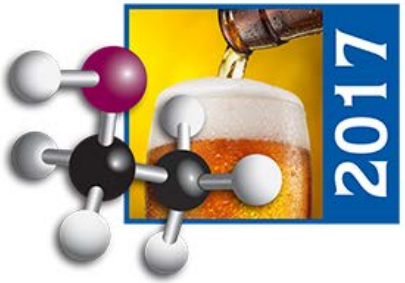


ASBC Annual Meeting

June 4–7 ■ Fort Myers, Florida

See what SCIENCE can brew for you

MetabAromics: A Semi-Targeted Approach to Beer Analysis

(better title for this talk: Quality Control in GCMS-based Metabolomics)

Luke Chadwick
Senior Scientist
Bell's Brewery, Inc.

"Beer isn't rocket science – it's much more complicated than that."
-Jeff Cornell

Metabolomics workflow

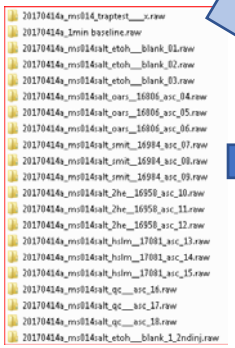
Collect/obtain samples

Prepare samples for analysis

Optimize and standardize instrumental parameters

Optimize and standardize sample collection and preparation

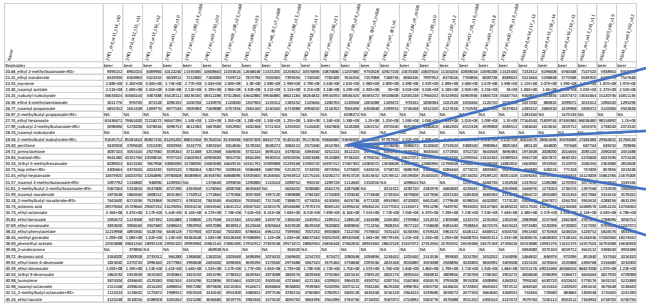
Run samples



Raw data

*

Today we will be comparing three different processing methods



Huge table

PCA

ANOVA

HCA

etc…

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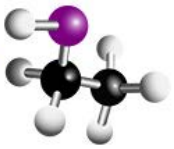


samples

compounds

Name	2HE1_ord_w11_t14_v10	2HE1_ord_w12_t15_v11	2HE1_ord_w13_t16_v12	2HE1_ran_w11_t40_v10	2HE1_ran_w15_t89_v14_noSIR	2HE1_ran_w21_t50_v20	2HE1_ran_w24_t98_v23_noSIR	2HE1_ran_w36_t65_v35	2HE1_ran_w8_t82_v7_noSIR	2HE2_ran_w14_t88_v13_noSIR	2HE2_ran_w18_t92_v17	2HE2_ran_w22_t96_v21	2HE2_ran_w25_t99_v24_noSIR	2HE2_ran_w6_t80_v5_noSIR	2HE2_ran_w7_
Replicates	beer	beer	beer	beer	beer	beer	beer	beer	beer	beer	beer	beer	beer	beer	beer
20.68_ethyl 2-methylbutanoate<RS>	9393152	8961024	8489984	10122240	11335680	10608640	11034624	12648448	11315200	10258432	10576896	10874880	11207680	9750528	10927
21.21_ethyl isovalerate	6343936	6504960	5522432	6630912	7212800	7169280	7539712	7873792	7503360	7393536	7740160	7782400	7616256	7257088	7188
22.53_myrcene	2.88E+08	3.26E+08	3.04E+08	2.74E+08	2.77E+08	3.04E+08	2.69E+08	2.8E+08	2.98E+08	2.99E+08	2.79E+08	2.63E+08	2.81E+08	2.98E+08	2.89E
23.30_isoamyl acetate	2.51E+08	2.69E+08	2.41E+08	3.04E+08	3.12E+08	3.19E+08	2.96E+08	3.33E+08	3.21E+08	3.11E+08	3.21E+08	3.18E+08	3.29E+08	2.92E+08	3E
24.23_isobutyl isobutyrate	36634624	34566144	3487488	35418112	38236160	38412288	37412864	43642880	39546880	38621184	38264832	39534592	38584320	36585472	39108
26.58_ethyl 4-methylpentanoate	1011776	976704	872128	1096192	1200768	1230976	1120000	1347904	1133312	1185152	1145664	1280704	1130048	1001088	1169
26.77_isoamyl propanoate	6852352	1651328	1499776	6977536	1905984	7269888	6707456	2364160	2140160	6713088	6956032	2116352	7063296	6350848	2199
26.97_2-methylbutyl propanoate<RS>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6198272	NA	NA
27.74_ethyl hexanoate	81436672	79462400	72118272	96567296	1.34E+08	1.12E+08	1.35E+08	1.28E+08	1.34E+08	1.25E+08	1.34E+08	1.34E+08	1.38E+08	1.2E+08	1.25E
27.99_isobutyl 2-methylbutanoate<RS>	5098496	5278208	5078016	5098752	4611584	5607680	5032960	5402368	5721344	5250304	5126400	5367808	5228288	5265152	4970
28.33_isoamyl isobutyrate	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
28.56_2-methylbutyl isobutyrate<RS>	91635712	85581824	85807104	81203200	88813568	93782016	81338368	94097408	86347776	91402240	95174656	95866880	93696000	91623424	97787
29.40_perillene	3420928	3709440	3315200	3303936	3243776	3403264	3014656	3178240	3638272	3066112	2575360	2614784	2935552	2894848	3178
29.72_prenylacetone	3087104	3055104	2927360	3339264	3711488	3251968	3469056	3732224	3695104	3478016	3394560	3252224	3412224	3577344	3494
29.84_linalool<RS>	9441280	10101760	10198016	9707520	11602944	10905600	9032704	8362496	9430016	10925056	10303488	9125888	9738240	8790016	10085
30.33_!ethyl 5-methylhexanoate	8289024	8413184	7667968	10884096	15748096	13682688	16649216	16161792	15909888	15239168	15390720	16397312	17467392	14083072	13636
30.75_hop ether<RS>	4305664	4373504	4104192	5355520	5784064	5281792	5209344	5586688	5687296	5212672	5742592	4973056	5273600	5449216	5758
31.45_ethyl heptanoate	14673920	14024704	13264896	19780608	36089856	26358784	36868096	33305600	35266560	32934912	32175104	33206272	35913728	31813632	32576
31.57_isoamyl 2-methylbutanoate<RS>	1097792	1116800	940096	1209920	NA	1376640	1093056	1290880	1110144	1098752	992192	1289728	1112640	1310016	NA
31.81_2-methylbutyl 2-methylbutanoate<RS>	5067264	5154816	5047808	4727296	4333568	5176064	4000768	4635648	NA	4626432	3508480	4562176	4287488	NA	4013
31.99_isoamyl isovalerate	1875648	1866560	1808512	1831360	1750912	1772864	1770368	2097408	1997120	1989888	1784320	1715008	1703040	1774848	1733
32.18_2-methylbutyl isovalerate<RS>	7641600	8174336	7923968	7619072	6785024	7843584	6562816	7920640	7517440	7388672	6774016	6150656	6676736	6771200	6952
33.74_octanoic acid	29579264	15729664	29407232	13229056	33033216	13634560	14641152	20587520	14181376	18546688	17970176	8069120	14396416	19636224	21377
34.73_ethyl octanoate	3.56E+08	3.47E+08	3.17E+08	5.44E+08	8.14E+08	6.17E+08	7.78E+08	7.62E+08	8.41E+08	7.64E+08	8.31E+08	7.89E+08	8.16E+08	7.51E+08	7.72E
35.83_ethyl benzoate	1092672	1123008	947392	1261888	1748800	1317568	1413184	1452288	1459776	1306240	1445952	1289152	1288128	1442688	1265
37.69_ethyl nonanoate	3833600	3906560	3367680	5498624	7892992	6937088	8038912	8123648	8305664	8419328	8629248	7820800	8089856	7712256	7808
38.10_ethyl phenylacetate	11219968	4891904	5428736	6448128	7707904	6071040	7302400	6786816	6961152	7599360	7007232	6903808	7222784	7576832	7431
38.44_methyl geranate	1.09E+08	1.08E+08	1.02E+08	1.14E+08	94363648	1.04E+08	84328448	97488896	1.12E+08	1.03E+08	88637440	93052928	89530368	1.04E+08	98779
38.90_phenethyl acetate	22053888	19641344	18992128	23992320	29993984	25862144	27882496	27942912	27829248	29571072	28882944	26856448	27682816	28943360	28822
39.08_2-undecanone	NA	379856	NA	NA	NA	460928	NA	NA	NA	356320	NA	NA	NA	NA	NA
39.72_decanoic acid	3264000	2300928	2733312	964288	1388480	1182016	1003648	1698496	1074432	1569600	1254720	873472	1080448	1056896	1234
39.92_ethyl trans-4-decenoate	1823040	2270720	1996160	2377984	2990848	2694400	3498496	3639296	3170560	2975488	3467520	3375360	3758848	2593536	2823
40.39_ethyl decanoate	1.03E+08	1.19E+08	1.13E+08	1.41E+08	1.64E+08	1.52E+08	1.57E+08	1.77E+08	1.65E+08	1.73E+08	1.78E+08	1.72E+08	1.75E+08	1.57E+08	1.76E
40.50_!ethyl 9-decenoate	1864192	1924928	1815040	2500864	2824192	2455296	2738432	2649344	2873088	2840576	2829568	2703360	2821632	2789120	2802
40.98_humulene	3874304	4336640	4129280	3381504	4535296	3120896	3821664	3429120	3500544	4721664	4221184	4109824	3864320	4330752	4287
41.98_isoamyl octanoate	2151168	2298624	2314240	4098816	9057280	5975808	10210304	9146672	8036864	9036800	7939584	9220096	10074112	8111104	8629
42.12_2-methylbutyl octanoate<RS>	1121024	1133632	1175872	1998912	3925504	2410240	3953408	3480832	2977536	3785216	3760384	3780352	3600128	3445248	3216
45.24_ethyl laurate	3125248	3510016	4188928	3435264	3521280	3636480	3019776	3982336	3275520	4065792	3863296	3562496	3764736	3724032	3587

Metabolomics workflow

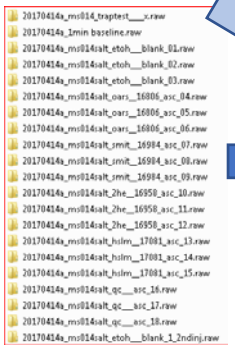
Collect/obtain samples

Prepare samples for analysis

Optimize and standardize instrumental parameters

Optimize and standardize sample collection and preparation

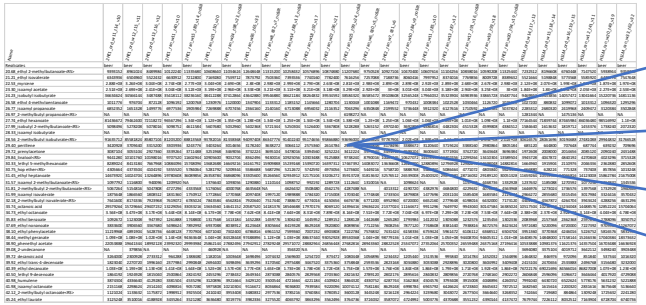
Run samples



Raw data

*

Today we will be comparing three different processing methods



Huge table

PCA

ANOVA

HCA

etc…

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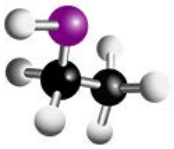
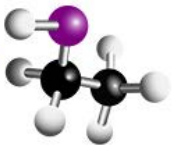


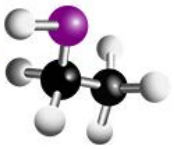
Outline

- State Goals and Objectives
- Define *targeted* vs *non-targeted* vs *semi-targeted* analysis
- Describe factors and endpoints
- Describe the dataset
- ~~Describe HS-trap-GC-MS Technique~~
  - Headspace-Trap sampling
  - Gas chromatography
  - Mass Spectrometry
- Quickly review chromatography
- Review standard addition samples
- Describe QC approach
- Apply and compare 3 different processing methods
- One example and one caveat
- Conclusions



Definitions used for this talk

Type of Analysis	Purpose
Targeted	Determine analytical concentrations (ppm,ppb,etc.) of specific compounds
Non-targeted	Identify significant 'features' in a sample set comprised of distinct groups (e.g. control, trial)
Semi-targeted	Compare peak areas of putatively identified components among a set of samples

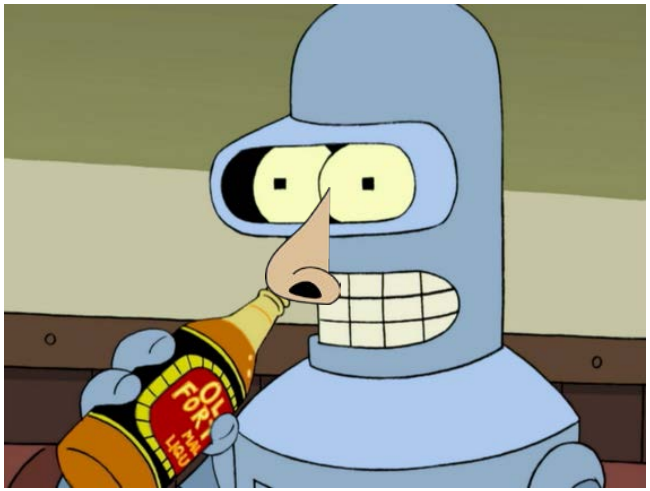


Goals

- Happy customers
- Consistent beers
- Predictable progression through shelf-life
- No defects

” you can't manage what you can't measure. ”

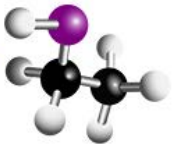
-Peter Drucker



“electronic nose”

Objectives

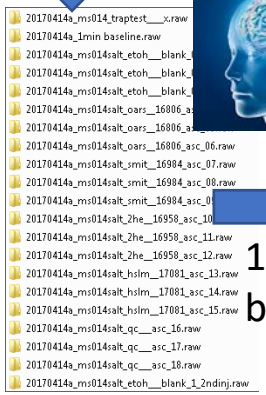
- Analytical measurement of aroma
  - Headspace-TRAP auto-sampling
  - Gas Chromatography (GC→ chemical separation)
  - Mass Spectrometry (MS→ chemical detection and characterization)
- Standardize our handling of GCMS data
  - Compare replicates
  - Compare samples to each other
  - **Raw data (set of mass chromatograms)→ single table of data**



Specific Objective

beer,hops

100s of decisions by human brain

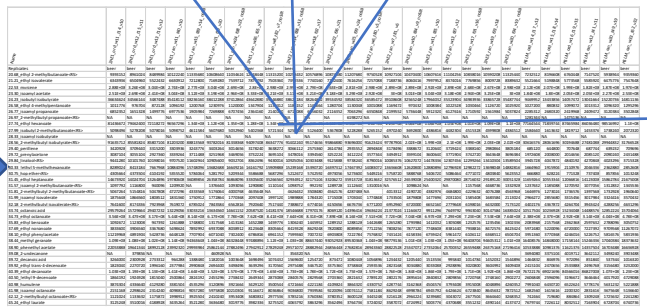


Raw data

*

100s of decisions by human brain

ANOVA PCA etc…



Huge table
(row for every compound in library, column for every mass chromatogram)

Compare replicate samples

	myrcene		2-methylbutyl isobutyrate		linalool	
sample	mean	CV	mean	CV	mean	CV
2HE1	3,267	1.4%	134	6.6%	76	79.2%
2HE2	3,065	4.5%	140	0.9%	120	1.8%
2HE3	3,120	4.3%	290	2.3%	157	7.8%
HSLM	2,084	1.2%	49	6.9%	39	0.9%
OARS	143	87.8%	8	0.7%	39	1.7%
SMIT	1,956	0.3%	39	3.1%	44	18.9%
QC__	1,240	1.8%	54	4.4%	40	1.0%

Averages and relative standard deviations of components in replicate samples

*

The objective is **to describe Quality Control procedures** related to this process

Today we will compare **three different processing methods**

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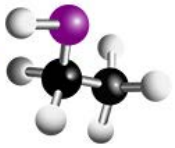
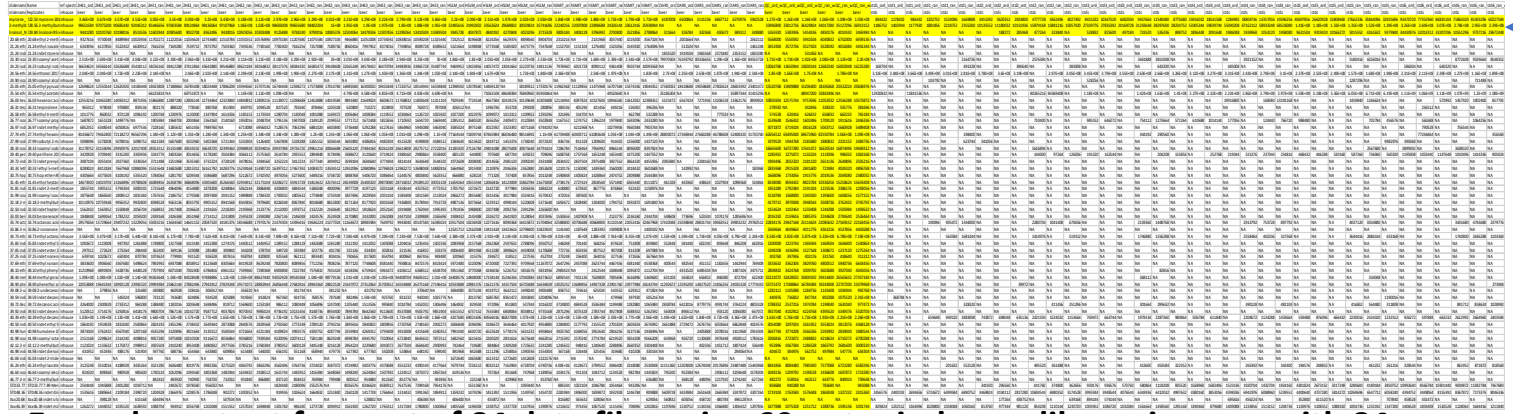


Example: AMDIS/Metab processing

The result is a table (.csv file) with X columns (samples) and Y rows (library entries)

(6 QC samples)

(3 targets)



Example: output from set of 84 data files using 60-entry in-house library is a .csv with 84 columns and 60 rows (+ headers)

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Factors investigated for this presentation

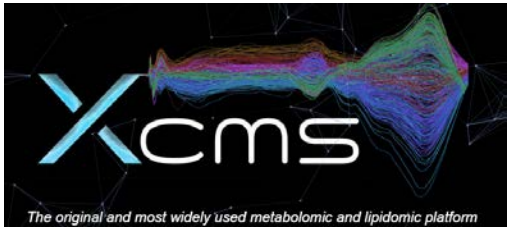
- Processing method
  - Turbomass (targeted)
  - AMDIS/Metab (semi-targeted)
  - MetaMS (non-targeted)
- ~~Target library~~
  - NIST subset (38 components)
  - In-house (60 components)

Overview of Processing Methods

	Turbomass (targeted)	AMDIS/Metab (semi-targeted)	metaMS (non-targeted)
human time	a few days per target compound	~30min for this dataset	~30min for this dataset
machine time	~instantaneous	~1 hr	~3 min for each group (beer/stds)
output	.qsc file (unparseable text file)	.csv file with one column for each sample, and one row for each library entry	in this case two .csv files (beers.csv, standards.csv) containing peak area and retention time info for library matches and 'unknowns'

U.S. Department of Commerce
Technology Administration

Automated
Mass Spectral
Deconvolution &
Identification
System



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Endpoints

- number (or percentage) of components with **RSD < 10% in randomized triplicate QC samples**

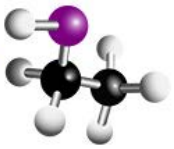
QC sample ≡ 1:1:1:1 mix of actual samples (beer/hops)

$$\text{RSD} = \text{relative standard deviation} = \frac{\text{standard deviation}}{\text{average}} \times 100\%$$

RSD Examples:

dataset	average	stdev	RSD
10,10,10	10	0.00	0%
9,10,11	10	1.00	10%
0,10,20	10	10.00	100%

*relative standard deviation is also known as the Coefficient of Variation (CV)



Samples

HopSteiner/Perkin-Elmer ASBC/EBC Collaborative Pre-Trial "Hop Aroma Compounds in Beer using HS-TRAP-GC-MS"

- Analytical standards
  - myrcene
  - 2-methylbutyl isobutyrate
  - linalool
  - geraniol

	myrcene	others
std1	4	2
std2	40	20
std3	80	40
std4	160	80
std5	400	200
std6	800	400

ppb in 7% EtOH

- Beer samples
  - Hoppy beers
    - 2HE1
    - SMIT
    - HSLM
  - "control beer"
    - OARS

QC = 1:1:1:1 mix 2HE1:SMIT:HSLM:OARS

  - Standard addition samples
    - 2HE+20 = 2HE1 spiked with 40/20 ppb standard mix
    - 2HE+200 = 2HE1 spiked with 400/200 ppb standard mix

Dataset

April 12 Sequence1	April 14 Sequence2
std1	OARS
std1	OARS
std1	OARS
std2	SMIT
std2	SMIT
std2	SMIT
std3	2HE1
std3	2HE1
std3	2HE1
std4	HSLM
std4	HSLM
std4	HSLM
std5	QC
std5	QC
std5	QC
std6	
std6	
std6	

April 15-17 Sequence3

Randomized triplicate beer samples and standards

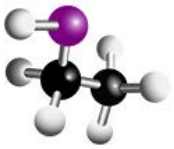
OARS
std6
std4
std5
std3
std1
2HE1
HSLM
QC
std2
SMIT
std4
SMIT
std5
std1
HSLM
2HE1
std3
OARS
std6
std2
QC
std1
std2
std4
QC
std3
SMIT
HSLM
OARS
std6
2HE1

April 17 Sequence4

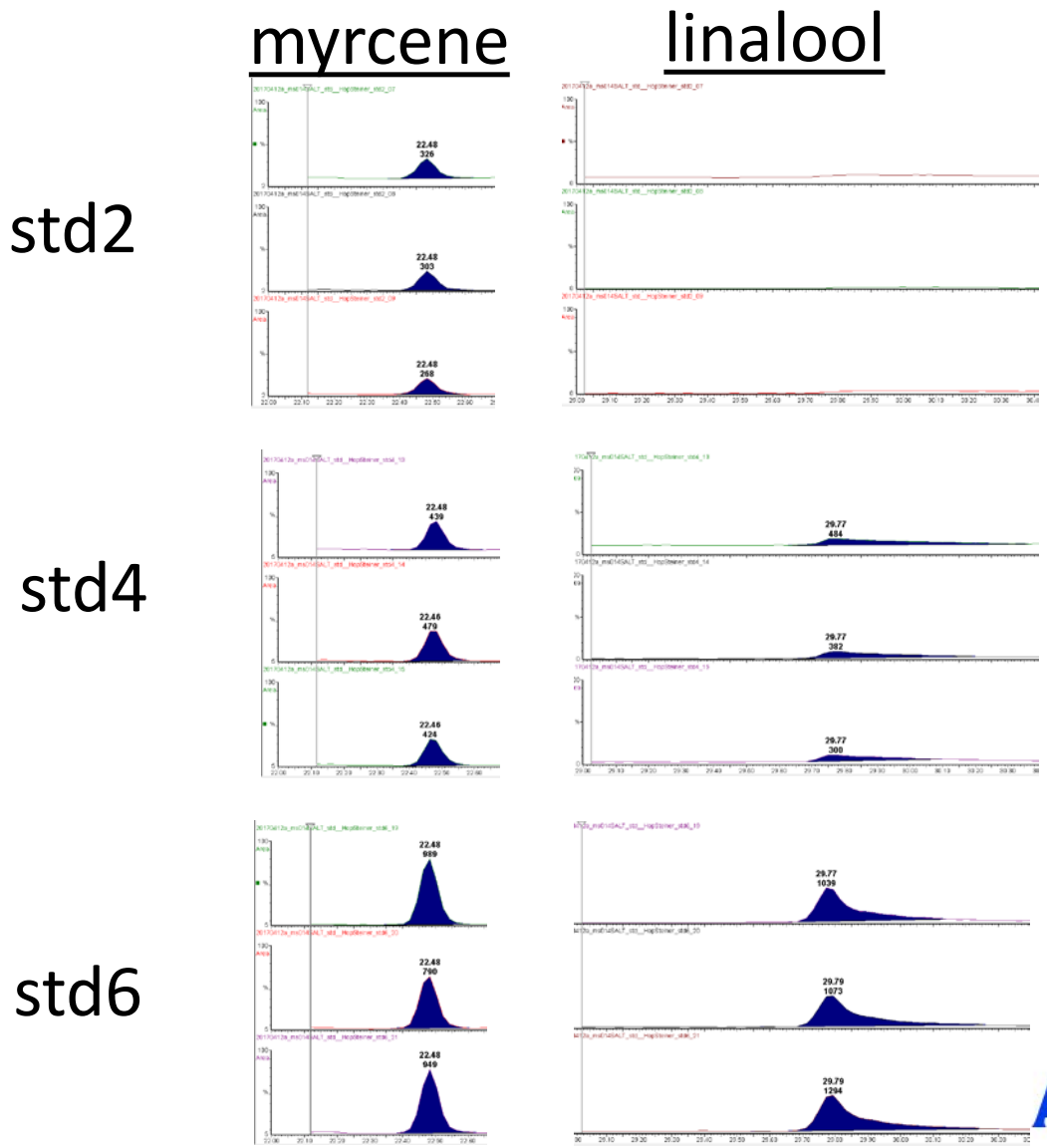
Standard addition samples

2HE+20NS
2HE+20
2HE1NS
blank
2HE+200NS
2HE+200
blank
2HE+200
2HE+20NS
2HE1NS
blank
2HE+200NS
2HE+20
2HE+200
2HE+200NS
blank
2HE+20
blank
2HE1NS
2HE+20NS

All stds prepped on 4/12/17
All beer samples prepped on 4/14/17



Quickly Review Chromatography



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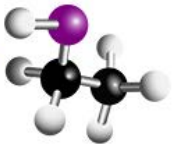
Endpoint: RSD of QC sample

- number (or percentage) of components with **RSD < 10% in randomized triplicate QC samples**

QC sample ≡ 1:1:1:1 mix of beer samples

Reference values (targeted processing)

		QC1	QC2	QC3	QC4	QC5	QC6	QC1,2,3 summary (seq. over 3 hours)			QC4,5,6 summary (random over 16 hours)		
time after preparation:		20hr	21hr	22hr	42hr	55hr	59hr						
		QC_ord_w	QC_ord_w	QC_ord_w	QC_ran_w	QC_ran_w	QC_ran_w	average	STDEV	RSD	average	STDEV	RSD
Re-integrated ppb values	myrcene ppb	1403.4	1399.2	1407.8	1224.9	1230.6	1265.5	1403.4	4.3	0.3%	1240.3	22.0	1.8%
	2mbib ppb	52.2	51.6	54.3	51.4	54.5	56.0	52.7	1.4	2.6%	54.0	2.4	4.4%
	linalool ppb	40.2	39.8	40.0	39.1	39.9	39.7	40.0	0.2	0.6%	39.6	0.4	1.0%
Raw peak areas	myrcene (T)	8,534,654	8,509,965	8,560,711	7,483,632	7,516,770	7,722,356	8,535,110	25,376.1	0.3%	7,574,252	129,326.9	1.7%
	2mbib(T)	1,847,489	1,822,215	1,932,330	1,812,048	1,941,975	2,005,260	1,867,345	57,680.7	3.1%	1,919,761	98,503.0	5.1%
	linalool(T)*	19,109	14,598	16,616	8,764	16,054	14,232	16,774	2,259.6	13.5%	13,017	3,793.8	29.1%



Review QC samples

Example: MetaMS with NIST subset library

		QC1	QC2	QC3	QC4	QC5	QC6	(random over 16 hours)	
	time after preparation:	20hr	21hr	22hr	42hr	55hr	59hr		
	(re-integrated target reference)	QC_ord_w	QC_ord_w	QC_ord_w	QC_ran_w	QC_ran_w	QC_ran_w	average	RSD
Reference values (targeted)	myrcene ppb	1403.35	1399.16	1407.78	1224.92	1230.55	1265.45	1,240.3	1.8%
	2mbib ppb	52.23	51.63	54.25	51.38	54.48	55.99	54.0	4.4%
	linalool ppb	40.24	39.76	39.98	39.14	39.92	39.72	39.6	1.0%
	myrcene (T)	8,534,654	8,509,965	8,560,711	7,483,632	7,516,770	7,722,356	7,574,252	1.7%
	2mbib(T)	1,847,489	1,822,215	1,932,330	1,812,048	1,941,975	2,005,260	1,919,761	5.1%
	linalool(T)*	19,109	14,598	16,616	8,764	16,054	14,232	13,017	29.1%
	metaMS/NIST	QC_ord_w	QC_ord_w	QC_ord_w	QC_ran_w	QC_ran_w	QC_ran_w30_t59_v29		

MetaMS with NIST subset library

Component Nar	QC_ord	QC_ord	QC_ord	QC_ran	QC_ran	QC_ran	averag	RSD
Unknown 4	0.74279	0.838457	0.835564	0.927877	0.949604	0.937963	1	1.2%
Unknown 7	0.737254	0.760752	0.832535	0.935249	0.901722	0.932082	1	2.0%
myrcene	128345.4	125502	140573.1	109772.3	114861.9	113424.1	112,686	2.3%
Unknown 2	1.055797	0.923347	1.007	1.047971	1.033919	1.093277	1	2.9%
Unknown 6	0	0	0	0.851607	0.892819	0.911999	1	3.5%
Unknown 26	0.840545	0.808091	0.878397	1	0.920156	1.03275	1	5.9%
isoamyl acetate	171338.2	149855.1	174734.2	196848.7	203310.7	220804.1	206,988	6.0%
Unknown 10	0.423632	0.411345	0.445313	0.373217	0.403094	0.424037	0	6.4%
Unknown 8	1.036621	1.082249	1.128436	1.183619	1.339433	1.357539	1	7.4%
!ethyl 9-decenoate	1487.51	1504.681	2339.679	2648.152	2301.293	2328.837	2,426	7.9%
octanoic acid	23586.81	29863.5	24690.16	24513.59	29056.6	25234.7	26,268	9.3%
ethyl hexanoate	57826.56	67764.06	69710.81	82649.7	98338.16	98248.01	93,079	9.7%
ethyl 2-methylbutanoate<RS>	3144.977	3358.81	3385.678	3475.022	4004.003	4245.834	3,908	10.1%
ethyl nonanoate	3634.329	3659.47	5148.65	4833.539	5607.5	6117.293	5,519	11.7%
Unknown 15	1.95204	1.193274	1.288069	1.28598	1.002642	1.136602	1	12.4%
Unknown 5	0	0.708217	0.736012	1.135227	0.867675	1.012424	1	13.3%
Unknown 13	1.128939	0.938446	0	0.877841	0.64571	0.773488	1	15.2%
Unknown 29	0.344551	0.385952	0.371215	0.366506	0.473844	0.49615	0	15.6%
ethyl laurate	7304.221	7645.642	7311.186	6261.523	4547.884	5928.227	5,579	16.3%
linalool<RS>	5268.566	5241.318	5421.251	4820.923	3785.514	3369.121	3,992	18.7%
methyl geranate	29660.87	37182.53	41081.63	36588.77	26435.03	27160.86	30,062	18.8%
phenethyl acetate	15569.54	26568.64	16644.34	20670.77	29152.33	29851.03	26,558	19.2%
Unknown 16	0.271113	0.266412	0.499533	0.412691	0.277398	0.348137	0	19.6%
ethyl octanoate	367280.5	409627.5	408822.2	474983.3	642680.8	710274.9	609,313	19.9%
humulene	3559.017	3333.648	3462.219	2921.154	1950.133	2089.557	2,320	22.6%
ethyl trans-4-decenoate	1736.916	1377.058	2107.539	2334.879	1517.101	1644.86	1,832	24.0%
ethyl decanoate	180842.7	175724.8	208198.7	205257.8	317714.8	330820.7	284,598	24.3%
Unknown 35	1	0	0	0.977747	1.177317	0.666702	1	27.4%
Unknown 22	2.023241	0.781452	1.113487	0.847436	1.21212	0.577511	1	36.2%
!ethyl trans-2-heptenoate	3250.625	3979.909	336.161	3704.513	685.7502	2849.634	2,413	64.5%
Unknown 18	0.57312	0	0	0.611703	0	0.607926	0	86.6%
Unknown 19	0	0	0.815771	0	0.900081	0.881904	1	86.6%
Unknown 1	1.030731	0.992326	0.989252	1.011203	1.049578	0	1	86.6%
Unknown 11	0	0	0	0.363465	0	0.387921	0	86.7%
ethyl phenylacetate	0	0	0	0	3837.557	4111.441	2,650	86.8%
ethyl isovalerate	2648.01	0	2856.049	2924.64	0	3727.35	2,217	88.5%
Unknown 21	0.877249	0	0.948575	1.176216	0	1.779213	1	91.9%
2-methylbutyl octanoate<RS>	0	0	0	0	0	1252.544	418	173.2%
Unknown 31	0	0	0.765222	0	0	0.867902	0	173.2%
Unknown 36	0.870101	0.926927	0.965112	0.715402	0	0	0	173.2%
Unknown 3	0.840971	0.905644	0	1.024106	0	0	0	173.2%
Unknown 27	0	1.071395	0	0	0	1.071301	0	173.2%
isobutyl isobutyrate	20100.29	13420.63	21542.23	0	0	16083.64	5,361	173.2%

12 components with RSD < 10%
(out of 38 total components in the library)

Preliminary conclusion:
A **10% relative standard deviation** (RSD) of a given component in the QC sample over a 16-hour timespan **is a reasonable filter** for multivariate analysis

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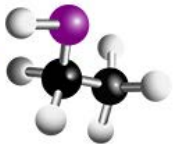


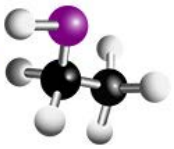
QC sample summary

#components with RSD < 10%

processing method	library	#components with RSD<10% / total components	%
targeted	in-house	(2/3)	67%
AMDIS/Metab	in-house	24/60	40%
AMDIS/Metab	NIST subset	16/38	42%
MetaMS	NIST subset	12/38	32%

Preliminary conclusions:

- Each method has distinct advantages.
- Each method has drawbacks.
- No clear winner overall.



QC sample RSD is useful to:

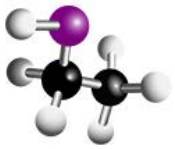
- **Evaluate acquisition and processing methods**
- **Filter out 'garbage' as we prepare data for multivariate analysis**

Name	2HE1_ord	2HE1_ord	2HE1_ord	2HE1_ran	2HE1_ran	2HE1_ran	2HE1_ran	2HE1_ran	2HE1_ran	2HE2_ran	2HE2_ran	2HE2_ran	2HE2_ran	2HE2_ran	2HE2_ran	2HE3_ran	2HE3_ran	2HE3_ran	2HE3
Replicates	beer	beer	beer	beer	beer	beer	beer	beer	beer	beer	beer	beer	beer	beer	beer	beer	beer	beer	beer
20.68_ethyl 2-methylbutanoate<RS>	9393152	8961024	8489984	10122240	11335680	10608640	11034624	12648448	11315200	10258432	10576896	10874880	11207680	9750528	10927104	10470400	10607616	11104256	1083
21.21_ethyl isovalerate	6343936	6504960	5522432	6630912	7212800	7169280	7539712	7873792	7503360	7393536	7740160	7782400	7616256	7257088	7188736	8060416	7997952	8374016	776
22.53_myrcene	2.88E+08	3.26E+08	3.04E+08	2.74E+08	2.77E+08	3.04E+08	2.69E+08	2.8E+08	2.98E+08	2.99E+08	2.79E+08	2.63E+08	2.81E+08	2.98E+08	2.89E+08	2.89E+08	2.92E+08	2.89E+08	2.68
23.30_isoamyl acetate	2.51E+08	2.69E+08	2.41E+08	3.04E+08	3.12E+08	3.19E+08	2.96E+08	3.33E+08	3.21E+08	3.11E+08	3.21E+08	3.18E+08	3.29E+08	2.92E+08	3E+08	3.01E+08	3.04E+08	3.18E+08	2.94
24.23_isobutyl isobutyrate	36634624	34566144	3487488	35418112	38236160	38412288	37412864	43642880	39546880	38621184	38264832	39534592	38584320	36585472	39108608	32565248	17964032	35323904	3498
26.58_ethyl 4-methylpentanoate	1011776	976704	872128	1096192	1200768	1230976	1120000	1347904	1133312	1185152	1145664	1280704	1130048	1001088	1169472	970432	1008384	1022528	103
26.77_isoamyl propanoate	6852352	1651328	1499776	6977536	1905984	7269888	6707456	2364160	2140160	6713088	6956032	2116352	7063296	6350848	2199552	5736448	5912320	6127616	171
26.97_2-methylbutyl propanoate<RS>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6198272	NA	NA	NA	NA	NA	NA
27.74_ethyl hexanoate	81436672	79462400	72118272	96567296	1.34E+08	1.12E+08	1.35E+08	1.28E+08	1.34E+08	1.25E+08	1.34E+08	1.34E+08	1.38E+08	1.2E+08	1.25E+08	1.06E+08	1.06E+08	1.05E+08	1.01
27.99_isobutyl 2-methylbutanoate<RS>	5098496	5278208	5078016	5098752	4611584	5607680	5032960	5402368	5721344	5250304	5126400	5367808	5228288	5265152	4970240	3692800	4386816	4482304	451
28.33_isoamyl isobutyrate	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
28.56_2-methylbutyl isobutyrate<RS>	91635712	85581824	85807104	81203200	88813568	93782016	81338368	94097408	86347776	91402240	95174656	95866880	93696000	91623424	97787904	2.02E+08	1.99E+08	2.1E+08	1.99
29.40_perillene	3420928	3709440	3315200	3303936	3243776	3403264	3014656	3178240	3638272	3066112	2575360	2614784	2935552	2894848	3178496	3388672	3120640	3719424	338
29.72_prenylacetone	3087104	3055104	2927360	3339264	3711488	3251968	3469056	3732224	3695104	3478016	3394560	3252224	3412224	3577344	3494912	3599104	3600640	3771904	374
29.84_linalool<RS>	9441280	[illegible]	[illegible]	9707520	11602944	[illegible]	9032704	8362496	9430016	10925056	10303488	9125888	9738240	8790016	10085376	10627072	14478336	12307456	1229
30.33_!ethyl 5-methylhexanoate	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	16161792	15909888	15239168	15390720	16397312	17467392	14083072	13636608	12620800	12880896	12796928	1298
30.75_hop ether<RS>	4305664	4373504	4104192	5355520	5784064	5281792	5209344	5586688	5687296	5212672	5742592	4973056	5273600	5449216	5758720	5888768	5406720	5086464	477
31.45_ethyl heptanoate	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	26539008	25400320	29870080	2871
31.57_isoamyl 2-methylbutanoate<RS>	1097792	1116800	940096	1209920	NA	1376640	1093056	1290880	1110144	1098752	992192	1289728	1112640	1310016	NA	1098624	NA	NA	115
31.81_2-methylbutyl 2-methylbutanoate<RS>	5067264	5154816	5047808	4727296	4333568	5176064	4000768	4635648	NA	4626432	3508480	4562176	4287488	NA	4013312	4190720	4382976	4684800	422
31.99_isoamyl isovalerate	[illegible]	1866560	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	1733504	1879808	1477696	2031104	158
32.18_2-methylbutyl isovalerate<RS>	7641600	8174336	7923968	7619072	6785024	7843584	6562816	7920640	7517440	7388672	6774016	6150656	6676736	6771200	6952960	6720000	6652160	2779648	619
33.74_octanoic acid	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	14641152	20587520	14181376	18546688	17970176	8069120	14396416	19636224	21377024	11164672	9911296	7649792	994
34.73_ethyl octanoate	3.56E+08	3.47E+08	3.17E+08	5.44E+08	8.14E+08	6.17E+08	7.78E+08	7.62E+08	8.41E+08	7.64E+08	8.31E+08	7.89E+08	8.16E+08	7.51E+08	7.72E+08	7.04E+08	6.97E+08	7.29E+08	7.23
35.83_ethyl benzoate	1092672	1123008	947392	1261888	1748800	1317568	1413184	1452288	1459776	1306240	1445952	1289152	1288128	1442688	1265280	1793984	1412032	1305088	123
37.69_ethyl nonanoate	3833600	3906560	3367680	5498624	7892992	6937088	8038912	8123648	8305664	8419328	8629248	7820800	8089856	7712256	7808256	7877120	7748608	8381440	793
38.10_ethyl phenylacetate	11219968	4891904	5428736	6448128	7707904	6071040	7302400	6786816	6961152	7599360	7007232	6903808	7222784	7576832	7431424	6158336	6759424	5961472	610
38.44_methyl geranate	1.09E+08	1.08E+08	1.02E+08	1.14E+08	94363648	1.04E+08	84328448	97488896	1.12E+08	1.03E+08	88637440	93052928	89530368	1.04E+08	98779136	1.01E+08	1.03E+08	1.05E+08	9440
38.90_phenethyl acetate	22053888	19641344	18992128	23992320	29993984	25862144	27882496	27942912	27829248	29571072	28882944	26856448	27682816	28943360	28822528	25347072	27352064	25700352	2655
39.08_2-undecanone	NA	379856	NA	NA	NA	460928	NA	NA	NA	356320	NA	NA	NA	NA	NA	NA	NA	NA	NA
39.72_decanoic acid	3264000	2300928	2733312	964288	1388480	1182016	1003648	1698496	1074432	1569600	1254720	873472	1080448	1056896	1234432	1205440	1513536	995840	101
39.92_ethyl trans-4-decenoate	1823040	2270720	1996160	2377984	2990848	2694400	3498496	3639296	3170560	2975488	3467520	3375360	3758848	2593536	2823168	3034880	3033088	2928896	302
40.39_ethyl decanoate	1.03E+08	1.19E+08	1.13E+08	1.41E+08	1.64E+08	1.52E+08	1.57E+08	1.77E+08	1.65E+08	1.73E+08	1.78E+08	1.72E+08	1.75E+08	1.57E+08	1.76E+08	1.84E+08	1.86E+08	1.79E+08	1.71
40.50_!ethyl 9-decenoate	1864192	1924928	1815040	2500864	2824192	2455296	2738432	2649344	2873088	2840576	2829568	2703360	2821632	2789120	2802176	2895616	2840832	2809856	272
40.98_humulene	3874304	4336640	4129280	3381504	4535296	3120896	3921664	3429120	3500544	4721664	4221184	4109824	3864320	4330752	4287744	3162368	4504576	3793408	391
41.98_isoamyl octanoate	2151168	2298624	2314240	4098816	9057280	5975808	10210304	9116672	8036864	9036800	7939584	9220096	10074112	7581184	8629248	6998784	6945792	6426624	672
42.12_2-methylbutyl octanoate<RS>	1121024	1133632	1175872	1998912	3925504	2410240	3953408	3480832	2977536	3785216	3760384	3780352	3600128	3445248	3216128	2964224	3239680	[illegible]	267
45.24_ethyl laurate	3125248	3510016	4188928	3435264	3521280	3636480	3019776	3982336	3275520	4065792	3863296	3562496	3764736	3724032	3587072	4724992	5003776	4370688	355

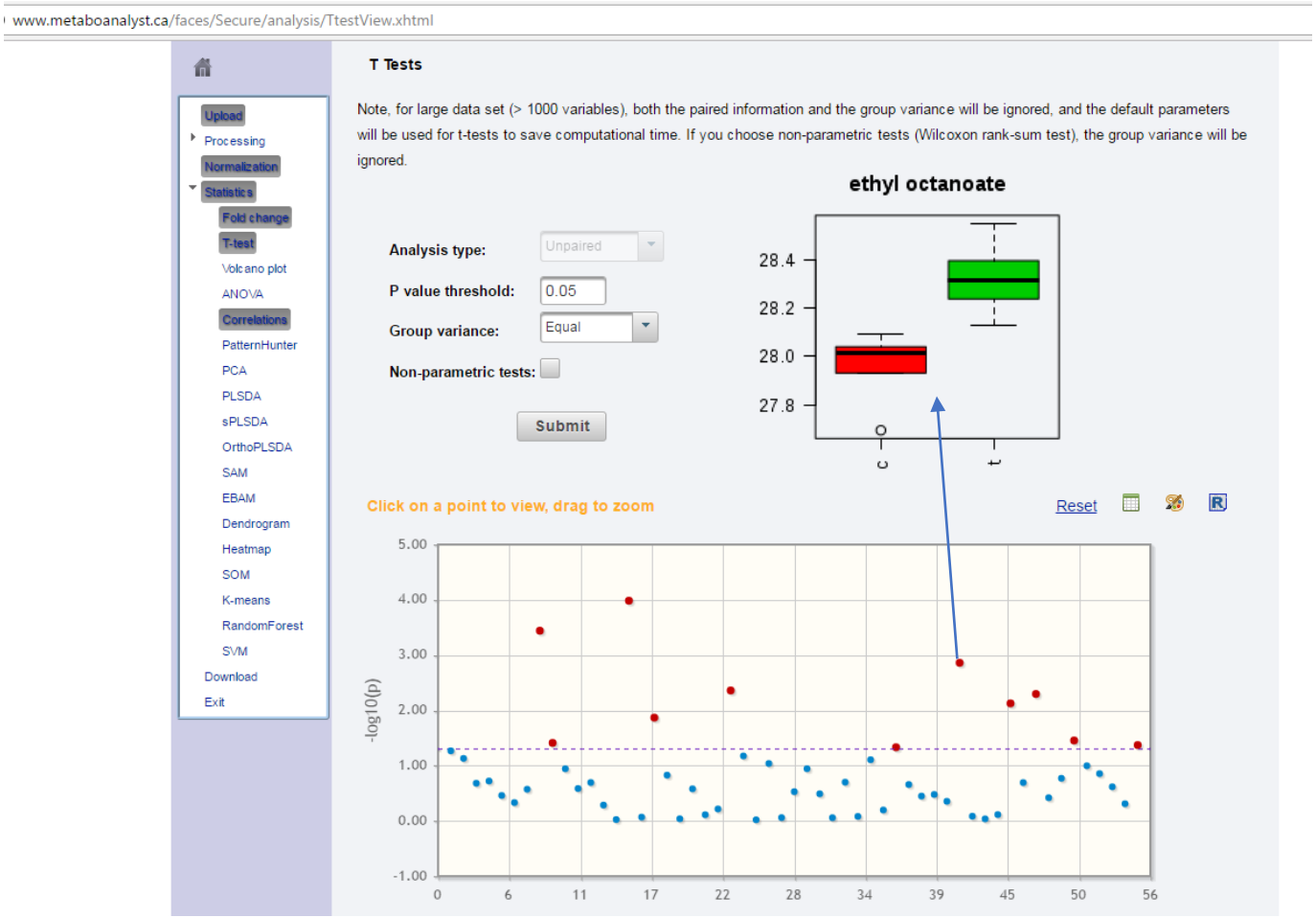
Summary Table of Pros

Pro	Targeted	Non-targeted*
Automation	+++	+
Mature software	+++	-
Manual re-integration capabilities	+++	-
Established framework for decision-making	+++	-/+
Stability over time	+	???
Make tables for multivariate analysis	-	+++
Compare a set of samples	+	+++

*MetaMS package has the additional pro of identifying key “features” in the dataset (regardless of whether or not they exist in the target library)



Example of a “false discovery”



Multi-component T-test viewer

http://www.metaboanalyst.ca/faces/Secure/analysis/TtestView.xhtml

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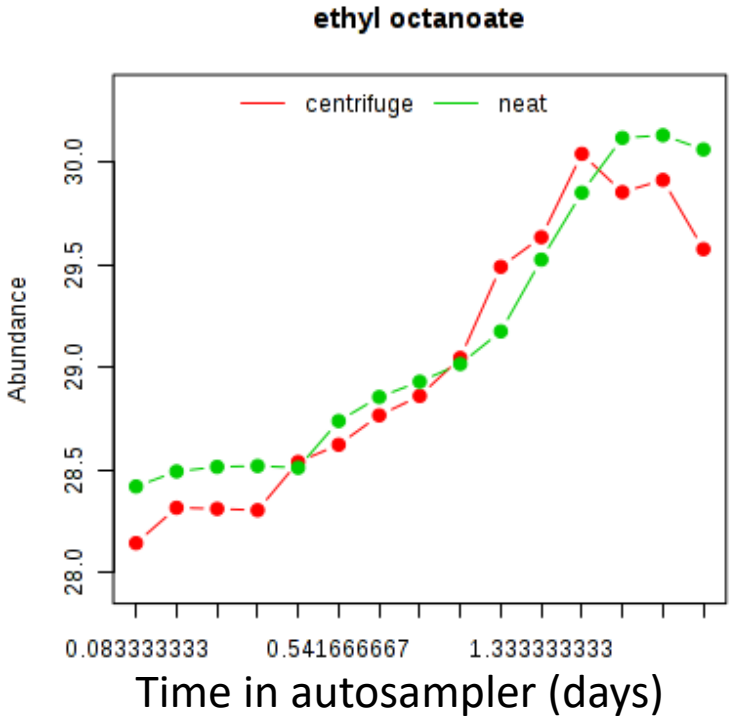
Follow-up experiment

Sample pairs over time in autosampler, comparing sample prep conditions (18 neat/filter pairs)

Punchline: **T**ime-in-autosampler was the primary factor in ethyl octanoate peak area.



- This goose chase would have been avoided had this QC approach been in place at the time

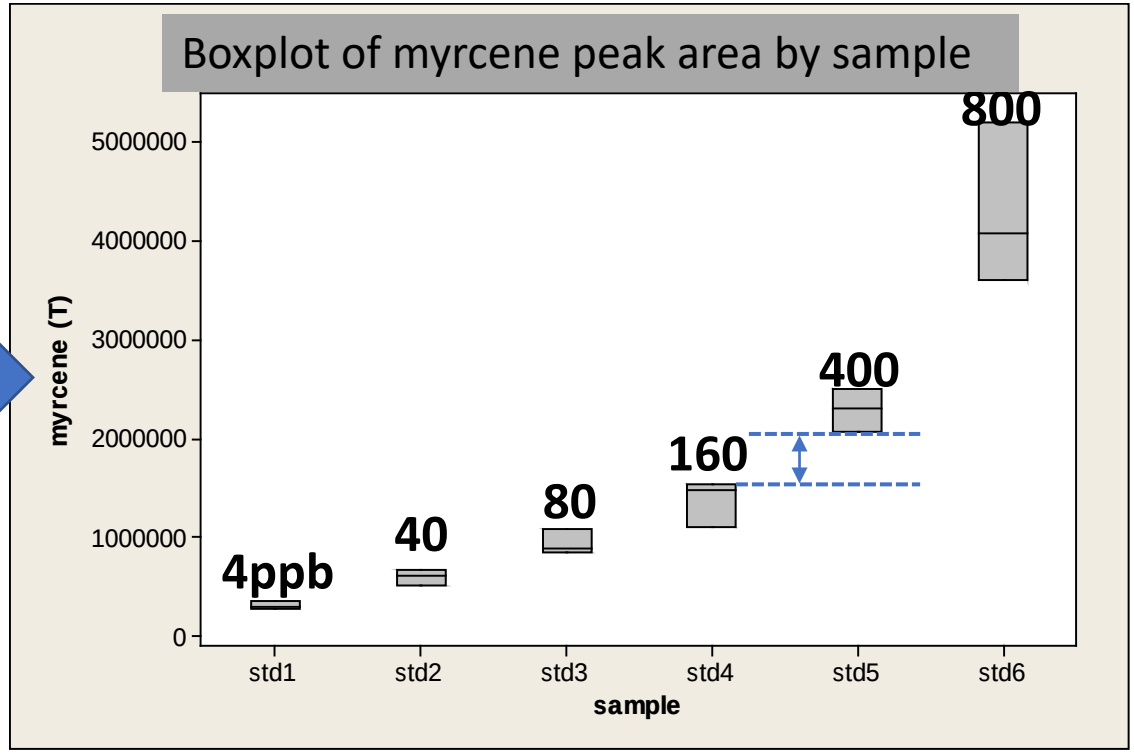
*Time-course profile from http://www.**metaboanalyst.ca**/faces/home.xhtml

Caveat!

- This approach will not work very well for ‘very different’ samples!

(describe boxplots)

sample	replicate	myrcene (T)
4ppb	std1_ran_w10_t39_v9	283,961
	std1_ran_w19_t48_v18	288,573
	std1_ran_w27_t56_v26	361,128
40ppb	std2_ran_w14_t43_v13	664,251
	std2_ran_w25_t54_v24	604,040
	std2_ran_w28_t57_v27	520,084
80ppb	std3_ran_w22_t51_v21	893,353
	std3_ran_w31_t60_v30	1,081,942
	std3_ran_w9_t38_v8	841,259
160ppb	std4_ran_w16_t45_v15	1,479,461
	std4_ran_w29_t58_v28	1,098,559
	std4_ran_w7_t36_v6	1,524,173
400ppb	std5_ran_w18_t47_v17	2,071,518
	std5_ran_w37_t66_v36	2,504,402
	std5_ran_w8_t37_v7	2,292,235
800ppb	std6_ran_w24_t53_v23	4,075,410
	std6_ran_w35_t64_v34	5,190,386
	std6_ran_w6_t35_v5	3,601,517



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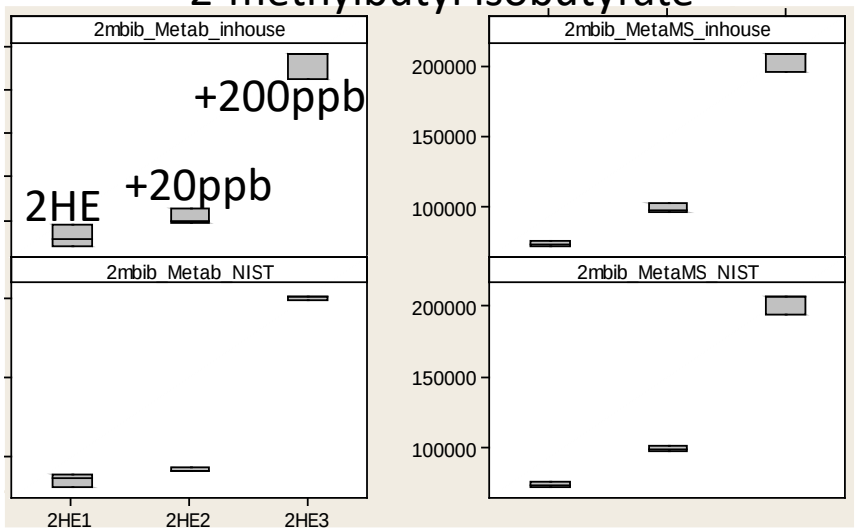
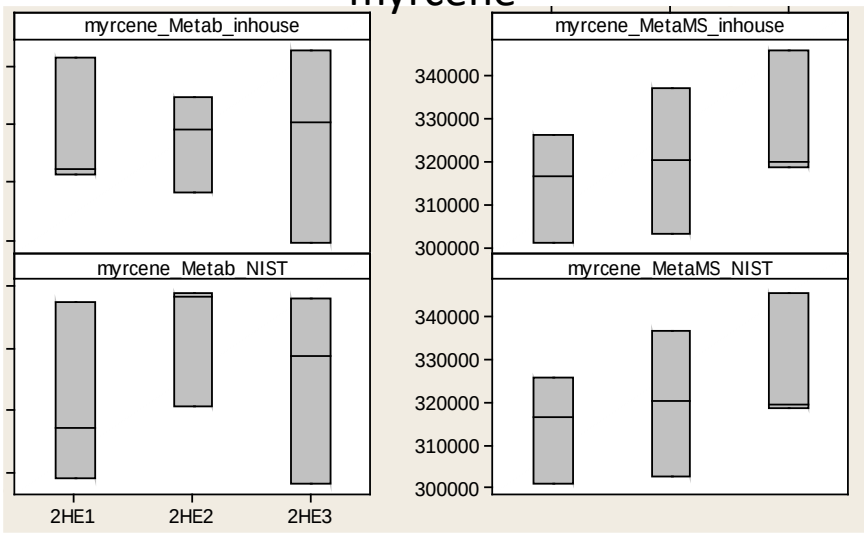
Boxplots of standard addition samples by processing method

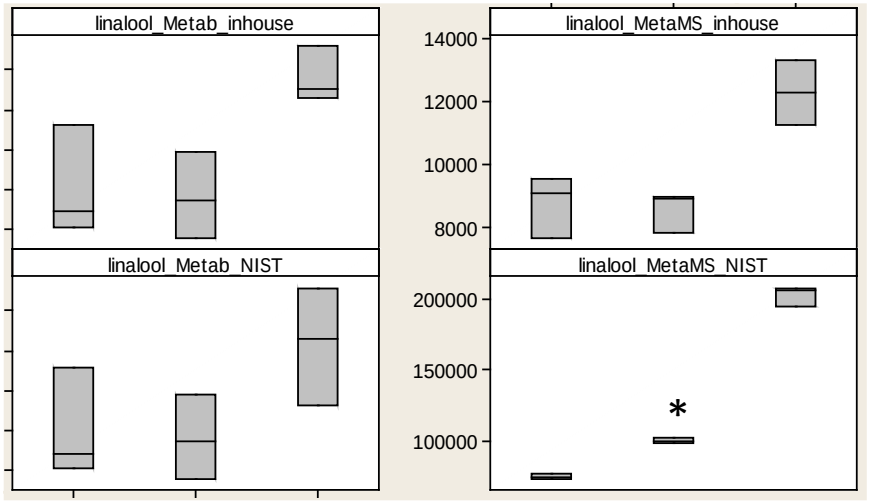
2-methylbutyl isobutyrate



myrcene



linalool



Preliminary conclusion: for these analytes under these conditions, **%recovery was a property of the analyte and sample matrix,** moreso than of processing method or library

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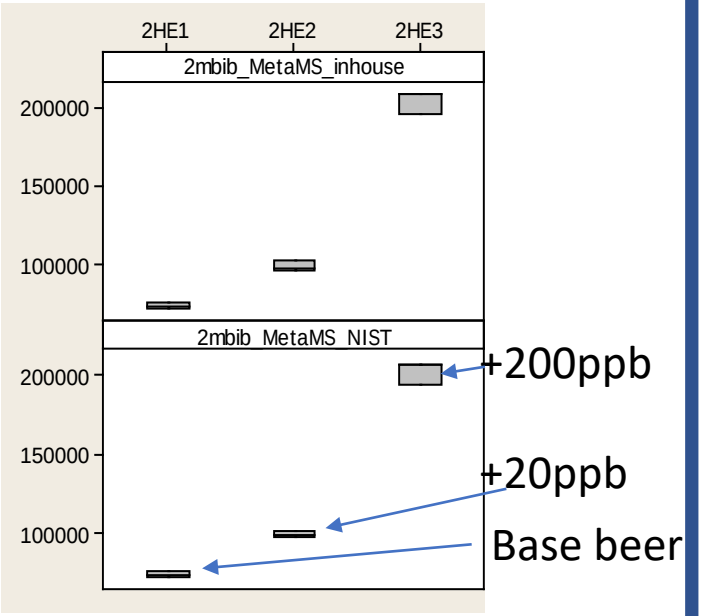
Caveat

GCMS data is highly matrix-dependent. **Be careful when comparing different brands!**

2-methylbutyl isobutyrate in
standard addition samples

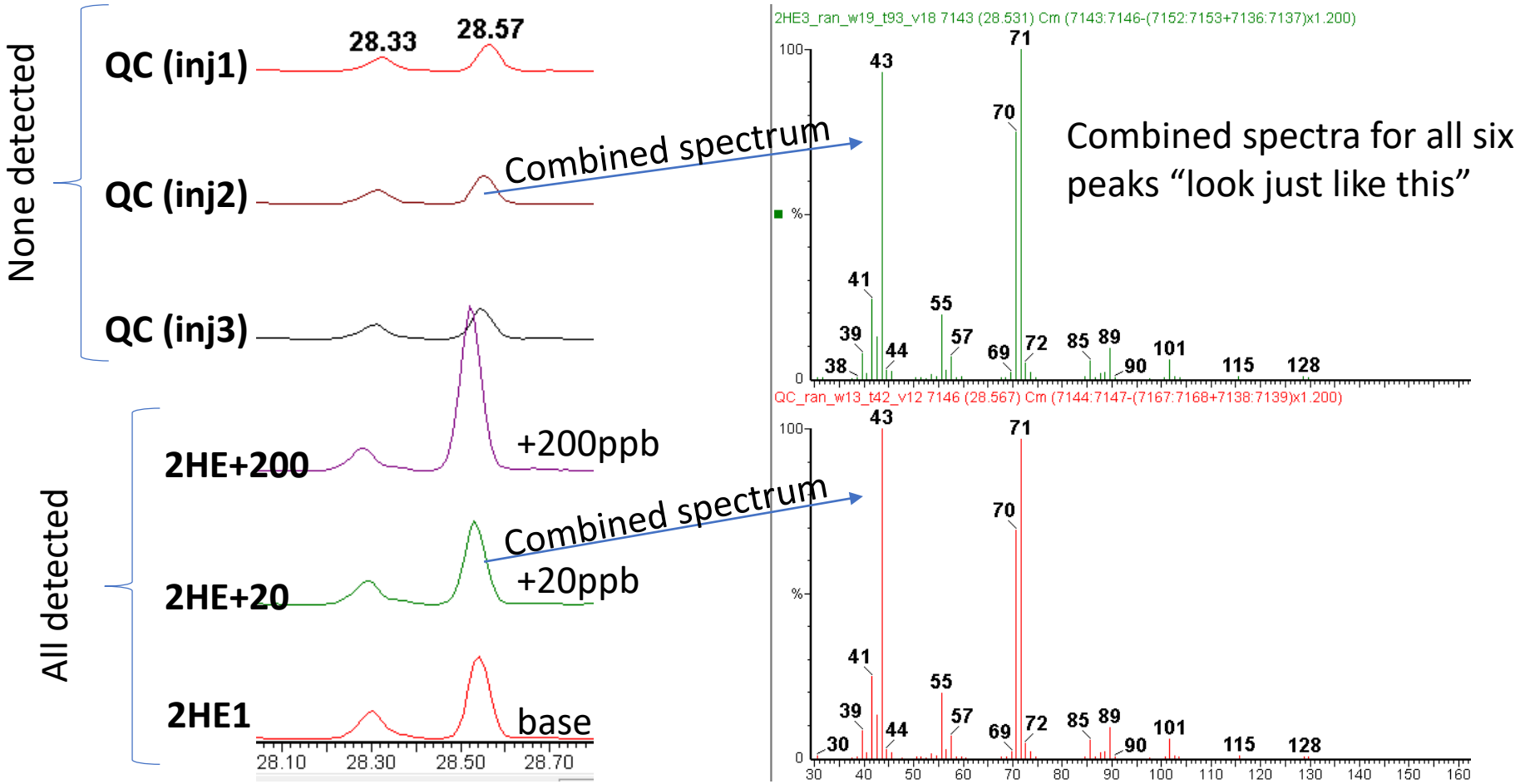


2-methylbutyl isobutyrate in
QC samples

Not detected

In this case, the QC sample approach would filter out this very real compound

Caveat



Manual inspection with *human chromatographer brain* can easily see this component in all six samples

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Conclusions

- The human **chemist brain is still relevant**
- **Each processing method has distinct advantages** and disadvantages
- Comparing **RSD for components in QC samples** (randomized triplicate injections) is a fast way to identify ‘opportunities’ in BIG DATA projects
- A **10% QC RSD** is a reasonable way to filter GCMS data for multivariate analysis

Thank you…

- Martin Biendl and Christina Schmidt (HopSteiner)
  - Analytical standards
- The R/metabolomics/xcms community and Scripps University
  - Methodology and [open source] software
- Mark Zunkel/ASBC Beer&Hop chemistry databases
- Erika Brockberg
  - Sample prep
- John Mallett, Laura Bell, Bell's Quality/Lab team
  - Support

Thank YOU for listening!!

- Questions?
- Comments?