# Comprehensive Analysis and Characterization of Tobacco Smoke Extracts

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## Background

The pyrolysis of tobacco produces harmful vapors that contain hundreds of compounds that are known to be toxic or carcinogenic. The Family Smoking Prevention and Tobacco Control Act (H.R. 1256) was passed in 2009 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. The FDA has established a list of harmful and potentially harmful constituents (HPHCs), including compounds in smoke, that tobacco companies must measure and report in each brand and sub-brand. Thus, it has become very important to analyze and characterize the compounds in tobacco smoke. As tobacco smoke is a complex mixture of chemical compounds, this is an analytical challenge. The list of 93 HPHCs that will be required to be measured was released in March 2012 and includes compounds such as benzene, nicotine, phenols, polyaromatic hydrocarbons (PAHs), inorganic compounds, and tobacco-specific nitrosamines (TSNAs) that span a range of chemical compound classes. The complexity of the smoke matrix has traditionally been dealt with by employing multiple methods along with considerable sample clean-up to target various classes of compounds individually. This poster shows the development of a comprehensive two-dimensional gas chromatography with time of flight mass spectrometry (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.

## Methods

### *Samples and Standards*

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. Upon completion of smoking, the constituents collected on the filter pad were extracted into 20 ml of methanol with 30 minutes on a bench-top shaker. Representative standards, TSNAs (from Sigma Aldrich St. Louis, MO, USA) and other compound class standards, including benzene, PAHs, phenols, nitrosamines, etc. (from Restek Bellefonte, PA, USA), were prepared at concentrations ranging from 1 ppb to 50 ppm in methanol. An internal standard, 1-pentanol, was added at 5 ppm (v/v) to each sample.

### *Instrumental Conditions*

GCxGC analyses were performed with LECO's Pegasus® 4D, consisting of an Agilent 7890 GC equipped with a GERSTEL MPS2 Auto Sampler and LECO's dual stage quad jet thermal modulator, secondary oven, and Pegasus 4D TOFMS.

**Table 1. Instrument Method Parameters**

| GCxGC-TOFMS (Pegasus 4D) Conditions | |
|---|---|
| Injection | 1.5 µL splitless with inlet @ 250°C |
| Carrier Gas | He @ 1.0 ml/min, corrected for constant flow |
| Column One | Rxi-5Sil MS, 30 m x 0.25 mm x 0.25 µm (Restek, Bellefonte, PA) |
| Column Two | Rtx-200, 1.5 m x 0.18 mm x 0.20 µm (Restek, Bellefonte, PA) |
| Temperature Program | 3 min at 45°C, ramped 8°C/min to 300°C, held 10 min; Secondary oven maintained +10°C relative to primary |
| Modulation | 3 s with temperature maintained +15°C relative to 2nd oven |
| Transfer Line | Temperature set to 280°C |
| Mass Range | 33-400 m/z |
| Acquisition Rate | 200 spectra/s |
| Source Temp | 250°C |
| Data processing | ChromaTOF® |

## Comprehensive 2D GC-TOFMS

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. Analytes with similar properties may coelute with a single dimension separation, but GCxGC offers a complementary second dimension separation to help resolve coeluting analytes. Cryo-focusing enhances detection as effluent from the first column is refocused prior to injection on the second column. Coupling GCxGC to TOFMS provides identification and quantification information with the full mass range data acquisition.

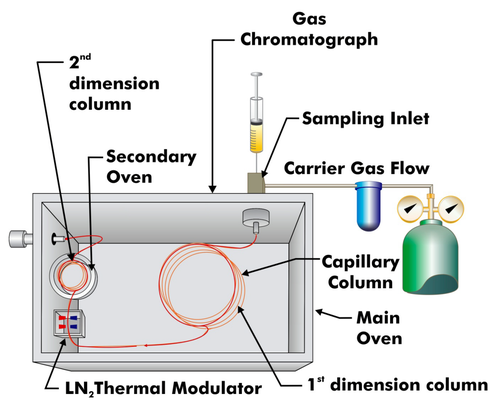



**Figure 1. Diagram showing the major GCxGC components**

Coeluting analytes in complex samples that appeared as one peak can often be resolved on the second dimension with GCxGC. With the column arrangement used here, analytes are primarily separated by boiling point in the first dimension and polarity in the second. An example of the increased peak capacity is shown in Figure 2. When the small region of 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. In the contour plot view, the x- and y-axes display the first and second dimension separations, respectively, with analyte peaks appearing in the 2D separation space as color spots.

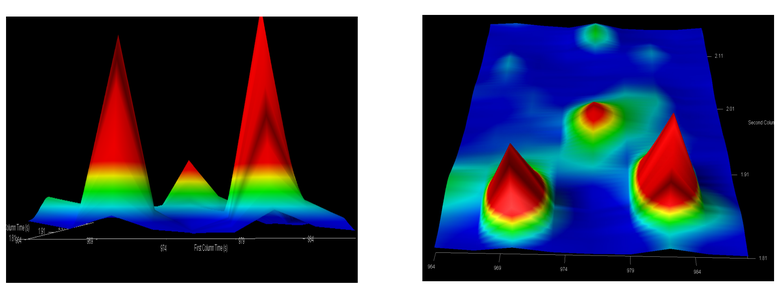


**Figure 2. Increased peak capacity can separate analytes that would coelute in a 1D separation.**

## Results

GCxGC-TOFMS provided a comprehensive analysis of analytes across several compound classes in tobacco smoke. A representative TIC contour plot is shown in Figure 3. The benefit of GCxGC (relative to GC) can be noted for this complex sample anywhere analytes are vertically aligned, as these would coelute in a comparable 1D separation.

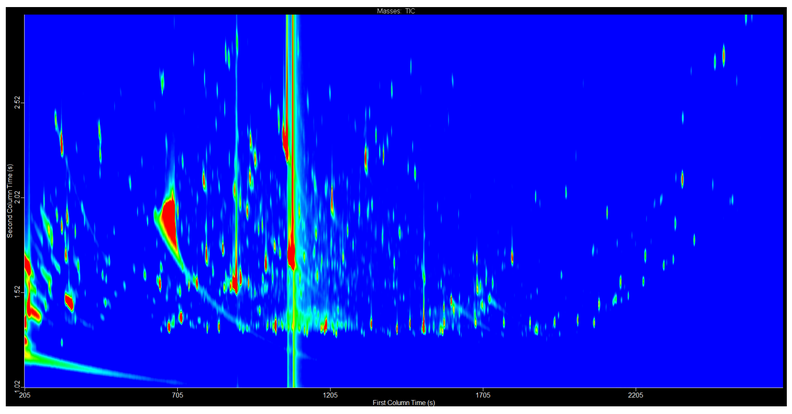
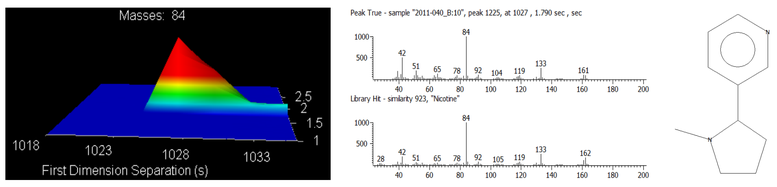



**Figure 3. GCxGC TIC plot showing effective separation of the tobacco smoke extract.**

The excellent peak capacity is augmented with powerful MS detection. The TOFMS acquired full mass range (33-400 m/z) data at 200 spectra/s and required neither specification of target analytes nor sacrifices to acquisition speed. ChromaTOF software automatically found and deconvoluted analyte peaks from noise and overlapping interferences and compiled identification and quantification information into Peak Tables, as shown in Figure 4.




| Peak # | Name | Formula | CAS | Similarity | R.T. (s) | Quant Masses | Quant S/N | Area |
|---|---|---|---|---|---|---|---|---|
| 1225 | Nicotine | C10H14N2 | 54-11-5 | 923 | 1027 , 1.790 | 84 | 65893 | 9255278 |

**Figure 4. Quantification (by unique mass) and identification (by library searching) information for each analyte is compiled into a Peak Table.**

Peak markers on the chromatogram indicate analyte retention times. Based on identification information, analytes were assigned to chemical compound classes using ChromaTOF's Classifications feature. The color of the peak marker in Figure 5 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.

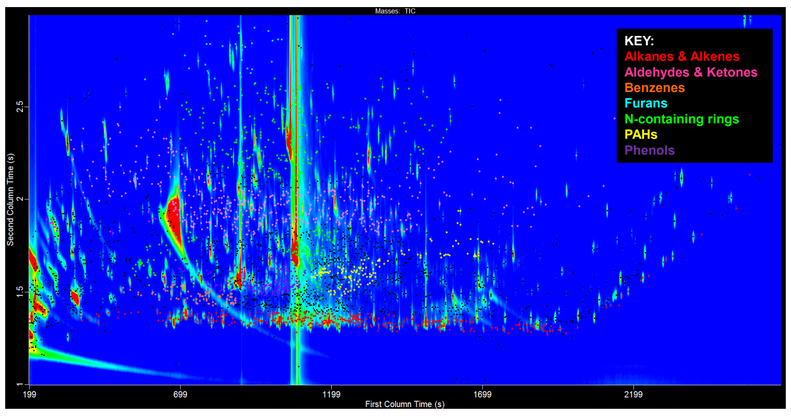



**Figure 5. Analytes can be grouped based on their compound class with ChromaTOF's Classification feature.**

## Calibration Data

ChromaTOF's Calibration feature was used to generate quantification and calibration information for these data. Reference compounds that are representative of the target analytes were analyzed as standards at concentrations from 10 ppb to 50 ppm and calibration data were compiled. The TSNAs were among the compound classes targeted and are provided as an example in Figure 6. For each standard, a Pearson's r value greater than 0.999 was determined, as shown in Figure 6 and Table 2. Similar calibration equations and r values were determined for other representative standards from the various target compound classes, with some examples listed in Table 2.

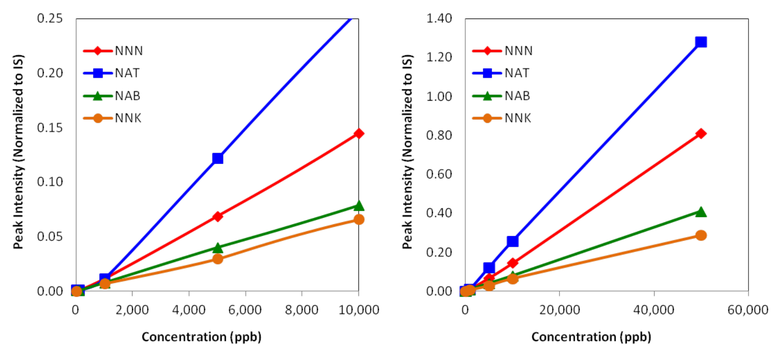



**Figure 6. The TSNAs calibration range is shown from 10 ppb to 50 ppm with Pearson r values greater than 0.999.**

The calibrations were then applied to the smoke extract data to determine the concentration of each analyte in the extract and the mass of each analyte extracted from the filter pad. The results are summarized in Table 2. The TSNAs are well resolved and can be detected with this methodology, as shown in the chromatographic separation in Figure 7, though not all were detected in the smoke samples, shown in Figures 3 and 5. When present at high enough levels, these analytes could be quantified with this method in other unknown samples.

## Calibration Data

**Table 2. Calibration Data. These compounds are intended to be representative and additional standards could readily be analyzed and added to the table.**

| | tR 1 (s) | Quant Mass | Standards in Calibration | Pearson r | conc (ppb) | ug on filter |
|---|---|---|---|---|---|---|
| **Nitrosamines (including TSNAs)** | | | | | | |
| N-Nitrosodimethylamine | 337 | 74 | 100 ppb - 50 ppm | 0.9997 | 435 | 10 |
| 1-Propanamine, N-nitroso-N-propyl- | 766 | 70 | 10 ppb - 50 ppm | 0.9996 | NF | NF |
| N-Nitrosonornicotine (NNN) | 1444 | 105 | 10 ppb - 50 ppm | 0.9998 | NF | NF |
| N-nitrosoanatabine (NAT) | 1489 | 159 | 10 ppb - 50 ppm | 0.9999 | NF | NF |
| N-nitrosoanabasine (NAB) | 1504 | 161 | 10 ppb - 50 ppm | 1.0000 | NF | NF |
| 4-methyl nitrosoamino-1-(3-pyridinyl)-1-butanone (NNK) | 1588 | 177 | 10 ppb - 50 ppm | 0.9996 | NQ | NQ |
| **PAHs** | | | | | | |
| Naphthalene | 910 | 128 | 10 ppb - 50 ppm | 0.9928 | 368 | 8 |
| Acenaphthene | 1219 | 154 | 10 ppb - 50 ppm | 0.9992 | NQ | NQ |
| Phenanthrene | 1483 | 178 | 10 ppb - 50 ppm | 0.9576 | NQ | NQ |
| Carbazole | 1522 | 167 | 100 ppb - 50 ppm | 0.9975 | 1629 | 36 |
| Fluoranthene | 1693 | 202 | 10 ppb - 50 ppm | 0.9982 | 1008 | 22 |
| Pyrene | 1732 | 202 | 10 ppb - 50 ppm | 0.9967 | 1262 | 28 |
| Benz[a]anthracene | 1948 | 228 | 100 ppb - 50 ppm | 0.9922 | 2746 | 61 |
| Benzo[k]fluoranthene | 2125 | 252 | 100 ppb - 50 ppm | 0.9919 | 2587 | 57 |
| **Aromatics (benzene, phenols, aromatic amines)** | | | | | | |
| Benzene | 232 | 78 | 1 ppb - 50 ppm | 0.9994 | NQ | NQ |
| Styrene | 532 | 104 | 1 ppm - 50 ppm | 0.9855 | 370 | 8 |
| Phenol | 646 | 94 | 1 ppb - 50 ppm | 0.9992 | 2758 | 61 |
| Aniline | 646 | 93 | 100 ppb - 50 ppm | 0.9966 | NF | NF |
| Phenol, 4-methyl- | 766 | 107 | 100 ppb - 50 ppm | 0.9990 | 3067 | 68 |
| 1,2-Benzenedicarboxylic acid, butyl octyl ester | 2083 | 149 | 10 ppm - 50 ppm | 0.9996 | NF | NF |
| **N-containing rings** | | | | | | |
| Pyridine | 346 | 52 | 1 ppm - 50 ppm | 0.9997 | 1251 | 28 |
| Azobenzene | 1345 | 77 | 10 ppb - 10 ppm | 0.9953 | NF | NF |

NQ = not quantified (present at levels too low for reliable quantification)
NF = not found

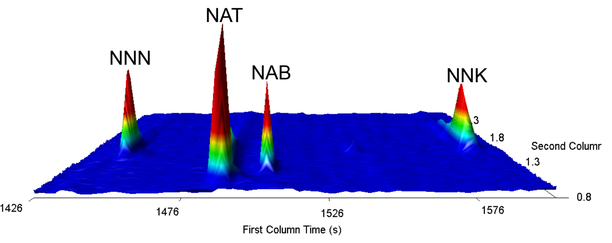



**Figure 7. The TSNA standards can be separated and detected with this method.**

The FDA released a "Guidance for Industry" draft document in which reporting guidelines are discussed to help prepare for regulations going into effect. An abbreviated list has been proposed to be the initial reporting requirement while methods are developed for all 93 HPHCs and laboratories are set-up to perform these methods. The draft abbreviated list for tobacco smoke is provided in Table 3. The presence or absence of the HPHCs in these and related samples detected with this method, based on mass spectral matching, is also indicated in Table 3. For further confirmation, standards can be run to verify both match and retention time.

**Table 3. Preliminary list of HPHCs to be monitored in tobacco smoke. The toxicology data is reported from the FDA and observations for these and related samples are reported.**

| Analyte | Toxicology | CAS | Molecular Weight | Observations for these data |
|---|---|---|---|---|
| Acetaldehyde | CA, RT, AD | 75-07-0 | 44 | Measured with similarity = 953 |
| Acrolein | RT, CT | 107-02-8 | 56 | Measured with similarity = 885 |
| Acrylonitrile | CA, RT | 107-13-1 | 53 | Measured with similarity = 640 |
| 4-Aminobiphenyl | CA | 92-67-1 | 169 | Measured with similarity = 633 |
| 1-Aminonaphthalene | CA | 134-32-7 | 143 | Not found in these data |
| 2-Aminonaphthalene | CA | 91-59-8 | 143 | Measured with similarity = 701 |
| Ammonia | RT | 7664-41-7 | 17 | All m/z below the range collected (33-400) |
| Benzene | CA, CT, RDT | 71-43-2 | 78 | Measured with similarity = 967** |
| Benzo[a]pyrene | CA | 50-32-8 | 252 | Not found in these samples** |
| 1,3-Butadiene | CA, RT, RDT | 106-99-0 | 54 | Not found in these samples |
| Carbon monoxide | RDT | 630-08-0 | 28 | All m/z below the range collected (33-400) |
| Crotonaldehyde | CA | 15798-64-8 | 70 | Measured with similarity = 887 |
| Formaldehyde | CA, RT | 50-00-0 | 30 | All m/z below the range collected (33-400) |
| Isoprene | CA | 1574-41-0 | 68 | Measured with similarity = 903 |
| Nicotine | RDT, AD | 54-11-5 | 162 | Measured with similarity = 936 |
| NNK | CA | 64091-91-4 | 207 | Found at levels too low to accurately report** |
| NNN | CA | 16543-55-8 | 177 | Not found in these samples** |
| Toluene | RT, RDT | 108-88-3 | 92 | Measured with similarity = 947** |

Toxicology Key: AD = addictive; CA = carcinogen; CT = cardiovascular toxicant; RDT = reproductive or developmental toxicant; RT = respiratory toxicant;
** : these analytes were run as standards, thus further confirmed with retention time

## Conclusions

The experiments described in this poster 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 database 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.