

Characterization of reference and commercial moist snuff samples by use of two GC-MS scan techniques

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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

- Some health experts are recommending that smokers, who refuse to quit or refuse to use NRT, switch to low TSNA smokeless tobacco products (STP)
 - US-style moist snuff is the most popular of the STP, but has attracted much criticism from those against STP use on account of toxicological and addiction concerns
 - Available analytical chemistry data has tended to focus on nicotine and pH values and trace-level (GothiaTek[®]) analytes but there are efforts to find more toxicants [e.g., PAHs, Stepanov *et al.*, 238th ACS Nat'l Meeting (2009), TOXI 3]
- Use of *in vitro* assays (e.g., Ames) to assess STP toxicity was of limited utility in distinguishing product types and brands within a type (Rickert *et al.*, 2009)

Objectives for the presentation

- Provide an updated report on research we first reported last year on use of Direct Silylation GC-MS scan (DS Scan) technique to characterize potential STP toxicants
- Provide an initial report of research we have done this year with the HFP (hexafluoroisopropanol) GC-MS scan (HFP Scan)
- Provide an initial report of the use of both scan techniques to analyze applicable compounds in the 2S3 reference moist snuff and nine brand-styles of moist snuff obtained at retail stores in Macon, GA
- Attract funding for analyses of additional types of STP

Materials and methods - 1

- Key references to prior work
 - 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 - 2

- Samples
 - 2S3 Reference moist snuff (Crop Science, NCSU)
 - Commercial samples (9 brand-styles) purchased from retail stores in Macon, GA (five cans per brand-style)
 - Brand-style breakdown (all long-cut unless indicated)
 - Tobacco flavor (straight, etc.) – 2
 - Wintergreen flavor – 2 (one long cut, one fine cut)
 - Fruit flavor – 3
 - Other flavor (vanilla, etc.) – 2
 - Not a representative sampling

Materials and methods - 3

- Sample handling and initial preparation
 - Strategy for initial sample preparation
 - Quickly equilibrate tobacco with ambient conditions to prevent spoilage due to microbial growth
 - Reduce water content to level that would not compromise silylation reactions needed for DS Scan
 - On receipt, two cans per brand-style were combined and tray-dried at ambient conditions for several days until tobacco appeared dry and had lost much of original odor
 - Samples were then packaged in Ziploc[®] bags and sent overnight to the GC-MS laboratory and kept refrigerated until the analytical samples were prepared
 - The retain samples (three cans per brand-style) were kept in frozen storage from date of receipt

Materials and methods - 4

- Preparation of analytical samples
 - DS Scan
 - The tobacco sample (100 mg) is weighed into a GC autosampler vial
 - BSTFA (800 μ L) and DMF (400 μ L) are added to the vial
 - Internal standards such as phenanthrene- d_{10} can be used
 - 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 μ L)
 - Internal standards such as phenanthrene- d_{10} can be used
 - 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 Total ion chromatograms
 - Typical TIC plots including all data points
 - Column charts based on chromatographic points used to pick 100 spectra per chromatogram and constructed using MS EXCEL 2003
 - Only peaks with retention times between 10 and 135 minutes used
 - Y-axis on column charts set to 10^7
 - MS Spectra
 - Spectra were provided as NIST (*.msp) format
 - Background was manually adjusted as necessary
 - Spectra were plotted using NIST MS Search 2.0
 - Interpretations were done manually using various spectral collections

Results

■ GC-MS Results

□ HFP Scans

- Ten samples including 2S3 reference moist snuff
- Analyses were not replicated

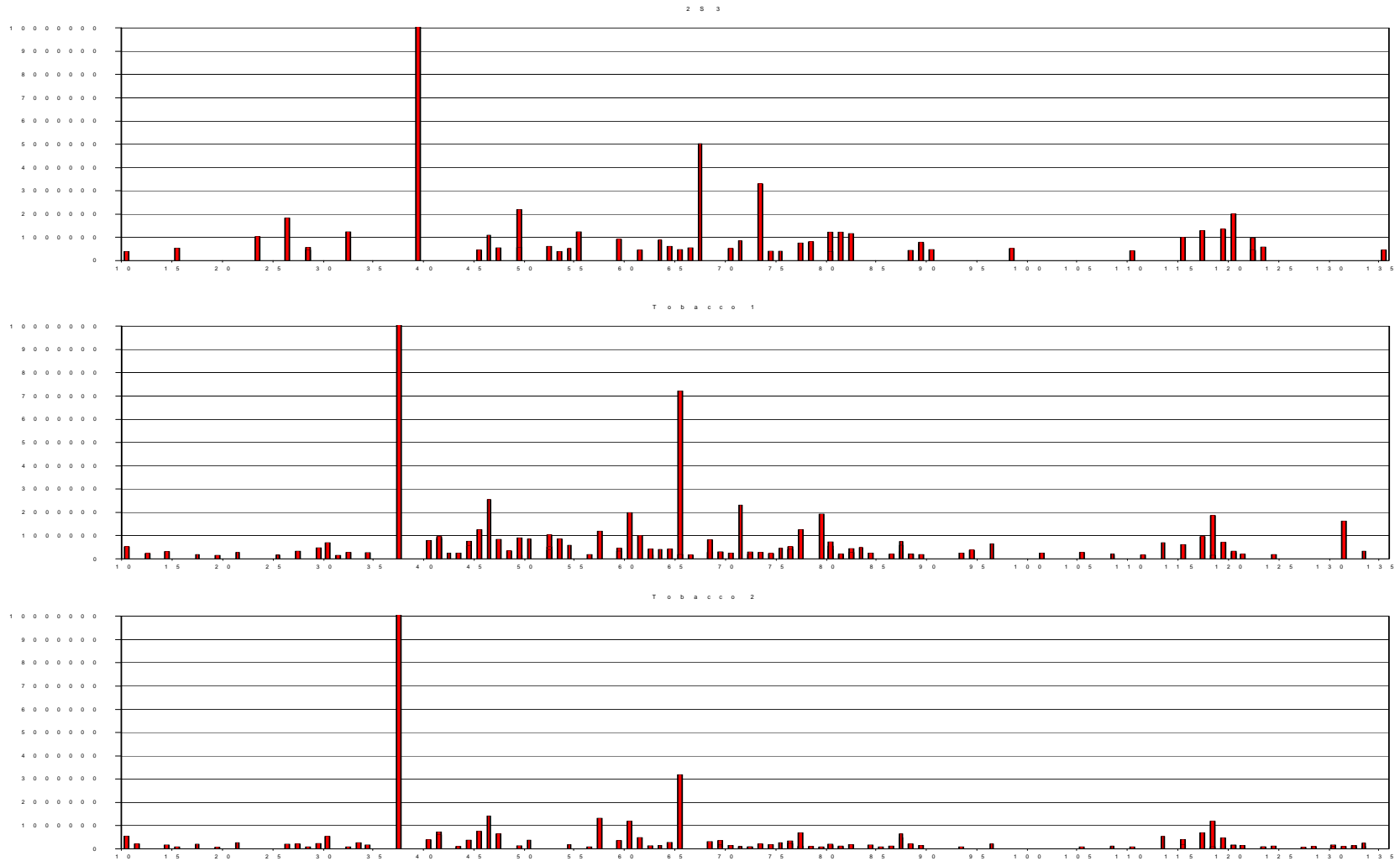
□ DS Scans

- Ten samples including 2S3 reference moist snuff
- Analyses were not replicated except in several cases where there were obvious experimental problems

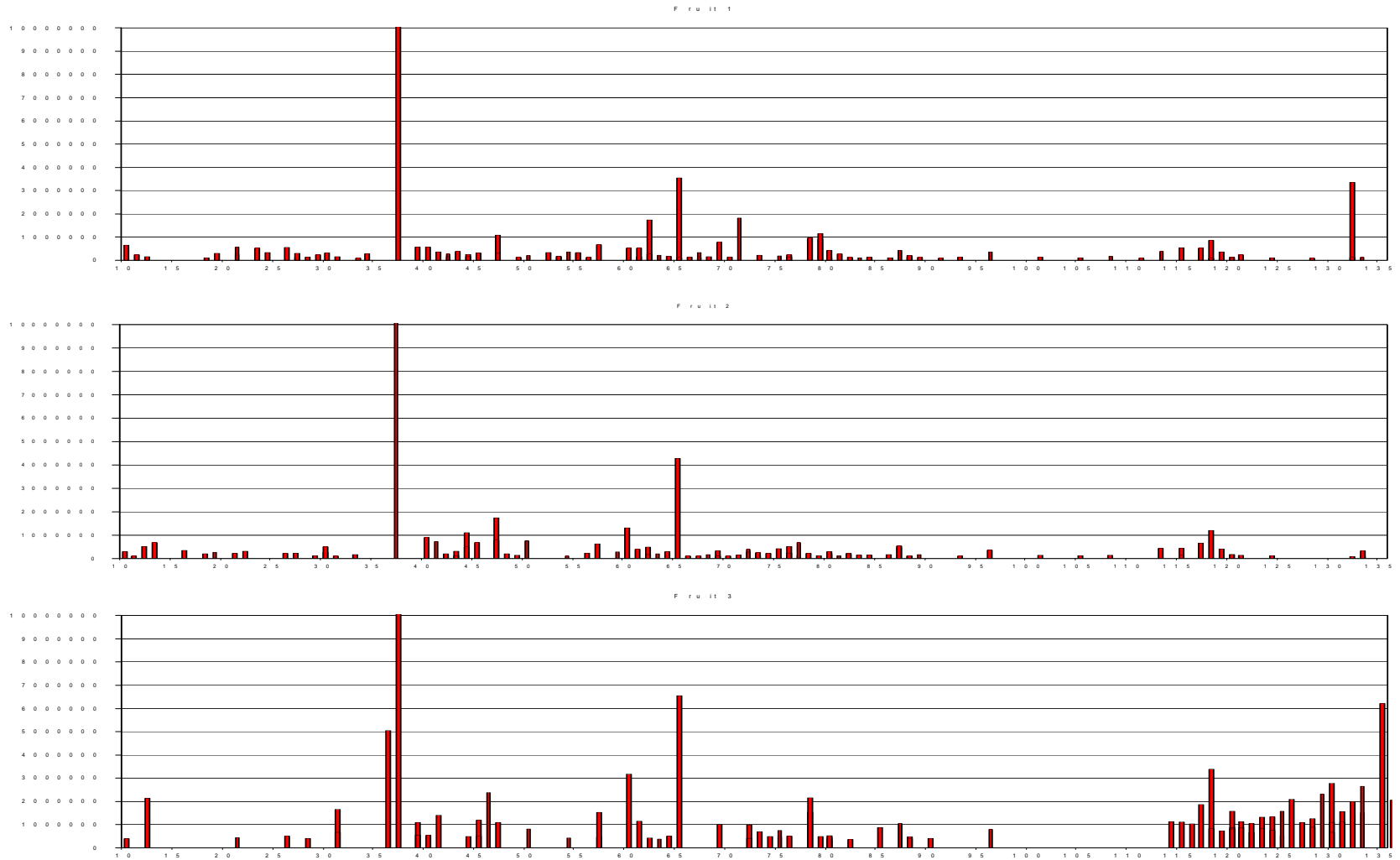
□ Data presentation

- Chromatographic data will be presented as MS EXCEL column charts

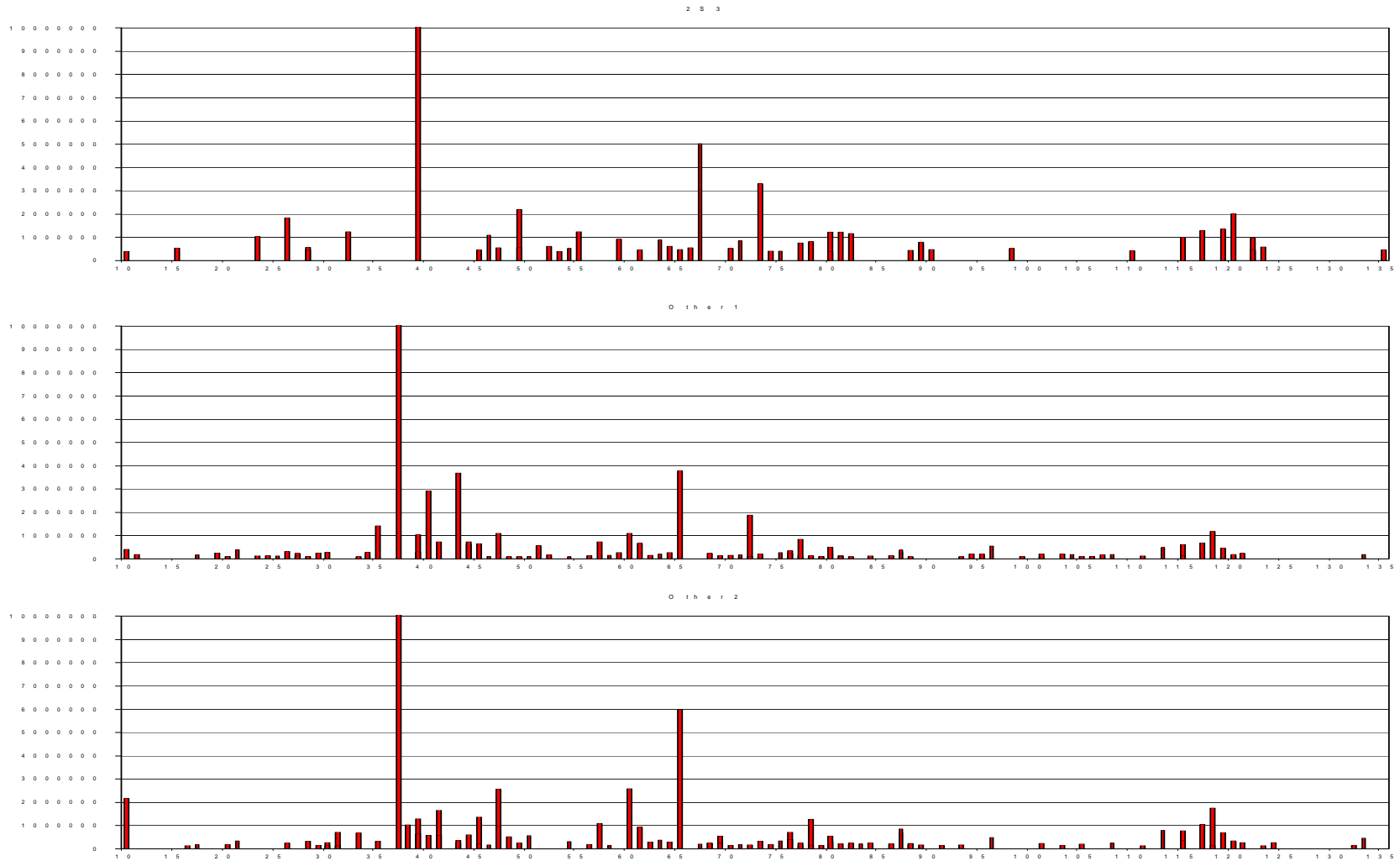
HFP Scan - 2S3 and tobacco-flavored samples



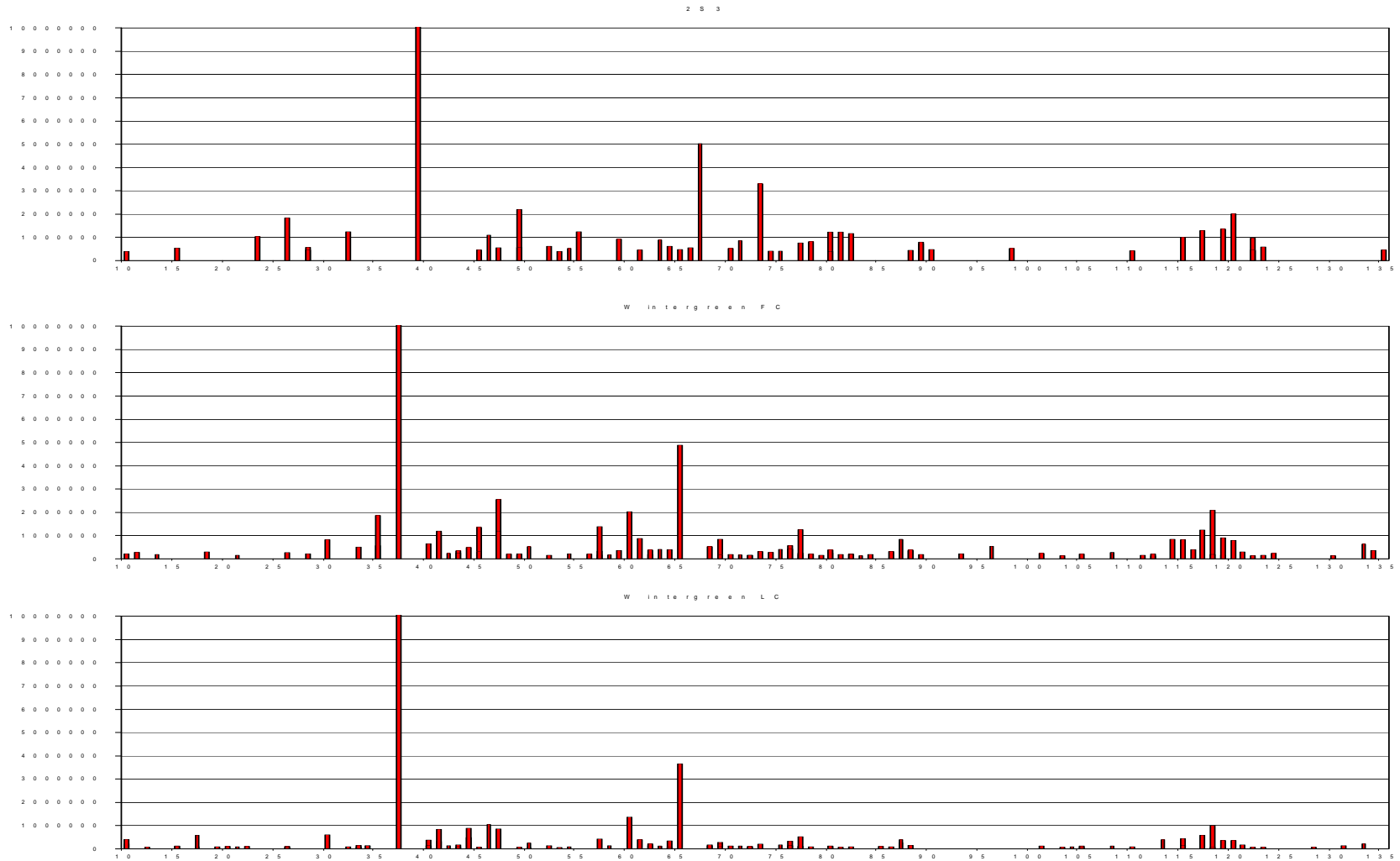
HFP Scan - fruit-flavored samples



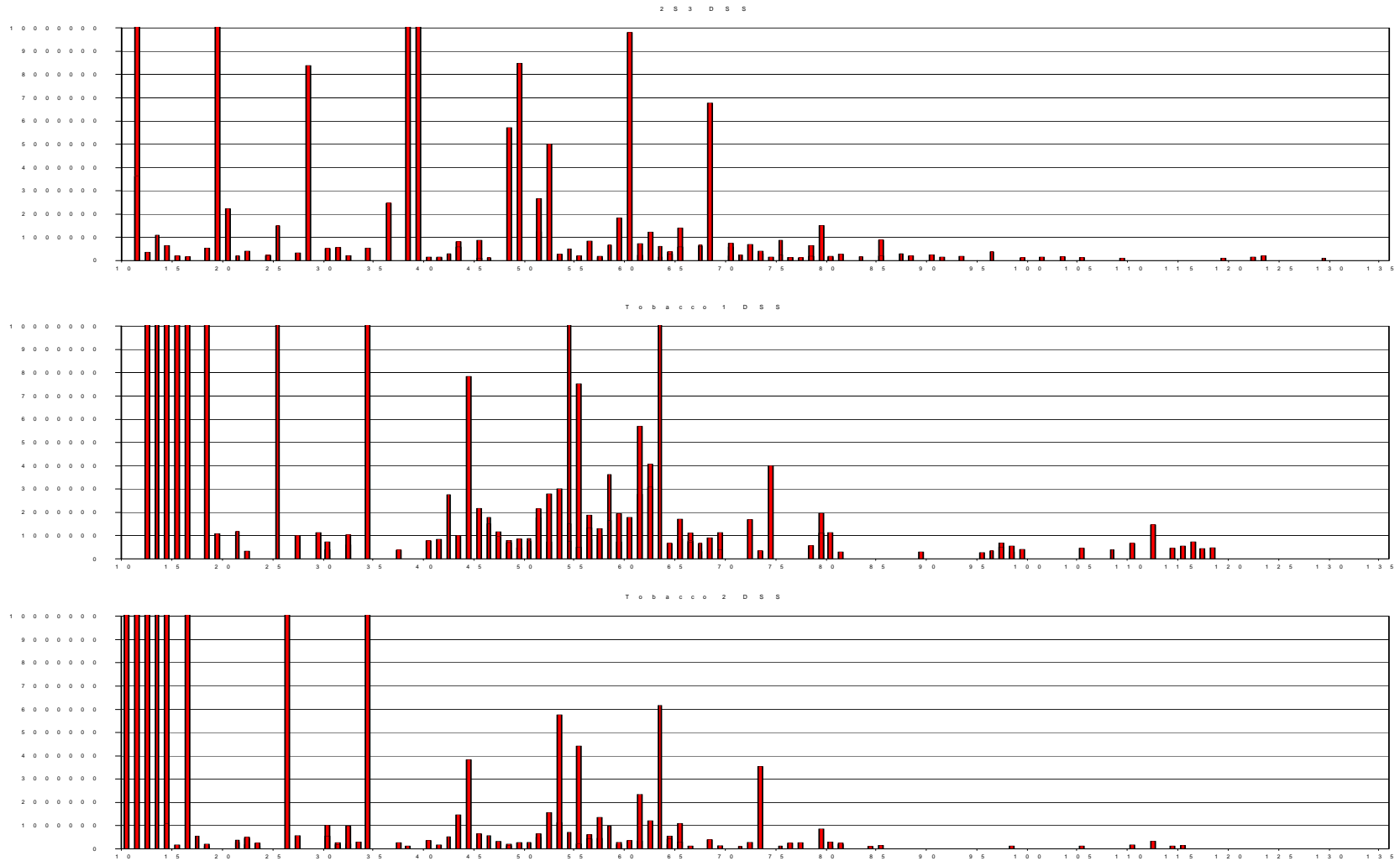
HFP Scan - 2S3 and "other" flavor samples



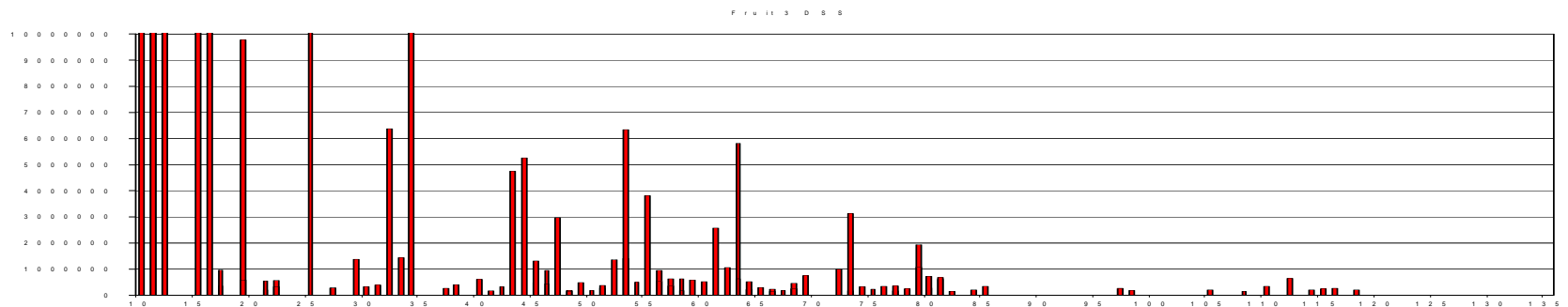
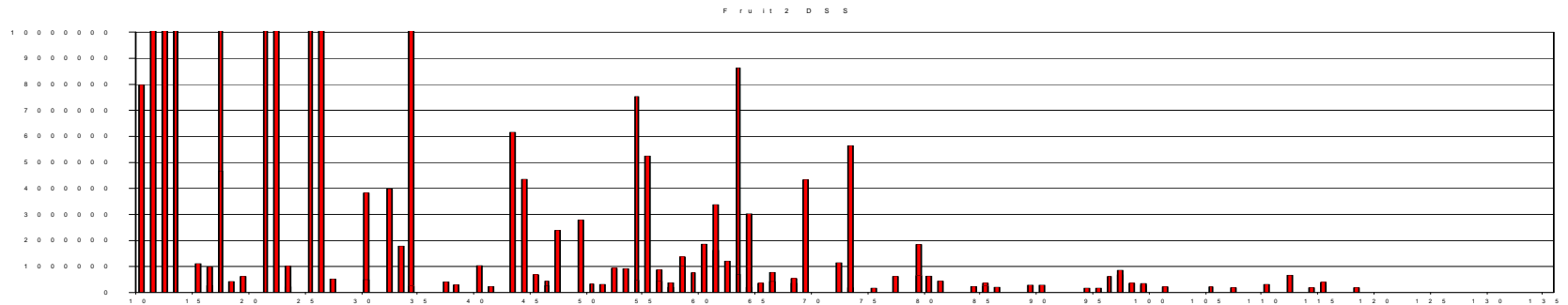
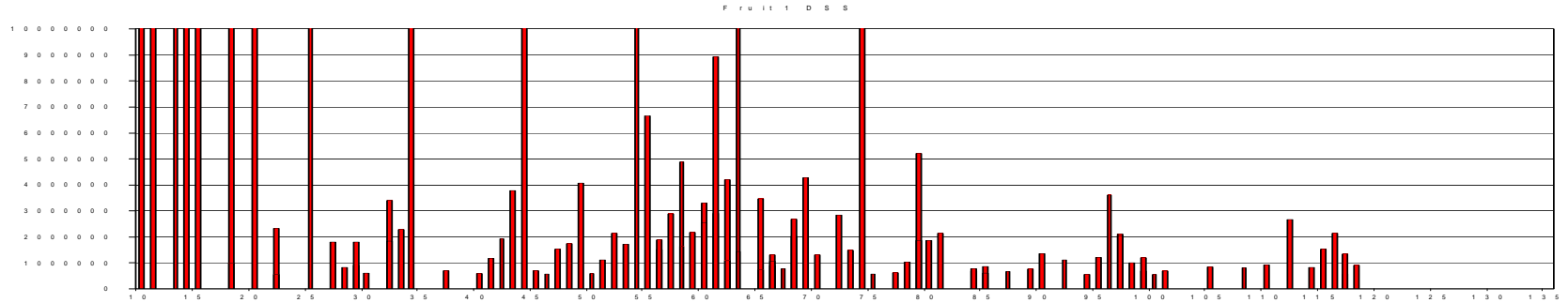
HFP Scan - 2S3 and wintergreen flavor samples



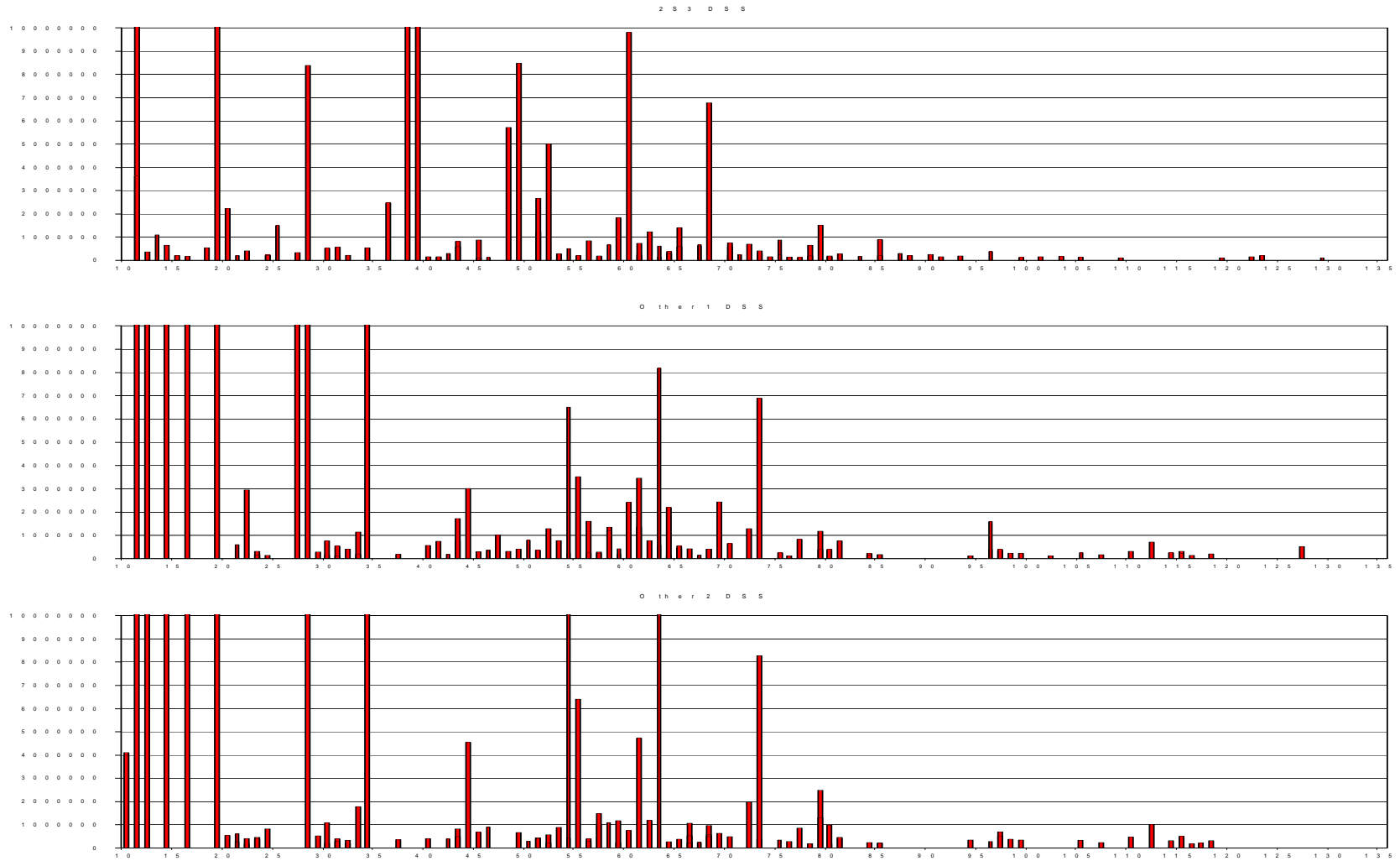
DS Scan - 2S3 and tobacco-flavored samples



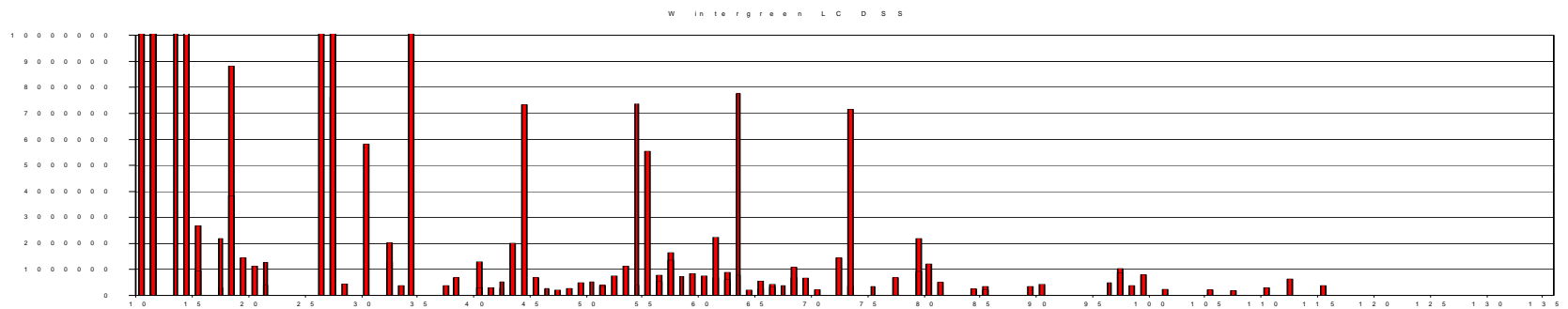
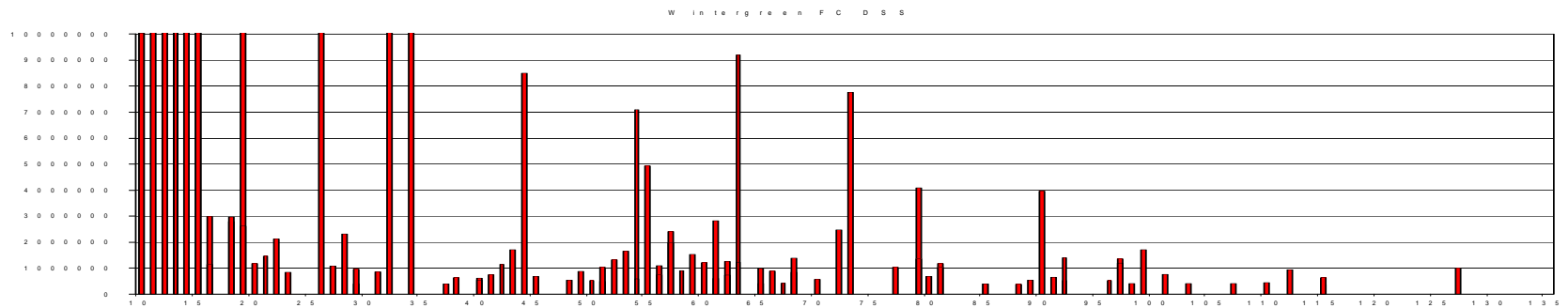
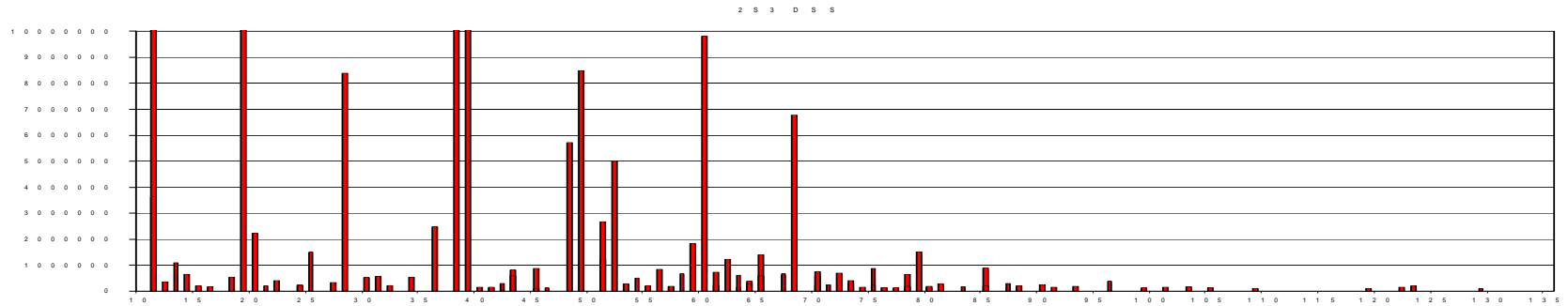
DS Scan - fruit-flavored samples



DS Scan - 2S3 and "other" flavor samples



DS Scan - 2S3 and wintergreen flavor samples



Conclusions

- We have extended use of both the HFP Scan and the DS Scan from reference samples of moist snuff to commercial, samples, including flavors other than tobacco and wintergreen
- Much work still needs to be done on interpretation of the mass spectra, particularly those from the DS Scan analyses
 - Many of the compounds not in mass spectra libraries
 - Some samples showed what appeared to be indicators of reactions of carbohydrates with other species
- We hope to apply these analytical techniques to other samples as resources permit