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Analytical Report

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| Title | Volatile Organic Compounds Profile by GC-MS in "Elderflower" Flavoring Concentrate |
| Report No. | 021816-025-8 |
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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. When inhaled, these compounds are believed to have deleterious effect on lung function and cause a serious lung disease. Thus it is imperative that no flavoring composition designed to be inhaled has any of the target compounds.

Samples

Sample arrived as somewhat viscous liquid with characteristic odor labeled "**Elderflower**". 4 ml of submitted material was placed in a 40 ml headspace collection vial followed by addition of internal standard (IS). The vial was held at 60C 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 5.

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.

| RT, min | Compound | Qualifier, m/z | Quantifier, m/z |
|----------------|-----------------|-----------------------|------------------------|
| 4.35 | Diacetyl | 86 | 43 |
| 6.44 | Acetylpropionyl | 100 | 43 |
| 7.28 | 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 (4.3,

6.4 and 7.2 min respectively) out of the GC column¹ A green arrow indicates a compound is not present at reporting cutoff and a red arrow indicates that level exceeds the cutoff. "*" designates internal standard. Table 2 lists target compound levels.

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

Table 2 Sample "Elderflower", 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 "Eiderflower" TIC chromatogram

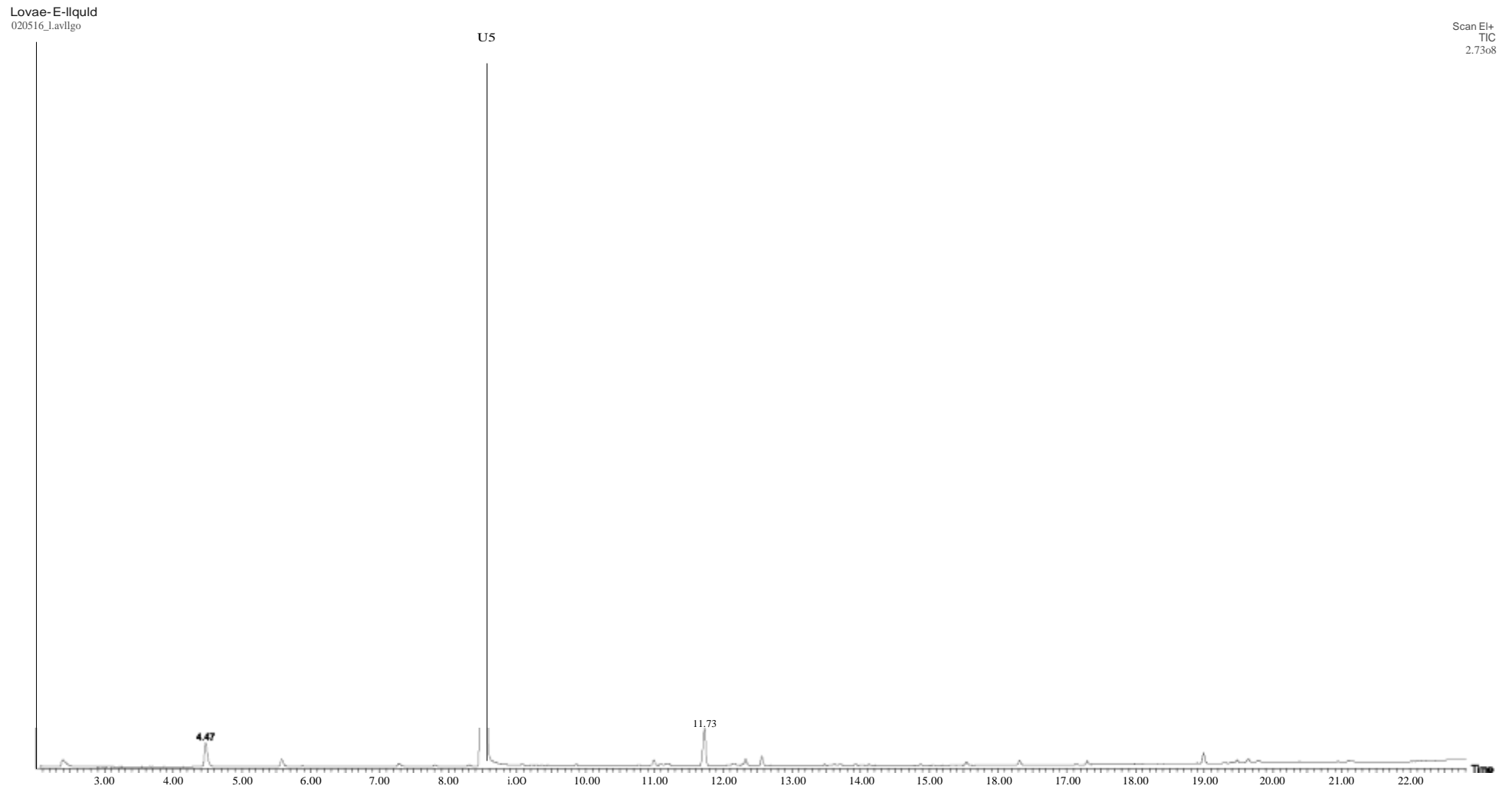


Figure 2. Sample "Eiderflower" Selected Ion Chromatograms

