Analysis of Multiple Classes of Cigarette Smoke Constituents by GCxGC-TOFMS

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1. Introduction

The pyrolysis of tobacco produces harmful vapors that the National Toxicology Program estimates contain over 250 compounds that are known to be toxic or carcinogenic, thus it has become very important to analyze and characterize the compounds in tobacco smoke. Furthermore, the Family Smoking Prevention and Tobacco Control Act (H.R. 1256) was recently passed to authorize the Food and Drug Administration (FDA) to regulate cigarettes, cigarette tobacco, and smokeless tobacco products. Among the areas of regulation is the monitoring of tobacco smoke constituents. Section 904(e) requires the FDA to establish "a list of harmful and potentially harmful constituents, including smoke constituents, to health in each tobacco product by brand and by quantity in each brand and subbrand" and the tobacco companies to report these constituents in their products.

As tobacco smoke is a complex mixture of chemical compounds, this is a challenge. The compounds of interest such as benzene, nicotine, phenols, polyaromatic hydrocarbons (PAHs), inorganic compounds, and tobacco specific nitrosamines (TSNAs), span a range of compound classes. The complexity of the smoke matrix has traditionally required multiple chromatography methods along with considerable sample clean-up to target each class of compounds individually.

Comprehensive two-dimensional gas chromatography (GCxGC) can be beneficial in the analysis of complex samples, such as smoke extracts, due to both an improved peak capacity offered by two dimensions of complementary separation and to a cryogenic focusing effect of thermal modulation. These features allow for the isolation of individual analyte components within a complex sample matrix and for low-level detection, respectively. Coupling GCxGC to Time-of-Flight Mass Spectrometry (TOFMS) provides identification and quantification information with the full mass range data acquisition.

This application note shows the development of a GCxGC-TOFMS method for the analysis of cigarette smoke extracts. This approach can comprehensively analyze tobacco smoke extracts across several compound classes while minimizing sample clean-up and the need for multiple methods of analysis.

2. Experimental Conditions

Samples

Smoke extracts were purchased from Arista Laboratories (Richmond, VA, USA). Smoke from five Kentucky 3R4F reference cigarettes was collected with an automated smoking machine (SM 450 Cerulean), per ISO smoking conditions. Cambridge filter pads were connected to a glass impinger filled with 20 ml of methanol and immersed in a dry ice/isopropyl alcohol bath. The cigarettes were smoked in environmental conditions of 22.9°C and 60% humidity. The total puff count was 43.4 and the puff volume was 35 ml. Upon completion, smoke constituents collected on the filter pad were extracted into 20 ml of methanol with 30 minutes on a bench-top shaker.

Representative standards were prepared at concentrations ranging from 1 ppb to 50 ppm in methanol. TSNAs were purchased from Sigma Aldrich (St. Louis, MO, USA) and other representative compound class standards—including benzene, PAHs, phenols, nitrosamines, etc.—were purchased from Restek (Bellefonte, PA, USA). An internal standard, 1-pentanol, was added at 5 ppm (v/v) to each sample.

Instrumental Conditions

Analyses were performed on an Agilent 7890 GC equipped with a GERSTEL MPS2 Auto Sampler and LECO's thermal modulator, secondary oven, and Pegasus® 4D TOFMS.

Injection:

1.5 µL splitless with inlet @ 250°C

Gas:

He @ 1.0 ml/min, corrected constant flow

Columns:

Rtx-5 Sil MS, 30 m x 0.25 mm x 0.25 µm for primary and Rtx-200, 1.5 m x 0.18 mm x 0.2 µm for secondary (Restek, Bellefonte, PA)

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Temperature Program:
Primary oven held 3 min at 45°C, ramped 8°C/min to 300°C and held 10 min; Secondary oven set +10°C relative to primary
Modulator Offset: +15°C (relative to 2nd oven)
Modulation Period: 3 s
Transfer Line Temp: 280°C
Saved Mass Range: 33-400 m/z
Acquisition Rate: 200 spectra/s
Source Temperature: 250°C

3. Results

GCxGC-TOFMS provided a comprehensive analysis of analytes extracted from tobacco smoke across several compound classes. A representative TIC contour plot of the tobacco smoke extract is shown in Figure 1. The x- and y-axes display the first and second dimension separations, respectively, with analyte peaks appearing in the two-dimensional separation space as color spots (intensity proportional to color scale.) The two separations occur simultaneously as effluent from the first column is collected and injected to the second column every 3 s, at the set modulation period. The complexity of this sample is evident with thousands of analytes appearing in the chromatogram. The benefit of GCxGC (relative to GC) can be noted anywhere analytes are vertically aligned as these analytes would co-elute in a comparable 1D separation. With this column arrangement, analytes are primarily separated by boiling point in the first dimension and analyte polarity in the second dimension. Hence, compounds with similar boiling points but different polarities that would be overlapped with GC can often be resolved in GCxGC.

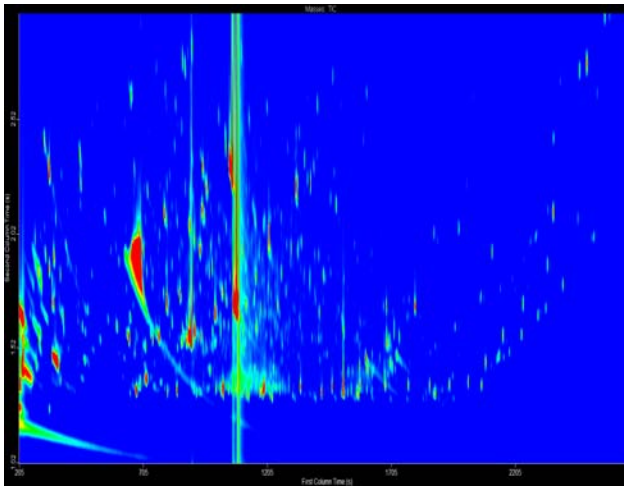

Figure 1. GCxGC TIC contour plot showing effective separation of a complex tobacco smoke extract.

An example of the increased peak capacity is highlighted with a small region of the chromatogram, shown in Figure 2. When the chromatogram is displayed to show the corresponding first dimension separation, it appears as if there are only three analytes present. However, when the chromatogram is tilted to display the additional second dimension separation, three additional analytes can be observed that coeluted in the first dimension.

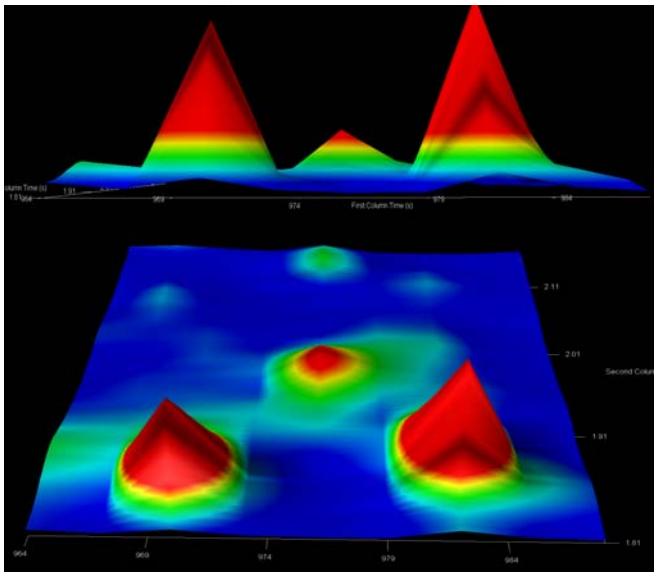

Figure 2. Increased peak capacity in the second dimension can separate analytes that would co-elute in a 1D separation.

The excellent peak capacity of the chromatography is augmented with powerful mass spectral detection. The TOFMS acquired data across a full mass range (33-400 m/z) at a rate of 200 spectra/s and required neither specification of target analytes nor speed to be sacrificed. ChromaTOF® software was used to rapidly and reliably process this data. A Deconvolution algorithm isolated each analyte peak from noise and overlapping interferences for both identification and quantification. The Automated Peak Find algorithm located analytes, identified based on mass spectral matching and quantified by peak area and/or height. This information was compiled in Peak Tables for user review and peak markers were added to the chromatogram to indicate retention times. Based on identification information, analytes were manually assigned to chemical compound classes using the Classifications feature in the ChromaTOF software. The color of the peak marker in Figure 3 corresponds to approximate class assignments. A range of target compounds were identified including alkanes, alkenes, aldehydes, ketones, benzene, substituted benzenes, phenols, PAHs, pyridines, pyrazines, nicotine, furans, etc.

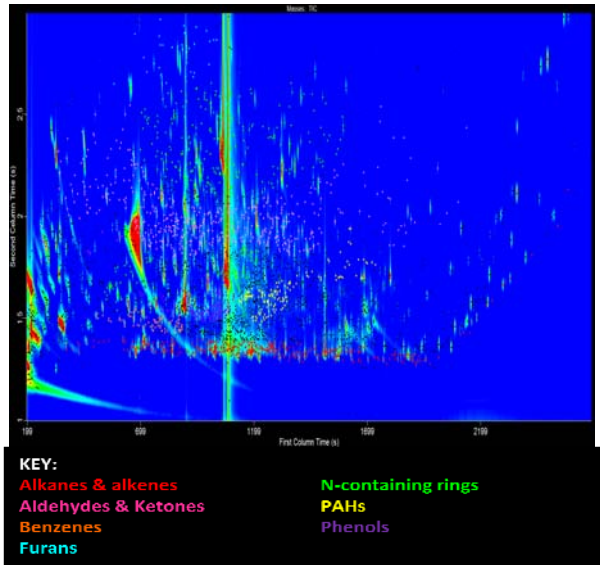

Figure 3. Analytes can be grouped based on their compound class, using the ChromaTOF Classification Feature.

In addition to peak identification and classification, ChromaTOF software also provided quantification and calibration information for this data. Reference compounds, listed in Table 1, that are representative of the target analytes were analyzed as standards, and calibration data were compiled using the Calibration feature in the ChromaTOF software. A range of compound classes were represented, including the TSNAs. The TSNA standards were analyzed at concentrations ranging from 10 ppb to 50 ppm. For each NNN, NAT, NAB, and NNK, an R^2 value greater than 0.999 was determined, as shown in Figure 4 and Table 1.

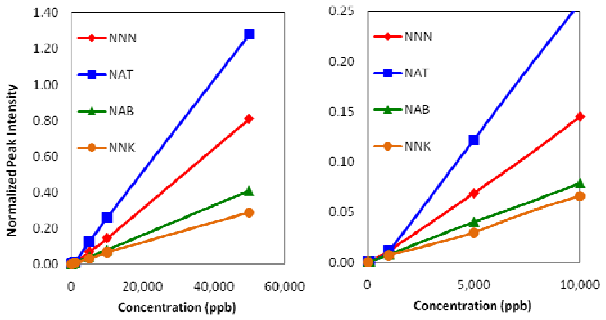

Figure 4. The TSNAs calibration range is shown from 10 ppb to 50 ppm with R^2 values greater than 0.999.

Similar calibration equations and R^2 values were determined for other representative standards from the various target compound classes. These were also quantified and calibration information was compiled in Table 1. These compounds are intended to be representative and additional standards could readily be added to the calibration.

Table 1. Calibration Data for Representative Analytes

	tR 1 (s)	Quant Mass	standards in calibration	R^2
Nitrosamines (including TSNAs)				
N-Nitrosodimethylamine	337	74	100 ppb - 50 ppm	0.9997
1-Propanamine, N-nitroso-N-propyl-	766	70	10 ppb - 50 ppm	0.9996
N-Nitrosonornicotine (NNN)	1444	105	10 ppb - 50 ppm	0.9998
N-nitrosoanatabine (NAT)	1489	159	10 ppb - 50 ppm	0.9999
N-nitrosoanabasine (NAB)	1504	161	10 ppb - 50 ppm	1.0000
4-methyl nitrosoamino-1-(3-pyridinyl)-1-butanone (NNK)	1588	177	10 ppb - 50 ppm	0.9996
PAHs				
Naphthalene	910	128	10 ppb - 50 ppm	0.9928
Acenaphthene	1219	154	10 ppb - 50 ppm	0.9992
Phenanthrene	1483	178	10 ppb - 50 ppm	0.9576
Carbazole	1522	167	100 ppb - 50 ppm	0.9975
Fluoranthene	1693	202	10 ppb - 50 ppm	0.9982
Pyrene	1732	202	10 ppb - 50 ppm	0.9967
Benz[a]anthracene	1948	228	100 ppb - 50 ppm	0.9922
Benzo[k]fluoranthene	2125	252	100 ppb - 50 ppm	0.9919
Aromatics (benzene, phenols, aromatic amines)				
Benzene	232	78	1 ppb - 50 ppm	0.9994
Styrene	532	104	1 ppm - 50 ppm	0.9855
Phenol	646	94	1 ppb - 50 ppm	0.9992
Aniline	646	93	100 ppb - 50 ppm	0.9966
Phenol, 4-methyl-	766	107	100 ppb - 50 ppm	0.9990
1,2-Benzenedicarboxylic acid, butyl octyl ester	2083	149	10 ppm - 50 ppm	0.9996
N-containing rings				
Pyridine	346	52	1 ppm - 50 ppm	0.9997
Azobenzene	1345	77	10 ppb - 10 ppm	0.9953

The calibrations were applied to the smoke extract data to determine the concentration of each analyte in the sample analyzed and the mass of each analyte extracted from the Cambridge filter pad. The results are summarized below in Table 2.

As shown in Table 2, not all of the TSNAs were detected in the smoke extract samples, shown in Figure 1 and 3. These analytes are well resolved and can be detected with this methodology as shown in the chromatographic separation of the TSNA standards in Figure 5. These analytes could be quantified with this method in other unknown samples, when present at high enough levels.

Delivering the Right Results

Table 2. Calculated values in smoke extract samples. Analytes labeled NF were "not found" while analytes with * were found at levels too low to reliably quantify.

	measured concentration (ppb)	µg on filter
Nitrosamines (including TSNAs)		
N-Nitrosodimethylamine	435	10
1-Propanamine, N-nitroso-N-propyl-	NF	0
N-Nitrosonornicotine (NNN)	NF	0
N-nitrosoanatabine (NAT)	NF	0
N-nitrosoanabasine (NAB)	NF	0
4-methyl nitrosoamino-1-(3-pyridinyl)-1-butanone (NNK)	*	*
PAHs		
Naphthalene	368	8
Acenaphthene	*	*
Phenanthrene	*	*
Carbazole	1629	36
Fluoranthene	1008	22
Pyrene	1262	28
Benz[a]anthracene	2746	61
Benzo[k]fluoranthene	2587	57
Aromatics (benzene, phenols, aromatic amines)		
Benzene	*	*
Styrene	370	8
Phenol	2758	61
Aniline	NF	0
Phenol, 4-methyl-	3067	68
1,2-Benzenedicarboxylic acid, butyl octyl ester	NF	0
N-containing rings		
Pyridine	1251	28
Azobenzene	NF	0

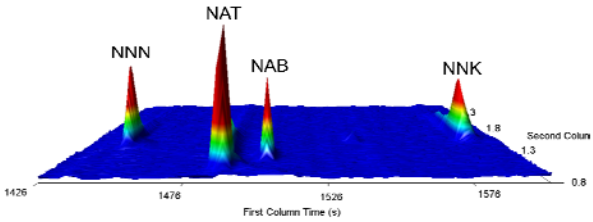

Figure 5. The TSNAs are separated and detected with this GCxGC-TOFMS method.

4. Conclusions

The experiments described in this application note demonstrate the use of the LECO Pegasus 4D GCxGC-TOFMS for the analysis of smoke extract samples. Individual smoke constituents were efficiently isolated from a complex tobacco smoke matrix. Sufficient peak capacity was provided to identify and quantify analytes with a single separation lasting less than 45 minutes. Representative analytes from many of the target compound classes were measured and identified with TOFMS detection. Full mass range acquisition allowed for positive confirmation of target compounds through mass spectral matching to data base standards. TOFMS detection was also used to quantitatively calibrate representative standard analytes from target compound classes, using the ChromaTOF software Calibration feature. Linear calibrations ranged from 1 ppb to 50 ppm, analyte dependent. This methodology reduces the need for time consuming sample clean up and/or repeat injections that individually target each compound class.

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