

Use of two GC-MS scan techniques for the characterization of tobacco fillers used in 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 how cigar and cigar-like products can be characterized with two GC-MS scan techniques
 - Direct Silylation GC-MS scan (DS Scan)
 - HFP (hexafluoroisopropanol) GC-MS scan (HFP Scan)
- Provide the results of the characterization studies
 - Tobacco fillers
 - Wrapper and binder tobaccos
- Encourage you to come to TSRC to see more
- 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

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
- 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

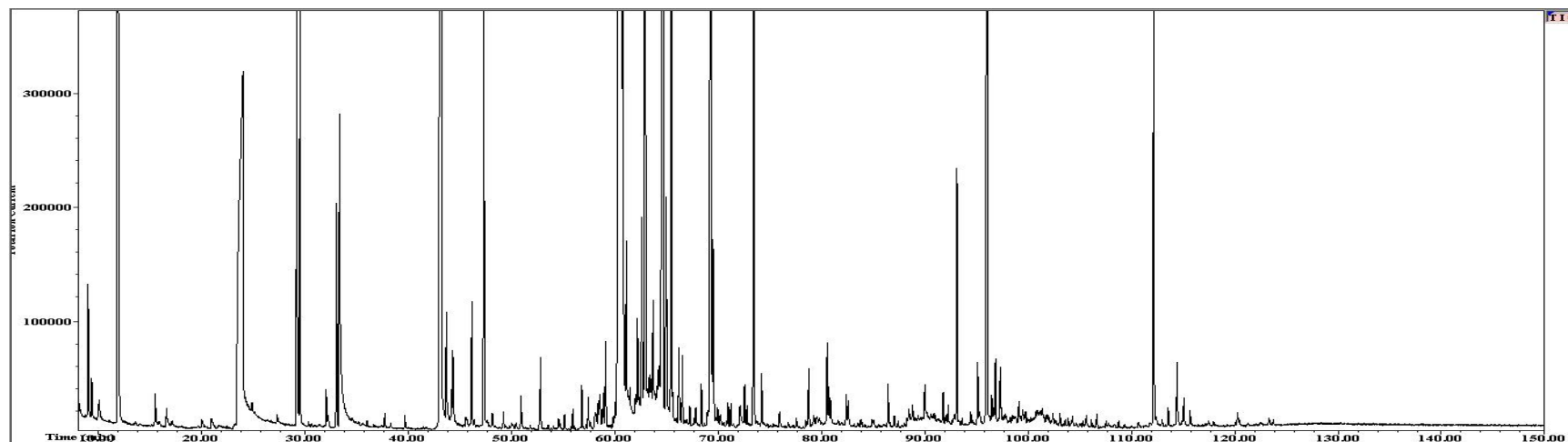
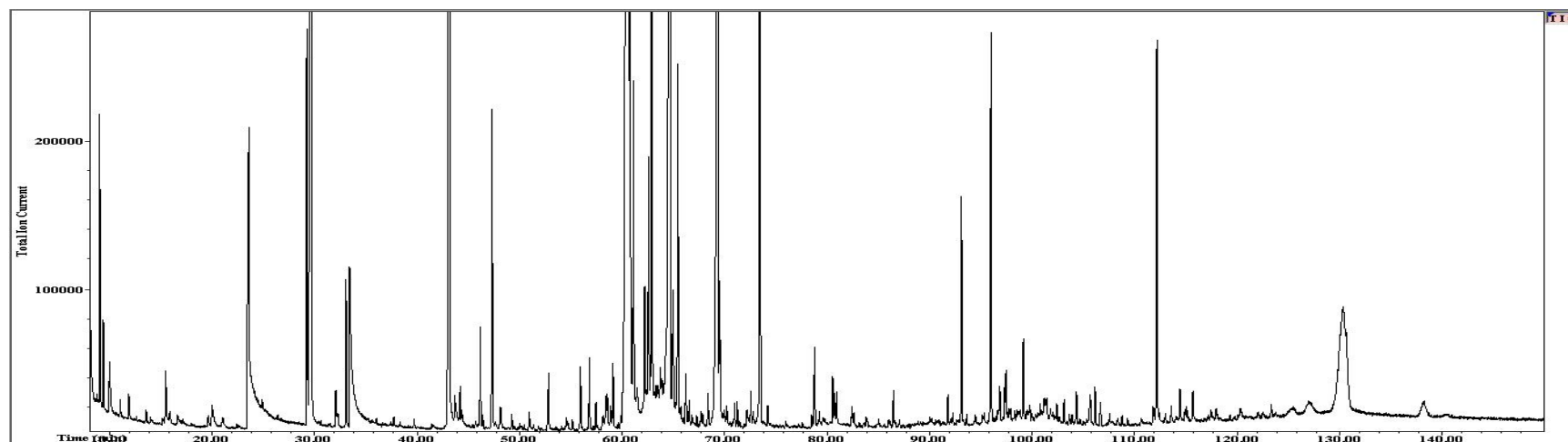
■ GC-MS Data reduction

- 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/>)

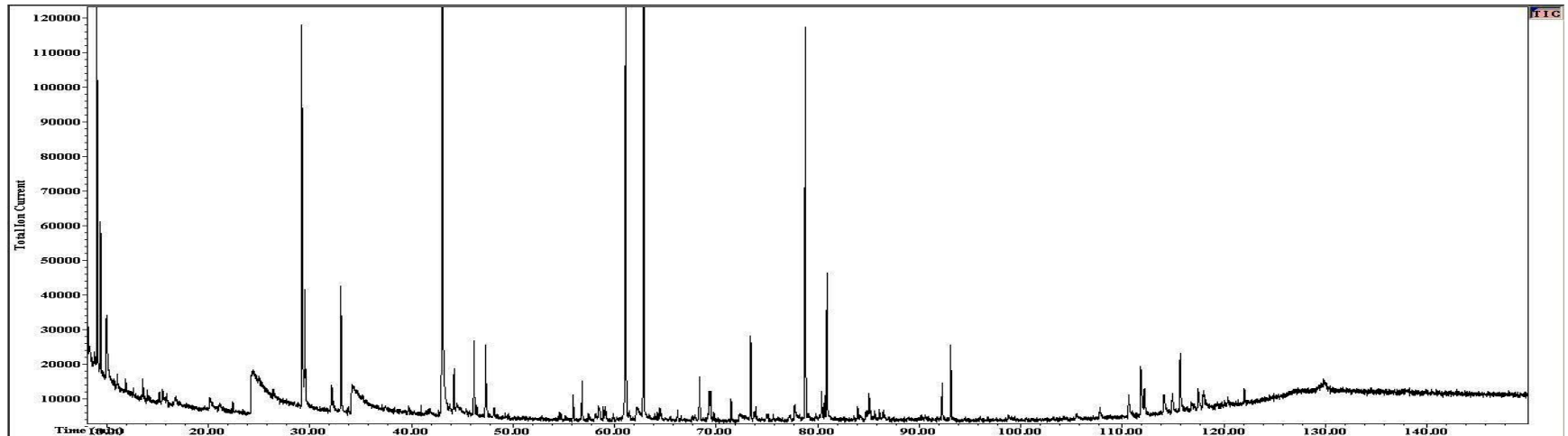
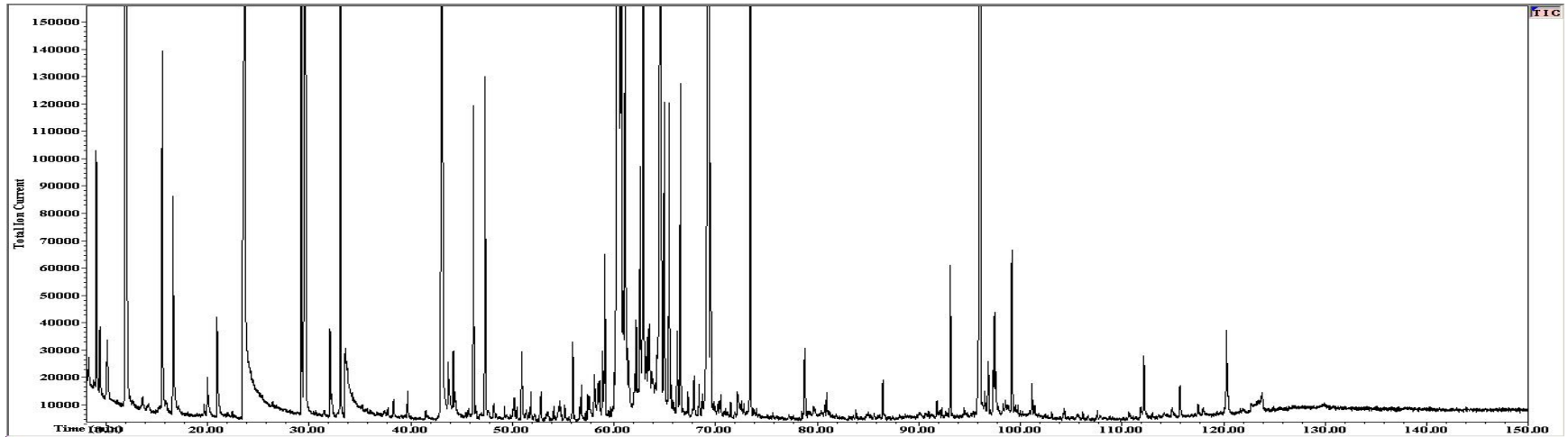
Samples used in this study

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

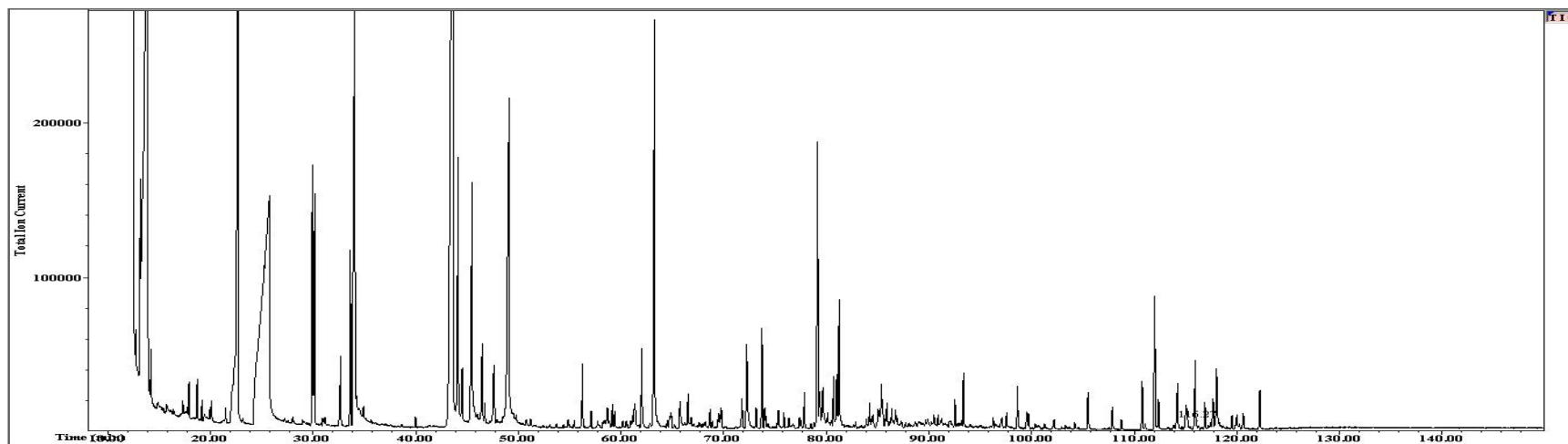
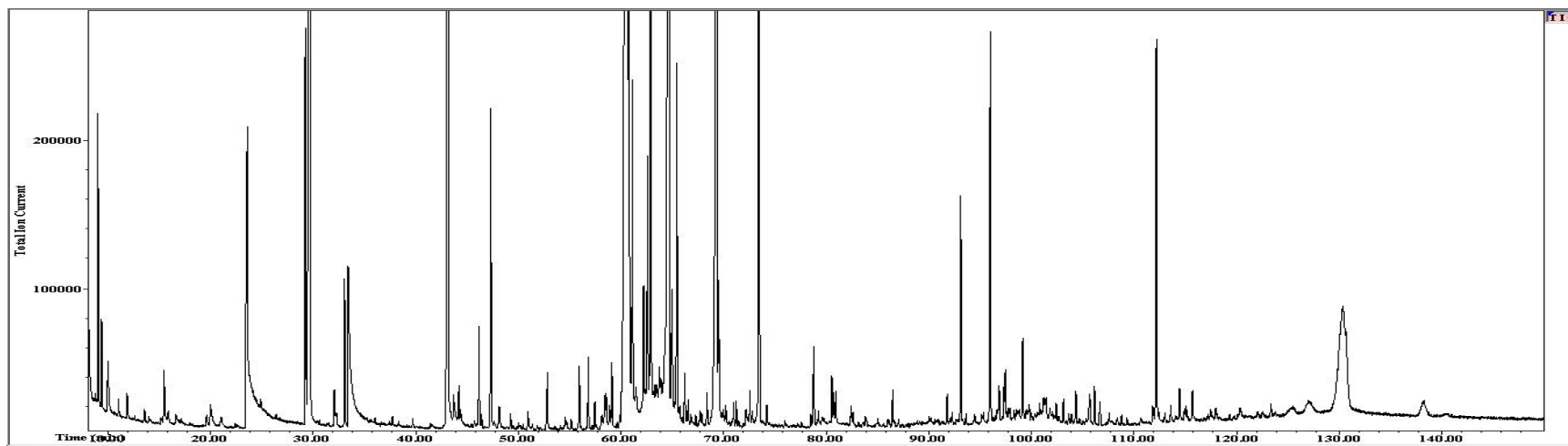
DS Scan KY3R4F (top) and flavored filter cigarillo (bottom)



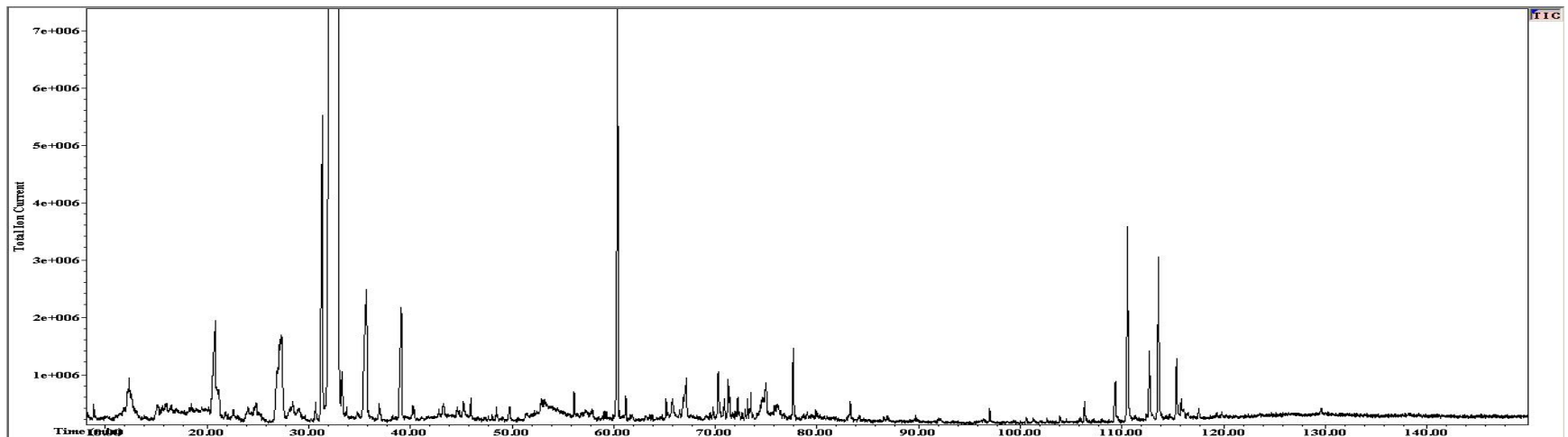
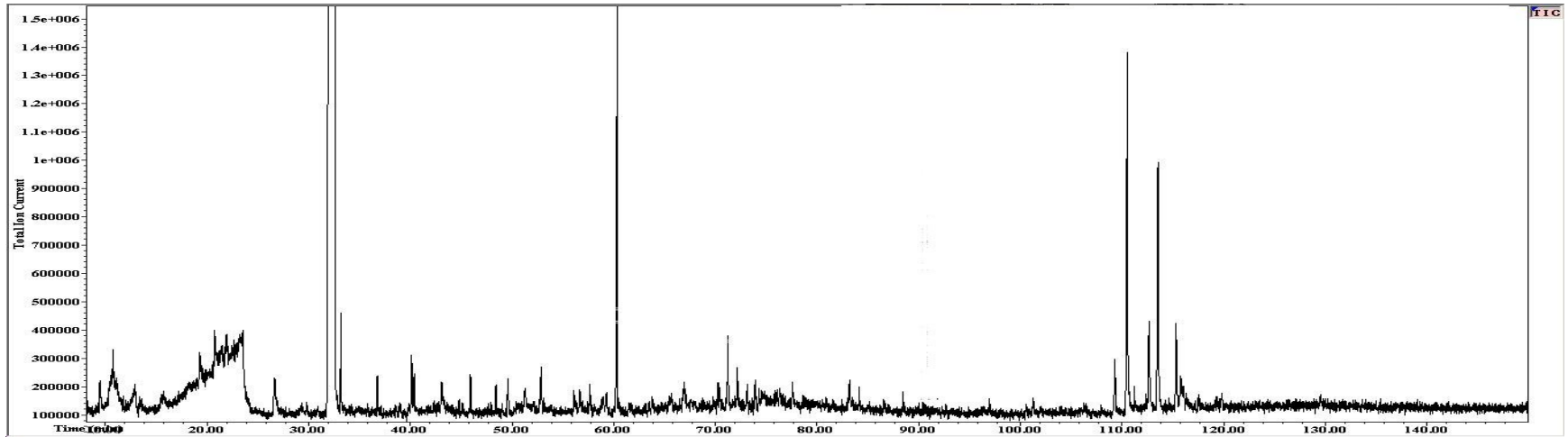
DS Scan pipe-tobacco cigarillo (top) and cigarillo (bottom)



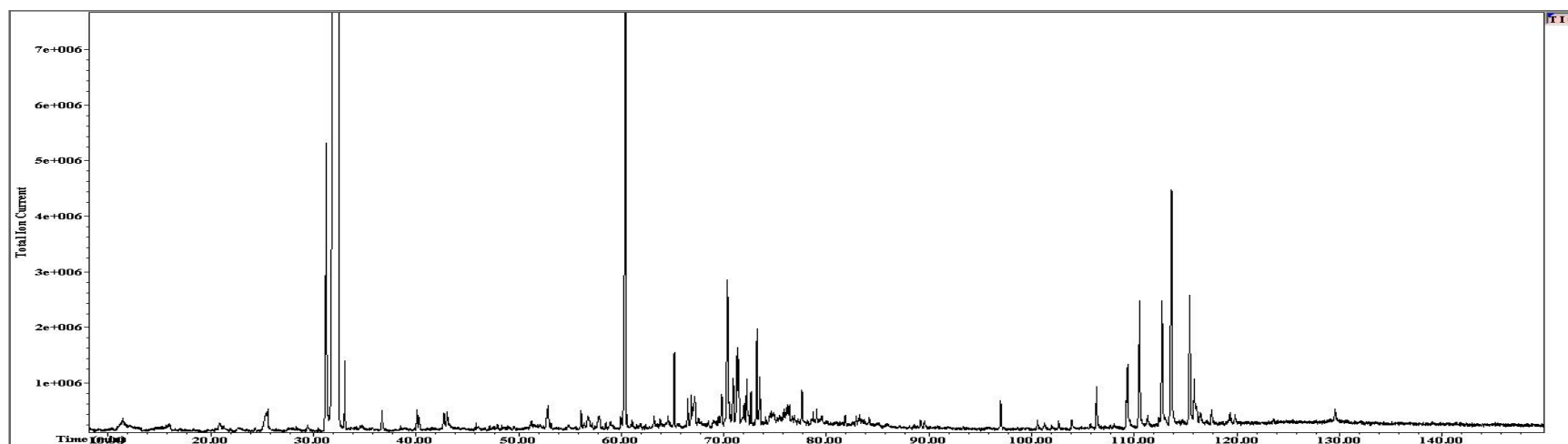
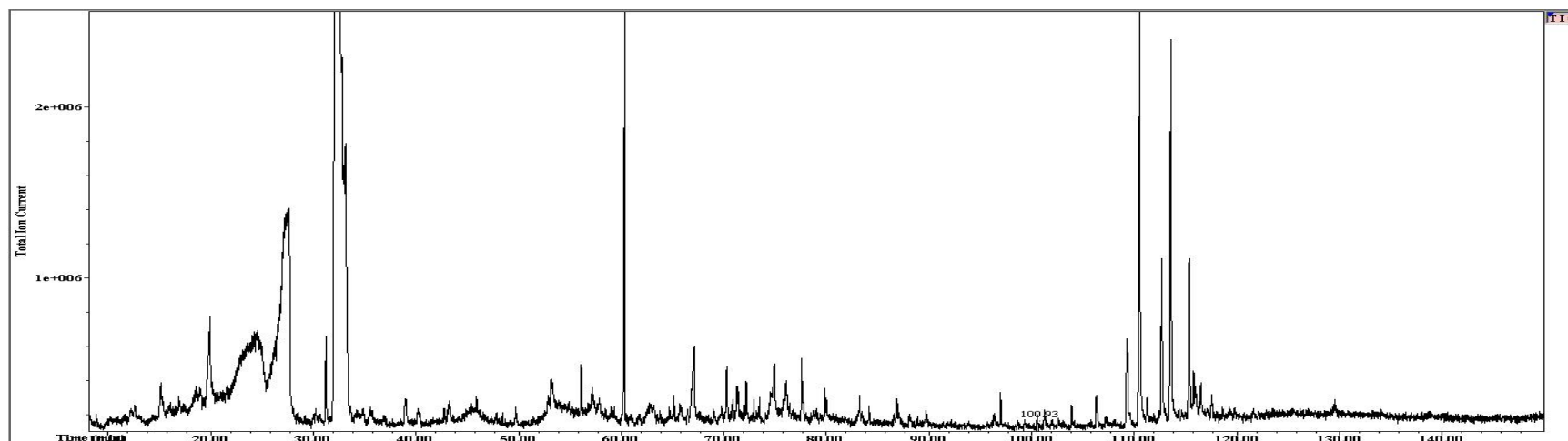
DS Scan KY3R4F (top) and flavored cigar tobacco (bottom)



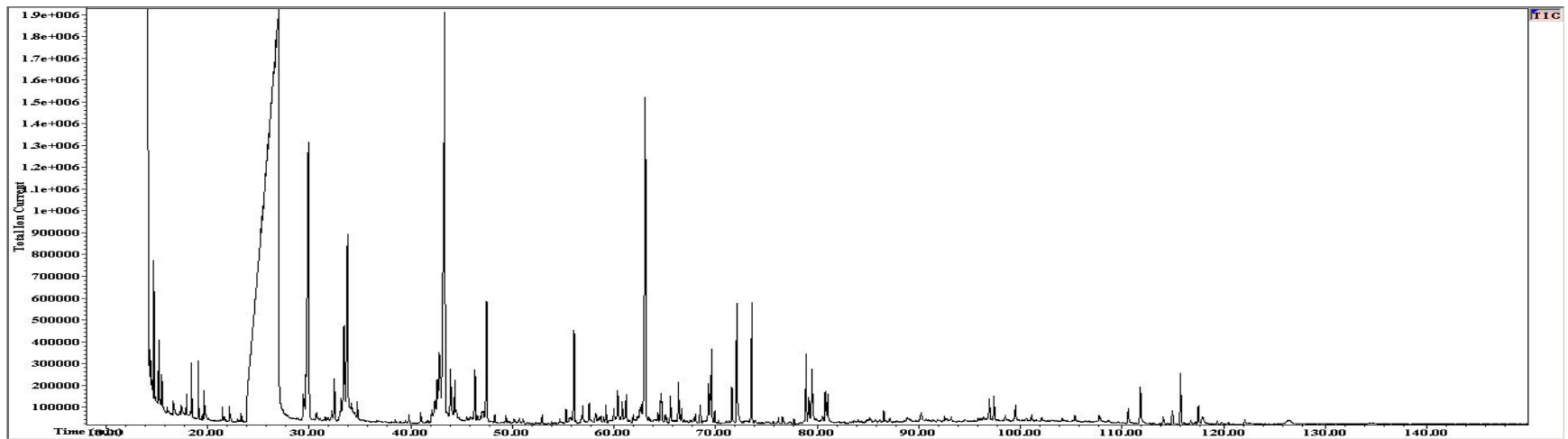
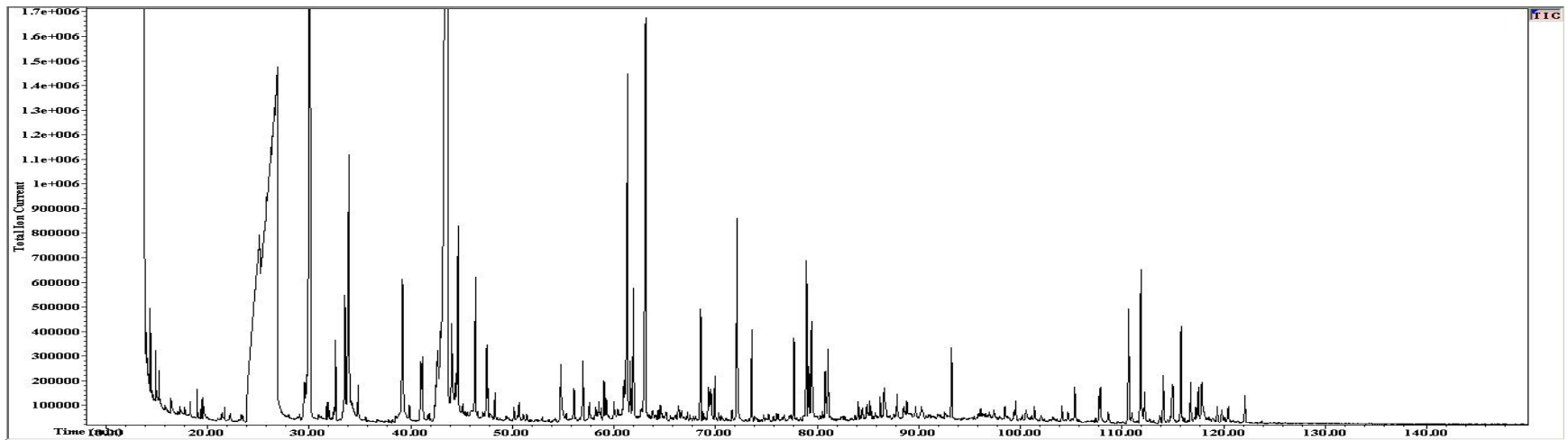
HFP Scan KY3R4F (top) and flavored filter cigarillo (bottom)



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



DS Scan cigarillo wrapper (top) and cigarillo binder (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 that use cigarette tobacco fillers can be easily identified
- Also, based on the limited set of samples analyzed, cigars that use a pipe-tobacco filler can be distinguished from those that use cigarette tobacco fillers and traditional cigar fillers