The Characterization of Flavored Tobacco with GCxGC-TOFMS

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Background

The characterization of flavor analytes in complex natural products, such as tobacco, is important. Information on naturally present flavors can aid in quality control and process optimization, and flavor additives are regulated by the FDA. Detecting and identifying individual flavor compounds, both naturally occurring and additives, within a complex tobacco matrix can be accomplished with chromatographic separations paired with mass spectral detection.

Methods were developed for sample preparation, chromatographic separation, and mass spectral detection to analyze natural flavor analytes and flavor additives in tobacco. Various tobacco products, including cigarettes and flavored tobacco, were analyzed. The volatile and semi-volatile compounds were sampled with headspace solid-phase micro-extraction (HS-SPME). Comprehensive two-dimensional gas chromatography (GCxGC) coupled to time-of-flight mass spectrometry (TOFMS) methods were developed to isolate and identify individual analytes within the complex tobacco matrix, including flavor compounds known to occur naturally in tobacco as well as flavor additives. Additional high resolution MS data were collected with LECO's Pegasus® GC-HRT for confirmation of analyte identities through accurate mass measurements and for gaining insight to analytes that were previously unknown. These techniques provide a reliable method to locate, identify, and quantify tobacco flavors, and offer rapid characterization of tobacco products and their flavor analytes.

Methods

Samples and Sample Preparation

Artificially flavored and natural tobacco samples (original and menthol flavored cigarettes; and raspberry and vanilla spice hookah tobacco) were analyzed. The samples were weighed into 10 mL vials and a saturated salt solution was added to each. The headspace was sampled by SPME with a DVB/Carboxen/PDMS (50/30 µm DVB/CAR/PDMS) fiber (Supelco, Bellefonte, PA, USA) for 20 min at 95°C immediately after a 5 min incubation period at the same temperature. Analytes were desorbed in the GC-inlet at 250°C for 2 min for injection.

Instrument Conditions

GCxGC analyses were performed with LECO's Pegasus 4D, consisting of an Agilent 7890 GC modified with LECO's dual stage quad jet thermal modulator and secondary oven, and paired with LECO's Pegasus TOFMS. The major GCxGC components are shown in Figure 1.

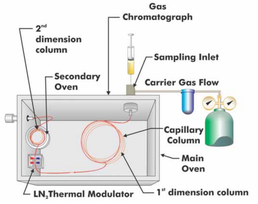


Figure 1. Diagram of the major GCxGC components.

Table 1. Instrument Parameters

GCxGC Conditions	
Carrier Gas	He @ 1.5 ml/min
Column One	Rxi-5Sil MS, 30 m x 0.25 mm x 0.25 µm (Restek, Bellefonte, PA)
Column Two	Rxi-17Sil MS, 1.25 m x 0.18 mm x 0.18 µm (Restek, Bellefonte, PA)
Temperature Program	2 min at 40°C, ramped 12°C/min to 300°C, held 2 min; Secondary oven maintained +5°C relative to primary
Modulation	2 s with temperature maintained +15°C relative to 2nd oven
Pegasus 4D Conditions	
Mass Range	35 to 500 m/z
Acquisition Rate	200 spectra/s
Source Temp	250°C

Benefits of GCxGC

GCxGC offers benefits relative to GC that lead to the isolation of more individual analytes and better characterization of a complex sample. The primary benefits of GCxGC, shown in Figures 2-3, are:

- **Increased Peak Capacity** – each sample is simultaneously and comprehensively separated on two complementary GC columns often isolating analytes in the second dimension that coelute in the first.
- **Lower Limit of Detection** – thermal modulation collects and refocuses effluent between the first and second column sharpening analyte peaks just prior to detection leading to more detectable analytes.
- **Structured Chromatograms** - analytes with similar functional groups elute in structured bands through the 2D space providing characterization information.

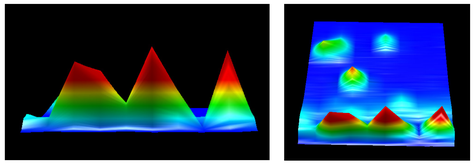


Figure 2. GCxGC increases the peak capacity relative to a 1D separation. (Standard sample shown.)

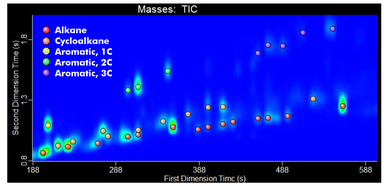
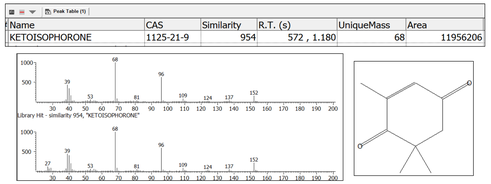


Figure 3. Analytes with similar functional groups elute in structured bands through the GCxGC space. (Standard sample shown.)

GCxGC-TOFMS

Coupling TOFMS detection with GCxGC allows for identification of the isolated analytes, accomplished through LECO's ChromaTOF® software. An example of the compiled information is demonstrated in Figure 4. Ketoisophorone has characteristic "musty, woody, sweet, tea, tobacco, and leaf" odor properties and was identified with a similarity of 954 with the automated data processing.



Name	CAS	Similarity	R.T. (s)	UniqueMass	Area
KETOISOPHORONE	1125-21-9	954	572 , 1.180	68	11956206

Figure 4. Representative analyte information provided by ChromaTOF's automated peak finding software. Ketoisophorone was identified in all samples.

Natural Tobacco Aroma and Flavor

A representative chromatogram for the original tobacco sample is shown in Figure 5. This sample is complex and benefits from the additional peak capacity of GCxGC. Peaks that align vertically in the GCxGC chromatogram would coelute in a comparable 1D separation.

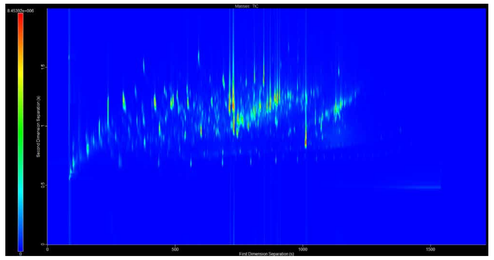


Figure 5. Representative TIC chromatogram of original tobacco.

A collection of analytes, tentatively identified with nominal mass library searching, are listed in Table 2 with their associated aroma properties. Many of these analytes have aromas typically associated with tobacco. Table 2 is intended to show representative examples of the types of flavor analytes detected and is not comprehensive.

Table 2. Naturally occurring flavors in the original cigarette sample

Name	CAS	Similarity	R.T. (s)	Odor Properties
pyrrole	109-97-7	958	222 , 1.050	sweet warm nutty ethereal
2-methyl pyrazine	109-08-0	959	294 , 1.055	nutty cocoa roasted chocolate peanut green
furfural	98-01-1	956	310 , 1.115	sweet woody almond fragrant baked bread
5-methyl-2(3H)-furanone	591-12-8	812	330 , 1.195	sweet solvent nutty tonka coumarin tobacco
1-(2-furanyl)-ethanone	1192-62-7	965	370 , 1.160	sweet balsam almond cocoa caramel coffee
dihydro-2(3H)-furanone	96-48-0	969	376 , 1.515	creamy oily fatty caramel
gamma-valerolactone	108-29-2	938	414 , 1.350	herbal sweet warm tobacco cocoa woody
limonene	138-86-3	904	480 , 0.830	citrus herbal terpene camphor
benzeneacetaldehyde	122-78-1	842	492 , 1.220	green sweet floral hyacinth clover honey cocoa
acetylfuran	1072-83-9	951	508 , 1.275	musty nutty coumarin licorice walnut bread
benzyl formate	104-57-4	959	518 , 1.155	floral fruity spicy almond cranberry black tea
3-acetyl pyridine	350-03-8	977	546 , 1.315	sweet nutty dry hawthorn phenolic woody popcorn
methyl nicotinate	93-60-7	802	570 , 1.195	warm herbal tobacco
ketoisophorone	1125-21-9	954	572 , 1.180	musty woody sweet tea tobacco leaf
safranal	116-26-7	919	618 , 1.095	fresh herbal phenolic metallic rosemary tobacco spicy
quinoline	91-22-5	928	650 , 1.310	medical musty tobacco rubber earthy
2-methoxy-4-vinylphenol	7786-61-0	902	698 , 1.190	spicy clove smoky phenolic peppery woody
(E) β-damascenone	23726-93-4	829	732 , 1.040	apple rose honey tobacco sweet
vanillin	121-33-5	954	756 , 1.400	sweet vanilla creamy chocolate
β-ionol	22029-76-1	885	812 , 1.090	sweet herbal floral violet tropical balsam woody
farnesol	4602-84-0	904	856 , 0.955	mild fresh sweet linden floral angelica

Flavor Additives

Each of the flavored tobacco samples was also analyzed with GCxGC-TOFMS and differences were apparent relative to the original tobacco sample. A TIC chromatogram for the menthol cigarette is shown in Figure 6.

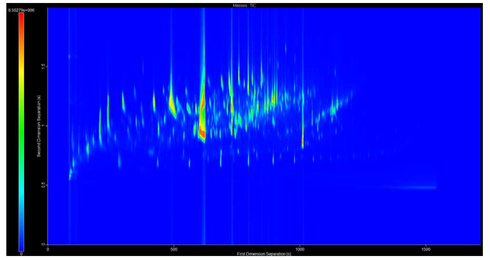


Figure 6. Representative TIC chromatogram menthol tobacco.

A collection of analytes that are characteristic of each flavored tobacco are listed in Tables 3-5. The similarity value and associated flavor properties are listed for each. These tables are representative and are not intended to be comprehensive.

Table 3. Analytes elevated in Menthol Cigarette

Name	CAS	Similarity	R.T. (s)	Odor Properties
benzenemethanol	100-51-6	904	504 , 1.100	floral rose phenolic balsamic
l-menthone	10458-14-7	896	584 , 0.995	minty
p-menthone	89-80-5	930	592 , 1.010	minty
d-menthol	15356-60-2	897	606 , 0.935	mint
methyl antranilate	134-20-3	900	722 , 1.305	fruity grape orangeflower neroli
vanillin	121-33-5	931	760 , 1.370	sweet vanilla creamy chocolate

Table 4. Analytes elevated in Raspberry Tobacco

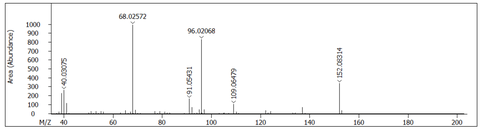
Name	CAS	Similarity	R.T. (s)	Odor Properties
2-methylpropyl ester acetic acid	110-19-0	959	242 , 0.845	sweet fruity ethereal banana tropical
ethyl butyrate	105-54-4	944	266 , 0.865	fruity juicy fruit pineapple cognac
acetic acid, phenylmethyl ester	140-11-4	863	586 , 1.130	sweet floral fruity jasmin fresh
methyl antranilate	134-20-3	935	718 , 1.325	fruity grape orangeflower neroli
raspberry ketone methyl ester	104-20-1	911	820 , 1.270	sweet dried raspberry rose cherry fruity cassie absolute
raspberry ketone	5471-51-2	939	850 , 1.420	sweet berry jam raspberry ripe floral
2(3H)-furanone, 5-heptyldihydro-	104-67-6	871	864 , 1.185	fruity peach creamy fatty lactonic apricot ketonic coconut

Table 5. Analytes elevated in Vanilla Spice Tobacco

Name	CAS	Similarity	R.T. (s)	Odor Properties
benzene, 1-methoxy-4-(1-propenyl)-	104-46-1	967	680 , 1.115	sweet anise licorice
piperonal	120-57-0	916	714 , 1.380	heliotrope flower sweet powdery coconut vanilla
methyl anthranilate	134-20-3	959	720 , 1.300	fruity grape orangeflower neroli
vanillin	121-33-5	863	756 , 1.440	sweet vanilla creamy chocolate
ethyl vanillin	121-32-4	925	794 , 1.420	sweet creamy vanilla caramel
2(3H)-furanone, 5-heptyldihydro-	104-67-6	957	864 , 1.170	fruity peach creamy fatty lactonic apricot ketonic coconut

High Resolution MS Data

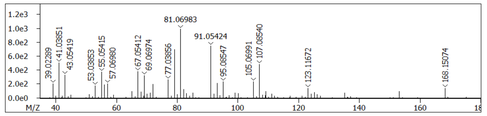
HR-TOFMS data that provide accurate masses for molecular and fragment ions which can be used for formula calculations were also collected. Ketoisophorone was tentatively identified through library searching of nominal mass data, shown in Figure 4, and the identification is supported with accurate mass data of the molecular ion and various fragment ions, as shown in Figure 7.



Formula	Observed Ion m/z	Expected Ion m/z	Mass Accuracy (ppm)
$C_6H_{12}O_2$	152.0831	152.0832	-0.29
$C_6H_4O_2$	96.0207	96.0206	1.03
C_4H_4O	68.0257	68.0257	0.86
C_7H_8O	109.0648	109.0648	-0.02

Figure 7. Accurate mass information supports the identification of ketoisophorone.

Accurate mass information is especially useful when library matches are not reliable, as shown in Figure 8. HR-TOFMS data are shown for an unknown analyte. The top nominal mass library matches had the formula $C_{10}H_{16}O_2$. With accurate mass information, the formula was determined to be $C_{11}H_{20}O$. $C_{10}H_{16}O_2$ and $C_{11}H_{20}O$. Both have a nominal mass of 168, but accurate masses of 168.1145 and 168.1509, respectively. Calculations of fragment formulas can suggest structural information.



Formula	Observed Ion m/z	Expected Ion m/z	Mass Accuracy (ppm)	Potential Loss
$C_{11}H_{20}O$	168.1507	168.1509	-0.78	--
$C_{10}H_{17}O$	153.1274	153.1274	0.29	CH_3
$C_{10}H_{15}$	135.1169	135.1168	0.39	CH_3+H_2O
C_9H_{15}	123.1167	123.1168	-0.83	C_2H_4OH
C_8H_{11}	107.0854	107.0855	-1.15	$C_2H_5OH+CH_3$
C_7H_7	91.0542	91.0542	0.11	
C_6H_6	81.0698	81.0699	-0.53	

Figure 8. Accurate mass information assists in identifying unknown analytes.

Conclusions

The experiments described in this poster demonstrate a food, flavor, and fragrance analysis for the characterization of the aroma and flavor analytes in tobacco. Natural flavors and additives were readily determined. HS-SPME sampling pre-concentrated the volatile and semi-volatile compounds and a GCxGC-TOFMS method separated and detected components in tobacco, including known additives and naturally occurring flavor analytes. The collection of full mass range TOFMS data allowed for measuring both targeted and non-targeted analytes. Identification was determined by matching the acquired full range mass spectral data to libraries of known spectra. Additional collection of HR-TOFMS data allowed for the confirmation of analyte identity and for determining information about analytes that were previously unknown. These techniques provide a reliable method to locate, identify, and quantify naturally occurring and added flavors in tobacco.