

Supplementary Materials: Development of A Method for Simultaneous Analysis of Allergenic Flavoring Agents in Cigarettes and Quantitative Risk assessment for Consumer Safety

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Table S1. Non-capsule cigarettes and capsule cigarettes selected for analysis.

No.	Brand	Category	Cigarette Size	Number of Capsules	Sub-Brand	Flavor Type
1	A	Non-capsule	King	None	Plus	-
2			King	None	Origin	-
3		Capsule	King	1 EA	Ice Kula	Mint
4			King	1 EA	Rula	Banana
5			King	1 EA (Random, 1 out of 5)	Mola	Fruit
6			King	1 EA	Ice Jack	Mint
7	B	Non-capsule	Slim	None	Prime	-
8			Slim	None	Soo 0.1	-
9			Slim	None	Soo	-
10			Slim	None	Sense 1	-
11			Slim	None	One	-
12			Slim	None	Classic	-
13		Capsule	Slim	1 EA	Change 1mg	Mint
14			Slim	1 EA	Change W	Wine
15			Slim	1 EA	Change Up	Orange
16			Slim	1 EA	Change LiNN	Mint
17			Slim	1 EA	Change Bing	Sweet
18			Slim	1 EA	Change Frozen	Mint
19			Slim	1 EA	Change Himalaya	Mint
20			Slim	1 EA (Random, 1 out of 5)	Change Secret	Fruit
21			Slim	1 EA	Change 4mg	Mint
22	C	Non-capsule	King	None	Gold	-
23			King	None	Silver	-
24			King	None	Medium	-
25			King	None	Red	-
26		Capsule	King	1 EA	Hybrid 1	Mint
27			King	1 EA	Ice Blast One	Mint

28			King	1 EA	Hybrid 5	Mint
29			King	2 EA	Zing Fusion Double	Orange
30			King	1 EA	Ice Blast	Mint
31	D	Non-capsule	King	None	6mg	-
32			Slim	None	Fine Cut Master 1mg	-
33			Slim	None	Fine Cut Master 3mg	-
34			King	None	1mg	-
35			Slim	None	Fine Cut 1mg	-
36			King	None	3mg	-
37		Capsule	King	1 EA	Switch one	Mint
38			Slim	1 EA	Fine Cut Switch	Mint
39			Slim	2 EA	Fine Cut Mellow Crush	Mellon, Mint
40			Slim	2 EA	Fine Cut Tropical Crush	Pineapple, Mint
41			King	2 EA	Summer Crush	Watermelon, Orange
42			King	1 EA	Switch 6mg	Mint
43	E	Non-capsule	King	None	Sky Blue	-
44			King	None	LSS One	-
45			King	None	One	-
46			King	None	Wind Blue	-
47			King	None	LSS Wind Blue	-
48			King	None	Original	-
49		Capsule	King	1 EA	LBS Yellow 1mg	Lemon
50			King	1 EA	LBS Blue	Mint
51			King	1 EA	LBS Mix Green	Fruit
52			King	1 EA	LBS Yellow 3mg	Lemon
53			King	1 EA	LBS Tropical Mix	Melon, Mint
54			King	1 EA	LBS Purple	Grape
Blank	C	Non-capsule	Slim	None	Zero Additive	-
Total number of tobacco or filter tips: 55 EA					Total number of capsules: 42 EA	

Table S2. Optimized GC-MS/MS conditions for analyzing flavoring agents in cigarettes.

Compound	Retention Time (min)	SRM Transition 1			SRM Transition 2		
		Precursor Ion(m/z)	Product Ion (m/z)	Collision Energy	Precursor Ion (m/z)	Product Ion (m/z)	Collision Energy
D-Limonene	6.39	68.1	53.1	10	93.1	77.0	12
Linalool	9.45	93.1	77.1	12	121.1	93.1	6
Methyl 2-octynoate	10.36	95.1	67.1	8	123.1	81.1	8
Citral	10.65; 11.11	94.1	79.1	8	69.1	41.1	6
Citronellol	11.32	95.1	67.1	8	69.1	41.1	6
Geraniol	12.26	69.1	41.1	6	93.1	77.0	12
α -Isomethyl ionone	12.53	150.1	107.1	6	135.2	91.1	14
Benzyl alcohol	12.71	108.1	79.1	12	79.1	77.1	10
Hydroxycitronellal	13.62	71.1	43.1	6	59.0	31.1	10
Methyl eugenol	14.89	178.1	107.1	14	163.1	107.1	6
Cinnamaldehyde	15.49	131.0	77.0	22	103.1	77.1	10
Lilial	15.72	189.1	131.1	10	204.1	189.2	8
Eugenol	18.83	164.1	131.1	10	131.1	103.1	10
α -Amylcinnamaldehyde	22.06	129.1	128.1	18	202.1	129.1	6
Anise alcohol	22.84	138.0	109.1	6	137.0	109.0	6
Cinnamyl alcohol	23.04	134.1	92.1	6	115.1	89.1	14
Isoeugenol	22.04; 26.45	164.1	131.0	10	149.1	121.1	6
α -Hexylcinnamaldehyde	26.93	216.2	129.1	6	117.1	115.1	8
Farnesol	26.07; 27.31	81.1	79.2	10	93.1	77.1	10
Coumarin	29.08	118.0	90.1	10	146.0	118.1	10
HICC	29.99; 30.23	105.1	79.0	10	136.1	79.1	8
α -Amylcinnamyl alcohol	31.25	133.1	55.0	8	91.1	65.0	14
Benzyl benzoate	32.16	105.0	77.1	12	91.1	65.1	12
Benzyl salicylate	33.48	228.1	91.1	6	91.1	65.1	14
Benzyl cinnamate	36.76	131.0	103.1	8	91.1	65.1	12
Menthol	10.25	95.1	67.1	8	123.1	81.1	8

Table S3. Method validation (MDL, LOQ, and linearity) for analyzing allergenic flavoring agents.

Compound	LOD (ng/ml)	MDL (ng/ml)		LOQ (ng/ml)			Calibration Range (ng/ml)	Linearity (R ²)
		Tobacco	Filter tip	Capsule	Tobacco	Filter tip		
D-Limonene	3.91	7.81	3.91	13.03	26.03	13.03	13.02 - 2,000	0.9999
Linalool	0.49	0.98	1.95	1.63	3.27	6.50	1.63 - 125; 125 - 2,000	0.9999; 0.9999
Methyl 2-octynoate	0.98	3.91	3.91	3.27	13.03	13.03	3.26 - 125	0.9998
Citral	0.98	1.95	0.98	3.27	6.50	3.27	3.26 - 1,000	0.9999
Citronellol	3.91	3.91	3.91	13.03	13.03	13.03	13.02 - 2,000	0.9999
Geraniol	1.95	3.91	3.91	6.50	13.03	13.03	6.51 - 62.5; 62.5 - 2,000	0.9998; 0.9997
α -Isomethyl ionone	0.24	0.49	3.91	0.80	1.63	13.03	0.81 - 125	0.9999
Benzyl alcohol	0.98	1.95	3.91	3.27	6.50	13.03	3.26 - 2,000	0.9999
Hydroxycitronellal	3.91	3.91	3.91	13.03	13.03	13.03	13.02 - 250	0.9996
Methyl eugenol	0.24	1.95	0.98	0.80	6.50	3.27	0.81 - 125	0.9999
Cinnamaldehyde	0.24	0.49	0.49	0.80	1.63	1.63	0.81 - 125	0.9999
Lilial	0.12	0.49	0.98	0.40	1.63	3.27	0.41 - 62.5	0.9998
Eugenol	0.98	0.98	3.91	3.27	3.27	13.03	3.26 - 250	0.9999
α -Amylcinnamaldehyde	0.98	0.98	1.95	3.27	3.27	6.50	3.26 - 125	0.9999
Anise alcohol	1.95	1.95	1.95	6.50	6.50	6.50	6.51 - 500	0.9999
Cinnamyl alcohol	0.49	0.98	0.49	1.63	3.27	1.63	1.63 - 250	0.9998
Isoeugenol	1.82	1.82	1.82	6.07	6.07	6.07	6.08 - 116.75	0.9999
α -Hexylcinnamaldehyde	0.98	0.98	0.98	3.27	3.27	3.27	3.26 - 125	0.9997
Farnesol	5.59	5.59	5.59	18.63	18.63	18.63	18.62 - 179	0.9999
Coumarin	0.98	1.95	3.91	3.27	6.50	13.03	3.26 - 125	0.9997
HICC	0.24	0.98	1.95	0.80	3.27	6.50	0.81 - 62.5	0.9998
α -Amylcinnamyl alcohol	1.95	1.95	3.91	6.50	6.50	13.03	6.51 - 125	0.9999
Benzyl benzoate	0.49	0.49	0.49	1.63	1.63	1.63	1.63 - 500	0.9998
Benzyl salicylate	3.91	3.91	3.91	13.03	13.03	13.03	13.02 - 250	0.9999
Benzyl cinnamate	0.49	2.44	0.98	1.63	8.13	3.27	1.63 - 125	0.9999
Menthol	0.49	0.98	1.95	1.63	3.27	6.50	1.63 - 156.25; 156.25 - 5,000	0.9999; 0.9999

MDL, Method detection limit; LOQ, Limit of quantitation.

Table S4. Recovery and reproducibility of analysis of allergenic flavoring agents in cigarette matrix.

Compound	Tobacco (<i>n</i> = 3, for individual concentrations)						Filter Tip (<i>n</i> = 3, for individual concentrations)					
	Recovery (%)			Reproducibility (% RSD)			Recovery (%)			Reproducibility (% RSD)		
	2.5 µg/g	5.0 µg/g	50 µg/g	2.5 µg/g	5.0 µg/g	50 µg/g	2.5 µg/g	5.0µg/g	50 µg/g	2.5 µg/g	5.0 µg/g	50 µg/g
D-Limonene	94.2 (2.2%)	91.0 (4.8%)	93.0 (1.0%)	1.81	3.95	0.81	92.9 (2.5%)	97.3 (2.3%)	93.1 (1.3%)	2.02	1.89	1.03
Benzyl alcohol	89.7 (4.8%)	101.4 (4.5%)	92.9 (1.8%)	3.88	3.68	1.49	94.3 (1.9%)	98.4 (2.2%)	93.2 (2.4%)	1.52	1.82	1.96
Linalool	95.5 (4.2%)	104.0 (0.8%)	102.7 (0.6%)	3.45	0.68	0.46	92.9 (1.2%)	99.2 (2.8%)	96.8 (1.5%)	0.95	2.31	1.22
Methyl 2-octynoate	89.0 (2.5%)	96.4 (1.1%)	106.4 (0.4%)	2.01	0.89	0.34	86.0 (6.8%)	90.6 (5.3%)	100.5 (1.5%)	5.56	4.35	1.24
Citronellol	103.2 (4.6%)	103.2 (5.4%)	108.3 (0.6%)	3.73	4.43	0.48	90.5 (0.7%)	99.6 (3.7%)	101.1 (1.6%)	0.60	3.05	1.32
Citral	89.5 (5.3%)	95.9 (4.3%)	105.1 (2.0%)	4.28	3.49	1.67	94.4 (0.8%)	104.4 (2.1%)	100.3 (1.7%)	0.65	1.72	1.40
Geraniol	88.3 (1.8%)	98.1 (2.9%)	99.5 (3.1%)	1.45	2.39	2.50	91.5 (2.1%)	97.2 (5.1%)	91.1 (3.0%)	1.72	4.19	2.45
Cinnamaldehyde	101.4 (8.0%)	102.6 (3.3%)	97.9 (0.6%)	6.56	2.71	0.45	100.1 (0.9%)	102.8 (2.7%)	96.6 (1.4%)	0.70	2.24	1.13
Anise alcohol	91.5 (3.1%)	93.8 (4.9%)	90.8 (5.0%)	2.51	3.96	4.11	87.0 (1.4%)	91.2 (4.1%)	97.0 (2.8%)	1.18	3.38	2.28
Hydroxycitronellal	101.3 (1.8%)	103.5 (3.0%)	105.2 (0.9%)	1.49	2.41	0.71	95.1 (1.5%)	103.3 (3.4%)	103.8 (0.9%)	1.20	2.76	0.70
Cinnamyl alcohol	89.3 (9.7%)	96.4 (5.2%)	99.3 (1.9%)	7.95	4.20	1.55	94.9 (1.0%)	92.3 (2.6%)	96.9 (1.4%)	0.77	2.13	1.12
Eugenol	102.9 (1.6%)	105.8 (1.1%)	101.0 (0.4%)	1.33	0.86	0.36	99.4 (2.0%)	102.8 (2.2%)	101.9 (3.9%)	1.66	1.76	3.14
Methyl eugenol	103.7 (2.7%)	104.8 (3.2%)	101.3 (0.2%)	2.18	2.61	0.14	97.9 (4.8%)	95.9 (1.6%)	97.1 (1.3%)	3.93	1.33	1.07
Coumarin	102.5 (3.3%)	98.7 (5.1%)	101.4 (0.2%)	2.72	4.16	0.12	100.7 (2.4%)	96.9 (0.8%)	98.9 (0.9%)	1.95	0.65	0.76
Isoeugenol	99.9 (4.5%)	100.9 (3.1%)	102.8 (2.0%)	3.68	2.53	1.62	95.9 (3.5%)	94.7 (1.4%)	91.2 (5.5%)	2.84	1.12	4.50
α-Isomethyl ionone	106.6 (2.4%)	101.9 (1.8%)	106.0 (0.3%)	0.39	1.40	0.69	101.1 (2.7%)	100.7 (2.1%)	101.1 (1.6%)	0.39	1.40	0.69

Lilial	93.4 (2.2%)	96.5 (4.1%)	101.2 (0.9%)	1.80	3.35	0.73	102.9 (1.0%)	95.1 (3.4%)	97.8 (1.4%)	0.83	2.81	1.12
α -Amylcinnamaldehyde	87.9 (8.4%)	100.6 (2.0%)	98.5 (2.2%)	6.82	1.59	1.79	85.9 (2.8%)	97.2 (1.4%)	96.8 (2.1%)	2.27	1.17	1.68
HICC	95.7 (5.3%)	101.3 (1.4%)	104.9 (3.3%)	4.30	1.13	2.67	97.3 (1.7%)	102.5 (5.6%)	103.9 (1.3%)	1.40	4.60	1.09
α -Amylcinnamyl alcohol	95.4 (4.3%)	104.2 (3.3%)	104.3 (1.5%)	3.53	2.71	1.25	92.8 (2.4%)	98.4 (4.6%)	103.1 (1.8%)	1.97	3.71	1.49
Farnesol	95.2 (5.6%)	91.7 (3.2%)	100.4 (2.2%)	4.55	2.60	1.75	94.1 (2.6%)	80.3 (4.6%)	87.7 (4.9%)	2.12	3.76	3.98
α -Hexylcinnamaldehyde	97.7 (2.1%)	100.6 (4.2%)	105.4 (1.8%)	1.71	3.41	1.45	95.1 (1.9%)	98.3 (4.1%)	100.8 (1.1%)	1.55	3.32	0.92
Benzyl benzoate	82.8 (5.7%)	90.9 (0.7%)	86.0 (3.0%)	4.66	0.58	2.48	92.1 (2.0%)	93.4 (1.1%)	83.8 (1.3%)	1.65	0.87	1.09
Benzyl salicylate	81.0 (3.0%)	82.8 (3.4%)	104.7 (1.7%)	2.44	2.74	1.37	90.2 (2.9%)	80.5 (3.4%)	97.0 (1.3%)	2.39	2.74	1.08
Benzyl cinnamate	85.6 (8.4%)	94.2 (4.8%)	102.6 (3.0%)	6.87	3.91	2.48	87.3 (8.7%)	90.9 (5.8%)	96.1 (1.8%)	7.09	4.70	1.43

Table S5. Concentration (µg/g) of flavoring agents in tobacco, filter tips, and capsules.

Compound	Tobacco (<i>n</i> = 54)						Filter tip (<i>n</i> = 54)						Capsule (<i>n</i> = 42)					
	N	Mean	Median	SD	Min	Max	N	Mean	Median	SD	Min	Max	N	Mean	Median	SD	Min	Max
D-Limonene	7	21.0	9.4	23.9	1.0	63.2	11	86.9	14.9	136.8	1.3	375.1	40	18,561.9	1,868.8	25,578.1	20.3	81,795.7
Linalool	21	1.9	0.1	5.5	0.1	25.1	44	21.7	0.5	81.3	0.2	472.0	41	2,152.3	213.3	3,893.0	2.0	14,558.8
Methyl 2-octynoate	0	-	-	-	-	-	0	-	-	-	-	-	0	-	-	-	-	-
Citral	54	0.7	0.5	1.6	0.2	12.4	16	88.0	0.7	331.6	0.4	1,372.1	29	1,254.2	203.3	2,994.2	3.1	11,931.8
Citronellol	0	-	-	-	-	-	2	19.6	19.6	13.5	6.1	33.0	25	180.6	22.7	289.4	12.2	1,237.6
Geraniol	0	-	-	-	-	-	6	13.7	7.3	12.6	2.8	36.4	26	548.8	103.3	1,297.8	7.7	6,323.2
α-Isomethyl ionone	12	0.04	0.04	0.01	0.03	0.07	8	0.2	0.2	0.1	0.1	0.4	1	1.3	1.3	-	1.3	1.3
Benzyl alcohol	52	5.5	1.3	10.4	0.2	55.6	54	138.4	14.4	304.3	1.7	1,761.9	21	2,151.7	11.1	6,544.7	1.8	23,269.9
Hydroxycitronellal	0	-	-	-	-	-	0	-	-	-	-	-	0	-	-	-	-	-
Methyleugenol	0	-	-	-	-	-	1	0.3	0.3	-	0.3	0.3	12	2.9	1.0	4.3	0.4	15.0
Cinnamaldehyde	0	-	-	-	-	-	7	2.5	1.1	2.5	0.5	8.2	10	2,035.1	4.3	3,231.1	0.6	8,523.5
Lilial	0	-	-	-	-	-	0	-	-	-	-	-	0	-	-	-	-	-
Eugenol	4	0.2	0.2	0.1	0.1	0.4	1	8.9	8.9	-	8.9	8.9	27	13.6	14.4	7.7	1.6	34.8
α-Amylcinnamaldehyde	0	-	-	-	-	-	0	-	-	-	-	-	0	-	-	-	-	-
Anise alcohol	4	0.8	0.5	0.6	0.4	1.8	2	4.8	4.8	1.1	3.7	6.0	0	-	-	-	-	-
Cinnamyl alcohol	0	-	-	-	-	-	0	-	-	-	-	-	3	11.5	14.4	6.0	3.1	16.9
Isoeugenol	5	0.6	0.4	0.3	0.3	1.1	0	-	-	-	-	-	5	17.3	3.1	19.9	0.8	48.2
α-Hexylcinnamaldehyde	0	-	-	-	-	-	0	-	-	-	-	-	0	-	-	-	-	-
Farnesol	0	-	-	-	-	-	0	-	-	-	-	-	2	10.6	10.6	0.2	10.4	10.8
Coumarin	0	-	-	-	-	-	0	-	-	-	-	-	0	-	-	-	-	-
HICC	0	-	-	-	-	-	0	-	-	-	-	-	0	-	-	-	-	-
α-Amylcinnamyl alcohol	0	-	-	-	-	-	0	-	-	-	-	-	0	-	-	-	-	-
Benzyl benzoate	31	0.7	0.1	1.8	0.1	8.1	9	1.3	0.6	1.1	0.3	3.7	7	14.8	6.8	12.7	1.6	36.5
Benzyl salicylate	2	0.9	0.9	0.1	0.7	1.0	0	-	-	-	-	-	0	-	-	-	-	-
Benzyl cinnamate	0	-	-	-	-	-	1	8.6	8.6	-	8.6	8.6	6	145.7	2.9	203.1	0.9	439.2
Menthol	53	463.8	2.4	1,211.4	0.1	5,949.5	53	3,633.5	17.6	7,678.9	1.2	31,006.5	42	98,948.6	96,829.4	31,755.3	42,307.9	168,826.6

n, The number of total samples; N, The number of sample containing flavoring agent; SD, Standard deviation.