

Millis Scientific, Inc

6400 Baltimore National Pike
 #201 Baltimore MD 21228
 Telephone: 877-844-2635
 Email: info@millisscientific.com

Analytical Report

Title	Volatile Organic Compounds Profile by GC-MS in "Key Lime Pie" E-liquid
Report No.	15392
Issue Date	August 11, 2015
Notebook reference	III-85-25
Contributors:	Alexei Gapeev, David Yeh
Quote No.	
Requester	TBD Liquids LLC

Specific Aim:

Determine concentration of Acetoin, Diacetyl and Acetylpropionyl 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 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 viscous liquid with characteristic odor labeled "Key Lime Pie". 1 ml of submitted material was placed in 40 ml headspace collection vial. 3 ml of DI

water was added followed by addition of internal standard (IS). Each 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: 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 ml/min 1:9 split.

4. Data treatment.

Methylethylketone (MEK) was used as an internal standard. Table 1 lists qualifier and quantifier ions for the target compounds. LLOQ¹ for acetoin was no higher than 500 ppb (0.5 ppm or 500 ng/ml). LLOQ for diacetyl and acetylpropionyl was no higher than 100 ppb.

RT, min	Compound	Qualifier, m/z	Quantifier, m/z
5.8	Diacetyl	86	43
7	Acetylpropionyl	100	43
10.55	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 retention times (RT) for diacetyl, acetylpropionyl and acetoin elution (5.8, 7 and 10.6 min

1 Lower limit of quantification

respectively) out of the GC column. Table 2 lists target compound levels.

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

Table 2 Sample “Key Lime Pie”, target compound levels. Concentration units are ppm or N/D, not detected.

APPENDIX I

Figure 1. Sample "Key Lime Pie" TIC chromatogram

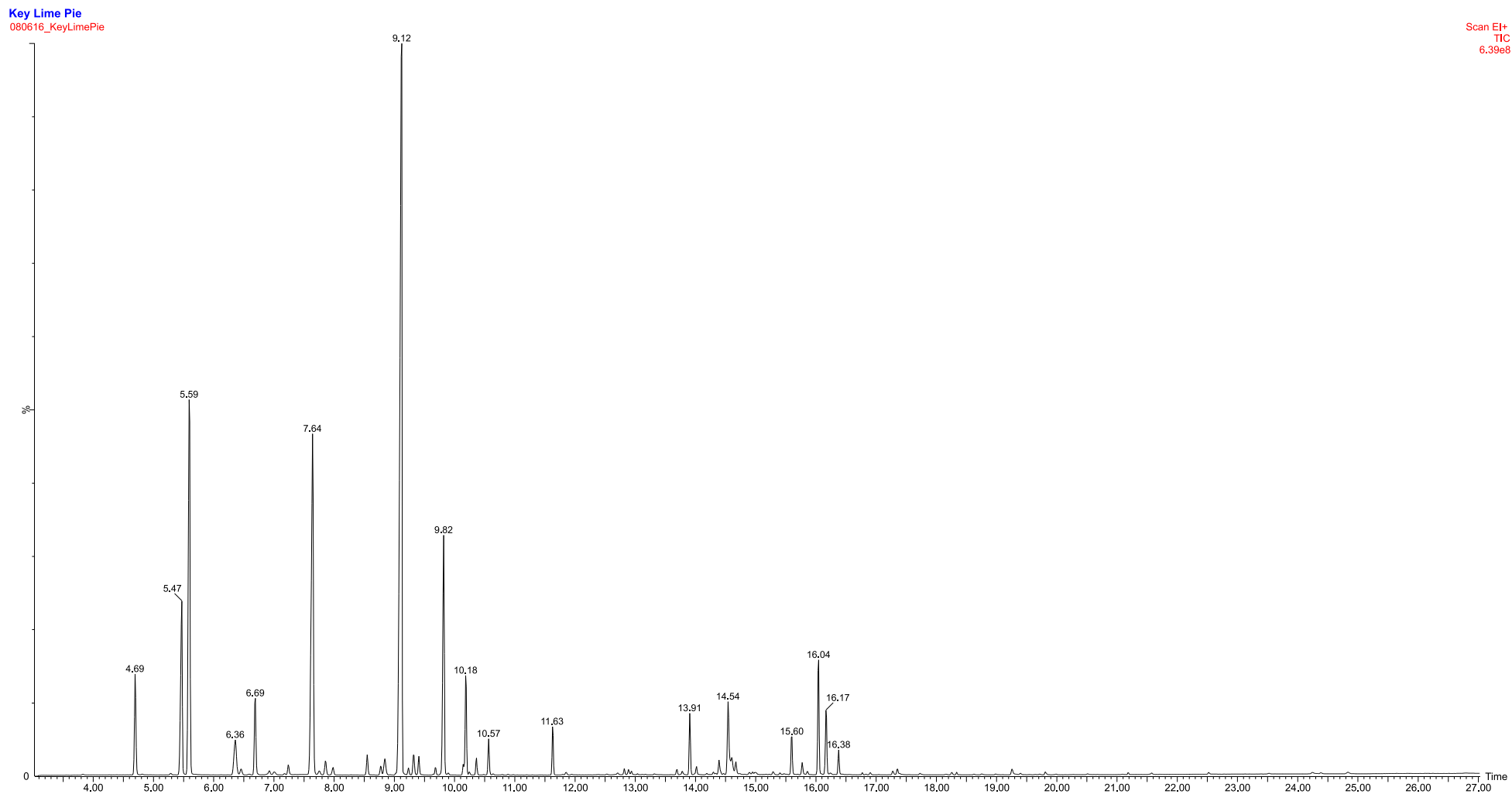


Figure 2. Sample "Key Lime Pie" Selected Ion Chromatograms

