

Analysis of Flavor Chemicals by Simultaneous GC-MS and GC-FID

Abstract

The Department of the Treasury's Alcohol and Tobacco Tax and Trade Bureau (TTB) is responsible for regulating the use of ethanol in products manufactured in the United States, and collecting revenue generated from such use. One area that falls within TTB's jurisdiction is the regulation of nonbeverage products. Nonbeverage products are medicines, medicinal preparations, food products, flavors, flavoring extracts, and perfumes which are manufactured using tax-paid distilled spirits, and which are unfit for beverage purposes. One such way to ensure that products are unfit for beverage purposes is for the manufacturer to add specific flavor agents to the ethanol base at levels determined by TTB.

A quantitative method¹ was previously developed to determine the concentration of various chemicals commonly used in flavors using gas chromatography (GC) coupled to both a mass spectrometer (MS) and a flame ionization detector (FID). The focus of the present study was to determine if additional compounds of interest could be successfully quantitated utilizing the same acquisition parameters. Target analytes studied included (-)-Borneol, 1-Hexanol, 1-Heptanol, 1-Octanol, 2-Octanol, 1-Octen-3-ol, 1-Nonanol, cis-6-Nonen-1-ol, 1-Decanol, 1-Dodecanol, Geraniol, and (E)-trans-Isoeugenol. The internal standard (ISTD) used for quantitation was 2-nonanol. Accuracy was determined, with recoveries above 95% for most of the compounds at the three levels tested.

Introduction

Domestic manufacturers who use distilled spirits in manufacturing nonbeverage products are eligible for drawback of most of the Federal excise tax paid on those spirits. A manufacturer wishing to receive drawback, in most instances, must first obtain formula approval from TTB.

It is TTB's responsibility to verify that these flavors and flavoring extracts are indeed nonbeverage products through examination of formulas and analysis of samples. When this examination reveals that a product is fit for beverage purposes, the formula is disapproved. Thus drawback of tax is denied. Denial of drawback can result in the loss of thousands of dollars for manufacturers.

The goal of this study is the quantitative determination of 12 commonly used flavoring agents. We investigated the use of a microfluidic two-way splitter. The splitter divides the effluent from the chromatographic column between two different detectors, a mass selective detector (MSD) and a flame ionization detector (FID). This splitter also allows backflushing, a procedure which reverses the column flow in the post-run to flush high-boiling contaminants or uneluted compounds out of the column via the split vent trap.

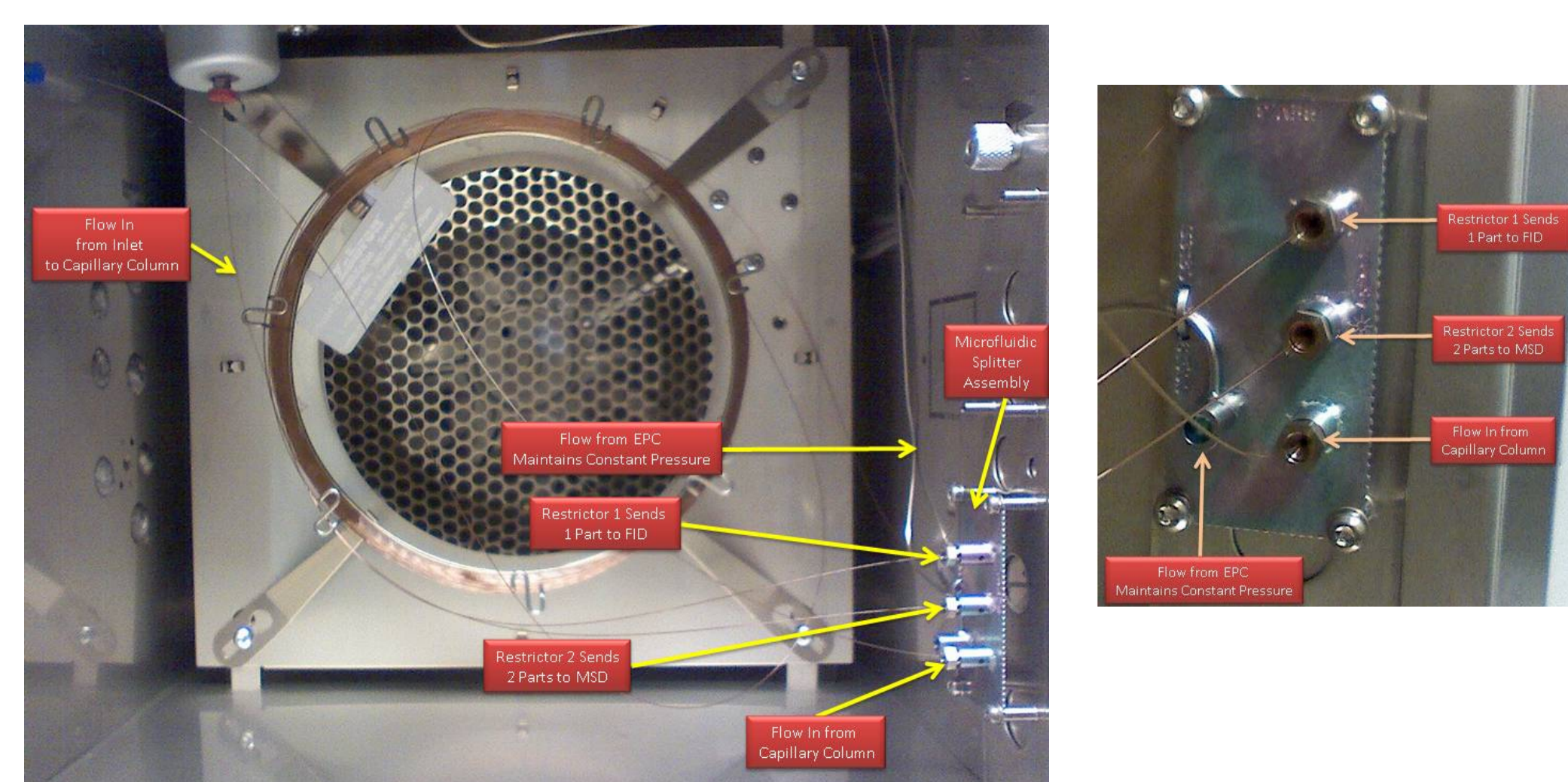


Figure 1. Two-way splitter for GC/MS/FID. Effluent split between 2 detectors and backflush allowed

Table 1. Flavoring agents[†]

Chemical Name	Retention Time	R ² from MSD*	R ² from MSD*
2-Nonanol (ISTD)	23.603	-----	-----
1-Hexanol	16.869	0.999	0.998
2-Octanol	19.638	0.998	0.998
1-Octen-3-ol	20.783	0.998	0.999
1-Heptanol	21.003	0.999	0.999
1-Octanol	25.041	0.999	0.998
1-Nonanol	28.922	0.999	0.999
(-)-Borneol	30.074	0.998	0.998
cis-6-Nonen-1-ol	30.940	0.999	0.997
1-Decanol	32.628	0.999	0.998
Geraniol	35.506	0.998	0.998
1-Dodecanol	39.571	0.998	0.997
(E)-trans-Isoeugenol	50.409	0.997	0.994

[†] All chemicals purchased from Sigma Aldrich (St. Louis, MO).

*R² calculated from 7 Point calibration Curve, Concentration Levels: 5 – 125 ppm

Table 2. Experimental conditions

Gas chromatograph	Agilent 6890
Autosampler	Gerstel MPS 2
Autosampler mode	Liquid
Injection volume	1 µL
Inlet	250 °C; 5:1 split
Oven profile	40 °C 2'; 3 °C/min 240 °C; 1.0'
Post run	240 °C for 5 min
Backflush inlet flow	0.1 mL/min
Backflush pressure splitter	60 PSI
Column	Phenomenex ZB-WAXplus
Column dimensions	30 m x 0.25 mm x 0.25 µm
Pressure at splitter	20 PSI
Restrictor 1 (to FID)	0.18 µm ID ; 2.128 m
Restrictor 2 (to MSD)	0.18 µm ID ; 2.886 m
Mode	Constant flow 1 mL/min
MSD Detector	Agilent MSD 5975 Inert
FID Temperature	300 °C
FID Detector flows	H ₂ 30 mL/min, air 400 mL/min
Solvent delay-(MSD)	4.67 min
Scan-(MSD)	30-300 amu

A. Standards Preparation

Each analyte in Table 1 was diluted with methanol to a concentration of 1% (wt/wt). A stock solution containing each analyte at 750 ppm was created by diluting the 12 individual 1% solutions with methanol. Seven calibration levels (5, 10, 25, 50, 75, 100 and 125 ppm) were prepared by diluting the stock solution with methanol.

B. Internal Standards Preparation

A stock solution containing three internal standards at 300 ppm each was prepared by diluting Deuterated Ethyl Butyrate-4,4,4-d₃; 2-Nonanol; and 3',4'-(Methylenedioxy)acetophenone with methanol. For this study only 2-Nonanol was used to quantify the target analytes.

C. Preparation of solutions for chromatographic analysis

1 ml of each of calibration level standard solution was transferred to a GC vial with a micropipette, followed by the addition of 200 µL Internal Standard Stock Solution.

Figure 2. Total Ion Chromatogram (for 125 ppm standard, 1 µl injection).

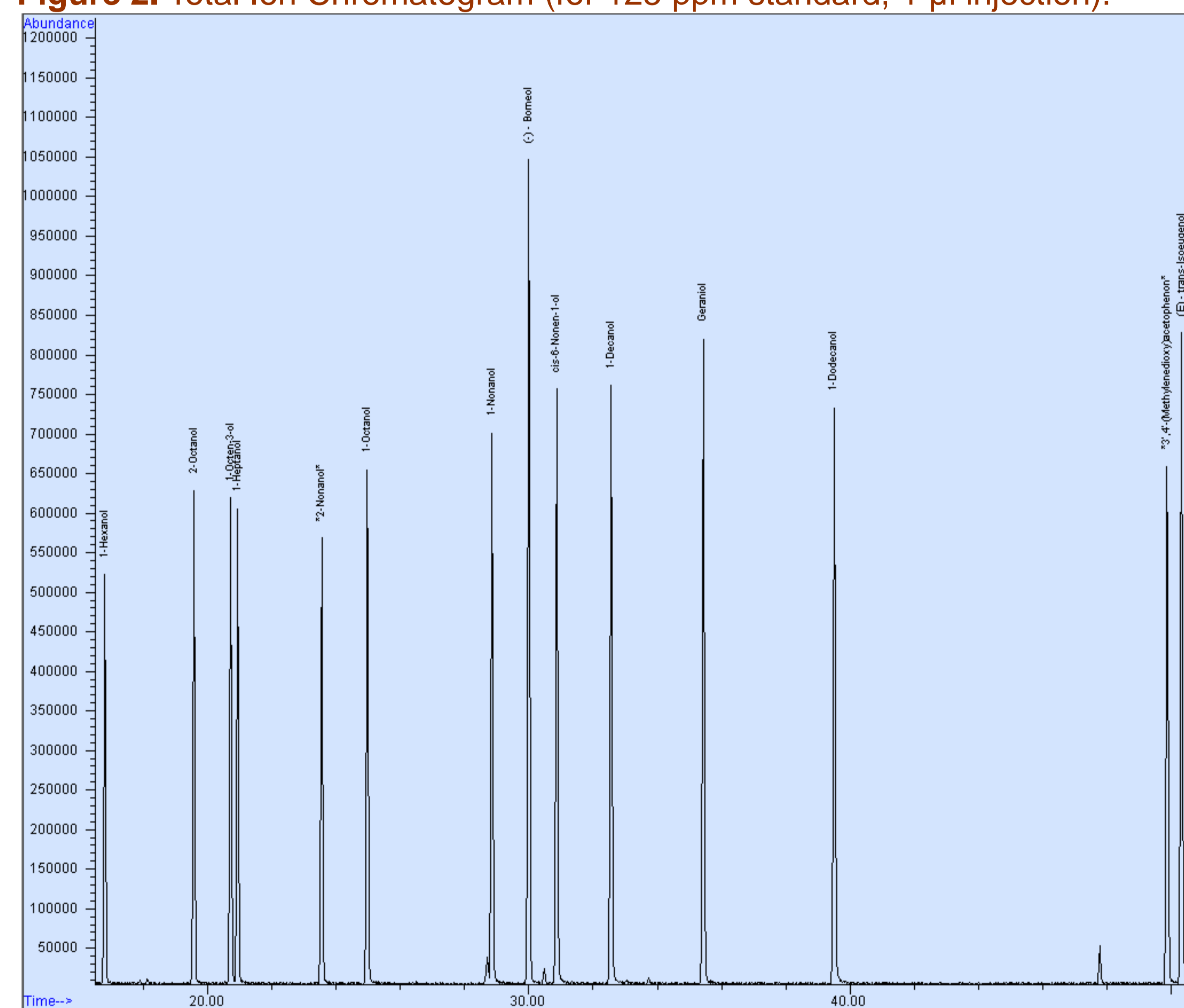


Figure 3. FID Chromatogram (for 125 ppm standard, 1 µl injection).

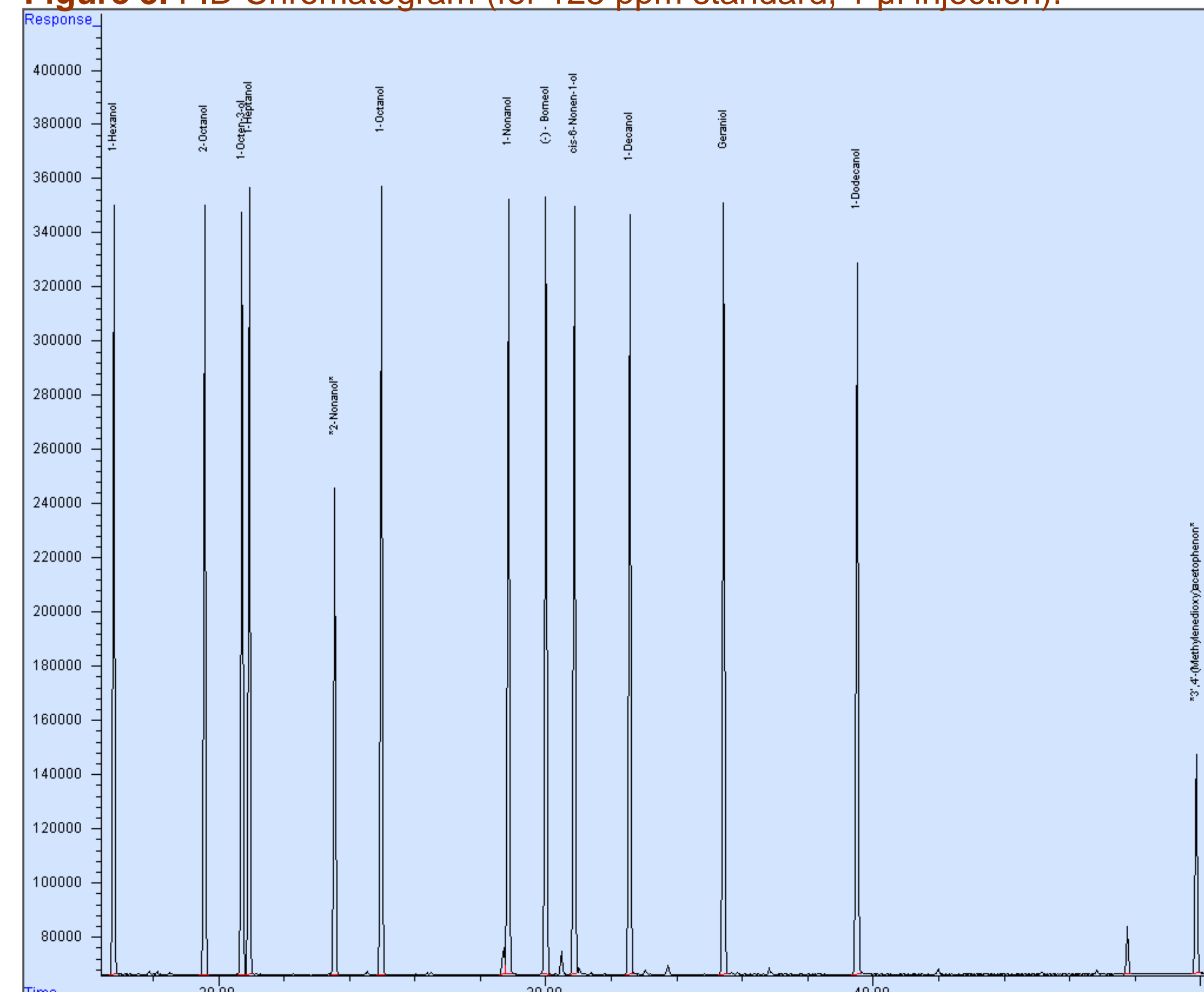
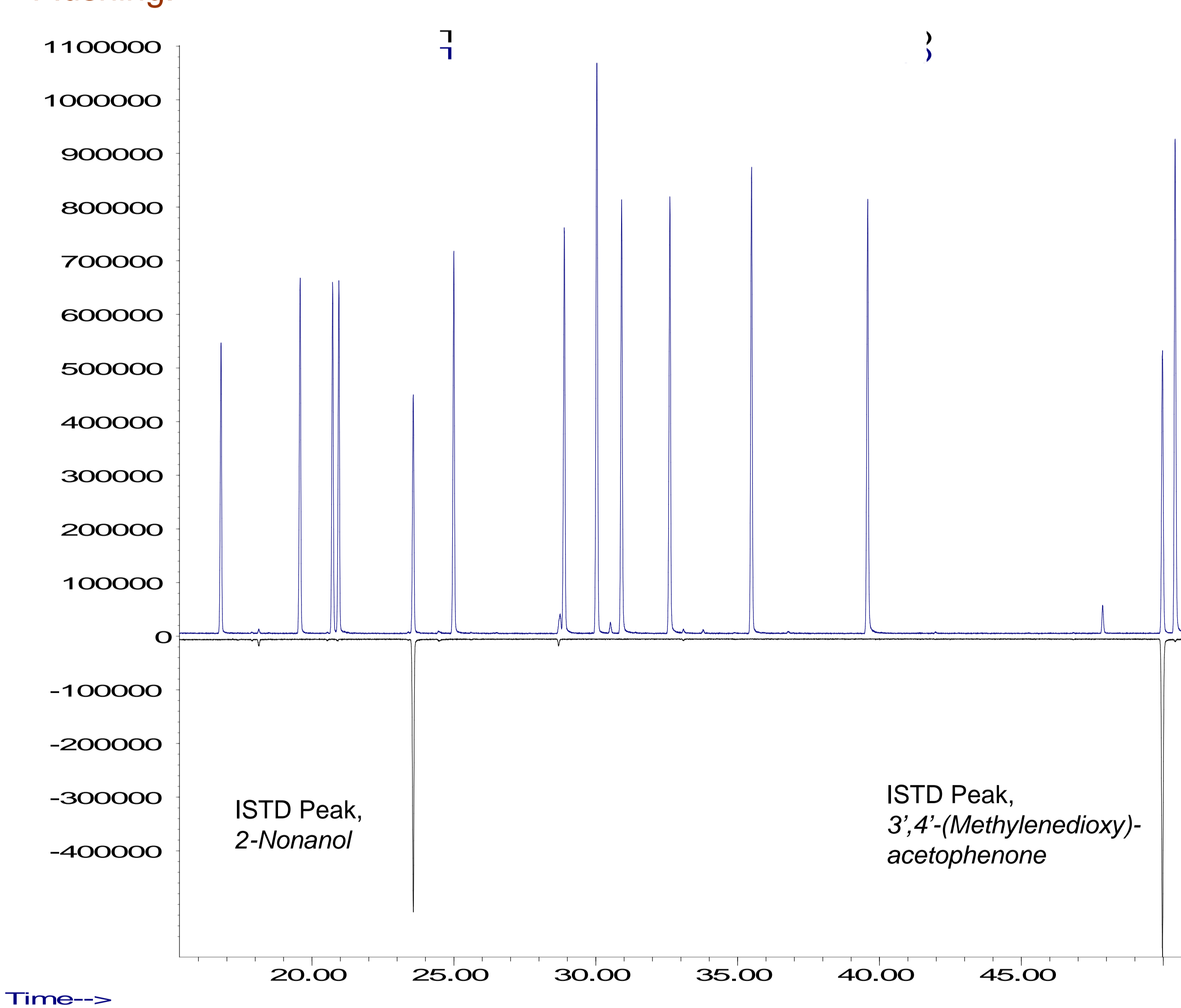


Table 3. Accuracy by FID & MSD – Average of 3 Responses

Actual Amounts:	10 PPM Level		50 PPM Level		100 PPM Level	
	Reported FID Amount	Reported MSD Amount	Reported FID Amount	Reported MSD Amount	Reported FID Amount	Reported MSD Amount
1-Hexanol	9.60	9.44	48.41	47.87	94.88	94.72
2-Octanol	9.76	9.95	48.57	47.60	95.02	94.70
1-Octen-3-ol	9.78	9.87	48.65	48.11	95.07	95.49
1-Heptanol	9.54	10.16	48.35	48.53	94.80	95.44
1-Octanol	9.64	12.31	48.42	49.18	94.93	94.62
1-Nonanol	9.46	9.51	48.53	48.46	94.88	95.93
(-)-Borneol	9.73	9.69	48.66	48.71	94.99	94.83
cis-6-Nonen-1-ol	9.68	10.10	48.60	47.19	95.19	93.59
1-Decanol	9.68	10.20	48.58	47.52	94.92	95.34
Geraniol	9.82	9.89	48.80	46.63	94.91	94.84
1-Dodecanol	9.70	10.54	48.63	46.26	94.77	94.51
(E)-trans-Isoeugenol	10.28	12.98	48.66	47.19	95.50	92.09

Figure 4. Total Ion Chromatogram of 125 ppm standard, 1 µl injection mirrored against subsequent 1 µl injection of Blank with ISTD; Post-Back Flushing.



Conclusions and future work

- Excellent linearity over the working range of 5 – 125 ppm for all compounds was observed using both the FID and MSD.
- Good accuracy was obtained for all 12 flavor compounds in both detectors with 1 µL injections and splitting 2 parts to MSD and 1 part to FID.
- The ability to accurately quantify several more compounds by the previously developed method has been shown.
- The use of back-flushing during the post-run time eliminated carry-over issues; no target analytes were detected on the subsequent blank injection immediately following the high standard.
- Future work must be done to investigate the potential of combining the target analytes from this study with those of the previous study.

References

1. Kinton, V. R., Limowski, E. R., Scalse, J. M. (2009). Quantitation of Flavoring Agents. 238th National ACS Meeting, Washington, DC.