

Use of two GC-MS scan techniques for the characterization of wrappers and binders taken from cigar products

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Outline for the presentation

- Background for the presentation
- Objectives for the presentation
- Materials and methods used for the analyses
- Results of the analyses
- Conclusions

Background for the presentation

- Trends in tobacco products driven by regulations
 - Increased regulation/taxation driving cigarette sales down
 - Flavored cigarettes have come under special attack
 - Alleged appeal to experimentation by youth
 - Alleged resistance to cessation
 - Resulted in increased sales of other tobacco products
 - Flavored moist snuffs
 - Flavored cigars
 - Flavored little cigars, cigarillos, filtered cigars
 - Cigar sales had already been on the increase, including younger adult users, much to the chagrin of tobacco regulators
 - However, what constitutes a cigar can often be a topic for debate - when is it a cigar and when is it a cigarette wrapped in reconstituted tobacco paper instead of cigarette paper?

Objectives for the presentation

- Give examples of cigar and cigar-like products
- Review prior work on cigar filler characterization
- Show our recent work on cigar filler characterization
- Provide the results of our latest work on the characterization of cigar wrappers and binders
- Attract funding for analyses of additional types of cigars, cigar-like products, and novel tobacco products

Examples of cigars, cigarillos and related products



Filtered little cigars and packaging



Photo adapted from UMDNJ-School of Public Health, 2006. Tobacco Surveillance Data Brief: Cigars and Smokeless Tobacco, Volume 1, Issue 4.

Classification of cigar fillers has been a continuing problem

- References to prior work on classification of cigar fillers
 - Clarke, *et al.*, 2006. Determination of carbohydrates in tobacco products by LC-mass spectrometry/mass spectrometry: a comparison with ion chromatography and application to product discrimination. *J. Agric. Food Chem.* 54, 1975-1981.
 - Ng, L.K., *et al.*, 2001. Characterization of cigar tobaccos by gas chromatographic/mass spectrometric analysis of nonvolatile organic acids: application to the authentication of Cuban cigars. *J. Agric. Food Chem.* 49, 1132-1138.
 - Zook, C.M., *et al.*, 1996. Characterization of tobacco products by high-performance anion exchange chromatography-pulsed amperometric detection. *J. Agric. Food Chem.* 44, 1773-1779.
 - ATF procedures 76-2 and 73-5 (<http://www.ttb.gov/procedures/index.shtml>)

Why just determining sugars does not work?

- Determination of sugars was thought to be a solution to the problem
 - ❑ Cigar fillers generally contain fermented, air-cured tobaccos that have very low sugar levels
 - ❑ Cigarette fillers generally contain flue-cured tobaccos and, in the case of US-blends, also contain sugar-cased burley tobaccos
- Would have worked except that with tobacco, there are always products that do not follow the rules
 - ❑ Cigars made with pipe tobacco fillers
 - ❑ Cigars made with cased air-cured tobaccos in their fillers
- DS and HFP scans should be able to distinguish those cigars from cigars made with cigarette tobacco fillers and cigars made with different types of wrappers, binders

Cigar wrappers and binders

- Natural leaf for both wrapper and binder
- Natural leaf wrapper with reconstituted tobacco binder
- Reconstituted tobaccos for both wrapper and binder
 - Band-cast
 - Paper-type
 - Spiral-wound versus rolled (as in cigarettes)
 - Minimum tobacco content and strength
- Reconstituted tobacco wrapper only
 - Generally only used for filters little cigars
 - Strength is important when used on same type of equipment used to make cigarettes
 - Minimum tobacco content important

Materials and methods - 1

■ Samples

- ❑ KY3R4F references cigarettes were obtained from the University of Kentucky, College of Agriculture, Reference Cigarette Program
- ❑ Commercial cigar samples purchased at retail in Macon, GA
 - Specialty tobacco stores
 - Convenience stores
 - Pharmacies
 - Big-box retailers
- ❑ Not a representative sampling for the KY3R4F or commercial cigar products
- ❑ Many of the products not readily available contrary to allegations of anti-tobacco lobbyists

Materials and methods - 2

- Key references to prior work for methodology
 - **DS Scan**
 - Moldoveanu *et al.*, "Comparative study of several methods for direct derivatization of tobacco," 46th Tobacco Chemists' Research Conference, 1992, Paper #28.
 - Alford, "Gas chromatography - mass spectrometry studies on popular old and new flue-cured varieties," 41st Tobacco Chemists' Research Conference, 1987, Paper #56.
 - **HFP Scan**
 - Dong *et al.*, "A parallel study between leaf chemistry and particulate-phase smoke chemistry," 47th Tobacco Chemists' Research Conference, 1993, Paper #16.

Materials and methods - 3

- Sample handling and initial preparation
 - Wrappers or wrapper/binder laminates were cut from the mouth end and slit
 - Tobacco filler was removed from wrapper or wrapper/binder
 - Wrapper was separated from the binder
 - Tobaccos ground or broken into small pieces prior to analysis

Materials and methods - 4

■ Preparation of analytical samples

□ DS Scan

- The tobacco sample (100 mg) is weighed into a GC autosampler vial
- BSTFA (800 μL) and DMF (400 μL) are added to the vial
- Phenanthrene- d_{10} (10 $\mu\text{g}/1200 \mu\text{L}$) is used as internal standard
- The vial is sealed and heated for 30 min at 76°C; after heating the supernatant liquid above the tobaccos, is ready for analysis
- If an autosampler is used, enough replicate samples can be prepared at one time for GC-MS system to run overnight

□ HFP Scan

- The tobacco sample (250 mg) is weighed into a GC autosampler vial
- Hexafluoroisopropanol (HFP, 1000 μL) is added to vial
- Phenanthrene- d_{10} (24 $\mu\text{g}/1000 \mu\text{L}$) is used as internal standard
- The vial is sealed and heated for 30 min at 76°C; after heating the supernatant liquid above the tobaccos is ready for analysis

Materials and methods - 5

■ GC-MS Analyses

□ Common to both techniques

- GC-MS: Agilent 6890 GC coupled with Agilent 5972 MS
- Column: J&W DB-5ms, 25 m x 0.25 mm ID x 0.25 μm film thickness
- Column: Agilent DB-5ms, 25 m x 0.25 mm ID x 0.50 μm film thickness
- Injection port liner: Sigma-Aldrich, 2055101, Jennings cup with 10% OV-1 on Chromosorb-W HP
- MS scan: 40 – 700 amu, EI+; Solvent delay: 8 min
- Injection port temperature: 300°C; transfer line temperature: 320°C
- Injection volume: 2 μL

□ DS Scan - GC oven temperature program

- Initial temperature: 50°C; initial time: 2 min; ramp rate: 2°C/min
- Final temperature: 300°C; hold time: 25 min; total run time: 150 min

□ HFP Scan - GC oven temperature program

- Initial temperature: 40°C; initial time: 0 min; ramp rate: 2°C/min
- Final temperature: 300°C; hold time: 22 min; total run time: 150 min

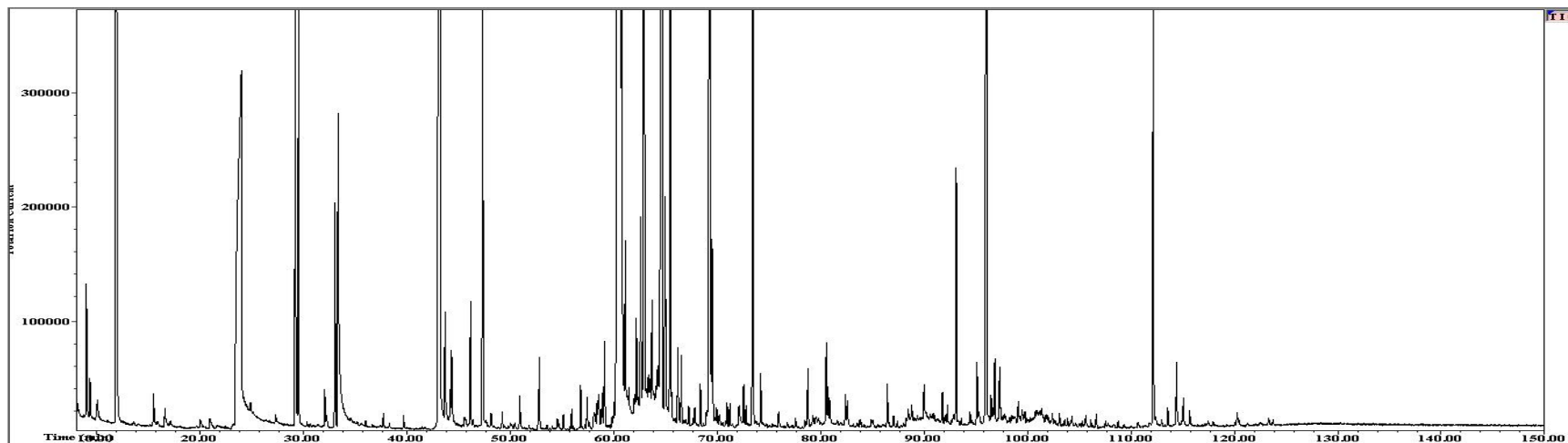
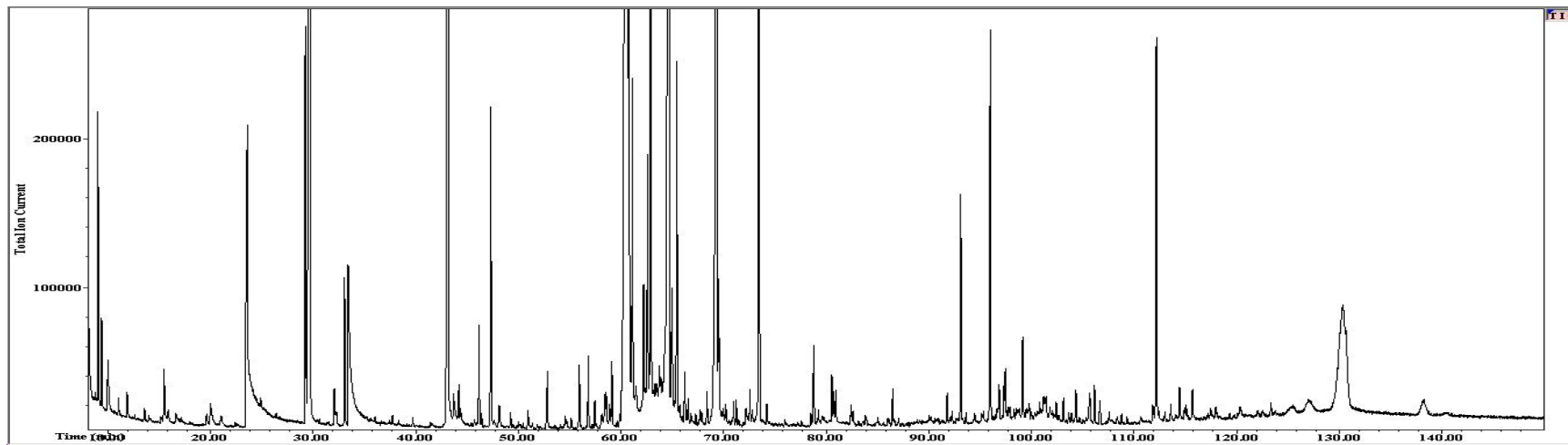
Materials and methods - 6

- GC-MS Data reduction
 - GC-MS data files were processed with WSearchPro (URL <http://www.wsearch.com.au>)
 - GC-MS Total ion chromatograms (TICs)
 - TICs were generated with the WSearchPro software
 - Typical TIC plots including all data points
 - Only peaks with retention times between 10 and 135 minutes used
 - Converted to jpg format for presentation
 - MS Spectra
 - Spectra were obtained the WSearchPro software
 - Interpretations were done manually
 - Comparison with spectra on MassBank (<http://www.massbank.jp/index.html?lang=en>)
 - Comparison with spectra and retention times in reports by Moldoveanu *et al.* in UCSF Legacy Tobacco Documents Library (<http://legacy.library.ucsf.edu/>)

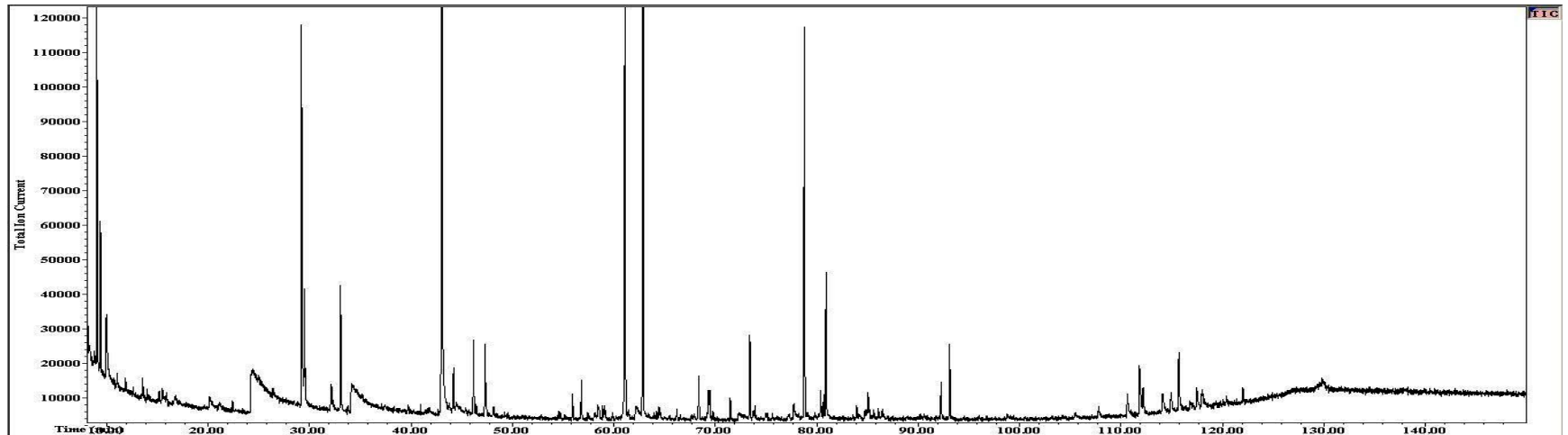
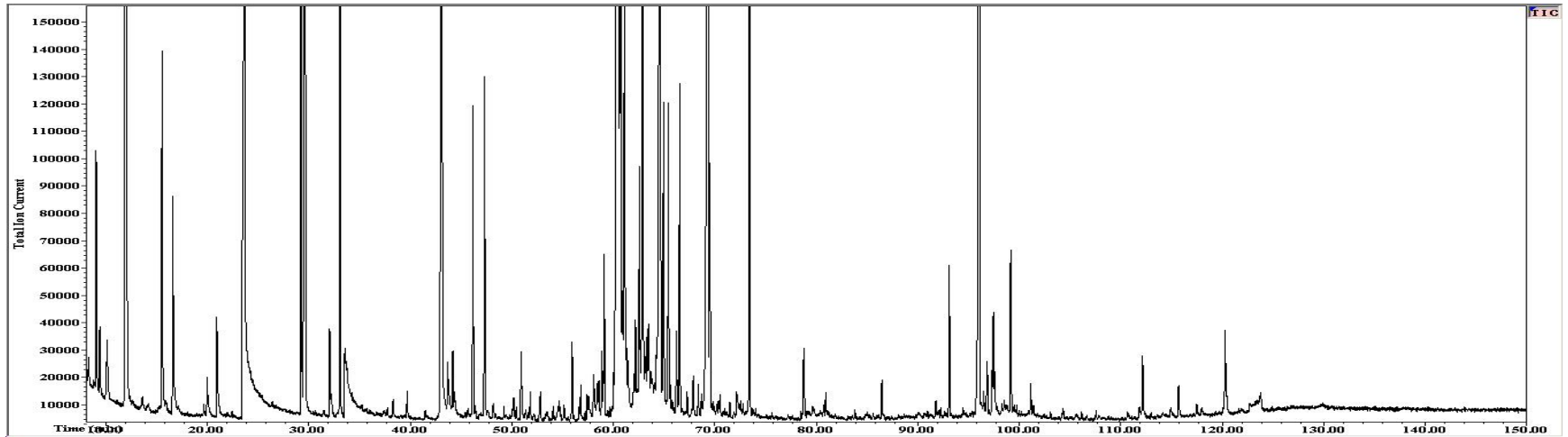
Samples used in this study

Sample	Description	Wrapper	Binder	Filler Appearance
1	KY3R4F KS reference cigarette	paper	none	cigarette
2	Flavored 95-mm filter cigarillo	reconstituted	none	cigarette
3	Tipped pipe-tobacco cigarillo	reconstituted	reconstituted	cigar
4	Cigarillo (no tip, no filter)	natural leaf	reconstituted	cigar
5	Flavored cigar (no tip, no filter)	reconstituted	reconstituted	cigar
6	Flavored cigarillo (no tip, no filter)	reconstituted	reconstituted	cigar

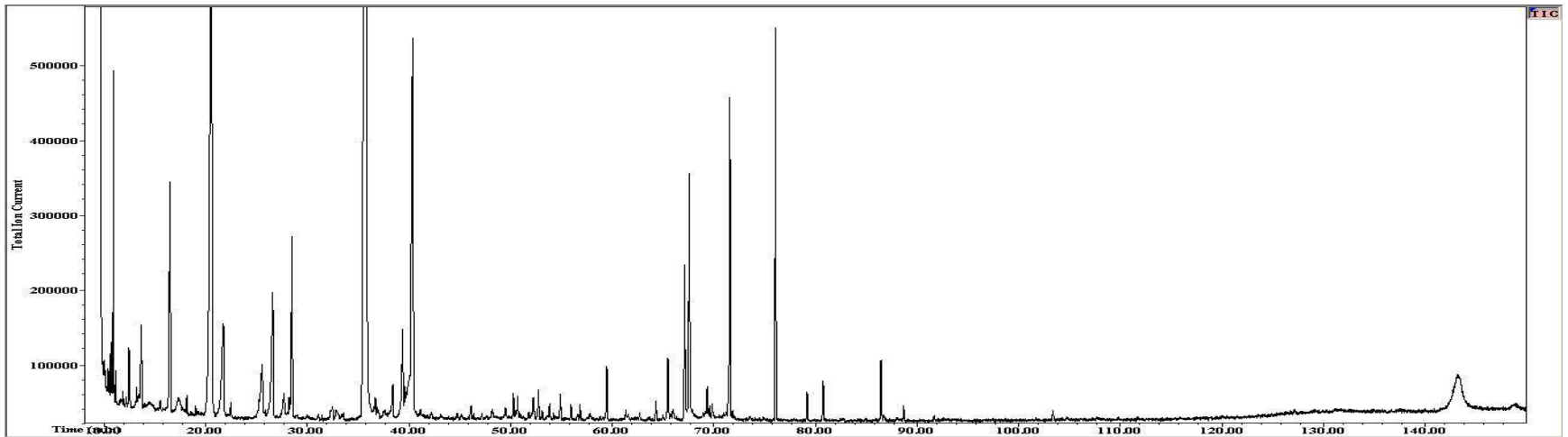
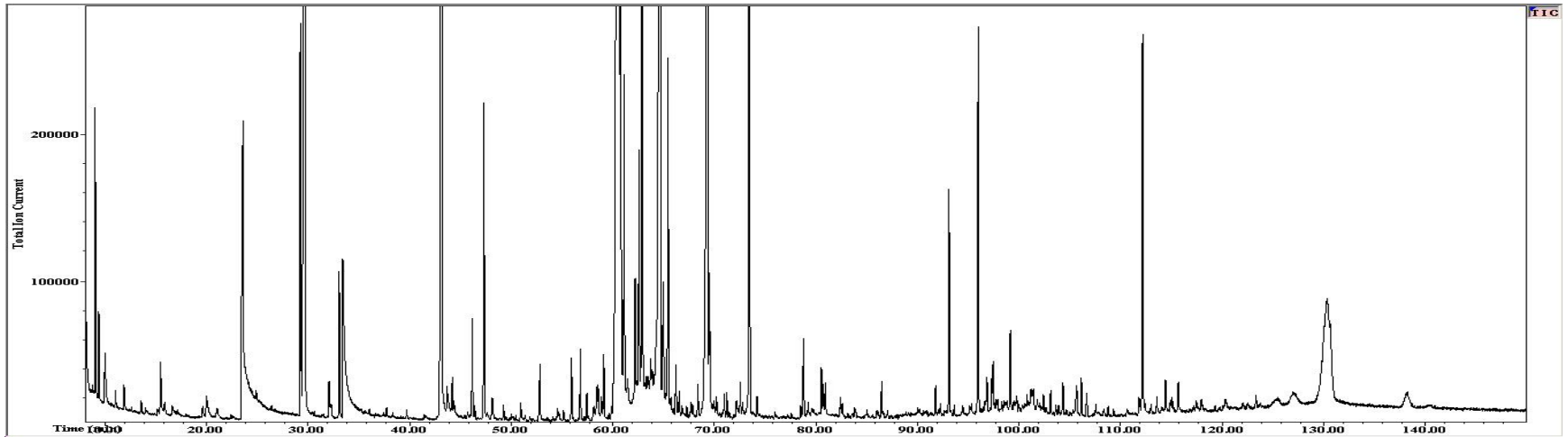
DS Scan KY3R4F (top) and flavored filter cigarillo (bottom)



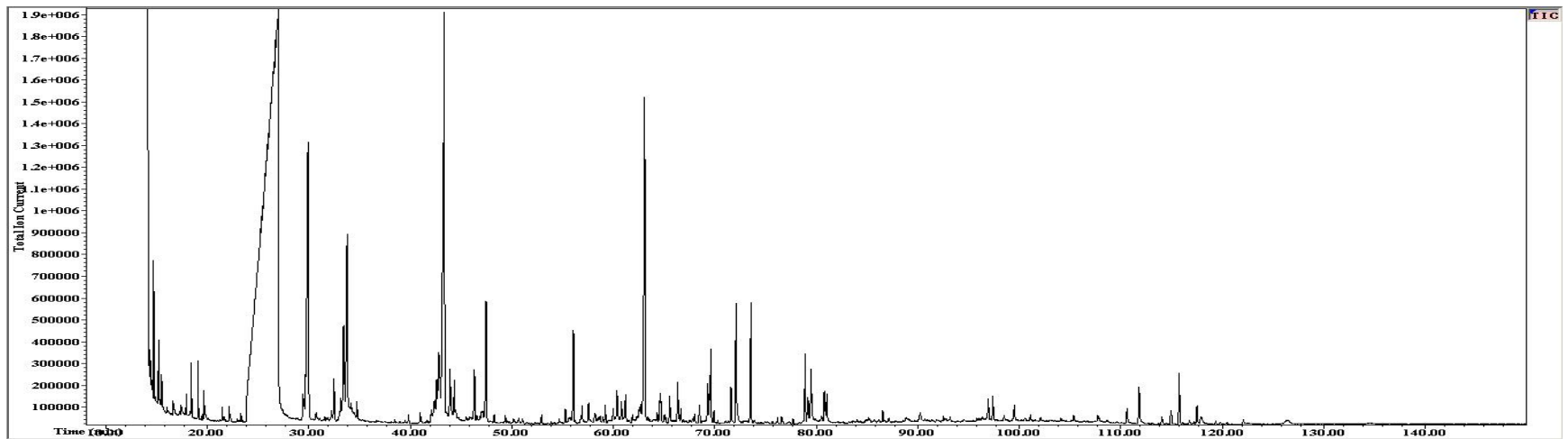
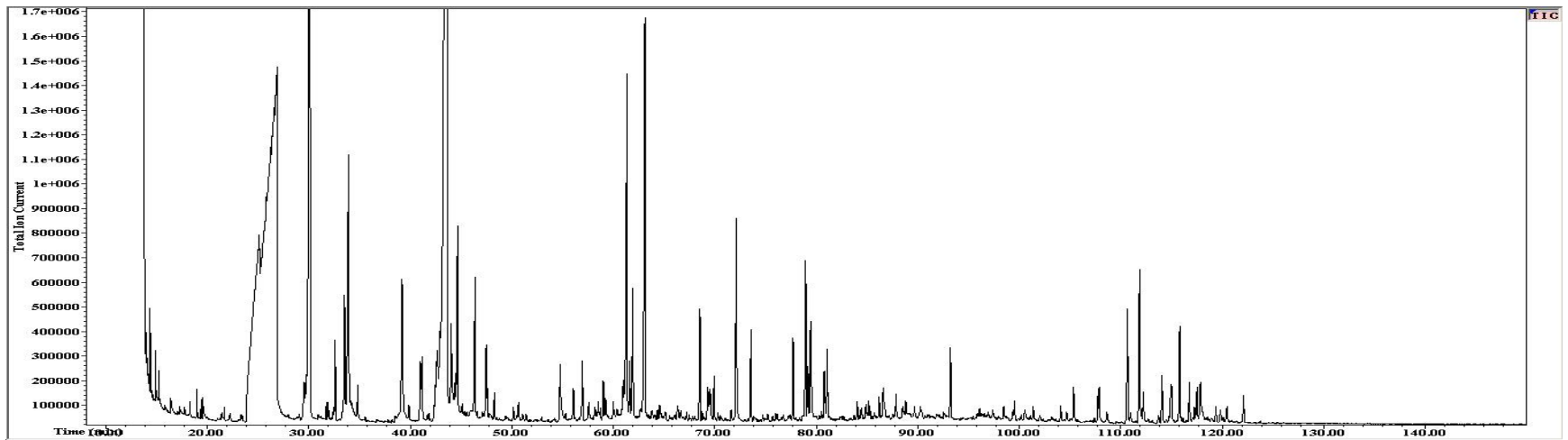
DS Scan pipe-tobacco cigarillo (top) and cigarillo (bottom)



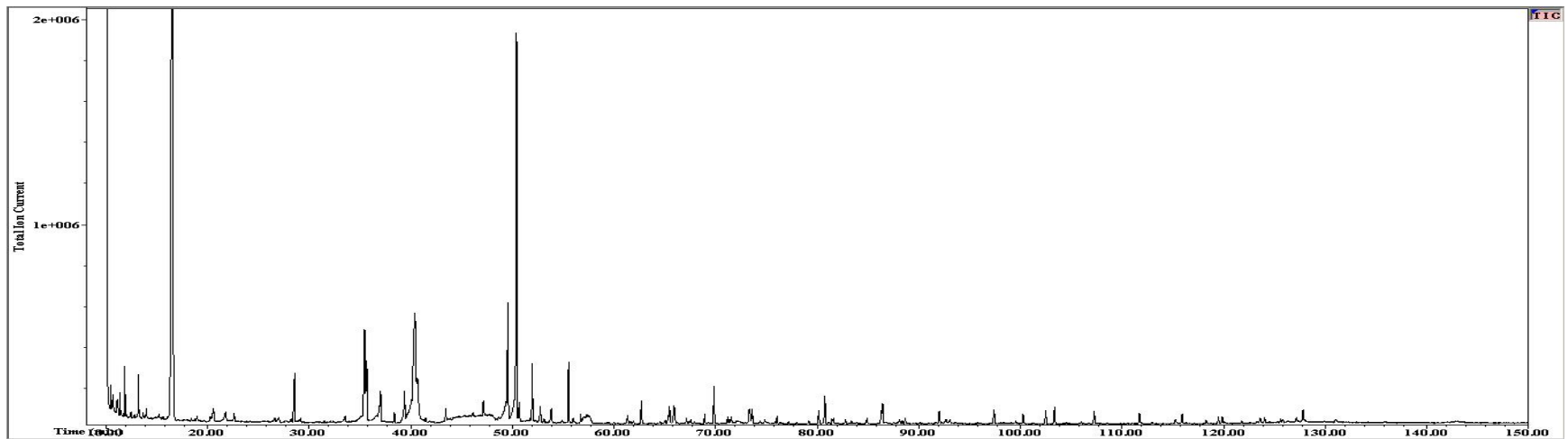
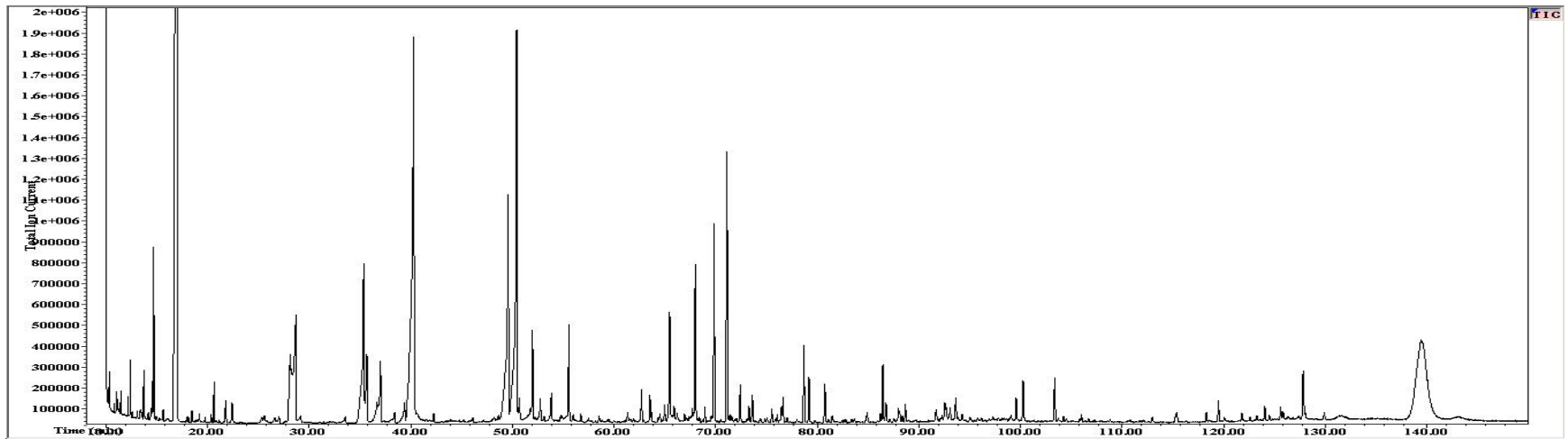
DS Scan KY3R4F tobacco (top) and cigarette paper (bottom)



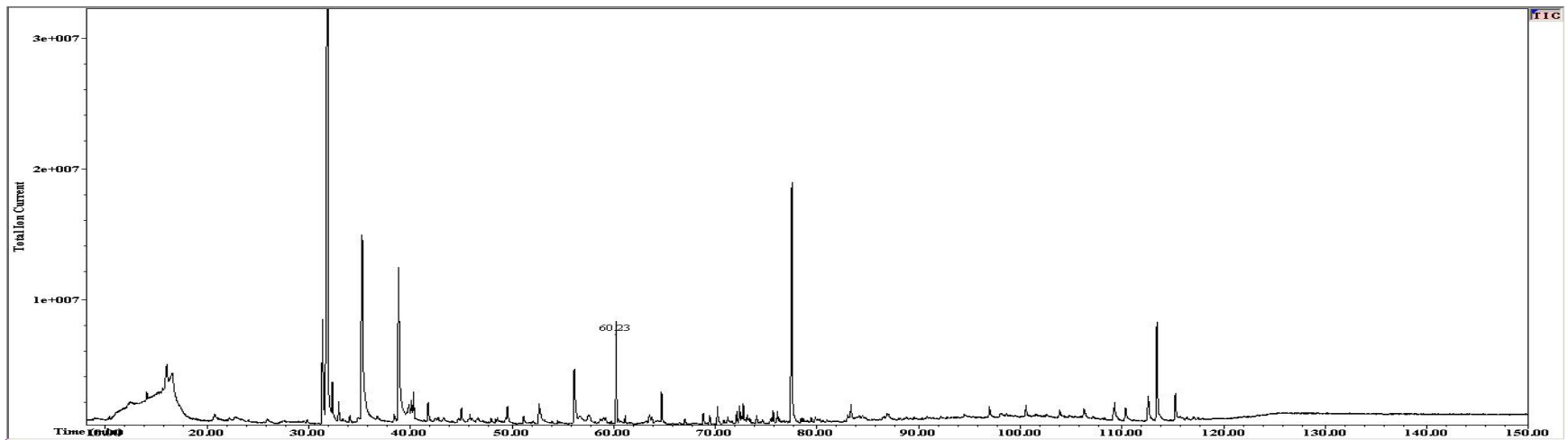
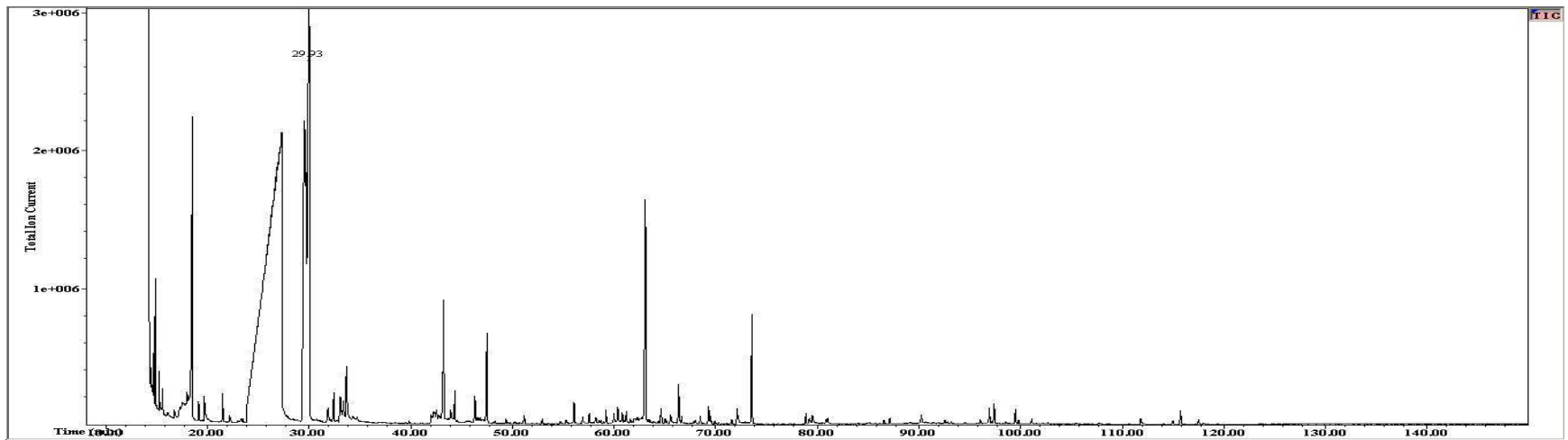
DS Scan cigarillo wrapper (top) and cigarillo binder (bottom)



DS Scan flavored cigarillo wrapper (top) and binder (bottom)



DS Scan single wrapper (top) and HFP scan (bottom)



Conclusions

- We have shown that use of the DS scan and HFP scan together has the potential to characterize tobacco fillers used in cigarettes and cigars
- Based on the limited set of samples analyzed, little cigars using cigarette tobacco fillers can be identified
- Also, with this limited set of samples, cigars that use a pipe-tobacco filler can be distinguished from those that use cigarette tobacco fillers or traditional cigar fillers
- We have shown that use of the DS scan and HFP scan together has the potential to characterize compounds in cigarette paper and cigar wrappers and binders