Supporting Information for “Identification of New and Distinctive Exposures from Little Cigars”

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Table S1. Package Information for Little Cigar Products Tested

Product Name	Complete Text on Front of Package
Swisher Sweets Original	Swisher Sweets; Little Cigars; Swisher Sweets; Filtered Little Cigars; 20; Smooth • Sweet • Satisfying
Swisher Sweets Cherry	Swisher Sweets; Little Cigars; Swisher Sweets; Sweet Cherry; Filtered Little Cigars; 20; Smooth • Sweet • Satisfying
Cheyenne Full Flavor	20 Cigars; Cheyenne; Full Flavor; 100’s; Made in U.S.A.
Cheyenne Menthol	20 Cigars; Cheyenne; Menthol; 100’s; Made in U.S.A.

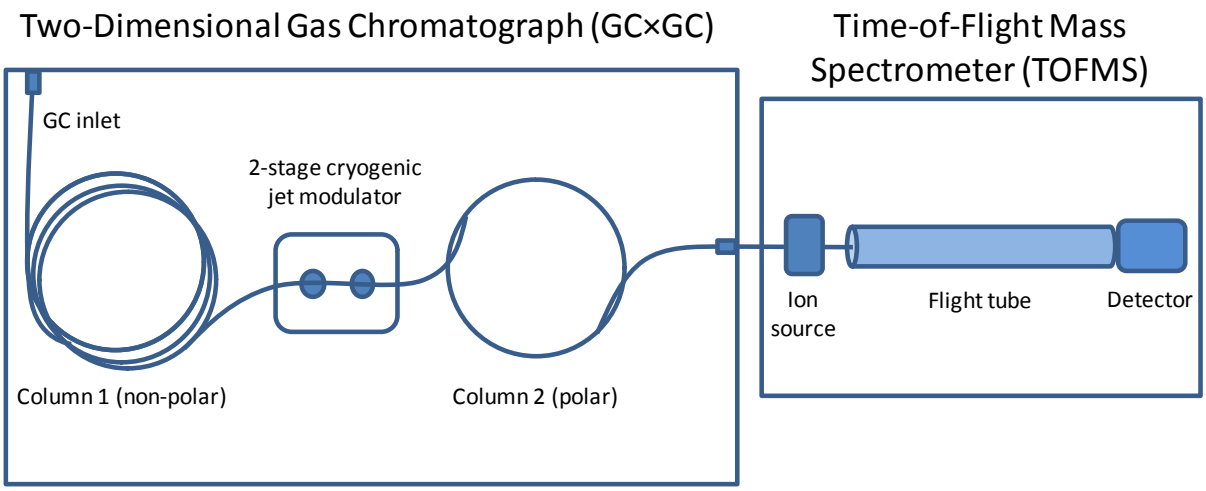

Figure S1. Schematic illustration of two-dimensional gas chromatograph–time-of-flight mass spectrometer.

Table S2. Acquisition Parameters for Two-Dimensional Gas Chromatography–Time-of-Flight Mass Spectrometry for Mainstream Smoke Analyses

Two-Dimensional Gas Chromatography	
1st-dimension column	100% dimethylpolysiloxane: 30 m, 250 µm i.d., 1.0 µm film
2nd-dimension column	50% phenyl polysilphenylene-siloxane: 1 m, 100 µm i.d., 0.1 µm film
Inlet temperature	250 °C
Main oven temperature program	45 °C (1.5 min hold) 45 °C – 100 °C @ 20 °C/min 100 °C – 270 °C @ 3 °C/min; hold 1 min 270 °C – 320 °C @ 20 °C/min, hold 16 min
Secondary oven temperature program	80 °C (1.5 min hold) 80 °C – 275 °C @ 3 °C/min 275 °C – 330 °C @ 20 °C/min, hold 11 min
Modulation temperature	20 °C above 1st-dimension column temperature
Modulation period	3 seconds
Carrier gas (He) flow rate	1.5 mL/min constant flow
Injection volume	1 µL splitless for 60 seconds
Time-of-Flight Mass Spectrometry	
Transfer line temperature	290 °C
Ion source temperature	200 °C
Detector voltage	Tune voltage + 200V
Data rate, range	100 spectra/sec, 35–600 amu
Mass resolution	Unit resolution

Methods for Confirmation Analysis

Confirmation analysis was performed for ambrox, 3-methylbutanenitrile, and 4-methylimidazole, the tentatively identified New/Distinctive Exposure Candidates that were selected as described in the "Results" section. To achieve this objective, aliquots of some concentrated extracts were spiked with authentic standards of the compounds and analyzed by either GC×GC-TOFMS or LC–MS/MS. GC×GC-TOFMS analysis was used for confirmation analysis of ambrox and 3-methylbutanenitrile, which have low to moderate polarity. LC–

MS/MS analysis was used for confirmation analysis of 4-methylimidazole, which is polar and alkaline.

Confirmation studies were performed by concurrent analyses of the spiked samples and non-spiked (i.e., "native") samples. The native samples were modified as needed using co-solvents and internal standards to yield compositions equivalent to the spiked extracts other than the inclusion of the authentic materials. A compound was considered a confirmed detection if the retention times, peak shapes, and mass spectra for the native extracts were consistent with those for the spiked extracts and spiked blanks. When the presence of a compound in a sample was confirmed, the peak responses for the paired spiked and native solutions were evaluated to provide a semi-quantitative estimate of the concentration in the native solution, assuming a linear response factor. In addition, an approximate detection limit was determined from the chromatogram for each spiked solution using interpolation to estimate the minimum concentration needed to yield a signal-to-noise ratio sufficiently large to enable compound detection. For a given compound, the largest value for any calculated approximate detection limit was reported as the estimated detection limit.

Concentrated stock solutions were prepared from authentic standards of the three compounds. For each of ten concentrated extracts—one from each of the eight tobacco products tested and two blanks—a 40-μL aliquot was spiked with the internal standard acenaphthene-d_{10} and stock solutions of ambrox and 3-methylbutanenitrile, yielding spiked concentrations of 0.5 μg/mL for ambrox and 1 μg/mL for 3-methylbutanenitrile. The spiked and native solutions were analyzed by GC×GC-TOFMS for confirmation analysis using the same method employed in the original sample analyses. Similarly, for each of ten concentrated extracts—one from each of the eight tobacco products tested and two blanks—an 8-μL aliquot was diluted 120-fold into

2/98 (volume/volume) methanol/water and spiked with a stock solution of 4-methylimidazole, yielding spiked concentrations of 7.5 ng/mL for cigarette samples and 30 ng/mL for little cigar samples and blanks. The spiked and native solutions were analyzed by LC–MS/MS for confirmation analysis using the analytical conditions indicated in Table S3.

Table S3. Acquisition Parameters for Liquid Chromatography–Tandem Mass Spectrometry for Mainstream Smoke Analyses

Ultra Perfomance Liquid Chromatography (UPLC)			
UPLC system	Waters Acquity H-Class		
UPLC column	Restek Pinnacle DB PFP (Pentafluorophenyl) Propyl, 2.1 × 100 mm, 1.9 µm		
Column temperature	40 °C		
Mobile phases	A: 2 mM each of formic acid and ammonium formate in water B: 2 mM each of formic acid and ammonium formate in methanol		
Gradient	Time (min)	Flow Rate (mL/min)	% Mobile Phase B
	0	0.4	2
	1	0.4	2
	5	0.4	42
	5.01	0.5	100
	6.5	0.5	100
	6.51	0.4	2
	9	0.4	2
Injection volume	25 µL		
Run time	9 minutes		
Tandem Mass Spectrometry			
Mass spectrometer	Micromass Quattro Premier, triple quadrupole		
Ionization method	Electrospray (Positive Mode)		
Capillary voltage	0.2 kV		
Source temperature	100 °C		
Desolvation temperature	500 °C		
Desolvation gas	Nitrogen, 1000 L/hr		
Collision cell pressure	~2×10^{-3} mbar		
Collision gas	Argon		
Multiple reaction monitoring (MRM) ion transitions	1° (quantifier): 83 > 56 2° (qualifier): 83 > 42		
Cone voltage	35 V		
Collision energies	15 eV for all ion transitions		

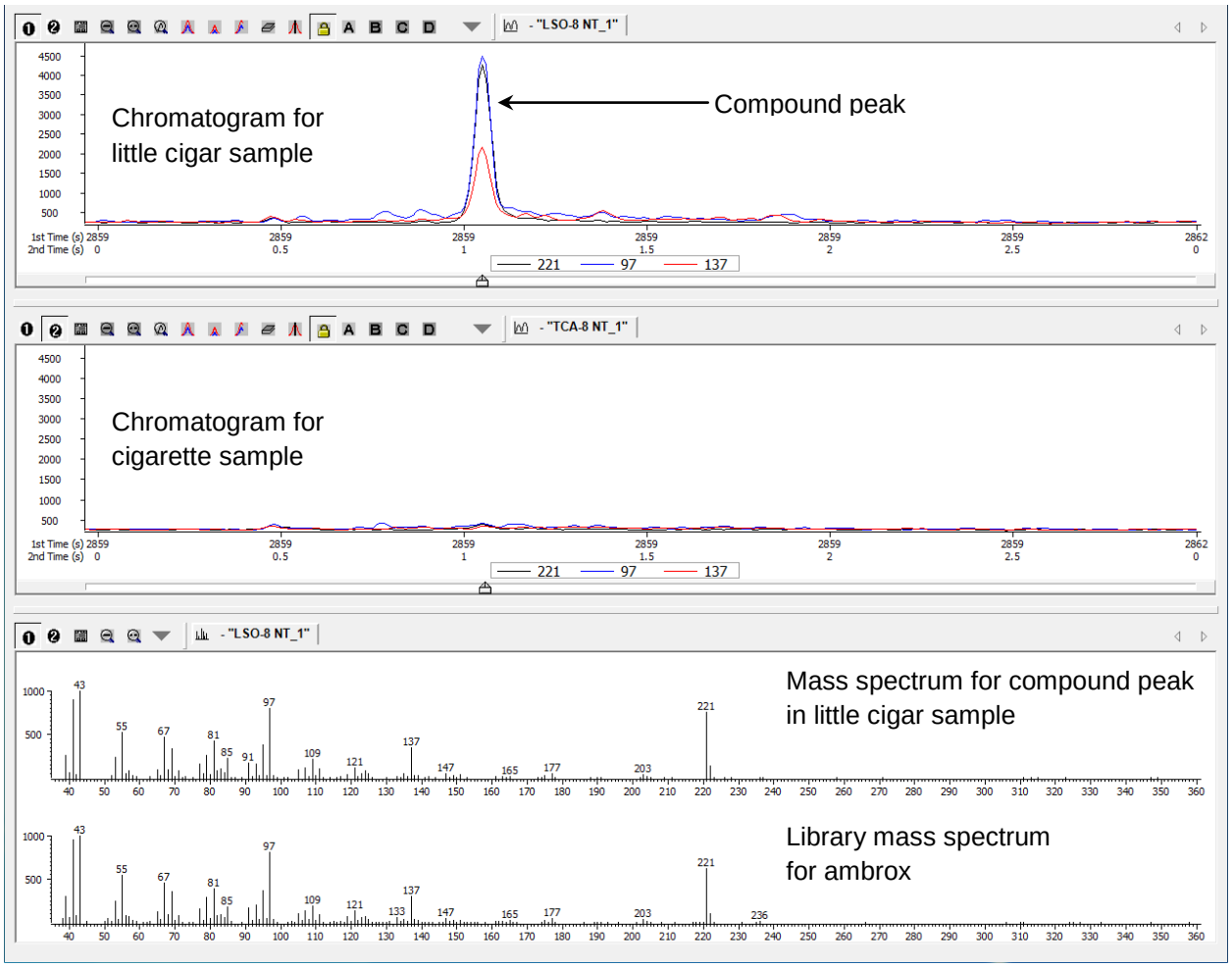

Figure S2. Example of extracted ion chromatograms (m/z 221, 97, and 137) and mass spectra for detection of ambrox by GC×GC-TOFMS.

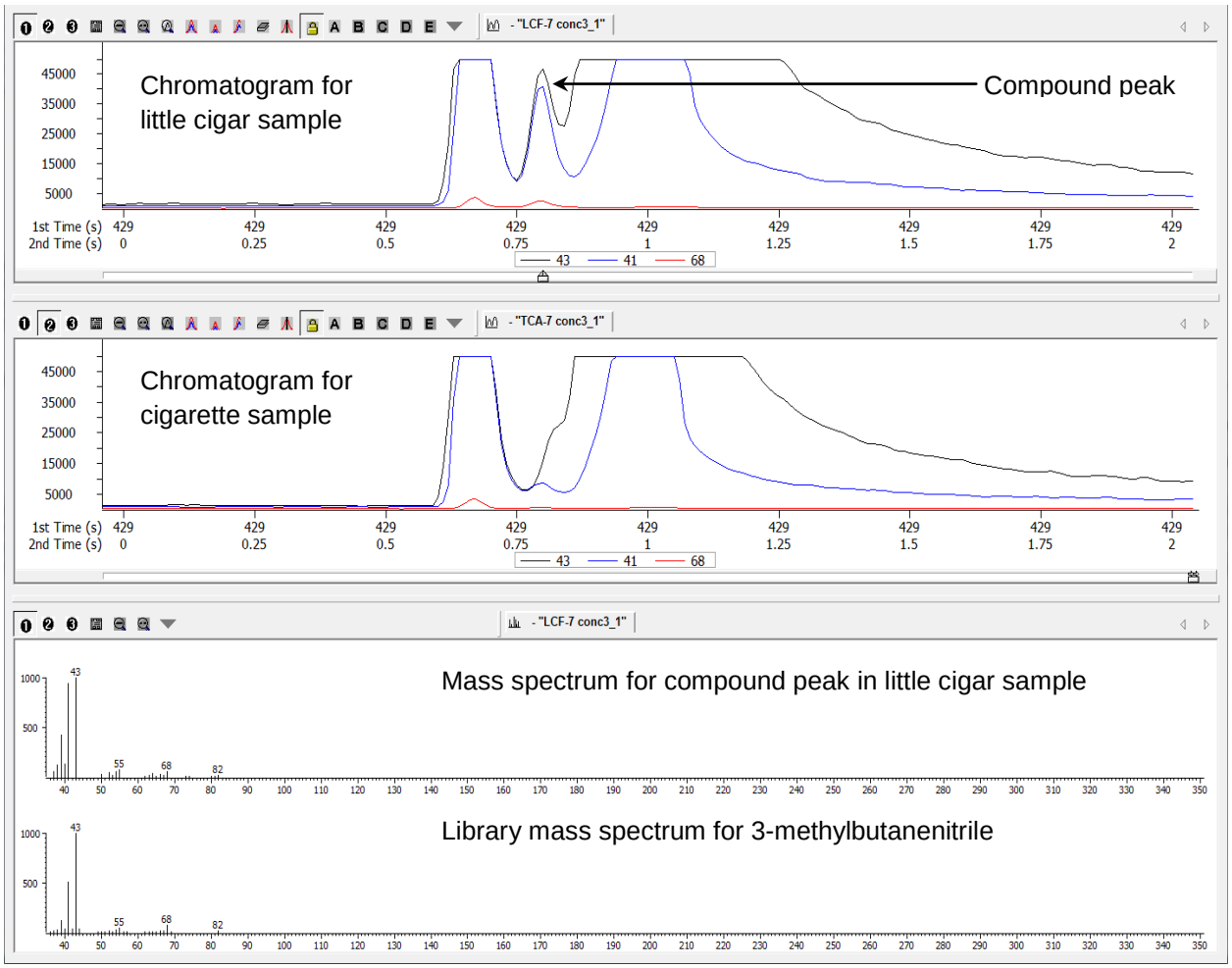

Figure S3. Example of extracted ion chromatograms (m/z 43, 41, and 68) and mass spectra for detection of 3-methylbutanenitrile by GC×GC-TOFMS.

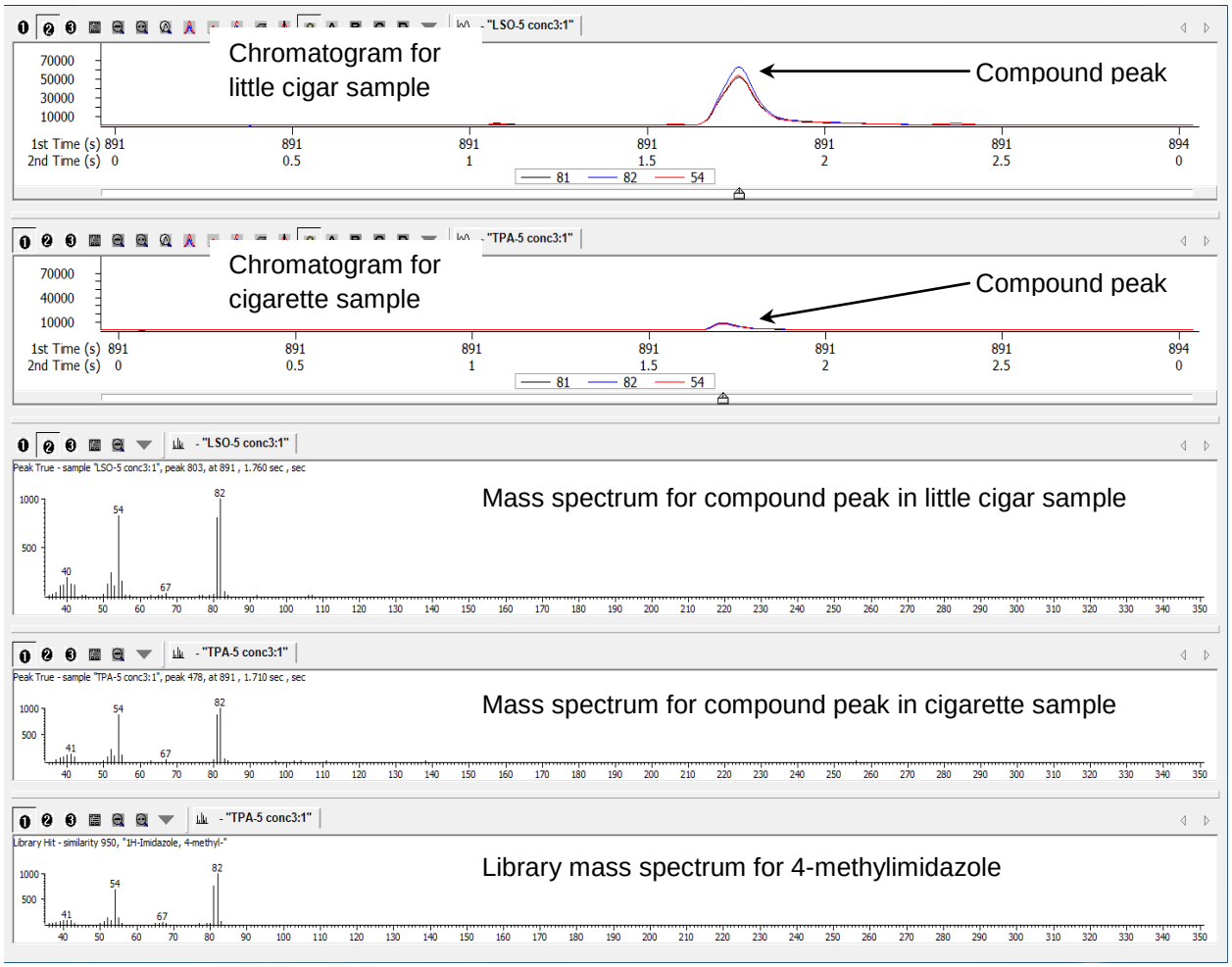

Figure S4. Example of extracted ion chromatograms (m/z 81, 82, and 54) and mass spectra for detection of 4-methylimidazole by GC×GC-TOFMS.