# Differences in detailed chemistries among moist snuff, snus, and novel smokeless tobacco products

John H. Lauterbach, PhD, DABT
Lauterbach & Associates, LLC
Macon, GA 31210-4708 USA
Deborah A. Grimm, Ph.D.
Tulane University Coordinated Instrumentation Facility
New Orleans, LA 70118-5698

# Outline for presentation

- Background
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- Results
- Conclusions
- Sponsorship and references

# Background

- There has been a continuing debate about the non-nicotine toxicology associated with oral nicotine sources
  - NRT – chewing gum, lozenges
  - Novel tobacco-based products such as tablets, pellets, sticks
  - Conventional northern European tobacco-based products such as snus, twists, English and German nasal snuffs
  - Conventional American tobacco-based products such as moist and dry snuff, chewing tobacco
  - Various products made in Asia and Africa
- There is uncertainty about how differences in product composition affect product toxicity
- Processing conditions can change product composition

# Objectives

- Review the analytical techniques used to characterize oral tobacco products
- Show differences in product chemistries among different types of novel oral tobacco products
- Compare our analytical approach and results with those recently published by Goodpaster *et al*., *JAFC* 2011; 59(6):2745-51, on dissolvable tobacco products
- Discuss some compounds of particular interest in oral tobacco products

# Experimental - 1

- Use GC-MS scan techniques to characterize samples
  - Long history of use with tobacco products
    - Cured tobaccos from the farms
    - Manufactured tobaccos
      - Cigarette, cigar blends, reconstituted tobaccos, expanded tobaccos
      - Conventional and novel oral tobacco products
  - Semivolatile and compounds that can be derivatized for GC
  - Simple sample preparation
    - Tray dry sample at ambient conditions if > ~ 20% moisture
    - Weigh sample (100 to 250 mg) into GC autosampler vial
    - Add solvent or solvent/derivatizing agent(s) with ISTD and seal
      - MeOH or hexafluoroisopropanol (1000 µL) ***or***
      - BSTFA (800 µL) and DMF (400 µL)
    - Heat for 30 minutes at 76º C in block heater

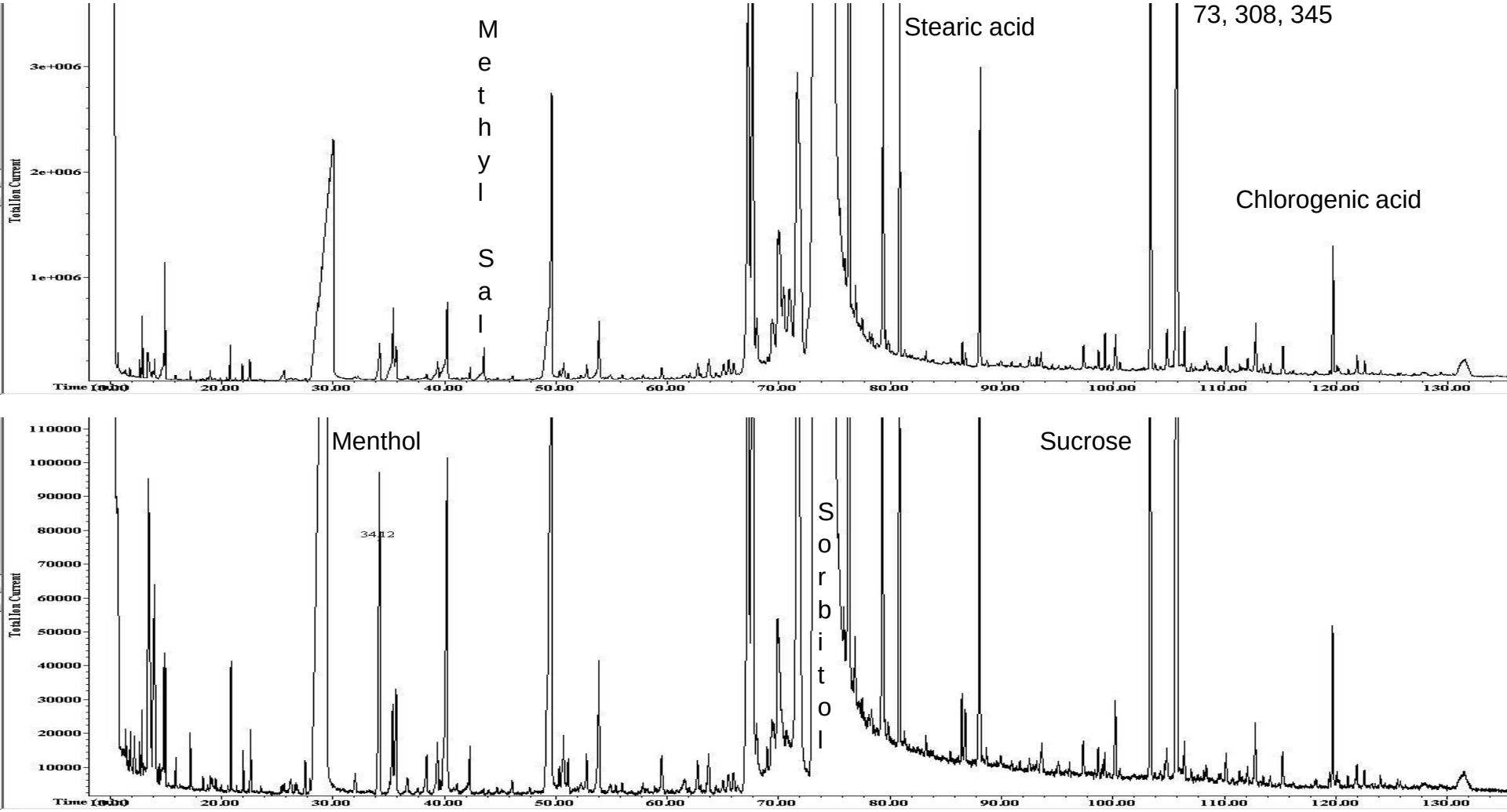
# Experimental – 2

- Simple instrumentation
  - Bench-top GC-MS with autosampler (e.g., 6890 GC/5972 MS)
  - DB-5 MS capillary column or equivalent
  - Jennings cup injection port liner
- Simple chromatography
  - Initial oven temperature hold at 40º or 50º C for 2 minutes
  - Ramp oven temperature at 2º C per minute
  - Hold at 300º for 25 minutes (~ 150 minutes run time)
  - MS scan range : 40 to 700 amu
- Simple data reduction and MS identification tools
  - WsearchPro (http://www.wsearch.com.au)
  - NIST Chemistry WebBook (http://webbook.nist.gov/chemistry/)
  - MassBank (http://www.massbank.jp/)
  - Literature references

# Results for tableted tobaccos

- DS Scan of tableted tobaccos
  - Total ion chromatograms (TICs) shown on next slide
    - Wintergreen flavored product shown in top TIC
    - Mint flavored product shown in bottom TIC
  - Both TICs are dominated by peak from sorbitol-TMS which shows as a very broad peak at ~ 74 to 75 minutes
  - Both TICs show peaks for sucrose-TMS at ~103 minutes and unidentified peak at ~106 minutes (m/e 73, 308, 343, 361)
  - The presence of the TMS derivative of chlorogenic acid at ~120 minutes indicates that these oral tobacco products are likely based on flue-cured, not the air-cured tobaccos used for most oral tobacco products

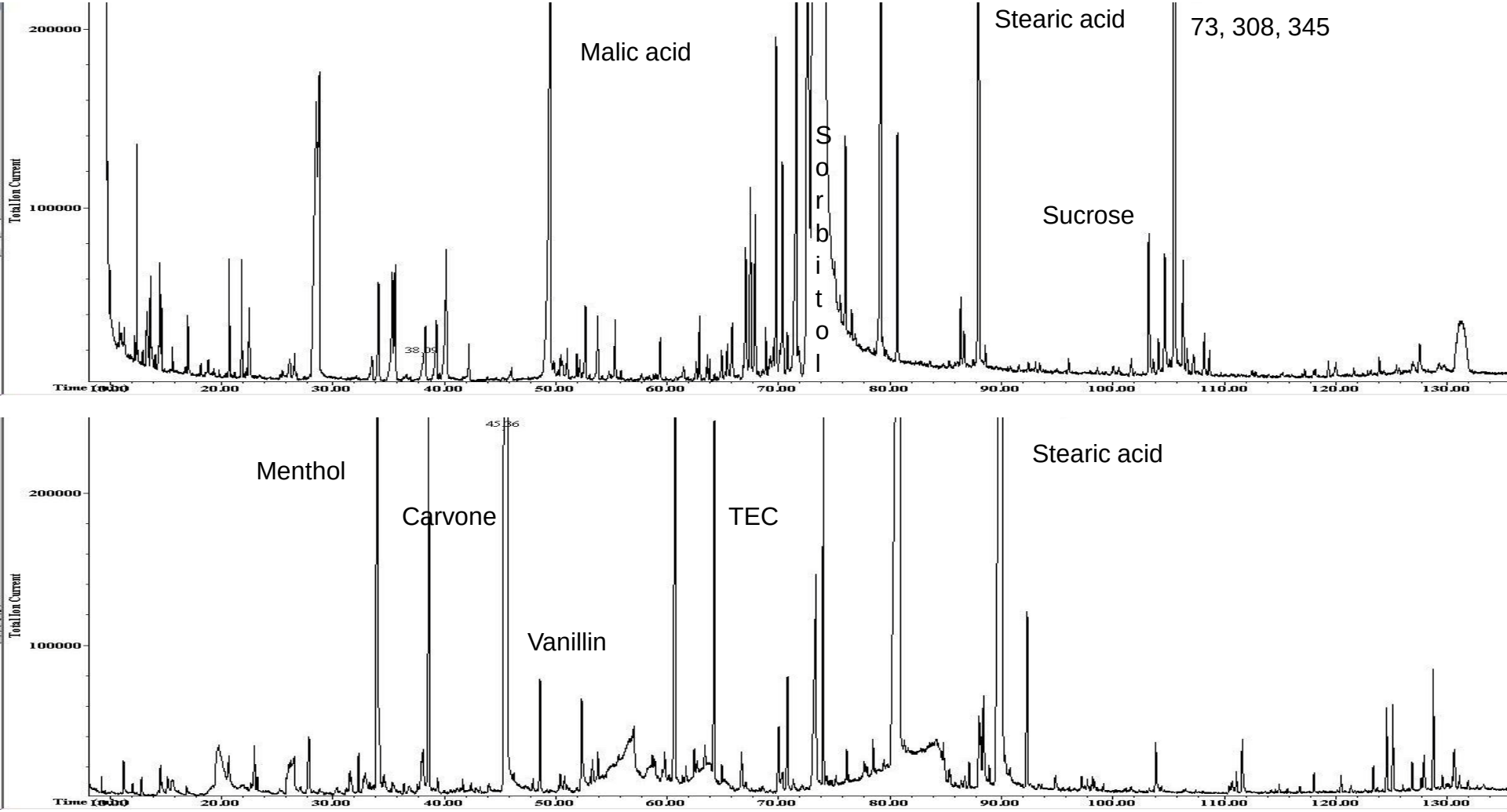
# Results – Tableted tobaccos – Wintergreen (top), Mint (bottom)



# Results for Camel Fresh Orbs

- DS Scan (top) showed much more detail than reported by Goodpaster *et al.*, particularly the previously unreported sugar-like TMS derivative at ~ 106 minutes
- DS Scan also showed numerous organic acids such as malic acid, citric acid, and quinic acid; but no chlorogenic acid found so probably not flue-cured tobaccos
- MeOH scan (bottom) showed same compounds as reported by Goodpaster *et al.*, but numerous tobacco constituents found as well

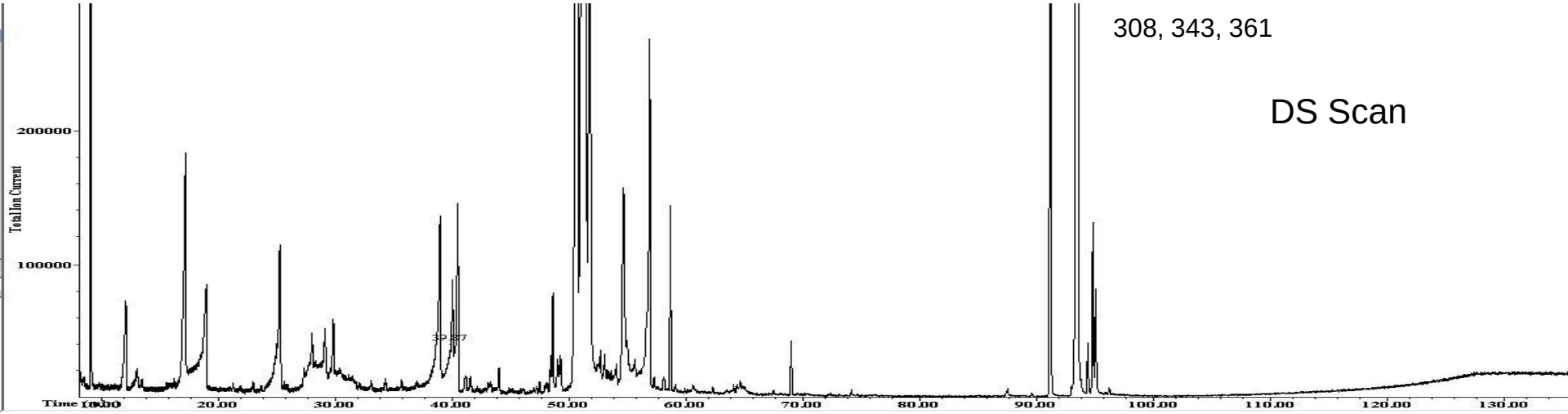
# Results – Camel Fresh Orbs – DS (top), MeOH (bottom)

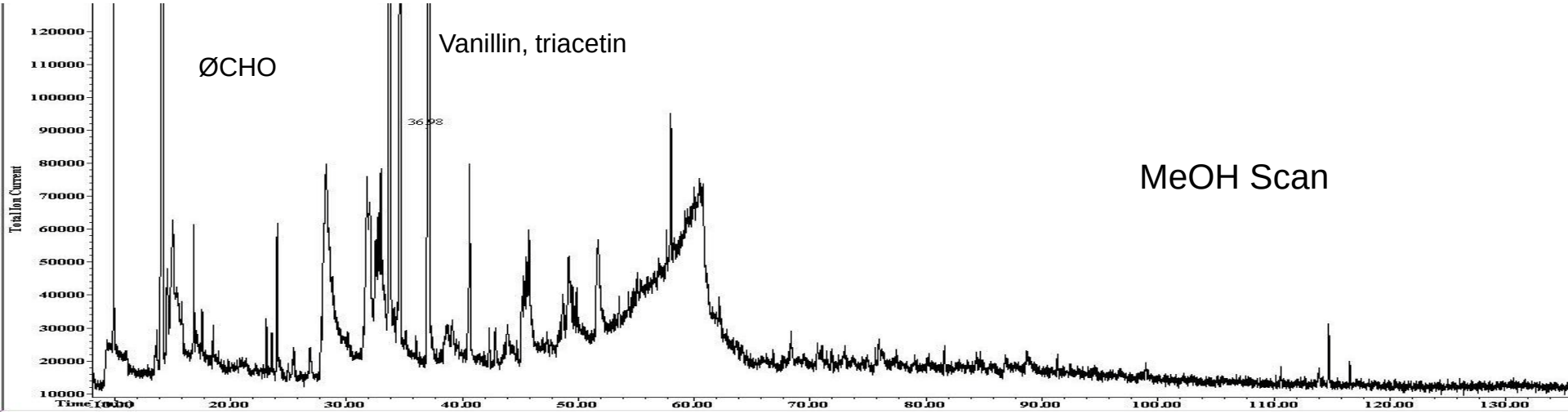


# Results – experimental cherry flavored oral tobacco product

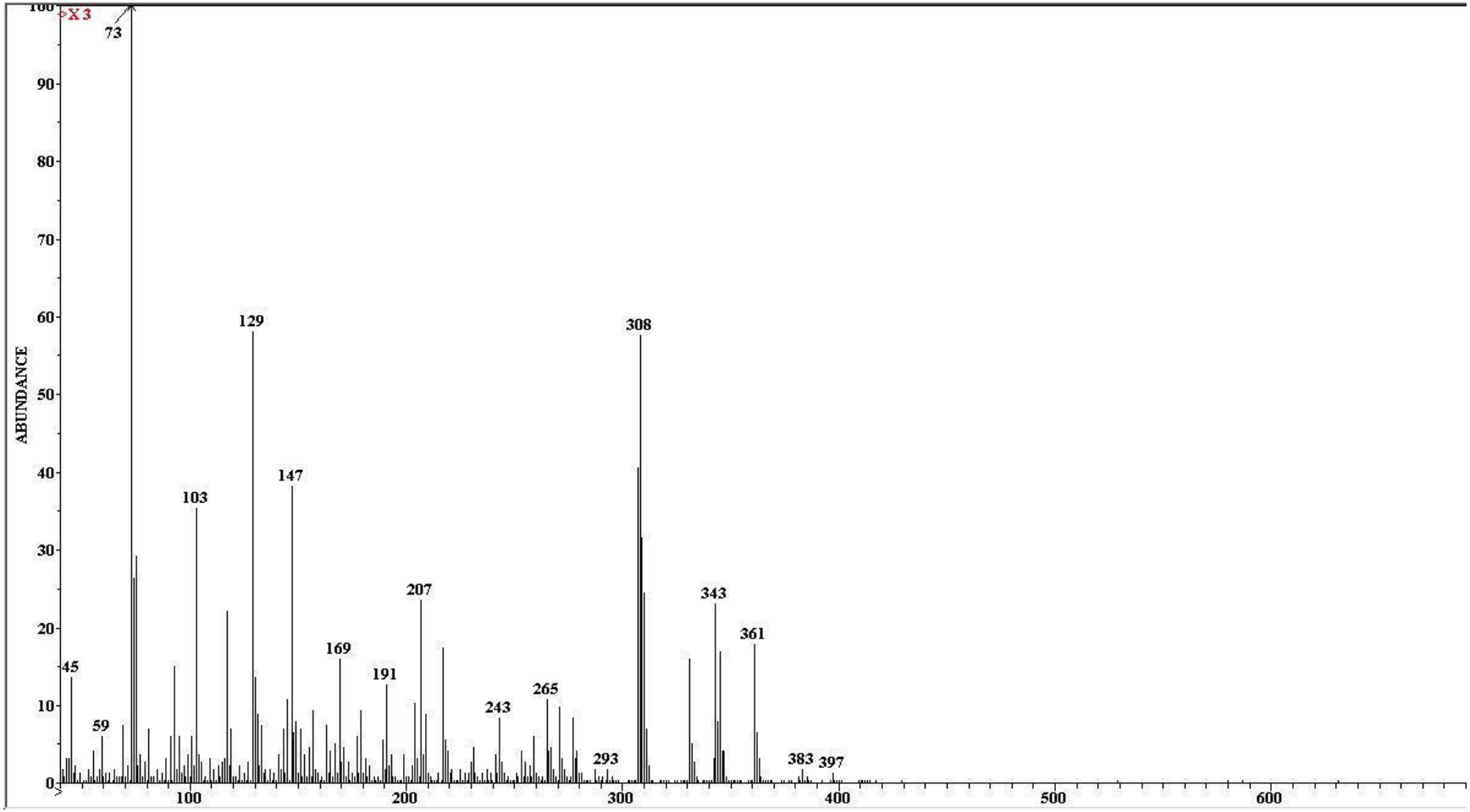
- Sample preparation was difficult with less than clear separation between product, cloudy middle layer, and extract
- DS Scan showed evidence of tobacco components such as phosphate, nicotine, and the major organic acids, but no evidence for chlorogenic acid
- DS Scan also showed use of sugar alcohols plus unknown peak with m/e 73, 308, 129, 147, 343, 361
- MeOH Scan showed evidence for cherry flavor, some tobacco components and probably a polyglycol humectant

# Results – experimental cherry flavored oral tobacco product



# Can you identify this spectrum?



# Conclusions

- GC-MS scan techniques were shown to be very suitable for characterizing novel oral tobacco products
- We showed how the scan techniques can distinguish products made with flue-cured tobaccos from those made with air-cured tobaccos
- Except for the apparently previously unreported carbohydrate TMS derivative found in the products studied, all other substances identified were reasonably expected to be in the products
- We found the same compounds in the Fresh Orb product that had been reported by Goodpaster *et al*., *JAFC* 2011; 59(6):2745-51

# Sponsorship and references

- Sponsorship
  - This research was funded entirely by Lauterbach & Associates, LLC
  - The travel and lodging expenses of the speaker were also funded entirely by Lauterbach & Associates, LLC
- References – Copies of this presentation and related presentations on oral tobacco products along with abstracts of our journal articles can be found at http://www.lauterbachandassociates.net/2101.html