

Detailed chemical comparisons of new and old series reference smokeless tobacco products (STP)

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Abstract

Some health experts recommend that smokers, who refuse to quit or refuse to use nicotine replacement therapies, switch to low TSNA smokeless tobacco products (STP). U.S.-style moist snuff is the most popular STP, but has attracted criticism because of toxicological concerns. Use of *in vitro* assays (e.g., Ames; Rickert et al., Regul. Toxicol. Pharmacology 2009 53:121-33) to assess STP toxicity was of limited utility in distinguishing product types and brands within a type; and use of the *in vitro* mammalian GreenScreen HC assay (GADD45a-GFP reporter hosted in TK6 cells) (Lauterbach et al., Toxicol. Sci. 2010 114(1-S):2590) did not yield data that correlated with levels of known genotoxicants in the old series reference STP. However, the genotoxicity may stem from other sources such as sugar-amine reaction products as indicated by detailed GC-MS analyses of the STP. A new series of reference STP has just been released, and the moist snuff (CRP2 replaces 2S3), dry snuff (CRP3 replaces 1S2), and chewing tobacco (CRP4 replaces 2S1) products use the same recipes as did the earlier samples. While the routine analytical data on the new products are similar to those of the old, the detailed analytical data [GC-MS analyses (BSTFA/DMF extract of STP or MeOH extract of STP) were performed on an Agilent 6890 GC coupled with an Agilent 5972 MS with DB-5MS capillary GC column (25 m X 0.50 µm film thickness and 0.25 mm ID)], showed differences in sugars, organic acids, and marker compounds for sugar-amine reaction products. Our findings indicate that toxicological properties of the new reference STP and their performance on toxicological assays may not be the same as those of the older reference STP with likely higher genotoxicity (based on levels of marker compounds for sugar-amine reaction products) found in the CRP2 and CRP3 samples than in their older counterparts.

Introduction

A recent LSRO report, Differentiating the Health Risks of Categories of Tobacco Products, called for the use of smokeless tobacco reference products (STRP) to help characterize the health risks associated with the use of smokeless tobacco products (STP). However, the available STRP were outdated and a new series of STRP were manufactured under the auspices of the CORESTA Smokeless Tobacco Sub-Group.

Experimental

The objective of this work was to show that two experimental GC-MS scan techniques we have used for characterizing other tobacco products could be used to determine if there were differences between the old series of reference smokeless tobacco products (STRP) and the new series of STRP. The new series of STRP also included four products: CRP1, a pouched Swedish-style snus; CRP2, a US-style moist snuff; CRP3, a US-style dry snuff; and CRP4, a US-style loose-leaf chewing tobacco. The CRP2, CRP3, and CRP4 products were made using recipes and processes that were similar to the original STRP series as shown in the table below.

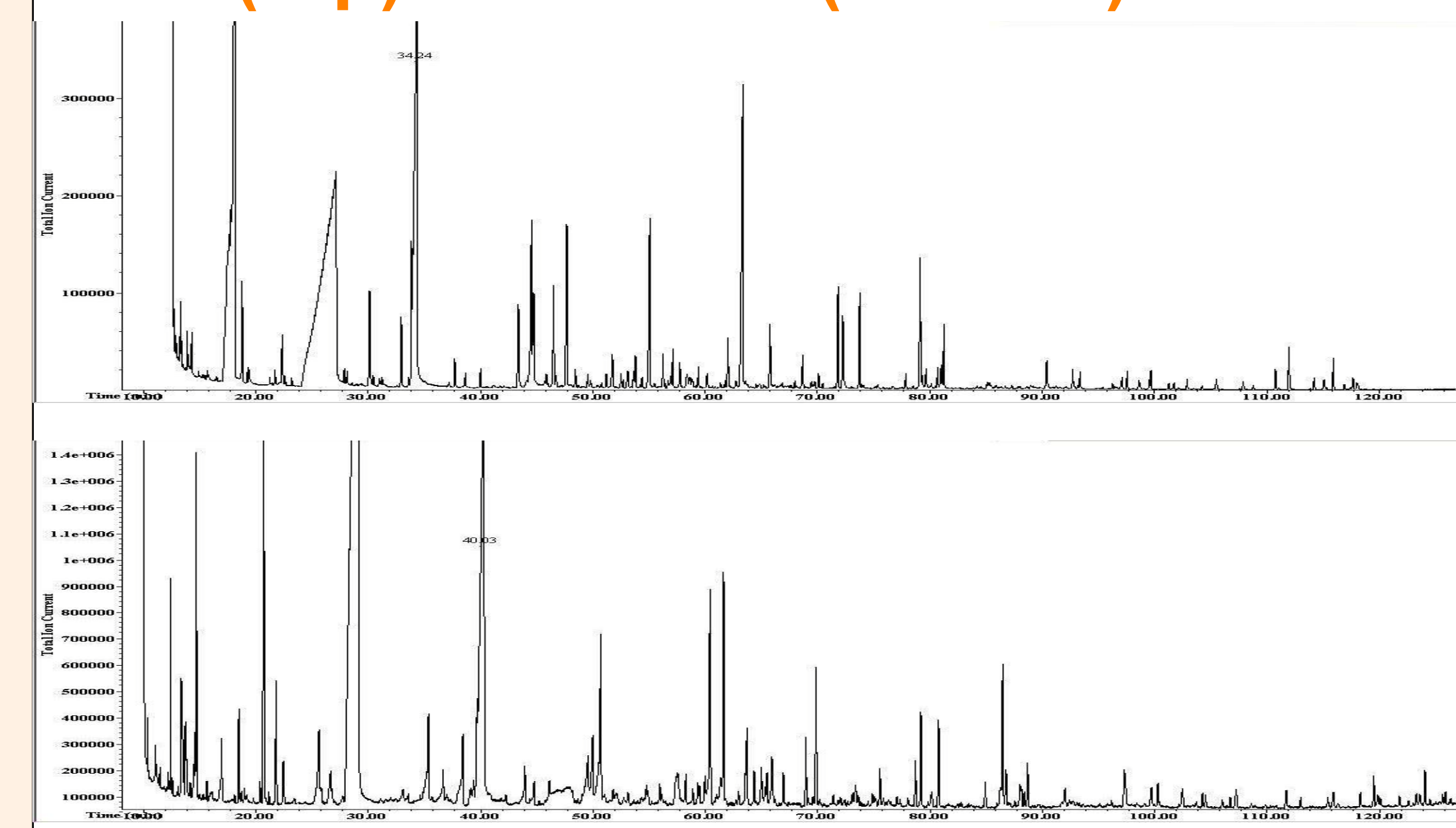
Designation	1S1	2S1	1S2	1S3	2S3
Product type	Loose-leaf chewing	Loose-leaf chewing	Dry snuff	Moist snuff	Moist snuff
Production date	2/86	8/98	2/86	1/86	8/98
Ingredient	%	%	%	%	%
Wisconsin air-cured tobacco	17.40	17.40			
Pennsylvania air-cured tobacco	15.47	15.47			
Dark fired tobacco			22.75	25.73	25.83
Air-cured tobacco				7.83	7.80
Fire-cured Virginia tobacco			19.86		
Air-cured stem			33.03		
Burley stem	5.80	5.80		3.73	3.72
Flue-cured stem			15.20		
Sodium chloride	1.60	1.60	0.36	7.40	7.32
Sodium carbonate				0.51	0.72
Sucrose	23.01	23.01			
Maltose	1.30	1.30			
Dextrose	1.70	1.70			
Other corn syrup solids	6.21	6.21			
Glycerin	3.75	3.75			
Sodium propionate	0.28	0.28			
Moisture	23.48	23.48	9.00	54.80	54.81

We used the same experimental procedures we reported at several previous meetings. The STRP samples were tray-dried at normal room conditions, ground, and then analyzed under conditions we have reported previously for our DS scan and HFP scan conditions (Lauterbach and Grimm, 2009)

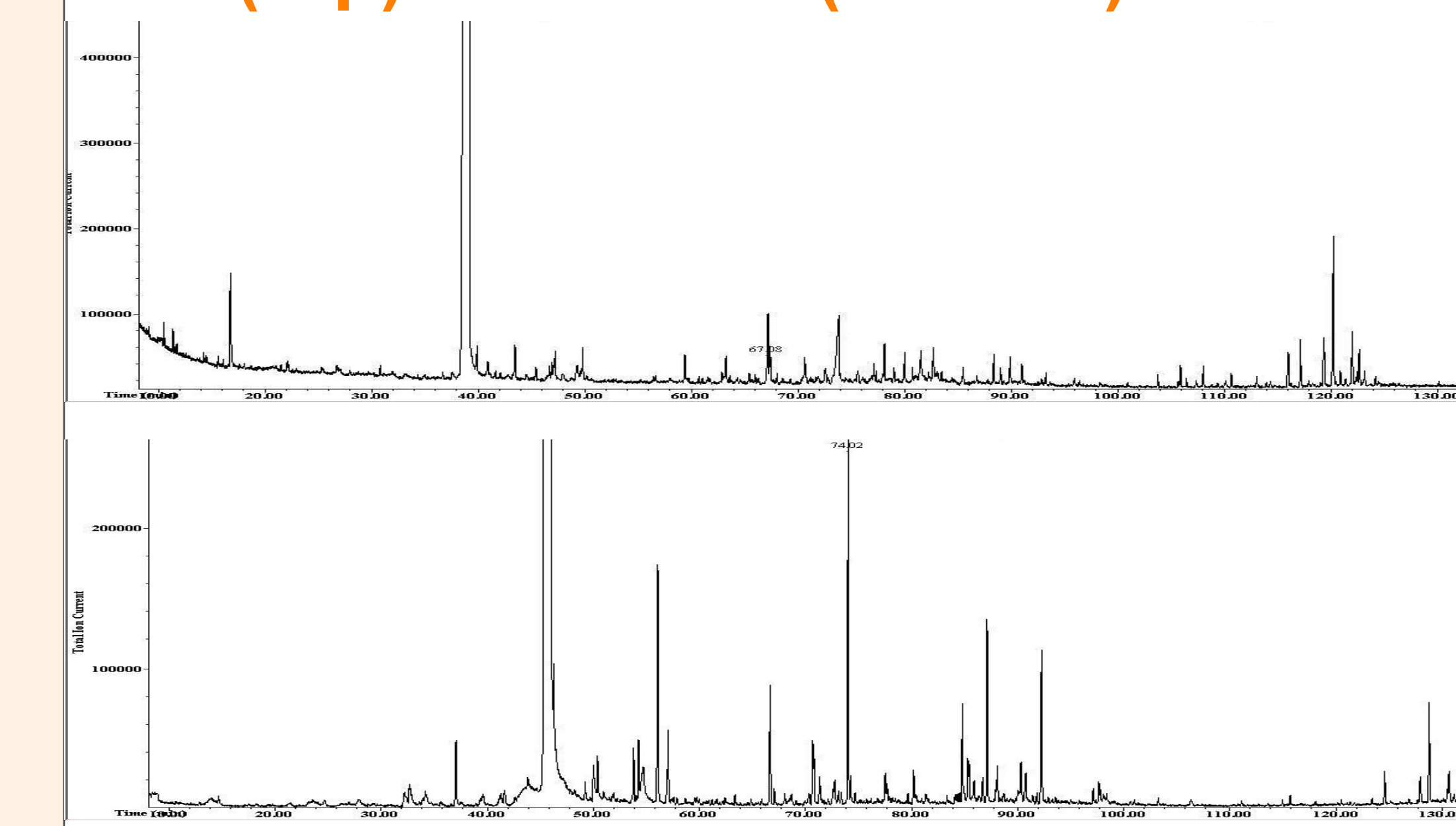
Results and discussion

Our results are shown in the TIC (total ion chromatogram) plots to the right of this panel. The TIC for each older style of STRP is shown directly above the TIC for its newer counterpart. The DS scan TICs are shown first followed by the ones for the HFP scan. While differences in retention times and peak shapes caused by column changes and amount of residual moisture in the samples, makes visual comparisons difficult in some cases, peak-by-peak comparisons using WSearchPro software, show differences between the TICs in each pair of chromatograms that are likely reflective of differences in sample age, leaf grades, and processing conditions. The 2S1 and 2S3 samples were produced in 1998 and the 1S2 sample was produced in 1986. Some of these changes may be indicative of changes in formation of Maillard compounds as indicated by levels of marker compounds for sugar-amine reaction products and may be reflected in genotoxicity of extracts the STRP.

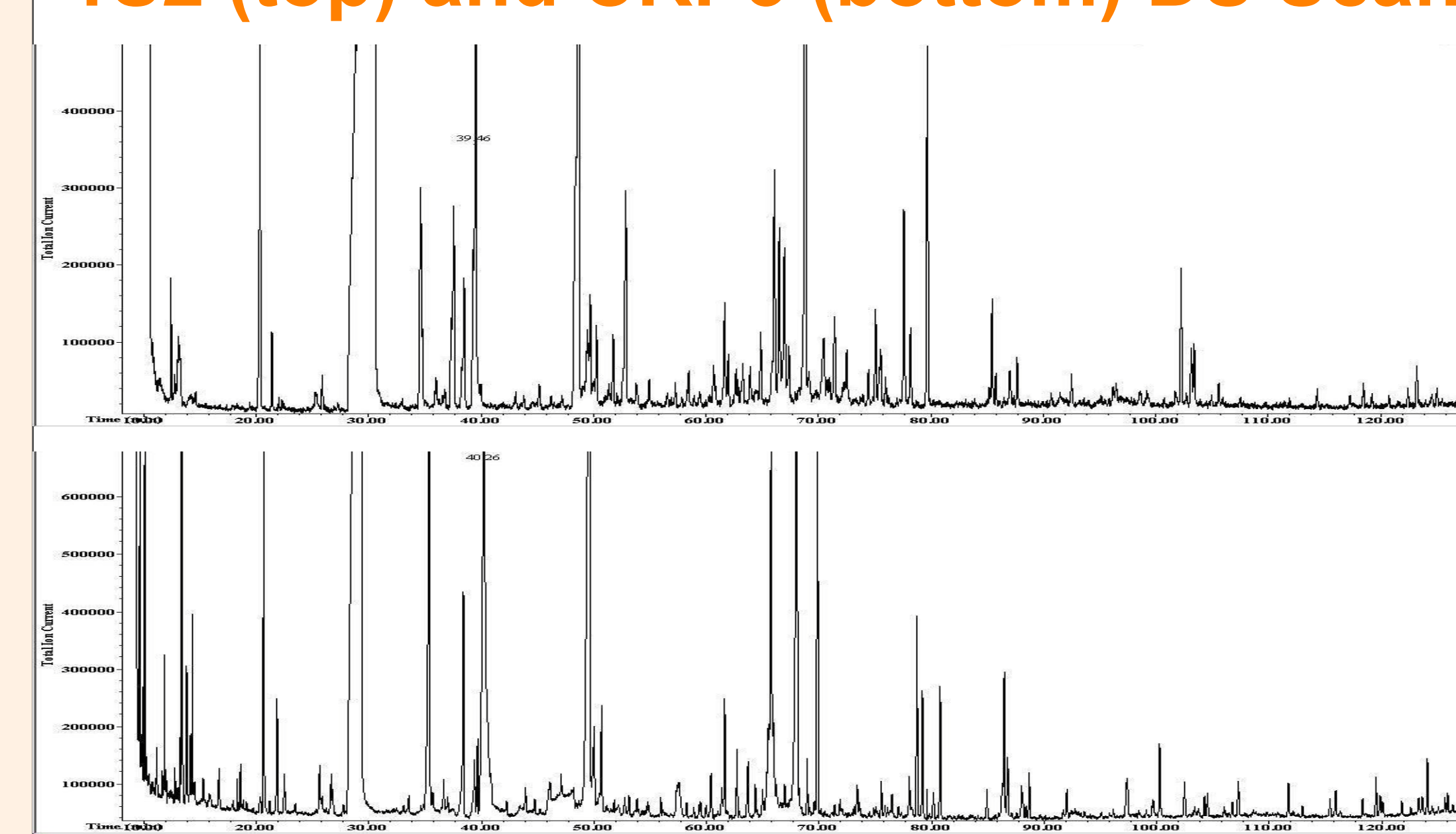
2S3 (top) and CRP2 (bottom) DS Scan



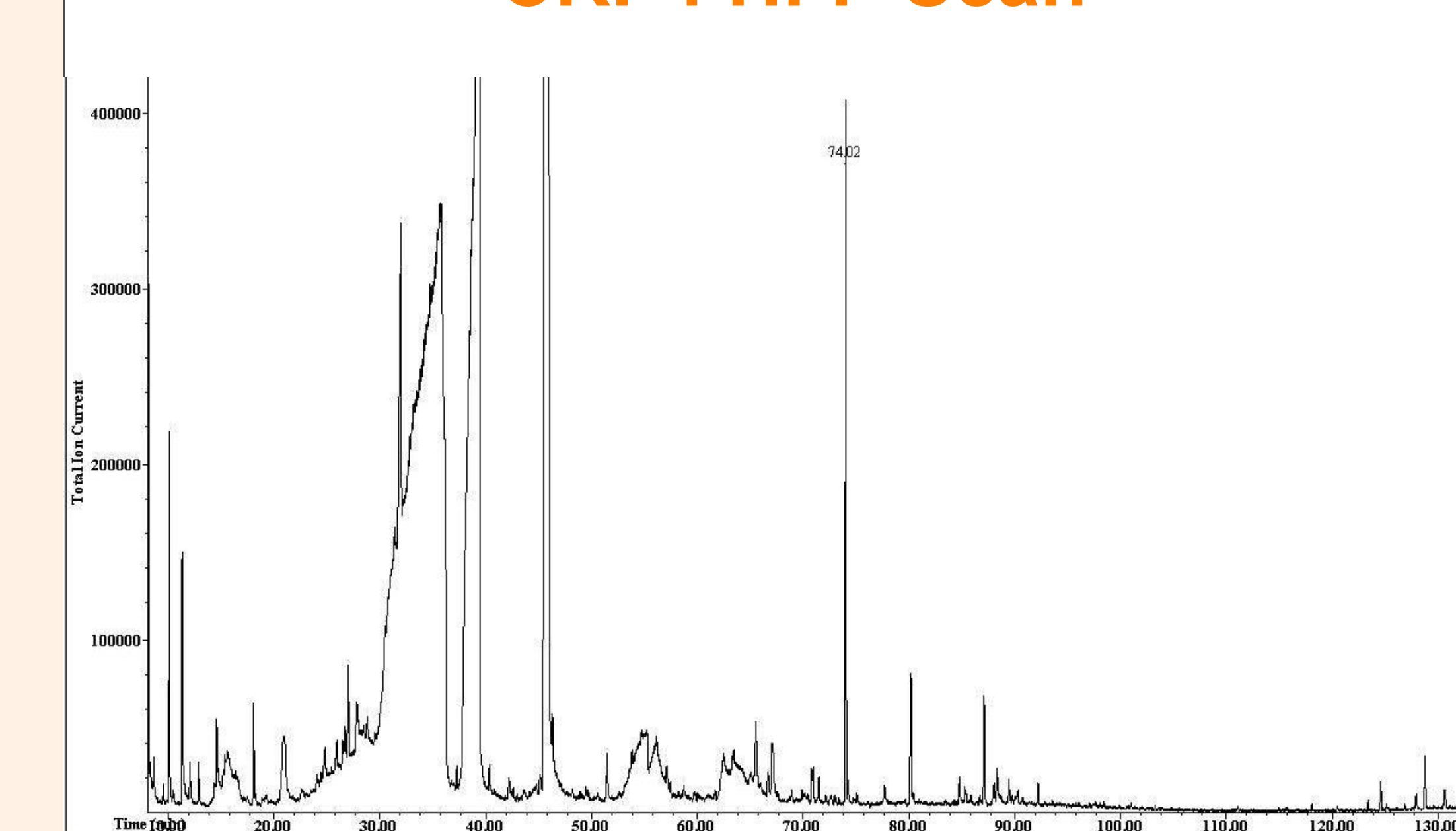
1S2 (top) and CRP3 (bottom) HFP Scan



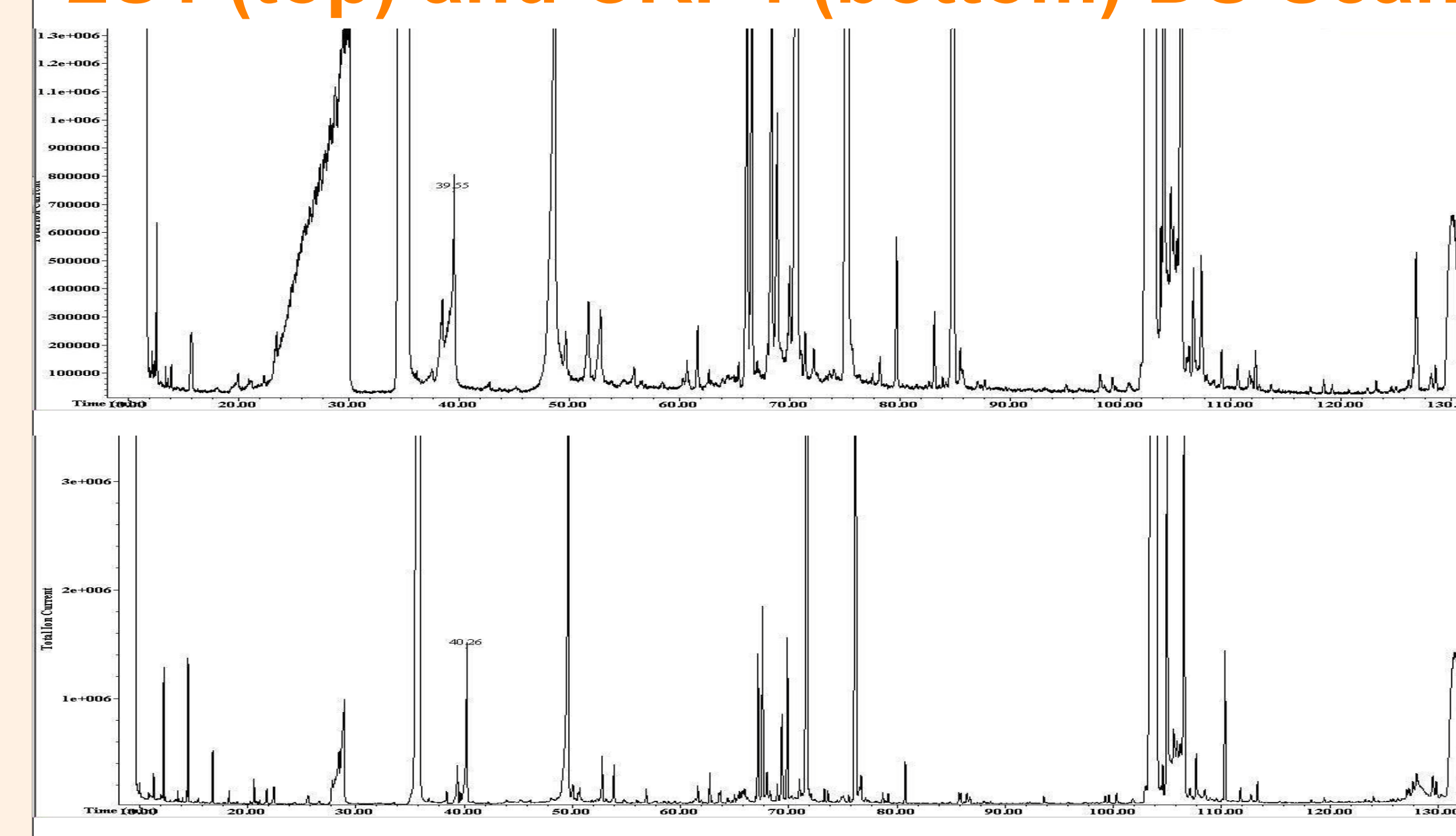
1S2 (top) and CRP3 (bottom) DS Scan



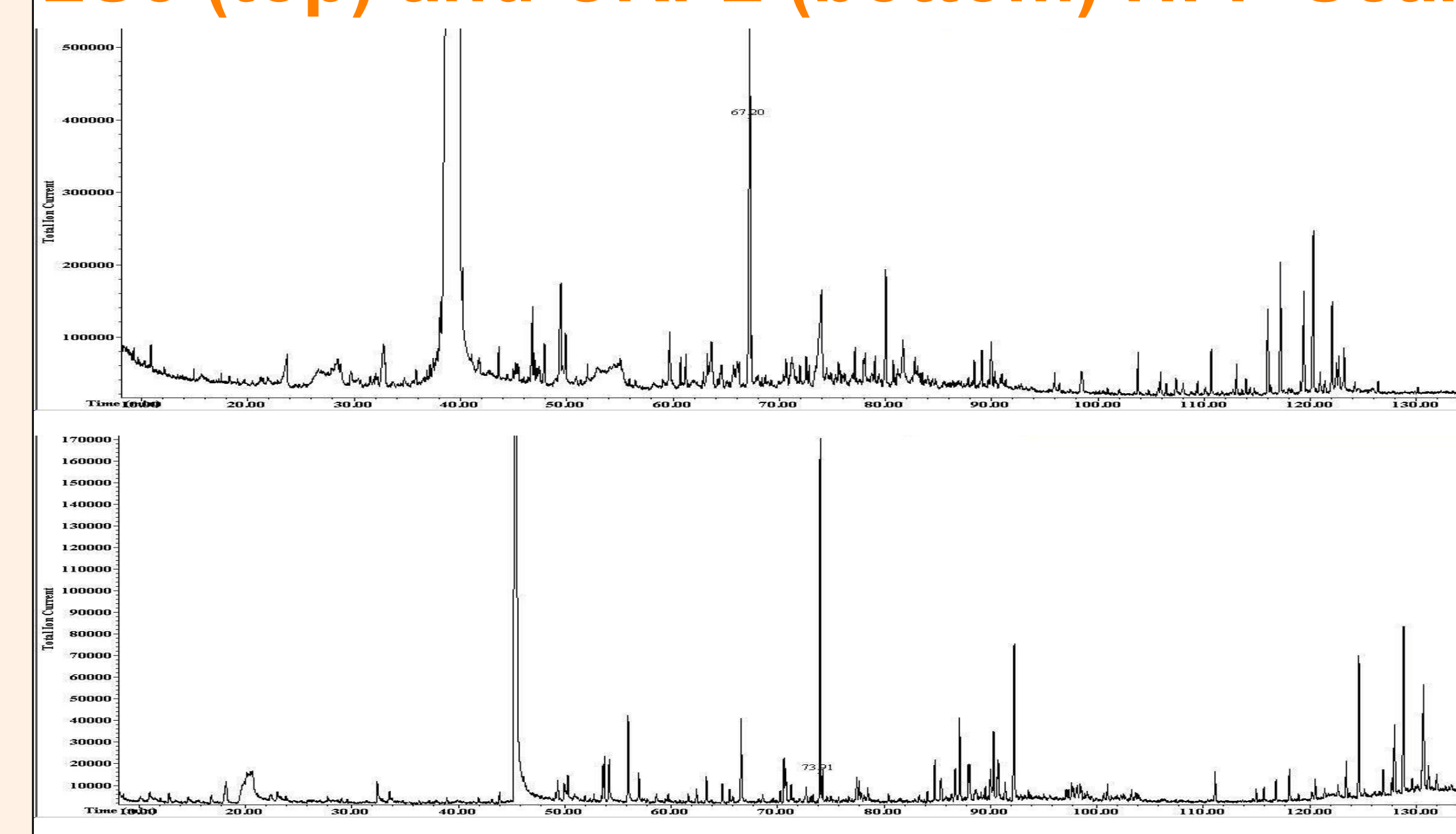
CRP4 HFP Scan



2S1 (top) and CRP4 (bottom) DS Scan



2S3 (top) and CRP2 (bottom) HFP Scan



Conclusions

The use of data from both the DS Scan and the HFP scan allows differentiation of smokeless tobacco products through both carbohydrates and acids (DS Scan) and through compounds soluble in methanol or HFP (HFP scan). The use of these two techniques showed that the new CRP products, although produced using recipes, leaf grades, and processes similar to original STRP are different and that such differences may be result in unexpected differences in the results from bioassays using the new CRP series of STRP.

Bibliography and literature cited

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