A Validated Method for Simultaneous Quantitation of 20 Common Flavoring Chemicals in e-Cigarette Refill Solutions

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Background

- Flavoring chemicals are added in differing concentrations to numerous flavored e-cigarette refill solutions
- Previous qualitative studies have identified commonly used flavoring chemicals among various flavors, including those with potential inhalation concern.
- 20 of these popular flavoring chemicals have been selected for quantitative identification among e-cigarette liquids.
- This study aimed to develop a validated quantitative method to measure the concentration of these chemicals (listed in Table 1).
- This method was used to measure concentrations of these 20 chemicals among 215 commercial e-cigarette refill solutions purchased in the United States.

Instrumentation

- Analysis was performed on an Agilent 7250 gas chromatograph quadrupole/time-of-flight mass spectrometer (GC/Q-TOF)
- **Column**: DB-624 UI 30 m x 0.25 mm x 1.4 µm
- **Injection Volume**: 1 µL
- **Injection Port Liner**: Universal with glass wool, 4 mm.
- **Injection**: Split 20:1
- **Injection Port Temp**: 320°C
- **Oven**: 60°C – hold 1 min then 30°C/min to 225°C – hold 4 min. Post run at 280°C for 1 min.
- **Equilibrium time**: 1.5 min
- **MS Transfer line**: 250°C
- **Total Run time**: 10.5 min

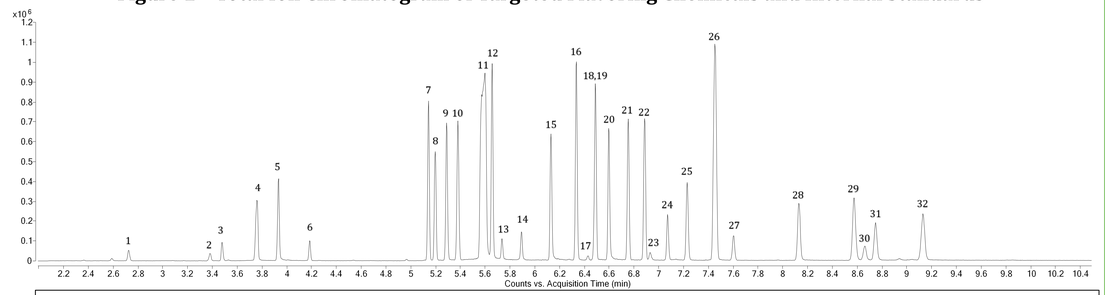
Figure 1. Agilent 7250 GC/Q-TOF

Sample Preparation

30µL of Calibration, QC and refill solutions → 30µL of Internal Standard (1mg/mL) → 3mL of Methanol Vortex 5 min → Analyze via GC/Q-TOF

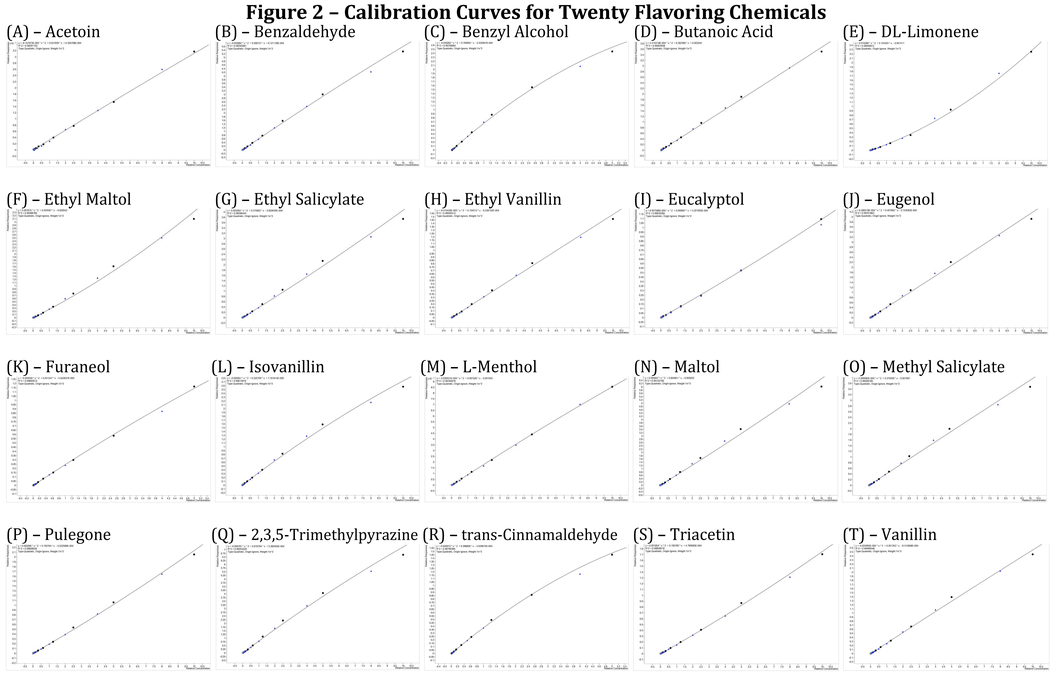
Chromatogram/Calibration

Figure 1 – Total Ion Chromatogram of Targeted Flavoring Chemicals and Internal Standards



1 – Benzene-d6	5 – Butanoic Acid	9 – D/L-Limonene	13 – Furaneol	17 – Unknown	21 –(+)Pulegone	25 – Triacetin	29 – Ethyl Vanillin
2 – Acetoin	6 – Chlorobenzene-d5	10 – Eucalyptol	14 – Unknown	18 – Naphthalene-d8	22 – Ethyl Salicylate	26 – Eugenol	30 – Acenaphthene-d10
3 – Pyridine-d6	7 – Benzaldehyde	11 – Vegetable Glycerin (VG)	15 – Maltol	19 – Methyl Salicylate	23 – Unknown	27 – Unknown	31 – Isovanillin
4 – Propylene Glycol (PG)	8 – 2,3,5-Trimethylpyrazine	12 - Benzyl Alcohol	16 – L-Menthol	20 – Ethyl Maltol	24 – trans-Cinnamaldehyde	28 – Vanillin	32 – Unknown

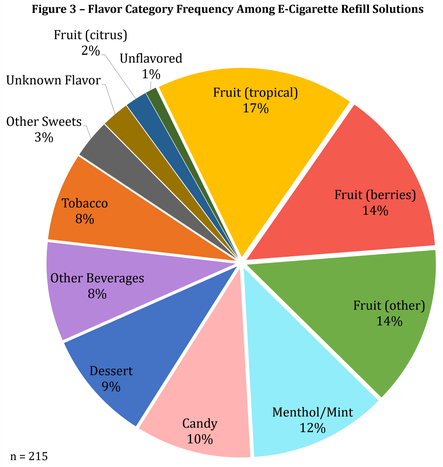
Figure 2 – Calibration Curves for Twenty Flavoring Chemicals



Circles (black) indicate calibration standards within the instrument linear range and are included in the calculation of the curve and corresponding r^2 value. Triangles (blue) indicate quality control standards within the instrument linear range.

Flavorings in E-Cigarette Refill Solutions

Figure 3 – Flavor Category Frequency Among E-Cigarette Refill Solutions



n = 215

Alcohol and Other Flavors account for <1% of the total e-cigarette liquids selected, which are not included here

Figure 4 – Percentage of Detections Among Flavoring Chemicals in E-Cigarette Liquids

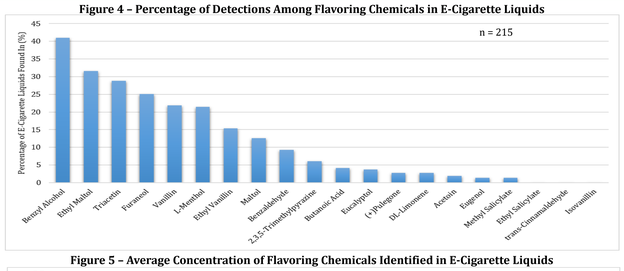
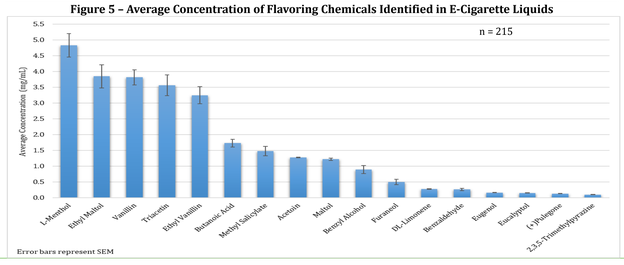


Figure 5 – Average Concentration of Flavoring Chemicals Identified in E-Cigarette Liquids



Error bars represent SEM

Method Validation

Table 1 – Method and Calibration Parameters

Compound	RT (min)	Quant Ion (±10ppm)	r^2 Value[1]	RSE (%)	Calibration Range[2] (mg/mL)	LOQ (mg/mL)
(+)Pulegone	6.754	81.0706	0.994	6.4	0.02-10.0	0.02
2,3,5-Trimethylpyrazine	5.203	122.0834	0.993	4.9	0.02-10.0	0.02
Acetoin	3.385	88.0530	0.996	8.0	0.31-10.0	0.31
Benzaldehyde	5.144	106.0409	0.993	8.5	0.02-10.0	0.02
Benzyl Alcohol	5.656	108.0566	0.994	8.6	0.02-5.0	0.02
Butanoic Acid	3.931	60.0211	0.998	8.5	0.16-10.0	0.63
DL-Limonene	5.289	93.0705	0.994	9.5	0.08-10.0	0.08
Ethyl Maltol	6.596	140.0471	0.991	9.7	0.08-10.0	0.08
Ethyl Salicylate	6.886	120.0204	0.993	8.7	0.02-10.0	0.02
Ethyl Vanillin	8.574	137.0244	0.995	6.8	0.04-10.0	0.31
Eucalyptol	5.380	139.1129	0.993	8.1	0.04-10.0	0.04
Eugenol	7.459	164.0830	0.995	8.3	0.04-10.0	0.04
Furaneol	5.737	128.0478	0.991	7.8	0.02-5.0	0.02
Isovanillin	8.749	152.0475	0.996	8.1	0.04-10.0	0.31
Maltol	6.131	126.0310	0.992	7.5	0.16-10.0	0.63
L-Menthol	6.336	123.1180	0.993	7.0	0.08-10.0	0.08
Methyl Salicylate	6.491	120.0206	0.993	5.6	0.08-10.0	0.08
trans-Cinnamaldehyde	7.071	131.0499	0.993	8.9	0.02-5.0	0.16
Triacetin	7.229	103.0398	0.998	7.2	0.04-10.0	0.08
Vanillin	8.132	152.0474	0.995	6.6	0.31-10.0	0.63

[1]Values quantitated using Quadratic $1/x^2$ calibration model (Internal Standard Method)
[2]Calibration levels prepared by serial dilution from 10mg/mL in 50:50 PG:VG

Table 2 – Method Validation Results

Compound	Within Batch[1] Average CV (%)	Between Batch[2] Average CV (%)	Average Bias (%)
(+)Pulegone	3.8	4.6	-1.3
2,3,5-Trimethylpyrazine	2.4	4.6	1.1
Acetoin	4.5	5.8	1.2
Benzaldehyde	3.6	5.0	-4.0
Benzyl Alcohol	4.2	8.0	1.4
Butanoic Acid	2.3	3.6	-1.4
DL-Limonene	3.6	6.6	-0.2
Ethyl Maltol	3.0	4.9	2.7
Ethyl Salicylate	2.9	3.7	0.7
Ethyl Vanillin	2.8	4.0	2.0
Eucalyptol	3.3	4.9	-1.0
Eugenol	2.6	3.4	0.7
Furaneol	6.2	6.7	3.8
Isovanillin	4.2	4.7	1.3
L-Menthol	4.4	5.5	-0.6
Maltol	2.2	3.9	3.5
Methyl Salicylate	2.2	3.7	1.4
trans-Cinnamaldehyde	4.9	6.6	-4.6
Triacetin	3.4	4.2	-2.6
Vanillin	2.1	6.1	1.3

[1]CV reported as the average of individual within-batch variation for standards prepared at concentrations of 0.04, 0.10, 0.88, 1.75, 3.50 and 7.00mg/mL (each analyzed in triplicate).
[2]CV reported as the average total variation (between-batch) across 7 batches for standards prepared at concentrations of 0.04, 0.10, 0.88, 1.75, 3.50 and 7.00mg/mL (each analyzed in triplicate per batch).

Conclusion

- Average coefficient of variation (CV) and bias among method validation samples were within 20%, when compared within and between batches.
- This developed method is precise and reliable when analyzed across multiple days.
- 20 common chemical additives to e-cigarette refill solutions can be accurately measured simultaneously when using this method.
- The large dynamic working range allows for a variety of concentrations to be rapidly measured in consumer products.

Disclosures

Dr. Goniewicz reports grants from Pfizer Inc. and served as an advisory board member to Johnson & Johnson, manufacturers of smoking cessation drugs, outside the submitted work.

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