Identification of Iris Scent Volatiles Using Dynamic Headspace with PDMS Foam Trapping and GC-TOFMS

The importance of when and how fragrance chemicals are extracted in order to accurately reconstitute the scent of a flower

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The alluring fragrances of flowers are the primary inspiration for new perfumes. In the quest to develop novel synthetic aroma chemicals, perfumers have increasingly relied upon the assistance of analytical chemists to help them identify major chemicals responsible for floral fragrances.

Gas chromatography with mass spectrometry detection (GC/MS) has been the method of choice for identifying fragrance chemicals in natural products. Gas chromatography is the tool for separating the numerous fragrance chemicals once they are extracted from floral scents, while mass spectrometry is used to identify the structures of the chemicals, usually by matching the mass spectrum of an eluting chromatographic peak with the mass spectra in an electronic database library. The challenge facing the analytical fragrance chemist is how to extract a representative profile of fragrance chemical that mimics the optimum aroma of the flower being studied.

How and when the fragrance chemicals are extracted profoundly affects the chemical profile obtained. Chemists have learned that picked flowers can have an entirely different fragrance profile than growing flowers.[1] Additional factors that determine the fragrance chemicals present include temperature, moisture and soil conditions, as well as the flower's stage of life (i.e., its maturity). The time of day in which sampling is performed is also critical. Optimum sampling time normally occurs when the plant's primary pollinator is most active. Floral scent analysis normally involves sampling at specific time intervals over a 24-hr period to determine maximum levels of key fragrance chemicals. The peak olfactive moment is defined as the maximum scent emission. The composition of aroma chemicals present at the peak olfactive moment is determined and is then the basis for formulating the reconstituted fragrance. Understanding the biorhythm of the particular flower being studied is fundamental to determining the peak olfactive moment and is critical to the successful artificial synthesis of the desired floral scent.

To accurately reconstitute the scent of a flower, it is necessary to capture, analyze and identify the most significant aroma-contributing chemicals that are present at the peak olfactive moment.

Methods Used to Isolate Floral Scent Chemicals

The analytical techniques used to extract/isolate fragrance chemicals can influence the types and quantities of chemicals collected for GC/MS analysis. Extracting picked flowers with solvents and/or distillation techniques is not advisable. Solvent extracts and floral distillates often lack the delicate aroma of the flower at the peak of its life cycle. Extraction techniques that require heat can generate artifacts by hydrolysis, oxidation or thermal breakdown of plant metabolites.

Headspace extraction techniques on living flowers are preferred. They can be classified as static headspace and dynamic headspace. The first headspace technique applied to collection of floral scents was static headspace and was initially applied by Dodson and Hill in 1966.[2] They were attempting to identify the aroma chemicals that attract Euglossine bees to several species of orchids. To extract the fragrance chemicals, the researchers placed orchids in a sealed jar for 30 min, then used a gas-tight syringe to withdraw a sample of the air and injected it into a GC. Static headspace methods suffer from poor sensitivity for less volatile floral scent chemicals. While crude compared to today's sophisticated sampling techniques, Dodson and Hill's approach helped direct fragrance scientists to the development of modern sensitive and accurate sampling methods.

Examples of the most common techniques currently used to extract fragrance chemicals from flowers include:

Dynamic headspace extraction: This technique usually involves enclosing the scent emitter in a suitably shaped glass vessel. With a vacuum pump, the scented air is then drawn through a Tenax trap (or a trap filled with some other adsorbing or absorbing material). The chemicals responsible for the scent are preconcentrated on the sorbent trap, eluted off the sorbent with heat (or sometimes with a small amount of organic solvent) and analyzed by GC/MS.

Solid-phase microextraction (SPME): SPME was developed in 1988 by Janusz Pawliszyn at the University of Waterloo in Ontario, Canada. The technique was commercialized in 1993 by Supelco. With SPME—a solvent-free sample preparation method—a fused silica fiber coated with a polymer film is exposed to the air

above a flower, causing an equilibrium distribution to be established between the stationary phase (the microfiber) and the gas phase.

Several types of SPME fibers with varying levels of polarity have been developed for SPME. Polydimethylsiloxane (PDMS) is an excellent fiber for capturing nonpolar volatiles. Carboxen/PDMS is slightly polar and works very well for extremely volatile, low-molecular-weight polar compounds. Polyacrylate (PA) works well for extracting midpolar volatiles. Carbowax/divinylbenzene is preferred for extracting polar volatiles. (Please note that the Carbowax/DVB is no longer available; it has been replaced by cross-linked PEG.) After the aroma volatiles are captured on the SPME fiber, they are thermally desorbed in the hot GC injector and into the GC capillary column for separation, followed by MS identification of resolved chemical components.

Mookherjee et al. were the first to apply SPME to floral fragrance extraction.[3] In their study of the orchid flower (*Dendrumbium superbum*) scent, SPME was a superior technique to classical headspace.

Zenith Trap: The Zenith Trap was developed and patented by Thomas McGee and Kenneth Purzycki of Givuadan.[4] The Zenith Trap consists of a bundle of fused silica capillary tubes whose inner surfaces are coated with different substrates with various degrees of absorption capacity for aroma chemicals of different polarities. The details explaining the creation of the Zenith Trap have been previously published.[5]

Advantages and Disadvantages of Various Extraction Techniques

All sample extraction techniques have strengths and weaknesses. Dynamic headspace extraction using adsorbent/absorbent traps has distinct advantages over other sample extraction techniques for analyzing floral fragrances. The choice of trapping resins and conditions used for trapping and desorbing fragrance chemicals from the medium are important. When preconditioned Chromosorb 101 and Lichrolut EN traps are desorbed and analyzed by GC/MS, a large number of background aromatic peaks are observed. Tenax (based on 2,6-diphenyl-p-phenyl oxide), one of the most popular trapping resins used when thermal desorption is employed, has numerous breakdown products, including benzene, toluene, benzaldehyde, acetophenone, styrene and other aromatic compounds. Since many natural products also contain these chemicals, it is difficult to determine if these chemicals, when observed in sample chromatograms, originate from the sample or are Tenax breakdown products. Furthermore, GC peaks from Tenax byproducts may coelute with fragrance chemicals, complicating the analysis. In contrast, PDMS sorbent has only a few breakdown products, which are polysiloxanes and have characteristic mass spectra that are readily distinguishable from the mass spectra of sample analytes.

The excellent stability of PDMS is one of its greatest advantages over Tenax TA and other types of adsorbent traps.[6]

Since SPME uses such small quantities of adsorbent material, it captures far fewer volatiles than dynamic headspace and usually requires extraction times of one to several hours to permit detection of a representative sampling of the fragrance chemicals responsible for the characteristic aroma of a flower. Lack of sensitivity is a major disadvantage of SPME for fragrance analysis. Another problem with SPME is analyte competition for active sites. Since most SPME fibers are adsorbents rather than absorbents, the more volatile components extracted from the flower can be displaced by less volatile components during extraction, thus skewing the recovery of extractables.

McGee and Purzycki reported the first comparative study of SPME and dynamic gas-sampled headspace for collected flower scents.[7] They compared different SPME polymers to dynamic headspace using Tenax and found the selectivity of the different fibers to be problematic. Each SPME polymer demonstrated a marked bias for the polarity of the aroma chemicals similar to the polymer's polarity, indicating that SPME is not ideal for collecting flower scents.

With the ability to use flow rates much higher than those possible with Tenax traps, it is possible to collect volatiles in far less time with the PDMS foam traps compared to the Tenax trap. For example, in research recently performed on beer samples, collection of beer flavor/aroma volatiles after 5 min extraction was shown to be equivalent to 30 min of extraction with Tenax TA.[8]

A disadvantage of the Zenith Trap is that it is not commercially available and reconstructing the trap to provide reproducible results could be challenging.

Comparison of Tenax and PDMS Foam Trapping

The present article attempts to show the advantages of PDMS foam traps for dynamic headspace sampling and the importance of the peak deconvolution capabilities of the Leco Pegasus GC-TOFMS for accurate and sensitive quantitation of coeluting fragrance chemicals.

A bearded iris with an unusually strong, pleasant fragrance (caramel, spicy, vanilla with citrus nuances) was selected to evaluate the efficiency of PDMS foam trapping compared to Tenax TA and the advantages of using the Leco Pegasus GC-TOFMS for detection.

The fragrant purple bearded iris is shown in **F-1**. A GERSTEL TDU desorption tube containing the PDMS foam sorbent is shown in **F-2**, and the PDMS foam tube inserted into the iris during fragrance extraction is shown in **F-3**. The extraction was conducted for 30 min at a flow of 120 mL/min. No enclosure was placed around the iris. A "blank" PDMS foam tube was prepared by analyzing garden air approximately 10 ft from the iris. This blank analysis allowed discrimination of chemicals extracted from the iris as opposed to chemicals extracted from the garden air or from decomposition products of the PDMS foam.

Fragrant, purple bearded iris **F-1**

PDMS foam trap in a GERSTEL desorption tube **F-2**

PDMS foam TDU tube inserted into the iris during extraction **F-3**

Following extraction of the flower with either PDMS foam or Tenax TA, the extraction tube containing the sorbent with the extracted fragrance chemicals was placed in the GERSTEL storage tray, as shown in **F-4A**. **F-4B** shows the GERSTEL MPS2 (multipurpose sampler) placing a desorption tube (taken from the storage tray) into the thermal desorbing unit (TDU) that is located above the GERSTEL CIS4 liquid nitrogen cooled inlet where desorbed volatiles are cyrofocused prior to injection into the GC capillary column.

F-5 compares two PDMS foam blank chromatograms to a chromatogram of the iris scent PDMS foam extract. The duplicate blank analyses showed few background chemicals compared to the iris extract.

Evidence that the PDMS foam trap was more efficient than Tenax TA at trapping fragrance chemicals appears in **F-6**. Some of the chemicals found in PDMS foam iris extracts and area percentages for these components can be seen in **T-1**. Several important fragrance chemicals detected in PDMS foam extracts but not in Tenax TA extracts are shown in **T-2**.

Instrumentation and Instrumental Conditions

Analyses were performed on a 6890 GC (Agilent Technologies) equipped with a CIS4 inlet and MPS2 robotic sampler with TDU option (GERSTEL) and a Pegasus GC-TOFMS (Leco).

The GC capillary column used for all determinations was a 30 m HP-5MS (Agilent) with an internal diameter of 0.32 mm and a film thickness of 0.25 µm. Chromatographic grade helium was used as the carrier gas with a head pressure of 1.6 psi and a constant flow of 1.5 mL/min. The oven ramping conditions were as follows: 40°C for 1 min, then heated at a rate of 10°C/min to 270°C and held at 270°C for 6 min.

Extracted volatiles were thermally desorbed from the PDMS foam and Tenax traps with the GERSTEL TDU using the following conditions: splitless desorption with an initial temperature of 20°C, then increased at a rate of 60°C/min and held at 260°C for 3 min.

The volatiles that were thermally desorbed from the sorbent traps were cryofocused in the GERSTEL CIS4 inlet at -60°C with a carrier gas flow of 50 mL/min. After cryofocusing was completed, the volatiles were released into the capillary column by heating the CIS4 inlet at a rate of 10°C/s to 300°C. The CIS4 was maintained at 300°C for 3 min and operated in the splitless mode for 1.5 min.

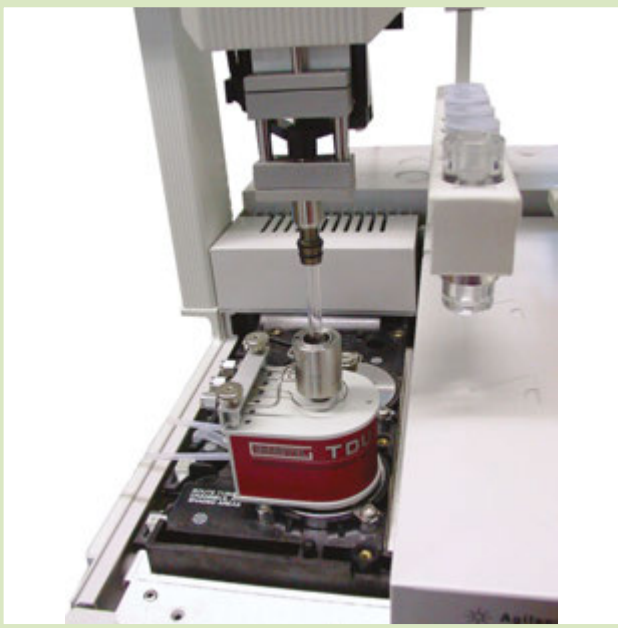
The Leco Pegasus TOFMS conditions were as follows: start/end mass was 40–300; the acquisition rate was 10 spectra/s; the detector voltage was 1300 volts; the electron energy was 70 volts; the ion source temperature was 200°C; and the signal-to-noise ratio was set at 50.0.

The Advantages of GC-TOFMS

The Leco Pegasus GC-TOFMS was found to be critical to accurate analysis of fragrance volatiles because of its ability to perform peak deconvolution of coeluting volatiles. **F-7** illustrates a region of the PDMS foam total ion chromatogram (730–736 s) where coelution of chemical components is occurring. While **F-7** indicates that there seem to be two chemical components eluting in this time frame, the deconvoluted chromatogram in **F-8** shows that there are actually seven chemicals eluting from 730–736 s.

Automated analysis of PDMS foam tubes with GERSTEL TDU and MPS2 **F-4A**

PDMS foam tubes automatically transferred by GERSTEL MPS2 from storage tray to GERSTEL TDU **F-4B**



GC-TOFMS chemical profile of fragrant bearded iris extracted by PDMS foam and analyzed by GC-TOFMS (area %) **T-1**

Compound	R.T. (s)	CAS	Area %
acetaldehyde	97.5	75-07-0	0.0122
methanethiol	99.6	74-93-1	0.0004
formic acid, propyl ester	104.7	110-74-7	0.0019
ethanol, 2-methoxy-, acetate	105.1	110-49-6	0.0141
2-hexanone, 4-methyl-	122.6	105-42-0	0.1089
2(5H)-thiophenone	137.2	3354-32-3	0.0243
acetic acid	159	64-19-7	0.3719
2-propanone, 1-hydroxy- / acetol	164.5	116-09-6	0.0423
benzyl methyl ketone	189.5	103-79-7	0.0206
2-hexenal	210.2	505-57-7	0.1452
hexanal	210.6	66-25-1	0.1452
4-heptenal, (Z)-	244.8	6728-31-0	0.0153
1H-pyrrole, 3-methyl-	245.7	616-43-3	0.0050
3-hexen-1-ol	256.4	544-12-7	0.0138
butanal, 2-ethyl-	263.1	97-96-1	0.0055
2,4-hexadien-1-ol	266	111-28-4	0.0199
3-heptanone	278.1	106-35-4	0.0019
heptanal	290.5	111-71-7	0.0958
1-butanone, 1-(2-furanyl)-	302	4208-57-5	0.0012
DL-2,3-butanediol	309.9	6982-25-8	0.0101
butyrolactone	310.5	96-48-0	0.0531
2(5H)-furanone	311.7	497-23-4	0.0570
2-butenal, (Z)-	317.8	15798-64-8	0.0761
3-carene	318.8	13466-78-9	0.0751
camphene	332.1	79-92-5	0.0601
2-decanol	337.6	1120-06-5	0.0653
4-octanone	353.5	589-63-9	0.0151
α-phellandrene	354.3	555-10-2	0.0033
α-thujene	357.2	28634-89-1	0.0008
5-hepten-2-one, 6-methyl-	366.5	110-93-0	0.2751
myrcene	369.4	123-35-3	0.0547
furan, 2-pentyl-	370	3777-69-3	0.0536
phenol	372.3	108-95-2	0.0135
2-methyl-3,7-diphenylindole	374.3	1863-20-3	0.1646
hexanoic acid	381.5	142-62-1	0.0316
4-hexen-1-ol, acetate, (Z)-	383.9	42125-17-7	0.2640
2-propyl-1-pentanol	405	58175-57-8	0.1221
furan-2-carboxylic acid, 3-formylphenyl ester	415	332411-91-3	0.0013
2(3H)-furanone, 5-ethenyldihydro-5-methyl-	416.8	1073-11-6	0.0509
5-nonen-4-one, 6-methyl-	418.3	7036-98-8	0.0093
3-nonen-1-ol, (E)-	419	10339-61-4	0.0094
benzene, 1-methyl-2-(1-methylethyl)-	431	527-84-4	0.0382
2-nonen-1-ol	439.6	22104-79-6	0.1053
1-octanol	442.4	111-87-5	0.0221
2-pentenoic acid, 4-hydroxy-	444.7	28525-83-9	0.0026
6-octen-1-ol, 7-methyl-3-methylene-	453.9	13066-51-8	0.0100
o-cymene	455.1	527-84-4	0.0004
cyclohexene, 3-methyl-6-(1-methylethylidene)-	457.8	586-63-0	0.0088
2-nonanone	460.4	821-55-6	0.0012
heptanoic acid	461	111-14-8	0.0041
4-nonenal, (E)-	462.3	2277-16-9	0.0633
nonanal	471.5	124-19-6	0.5159
trans-decalin, 2-methyl-	477.6	–	0.0222

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Compound	R.T. (s)	CAS	Area %
benzene, 1-methoxy-2-(1-methylethenyl)-	482.5	10278-02-1	0.0549
benzeneethanol, dimethyl-	487.4	100-86-7	0.0065
octanoic acid, methyl ester	488.6	111-11-5	0.1953
trans-undec-4-enal	498.5	68820-35-9	0.0421
3-hydroxymandelic acid, ethyl ester, di-TMS	502.2	–	0.0025
trans-cinnamyl bromide	503.6	26146-77-0	0.0004
2,3-dimethylanisole	504.3	2944-49-2	0.0037
4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	512.7	28564-83-2	0.0107
2-benzylpiperazine	513.4	84477-71-4	0.0450
(R)-(+)-citronellal	514.7	2385-77-5	0.4362
thiophene, 2-(1-methylethyl)-	519.1	4095-22-1	0.0408
2-decen-1-ol	528.5	22104-80-9	0.2035
m-menth-6-ene, (R)-(+)-	528.9	13837-70-2	0.2050
octanoic acid, methyl ester	532.5	111-11-5	0.0107
chrysanthemumic acid 2,4-dimethylbenzyl ester	534	70-38-2	0.0335
thiazole, 5-butyl-	541.8	52414-90-1	0.0049
acetophenone, 4'-methyl-	543.9	122-00-9	0.0045
methyl 6-methyl heptanoate	548.9	2519-37-1	0.0011
p-methylguaiacol	551.2	93-51-6	0.1356
salicylic acid, methyl ester	552.5	119-36-8	0.0168
1,3,5-cycloheptatriene, 3,7,7-trimethyl-	559.6	3479-89-8	0.6462
decanal	560.1	112-31-2	0.6462
6-octen-1-ol, 7-methyl-3-methylene-	572.2	13066-51-8	0.2608
3-nonenoic acid, methyl ester	572.7	13481-87-3	0.2608
nonanoic acid, methyl ester	574.7	1731-84-6	0.0088
8-nonenoic acid, methyl ester	576.9	20731-23-1	0.0058
3-carene	580.2	13466-78-9	0.3510
(R)-(+)-α-citronellol	581.7	1117-61-9	1.7476
1-penten-3-one	585.2	1629-58-9	0.0994
cis-citral	591.3	106-26-3	0.0781
isogeraniol	593.4	5944-20-7	0.1342
octanoic acid	593.7	124-07-2	0.1342
(+)-carvone	594.7	2244-16-8	0.0294
trans-geraniol	602.9	106-24-1	0.4467
oxalic acid, allyl ethyl ester	603.3	–	0.6280
4-hydroxy-2-methylbenzaldehyde	604	41438-18-0	1.0200
cis-geraniol	605.3	106-25-2	2.6724
1,3-cyclohexadiene-1-methanol, 4-(1-methylethyl)-	605.8	1413-55-4	2.6804
6-octenoic acid, 3,7-dimethyl-, methyl ester	606.4	2270-60-2	0.2631
trans-tagetone	607	6752-80-3	0.1561
trans-2-caren-4-ol	611.5	4017-82-7	0.0269
bicyclo[4.1.0]heptan-3-ol, 4,7,7-trimethyl-, (1à,3à,4à,6à)-	611.8	52486-23-4	0.0269
trans-citral	616	141-27-5	0.3166
2,6-octadienoic acid, 3,7-dimethyl-, methyl ester	623.1	2349-14-6	0.0761
nonanoic acid	623.8	112-05-0	0.0758
1-methylverbenol, methyl ether	624.9	–	0.0005
(-)-bornyl acetate	629.3	5655-61-8	0.0214
1,3-dioxolane, 2-ethenyl-	629.9	3984-22-3	0.1106
2-undecanone	633.1	112-12-9	0.0250
indole	638.7	120-72-9	0.0002
geraniol formate	640.1	105-86-2	0.0006
undecanal	644	112-44-7	0.0247
methyl cinnamate	644.8	103-26-4	0.0501

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Compound	R.T. (s)	CAS	Area %
4-decenoic acid, methyl ester	645.4	7367-83-1	0.0271
2-methoxy-4-vinylphenol	652.7	7786-61-0	0.0010
decanoic acid, methyl ester	657.7	110-42-9	0.9852
methyl geranate	658.3	2349-14-6	0.9852
camphene	666.7	79-92-5	0.0016
benzoic acid, 2-amino-, methyl ester	675.4	134-20-3	0.0258
α-cubebene	680.8	17699-14-8	0.0453
acetophenone, 4'-methoxy-	683.8	100-06-1	0.0131
Z-8-octadecen-1-ol acetate	685	–	0.0072
isoeugenol	686.9	97-54-1	0.0098
2-decenoic acid, methyl ester, (E)-	695	7367-85-3	0.0042
n-decanoic acid	699.5	334-48-5	0.0518
ylangene	702.8	14912-44-8	0.3456
7-octen-4-ol, 2-methyl-6-methylene-, (S)-	703.9	35628-05-8	0.0260
methyl cinnamate	707.2	103-26-4	0.0578
undecanoic acid, methyl ester	712.6	1731-86-8	0.0803
vanillin	721.6	121-33-5	0.0025
decanal	723.4	112-31-2	0.0153
limonen-6-ol, pivalate	727.8	–	0.0035
acetophenone, 4'-tert-butyl-	730.5	943-27-1	0.0056
bergamotene	731.8	17699-05-7	0.1331
valeric acid, 3,5-dihydroxy-2,4-dimethyl-, lactone	735.2	109717-37-5	0.0189
sesquiphellandrene	752.3	20307-83-9	0.0418
geranyl acetone	758	689-67-8	0.2072
lilac aldehyde C	767.4	53447-47-5	0.0000
2-propenoic acid, 3-phenyl-, ethyl ester, (E)-	769.3	4192-77-2	0.0353
cyclopropane, nonyl-	773.7	74663-85-7	0.3414
10-undecenoic acid, methyl ester	798.1	111-81-9	0.0083
α-farnesene	798.9	502-61-4	0.0083
dibenzofuran	808.2	132-64-9	0.0217
dodecanoic acid, methyl ester	809.4	111-82-0	0.5105
1,3-cyclohexanedione, 2-(2-propenyl)-	813.7	42738-68-1	0.1068
octanoic acid, 4-methylpentyl ester	828.6	–	0.0124
3,7-octadien-2-ol, 2,6-dimethyl-	835.4	62911-76-6	0.0100
dodecanoic acid	840.7	143-07-7	0.0573
2-decenoic acid, methyl ester, (E)-	843.9	7367-85-3	0.0070
cis-Z-α-bisabolene epoxide	846.9	–	0.0781
diethyltoluamide	853.1	134-62-3	0.0264
caryophyllene oxide	858.7	1139-30-6	0.0029
tridecanoic acid, methyl ester	859.3	1731-88-0	0.0029
2,6-heptadien-1-ol, 2,4-dimethyl-	874.3	80192-56-9	0.0927
decanoic acid, 3-methyl-	883.3	60308-82-9	0.0036
benzophenone	887.7	119-61-9	0.0166
caryophyllene oxide	890.1	1139-30-6	0.7754
decanoic acid, octyl ester	892.4	2306-92-5	0.0922
2-nonadecanone	929.8	629-66-3	0.2124
methyl tetradecanoate	946.7	124-10-7	0.5498
geranyl butyrate	965.6	106-29-6	0.0332
tetradecanoic acid	978.7	544-63-8	0.4216
tetradecanoic acid, 12-methyl-, methyl ester	992.2	5129-66-8	0.1781
4-nonenoic acid, methyl ester	999.6	20731-19-5	0.0073
benzoic acid, 2-hydroxy-, pentyl ester	1001.7	2050-08-0	0.0053
tetradecanoic acid, 12-methyl-, methyl ester	1010	5129-66-8	0.0768

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Compound	R.T. (s)	CAS	Area %
pentadecanoic acid	1041.1	1002-84-2	0.2805
benzyl salicylate	1042.8	118-58-1	0.0050
hexadecen-1-ol, *trans*-9-	1045.2	64437-47-4	0.5747
homomenthyl salicylate	1052.8	52253-93-7	0.0056
caryophyllene oxide	1053.9	1139-30-6	0.1100
hexadecanoic acid, methyl ester	1075.9	112-39-0	2.3590
oxybenzone	1138.3	131-57-7	0.2105
hexadecen-1-ol, *trans*-9-	1162	64437-47-4	0.1921
octadecanoic acid, methyl ester	1185.3	112-61-8	0.6880
myristoleic acid	1197.6	544-64-9	0.6734
n-hexadecanoic acid	1208.8	57-10-3	1.1225
2-ethylhexyl p-methoxycinnamate	1209.7	5466-77-3	1.1225
hexadecanoic acid, butyl ester	1216.7	111-06-8	0.0897
benzoic acid, octyl ester	1226.4	94-50-8	0.2436
2-ethylhexyl *trans*-4-methoxycinnamate	1262.6	83834-59-7	0.0050
benzoic acid, undecyl ester	1279.7	6316-30-9	0.3622
hex-1-en-3-one, 1-(4-methoxyphenyl)-6-methyl-	1289.4	72178-64-4	0.9801
benzoic acid, 2-methylpropyl ester	1295.8	120-50-3	0.0153
1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester	1397.1	4376-20-9	1.2709
citronellol epoxide (R or S)	1504.7	–	0.0019
tetradecanoic acid, tetradecyl ester	1620.3	3234-85-3	0.2346
9-octadecenal	1654	5090-41-5	0.0177
oxalic acid, allyl dodecyl ester	1670.2	–	0.0083
andrographolide	1757.3	5508-58-7	0.3843

Another example where coelution is occurring in the chromatogram of the PDMS foam iris extract is shown in **F-9**. Between 600 and 608 s, there appear to be two coeluting peaks in the TIC. However, **F-10** shows that there are actually eight chemicals that elute in this region. Detecting these chemicals would be challenging without the peak deconvolution capabilities of the Pegasus GC-TOFMS. Note the ability of the ChromaTOF software to deconvolute minor trace peaks even when coelution with an extremely large volume of eluting chemical (in this case, *cis*-geraniol) is occurring. It is often these minor peaks that are among the most significant fragrance chemicals in a floral scent.

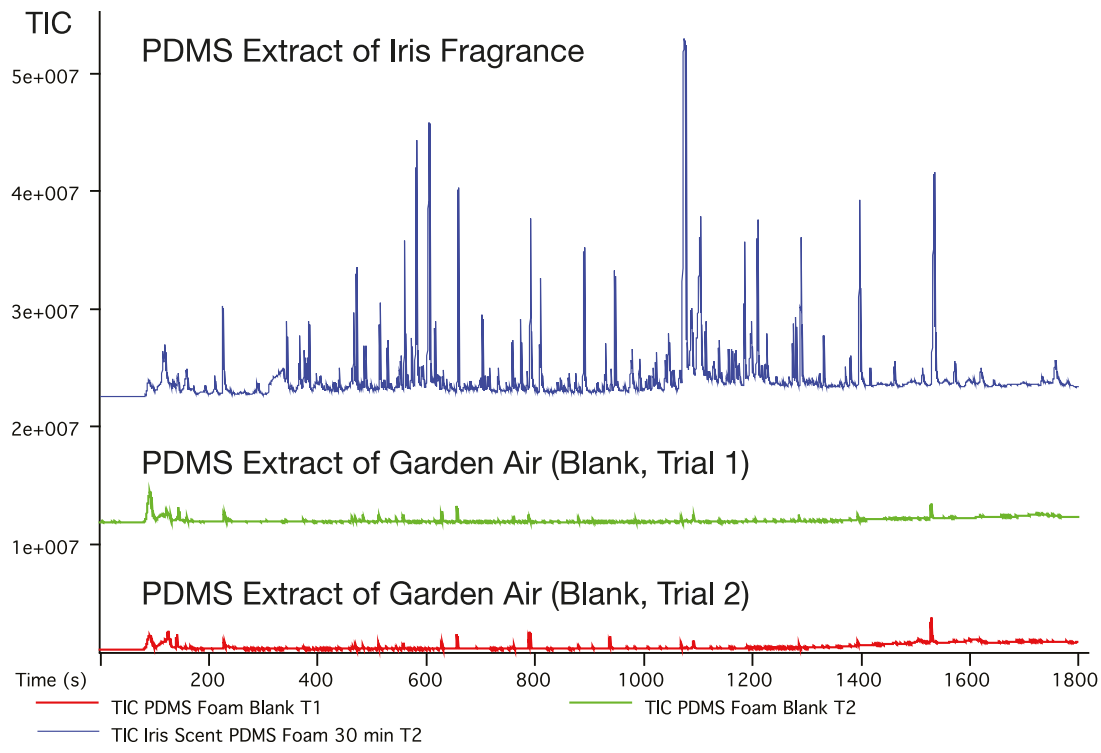
The region of the chromatogram between 680 and 690 s shown in **F-11A** is crowded with coeluting chemicals. For example, peak apexes for octadecen-1-ol acetate (peak no. 518) and bis(trimethylsilyl) mercaptoacetic acid (peak no. 519) are separated by less than 250 ms, yet they are easily deconvoluted for accurate identification and quantitation with ChromaTOF. One important fragrance compound that could easily be undetected without the ChromaTOF peak deconvolution and auto peak find algorithms is isoeugenol (peak no. 520 at 686.9 s).

F-11B shows the caliper (undeconvoluted) mass spectrum, true (deconvoluted) mass spectrum and the library mass spectrum match for peak no. 520. Clearly, attempting to find a high quality library match for isoeugenol in this sample using the caliper mass spectrum (total ion chromatogram mode) would be highly unlikely. The true spectrum for this peak offers a high similarity library match of 759 for isoeugenol.

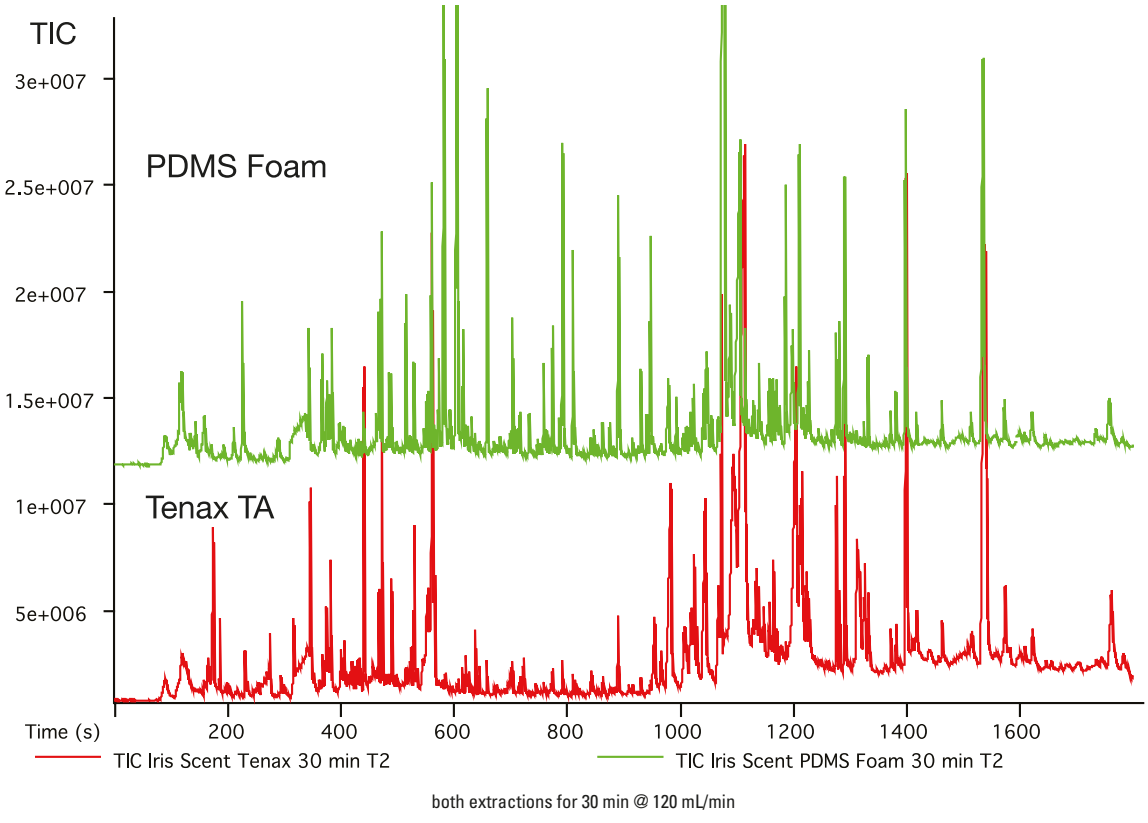
Chemicals not detected in Tenax TA extract but detected in PDMS foam extract **T-2**

p-methylguaiacol
salicylic acid, methyl ester
citronellol
isogeraniol
cis-citral
cis-geraniol
methyl geranate
isoeugenol
ylangene
vanillin
bergamotene
lilac aldehyde C
benzyl salicylate

PDMS foam iris extract compared to duplicate analysis of blank PDMS foam tubes after 30 min extraction of garden air **F-5**



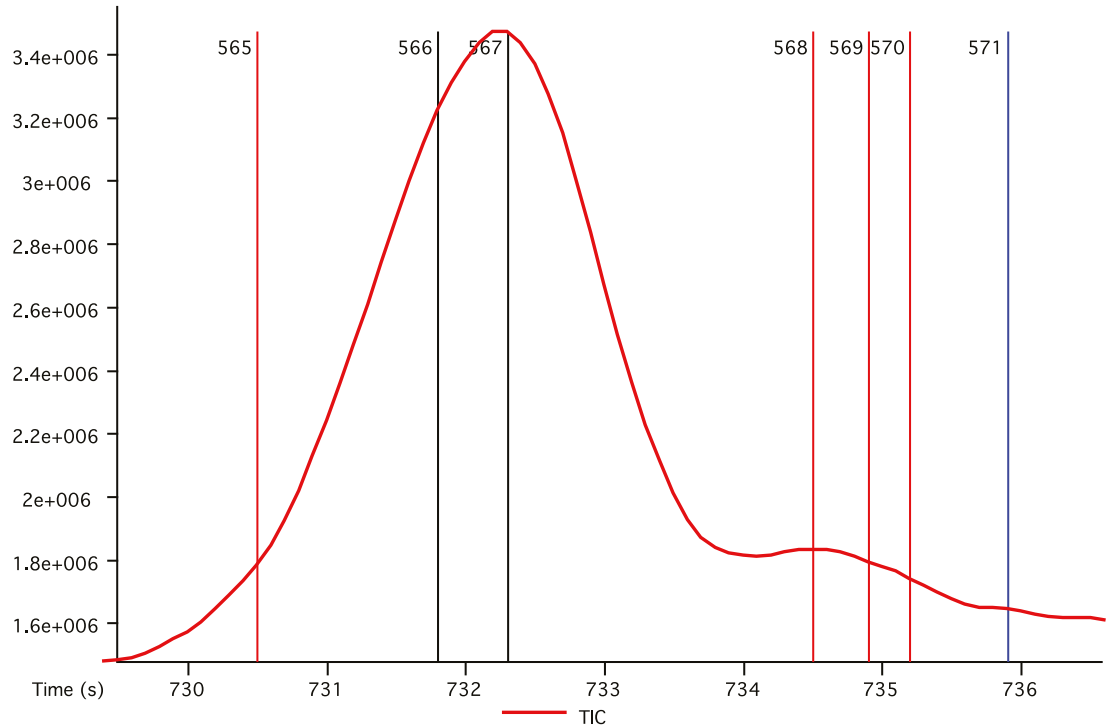
Comparison of iris extraction with PDMS foam vs. Tenax TA **F-6**



both extractions for 30 min @ 120 mL/min

Peak deconvolution potential of Pegasus GC-TOFMS (undeconvoluted TIC)—Example 1

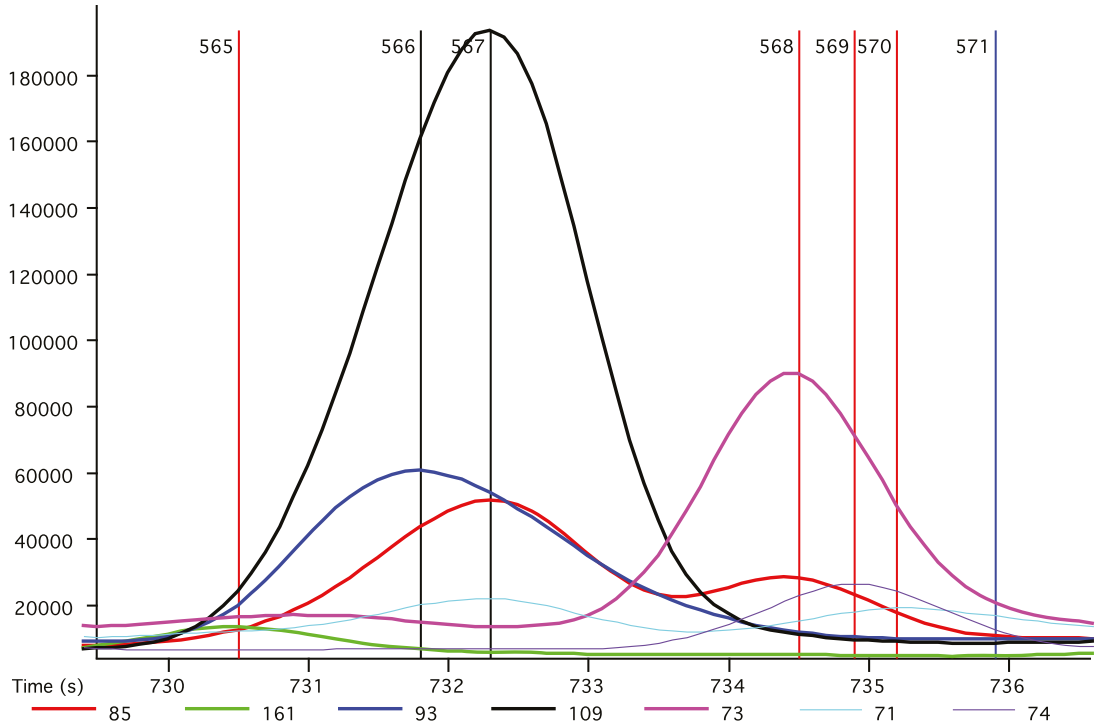
F-7



The region of chromatogram (TIC) from 730 s to 735 s appears to contain two partially resolved peaks. However, the Pegasus ChromaTOF software indicates seven detectable peaks in this region of the chromatogram.

After peak deconvolution—Example 1

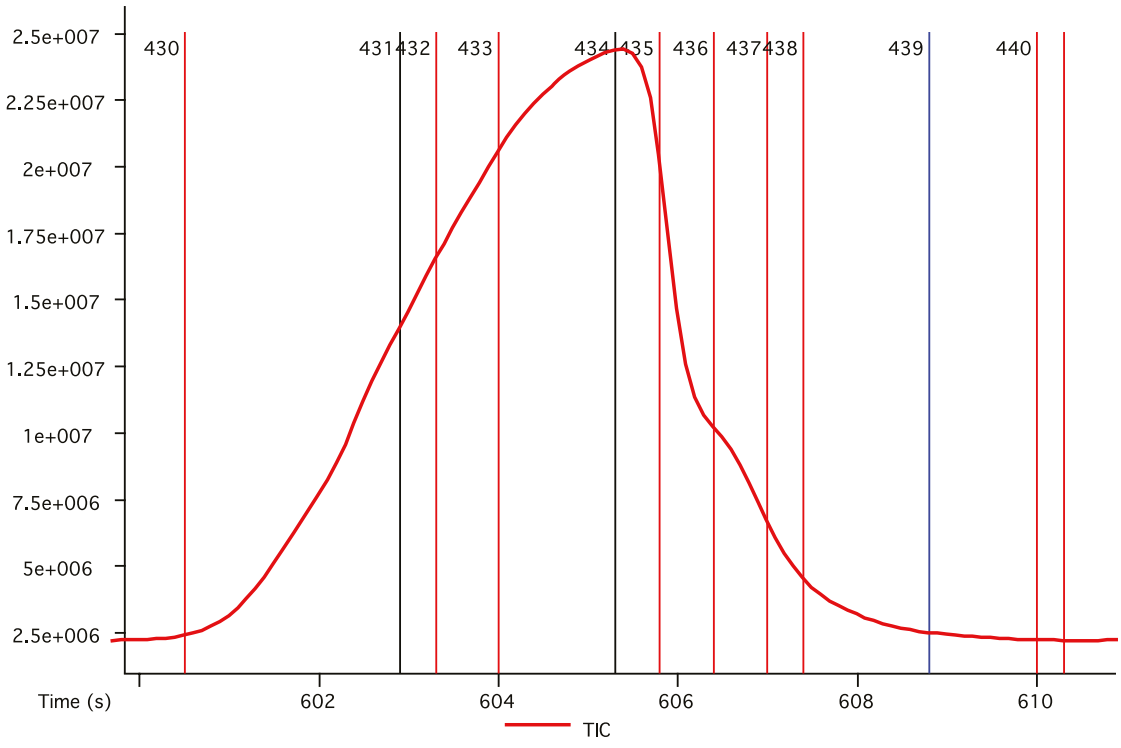
F-8



565 = 4′-tert-butyl acetophenone; 566 = bergamotene; 567 = 2,4,7,9-tetramethyl-5-decyn-4,7-diol; 568 = blank (from a polysiloxane from PDMS); 569 = tridecanoic acid, methyl ester; 570 = valeric acid, 3,5-dihydroxy-2,4-dimethyl, lactone; 571 = 1,2-dimethyl naphthalene

Peak deconvolution potential of Pegasus GC-TOFMS (undeconvoluted TIC)—Example 2

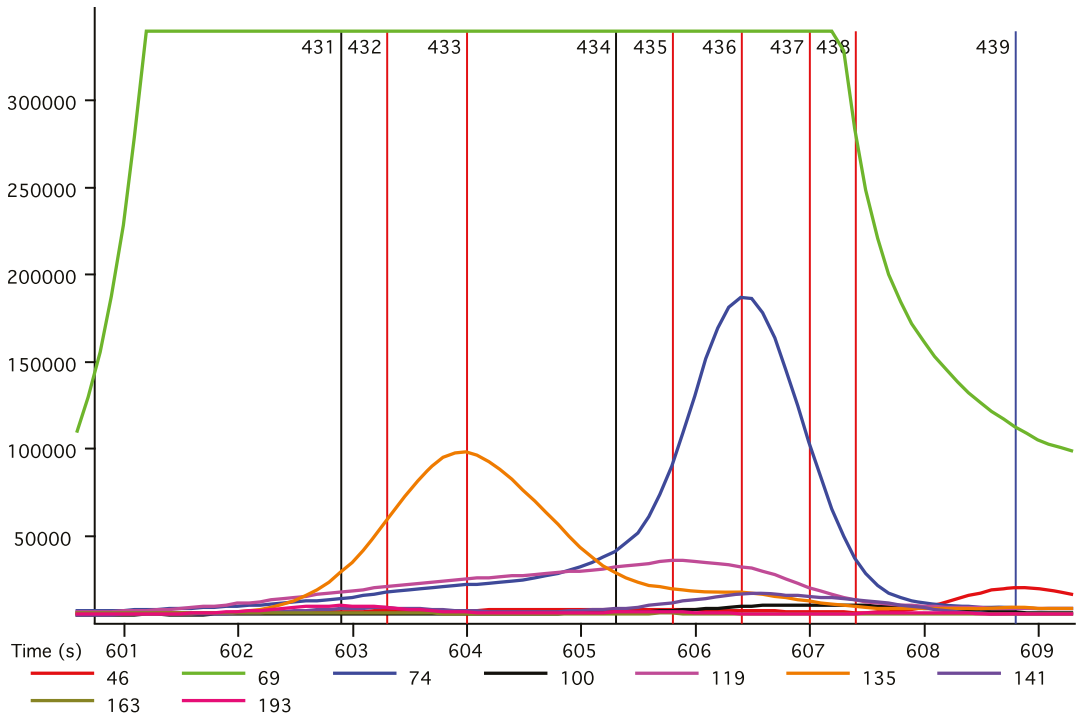
F-9



the region of the chromatogram (TIC) from 600 s to 608 s appears to contain two partially resolved peaks

After peak deconvolution—Example 2

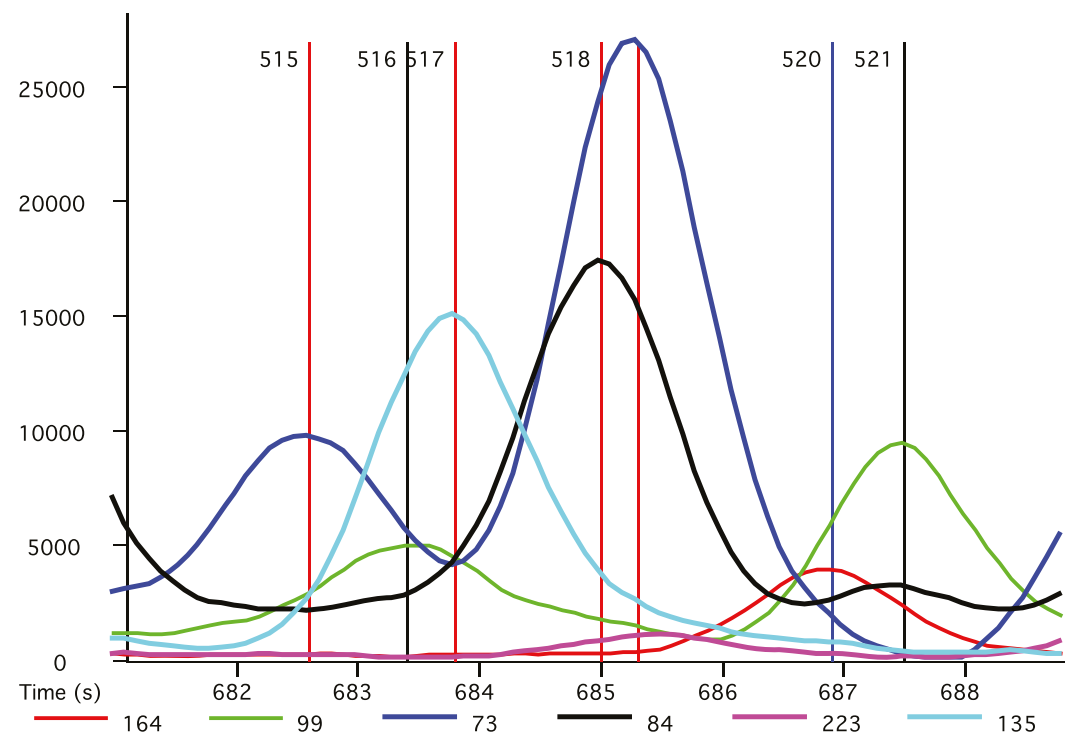
F-10



431 = t-geraniol; 432 = oxalic acid, allyl ethyl ester; 433 = 4-hydroxy-2-methylbenzaldehyde; 434 = c-geraniol; 435 = cyclohexadiene-1-methanol, 4-(1methylethyl); 436 = 6-octenoic acid, 3,7-dimethyl, methyl ester; 437 = t-tagetone; 438 = 2,3,6-trimethyl decane

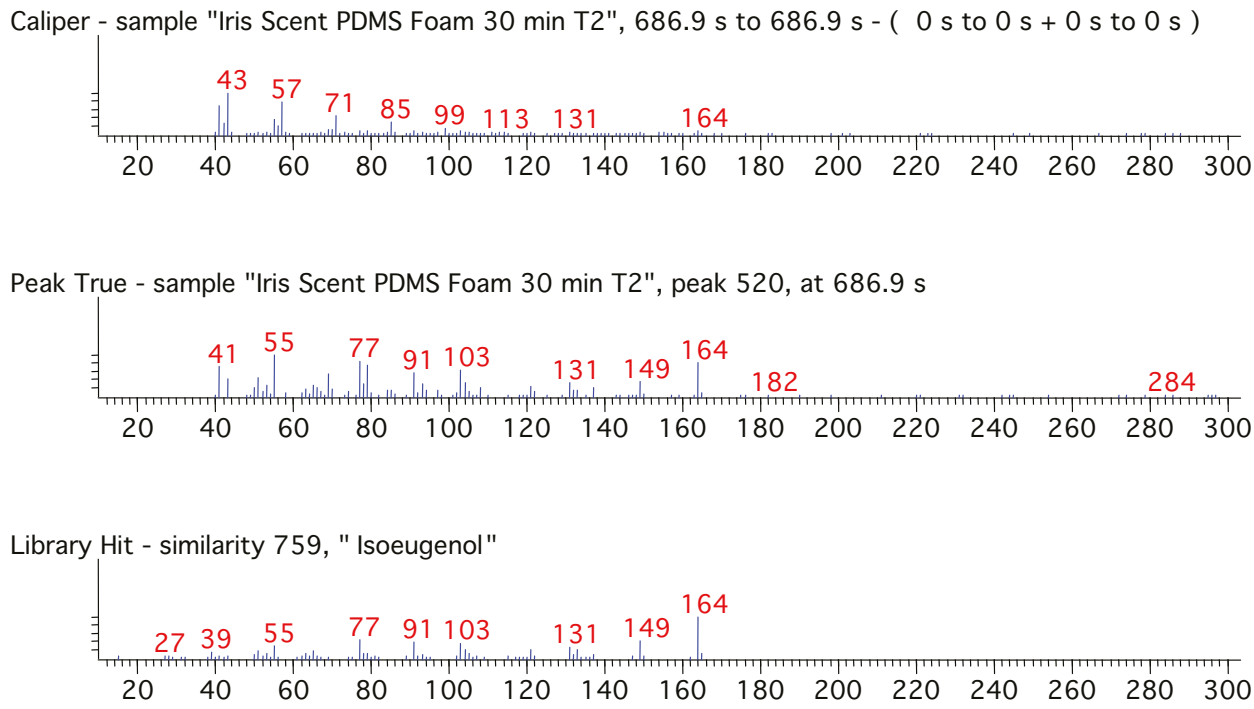
Peak deconvolution of isoeugenol

F-11A



515 = 3-methyl-3-(1-ethoxyethoxy)-1-butene; 516 = 4-methyl tridecane; 517 = 4'methoxy acetophenone; 518 = 8-octadecen-1-ol acetate; 519 = mercaptoacetic acid, bis(trimethylsilyl) background peak; 520 = isoeugenol; 521 = 3-methyl dodecane

Peak deconvolution for isoeugenol showing caliper, true and library mass spectra **F-11B**



Without peak deconvolution, it would be difficult to detect several important fragrance contributors in the iris extract. It is the experience of the authors that the serious problem of peak coelution is far more common than most chromatographers realize. It frequently occurs in complex natural products. The advantages of GC-TOFMS for flavor and fragrance testing have been previously described.[9]

Conclusion

This work shows that extraction with PDMS foam absorbent is able to detect more iris fragrance chemicals than Tenax TA. Furthermore, the use of the Leco Pegasus GC-TOFMS was found critical for accurate quantitation of coeluting chemicals, many of which were important fragrance compounds.

With the development of more sensitive extraction techniques—such as stir bar sorptive extraction (SBSE) and dynamic headspace extraction with PDMS foam sorbent—analytical chemists are extracting more analytes compared to older extraction methods. While this is highly desirable for improving detection limits of potentially important fragrance compounds, it increases the likelihood of analyte peak coelution, which necessitates the need for more powerful mass spectrometry instrumentation and algorithms capable of performing peak deconvolution.

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