### Analytical Report

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<th>Volatile Organic Compounds Profile by GC-MS in “Creamy Tiramisu” E-liquid</th>
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<td>Report No.</td>
<td>SE-36406a</td>
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<td>Issue Date</td>
<td>July 9, 1 2015</td>
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<td>Notebook reference</td>
<td>III-85-09</td>
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The Clean Vape |

**Specific Aim:**
Determine if Acetoin, Diacetyl or Acetylpropionyl are present in submitted material.

**Background of the matter**
Diacetyl (2,3-butanedione, CAS 431-03-8) is a volatile liquid with intense buttery flavor occurring naturally in diary 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 viscous liquid with characteristic odor labeled as “Creamy Tiramisu”. 5 ml of submitted material was placed in 40 ml headspace collection vials. Each vial was held at 60C for 4 hours before headspace sampling. Volatile compounds were extracted out of headspace with Carboxen/Polydimethylsiloxane SPME fiber followed by desorption into the GC injector splitless. Extraction time was 30 min for each sample.

**Experimental:**

1. GC conditions:
   - Injector temperature: 250 C
   - Initial oven temperature: 0 C
   - Ramp I: 20 C/min
   - Final temperature I: 40 C
   - Ramp II: 10 C/min
   - Final temperature II: 220 C

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 0.25 mm ID DB-WAX column was used to separate components. Carrier gas was helium at 1.1 ml/min splitless.

   Methylethylketone (MEK) was used as an internal standard. Limit of quantification for each of the target compounds was no lower than 50 ppb (0.05 ppm or 50 ng/ml).

**Results:**
The GC-MS chromatogram is shown in Appendix I. Arrows designate retention times (RT) for diacetyl, acetylpropionyl and acetoin elution (5.3, 6.4 and 9.8 min respectively) out of the GC column. Target compounds were not detected\(^1\).

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\(^1\) If present, their levels < 0.05 ppm
Appendix I. “Creamy Tiramisu” E-liquid GC-MS profile