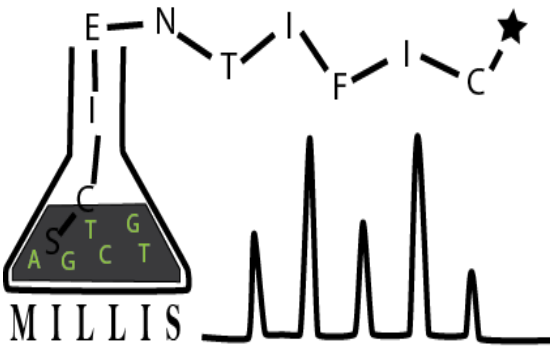


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|---|---|
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|---|---|

### Analytical Report

|                    |   |
|--------------------|---|
| Title              | Volatile Organic Compounds Profile by GC-MS in "Banana" E-liquid Flavor Concentrate |
| Report No.         | SE-37974-1  |
| Issue Date         | September 23, 2015  |
| Notebook reference | III-85-52   |
| Contributors:      | Alexei Gapeev, David Yeh  |
| Quote No.          |   |
| Requester          | Flavorah<br>PO Box 2624<br>Woodinville, WA 98072                                    |

**Specific Aim:**

The goal is to determine levels of Acetoin, Diacetyl and Acetylpropionyl with reporting cutoff 5 ppm

**Background of the matter**

Diacetyl (2,3-butanedione, CAS 431-03-8) is a volatile liquid with intense buttery flavor occurring naturally in dairy and fermented foods. It is extensively used as a flavoring agent to impart a buttery flavor.

Acetoin (3-Hydroxy-2-butanone, CAS 513-86-0), Acetylpropionyl (2,3-Pentadione, CAS 600-14-6) are related compounds with somewhat similar custard flavor also used as flavoring agents.

These compounds find use in flavoring compositions designed to impart certain flavors to foodstuffs. They are also common products of fermentation and present in beers, wines and dairy products albeit in low (tens of ppb) levels.

## **Samples**

Sample arrived as viscous liquid with characteristic odor labeled "Banana 100010". 5 ml of submitted material was placed in 40 ml headspace collection vial followed by addition of internal standard (IS). The vial was held at 50C for 4 hours before headspace sampling. Volatile compounds were collected out of headspace with the aid of 1 ml gastight syringe. 0.2 ml of collected headspace was injected at split ratio of 10.

## **Experimental:**

### 1. GC conditions:

|                           |          |
|---------------------------|----------|
| Injector temperature:     | 250 C    |
| Initial oven temperature: | 40 C     |
| Hold I                    | 2 min    |
| Ramp I                    | 10 C/min |
| Final temperature I       | 220 C    |
| Hold II                   | 5 min    |

### 2. MS parameters

|                             |             |
|-----------------------------|-------------|
| Ionization and ion polarity | EI+         |
| Scan rate                   | 2 scans/sec |
| Mass range                  | 35-300 Da   |
| Ion source temperature      | 150         |
| Transfer line temperature   | 220C        |

3. GC-MS analysis. Waters/Micromass Quatro GC mass spectrometer interfaced to a Thermo Electron Trace gas chromatograph was utilized for the analysis. 30M 0.32 mm ID DB-624 column was used to separate components. Carrier gas was helium at 2 ml/min.

### 4. Data treatment.

Methylethylketone (MEK) was used as an internal standard. Table 1 lists qualifier and quantifier ions for the target compounds.

| <b>RT, min</b> | <b>Compound</b> | <b>Qualifier, m/z</b> | <b>Quantifier, m/z</b> |
|----------------|-----------------|-----------------------|------------------------|
| 4.25           | Diacetyl        | 86                    | 43                     |
| 6.35           | Acetylpropionyl | 100                   | 43                     |
| 7.2            | Acetoin         | 88                    | 45                     |

*Table 1 Retention times and m/z ratios of the target compounds*

## **Results:**

TIC GC-MS chromatogram of the submitted sample is shown in Appendix I, figure 1. Displayed on figure 2 are selected ion chromatograms for ions used to quantify the target compounds. Arrows designate expected retention times (RT) for diacetyl, acetylpropionyl and acetoin elution (4.25,

6.35 and 7.2 min respectively) out of the GC column<sup>1</sup> Green arrow indicates a compound is not present at reporting cutoff and red arrow indicates that level exceeds the cutoff. Table 2 lists target compound levels.

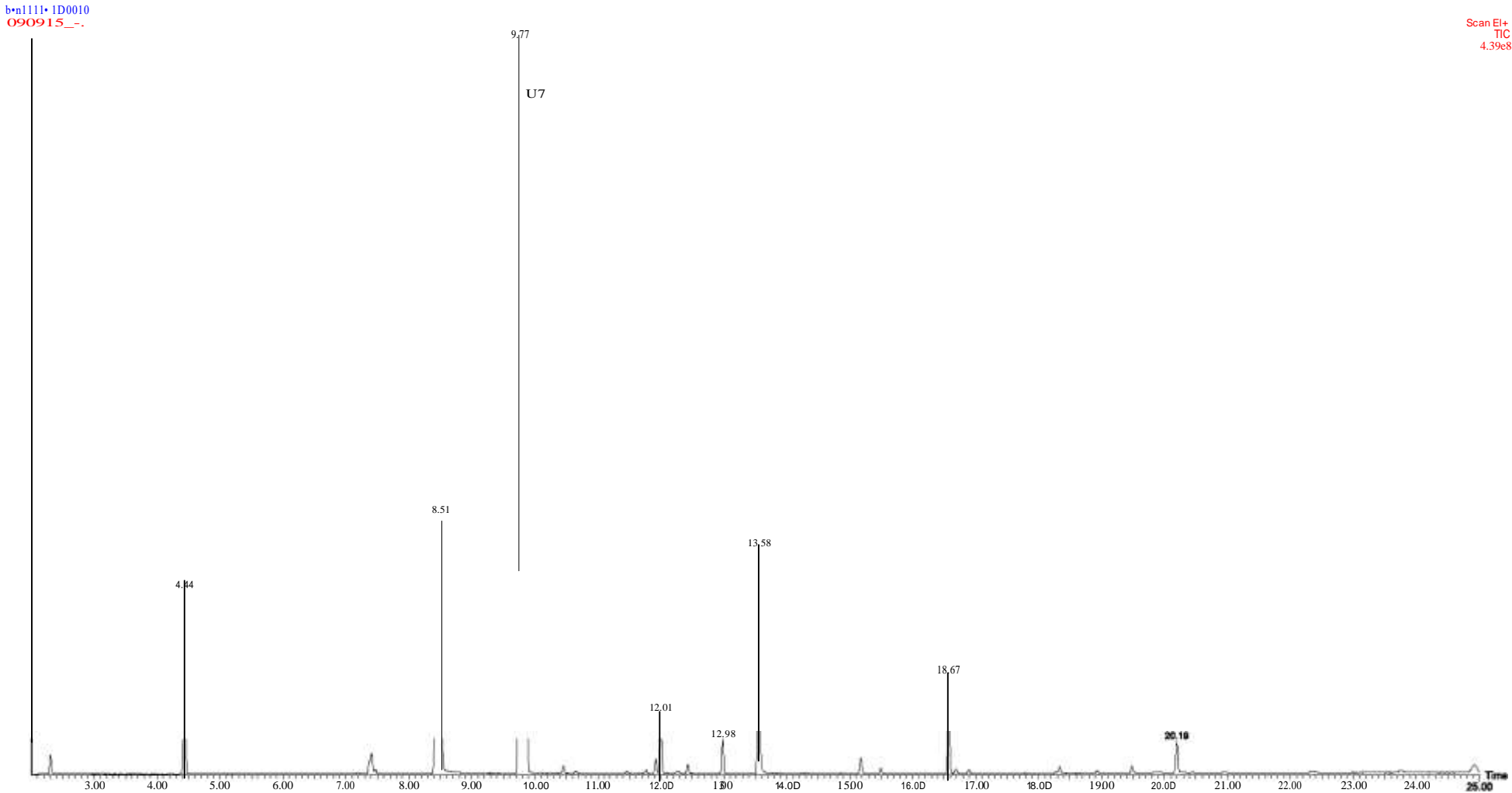
| <b>Compound</b> | <b>Concentration, ppm</b> |
|-----------------|---------------------------|
| Diacetyl        | N/D                       |
| Acetylpropionyl | N/D                       |
| Acetoin         | N/D                       |

*Table 2 Sample "Banana", target compound levels. Concentration units are ppm or N/D, not detected.*

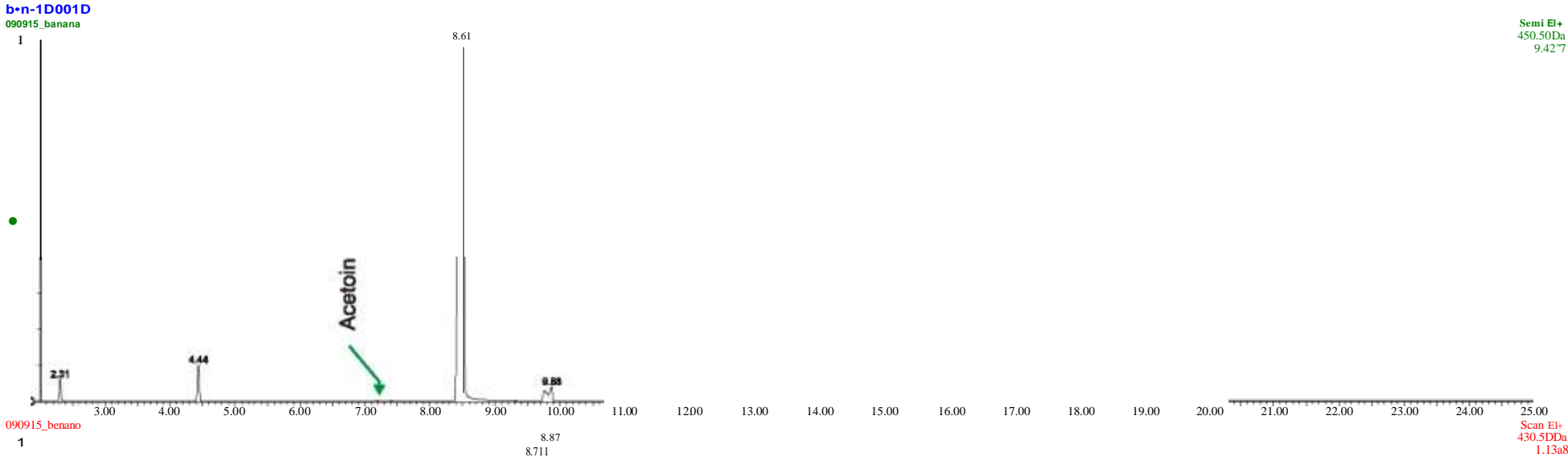
1 Presence of a peak at designated RT does not necessary signify that a target compound detected. If stated as N/D the qualifier ion is not detected.

## **APPENDIX I**

# Figure 1. Sample "Banana 10001 O" TIC chromatogram



# Figure 2. Sample "Banana 10001O" Selected Ion Chromatograms



Acetylpropionyl

3.00 4.00 5.00 6.00 7.00 8.00 9.00 10.00 11.00 12.00 13.00 14.00 15.00 16.00 17.00 18.00 19.00 20.00 21.00 22.00 23.00 24.00