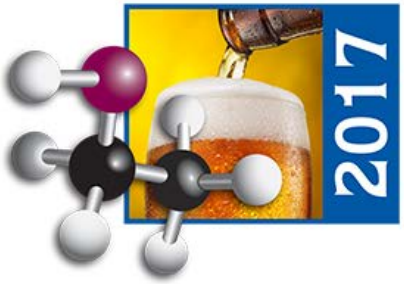


ASBC Annual Meeting
June 4–7 ■ Fort Myers, Florida
See what SCIENCE can brew for you

What You Can Do With a Gas or Liquid Chromatograph in a Brewing Lab?

Joe Palausky
Quality Laboratory Manager
Boulevard Brewing
Kansas City, Missouri

The Black Box

Chromatography 1st Questions

- What do I want to look for?
- Gas Phase or Liquid Phase?
- Qualitative or Quantitative? Presence/Absence or Quality Control?

Functionality or Style?

ASBC Methods of Analysis

- The Good
  - Tested by multiple laboratories
  - Precision/Accuracy established

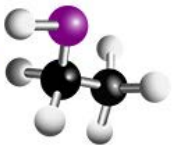
- The not so Good
  - Technology changes
  - New compounds of interest

ASBC HPLC Methods

ASBC Method	Test	Detector
Sugars and Syrups 17-B	Fermentable Saccharides	RI
Sugars and Syrups 18	Fermentable Carbohydrates	RI
Hops 9-C/D	Iso alpha-acids	UV
Hops 14	alpha- acids and beta- Acids in Hops and Hop Extracts	UV
Hops 15	Iso alpha-acids in Isomerized Hop Pellets	UV
Hops 16	Iso-alpha, alpha - and beta-Acids in Hop Extracts and Isomerized Hop Extracts	UV
Wort 14-B	Fermentable Saccharides	RI
Wort 19	Fermentable Carbohydrates	RI
Wort 22	Wort and Beer Fermentable and Total Carbohydrates	RI
Wort 23-C	Iso alpha-Acids in Wort	UV
Beer 23 –C	Iso alpha-Acids	UV
Beer 41	Total Carbohydrate	RI

ASBC GC Methods

ASBC Method	Test	Detector
Barley 11/ Malt 13	Deoxynivalenol	Electron Capture
Malt 14	Dimethyl Sulfide Precursor	Flame Photometric/Sulfur Chemiluminescence
Sugars and Syrups 17-A	Fermentable Saccharides	Flame Ionization
Hops 17	Hop Essential Oils	Flame Ionization
Wort 14-A	Fermentable Saccharides	Flame Ionization
Beer 4 -D	Alcohol	Flame Ionization
Beer 25-F	Diacetyl	Electron capture
Beer 29	Lower Boiling Volatiles	Flame Ionization
Beer 40	N-Nitrosoamines	Thermal Energy/Hall
Beer 44	Dimethyl Sulfide	Sulfur Chemiluminescence
Beer 48	Beer Volatiles	Flame Ionization



Fermentable Saccharides by ASBC Wort Method 14B-HPLC

Method

Calibration standard: Dissolve 1.0000 g glucose (re- agent d), 0.1000 g fructose (reagent f), and 3.0000 g *anhydrous* maltose (reagent e) in water and dilute to 50 mL. (Actual amount of maltose used must be calculated from moisture content and purity of the particular lot.)

Worts: Dilute worts to approximately 12°P, if necessary.

To 10.0 mL wort or calibration standard, add 200 ± 5 mg AG 501-X8 (reagent c) and shake 15 min on a wrist- action shaker. Prepare Sep-Pak Classic C_{18} cartridge (app. d) by pumping 2 mL water through the cartridge with a syringe. Fit syringe with the Swinnex containing the 0.45-µm filter pad and follow with the Sep-Pak. Filter the wort or calibration standard through this combination. Discard the first few mL and retain the remainder for analysis.

Operating conditions for HPLC:

Column temperature:	85°C (Insulate connectors and fittings to help maintain a uniform temperature into and out of the column. Connection to the refractometer detector should be as short as possible.)
Column eluent:	Distilled, deionized, Millipore- filtered, degassed water. (Best results are obtained by heating the water to near boiling for de-gassing and maintaining 85°C or higher in the reservoir.)
Refractometer temperature:	Ambient
Flow rate:	0.2 mL/min
Sample size:	10 µL for calibration standard and each wort sample
Refractometer attenuation:	Set by the analyst

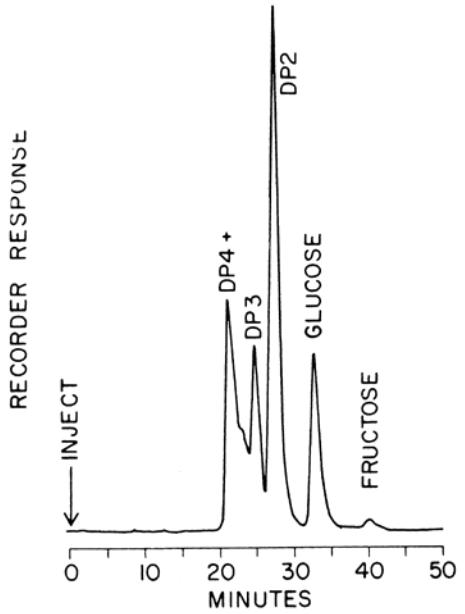


Fig. 2. Sample chromatogram for liquid chromatography (HPLC) of wort fructose, glucose, and di- and trisaccharides, with degree of polymerization indicated by "DP2" (primarily maltose) and "DP3" (primarily maltotriose).

Fermentable Saccharides by ASBC Wort Method 14A-GC

Preparation of worts: Pipet 0.5 mL wort into 10-mL serum bottle, add one drop ammonium hydroxide (reagent j), and freeze-dry. To the freeze-dried sample, add 40 mg Drierite (reagent i), 3.0 mL internal standard solution, 0.9 mL hexamethyldisilazane (reagent b), and 0.1 mL trifluoroacetic acid (reagent c). Shake for 1 h on wrist-action shaker. Allow Drierite to settle and transfer supernatant to another vial.

Chromatograph the calibration standard three times and the wort samples once using the following conditions:

Injection port temperature:	325°C
Detector temperature:	350°C
Column temperature:	150–325°C at 10°C/min, hold 10 min
Carrier gas: helium or nitrogen at	40 mL/min
Sample size:	10 µL

Measure peak areas for α-fructose and β-glucose, internal standard sucrose, and α- and β-maltose for the standard chromatograms. For worts, measure the previous and also α- and β-isomaltose and α- and β-maltotriose. Approximate retention times are shown in Fig. 1. Sum the areas of α- and β-maltose, α- and β-maltotriose, and α- and β-isomaltose.

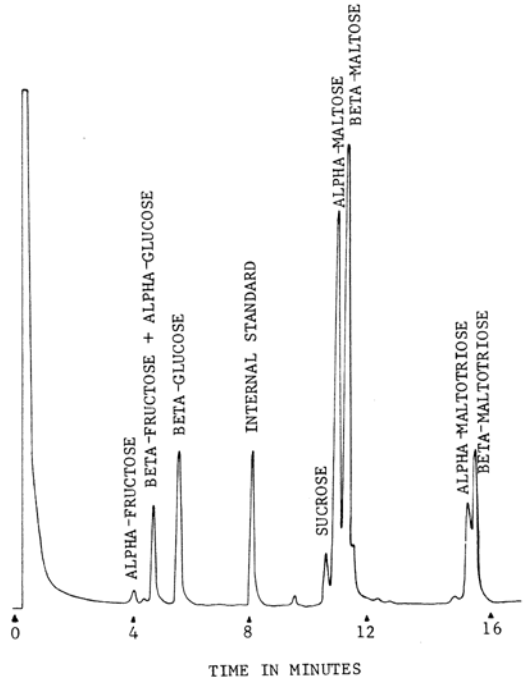
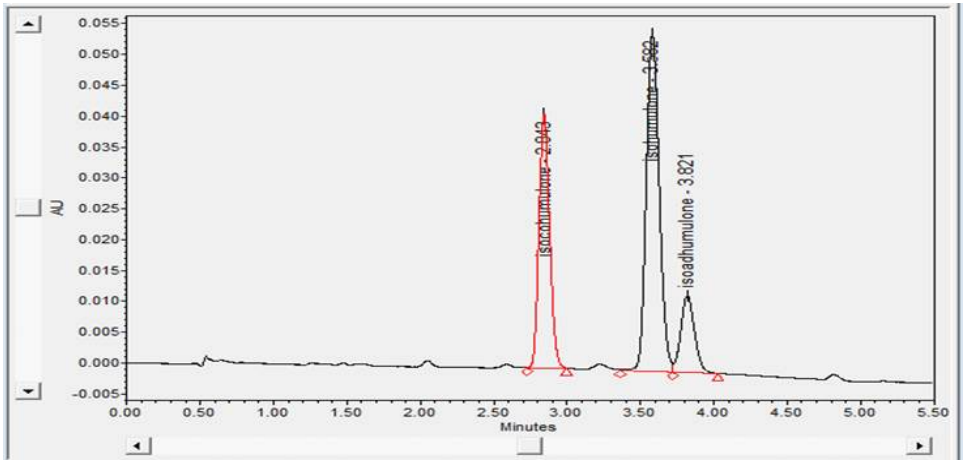


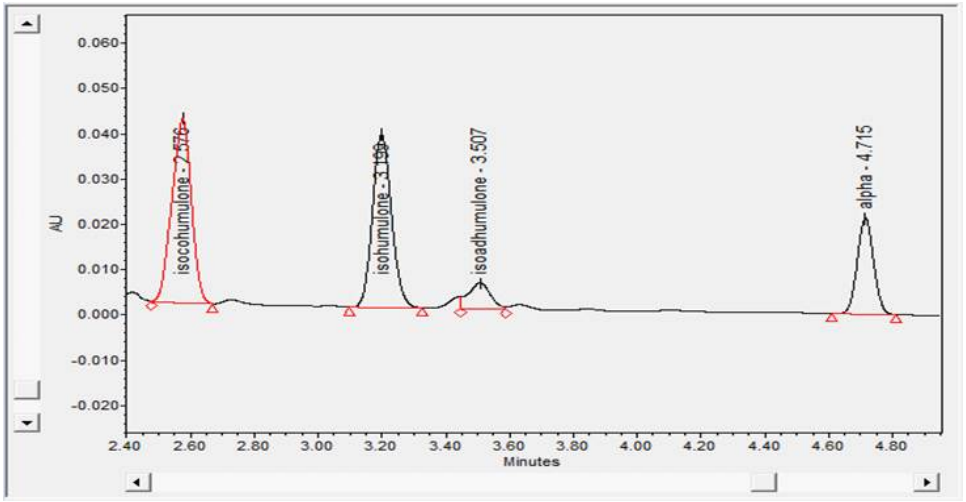
Fig. 1. Sample chromatogram from gas chromatography (GC) of trimethylsilyl derivatives of fermentable mono-, di-, and trisaccharides of wort, showing approximate retention times.

Beer 23C, Iso-Alpha Acids- uHPLC

Iso-Alpha Standard



Iso-Alpha Acids Profile in 40 IBU Brand



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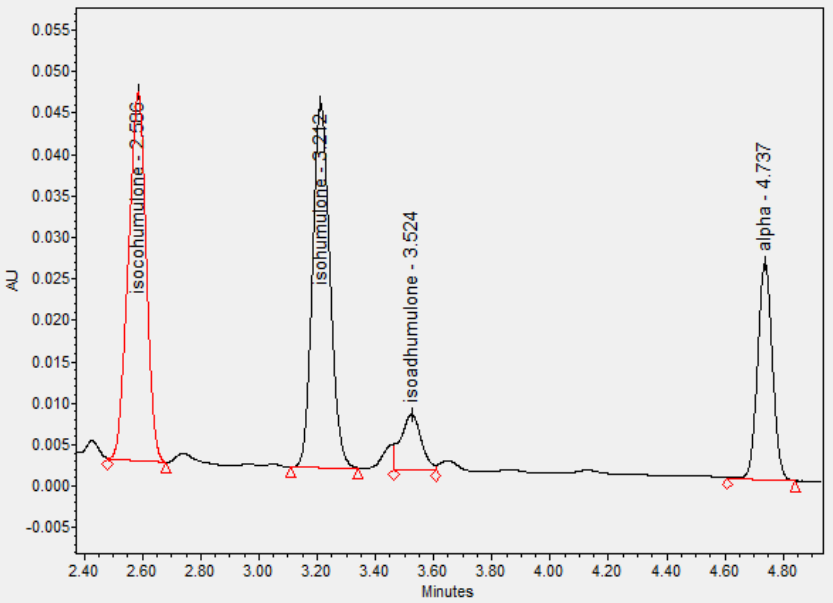
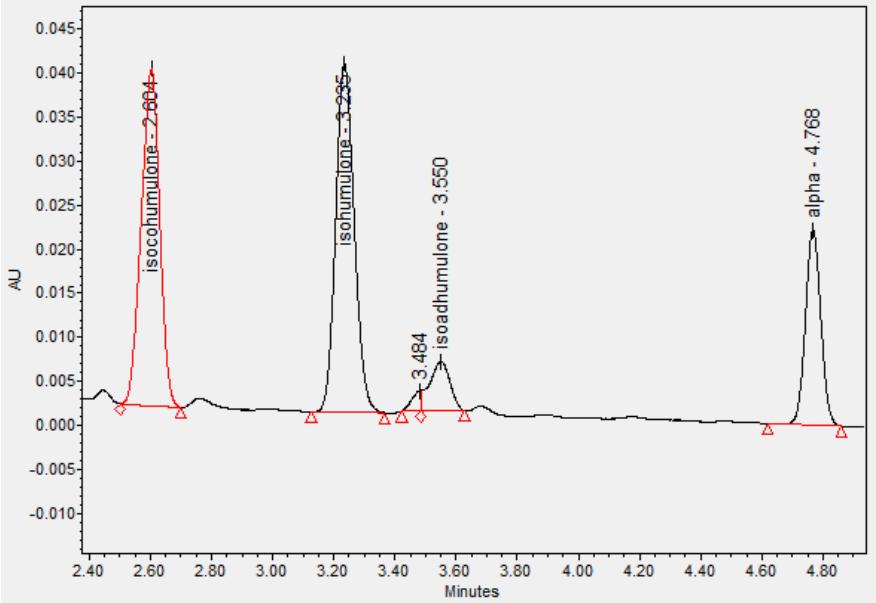
Beer 23C, Iso-Alpha Acid Aging



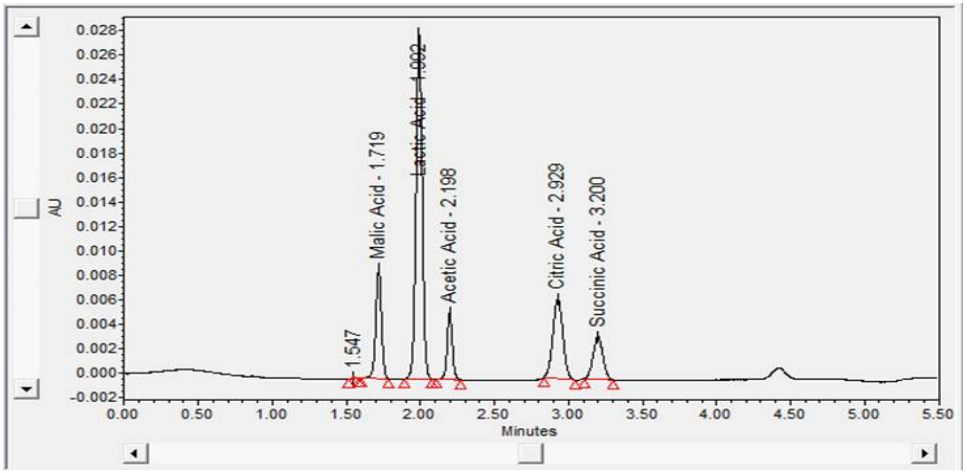
Fresh 40 IBU/25 IAA sample



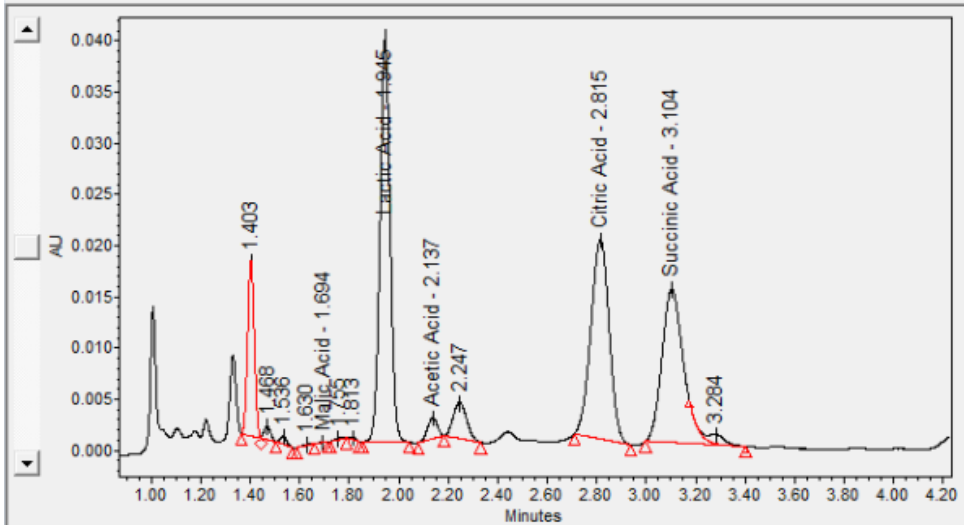
Same sample, Warm 30 days/Cold 30 days

Organic Acids - uHPLC

Organic Acids Standard

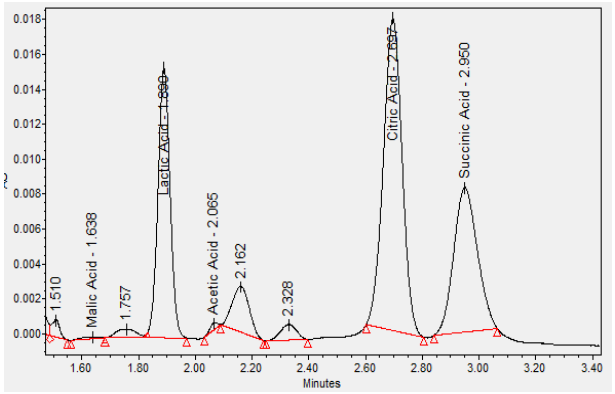


Organic Acids Profile in Aged Foeder

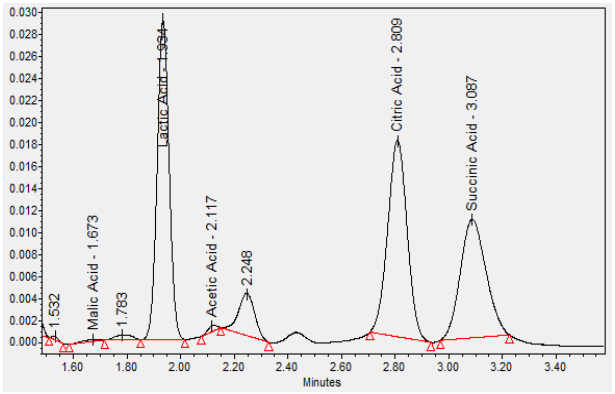


Organic Acids Aging

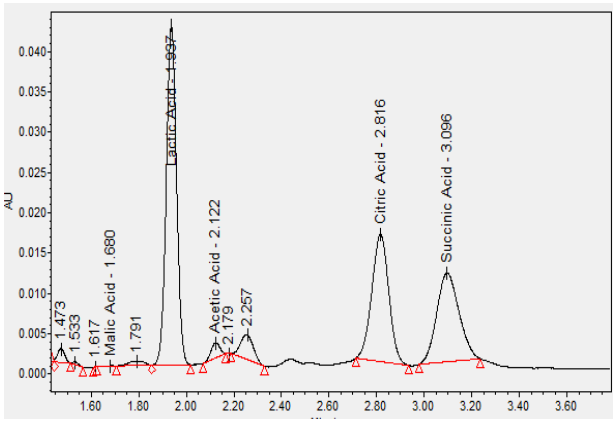
1 Month



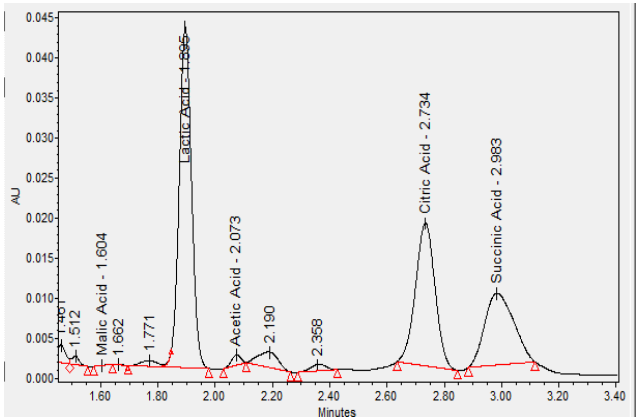
4 Months



8 Months



12 Months



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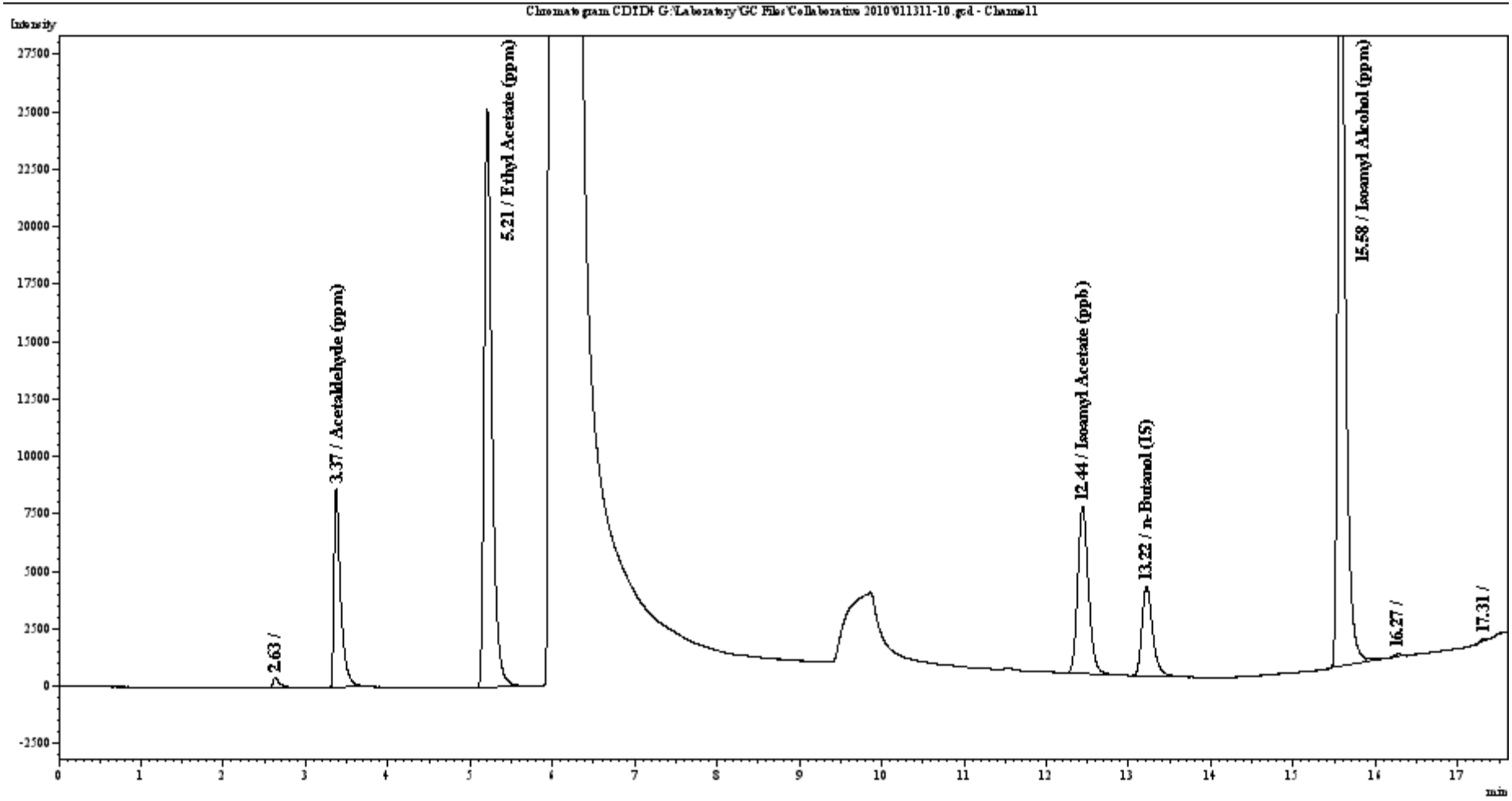
Beer 48, Beer Volatiles – FID Standard



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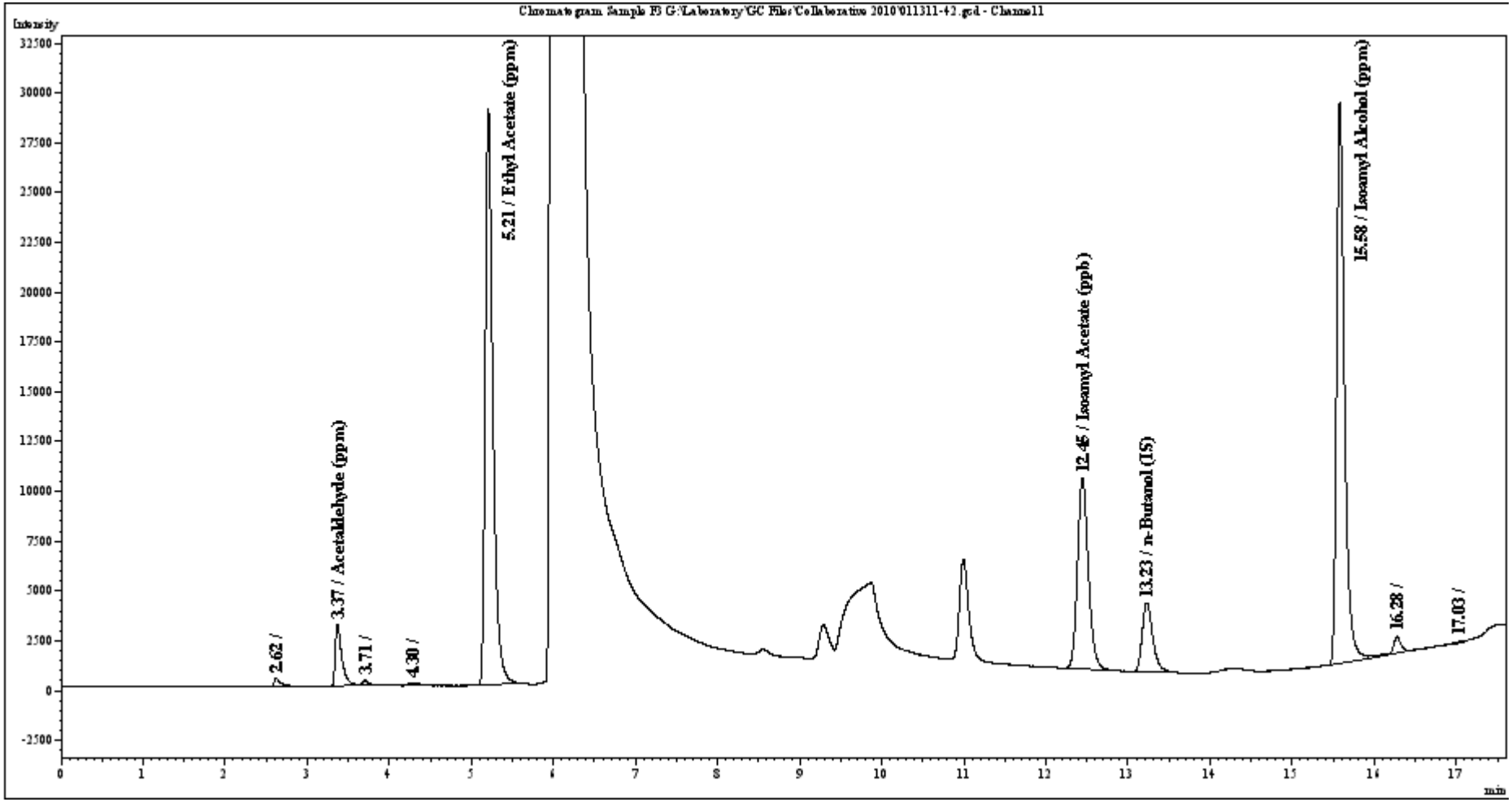
Beer 48, Beer Volatiles –Beer Sample



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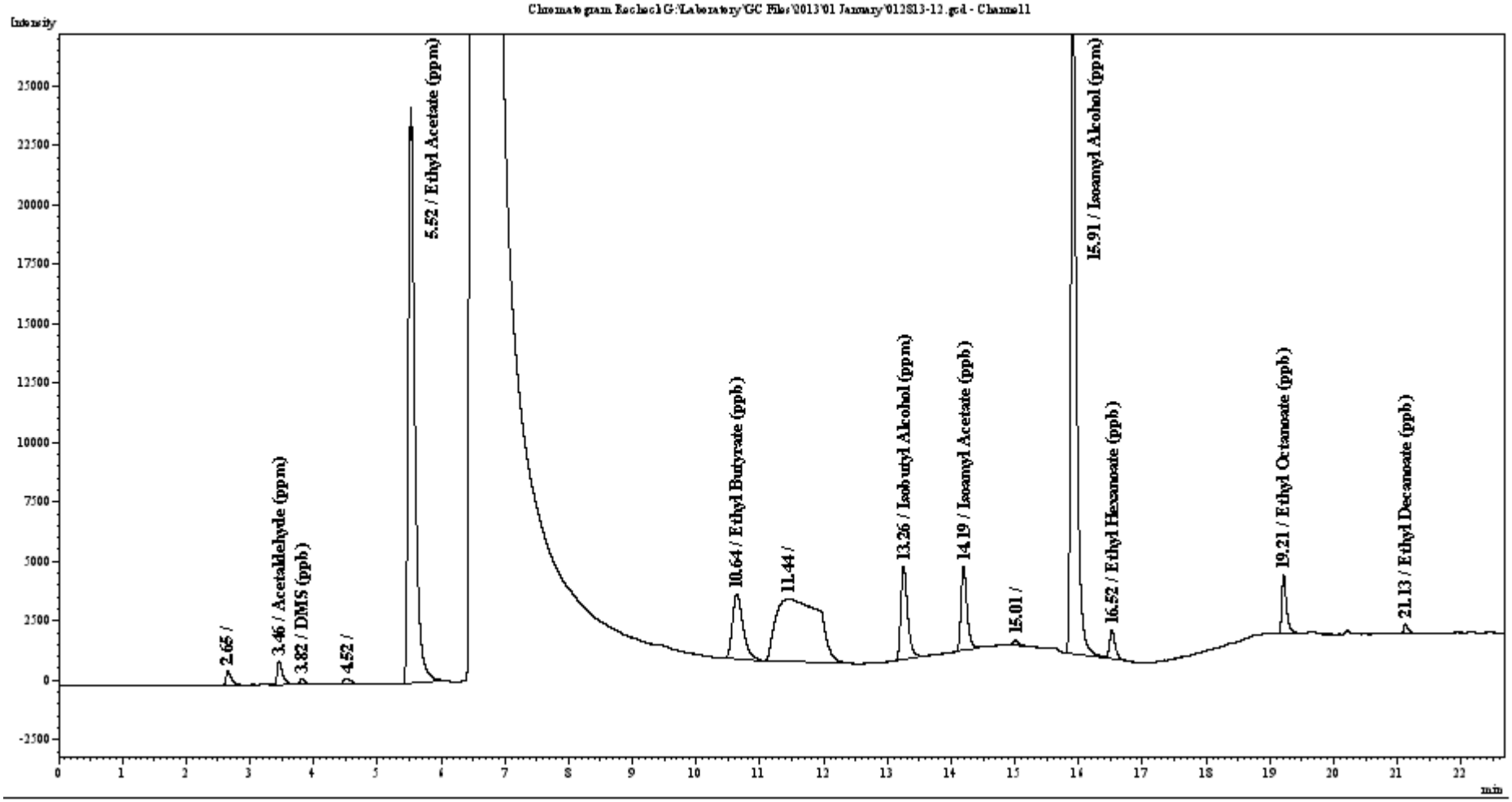
Beer Volatiles – FID Completing the View



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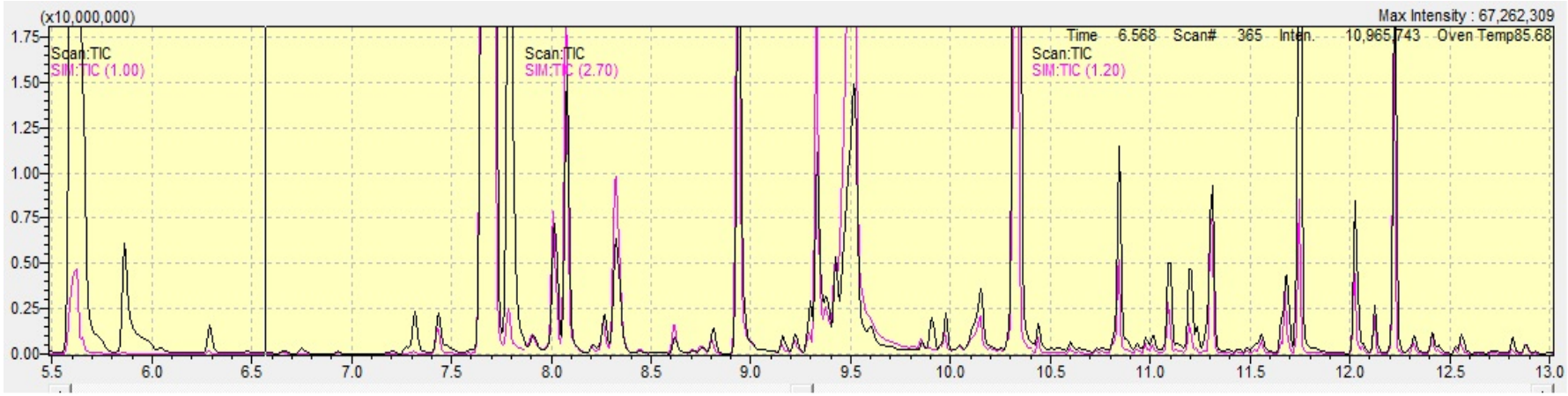
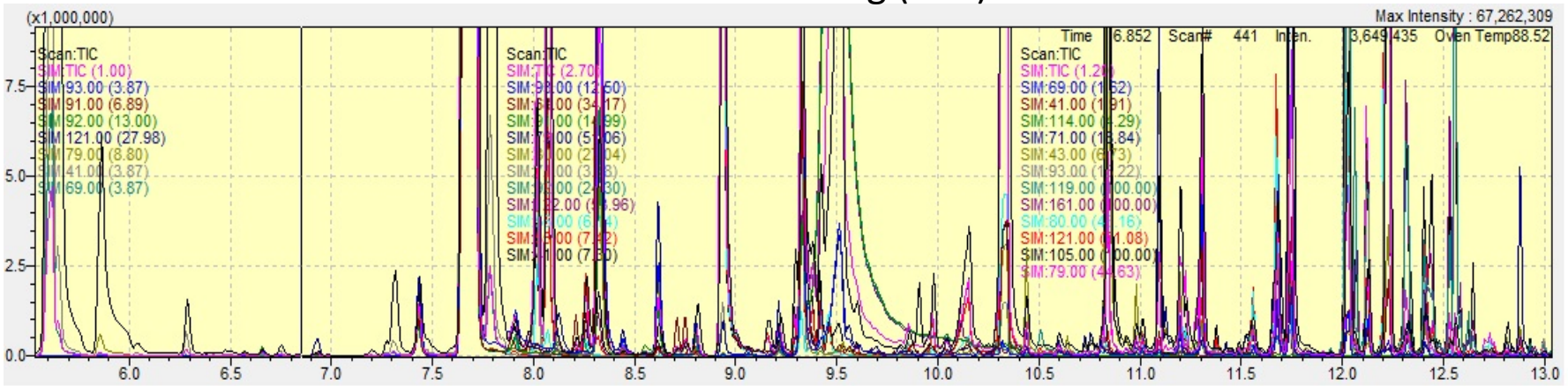
GC/MS - Scan vs. SIM

Scan View



Selective Ion Monitoring (SIM) View



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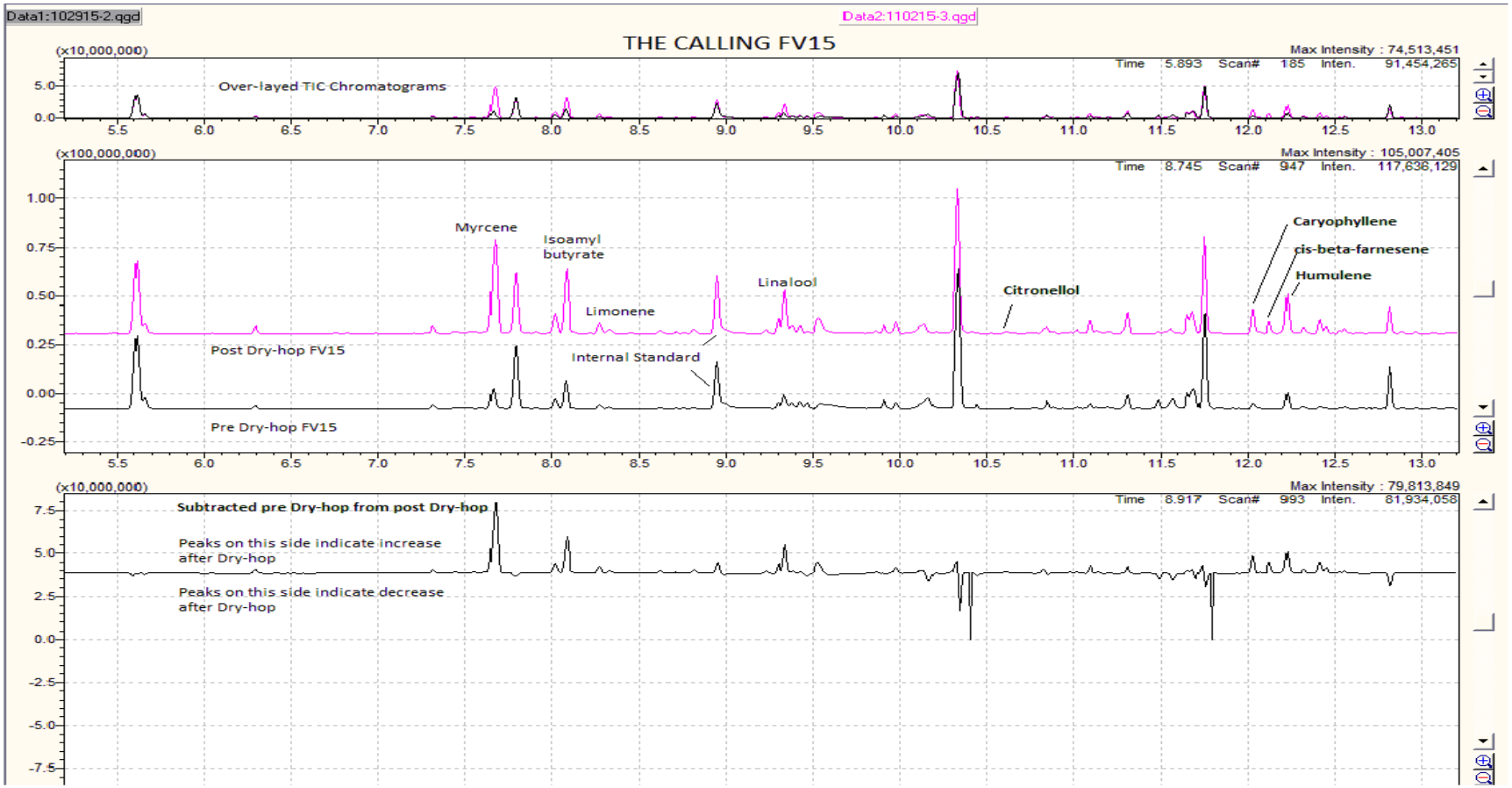
GC/MS Hop Compounds



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Chromatography Considerations

- TIME
- Analyst Training

    - Sample Type
    - Interferences

        - Introduction Techniques
        - Separation/Column(s)
        - Detectors
        - Data Collection

SPECIAL THANKS

Jeff Irby, Dana Sedin- New Belgium Brewing Company

ASBC Technical Committee