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Rapid collection of floral fragrance volatiles using a headspace volatile collection technique for GC-MS thermal desorption sample analysis

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TITLE:
Rapid Collection of Floral Fragrance Volatiles using a Headspace Volatile Collection Technique for GC-MS Thermal Desorption Sampling

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KEYWORDS:
charcoal filter, semiochemicals, portable, vacuum, scent, flower

SUMMARY:
Here, we present a protocol for collecting the floral fragrance volatiles from blooming flowers, using a non-destructive sampling procedure.

ABSTRACT:
Fragrances of many flower families have been sampled and the volatiles analyzed. Knowing the compounds that make up the fragrances can be an important step to conservation of flowers that are threatened or endangered. Because floral fragrance is critical for attracting pollinators, this method could be used to better understand or even enhance pollination. We present a protocol using a portable charcoal air filter and vacuum to collect floral fragrance volatiles, which are then analyzed by a GC-MS. By using this method, fragrance volatiles can be sampled using a non-destructive method with a machine that is easily transported. This methodology uses a rapid sampling procedure, cutting sampling time down from 2-3 hours to approximately 10 minutes. Using GC-MS, the fragrance compounds can be identified individually, based on authentic standards. The steps used for collecting fragrance and control data are presented, from material setup to collecting the data output.

INTRODUCTION:
Flowers typically produce a fragrance used to attract pollinators. These fragrances are made up of many chemical compounds all acting together as a floral blend[1-3]. Without these fragrances, flowers would be less likely to pass on their genetic information using pollinators. Floral fragrance has been documented in many flowering plant families, with *Orchidaceae* being one of the more

common families studied[4]. To understand the role of floral fragrance in pollination, it is important to nondestructively collect and analyze the chemical compounds being emitted from the flowers at different times of the day and during the several days to weeks the flower blooms are open, as fragrance can vary over time[5].

An early protocol for this kind of sampling was developed by Heath and Manukian[6]. The goal of their sampling methods was to reduce stress on the specimen (e.g., plants, insects) being studied. Earlier papers documented that destructive procedures to the plant were required, such as removing blooming flowers in order to collect the fragrance. More recent floral fragrance publications by Cancino and Damon[7,8] used similar methods. This study put the flowers in glass chambers and passed purified air over them; then fragrance compounds from the chamber were absorbed onto porous polymer adsorbents in clear Pasteur pipettes. The fragrances were collected for at least two hours during this study. Sadler et al.[9] carried out floral fragrance studies on an epiphytic orchid in south Florida, much like the original study[10]. Again, this study required the flowers to be sampled for over two hours to collect the fragrance volatiles, with fragrance collected onto the porous polymer adsorbent. The paper here presents a non-destructive method that allows for much quicker sampling, lasting only 10 minutes. Also, instead of using a glass chamber oven baking bags are used, which allow for more flexible movement of the chamber and reduce the chances of damage to the flowers. These bags come in several sizes allowing the option to select the size of bag that will easily fit individual samples without damaging the sample or the surrounding material. The adsorbent used in this study was Tenax Porous Polymer Adsorbent. This differs from Porapak, because the sample can be thermally desorbed onto the GC-MS column for analysis, eliminating the use of a chemical solvent.

The methods in this study provide a way to quickly sample fragrance volatiles produced by flowers and could be used to sample volatiles from other specimens as well, such as insect pheromones, or mushroom volatiles. The reduced time for sampling means there is less stress on the sample and the ability to collect many samples in a short period of time. For example, in Sadler et al.[9], the flower was only fragrant at night, so only two or three samples could be collected each night. With the method here, samples could be taken all night at 15-20-minute intervals from the same flower. Additionally, by using bags instead of glass chambers, the headspace can be suspended easier for sampling in the field for in situ collection on endangered or threatened plant species. Using the method presented here, we were able to sample flowers 1.5 to 2 meters above ground. These methods are incredibly useful for fragrance collection in the laboratory and the field, and provides researchers with a sampling technique that is fast and non-destructive to the sample.

PROTOCOL:

NOTE: Perfumes or scented lotions and products must not be worn during any of these procedures.

1. Flower selection

NOTE: Flowers used can be either naturally growing in the environment or kept under artificial environmental conditions. Temperature, humidity, and light level during collection can vary based on the specific flower species used, and what type of data is being collected. For example, data has been collected during the day and at night for the same flower to determine if fragrance varies over time of day, and collected from both in situ and greenhouse flowers.

1.1. Select a flower that is initially unopened, to standardize sample collection time. This controls for a flower changing fragrance over time.

1.2. Depending on the duration of the blooming time, if possible, wait at least 24 hours after blooming to collect the sample, setting a standard time for all samples.

1.3. If there are multiple blooming flowers on a plant, mark the one that will be used with flagging tape or something similar to ensure repeated sampling of the same flower.

2. **Material preparation**

2.1. Use oven bags (approximately 40.5 cm × 44.5 cm) and corrugated PTFE tubing.

2.2. Initially, boil oven bags in water for ~30 min to remove residual plastic compounds. To dry, bake in an oven at 175 °C.

2.3. Once the bags have dried, add a polypropylene bulkhead union to each corner of the closed end of the oven bags. These attachments allow connection of tubes to push charcoal filtered air in and pull fragrance out of the headspace.

2.4. Rinse all bags and tubes with 75% ethanol. Let both air dry after rinsing.

2.5. After the oven bags have dried, bake bags and tubes in an oven at low heat, approximately 74-85 °C for 30 min.

3. **Volatile collection**

NOTE: Sterile neoprene gloves need to be worn throughout this process, as contacting the bag or filter cartridges can contaminate the samples.

3.1. Cover the selected flower with a baked oven bag. Cinch the bag together tightly with a plastic zip tie below the flower to prevent unwanted airflow into the bag.

3.2. Attach a tube from the air outlet of the collection equipment and connect it to one of the bulkhead unions on the oven bag.

3.3. On the other bulkhead union, attach a glass filter cartridge containing porous polymer adsorbent.

3.4. Attach a second tube to the collection equipment on the vacuum input. Connect end of the second tube onto the glass volatile collection filter cartridge.

3.5. Turn on both the air pump and vacuum at the same time set at ~0.05 L/min. The headspace around the flower will fill with air, but not become overinflated. The system will pull air from the bag through the filter, trapping the floral volatiles.

3.6. Allow the machine to run for 10 min and then turn off both the air pump and vacuum.

NOTE: Flower species that produce/emit a smaller amount of fragrance may need to be sampled for a longer period of time.

3.7. Disassemble the tubes and the glass filter cartridge. Place the filter into a glass vial with a screw-on cap. Once the cap is on, seal the vial with PTFE pipe thread tape.

3.8. Store samples in a freezer (-80 °C) until analyzed using GC-MS.

3.9. Repeat this process with a clean oven bag and glass filter, this time with an empty oven bag, to collect a blank air sample as a control. This allows any background volatiles collected to be identified.

NOTE: Repeating sample collection needs to be done at approximately the same time each day, as some flowers produce varying fragrance levels over the course of a day.

4. **GC-MS**

4.1. Remove the glass filter cartridge from the freezer and place into a GC-MS in the injector port.

4.2. Release headspace volatiles collected on porous polymer adsorbent from the adsorbent by heating in the thermal collection trap (TCT) to 220 °C for 8 min within a flow of helium gas (rate: 1.2 mL/min).

4.3. Collect desorbed compounds in the TCT cold trap unit at -130 °C. The cold trap temperature is regulated by the GC-MS program.

4.4. Flash heat the TCT cold trap unit to inject the compounds into the capillary column of the gas chromatograph to which the TCT cold trap unit was connected. The method for the TCT begins at -20 °C and ends at 150 °C.

4.5. Program the GC-MS to rise from 40 °C to 280 °C at 15 °C/min, with a 5 min hold at 40 °C.

5. Data analysis

5.1. For identification, compare the mass spectra of the sample to those from mass spectra libraries (NIST and Department of Chemical Ecology, Goteborg University, Sweden[11]), as well as retention times of the volatiles to times of authentic compound standards[12].

5.2. Compare chromatograms of collected volatiles to identify common reoccurring peaks.

5.3. After identifying the peak volatiles, use Pherobase (online database of semiochemicals and pheromones) to determine if they have been previously described in floral fragrances[10].

REPRESENTATIVE RESULTS:

Representative data from the GC-MS are shown as a chromatogram in **Figure 1**. In addition to the chromatogram, a data file of results is also provided (**Supplementary File 1**). This data file provides the retention time for each peak (RT), and an identification of what compound that peak is (Library/ID). Peaks between 10:00 and 15:00 minutes are floral volatiles, due to the molecular weight of the compounds[10]. The numbers above the peaks signify the retention times of the identified compounds which are referenced the data file of the results (**Supplementary File 1**). By obtaining the chromatogram and data file for each fragrance sample, the compounds can be compared and those that are reoccurring for each flower sample can be identified. Collections can be identified from this document under the category "Sample", named to represent the flower sampled, and the time and date of the collection (example: UF1 8AM 03/16/15). Page 1 of this document also shows the identification of specific compounds identified from the sample (LibraryID), which peak retention time from **Figure 1** the compound corresponds to (Pk#), and the percentage of the total fragrance that each volatile comprises (Area %). All the collected volatiles listed under "Library/ID" can be referenced in Pherobase to determine if they have previously described in a floral fragrance. For example, in the **Supplementary File 1**, compound #21, with a retention time (RT) of 10.311 has been identified as benzaldehyde. In future samples, if benzaldehyde is present, it can be referenced on Pherobase to determine if it is a likely floral compound for the flower. In **Figure 2**, benzaldehyde was searched on Pherobase. Once a compound has been selected, the page shows a list of all flower species, organized by plant family, from which that fragrance compound has been identified. Highlighted in the bottom right corner of **Figure 2** is a small subset of the orchid species (Orchidaceae) from which benzaldehyde has been determined to be present in the floral fragrance.

Figure Legends:

Figure 1. GC-MS volatile peak results. Graphical results showing the peak volatiles of the floral fragrance sample. Numbers above the peaks correspond to a list of all collected volatile compounds, identifying the peak to the specific volatile. Peaks between 10:00 and 15:00 minutes are most likely to be volatiles from a floral fragrance.

Figure 2. Pherobase example results. An example of results from a Pherobase search for a fragrance compound. In this figure Benzaldehyde was searched, and the results show a list of all flower species from which this fragrance has been identified.

DISCUSSION:

Though this technique is incredibly valuable for its sampling speed and portability, one limitation is using it for epiphytic species, or those growing on trees and not from the ground. In the original study[10], one of the flowers sampled was epiphytic. Because the machine is too heavy to hang freely, a stable, elevated base must be made for sampling. Additionally, the machine can either be plugged in to an electrical outlet or battery powered, so if there is prolonged field sampling, there must be a power source to charge the batteries when the machine is not in use.

The methods here allow for an in situ non-destructive sampling, with rapid repeated sampling and a much quicker sampling time. While some floral fragrance studies require the fragrance to be collected for 2-3 hours for one sample, the presented method can accurately collect the volatiles in approximately 10 min due to the collection material (porous polymer adsorbent) used in the glass filter.

These collection methods provide a way to quickly and safely sample the fragrance produced by flowers, without destroying or harming the flower. With so many flowers, especially those in the family *Orchidaceae*, being categorized as threatened or endangered, analyzing the fragrances they are producing in a non-destructive manner is critical as work is conducted to understanding their pollination biology. The information gained from these studies could potentially be used to boost pollination using synthetic blends based on peak chemicals found to attract more pollinators to areas with blooming orchids.

DISCLOSURES:

The authors declare no conflicts of interest.

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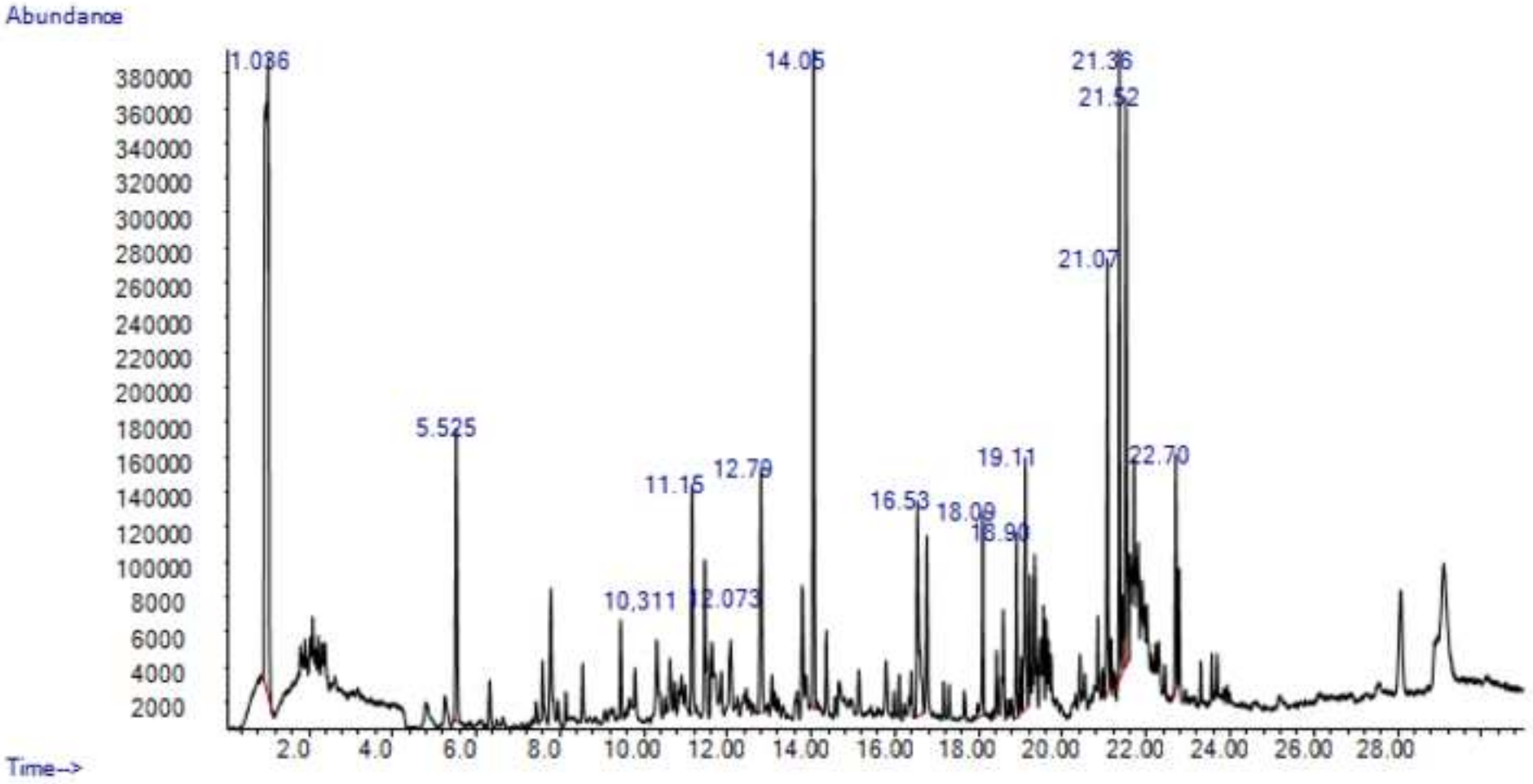

Figure 2

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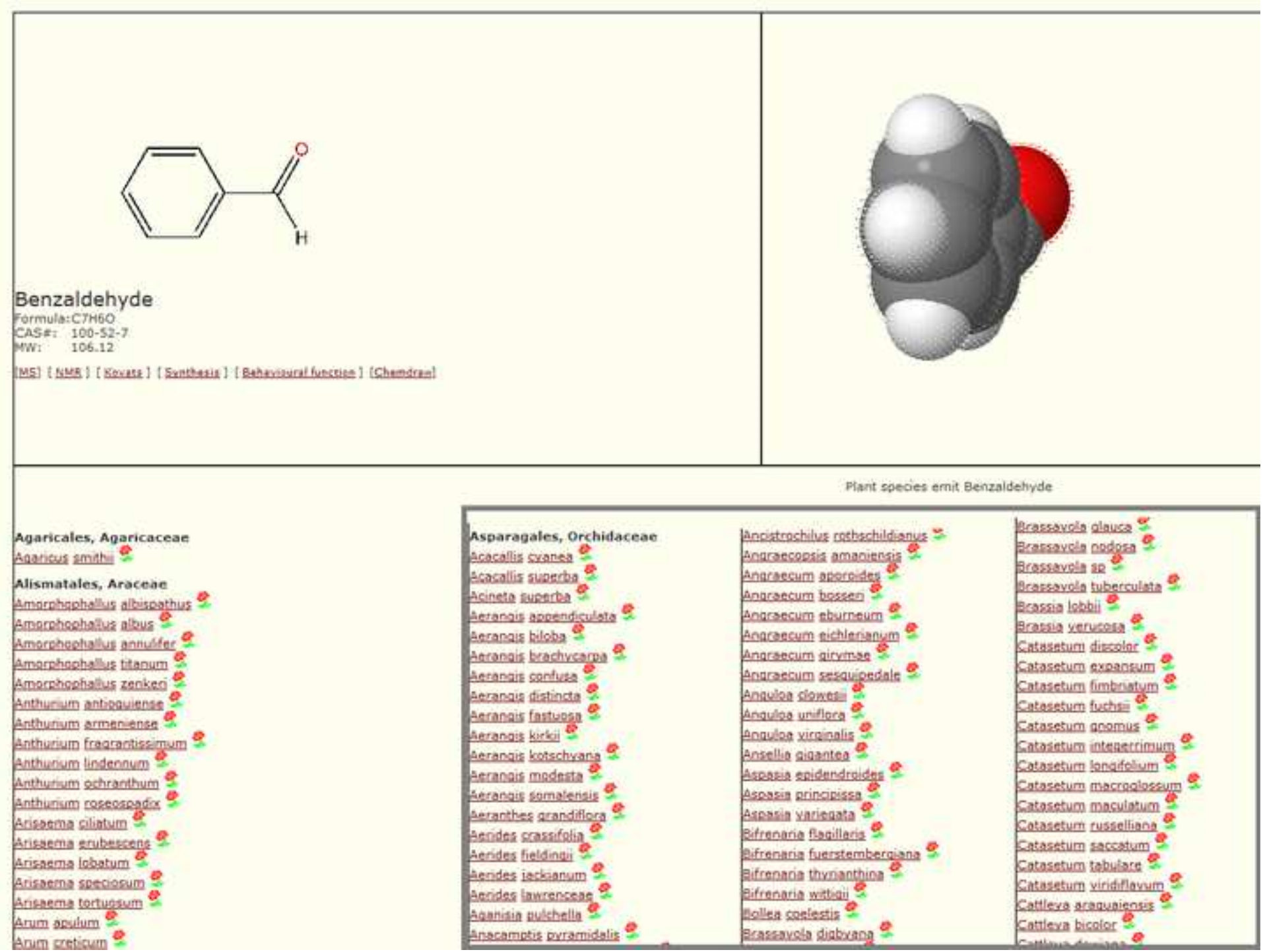

Name of Material/ Equipment	Company	Catalog Number	Comments/Description
Bulkhead Union	Cole-Palmer	UX-06390-10	
FEP tubing	Cole-Palmer	UX-06407-60	
Gas Chromatography	Hewlett Packard	6890	
Glass Wool, Silanized	Sigma-Aldrich	20411	
Inlet liner	Agilent	5062-3587	
Mass Spectrometer	Hewlett Packard	5973	
Reynolds oven bag	Reynolds Consumer Products	Turkey size	
Tenax Porous Polymer Adsorbent	Sigma-Aldrich	11982	

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JoVE Editorial Board,

We would like to submit the revisions for our paper, "Rapid collection of floral fragrance volatiles using a headspace volatile collection technique for GC-MS thermal desorption sample analysis". The comments from the reviewers and/or editor have been addressed, and the manuscript submitted shows the "Track Changes" for the revisions made. I have also uploaded a document titled "JoVE Final", which is a clean version of the document with the aforementioned changes accepted. In addition, we have added a figure (Figure 2), as well as a supplementary file, in order to better explain the results of these methods. Please let me know if there is anything else that I can do.

Thank you,

Haleigh A. Ray
University of Florida
Department of Entomology and Nematology
hray12@ufl.edu

Library Search Report

Data Path : D:\msdata\CStuhl\Haleigh\
Data File : UF1 8 AM 031615.D
Acq On : 17 Mar 2015 8:03
Operator :
Sample : UF1 8 am 031615.D
Misc :
ALS Vial : 9 Sample Multiplier: 1

Search Libraries: C:\Database\NIST14.L Minimum Quality: 80

Unknown Spectrum: Apex
Integration Events: RTE Integrator - LSCINT.P

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1	0.967	5.52	C:\Database\NIST14.L			
			Propane	80	000074-98-6	3
			Propane	79	000074-98-6	3
			Propane	78	000074-98-6	3
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			TATP	86153	017088-37-8	25
			Acetone	218	000067-64-1	9
			Acetone	214	000067-64-1	9
3	1.907	0.20	C:\Database\NIST14.L			
			Acetone	215	000067-64-1	10
			Acetone	214	000067-64-1	10
			Acetone	218	000067-64-1	10
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			Acetone	218	000067-64-1	22
			Acetone	217	000067-64-1	12
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5	2.094	0.48	C:\Database\NIST14.L			
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6	2.153	0.25	C:\Database\NIST14.L			
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			Acetone	217	000067-64-1	10
			Acetone	215	000067-64-1	10
7	2.228	0.39	C:\Database\NIST14.L			
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			TATP	86153	017088-37-8	56
			O-Methylisourea	782	002440-60-0	39
			Acetone	218	000067-64-1	14

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 9   4.803  0.37 C:\Database\NIST14.L
              Acetone                                  214 000067-64-1 43
              Acetic acid, sodium salt                1146 000127-09-3 38
              Methyl glyoxal                           634 000078-98-8 37

10   5.246  0.22 C:\Database\NIST14.L
              Silanol, trimethyl-                     2269 001066-40-6 64
              Propanoic acid, TMS derivative         22476 016844-98-7 53
              Isopropyl alcohol, TMS derivative      14712 001825-64-5 45

11   5.524  3.68 C:\Database\NIST14.L
              Ethyl Acetate                           2042 000141-78-6 91
              Ethyl Acetate                           2038 000141-78-6 90
              Ethyl Acetate                           2040 000141-78-6 86

12   6.325  0.62 C:\Database\NIST14.L
              Pentanal                                1727 000110-62-3 80
              Pentanal                                1728 000110-62-3 59
              Butanal, 3-methyl-                      1765 000590-86-3 56

13   7.426  0.28 C:\Database\NIST14.L
              1,3,5-Cycloheptatriene                  2495 000544-25-2 42
              Toluene                                 2481 000108-88-3 38
              1,3,5-Cycloheptatriene                  2496 000544-25-2 38

14   7.586  0.76 C:\Database\NIST14.L
              2-Propanone, 1-hydroxy-                  827 000116-09-6 45
              2-Propanone, 1-hydroxy-                  825 000116-09-6 45
              2-Propanone, 1-hydroxy-                  826 000116-09-6  9

15   7.784  1.92 C:\Database\NIST14.L
              Hexanal                                 3832 000066-25-1 80
              Hexanal                                 3833 000066-25-1 76
              Hexanal                                 3830 000066-25-1 64

16   7.955  0.30 C:\Database\NIST14.L
              Cyclopropane, pentyl-                   6813 002511-91-3 87
              cis-1-Butyl-2-methylcyclopropane        6920 038851-69-3 81
              trans-1-Butyl-2-methylcyclopropane      6934 038851-70-6 81

17   8.136  0.37 C:\Database\NIST14.L
              Heptane, 2,4-dimethyl-                 12973 002213-23-2 50
              Sulfurous acid, dodecyl hexyl este    191786 1000309-13-4 50
              Octane                                  7760 000111-65-9 49

18   8.542  0.62 C:\Database\NIST14.L
              Cyclotrisiloxane, hexamethyl-          85992 000541-05-9 90
              Cyclotrisiloxane, hexamethyl-          85994 000541-05-9 86
              Cyclotrisiloxane, hexamethyl-          85993 000541-05-9 64

19   9.450  1.07 C:\Database\NIST14.L
              Heptanal                                7559 000111-71-7 96
              Heptanal                                7563 000111-71-7 94
              Heptanal                                7562 000111-71-7 90

20   9.792  0.49 C:\Database\NIST14.L
              Nonane                                 12937 000111-84-2 76
              Nonane                                 12939 000111-84-2 76
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			Nonane	12938	000111-84-2 64
21	10.311	1.15	C:\Database\NIST14.L		
			Benzaldehyde	5152	000100-52-7 94
			Benzaldehyde	5148	000100-52-7 94
			Benzaldehyde	5151	000100-52-7 93
22	10.498	0.24	C:\Database\NIST14.L		
			Benzene, propyl-	9586	000103-65-1 68
			Benzene, propyl-	9585	000103-65-1 58
			Benzene, propyl-	9587	000103-65-1 52
23	10.620	0.71	C:\Database\NIST14.L		
			Benzene, 1-ethyl-2-methyl-	9606	000611-14-3 93
			Benzene, 1-ethyl-2-methyl-	9603	000611-14-3 93
			Benzene, 1-ethyl-3-methyl-	9604	000620-14-4 87
24	10.743	0.37	C:\Database\NIST14.L		
			Mesitylene	9581	000108-67-8 76
			2,4-Nonadiyne	9583	063621-15-8 76
			Benzene, 1,2,3-trimethyl-	9599	000526-73-8 76
25	10.839	0.25	C:\Database\NIST14.L		
			Nonane, 4-methyl-	19664	017301-94-9 87
			Nonane, 4-methyl-	19658	017301-94-9 81
			Nonane, 4-methyl-	19665	017301-94-9 58
26	10.898	0.43	C:\Database\NIST14.L		
			Hexadecane	89841	000544-76-3 43
			Decane, 6-ethyl-2-methyl-	51469	062108-21-8 43
			2,2-Dimethyl-3-octanone	29209	005340-64-7 43
27	10.989	0.23	C:\Database\NIST14.L		
			Phenol	2619	000108-95-2 53
			3-Methylpyridazine	2605	001632-76-4 49
			2-Vinylfuran	2622	001487-18-9 49
28	11.155	2.87	C:\Database\NIST14.L		
			Mesitylene	9578	000108-67-8 74
			Benzene, 1,2,3-trimethyl-	9592	000526-73-8 74
			Benzene, 1-ethyl-3-methyl-	9609	000620-14-4 70
29	11.459	1.70	C:\Database\NIST14.L		
			Decane	19648	000124-18-5 96
			Decane	19649	000124-18-5 95
			Decane	19651	000124-18-5 94
30	11.513	0.46	C:\Database\NIST14.L		
			Cyclotetrasiloxane, octamethyl-	155949	000556-67-2 90
			Cyclotetrasiloxane, octamethyl-	155952	000556-67-2 86
			2,5-Dihydroxyacetophenone, 2TMS de rivative	155021	1000352-83-7 59
31	11.619	1.22	C:\Database\NIST14.L		
			Benzeneacetaldehyde	9549	000122-78-1 55
			Benzeneacetaldehyde	9551	000122-78-1 55
			Benzeneacetaldehyde	9550	000122-78-1 55

```
32  11.849  0.54 C:\Database\NIST14.L
              D-Limonene                              16046 005989-27-5 50
              1-Cyclohexyl-2-buten-1-ol (c,t)         27614 079605-62-2 46
              Limonene                                16027 000138-86-3 46

33  12.073  1.21 C:\Database\NIST14.L
              .beta.-Ocimene                          16062 013877-91-3 60
              1,3,6-Octatriene, 3,7-dimethyl-, (      16175 003338-55-4 60
              Z)-
              3-Carene                                16037 013466-78-9 53

34  12.447  0.19 C:\Database\NIST14.L
              Dodecane, 2,6,11-trimethyl-             76619 031295-56-4 90
              Dodecane, 2,6,11-trimethyl-             76621 031295-56-4 81
              Decane, 3,7-dimethyl-                   39995 017312-54-8 81

35  12.795  3.19 C:\Database\NIST14.L
              Nonanal                                 20435 000124-19-6 86
              Nonanal                                 20436 000124-19-6 83
              Nonanal                                 20437 000124-19-6 80

36  13.062  0.47 C:\Database\NIST14.L
              Undecane                                29356 001120-21-4 97
              Undecane                                29357 001120-21-4 93
              Undecane                                29354 001120-21-4 87

37  13.126  0.19 C:\Database\NIST14.L
              Decane, 3,6-dimethyl-                   40000 017312-53-7 80
              Hexadecane, 7,9-dimethyl-              115564 021164-95-4 72
              Octane, 5-ethyl-2-methyl-               29382 062016-18-6 68

38  13.660  0.32 C:\Database\NIST14.L
              Butylphosphonic acid, di(4-methoxy 194995 1000323-49-3 53
              -2-methylbutyl) ester
              Thiocyanic acid, propyl ester            4121 004251-16-5 53
              Succinic acid, octyl propyl ester  132412 1000324-95-5 47

39  13.783  1.87 C:\Database\NIST14.L
              Benzeneacetic acid, methyl ester    25201 000101-41-7 91
              Benzeneacetic acid, methyl ester    25199 000101-41-7 91
              Benzeneacetic acid, methyl ester    25197 000101-41-7 91

40  13.879  0.35 C:\Database\NIST14.L
              1-(1-Methoxypropan-2-yloxy)propan-  56551 1000367-08-6 64
              2-yl acetate
              Silane, trimethyl-                    808 000993-07-7 59
              1-(1-Methoxypropan-2-yloxy)propan- 120926 1000367-13-1 59
              2-yl heptanoate

41  14.055  6.75 C:\Database\NIST14.L
              Cyclopentasiloxane, decamethyl-    220036 000541-02-6 91
              N-(Trifluoracetyl)-O,O',O''-tris(t 265635 054135-51-2 38
              rimethylsilyl)epinephrine
              N-(Trifluoroacetyl)-O,O',O''-tris( 263373 1000072-26-3 38
              trimethylsilyl)norepinephrine

42  14.360  1.00 C:\Database\NIST14.L
              Decanal                             29131 000112-31-2 86
```

```
                Decanal                                29132 000112-31-2 80
                13-Methyltetradecanal                  89788 075853-51-9 52

43  14.574  0.26 C:\Database\NIST14.L
                Octane                                  7759 000111-65-9 80
                Carbonic acid, decyl vinyl ester       91176 1000383-25-7 64
                Hexane, 3,3-dimethyl-                  7774 000563-16-6 59

44  14.664  0.70 C:\Database\NIST14.L
                Caprolactam                             7051 000105-60-2 90
                Caprolactam                             7052 000105-60-2 87
                Caprolactam                             7050 000105-60-2 87

45  15.129  0.51 C:\Database\NIST14.L
                1-Dodecene                             38260 000112-41-4 58
                1-Pentadecene                          74570 013360-61-7 53
                2-Decenal, (E)-                        27514 003913-81-3 52

46  15.786  0.77 C:\Database\NIST14.L
                Octane, 2-methyl-                      12947 003221-61-2 38
                Pentadecane, 2,6,10,14-tetramethyl    128855 001921-70-6 38
                Pentadecane, 2,6,10,14-tetramethyl    128857 001921-70-6 35

47  15.979  0.26 C:\Database\NIST14.L
                Tridecane                              51395 000629-50-5 87
                Tridecane                              51393 000629-50-5 87
                Hexadecane                             89842 000544-76-3 86

48  16.101  0.45 C:\Database\NIST14.L
                1-Undecene, 7-methyl-                  38300 074630-42-5 64
                2-Methyl-Z-4-tetradecene               74578 1000130-78-3 52
                Hexadecanoic acid, 2-hydroxy-, met    146257 016742-51-1 52
                hyl ester

49  16.219  0.18 C:\Database\NIST14.L
                Octane, 4-methyl-                      12945 002216-34-4 52
                Diglycolic acid, isohexyl 2-methyl    147767 1000381-81-5 47
                butyl ester
                Cyclohexane, 1,1-dimethyl-2-propyl     27977 081983-71-3 46

50  16.336  0.21 C:\Database\NIST14.L
                Cyclopentane, hexyl-                   27934 004457-00-5 49
                Cyclohexane, 1,2,4-trimethyl-          11829 002234-75-5 45
                1-Pentadecene                          74570 013360-61-7 38

51  16.385  0.44 C:\Database\NIST14.L
                Nonane, 1-iodo-                       115703 004282-42-2 72
                Hexadecane, 1-iodo-                   206779 000544-77-4 64
                Tetradecane, 1-iodo-                  181794 019218-94-1 64

52  16.534  2.89 C:\Database\NIST14.L
                2-Butyramido-2-[4-chloro-2-(triflu    250555 1000224-20-4 32
                oromethyl)anilino]-1,1,1,3,3,3-hex
                afluoropropane
                Cyclohexasiloxane, dodecamethyl-      254923 000540-97-6 25
                Cyclohexasiloxane, dodecamethyl-      254924 000540-97-6 22

53  16.759  1.96 C:\Database\NIST14.L
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			Butanoic acid, butyl ester	21449	000109-21-7	74
			Butanoic acid, hexyl ester	41263	002639-63-6	64
			Propanoic acid, 2-methyl-, 3-hydro xy-2,2,4-trimethylpentyl ester	79620	000077-68-9	64
54	17.159	0.40	C:\Database\NIST14.L			
			Tridecanal	63515	010486-19-8	76
			Undecanal	39874	000112-44-7	74
			Bacchotricuneatin c	199015	066563-30-2	58
55	17.293	0.30	C:\Database\NIST14.L			
			Pentadecane	76609	000629-62-9	46
			Sulfurous acid, butyl decyl ester	137914	1000309-17-7	43
			Sulfurous acid, butyl undecyl este	151433	1000309-17-8	43
56	17.651	0.32	C:\Database\NIST14.L			
			Hexasiloxane, tetradecamethyl-	258608	000107-52-8	50
			Trisiloxane, 1,1,1,5,5,5-hexamethy l-3,3-bis[(trimethylsilyl)oxy]-	229156	003555-47-3	43
			Benzyloxyamine, 2TMS derivative	127249	079208-41-6	38
57	18.094	1.69	C:\Database\NIST14.L			
			4-(Adamantan-1-ylamino)-2,5,6-tric hloro-nicotinonitrile	209305	339060-92-3	50
			1-Adamantanecarboxamide, N,N-dimet hyl-,	71591	001502-00-7	40
			1,2-Benzisothiazole-3-propanoic ac id	71226	050565-45-2	40
58	18.425	0.85	C:\Database\NIST14.L			
			2,4-Di-tert-butylphenol	70634	000096-76-4	96
			2,4-Di-tert-butylphenol	70632	000096-76-4	95
			Phenol, 2,5-bis(1,1-dimethylethyl)	70651	005875-45-6	87
59	18.537	0.43	C:\Database\NIST14.L			
			Eicosane	142239	000112-95-8	87
			Hexadecane	89842	000544-76-3	87
			Tetracosane	195673	000646-31-1	86
60	18.580	1.07	C:\Database\NIST14.L			
			Heptadecane	102600	000629-78-7	72
			Tridecane, 1-iodo-	168440	035599-77-0	64
			Undecane, 2-methyl-	39986	007045-71-8	64
61	18.692	0.14	C:\Database\NIST14.L			
			Octadecane	115546	000593-45-3	83
			Nonadecane	128834	000629-92-5	72
			Tetradecane, 1-iodo-	181793	019218-94-1	72
62	18.773	0.15	C:\Database\NIST14.L			
			6,6-Diethylhoctadecane	169414	1000360-41-8	80
			Tricosane, 2-methyl-	195680	001928-30-9	74
			Eicosane	142240	000112-95-8	74
63	18.901	1.77	C:\Database\NIST14.L			
			Cyclohexane, 1,2,4-trimethyl-	11827	002234-75-5	45
			Cyclohexane, 1,2,4-trimethyl-	11829	002234-75-5	43
			Cyclopentane, (2-methylbutyl)-	18472	053366-38-4	38

64 19.008 0.48 C:\Database\NIST14.L

Cyclohexane, 1,2,4-trimethyl-	11829	002234-75-5	30
Decane, 3,7-dimethyl-	39995	017312-54-8	30
Oxalic acid, 6-ethyloct-3-yl hepty l ester	186278	1000309-34-5	27

65 19.109 2.99 C:\Database\NIST14.L

Octadecane, 1-iodo-	226953	000629-93-6	80
Octane, 2-methyl-	12950	003221-61-2	68
Heptacosane	227469	000593-49-7	64

66 19.227 1.24 C:\Database\NIST14.L

Octane, 5-ethyl-2-methyl-	29382	062016-18-6	76
Tetracosane, 1-iodo-	260060	1000406-32-0	72
Heptadecane	102599	000629-78-7	64

67 19.285 0.15 C:\Database\NIST14.L

Tetratetracontane	273584	007098-22-8	80
Triacontane, 1-iodo-	270745	1000406-32-3	80
2-methyloctacosane	242320	1000376-72-8	72

68 19.333 1.27 C:\Database\NIST14.L

Hexadecane	89844	000544-76-3	86
Heptacosane	227469	000593-49-7	86
Eicosane, 1-iodo-	241993	1000406-31-8	83

69 19.381 0.34 C:\Database\NIST14.L

Hexacosane, 1-iodo-	265262	1000406-32-1	72
Hexacosane	217890	000630-01-3	72
Docosane, 1-iodo-	252495	1000406-31-9	64

70 19.462 0.58 C:\Database\NIST14.L

Tetracosane	195673	000646-31-1	80
Tetracosane	195672	000646-31-1	72
Hexadecane	89844	000544-76-3	72

71 19.542 0.85 C:\Database\NIST14.L

Sulfurous acid, hexadecyl 2-pentyl ester	224830	1000309-16-5	64
Sulfurous acid, dodecyl 2-pentyl e ster	178523	1000309-16-1	64
2,2,4-Trimethyl-1,3-pentanediol di isobutyrate	146067	006846-50-0	58

72 19.606 0.68 C:\Database\NIST14.L

Heneicosane	155888	000629-94-7	80
Docosane	169409	000629-97-0	72
Octadecane, 1-iodo-	226952	000629-93-6	59

73 19.665 0.49 C:\Database\NIST14.L

2H-Pyran, tetrahydro-2-(12-pentade cynyloxy)-	167395	056666-38-7	92
Octadecane	115545	000593-45-3	91
Heptadecane	102600	000629-78-7	87

74 19.718 0.41 C:\Database\NIST14.L

Hexadecane	89843	000544-76-3	94

			Hexadecane	89840	000544-76-3 94
			Hexadecane	89841	000544-76-3 94
75	20.407	0.53	C:\Database\NIST14.L		
			(4aS,8aS)-8-Isopentyl-4,4,7,8a-tetramethyl-1,2,3,4,4a,5,6,8a-octahydronaphthalene	123189	220766-79-0 38
			Silane, 1,3-decadiynyltrimethyl-	70601	084751-17-7 38
			Oxirane, [[4-(1,1-dimethylethyl)phenoxy]methyl]-	70543	003101-60-8 35
76	20.530	0.20	C:\Database\NIST14.L		
			Nonahexacontanoic acid	276185	040710-32-5 49
			Dodecane, 3-methyl-	51413	017312-57-1 43
			Sulfurous acid, butyl tetradecyl ester	191788	1000309-18-1 38
77	20.845	0.58	C:\Database\NIST14.L		
			Heptadecane	102599	000629-78-7 94
			Tridecane, 1-iodo-	168440	035599-77-0 93
			Hexadecane	89842	000544-76-3 90
78	20.963	0.27	C:\Database\NIST14.L		
			Hentriacontane	252712	000630-04-6 87
			Pentadecane, 2,6,10,14-tetramethyl	128856	001921-70-6 86
			Tetracosane	195673	000646-31-1 86
79	21.070	4.36	C:\Database\NIST14.L		
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			Heptacosane	227469	000593-49-7 90
			Octacosane	235614	000630-02-4 86
80	21.166	0.44	C:\Database\NIST14.L		
			1-Iodo-2-methylundecane	154846	073105-67-6 90
			Docosane, 1-iodo-	252495	1000406-31-9 87
			2-Bromotetradecane	136205	074036-95-6 86
81	21.219	0.20	C:\Database\NIST14.L		
			Heptadecane	102599	000629-78-7 91
			Octadecane, 1-iodo-	226952	000629-93-6 86
			Hexacosane, 1-iodo-	265262	1000406-32-1 86
82	21.358	6.04	C:\Database\NIST14.L		
			Triacontyl pentafluoropropionate	272551	1000351-80-0 52
			Octacosyl heptafluorobutyrate	273239	1000351-83-6 52
			Hexacosyl pentafluoropropionate	269275	1000351-81-2 50
83	21.454	0.72	C:\Database\NIST14.L		
			Cyclohexane, 1-ethyl-2-propyl-	27962	062238-33-9 41
			Pentadecane	76609	000629-62-9 38
			Hexadecane	89842	000544-76-3 30
84	21.518	5.49	C:\Database\NIST14.L		
			Heneicosane	155888	000629-94-7 72
			2-Bromo dodecane	109279	013187-99-0 64
			Hexane, 2,3,4-trimethyl-	12990	000921-47-1 52
85	21.615	0.89	C:\Database\NIST14.L		

			2-Bromotetradecane	136205	074036-95-6 86
			Decane, 3,8-dimethyl-	40006	017312-55-9 80
			2-methyloctacosane	242320	1000376-72-8 78
86	21.711	1.11	C:\Database\NIST14.L		
			Heptacosane	227469	000593-49-7 91
			2-Bromo dodecane	109279	013187-99-0 90
			Hexadecane	89838	000544-76-3 90
87	21.764	0.43	C:\Database\NIST14.L		
			Hexadecyl octyl ether	209173	1000406-38-6 64
			11-Methyltricosane	195679	027538-41-6 64
			Carbonic acid, decyl nonyl ester	186528	1000383-15-8 64
88	21.807	0.90	C:\Database\NIST14.L		
			Hexadecane, 2,6,11,15-tetramethyl-	142256	000504-44-9 90
			2-Bromotetradecane	136205	074036-95-6 80
			Hexacosane	217890	000630-01-3 80
89	21.898	0.82	C:\Database\NIST14.L		
			Hentriacontane	252712	000630-04-6 80
			Tetracosane	195673	000646-31-1 74
			Dotriacontane, 1-iodo-	272203	1000406-32-4 72
90	21.951	0.24	C:\Database\NIST14.L		
			Heptadecane	102599	000629-78-7 94
			Pentacosane	207499	000629-99-2 91
			Eicosane	142238	000112-95-8 91
91	22.010	0.22	C:\Database\NIST14.L		
			Tetratriacontane, 17-hexadecyl-	275157	055256-07-0 58
			Carbonic acid, octadecyl vinyl est er	197481	1000382-54-4 58
			Hexacosane	217890	000630-01-3 49
92	22.229	0.20	C:\Database\NIST14.L		
			Triacontane, 1-bromo-	266454	004209-22-7 72
			Tetratetracontane	273584	007098-22-8 72
			Decane, 3,8-dimethyl-	40006	017312-55-9 70
93	22.293	0.78	C:\Database\NIST14.L		
			Ethanone, 1-(5,6,7,8-tetrahydro-2, 8,8-trimethyl-4H-cyclohepta[b]fura n-5-yl)-	83411	071596-88-8 52
			2,5-di-tert-Butyl-1,4-benzoquinone	83301	002460-77-7 42
			3-Acetylphenanthrene	83713	002039-76-1 25
94	22.448	0.32	C:\Database\NIST14.L		
			Homosalate	122907	000118-56-9 93
			Homosalate	122906	000118-56-9 81
			3,3,5-Trimethylcyclohexyl acrylate	61569	087954-40-3 22
95	22.704	2.16	C:\Database\NIST14.L		
			Homosalate	122906	000118-56-9 99
			Homosalate	122907	000118-56-9 98
			Homosalate	122908	000118-56-9 76
96	22.784	1.24	C:\Database\NIST14.L		

			7,9-Di-tert-butyl-1-oxaspiro(4,5)d eca-6,9-diene-2,8-dione	136515	082304-66-3 95
			7,9-Di-tert-butyl-1-oxaspiro(4,5)d eca-6,9-diene-2,8-dione	136513	082304-66-3 93
			Ethanone, 1-(5,6,7,8-tetrahydro-2, 8,8-trimethyl-4H-cyclohepta[b]fura n-5-yl)-	83411	071596-88-8 56
97	23.297	0.35	C:\Database\NIST14.L		
			Hentriacontane	252711	000630-04-6 90
			Octacosane	235614	000630-02-4 87
			Docosane	169409	000629-97-0 86
98	23.564	0.36	C:\Database\NIST14.L		
			Sulfurous acid, butyl octadecyl es ter	233252	1000309-18-5 46
			Cyclohexane, 1-ethyl-2-propyl-	27962	062238-33-9 38
			Octadecane, 1-(ethenyloxy)-	155862	000930-02-9 30
99	23.693	0.36	C:\Database\NIST14.L		
			2-Methylhexacosane	227470	001561-02-0 90
			Docosane, 1-iodo-	252495	1000406-31-9 87
			Eicosane, 1-iodo-	241993	1000406-31-8 86
100	28.068	1.45	C:\Database\NIST14.L		
			Eicosane	142239	000112-95-8 86
			Eicosane, 1-iodo-	241993	1000406-31-8 81
			Hentriacontane	252712	000630-04-6 81

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