

concentration, high sensory organics that contribute to flavor and aroma. By automating the GC–GC/MS process, portions of the sample from pre-determined time intervals are injected from the first column onto the second column. In contrast to studies that transfer multiple heartcuts onto the second column, we chose to separate only one portion of the sample per injection. Subsequent injections are made only after the preceding heartcut has completely eluted from the second column. Automated sequential, multidimensional GC/MS offers higher resolution over other separation choices but it is time-consuming, since total analysis time is a function of the number of heartcuts and cumulative GC runtimes:

$$T(\text{min}) = \sum_{n=1}^{x} [(n + t_{\text{heartcut}}) + t_1] + t_2$$

where n is the first heartcut, t_{heartcut} is the time-period, t_1 and t_2 are the first and second column GC runtimes, and x is the total number of heartcuts defined by t_1/t_{cut}. Automated sequential GC–GC/MS offers the best opportunity for obtaining a pure mass spectrum for each compound in the sample. It is not our intention to compare different multidimensional techniques in this paper; for that see historical [12,13] and updated reviews [14–22].

Previously, we separated a mixture of several essential oils used to make gin by GC–GC/MS to determine if it was possible to detect an adulterant using spectral deconvolution software [23]. Although the mixture contained 101 compounds based on the experimental conditions employed, the goal was not to produce comprehensive matrix-specific libraries. Nonetheless, spectral deconvolution of the data correctly identified 23 compounds unique to nutmeg oil, which suggested it might be possible to track an essential oil through the gin distillation process.

In this study, we used spectral deconvolution software to identify essential oil compounds when GC–GC/MS could not produce pure spectra during the library-building process and when oil components were tracked from raw material to final product by GC/MS. The model mixture selected to study was juniper berry because it is the main ingredient in gin and is added to grain spirit as a botanical, oil, or both. In addition to gin, juniper berry is used to flavor tea, beer, brandy and marinades for meat, poultry and fish. Establishing the chemical signature of juniper berry is difficult, since its chemical content is dependent on which of the six edible plant species the oil is made from [24,25] as well as the geographic growing environment, age, size, ripeness, and isolation method [26,27]. Other plant materials that become part of the isolation process will also contribute to chemical content [28–30]. The objective of this research is to address the following two questions. Is it possible to identify every detectable compound in juniper berry oil found by GC–GC/MS by GC/MS? Is it possible to track juniper berry content through the manufacturing process and differentiate one gin from another based on the gin's juniper berry signature? No methods exist to accomplish these tasks.

2. Experimental

2.1. Juniper berry oil samples

Juniper berry oils from the same manufacturer but different batch lots were refrigerated and analyzed as received by GC–GC/MS. A sensory expert examined each sample prior to analysis to determine their usability to make gin.

2.2. Distillate and gin samples

Prior to distillation, both the oil and berries were soaked in ethanol for two hrs and tested by a sensory expert for manufacturing acceptability. The oil mixture contained 47 μL oil, 493 mL

Table 1
GC–GC/MS columns, temperature and pressure programs.

Gas chromatograph
Injector and oven temperatures 240 °C
2 μL splitless injection
Cryotrap/thermal desorption unit (freeze trap −150 °C, ramp 25 °C/s to 240 °C (10 min))
Column 1 – Rtx-Wax (polyethylene glycol), 30 m × 0.25 mm I.D., 25 μm film thickness
Pre-heartcut
Initial temp 60 °C (2 min), ramp 4 °C/min to 220 °C (10 min)
Initial pressure 31.37 psi (2 min), ramp 0.45 psi/min to 49.45 psi (10 min)
Nominal initial helium flow rate 0.7 mL/min,
Post-heartcut
Initial temp 220 °C, ramp 80 °C/min to 60 °C (2 min), then 3 °C/min to 220 °C (10 min)
Initial pressure 49.45 psi, ramp 8.92 psi/min to 31.37 psi (2 min), then ramp 0.33 psi/min to 49.45 psi (10 min)
Average velocity 29 cm sec
Column 2 – Rxi-5MS (95% dimethyl/5% diphenyl polysiloxane), 30 m × 0.25 mm I.D. column, 25 μm film thickness
Pre-heartcut
Initial temp 60 °C (2 min), ramp 4 °C/min to 220 °C (10 min)
Initial pressure 26.10 psi (2 min), ramp 0.38 psi/min to 41.60 psi (10 min)
Helium initial flow 1.5 mL/min
Post-heartcut
Initial temp 220 °C, ramp 80 °C/min to 60 °C (2 min), then 3 °C/min to 240 °C (10 min)
Initial pressure 41.60 psi, ramp 7.60 psi/min to 26.10 psi (2 min), then ramp 0.29 psi/min to 43.90 psi (10 min)
Average velocity 49 cm/s
Mass spectrometer
Solvent delay 50 min
Heated transfer line 280 °C
Source 230 °C and quadrupole 150 °C temperatures
Scan range 30–350 m/z at 8 scans/s
EM voltage 1952

spirit, and 267 mL water. Berries (18.4 g) were soaked overnight in 493 mL spirit and 267 mL water. The distillates contained 666 mL of final product. The analysis of four gins produced by the same manufacturer over a two-year period produced manufacturing precision data. The analysis of gins obtained from four different manufacturers produced the data to address the question of whether it is possible to differentiate one manufacturer's gin from another based on juniper berry content. In these experiments phenanthrene-d10 served as the internal standard (Mix #31006 from Restek Corporation, Bellefonte, PA, USA). All samples were analyzed by GC/MS.

2.3. Juniper berry oil analysis

Table 1 lists the columns and instrument operating conditions for the GC–GC/MS work. Agilent (Little Falls, DE, USA) models 6890N/5975C GC/MS were used with a Gerstel (Mülheim an der Ruhr, Germany) MPS 2 autosampler. The GC door was modified to house two low thermal mass column heaters. The columns, wrapped with heater and sensor wires and resistively heated by a separate control module, were connected to one another by a Dean's switch located inside the GC. The oven was held at 240 °C to heat the transfer lines that connected the injector to the first column, the first column to the Dean's switch, the Dean's switch to the second column or the flame ionization detector, as well as the second column to the MS. A Gerstel cryotrap/thermal desorber unit was used to condense the sample cut by the Dean's switch.

Also shown in the table are the operating conditions for the GC–GC/MS analysis. To obtain precise heartcuts, both columns followed the same temperature program, which ensured the pressures inside the columns were balanced. A 50-min solvent delay was employed so that the MS started with injection of the sample onto the second column. The total runtime for each heartcut was 130 min, which included the time to rinse the syringe prior to injec-

tion. Based on thirty-nine, 1-min heartcuts, the total analysis time to profile juniper berry was 3.5 days.

The same 30 m × 0.25 mm × 0.25 μm Rtx-wax and Rxi-5MS columns were used for GC/MS as for GC–GC/MS. The initial oven temperature was 60 °C (2 min) followed by a temperature program of 4 °C/min to 220 °C (10 min) for the wax column and 3 °C/min to 240 °C (10 min) for the Rxi-5 column. Helium was used as the carrier gas at 1 mL/min constant flow. A split ratio of 100:1 was used for each 1 μL injection.

2.4. Distillate analysis

Distillate samples were extracted using stir bar sorptive extraction (SBSE) and analyzed by GC/MS, equipped with a programmable temperature vapor inlet (CIS 4) and thermal desorption unit (TDU) from Gerstel. Except for the commercial gins, 2 mL of each distillate sample was diluted with 8 mL of deionized water. A 0.5 mm thick × 10 mm long PDMS coated stir bar (Twister, Gerstel) was submerged in the sample and stirred for 2 h at room temperature prior to analysis. The following sample injection conditions were employed: TDU, solvent vent 0.5 min, temperature program 50 °C, ramp 200 °C/min to 250 °C (3 min) and CIS, splitless, temperature program −150 °C (0.1 min), ramp 12 °C/s to 250 °C (3 min). The same GC/MS operating conditions employed for the oils were used for the distillates.

2.5. Data analysis software

The Ion Signature Technology (North Smithfield, RI, USA) data analysis software incorporates the spectral deconvolution algorithms developed at the university [31–33]. New developments by one of the authors (AR) improve on the previous algorithms by incorporating compound-specific filters, minimum number of consecutive peak scans for compound identification, ion background subtraction routines, and peak scan comparison algorithms. These new algorithms were tested to evaluate whether individual berry components could be identified using wide and narrow retention windows for unknowns and target compounds for the wax and Rxi-5 columns, respectively. Also compared were data analysis software made by Agilent (Chemstation) and the National Institute of Standards and Technology (Automated Mass Spectral Deconvolution and Identification System, AMDIS). A match factor of 70 between library and sample extracted ion ratios was used to determine positive identification.

3. Results and discussion

The findings described below show automated sequential GC–GC/MS produced a comprehensive library of retention times and mass spectrometry patterns for essential oils. We selected juniper berry oil as the model matrix because it's chemical content is complex and serves as a useful indicator of product differentiation.

3.1. GC–GC/MS analysis

In order to make the library we purposely overloaded the first column, wax, by injecting 2 μL of oil in splitless mode. The flame ionization chromatogram in Fig. 1 (top trace) shows the separation. Also shown in the figure are four example total ion current (TIC) chromatograms of 1-min heartcuts separated on Rxi-5 (bottom traces). This column served as the analytical column for GC/MS analysis. Evident are the wide unresolved peaks between 5–12 min and 19–27 min on the wax phase compared to the sharp peaks observed on Rxi-5. Detection of more than 20 peaks was observed for each 1-min heartcut. In contrast, sample portions 13, 14, 27,

and 30, which seemed uninteresting on wax produced 10 or more peaks on Rxi-5. This finding was surprising for sample portion 14, since the wax signal looked like instrument noise, and for sample portions 13, 27, and 30 where only a few peaks appeared on wax. For most critical pairs, compounds that coeluted on one phase separated on the other.

Every peak was inspected to meet the following criteria. First, the relative ion abundances must be constant, i.e., ≤20%, for five consecutive scans. Second, the criterion used to determine peak identity was established by selecting an acceptable scan-to-scan variance (relative error, RE). RE is the measure of how well each scan's target ions compare to all other scans in the peak. The closer the calculated value is to zero, the smaller the difference among the spectra. Third, identity filters such as Q-value, Q-ratio, and best retention time for isomers were established. The Q-value is an integer between 1 and 100. It measures the total ratio deviation of the absolute value of the expected minus observed ion ratios divided by the expected ion ratio times 100 for each ion across the peak. The closer the value is to 100, the higher is the certainty between library and sample spectra. The Q-ratio is the measure of the molecular (or base) ion to qualifier ion peak area ratios. To assert compound presence these ratios must be within 20% of the target ion ratios [13,21–23]. Table 2 lists the retention time and retention window and at least four ions and their relative abundances for 190 juniper berry compounds. Although another 20 peaks were detected, peak signals failed to meet the acceptance criteria, which is unfortunate since some of these compounds may be of sensory importance.

Fig. 2 is an expanded view of the poorly resolved peaks shown in Fig. 1, where the selectivity factor is <1.05. When four clean fragmentation patterns obtained from the left-hand side of Peak A are averaged (see mass spectrum) and used as library ions, the spectra for all Peak A scans are deconvolved from Peak B. Similarly, when clean mass spectra from the right-hand side of Peak C are averaged, the spectrum for this compound is easily deconvolved from Peak B. The reconstructed ion current (RIC) chromatograms for each compound are shown below its TIC trace. Evident from the RIC's are seven clean Peak B spectra, three on either side of the peak maximum. By averaging these spectra, the mass spectrum for compound B is deconvolved from the other two compounds. Identification occurs when the normalized ion ratios at each scan for the quantitative ion (in blue) and confirming ions (in green, aqua blue, red, and purple) appear at the same height. This occurs when the RE, Q-value, Q-ratio, and retention time meet the identity filter criteria set by the analyst. (For interpretation of the references to color in this text, the reader is referred to the web version of the article.)

Confirming ions are normalized to the main ion and are shown as a histogram at each scan in the peak, which provides an easy to interpret visual of the compound's presence/absence in the sample. When the scan-to-scan peak variance, RE, is zero, every scan is like every other scan in the peak. Only those scans that fall within the acceptance criteria appear as histograms. When this occurs, the main ion is used to quantify the analyte in the sample. The average scan-to-scan RE for compounds A and B are 1.4 and 0.8, respectively. Compared to the acceptance criterion of 5, little difference exists in the ion ratios for each scan in the peak. Without deconvolution, m/z 55 from compound B would have interfered with the right-hand scans of compound A. For example, the ion signal on the right-hand side of the peak is higher than the left-hand side; see purple bars for m/z 55. Similarly, compound C's major ions at m/z 93 and 89 as well as the minor ions at m/z 79 and 77 would have interfered with compound B's right-hand scans. The deconvolution algorithms eliminate additive ion currents to produce ion ratios that fall within the RE, Q-ratio, and Q-value criteria. This results in more accurate concentration estimates, since peak areas no longer include signal from other compounds in the matrix.

Table 2
Compounds identified in juniper berry essential oil analyzed by GC–GC/MS.

Rxi-5 RT, min	Name	Main ion	Confirming ions			
			1	2	3	4
0.951	n-Hexane[a]	57	56 (86)	41 (57)	43 (20)	
1.046	Methylcyclopentane[a]	56	69 (65)	84 (18)	41 (32)	
1.644	Methylbenzene[a]	91	92 (59)	65 (8)		
1.880	Ethyl butyrate[a]	71	88 (100)	60 (20)	73 (18)	
2.013	2,3-Butanediol[a]	45	75 (17)	43 (8)		
2.234	Furfural[b]	96	95 (98)	39 (23)		
2.286	3,3,5-Trimethyl-Cyclohexene[b]	109	67 (26)	124 (24)	81 (9)	
2.750	p-Xylene[b]	91	106 (53)	104 (29)		
2.750	o-Xylene[b]	91	106 (51)	105 (22)		
2.839	Isopentyl acetate[b]	70	55 (68)	87 (21)	61 (20)	
3.090	Styrene[b]	104	103 (46)	78 (39)	77 (19)	
3.680	Tricyclene[b,c]	93	121 (49)	136 (28)	105 (22)	79 (19)
3.798	α-Thujene[b,c]	93	92 (36)	77 (33)	136 (15)	
4.299	α-Pinene[b,c]	121	105 (91)	94 (66)	136 (64)	53 (53)
4.447	Camphene[b]	93	121 (73)	107 (29)	67 (25)	
4.521	Thuja-2,4(10)-diene[b]	91	92 (47)	119 (22)	77 (16)	
4.757	Verbenene[b]	91	119 (59)	77 (38)	105 (35)	134 (24)
5.133	Sabinene[b,c]	93	91 (44)	77 (33)	136 (19)	94 (13)
5.207	β-Pinene[b,c]	93	91 (27)	79 (22)	77 (20)	
5.273	**1**[b,c]	93	79 (65)	121 (53)	107 (45)	67 (25)
5.354	6-Methyl-5-hepten-2-one[b]	108	69 (57)	111 (41)	55 (35)	
5.738	Myrcene[b,c]	93	69 (64)	41 (61)	67 (12)	53 (11)
5.753	cis-2,6-Dimethyl-2,6-octadiene[b]	69	95 (33)	67 (14)	123 (11)	
5.812	Ethyl hexanoate[b]	88	99 (61)	101 (31)	73 (28)	
5.907	Pseudolimonene[b,c]	93	79 (36)	136 (22)	77 (16)	67 (14)
6.040	α-Phellandrene[b,c]	93	91 (59)	77 (40)	92 (35)	136 (26)
6.040	δ-3-Carene[b,c]	93	77 (28)	121 (24)	136 (22)	
6.099	**2**[b]	117	132 (87)	115 (70)		
6.180	Isoeucolyptol[b]	111	125 (58)	71 (36)	154 (45)	55 (12)
6.210	α-Terpinene[b]	121	93 (85)	136 (52)	91 (45)	
6.549	p-Cymene[b,c]	119	134 (29)	117 (14)	115 (11)	
6.844	β-Phellandrene[b]	93	77 (34)	136 (26)	79 (24)	121 (10)
6.844	Limonene[b,c]	93	68 (99)	107 (28)	94 (35)	
6.859	Eucalyptol[b]	111	139 (83)	71 (72)	84 (59)	
6.962	o-Cymene[b,c]	119	134 (31)	91 (23)	117 (13)	
6.962	(Z)-β-Ocimene[b]	93	80 (36)	92 (13)	121 (23)	136 (25)
7.250	(E)-β-Ocimene[b]	93	79 (30)	77 (27)	92 (25)	80 (16)
7.265	**3**[c]	93	79 (45)	107 (11)	121 (22)	
7.619	γ-Terpinene[b,c]	93	91 (57)	121 (39)	77 (29)	136 (52)
7.744	Ethyl levulinate[b]	99	129 (46)	101 (31)	74 (22)	144 (9)
8.024	**4**[b]	79	137 (61)	94 (50)	152 (45)	
8.556	Fenchone[b]	81	69 (35)	152 (25)		
8.637	Terpinolene[b,c]	121	136 (97)	93 (85)	105 (26)	
8.666	p-Cymenene[b,c]	132	117 (93)	115 (54)		
8.851	**5**[b]	79	59 (41)	67 (35)	85 (19)	
8.932	α-Pinene oxide[b]	67	109 (95)	137 (65)	83 (47)	95 (41)
9.116	Ethyl heptanoate[b]	88	113 (55)	101 (40)	73 (20)	
9.116	Perillene[b]	69	150 (80)	81 (80)	53 (27)	
9.131	Linalool[b]	93	55 (45)	121 (35)	80 (31)	
9.264	cis-Thujone[b]	81	110 (83)	55 (35)	152 (13)	
9.271	**6**[b]	91	92 (40)	65 (16)	63 (10)	
9.492	cis-Rose oxide[b]	139	69 (69)	83 (54)		
9.515	endo-Fenchol/exo-Fenchol[b]	81	80 (62)	93 (21)	111 (19)	
9.625	trans-Thujone[b]	110	95 (56)	81 (24)	67 (23)	
9.824	trans-Rose oxide[b]	139	69 (41)	55 (32)	83 (27)	
9.900	α-Campholenal[b]	108	93 (75)	95 (31)	67 (24)	
10.083	**7**[a]	98	111 (45)	55 (45)	83 (40)	84 (30)
10.459	**8**[b]	91	119 (98)	134 (92)	105 (36)	
10.473	trans-Pinocarveol[b]	92	91 (89)	55 (65)	83 (52)	
10.473	**9**[b]	91	119 (59)	134 (42)	105 (23)	117 (6)
10.591	**10**[a]	91	92 (94)	134 (24)		
10.599	1-Terpineol[a]	81	121 (54)	93 (50)	107 (30)	
10.643	Camphor[b,c]	95	81 (68)	108 (46)	152 (33)	
10.820	**11**[b]	79	110 (77)	95 (53)	109 (43)	
10.940	Camphene hydrate[b]	71	96 (44)	86 (42)	121 (21)	
11.668	Borneol[b]	95	110 (21)	67 (11)	139 (9)	
11.870	(3Z,5E)-1,3,5-Undecatriene[b]	79	80 (66)	150 (66)	78 (24)	
12.347	Terpinen-4-ol[b,c]	71	111 (92)	154 (36)	136 (29)	86 (28)
12.443	**12**[b]	119	134 (31)	167 (17)		
	Verbenyl ethyl ether[d]	100	119 (79)	137 (50)		
12.576	p-Cymen-8-ol[b]	135	43 (19)	91 (16)	150 (13)	
12.782	α-Terpineol[b]	121	136 (88)	59 (55)	81 (46)	
12.886	Myrtenal[b]	79	107 (72)	121 (37)	135 (32)	

Table 2 (*Continued*)

Rxi-5 RT, min	Name	Main ion	Confirming ions			
			1	2	3	4
12.974	Myrtenol[b]	79	91 (76)	108 (51)	119 (34)	
13.158	Ethyl octanoate[b,c]	88	101 (50)	127 (42)		
13.291	**13**[b]	95	93 (48)	121 (29)		
13.358	**14**[b]	112	97 (71)	167 (29)		
13.446	Verbenone[b]	107	135 (84)	91 (63)	150 (42)	
13.800	**15**[b,c]	93	139 (100)	86 (91)	111 (39)	
13.926	endo-Fenchyl acetate[b,c]	81	121 (51)	80 (39)	107 (20)	
14.471	Citronellol[b]	95	81 (93)	67 (91)	55 (70)	123 (49)
14.582	**16**[b,c]	112	97 (77)	83 (62)		
14.626	**17**[b]	119	137 (89)	117 (30)	152 (27)	
14.663	**18**[a]	137	163 (33)	152 (37)		
14.663	**19**[b]	163	133 (68)	105 (61)	135 (48)	
14.914	Carvone[b,c]	82	108 (45)	107 (40)	54 (27)	
15.017	Carvacrol, methyl ether[b,c]	149	164 (32)	150 (13)	119 (12)	
15.054	Hexyl isovalerate[b]	85	103 (98)	84 (54)	56 (47)	
15.312	**20**[a]	109	81 (40)	127 (39)	55 (19)	
15.644	**21**[b,c]	93	121 (37)	80 (27)	136 (15)	
15.880	Methyl citronellate[b,c]	69	110 (76)	95 (75)	82 (36)	
16.109	trans-Ascaridol glycol[b]	109	127 (45)	81 (20)	95 (17)	
16.854	Bornyl acetate[b,c]	95	136 (60)	121 (55)	108 (25)	154 (15)
16.950	**22**[b]	109	127 (41)	81 (34)		
17.068	Carvacrol[b]	135	150 (52)	79 (18)		
17.333	2-Undecanone[b,c]	58	71 (49)	59 (24)	85 (16)	
17.503	Terpinen-4-ol acetate[b]	121	93 (100)	136 (100)		
17.831	Tridecane[b]	57	71 (74)	86 (42)	121 (21)	
18.425	**23**[b]	112	97 (86)	83 (47)		
18.600	Myrtenyl acetate[b]	91	119 (40)	92 (30)	134 (12)	
18.683	**24**[a]	140	97 (71)	69 (59)	111 (45)	
19.082	δ-Elemene[b,c]	121	93 (68)	136 (56)	161 (36)	
19.657	α-Cubebene[b,c]	161	105 (88)	119 (84)	204 (25)	
20.048	Citronellyl acetate[b]	95	81 (81)	123 (78)	138 (48)	
20.498	α-Ylangene[b,c]	105	120 (76)	93 (59)		
20.771	α-Copaene[b,c]	161	119 (79)	105 (72)	204 (20)	
	Geranyl acetate[d]	69	68 (35)	121 (34)	136 (20)	
21.177	**25**[b,c]	93	161 (71)	81 (56)	189 (43)	
21.405	β-Cubebene[b,c]	161	105 (38)	91 (33)	119 (26)	
21.575	β-Elemene[b,c]	93	67 (67)	147 (55)	119 (37)	
21.892	Longifolene[b,c]	161	105 (62)	189 (55)	133 (46)	
22.062	Ethyl decanoate[b,c]	88	101 (60)	157 (39)	155 (36)	
22.202	α-Cedrene[b]	119	204 (36)	161 (16)	105 (16)	93 (16)
22.792	β-Caryophyllene[b,c]	133	93 (99)	91 (91)	120 (51)	
23.065	β-Copaene[b,c]	161	119 (83)	105 (70)	91 (48)	133 (41)
23.345	γ-Elemene[b,c]	121	93 (60)	107 (43)	105 (37)	
23.530	cis-Thujopsene[b]	119	105 (62)	123 (54)	133 (39)	
24.171	α-Humulene[b,c]	93	121 (38)	80 (27)	107 (18)	
24.363	**26**[b,c]	91	107 (92)	148 (66)	189 (50)	204 (43)
24.363	**27**[b,c]	161	105 (73)	147 (56)	204 (52)	189 (35)
24.584	(E)-β-Farnesene[b,c]	69	93 (71)	133 (37)		
25.285	Germacrene D[b,c]	161	105 (59)	119 (37)	79 (31)	81 (29)
25.285	**28**[b,c]	161	105 (53)	204 (38)	133 (16)	
25.536	β-Selinene[c]	105	107 (78)	189 (68)		
25.698	trans-Muurola-4(14),5-diene[b,c]	161	105 (32)	91 (25)	204 (25)	133 (14)
25.927	γ-Muurolene[b,c]	161	105 (84)	119 (57)	204 (44)	
25.927	**29**[b,c]	161	105 (69)	119 (56)	91 (54)	
26.237	α-Muurolene[b,c]	105	161 (63)	204 (39)	93 (34)	
26.296	Cuparene[b,c]	132	131 (42)	145 (35)	202 (27)	
26.775	γ-Cadinene[b,c]	161	105 (35)	119 (31)	204 (28)	133 (22)
27.115	trans-Calamenene[b,c]	159	160 (15)	202 (14)		
27.328	δ-Cadinene[b,c]	161	119 (58)	204 (58)	134 (50)	
27.520	trans-Cadina-1,4-diene[b,c]	119	105 (64)	161 (50)	121 (20)	
27.579	**30**[b,c]	161	133 (58)	135 (37)	147 (20)	
27.727	α-Cadinene[b,c]	105	91 (38)	119 (35)	133 (25)	
27.808	Selina-3,7(11)-diene[b,c]	161	204 (60)	133 (50)	107 (48)	
27.882	α-Calacorene[b,c]	157	142 (49)	141 (29)	200 (20)	156 (17)
28.236	Elemol	93	121 (56)	107 (51)	59 (35)	
28.258	**31**[b]	79	96 (96)	138 (61)	109 (43)	
28.280	**32**[b]	79	96 (93)	109 (57)	123 (46)	
28.457	Germacrene B[b,c]	121	93 (66)	105 (59)	161 (39)	
28.671	β-Calacorene[b,c]	157	142 (42)	141 (26)	200 (21)	158 (15)
28.929	Caryophyllenyl alcohol[b]	111	123 (35)	161 (34)		
29.018	**33**[b,c]	93	69 (66)	107 (65)		
29.409	**34**[b]	159	187 (64)	145 (55)	202 (35)	
29.534	Caryophyllene oxide[b,c]	79	93 (92)	91 (82)	107 (61)	
29.659	Gleenol[b]	121	222 (46)	108 (39)		

Table 2 (*Continued*)

Rxi-5 RT, min	Name	Main ion	Confirming ions			
			1	**2**	**3**	**4**
29.859	Salvial-4(14)-en-1-one[b]	128	81 (52)	91 (25)	159 (21)	
30.065	**35**[b]	93	121 (43)	107 (24)		
30.235	**36**[b]	107	122 (94)	105 (79)	189 (75)	147 (50)
30.249	Rosifoliol[b]	149	108 (74)	204 (53)	164 (24)	
30.249	**37**[b]	93	121 (104)	79 (79)	133 (65)	
30.316	Ethyl dodecanoate[b]	88	101 (53)	157 (24)	183 (32)	
30.508	Humulene epoxide II[b,c]	109	96 (90)	138 (88)	67 (53)	
30.699	**38**[b]	131	105 (83)	159 (77)	145 (60)	
30.781	1,10-di-epi-Cubenol[c]	161	119 (55)	179 (55)	105 (38)	
30.803	Junenol[b]	109	161 (71)	204 (56)	179 (44)	
30.803	**39**[b,c]	93	123 (29)	163 (18)		
31.083	α-Corocalene[c]	185	200 (66)	143 (28)		
31.238	**40**[b]	166	81 (50)	95 (37)	123 (31)	189 (21)
31.290	1-epi-Cubenol[b,c]	119	161 (82)	105 (52)	105 (41)	
31.437	γ-Eudesmol[b]	189	161 (67)	204 (65)	133 (49)	
31.592	Tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-ol, 4,4-dimethyl-[a]	136	131 (16)	91 (13)	109 (10)	
31.865	τ-Muurolol[b,c]	161	95 (56)	105 (52)	121 (47)	
31.880	α-Cadinol[b,c]	204	95 (65)	121 (65)	105 (37)	
32.071	α-Muurolol[b,c]	161	119 (51)	105 (46)	204 (31)	
32.271	**41**[b]	159	177 (50)	117 (41)	220 (38)	131 (35)
32.381	**42**[b,c]	121	95 (89)	204 (80)	161 (77)	
32.558	cis-Calamenen-10-ol[b]	157	175 (38)	203 (36)	142 (26)	158 (16)
32.883	trans-Calamenen-10-ol[b]	157	200 (29)	142 (25)	158 (14)	
32.993	**43**[b]	93	105 (77)	119 (46)	136 (38)	
33.060	Cadalene[b,c]	183	198 (42)	168 (33)	165 (22)	153 (19)
33.259	**44**[a]	159	177 (44)	131 (35)	220 (31)	
33.510	Amorpha-4,9-dien-2-ol[b]	159	220 (50)	131 (33)	177 (26)	145 (21)
33.613	E-Asarone[b]	208	193 (41)	165 (30)		
33.834	Eudesm-7(11)-en-4-ol[b]	189	204 (55)	222 (43)		
34.314	**45**[a]	107	147 (57)	135 (56)	162 (51)	
34.793	**46**[b]	162	220 (78)	149 (69)	187 (57)	202 (45)
36.564	Amorpha-4,7(11)-diene <2-α-hydroxy->[b]	220	159 (66)	187 (48)		
37.884	Ethyl tetradecanoate[b]	88	101 (70)	213 (28)		
41.292	**47**[b]	69	93 (78)	133 (62)	229 (50)	
42.200	Sclarene[b]	257	81 (94)	93 (79)	55 (49)	
43.020	**49**[b]	135	272 (84)	107 (75)	95 (69)	
43.040	**50**[a]	175	157 (46)	193 (22)		
43.195	**51**[b]	69	91 (68)	119 (57)	41 (29)	
44.102	**52**[c]	79	81 (96)	201 (31)	135 (60)	
44.287	**53**[b]	69	93 (91)	91 (65)	229 (46)	
44.707	Ethyl hexadecanoate[b]	88	101 (61)	241 (28)		
46.190	Abietatriene[b,c]	255	159 (63)	173 (62)	270 (33)	

[a] Compounds with 5 scans in 2D GC–GC/MS.
[b] Compounds detected on Rxi-5, 1 μL splitless injection.
[c] Compounds that survived distillation, found in oil/botanical distillates.
[d] Compounds found in gin due to enhancement from other essential oils/botanicals.

3.2. GC/MS analysis of juniper berry oil

Unlike the 2D experiments, where the objective was to produce a library of as many compounds as possible, the GC/MS work focused on assessing if it were possible to trace these components from starting material to final product and to determine if the data supported gin authentication. To accomplish these tasks, we injected 200-times less mass onto the column to ensure neither the stationary phase nor the detector overloaded. A total of 166 compounds was identified after deconvolution. The difference, 166 vs. 200, was the loss of analytes whose scans per peak by GC–GC/MS was six or less.

Fig. 3 illustrates the elution behavior on the Rxi-5 and wax columns; see Table 2 for compound identity. Regression of the retention times produced a Pearson cross-correlation coefficient, r, of 0.89. No compounds eluted early or late on one column concomitant with late or early elution on the other column ($r = -1.0$). Upon closer inspection, 91 of the 166 compounds detected produced elution behavior that yielded $r = 0.99$, with another 62 producing a less strong but positive relationship, $r = 0.83$. Moderate elution behavior was observed for only 13 components, $r = 0.41$.

The IST software found the same compounds on both columns even though the Rxi-5 analysis was approached as target compound analysis (i.e., with known mass spectral patterns, peak retention times, and retention windows) while the wax analysis was not (i.e., no retention time information was input into the software). The IST software correctly identified minor components in the presence of high concentration oil components such as limonene, which eluted at 6.844 min and had a TIC peak signal of 10^7 compared to β-phellandrene, eucalyptol, o-cymene, and (Z)-β-ocimene at <10^4. Table 3 shows the results of the Chemstation and AMDIS software comparison versus IST. AMDIS is the deconvolution software made by NIST. It extracts spectra by fitting a least-square regression model to the ion chromatogram from which the spectrum is deconvolved [34]. Although AMDIS and Chemstation can operate as one, findings are based on using each independently. Chemstation reported 175 compounds in the oil based on a 300 compound library. Of these, 154 are in agreement with IST, which means there are 21 false positives and 12 false negatives. In contrast, the AMDIS deconvolution software reported 96 compounds; 70 of them identified correctly, which means there are 26 false positives and 96 false negatives.
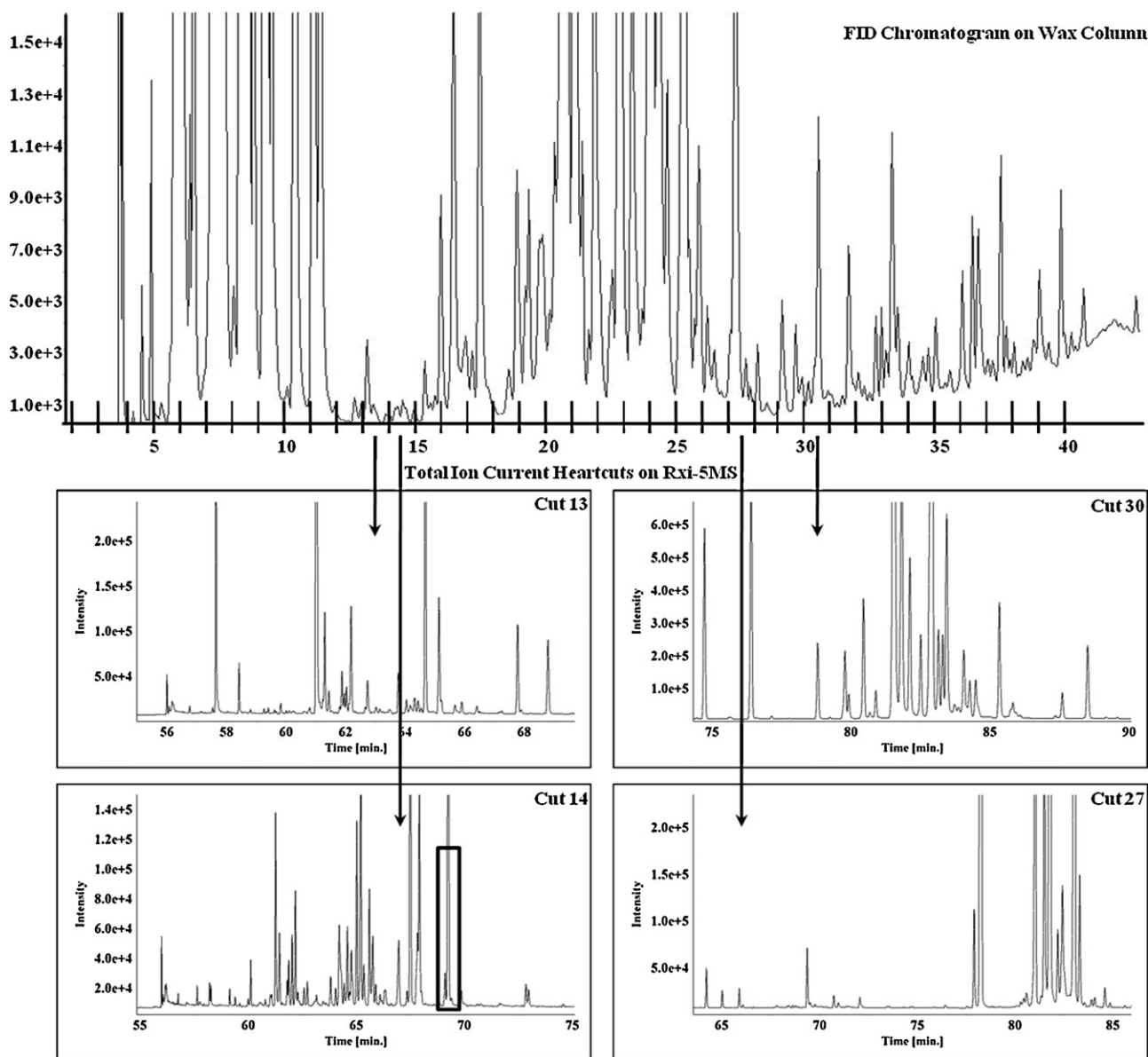
Fig. 1. GC–GC/MS of juniper berry oil and four example heartcuts.

When comparing AMDIS vs. IST peak areas, the relative percent difference (RPD) was ≤15% for 15 compounds, 13 were between 16 and 30%, with the remaining 42 compounds >30%. The peak area comparison between Chemstation and IST yielded 59 com-

pounds with RPD's ≤15%, 40 between 16 and 30%, and 50 analytes with RPD's >30%. Chemstation produced 11 positive RPD's (under-estimation) and 143 negative RPD's (overestimation). In contrast, AMDIS reported 43 and 27 underestimated and overestimated peak areas, respectively. The notably larger number of negatively biased RPD's makes it obvious Chemstation has no facility to deconvolve spectra and remove ion current signal due to the matrix, as does AMDIS when extracting target compound ion signals. Nonethe-less, Chemstation outperformed AMDIS in identifying juniper berry compounds in the oil but it will overestimate its concentration in the sample compared to AMDIS and IST.

Table 3
Juniper berry oil analysis ion signature metrics compared to Chemstation and AMDIS.

Metrics	IST	Chemstation	AMDIS (70 match factor)
Target compounds	166	154	70
RPD			
≤15%		59	15
16–30%		40	13
>30%		55	42
False positives		22	3
False negatives		12	96
Bias			
+		11	43
−		143	27

$\mathrm{RPD} = \left(\frac{(\mathrm{IST}_{\mathrm{PA}} - x_{\mathrm{PA}})}{(\mathrm{IST}_{\mathrm{PA}} + x_{\mathrm{PA}})/2} \right) \times 100$, where IST = Ion Signature Technology, x = Chemstation or AMDIS, and PA is the peak area determined by one of those data analysis software.

3.3. Oil, botanical and gin distillates

To determine which components survived distillation, oil and berry ethanol extracts were prepared, distilled, and analyzed by GC/MS. Analysis of juniper berry oil from the same manufacturer but different batch lot contained 141 compounds. Analysis of the chromatograms shown in Figs. 4 and 5 for the oil and berry distil-lates yielded 108 and 111 compounds, respectively, with 90 of them
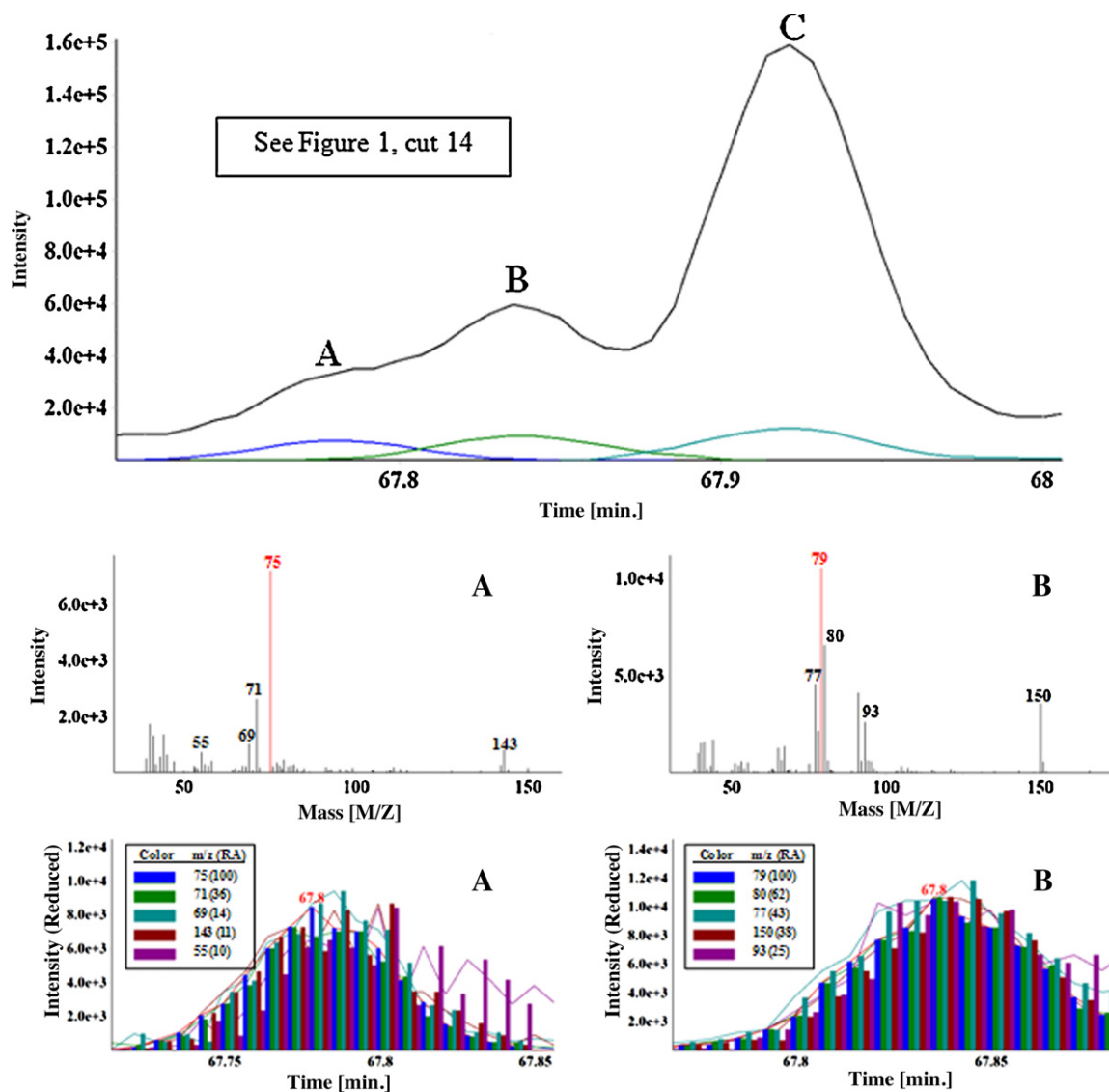
Fig. 2. Expansion of total and reconstructed ion current chromatograms for three coeluting peaks as well as the averaged mass spectra and deconvolved ion signals for peaks A, B, and C.
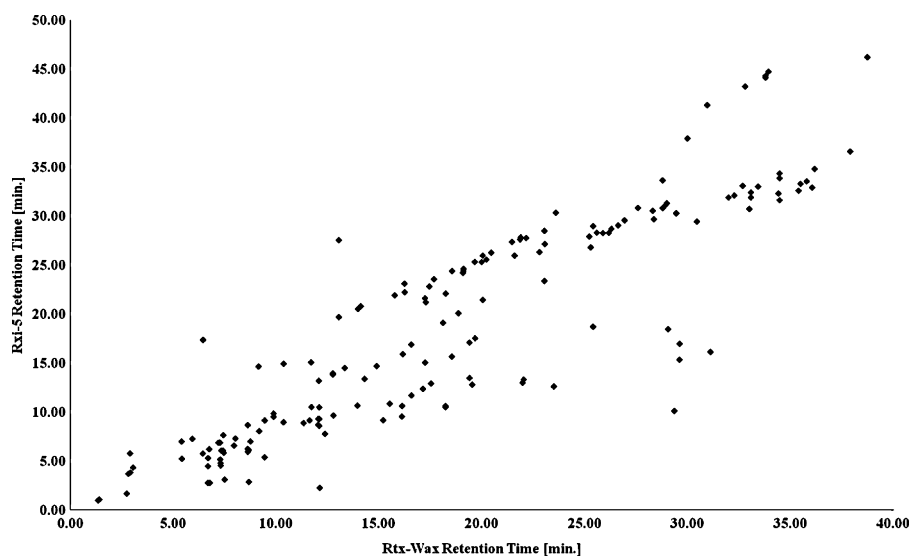

Fig. 3. Distribution of juniper berry oil components by retention time on wax and Rxi-5 stationary phases.
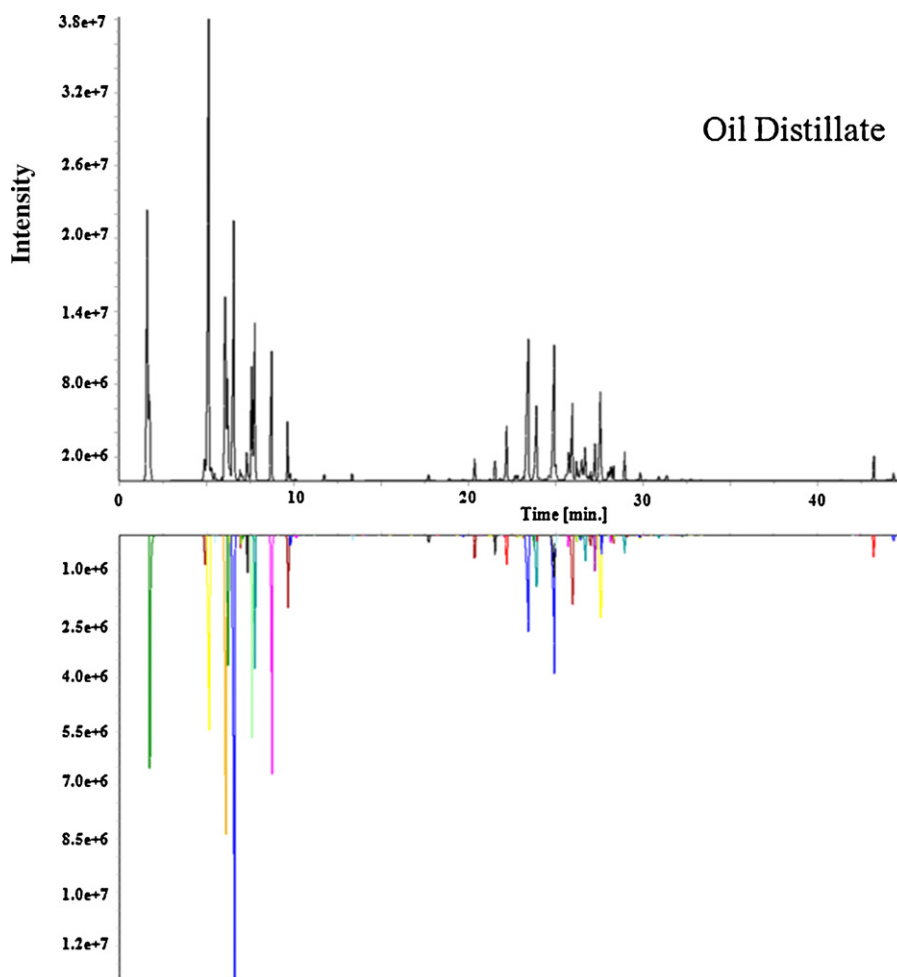
Fig. 4. Total ion current and reconstructed ion current chromatogram of juniper berry oil distillate.

common to both samples. Either the missing distillate compounds were in the heads or tails (which we did not collect) or diluted to non-detectable levels.

3.4. Gin from the same company

Analysis of four gins by the same manufacturer over a 2-year period produced relatively consistent juniper berry content. Fig. 6 shows each gin's component profile, see Table 2 for identities. We detected 69 of the 90 distilled juniper berry compounds in these gins. Only five compounds in gin are due solely to oil. Only four compounds are due solely to berries. The question of whether these few compounds influence gin flavor is outside the scope of this paper. In addition, the gins contained two juniper berry compounds, viz., verbenyl ethyl ether and geranyl acetate, not found in either the oil/berry distillates or residuals when analyzed in splitless mode. We suspect their presence is due to enrichment from other botanicals and oils used to make the gin. Fig. 7 illustrates the samples analyzed and where the juniper berry compounds that contribute to gin originate.

Trans-cadina-1,4-diene was selected as the reference compound to assess the consistency of each compound's concentration in the gins. The peak ratio for each component was computed by dividing the peak area of the compound by the reference compound peak area. For analytes found in all four gins, the peak area ratio relative standard deviation (RSD) was ≤50% for 39 compounds, between 51 and 100% for 28 components, and >100% for 9 others. High concentration volatiles produced the poorest % RSD's. Either

these compounds overloaded the column or the MS due to the time required to extract the higher boiling point organics. Work is in progress to optimize extraction conditions and to assess large volume total desorption dynamic headspace as an alternative sample injection technique, since twister extraction is selective and its efficiency is compound dependent. Nonetheless, these results suggested a manufacturing precision of 100% could account for 90% of the distilled juniper berry content in gin, which we used to distinguish juniper berry gin content produced by other manufacturers.

3.5. Gins from different companies

Since manufacturers use different essential oils, botanicals, and concentrations for gin production, we added phenanthrene-d10 to each gin prior to analysis. The internal standard served as a common reference when calculating juniper berry peak area ratios. We calculated the peak area ratio for each compound by dividing that compound's peak area by the internal standard peak area. Then, we divided the peak area ratio for each compound in gins 1–3 by the peak area ratio for the same compound in gin 4. Samples from four different manufacturers served as the basis of comparison.

A total of 80 ± 5 compounds were detected in these gins. Although the range was somewhat wider than that produced by the manufacturer above, this finding was not surprising since the final product was a function of the botanicals and/or oils to make the gin. Differences in juniper berry content was substantiated using the manufacturing precision criterion. For example, if the peak area ratio of gin 1 compounds divided by gin 4 compounds is 1 for all
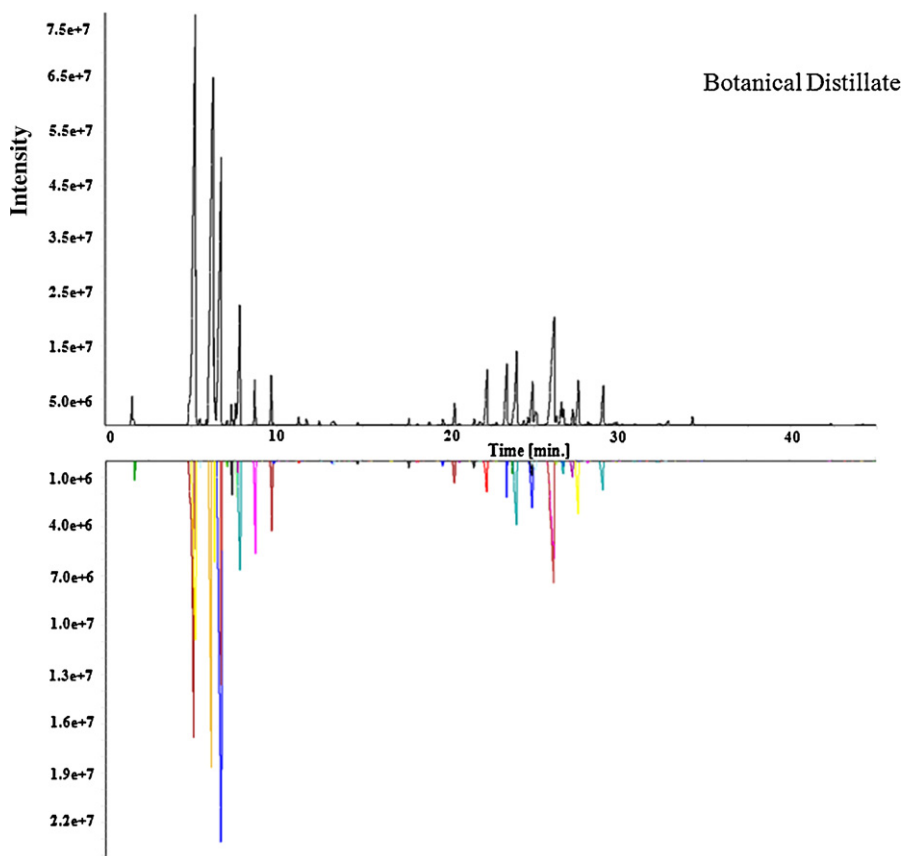
Fig. 5. Total ion current and reconstructed ion current chromatogram of juniper berry botanical distillate.

components, the juniper berry content in the two gins is the same. If, on the other hand, the ratio is >2 the concentration of juniper berry in gin 1 is materially different from gin 4. Table 4 lists the peak area ratios for gins 1–3 compared to gin 4. A total of 55 compounds in gin 1 have peak area ratios greater than gin 4, which indicates juniper berry concentration in gin 1 is much greater than gin 4, especially since 49 of the 55 compounds are more than 3.5-times gin 4.

Similarly, gin 3 contains much more juniper berry than gin 4. Of the 40 compounds, whose ratios exceed 2, 24 are greater than 3.5. In fact, gin 1 contains more juniper berry than does gin 3, since 31 of 43 compounds are 3.5-times gin 3. Gin 2 also contains more juniper berry than gin 4. However, an equal number of compounds fall above 2 and below 0.5 when gins 2 and 3 are compared, which, most likely means both gins contain about the same amount of juniper berry with additions of other botanicals and oils account-
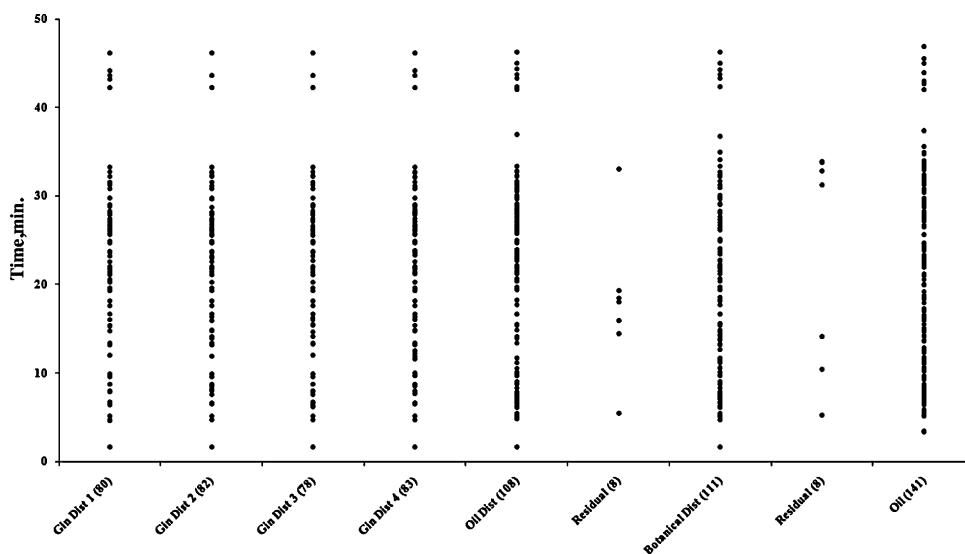

Fig. 6. Comprehensive component analysis of juniper berry oil, its distillate, the botanical distillate, their residuls, and commercial gin.

Table 4
Relative peak area comparison of four gins manufactured by different suppliers based on internal standard, phenanthrene-d10.

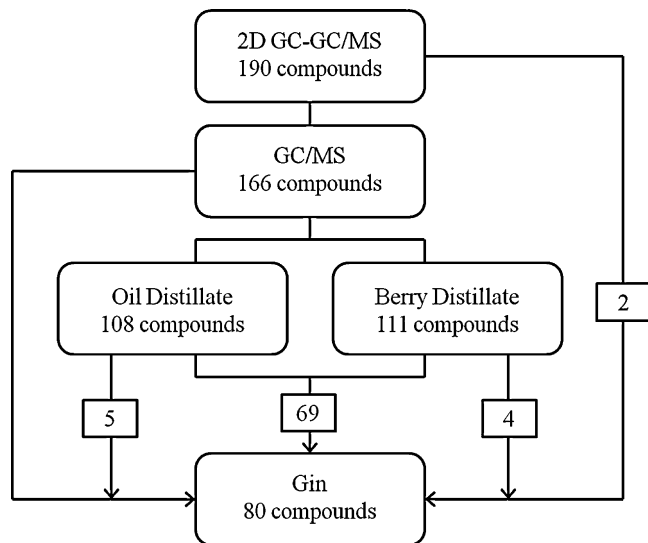|	Gin 1/Gin 4	Gin 2/Gin 4	Gin 3/Gin 4	Gin 1/Gin 3	Gin 2/Gin 3
≤0.499	5	7	5	5	26
0.5–2.0	6	34	25	20	25
>2.0	55	30	40	43	23

Fig. 7. Flowchart of samples analyzed, illustrating the juniper berry content tracked from starting oil through to the final gin product.

ing for the differences in the concentration in each gin. Work is in progress to analyze other typical gin additives to evaluate this supposition.

4. Conclusion

Experience indicates food, flavor, and personal care companies often compare products based on the ten largest peaks in the chromatogram. This type of analysis leads to erroneous conclusions due to the fact that many compounds coelute and remain unidentified in the sample. Essential oil companies trade on these perceived differences that until now were unsubstantiated. Automated sequential multidimensional GC/MS is capable of producing matrix-specific libraries of complex natural products. Spectral deconvolution of GC/MS data based on these libraries provides a reliable, unambiguous means of tracking the genealogy of juniper berry content from raw materials to final products and provides a more rationale means for detecting adulterants. Work is in progress to develop the mathematics to subtract full spectrum target compounds from the TIC trace to identify and quantify these compounds quickly and accurately.

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