# Volatile Constituents of the Giant Puffball Mushroom (*Calvatia gigantea*)

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**KEYWORDS**:

***Calvatia gigantea,* GC-MS, Headspace Volatiles, Anthranilates, VOCs**

**ABSTRACT:**

The volatile compounds of fresh and air dried fruiting bodies of the wild edible giant puffball mushroom (*Calvatia gigantea*) were investigated by high resolution GC-MS using a sequential purge & trap technique. A total of 235 compounds were identified in this study with significant changes occurring during the ageing & sequential purging of the samples. Major volatiles in the "fresh" samples were anisole (methoxybenzene), 3-octanone, 3-methylbutan-1-ol, 3-octanol, methyl N,N-dimethylanthranilate and methyl N-methylanthranilate. Major volatiles in the "air dried" samples initially were 3-methylbutan-1-ol, 3-methylbutanal and 2-Methylbutanal; however, after the final 48 hour purge of the air dried sample, the 3-methylbutan-1-ol, 3-methylbutanal and 2-Methylbutanal had decreased dramatically with substantial increases of isovaleric acid, 2-methylbutyric acid, 2-Phenylethyl alcohol, phenol, methyl N,N-dimethylanthranilate, several lactones, methionol and 4-Hydroxy-2-pentanone. The isolation of the two aforementioned anthranilate esters along with a lesser amount of methyl anthranilate appears to be the first report of anthranilate esters in a mushroom species. Potential environmental contaminants, including VOC air pollutants in such analyses are discussed.

## INTRODUCTION

The giant puffball (*Calvatia gigantea*) is one of the largest of the edible mushroom species. A member of the fungi family of the class Homobasidiomycetes and family Lycoperdaceae, it is usually found in late summer and autumn in meadows, fields, and forests worldwide. It is notable, and noticeable, because of its size and shape. Resembling a white soccer ball, this puffball has been known to reach a size as big as 150 cm in diameter, although more commonly they are 20-60 cm in diameter.

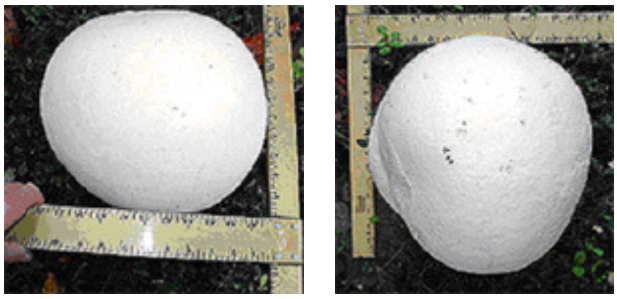
The white fruiting body develops rapidly over a period of several weeks after which it quickly turns brown and releases millions of spores.

This species is only edible when fresh and is usually consumed within 24 hours of harvest as the flavor becomes disagreeable on aging. Normally, it is sliced and pan-fried in butter, sometimes in batter, and optionally with garlic, herbs and spices. The consistency of the cooked slices resembles tofu and its inherent flavor is relatively mild.

Only a few references to the volatile constituents of *Calvatia gigantea* have been reported. Dijkstra reported that 1-octen-3-ol was the major volatile both in an extract of the fresh puffball and in a fermented submerged culture of the mycelium [1,2]. Subsequently, Overton reported that the giant puffball *Calvatia* contained a high concentration of methoxybenzene (Anisole) but did not find 1-octen-3-ol [3]. In addition, a few lipid components have been identified by Buckley [4]. A review of the ethnomycology and biotechnological potential of the genus *Calvatia* ('Gasteromycetes', Lycoperdaceae), including *C. gigantea* has recently been published [5].

Herein, we report the GC-MS study of the volatiles of both fresh and air-dried samples of this puffball mushroom by a sequential purge & trap technique.

Our interest in the giant puffball was generated by the observation of the powerful force generated by the growth of one of these puffballs under a slab of asphalt driveway. As the puffball grew, it lifted a circular area of the driveway to form a large hemispherical asphalt mound about 15 cm in height, beneath which was a giant puffball. While this obviously was not a candidate for analysis because of potential hydrocarbon contamination, several pristine puffballs averaging between 28-42 cm in diameter were harvested more than a hundred feet from the asphalt driveway in a meadow near a tree line. Nevertheless, as these were harvested in an urban area, part of this study included, not only identification of the total volatiles, but assessment of potential environmental contaminants – including VOC air pollutants.



**Photos of the *Calvatia gigantea* Samples**

## MATERIALS & EXPERIMENTAL METHODS

***Calvatia* Samples:** The *Calvatia gigantea* puffball mushrooms were harvested in a suburban area of Louisville Kentucky during late September 2005.

**Sample Preparation:** Four analyses of "fresh" slices of *Calvatia gigantea* were conducted by sequential dynamic purge and trap as described below. Similarly, three analyses of the "air dried" slices of *Calvatia gigantea* were conducted by sequential dynamic purge and trap. All samples were purged at air-conditioned room temperature of about 25-26$^{o}$ C.

A) A 4-5 gram sample of sliced "fresh" *Calvatia gigantea* was placed in 16 fl. oz. glass jar fitted with a metal lid having both inlet and outlet gas tubes. The inlet tube was connected to a helium tank and the outlet tube connected to a stainless tube with Swagelok nuts & ferrules attached. The top of the tube was fitted with a 1/4'" Swagelok union with the top nut hole diameter increased to allow the easy introduction of a Hewlett-Packard injection port liner (HP part

#5181-3316) and sealed with an injection port O-Ring. The injection port liner had been packed with 100 mg of Tenax TA (20-35 mesh) (glass-wool plugs on top and bottom) and previously baked out at 260C for 2.5 hours. Helium (50 cc/min) was introduced into the bottom of the glass jar containing the *Calvatia* slices. The sample was purged trapping the volatiles on the Tenax injection port liner. This was successively done four times at room temperature for periods of (FR-1) 9 hrs, (FR-2) 3 hrs, (FR-3) 9 hrs and (FR-4) 24 hrs, changing the Tenax trap between each purge. The Tenax liner was then placed directly into the GC injection port for thermal desorption of the volatiles onto the GC column, for each analysis.

B) In a similar manner, a sample of 4-5 grams of sliced *Calvatia gigantea* that had been air-dried for 48 hours was sequentially purged for (AD-1) 6 hrs, (AD-2) 24 hrs and (AD-3) 48 hrs for each analysis.

**GC and GC-MS:** The GC-MS was a Hewlett-Packard 6890/5973 High Performance combination. A Varian 60m X 0.32mm I. D. fused silica column coated with a 0.25 micron film thickness of VF-5MS (Varian part No. CP8961), a DB-5 equivalent column, was used in all analyses. The column was held isothermally at 30$^{o}$C for 2 minutes, and then programmed from 30$^{o}$C to 260$^{o}$C at 2$^{o}$C/min, with a final hold time of 28 minutes to give a total analysis time of 145 minutes. The Injection port was held at 260$^{o}$C. Helium Carrier Gas was used with a column flow rate of 2.9 ml/min. The Mass spectrometer was scanned in the EI mode from 26m/z to 350m/z using 70eV ionizing voltage. Additionally, FID spectra and sulfur detection using a PFPD (Pulsed Flame Photometric Detector) were employed. Percentages, corrected for known system artifacts, are FID percentages without correction for response factors.

**Component Identifications:** Identifications were based on mass spectra from the Wiley and NIST MS libraries as well as from the authors MS library. Where available, retention time comparisons were used employing primarily the Retention Indices compilations of the NIST Mass Spec Data Center [6], as well as those of Boelens [7] and Adams [8]. Initial analysis was done on the Agilent MS Enhanced Chemstation program employing both normal and selective ion modes. The NIST AMDIS deconvolution program (Ver. 2.65) was also employed in both normal and high resolution modes using the MSP file format from data imported from the Wiley and NIST MS libraries, as well as the authors library. For purposes of comparison, RI values for DB-5, HP-5, HP5-MS, etc. were considered valid (and were comparable to our prior experience using values obtained using an HP-5 column).

**Retention Indices:** Kovats RI Indices were derived using the classic (isothermal) formula of Kovats [9]:

$$RIx = 100n + 100[\log(tx) - \log(tn)] / [\log(tn+1) - \log(tn)]$$

LRI Indices (often referred to as Linear Temperature Programmed Retention indices [LTPRI] or LRI) were derived by the formula of van den Dool and Kratz [10]:

$$LRIx = 100n + 100(tx-tn) / (tn+1 - tn)$$

where $t_n$ and $t_{n+1}$ are retention times of the reference n-alkane hydrocarbons eluting immediately before and after chemical compound "X"; $t_x$ is the retention time of compound "X".

Both the calculated Kovats and the LRI retention indices are provided in our tables because much of the reported literature does not specify which RI calculations were employed. The NIST values used [6] for comparison were primarily the van den Dool and Kratz temperature programmed LRI values while the Adams values [8] used the Kovats isothermal equation even though temperature programming was utilized in their analyses. The Boelens values in the ESO 2000 (Update 2006) [7] that were derived from the literature do not specify the method of RI calculation. In most cases the RI values by both methods are quite similar, but some varied by as much as 6-7 RI units. As with all such reported RI values, those between 400-700 must be considered as somewhat approximate as such values are subject to some instrumental experimental fluctuations between laboratories.

## RESULTS & DISCUSSION

The volatile compounds identified by sequential dynamic headspace analyses of both fresh and air dried *Calvatia gigantea* consisted of 34 aromatic hydrocarbons, 33 alkanes, 29 alcohols, 28 monoterpenoids, 23 ketones, 16 acids, 15 aldehydes, 11 esters, 9 lactones, 6 sulphur compounds, 5 furans, 6 sesquiterpenes, 4 alkenes, 4 phenolics, 4 aromatic ethers, 3 anthranilate esters, 2 aniline formamides (tentative identifications), 1 pyrrole and 1 cycloalkane.

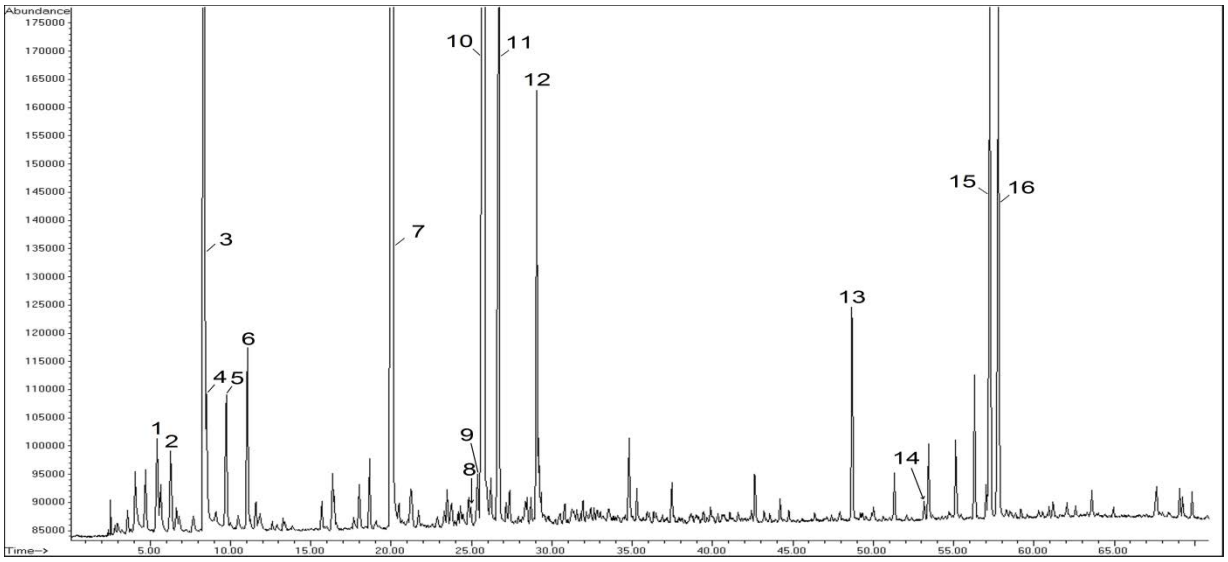



**Fig. 1. Headspace Chromatogram of Fresh (FR-1) *Calvatia gigantea***

(1) 3-Methylbutanal (2) 2-Pentanone (3) Isoamyl alcohol (4) 2-Methyl-1-butanol (5) Toluene (6) 1-Octene (7) Anisole (8) 1-Octen-3-one (9) 1-Octen-3-ol (10) 3-Octanone (11) 3-Octanol (12) Limonene (13) N-(2-methylphenyl)formamide[tent] (14) Methyl anthranilate (15) Methyl N,N-dimethylanthranilate (16) Methyl N-methylanthranilate

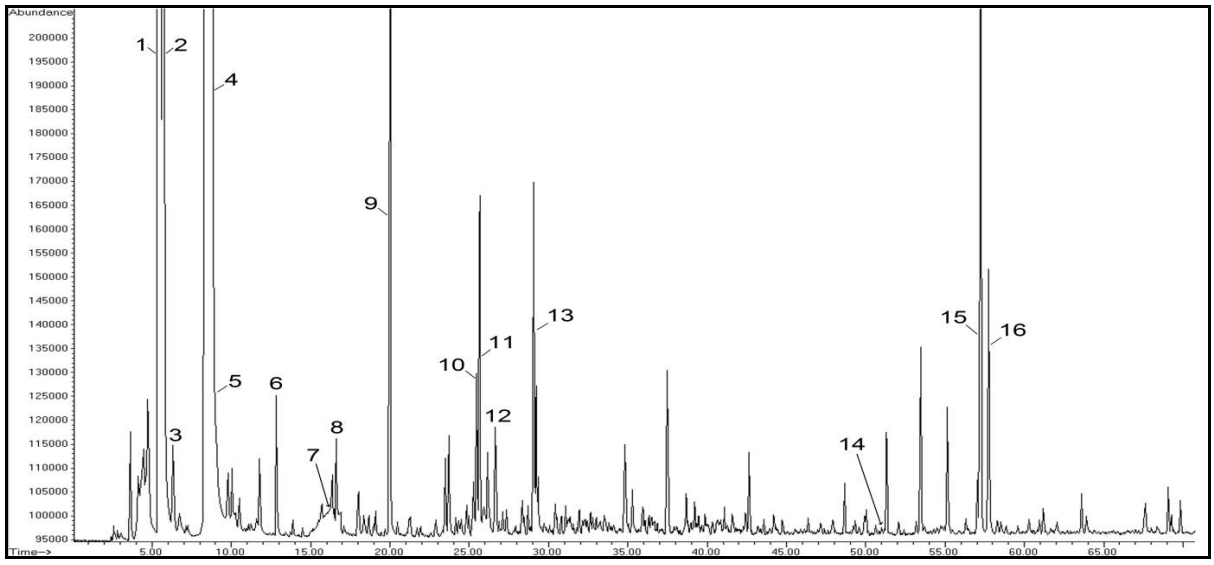



**Fig. 2. Headspace Chromatogram of Air-dried (AD-1) *Calvatia gigantea***

(1) 3-Methylbutanal (2) 2-Methylbutanal (3) 2-Pentanone (4) Isoamyl alcohol (5) 2-Methyl-1-butanol (6) 4-Hydroxy-2-pentanone (7) Isovaleric acid (8) 1-Hexanol (9) Anisole (10) Phenol (11) 3-Octanone (12) 3-Octanol (13) Limonene (14) Methyl anthranilate (15) Methyl N,N-dimethylanthranilate (16) Methyl N-methylanthranilate

Table 1 summarizes these 235 volatile compounds and their retention indices.

**Table 1. Volatiles of *Calvatia gigantea* by Sequential Purge & Trap Dynamic Headspace Analysis**

| | | | | | Fresh | | | | Air-Dried (48 Hours) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | FR-1 | FR-2 | FR-3 | FR-4 | AD-1 | AD-2 | AD-3 | |
| Actual R.T. | VF-5MS Kovats RI | VF-5MS LRI | Compounds | Purge Time---> | 9 Hr % | 3 Hr % | 9 Hr % | 24 Hr % | 6 Hr % | 24 Hr % | 48 Hr % | Ident. |
| 2.550 | 412 | 412 | Acetaldehyde[e] | | 0.05 | 0.10 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | MS,RI |
| 2.793 | 429 | 426 | Ethanol[e] | | 0.04 | 0.11 | 0.16 | 0.21 | 0.01 | 0.09 | ND | MS,RI |
| 3.000 | 496 | 496 | 2-Propanone[e] | | 0.05 | 0.16 | 0.15 | 0.21 | 0.02 | 0.03 | 0.04 | MS,RI |
| 3.572 | 553 | 549 | 1-Propanol[e] | | 0.05 | 0.14 | 0.11 | 0.10 | trace | ND | ND | MS,RI |
| 3.623 | 558 | 554 | Isobutanal[e] | | ND | ND | ND | ND | 0.23 | 0.16 | trace | MS,RI |
| 3.634 | 559 | 555 | 2-Methylpentane[d] | | 0.10 | 0.14 | ND | ND | ND | ND | ND | MS,RI |
| 3.983 | 588 | 587 | Acetic acid[e] | | 0.01 | 0.01 | 0.02 | 0.01 | 1.10 | 0.82 | 1.40 | MS,RI |
| 4.077 | 592 | 592 | 2-Butanone[e] | | 0.39 | 0.58 | 0.23 | 0.06 | 0.34 | trace | ND | MS,RI |
| 4.189 | 598 | 596 | 2-Butanol[e] | | 0.08 | 0.31 | 0.12 | 0.10 | ND | ND | ND | MS,RI |
| 4.390 | 607 | 604 | Ethyl acetate[e] | | ND | ND | trace | 0.20 | ND | ND | ND | MS,RI |
| 4.634 | 618 | 614 | Tetrahydrofuran[d?] | | 0.19 | 0.34 | 0.17 | 0.04 | ND | ND | ND | MS,RI |
| 4.705 | 621 | 616 | Isobutanol[e] | | 0.21 | 0.40 | 0.19 | 0.16 | 0.47 | 0.15 | ND | MS,RI |
| 5.436 | 651 | 645 | 3-Methylbutanal[e] | | 0.82 | 0.49 | 0.17 | 0.02 | 30.71 | 7.29 | 1.24 | MS,RI |
| 5.654 | 659 | 653 | 2-Methylbutanal[e] | | 0.44 | 0.45 | 0.09 | 0.07 | 12.13 | 5.17 | 0.30 | MS,RI |
| 6.279 | 680 | 678 | 2-Pentanone[e] | | 0.72 | 0.69 | 0.82 | 0.30 | 0.33 | 0.19 | 0.02 | MS,RI |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 6.643 | 700 | 700 | Heptane[e] | 0.18 | 0.24 | ND | ND | ND | ND | ND | MS,RI |
| 6.739 | 703 | 702 | Pentanal[e] | ND | ND | ND | ND | 0.10 | 0.14 | 0.02 | MS,RI |
| 6.798 | 704 | 703 | 2-Pentanol[e] | 0.14 | 0.22 | 0.23 | 0.38 | 0.04 | trace | ND | MS,RI |
| 7.114 | 712 | 710 | 1,2-Ethanediol | ND | ND | ND | ND | 0.03 | trace | ND | MS |
| 7.190 | 714 | 711 | Acetoin[e] | ND | ND | ND | ND | ND | ND | 0.03 | MS,RI |
| 7.470 | 721 | 717 | Propanoic acid | ND | ND | ND | ND | ND | ND | 0.04 | MS |
| 7.692 | 726 | 721 | Methylcyclohexane[d] | 0.15 | 0.27 | ND | ND | ND | ND | ND | MS,RI |
| 8.369 | 742 | 735 | Isoamyl alcohol[e] | 11.78 | 29.76 | 40.48 | 32.14 | 35.71 | 52.03 | 3.72 | MS,RI |
| 8.502 | 744 | 738 | 2-Methyl-1-butanol[e] | 0.95 | 3.02 | 3.77 | 3.21 | 3.32 | 3.04 | 0.78 | MS,RI |
| 8.572 | 746 | 739 | Dimethyl disulfide[e] | trace | trace | trace | trace | trace | trace | trace | MS,RI |
| 9.098 | 757 | 750 | Pyrrole[e] | trace | 0.23 | 0.61 | 0.25 | trace | ND | ND | MS,RI |
| 9.452 | 764 | 757 | Ethyl isobutyrate | ND | ND | trace | 0.30 | ND | ND | ND | MS,RI |
| 9.482 | 764 | 758 | Propylene glycol[e] | ND | ND | ND | ND | 0.09 | trace | trace | MS,RI |
| 9.735 | 769 | 763 | Toluene[a,d,e] | 1.00 | 0.11 | ND | ND | 0.23 | trace | trace | MS,RI |
| 9.990 | 773 | 768 | 1-Pentanol[e] | trace | trace | 0.11 | 0.11 | 0.08 | 0.30 | 0.02 | MS,RI |
| 10.290 | 779 | 774 | Isobutyric acid[e] | ND | ND | ND | ND | 0.18 | 0.17 | 0.25 | MS,RI |
| 10.471 | 782 | 778 | 2,4-Pentanedione[e] | 0.11 | 0.10 | 0.23 | 0.23 | 0.09 | 0.43 | 0.18 | MS,RI |
| 11.030 | 791 | 789 | 2-Hexanone[e] | ND | ND | trace | trace | trace | ND | 0.04 | MS,RI |
| 11.050 | 792 | 789 | 1-Octene[d,e] | 1.14 | 0.09 | ND | ND | ND | ND | ND | MS,RI |
| 11.215 | 794 | 793 | Cyclopentanone | trace | trace | 0.07 | 0.05 | ND | ND | ND | MS,RI |
| 11.574 | 800 | 800 | Octane[e] | 0.19 | 0.09 | 0.07 | 0.08 | 0.03 | 0.03 | 0.05 | MS,RI |
| 11.757 | 803 | 803 | Butyric acid[e] | ND | ND | ND | ND | 0.02 | 0.06 | 0.02 | MS,RI |
| 11.822 | 804 | 803 | Ethyl butanoate[e] | ND | ND | 0.06 | 0.07 | ND | ND | ND | MS,RI |
| 11.849 | 805 | 804 | Hexanal[e] | 0.20 | 0.28 | ND | 0.01 | 0.17 | 0.09 | 0.24 | MS,RI |
| 12.836 | 822 | 818 | 4-Hydroxy-2-pentanone | ND | trace | 0.06 | 0.16 | 0.26 | 1.22 | 8.10 | MS |
| 13.773 | 836 | 831 | Furfural[e] | trace | trace | trace | trace | 0.04 | trace | 0.01 | MS,RI |
| 14.680 | 850 | 844 | 2-Methylthioethanol | ND | ND | ND | ND | ND | 0.01 | 0.02 | MS |
| 14.710 | 850 | 844 | 4-Hydroxy-4-methyl-2-pentanone[e] | ND | ND | ND | ND | 0.02 | 0.06 | 0.21 | MS,RI |
| 15.374 | 859 | 854 | Ethyl isovalerate | ND | ND | trace | 0.04 | ND | ND | ND | MS,RI |
| 15.490 | 861 | 855 | Furfuryl alcohol[e] | ND | ND | ND | ND | 0.02 | trace | 0.41 | MS,RI |
| 15.686 | 864 | 858 | Ethylbenzene[d] | 0.17 | ND | trace | trace | 0.07 | trace | trace | MS,RI |
| 16.330 | 872 | 867 | Isovaleric acid[e] | ND | ND | ND | 0.01 | 0.51 | 1.71 | 19.15 | MS,RI |
| 16.354 | 872 | 867 | p-Xylene[d,e] | 0.32 | 0.09 | 0.06 | 0.02 | 0.10 | trace | trace | MS,RI |
| 16.455 | 874 | 869 | m-Xylene[d,e] | 0.16 | 0.06 | 0.01 | ND | 0.05 | ND | ND | MS,RI |
| 16.623 | 876 | 871 | 1-Hexanol[e] | trace | trace | 0.07 | 0.12 | 0.20 | 1.26 | 0.89 | MS,RI |
| 16.869 | 879 | 875 | 2-Methylbutanoic acid[e] | ND | ND | ND | ND | 0.07 | 0.64 | 5.58 | MS,RI |
| 17.196 | 883 | 879 | Isoamyl acetate[e] | ND | ND | ND | 0.03 | 0.01 | 0.30 | 0.29 | MS,RI |
| 17.353 | 885 | 882 | Allyl isothiocyanate | ND | ND | ND | ND | trace | 0.02 | 0.03 | MS,RI |
| 17.683 | 889 | 886 | 3-Heptanone | 0.06 | ND | 0.02 | 0.03 | trace | ND | ND | MS,RI |
| 17.919 | 891 | 889 | Cyclohexanol | ND | ND | ND | trace | 0.02 | ND | ND | MS,RI |
| 17.961 | 892 | 890 | 2-Heptanone[e] | ND | ND | ND | trace | 0.02 | trace | ND | MS,RI |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 17.983 | 892 | 890 | Styrene[d] | 0.10 | 0.05 | 0.03 | 0.05 | 0.04 | ND | trace | MS,RI |
| 18.023 | 893 | 891 | o-Xylene[d,e] | 0.21 | 0.09 | 0.05 | 0.10 | 0.06 | ND | ND | MS,RI |
| 18.365 | 897 | 896 | Cyclohexanone | ND | ND | ND | ND | 0.04 | ND | ND | MS,RI |
| 18.484 | 898 | 897 | Pentanoic acid[e] | ND | ND | ND | ND | 0.03 | 0.03 | 0.21 | MS,RI |
| 18.664 | 900 | 900 | Nonane[e] | 0.41 | 0.12 | 0.06 | 0.04 | 0.04 | ND | ND | MS,RI |
| 18.980 | 905 | 904 | Heptanal[e] | ND | ND | ND | trace | 0.02 | trace | ND | MS,RI |
| 19.092 | 906 | 905 | 2-Butoxyethanol[d,e] | ND | ND | ND | 0.01 | 0.06 | 0.19 | 0.09 | MS,RI |
| 19.460 | 912 | 910 | Methional[e] | ND | ND | ND | ND | trace | trace | trace | MS,RI |
| 19.514 | 912 | 911 | 2-Acetylfuran[e] | ND | ND | ND | ND | trace | trace | ND | MS,RI |
| 19.684 | 915 | 913 | gamma-Butyrolactone[e] | ND | ND | ND | ND | 0.02 | 0.04 | 0.16 | MS,RI |
| 20.094 | 921 | 918 | Anisole[a,e] | 23.65 | 18.92 | 15.08 | 11.72 | 1.64 | 0.43 | 0.37 | MS,RI |
| 20.481 | 926 | 923 | Isopropylbenzene[d,e] | 0.13 | 0.07 | 0.07 | 0.05 | 0.03 | ND | ND | MS,RI |
| 20.678 | 929 | 925 | alpha-Thujene | trace | trace | ND | ND | trace | trace | ND | MS,RI |
| 21.216 | 936 | 932 | alpha-Pinene[e] | 0.21 | 0.20 | 0.02 | trace | 0.03 | trace | ND | MS,RI |
| 21.284 | 937 | 933 | 2-Methyl-5-isopropenylfuran | 0.07 | 0.03 | 0.08 | 0.10 | 0.02 | 0.03 | 0.06 | MS,RI |
| 21.286 | 937 | 933 | 2,6-Dimethyloctane[d] | 0.14 | 0.01 | ND | ND | 0.02 | ND | ND | MS,RI |
| 21.719 | 942 | 938 | 3-Ethyl-2-methylheptane[d] | 0.11 | ND | ND | ND | 0.02 | ND | ND | MS,RI |
| 21.933 | 945 | 941 | 1-Butoxy-2-propanol[d] | ND | ND | ND | trace | 0.02 | 0.01 | ND | MS,RI |
| 22.618 | 954 | 949 | 2-Methylcyclohexanone[e] | ND | ND | ND | ND | ND | 0.01 | 0.02 | MS,RI |
| 22.904 | 957 | 953 | Propylbenzene[d] | 0.11 | trace | 0.02 | 0.01 | 0.26 | ND | ND | MS,RI |
| 22.940 | 958 | 953 | gamma-Valerolactone[e] | ND | ND | trace | 0.01 | 0.26 | 0.29 | 1.80 | MS,RI |
| 23.318 | 962 | 958 | 4-Methylnonane[d] | 0.12 | ND | trace | trace | 0.02 | ND | ND | MS,RI |
| 23.494 | 964 | 960 | 1-Ethyl-3-methylbenzene[d] | 0.28 | 0.11 | 0.09 | 0.09 | 0.17 | ND | trace | MS,RI |
| 23.548 | 965 | 961 | delta-Valerolactone | trace | 0.01 | 0.02 | 0.03 | 0.08 | 0.47 | 4.60 | MS,RI |
| 23.726 | 967 | 963 | Benzaldehyde[e] | 0.04 | 0.08 | 0.03 | 0.03 | 0.20 | 0.59 | 0.30 | MS,RI |
| 23.748 | 967 | 963 | 1-Ethyl-4-methylbenzene[d] | 0.08 | 0.06 | 0.04 | 0.04 | 0.04 | ND | ND | MS,RI |
| 23.801 | 968 | 964 | 2-Methylnonane[d] | 0.14 | ND | ND | ND | ND | ND | ND | MS,RI |
| 24.139 | 972 | 968 | Dimethyl trisulfide[e] | trace | trace | 0.01 | 0.02 | trace | 0.02 | 0.02 | MS,RI |
| 24.176 | 972 | 969 | 1,3,5-Trimethylbenzene[d] | 0.08 | 0.04 | 0.03 | 0.02 | 0.04 | 0.01 | ND | MS,RI |
| 24.572 | 977 | 973 | 1-heptanol[e] | ND | ND | ND | 0.03 | 0.05 | 0.08 | ND | MS,RI |
| 24.326 | 974 | 970 | 3-Methylnonane[d] | 0.13 | ND | ND | 0.03 | 0.08 | ND | ND | MS,RI |
| 24.464 | 976 | 972 | Sabinene[e] | trace | trace | 0.02 | 0.03 | 0.06 | 0.06 | ND | MS,RI |
| 24.830 | 980 | 977 | beta-Pinene[e] | 0.09 | 0.06 | 0.02 | 0.01 | 0.01 | trace | ND | MS,RI |
| 24.900 | 980 | 978 | 1-Ethyl-2-methylbenzene[d] | 0.15 | 0.07 | 0.03 | 0.02 | 0.07 | 0.08 | ND | MS,RI |
| 24.979 | 981 | 979 | 1-Octen-3-one[e] | 0.11 | ND | ND | ND | 0.04 | ND | 0.01 | MS,RI |
| 25.173 | 984 | 981 | Methionol | ND | ND | ND | trace | 0.01 | 0.88 | 4.96 | MS,RI |
| 25.396 | 986 | 984 | 1-Octen-3-ol[b,e] | 0.45 | trace | trace | trace | 0.15 | trace | ND | MS,RI |
| 25.650 | 989 | 987 | Phenol[e] | trace | 0.70 | 2.24 | 4.61 | 0.33 | 2.81 | 6.69 | MS,RI |
| 25.799 | 990 | 989 | 3-Octanone[e] | 25.75 | 15.45 | 12.68 | 9.20 | 0.70 | 0.12 | 0.16 | MS,RI |
| 25.932 | 992 | 990 | beta-Myrcene[e] | 0.19 | trace | trace | trace | 0.01 | 0.08 | ND | MS,RI |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 26.010 | 993 | 991 | 2-Pentylfuran[e] | 0.19 | trace | 0.02 | trace | 0.11 | trace | trace | MS,RI |
| 26.046 | 993 | 992 | 2-Octanone[e] | ND | ND | trace | trace | 0.01 | 0.01 | ND | MS,RI |
| 26.227 | 995 | 994 | 1,2,4-Trimethylbenzene[d] | 0.38 | 0.25 | 0.19 | 0.20 | 0.22 | 0.22 | ND | MS,RI |
| 26.296 | 996 | 995 | Hexanoic acid[e] | ND | ND | ND | 0.02 | 0.07 | 0.06 | 0.02 | MS,RI |
| 26.698 | 1000 | 1000 | 3-Octanol[e] | 4.97 | 5.06 | 4.60 | 8.04 | 0.21 | 0.09 | 0.03 | MS,RI |
| 26.706 | 1000 | 1000 | Decane[e] | 0.06 | trace | trace | ND | 0.11 | 0.05 | trace | MS,RI |
| 27.153 | 1006 | 1006 | Octanal[e] | 0.13 | 0.14 | 0.07 | 0.10 | 0.05 | 0.06 | 0.01 | MS,RI |
| 27.196 | 1007 | 1006 | alpha-Phellandrene[e] | trace | ND | 0.07 | trace | trace | trace | ND | MS,RI |
| 27.372 | 1009 | 1008 | delta-3-Carene | 0.22 | 0.09 | 0.10 | 0.07 | 0.08 | 0.03 | trace | MS,RI |
| 27.821 | 1015 | 1014 | Isoamyl isobutyrate | ND | ND | ND | ND | 0.02 | 0.02 | ND | MS,RI |
| 28.311 | 1022 | 1020 | meta-Cymene | 0.03 | 0.01 | 0.01 | 0.02 | 0.01 | 0.01 | trace | MS,RI |
| 28.355 | 1023 | 1020 | 1,2,3-Trimethylbenzene[d] | 0.12 | 0.03 | 0.09 | 0.08 | 0.06 | 0.01 | trace | MS,RI |
| 28.472 | 1024 | 1022 | 2,6-Dimethylnonane[d] | 0.17 | ND | 0.11 | trace | 0.04 | ND | ND | MS,RI |
| 28.717 | 1027 | 1025 | para-Cymene[e] | 0.14 | 0.09 | 0.05 | 0.05 | 0.06 | 0.01 | 0.02 | MS,RI |
| 29.085 | 1032 | 1029 | Limonene[a,e] | 2.31 | 1.24 | 0.85 | 0.72 | 0.68 | 0.28 | 0.16 | MS,RI |
| 29.199 | 1034 | 1031 | beta-Phellandrene[e] | 0.01 | 0.08 | 0.09 | 0.06 | 0.07 | 0.03 | trace | MS,RI |
| 29.209 | 1034 | 1031 | 2-Ethyl-1-hexanol[e] | 0.43 | 0.34 | 0.67 | 1.53 | 0.23 | 1.31 | 3.23 | MS,RI |
| 29.333 | 1035 | 1032 | 1,8-Cineole[e] | 0.13 | trace | 0.03 | 0.02 | 0.16 | 0.17 | ND | MS,RI |
| 29.554 | 1038 | 1035 | ortho-Cymene | trace | ND | trace | trace | ND | trace | ND | MS,RI |
| 29.681 | 1040 | 1037 | Benzyl alcohol[e] | ND | trace | 0.03 | 0.05 | 0.05 | 0.30 | 1.41 | MS,RI |
| 29.880 | 1042 | 1039 | 5-Methyl-5-vinyldihydrofuran-2(3H)-one | ND | ND | ND | ND | trace | 0.06 | 0.01 | MS,RI |
| 30.428 | 1049 | 1046 | Phenylacetaldehyde[a,e] | ND | ND | ND | ND | 0.06 | 0.08 | ND | MS,RI |
| 30.440 | 1049 | 1046 | 2-Hydroxybenzaldehyde[e] + unknowns | trace | trace | trace | 0.01 | ND | trace | trace | MS,RI |
| 30.528 | 1050 | 1047 | 1,3-Diethylbenzene[d] + unknown | 0.11 | 0.10 | 0.06 | ND | 0.04 | 0.04 | trace | MS,RI |
| 30.811 | 1054 | 1051 | 1-Methyl-3-propylbenzene[d] | 0.14 | 0.06 | 0.03 | 0.03 | 0.04 | 0.05 | trace | MS,RI |
| 31.100 | 1057 | 1054 | gamma-hexalactone[e] | ND | ND | ND | trace | 0.06 | 0.07 | 2.41 | MS,RI |
| 31.267 | 1059 | 1056 | 1-Methyl-4-propylbenzene[d] + unknown | 0.12 | 0.05 | 0.04 | 0.06 | 0.04 | 0.05 | trace | MS,RI |
| 31.356 | 1061 | 1057 | 1-Ethyl-3,5-dimethylbenzene[d] | 0.08 | 0.06 | 0.06 | 0.07 | 0.06 | 0.05 | trace | MS,RI |
| 31.477 | 1062 | 1059 | gamma-Terpinene[e] | 0.03 | trace | 0.02 | trace | trace | 0.02 | trace | MS,RI |
| 31.581 | 1063 | 1060 | 4-Methyldecane[d] | 0.08 | 0.05 | 0.02 | trace | 0.02 | 0.03 | ND | MS,RI |
| 31.800 | 1066 | 1063 | 1-Phenylethanol[e] | trace | ND | trace | 0.02 | 0.01 | 0.02 | 0.08 | MS,RI |
| 31.940 | 1067 | 1065 | 1-Methyl-2-propylbenzene[d] | 0.13 | 0.08 | 0.04 | 0.03 | 0.05 | 0.06 | trace | MS,RI |
| 32.156 | 1070 | 1067 | Acetophenone[e] | 0.07 | 0.07 | 0.04 | 0.05 | 0.02 | 0.04 | 0.11 | MS,RI |
| 32.421 | 1073 | 1070 | 3-Methyldecane[d] | 0.08 | trace | trace | ND | 0.04 | 0.05 | ND | MS,RI |
| 32.500 | 1074 | 1071 | cis-Linalool oxide (furanoid)[e] | ND | ND | ND | trace | ND | 0.03 | ND | MS,RI |
| 32.648 | 1076 | 1073 | 1-Octanol[e] | 0.06 | 0.05 | 0.04 | 0.02 | 0.03 | 0.06 | 0.18 | MS,RI |
| 32.660 | 1076 | 1073 | Heptanoic acid[e] | ND | ND | 0.01 | 0.01 | 0.01 | 0.02 | ND | MS,RI |
| 32.815 | 1078 | 1075 | 1-Ethyl-2,4-dimethylbenzene[d] | 0.06 | ND | 0.04 | 0.03 | 0.04 | 0.05 | trace | MS,RI |
| 33.539 | 1086 | 1084 | 1-Ethyl-2,5-dimethylbenzene[d] | 0.09 | 0.08 | 0.04 | 0.02 | 0.04 | 0.05 | trace | MS,RI |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| 33.653 | 1087 | 1086 | Terpinolene[e] | 0.04 | trace | 0.02 | trace | trace | 0.04 | trace | MS,RI |
| 33.760 | 1088 | 1087 | Guaiacol[e] | ND | ND | trace | trace | trace | trace | trace | MS,RI |
| 33.793 | 1089 | 1087 | trans-Linalool oxide (furanoid)[e] | ND | ND | ND | trace | trace | 0.04 | trace | MS,RI |
| 33.842 | 1089 | 1088 | 1-Ethyl-3,4-dimethylbenzene[d] (or isomer) | 0.05 | 0.03 | trace | trace | 0.02 | trace | ND | MS,RI |
| 33.890 | 1090 | 1089 | α,α-Dimethylbenzyl alcohol | ND | ND | trace | trace | 0.02 | 0.06 | 0.17 | MS,RI |
| 33.981 | 1091 | 1090 | Fenchone[e] | trace | ND | trace | 0.01 | 0.02 | trace | ND | MS,RI |
| 34.086 | 1092 | 1091 | 1-isopropenyl-4-methylbenzene | ND | ND | ND | 0.01 | 0.02 | 0.01 | trace | MS,RI |
| 34.160 | 1093 | 1092 | 2-Nonanone[e] | ND | ND | ND | 0.01 | 0.02 | 0.01 | 0.02 | MS,RI |
| 34.364 | 1095 | 1094 | 5-Ethyl-5-methyldihydrofuran-2(3H)-one[tent] | ND | ND | ND | ND | trace | 0.01 | 0.03 | MS |
| 34.459 | 1096 | 1096 | Methyl benzoate[e] | ND | ND | ND | 0.02 | 0.01 | 0.01 | 0.05 | MS,RI |
| 34.820 | 1100 | 1100 | Undecane[e] | 0.43 | 0.37 | 0.14 | 0.10 | 0.17 | 0.12 | 0.02 | MS,RI |
| 34.869 | 1101 | 1101 | Linalool[e] | 0.14 | trace | 0.08 | 0.13 | 0.11 | 0.18 | 0.13 | MS,RI |
| 35.314 | 1107 | 1106 | Nonanal[a,e] | 0.22 | 0.28 | 0.07 | 0.10 | 0.09 | 0.11 | ND | MS,RI |
| 35.942 | 1116 | 1114 | 2-Phenylethyl alcohol[e] | trace | 0.05 | 0.05 | 0.13 | 0.06 | 1.99 | 7.42 | MS,RI |
| 36.079 | 1118 | 1116 | 1,2,4,5-Tetramethylbenzene[d] | 0.06 | 0.04 | 0.03 | 0.04 | 0.04 | 0.06 | trace | MS,RI |
| 36.338 | 1121 | 1119 | 1,2,3,5-Tetramethylbenzene[d] | 0.04 | 0.09 | 0.03 | trace | 0.04 | 0.06 | trace | MS,RI |
| 36.477 | 1123 | 1121 | exo-Fenchyl alcohol | trace | trace | trace | 0.02 | 0.02 | 0.03 | trace | MS,RI |
| 36.625 | 1125 | 1123 | Isophorone[e] | ND | ND | ND | trace | trace | 0.03 | 0.07 | MS,RI |
| 36.680 | 1126 | 1124 | 2-Ethylhexanoic acid[e] | ND | ND | 0.01 | 0.02 | 0.05 | 0.03 | 0.24 | MS,RI |
| 38.091 | 1144 | 1142 | Benzeneacetonitrile | ND | ND | ND | ND | ND | 0.04 | 0.06 | MS,RI |
| 38.473 | 1149 | 1147 | 4-Ketoisophorone | ND | ND | ND | trace | ND | 0.01 | 0.04 | MS,RI |
| 38.669 | 1152 | 1149 | Camphor[e] | 0.04 | trace | 0.06 | 0.07 | 0.11 | 0.04 | 0.02 | MS,RI |
| 38.777 | 1153 | 1151 | 1,2,3,4-Tetramethylbenzene[d] | 0.04 | 0.02 | 0.03 | 0.03 | 0.03 | 0.02 | 0.04 | MS,RI |
| 39.094 | 1157 | 1155 | gamma-heptalactone | ND | ND | ND | ND | ND | trace | 0.03 | MS,RI |
| 39.336 | 1160 | 1158 | Menthone[e] | 0.03 | trace | 0.06 | 0.02 | 0.02 | trace | 0.01 | MS,RI |
| 39.638 | 1164 | 1162 | 1,2,3,4-Tetrahydronaphthalene | ND | ND | trace | 0.03 | 0.02 | 0.02 | trace | MS,RI |
| 39.853 | 1167 | 1165 | Benzyl acetate[e] | 0.08 | 0.02 | 0.03 | 0.03 | trace | 0.01 | 0.01 | MS,RI |
| 40.529 | 1175 | 1173 | 1-Nonanol[e] | ND | 0.02 | 0.02 | 0.03 | ND | 0.05 | 0.17 | MS,RI |
| 40.614 | 1176 | 1174 | Borneol | 0.03 | 0.04 | 0.04 | 0.04 | 0.02 | 0.04 | 0.08 | MS,RI |
| 40.738 | 1178 | 1176 | Octanoic acid[e] | 0.06 | 0.18 | 0.06 | 0.05 | 0.05 | 0.01 | ND | MS,RI |
| 41.069 | 1182 | 1180 | Menthol[e] | trace | 0.05 | 0.05 | 0.08 | 0.07 | 0.10 | 0.24 | MS,RI |
| 41.254 | 1184 | 1182 | 4-Terpineol | ND | ND | 0.01 | 0.02 | 0.02 | 0.03 | 0.04 | MS,RI |
| 41.562 | 1188 | 1186 | Naphthalene[e] | trace | 0.02 | 0.06 | 0.05 | 0.07 | 0.04 | 0.06 | MS,RI |
| 42.078 | 1194 | 1193 | 2-Decanone[e] | ND | ND | ND | 0.03 | trace | trace | ND | MS,RI |
| 42.157 | 1195 | 1194 | Methyl salicylate | trace | trace | trace | 0.01 | trace | 0.01 | 0.01 | MS,RI |
| 42.406 | 1198 | 1197 | alpha-Terpineol[e] | 0.03 | 0.05 | 0.04 | 0.05 | 0.03 | 0.08 | 0.19 | MS,RI |
| 42.620 | 1200 | 1200 | Dodecane[e] | 0.25 | 0.24 | 0.14 | 0.11 | 0.15 | 0.04 | 0.02 | MS,RI |
| 42.634 | 1200 | 1200 | Methyl chavicol | 0.02 | 0.01 | 0.02 | 0.03 | 0.02 | 0.02 | 0.04 | MS,RI |
| 43.202 | 1208 | 1208 | Decanal[e] | 0.08 | 0.12 | 0.03 | 0.03 | 0.03 | 0.02 | trace | MS,RI |
| 43.353 | 1211 | 1210 | Verbenone | ND | ND | ND | 0.02 | 0.02 | 0.02 | 0.07 | MS,RI |
| 44.185 | 1223 | 1221 | N-Phenylformamide[tent] | 0.11 | 0.06 | 0.04 | 0.06 | 0.01 | 0.03 | 0.01 | MS |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| 44.207 | 1223 | 1221 | 2-Phenoxyethanol[d] | 0.04 | 0.04 | 0.04 | 0.08 | 0.04 | 0.05 | 0.15 | MS |
| 46.109 | 1249 | 1247 | Carvone | trace | trace | trace | 0.02 | 0.01 | 0.02 | 0.03 | MS,RI |
| 46.349 | 1252 | 1250 | Linalyl acetate[e] | trace | 0.09 | 0.03 | 0.06 | 0.04 | 0.08 | 0.05 | MS,RI |
| 47.000 | 1261 | 1259 | gamma-Octalactone[e] | trace | ND | trace | 0.03 | 0.01 | 0.02 | 0.04 | MS,RI |
| 47.392 | 1266 | 1265 | (E)-2-decenal[a,e] | trace | 0.06 | 0.02 | ND | ND | 0.01 | ND | MS,RI |
| 47.907 | 1273 | 1272 | Nonanoic acid[e] | trace | 0.17 | 0.08 | 0.03 | 0.05 | ND | ND | MS,RI |
| 48.079 | 1275 | 1274 | 1-Decanol[e] | trace | ND | trace | 0.03 | 0.01 | 0.02 | 0.01 | MS,RI |
| 48.671 | 1283 | 1282 | N-(2-methylphenyl)formamide[tent] | 1.24 | 0.75 | 0.56 | 0.60 | 0.10 | 0.40 | 0.64 | MS |
| 48.997 | 1287 | 1286 | Isobornyl acetate | trace | trace | trace | trace | trace | trace | 0.01 | MS,RI |
| 49.188 | 1290 | 1289 | Bornyl acetate | 0.03 | trace | 0.02 | 0.02 | 0.01 | 0.02 | 0.03 | MS,RI |
| 49.232 | 1290 | 1289 | (E)-Anethole | 0.01 | trace | 0.01 | 0.02 | 0.01 | 0.02 | 0.05 | MS,RI |
| 49.557 | 1294 | 1294 | 2-undecanone[e] | 0.03 | trace | 0.02 | 0.03 | 0.02 | 0.04 | 0.05 | MS,RI |
| 49.903 | 1299 | 1299 | 2-methylnaphthalene[e] | 0.03 | 0.04 | 0.04 | 0.10 | 0.04 | 0.13 | 0.20 | MS,RI |
| 50.012 | 1300 | 1300 | Tridecane[e] | 0.08 | 0.07 | 0.06 | 0.08 | 0.05 | 0.04 | trace | MS,RI |
| 50.978 | 1315 | 1314 | 1-methylnaphthalene | 0.03 | 0.03 | 0.03 | 0.05 | 0.02 | 0.04 | 0.06 | MS,RI |
| 51.301 | 1319 | 1318 | 2,2,4,4,6,8,8-Heptamethylnonane[d?] | 0.28 | 0.23 | 0.19 | 0.27 | 0.22 | 0.22 | 0.14 | MS,RI |
| 53.155 | 1346 | 1345 | Methyl anthranilate | 0.11 | 0.13 | 0.12 | 0.28 | 0.03 | 0.11 | 0.21 | MS,RI |
| 53.870 | 1357 | 1355 | Eugenol[e] | ND | ND | ND | trace | trace | trace | trace | MS,RI |
| 54.460 | 1365 | 1363 | gamma-Nonalactone[e] | trace | trace | trace | 0.03 | 0.01 | 0.04 | 0.08 | MS,RI |
| 54.710 | 1368 | 1367 | Decanoic acid[e] + unknown | 0.06 | 0.08 | 0.06 | 0.07 | 0.02 | ND | ND | MS,RI |
| 55.429 | 1378 | 1377 | alpha-Copaene | ND | ND | trace | 0.05 | trace | 0.02 | trace | MS,RI |
| 56.290 | 1390 | 1390 | beta-Cubebene | 0.82 | 0.62 | 0.50 | 0.77 | 0.04 | 0.09 | 0.11 | MS,RI |
| 56.487 | 1393 | 1392 | 1-Tetradecene | 0.01 | ND | trace | 0.02 | 0.01 | 0.02 | 0.02 | MS,RI |
| 57.020 | 1400 | 1400 | Tetradecane[e] | 0.18 | 0.20 | 0.15 | 0.20 | 0.10 | 0.16 | 0.20 | MS,RI |
| 57.266 | 1404 | 1404 | Methyl N,N-dimethylanthranilate | 4.84 | 4.28 | 4.08 | 7.11 | 1.33 | 4.51 | 9.93 | MS |
| 57.307 | 1405 | 1404 | Diphenyl ether[d?] | 0.04 | 0.08 | 0.06 | 0.15 | 0.03 | 0.12 | 0.21 | MS,RI |
| 57.330 | 1405 | 1405 | Vanillin | ND | ND | trace | trace | trace | trace | 0.01 | MS,RI |
| 57.792 | 1412 | 1412 | Methyl N-methylanthranilate | 4.19 | 3.89 | 3.79 | 7.01 | 0.64 | 2.15 | 3.67 | MS,RI |
| 62.041 | 1477 | 1476 | 1-Dodecanol[e] | 0.11 | 0.18 | 0.10 | 0.17 | 0.04 | 0.05 | 0.09 | MS,RI |
| 62.580 | 1485 | 1485 | Germacrene D | 0.06 | trace | 0.06 | 0.11 | trace | trace | trace | MS,RI |
| 63.595 | 1500 | 1500 | Pentadecane[a,e] | 0.17 | 0.22 | 0.14 | 0.19 | 0.09 | 0.19 | 0.30 | MS,RI |
| 63.697 | 1502 | 1502 | alpha-Muurolene | 0.01 | ND | trace | 0.01 | trace | trace | trace | MS,RI |
| 63.867 | 1505 | 1504 | 2,6-di-tert-butyl-4-methylphenol (BHT)[d,e] | ND | trace | 0.01 | 0.03 | 0.05 | 0.05 | 0.06 | MS,RI |
| 64.941 | 1522 | 1522 | delta-Cadinene[e] | 0.06 | trace | 0.04 | 0.06 | ND | 0.01 | 0.02 | MS,RI |
| 67.644 | 1566 | 1565 | Dodecanoic acid[e] | 0.27 | 0.38 | 0.13 | ND | ND | ND | ND | MS,RI |
| 69.839 | 1600 | 1600 | Hexadecane[e] | 0.15 | 0.18 | 0.13 | 0.17 | 0.07 | 0.12 | 0.25 | MS,RI |
| 70.797 | 1617 | 1616 | Cedrol | 0.03 | 0.07 | trace | 0.04 | 0.01 | 0.01 | 0.07 | MS,RI |
| 71.949 | 1637 | 1636 | Benzophenone | 0.03 | trace | 0.03 | 0.04 | trace | 0.01 | 0.06 | MS,RI |
| 75.756 | 1700 | 1700 | Heptadecane[e] | 0.17 | 0.14 | 0.05 | 0.13 | 0.03 | 0.07 | 0.11 | MS,RI |
| 76.524 | 1714 | 1714 | 2,3-dihydro-1,1,3-trimethyl-3-phenyl-1H-Indene[d] | 0.03 | 0.05 | trace | 0.04 | 0.01 | 0.03 | 0.05 | MS |

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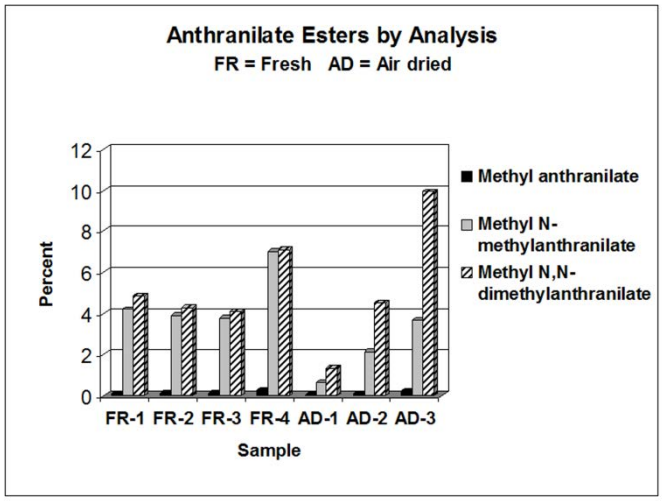
|---|---|---|---|---|---|---|---|---|---|---|---|
| 79.259 | 1763 | 1762 | Tetradecanoic acid[e] | 0.18 | 0.36 | 0.14 | 0.13 | 0.12 | 0.05 | ND | MS,RI |
| 81.380 | 1800 | 1800 | Octadecane[e] | 0.07 | 0.10 | 0.05 | 0.07 | 0.02 | 0.03 | 0.04 | MS,RI |
| 85.821 | 1883 | 1883 | 1-Nonadecene | 0.16 | 0.30 | 0.04 | 0.03 | 0.02 | ND | 0.02 | MS,RI |
| 86.740 | 1900 | 1900 | Nonadecane[e] | trace | 0.06 | 0.04 | 0.04 | 0.01 | 0.02 | 0.04 | MS,RI |
| 88.095 | 1927 | 1927 | Methyl palmitate[e] | trace | trace | trace | 0.01 | ND | trace | trace | MS,RI |
| 89.902 | 1963 | 1962 | Palmitic acid[c,e] | trace | 0.19 | 0.21 | 0.35 | 0.03 | ND | ND | MS,RI |
| 91.840 | 2000 | 2000 | Eicosane[e] | trace | 0.09 | 0.03 | 0.04 | 0.01 | 0.02 | 0.03 | MS,RI |
| 92.984 | 2024 | 2023 | Isopropyl palmitate | 0.11 | 0.27 | 0.04 | 0.06 | 0.03 | 0.03 | 0.06 | MS,RI |
| 96.090 | 2087 | 2087 | 1-octadecanol | trace | 0.04 | 0.01 | ND | ND | ND | ND | MS,RI |
| 96.719 | 2100 | 2100 | Heneicosane[e] | 0.06 | 0.12 | 0.03 | 0.06 | 0.01 | 0.02 | 0.04 | MS,RI |
| 101.392 | 2200 | 2200 | Docosane[e] | 0.10 | 0.16 | 0.03 | 0.05 | 0.01 | 0.02 | 0.03 | MS,RI |
| 105.867 | 2300 | 2300 | Tricosane[e] | 0.11 | 0.19 | trace | 0.02 | trace | 0.01 | trace | MS,RI |
| 110.161 | 2400 | 2400 | Tetracosane[e] | 0.10 | 0.17 | trace | trace | trace | ND | ND | MS,RI |
| 114.290 | 2500 | 2500 | Pentacosane[e] | 0.10 | 0.16 | trace | trace | trace | ND | ND | MS,RI |
| 118.266 | 2600 | 2600 | Hexacosane[e] | 0.11 | 0.17 | trace | trace | trace | ND | ND | MS,RI |
| 122.094 | 2700 | 2700 | Heptacosane[a,e] | 0.11 | 0.13 | 0.01 | 0.02 | trace | ND | ND | MS,RI |
| 125.824 | 2800 | 2800 | Octacosane[e] | 0.09 | 0.11 | trace | 0.04 | 0.01 | ND | ND | MS,RI |
| 126.362 | 2813 | 2813 | 10-Demethylsqualene | 0.24 | 0.25 | 0.11 | 0.11 | 0.57 | 0.01 | 0.01 | MS |
| 129.925 | 2900 | 2900 | Nonacosane[e] | 0.08 | 0.06 | 0.05 | 0.05 | 0.02 | ND | ND | MS,RI |
| | | | Total % Identified | 99.27 | 99.08 | 98.59 | 96.85 | 99.66 | 97.64 | 96.68 | |

[a] Previously identified in *Calvatia gigantean* by Overton [3]
[b] Previously identified in *Calvatia gigantea* by Dijkstra [1,2]
[c] Previously identified in *Calvatia gigantea* by Bentley [4]
[d] Probable or potential environmental contaminant, including VOC air pollutants [11,12, 13,14,15]
[e] Previously identified in other mushroom varieties [16,17]
ND = not detected; trace = <0.01%; *tent* = tentative identification

## Major Headspace Constituents and Changes with Sequential Purges & Sample Age

Perhaps the most surprising finding was the presence of Methyl anthranilate, Methyl N,N-dimethylanthranilate and Methyl N-methylanthranilate, with the latter two comprising 4.8-7.1% and 4.2-7.0% respectively in the fresh samples, and 1.3-9.9% & 0.6-3.7% respectively in the air-dried samples. The variations by analysis are shown in Fig. 3.



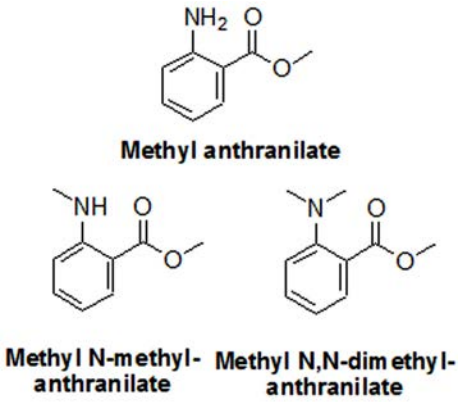




**Fig. 3 Anthranilate Esters - % by Analysis**

While methyl anthranilate has been produced in the laboratory by the basidiomycete *Pycnoporus cinnabarinus* in liquid cultures using maltose and diammonium tartrate as the carbon and nitrogen source [18], we believe that none of the three anthranilate esters identified in our analyses have ever previously been reported as naturally occurring in mushroom species.

Methyl N-methylanthranilate has been shown to undergo N-demethylation to methyl anthranilate using both fungi cultures of *Polyporus zonalis* ATCC 10089 [19] as well as by the topsoil bacterium *Bacillus megaterium* [20]. As no significant increase of methyl anthranilate was observed either between the air-dried vs. fresh sample analyses, there is nothing to suggest that such N-demethylation was occurring in our samples.

As to occurrence in natural products, methyl N-methylanthranilate primarily is found in citrus petitgrain oils (e.g. mandarin petitgrain) where it occurs up to a 47-65% level [21,22] while methyl anthranilate is known to be important to concord grape [23], genét [24] and bitter gourd [25]. We believe the only prior reported isolation of methyl N,N-dimethylanthranilate from a natural product was from an Italian mandarin petitgrain oil at a 0.02-0.4% level [22].

Considerable changes occur, both in composition and amounts, between analyses, presumably due to ***both*** catabolic reactions during the rapid senescence of the mushroom as it ages as well as by the expected depletion of certain constituents during the sequential purge analysis process. For example, 1-octen-3-ol which is considered to be the typical odor constituent of many mushroom species, was "only" found in measurable quantities in the initial fresh sample (FR-1) and the initial air-died sample (AD-1) at levels of 0.45% and 0.15%, respectively. Anisole, which is one of the major components in the fresh *Calvatia* declines with each purge as the samples age while phenol (which was less than 0.01% in the initial fresh *Calvatia*) increases over time in the subsequent purges as shown in Fig. 4. Whether anisole is converted to phenol by microbial O-demethylation in our studies is unclear [26]. Similarly, Fig. 5 shows that isoamyl alcohol tends to initially increase with sample age in the fresh *Calvatia*, while in the air-dried samples a large amount 3-methylbutanal has appeared that then declines rapidly in subsequent purges. The isoamyl alcohol also tends to initially increase followed by a precipitous decrease in the air-dried samples.

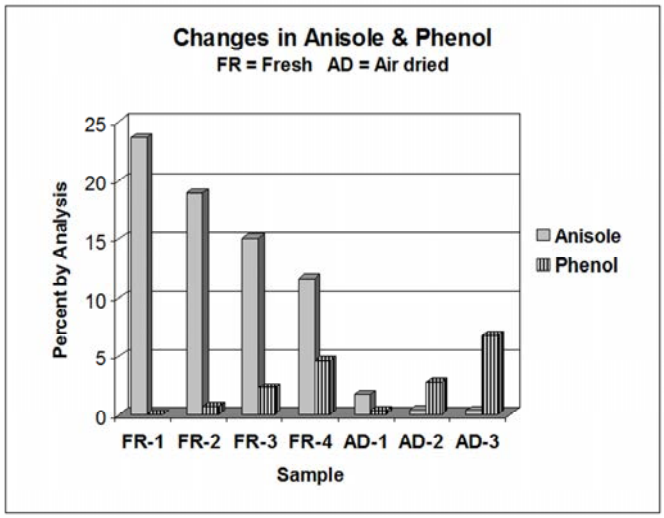



**Fig. 4. Changes in Anisole & Phenol by sequential purge analysis**

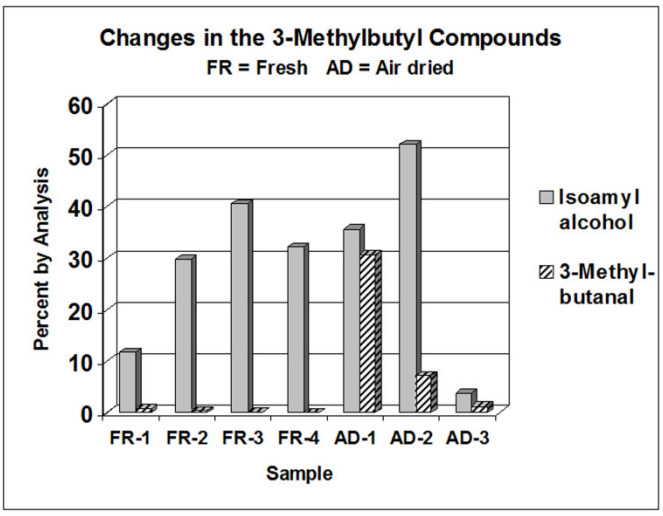



**Fig. 5. Changes in Isoamyl alcohol & 3-methylbutanal by sequential purge analysis**

Similarly, 2-Methyl-1-butanol increases with each successive purge in the fresh *Calvatia* while in the air-dried samples a large amount 2-methylbutanal had appeared that then declines rapidly.

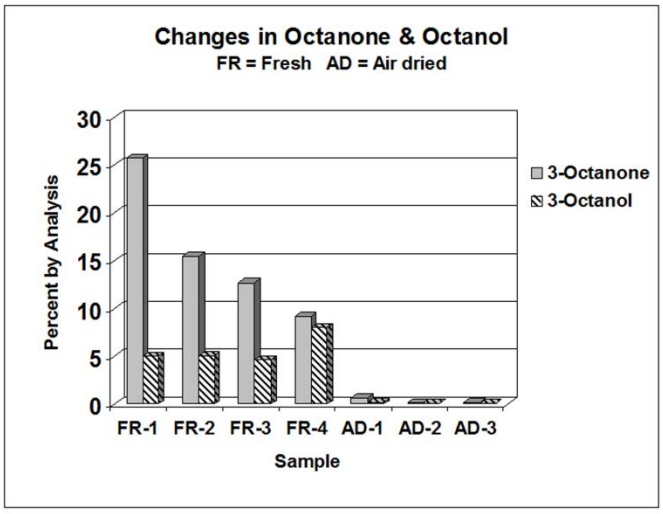



**Fig. 6. Changes in 3-Octanone & 3-Octanol**

Figure 6 shows that 3-Octanone, which was initially the largest component in the fresh sample, decreases with each purge while 3-octanol increases in the FR-4 analysis. Both were significantly decreased in the air-died samples.

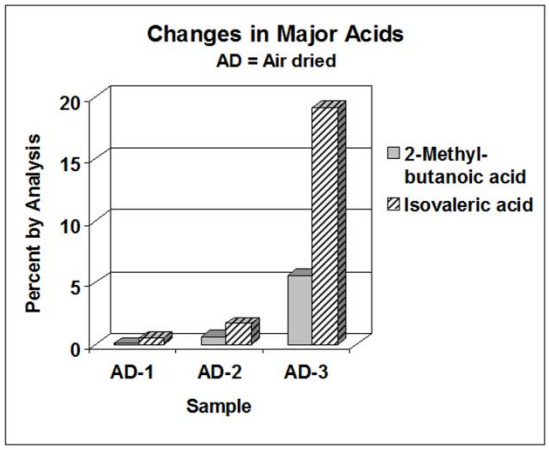



**Fig. 7. Changes in Major acids**

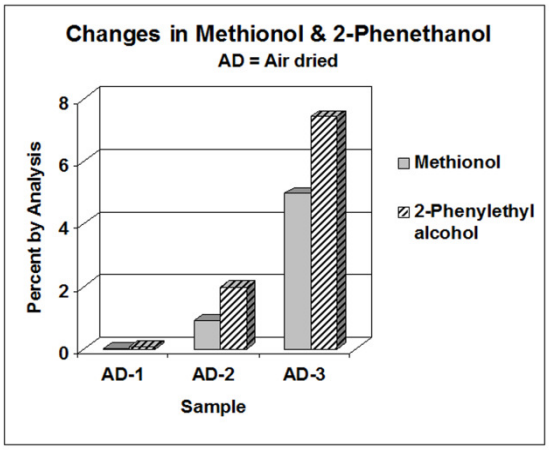



**Fig. 8. Changes in Methionol & 2-Phenethanol**

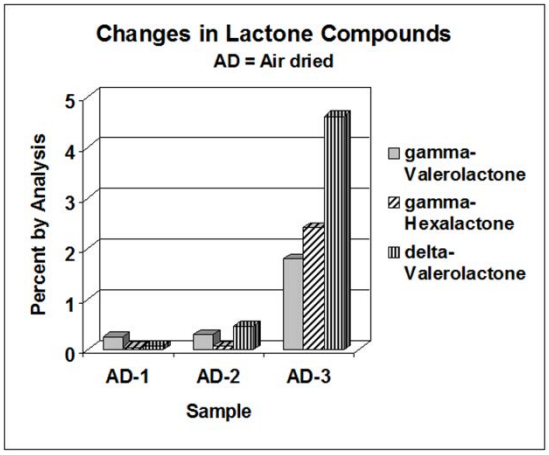



**Fig. 9. Changes in Lactones**

Figures 7, 8 & 9 show other major changes in the air-dried samples for compounds that were either absent or very minor constituents in the fresh *Calvatia* samples.

The finding that Anisole was the major constituent in the fresh *Calvatia* sample is consistent with that reported by Overton [3], but we also found many other major constituents not found in his analysis. While Dijkstra reported that 1-octen-3-ol was the major volatile [1,2], we only found modest amounts in both the "fresh' and "air-dried" samples.

We previously noted that this mushroom is only edible when fresh and becomes disagreeable on aging. In view of the major compositional changes that occur in the sequential purges and aging, especially of the air-dried samples, such an observation is not unexpected. The significant increase in the amounts of isovaleric & 2-methylbutanoic acids, as well of methionol, phenol, and lactones (Figures 7-9) would be expected to add a disagreeable (spoiled) flavor. See Table 2 for the odor descriptions of some important volatiles.

## Potential Environmental Contaminants

As mentioned, the concern about potential environmental contaminants in an urban area, including VOC air pollutants was a potential issue, especially because of the high surface area of fungi species and the potential affinity of such fungi to absorb volatile organic compounds (VOCs). Accordingly, a number of potential or probable environmental contaminants are noted in our tables. Potential environmental biogenic VOCs such as terpenoids and sesquiterpenoids from trees and other vegetation are more difficult to assess and are not addressed in our analyses.

There has been debate [27,28] as to whether or not items such as 2,6-di-tert-butyl-4-methylphenol (BHT), a prior mushroom isolate and a reported isolate of at least 38 other natural products [7,16], is "natural" and the concerns about how to handle such items which are known environmental contaminants in publications. We are aware that at least one paper has addressed this problem by noting potential environmental contaminants in their tabulations [11] and we have adopted a similar protocol.

In this study, we identified 44 compounds that are probable or potential environmental contaminants [11,12,13,14,15], including 25 substituted (C1-C4) benzenes, 9 branched alkanes and 10 miscellaneous.

## Odor Descriptions of Important *Calvatia gigantea* Constituents

Finally, in Table 2 we provide the organoleptic odor descriptions for the important constituents found in these analyses using the descriptors of Boelens "Perfumery Materials and Performance 2001" database [29], which has been ranked as the most reliable of the available large olfactory databases [30].

**Table 2. Odor Descriptors (Boelens 2001) of Important *Calvatia gigantea* Constituents**

| Compound | Odor Descriptors |
|---|---|
| 3-Methylbutanal | fruity, peach- & cocoa-like in dilution |
| 2-Methylbutanal | fresh, fruity, apple-like; somewhat herbal, fermented |
| Isoamyl alcohol | fresh, ethereal, fusel-like, fermented & yeasty |
| 2-Methyl-1-butanol | soft ethereal, somewhat fermented, apple-like |
| Isovaleric acid | acidic, caprylic, sweat-like, somewhat cheesy |
| 2-Methylbutanoic acid | acidic, caprylic, sweat-like, somewhat cheesy |
| Anisole | sweet, balsamic, gasoline, anise, somewhat phenolic |
| gamma-Valerolactone | fruity, lactonic, buttery; slightly fatty |
| delta-Valerolactone | lactonic, fruity |
| gamma-Hexalactone | herbaceous, tobacco-like, creamy |
| Methionol | sulfurous, meaty, soup-like aroma |
| Phenol | strongly phenolic, medicinal, antiseptic |
| 1-octen-3-ol | earthy, mushroom-like |
| 1-Octen-3-one | aromatic, mushroom-like aroma |
| 3-Octanone | herbaceous-fruity; slightly spicy, buttery; top note of lavender |
| 3-Octanol | oily, herbaceous, somewhat nut-like |
| Limonene | harsh, terpene-like; citrusy, fruity, orange, berry-like, tarty |
| 2-Ethyl-1-hexanol | mild ethereal, somewhat yeasty & fermented |

| | |
|---|---|
| 2-Phenylethyl alcohol | fresh, sweet aromatic floral, strongly reminiscent of rose with a hyacinth nuance |
| Methyl anthranilate | orangeflower-like, sweet fruity, tangerine & grape-note |
| Methyl N,N-dimethylanthranilate | sweet aromatic, citrusy, mandarin & tangerine note |
| Methyl N-methylanthranilate | sweet aromatic, citrus-fruity, musty; reminiscent of mandarin & tangerine |

## CONCLUSIONS

A sequential purge and trap technique was applied to the GC-MS analyses of both fresh and air-dried samples of the giant puffball mushroom (*Calvatia gigantea*) to examine the potential constituents contributing to its flavor. The presence of Methyl anthranilate, Methyl N,N-dimethylanthranilate and Methyl N-methylanthranilate are reported for the first time in a mushroom species. Consideration of probable, or potential, environmental contaminants, including VOC air pollutants, allowed us to identify 44 compounds that met that criteria.

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