NMRProcFlow

Targeted Metabolomics

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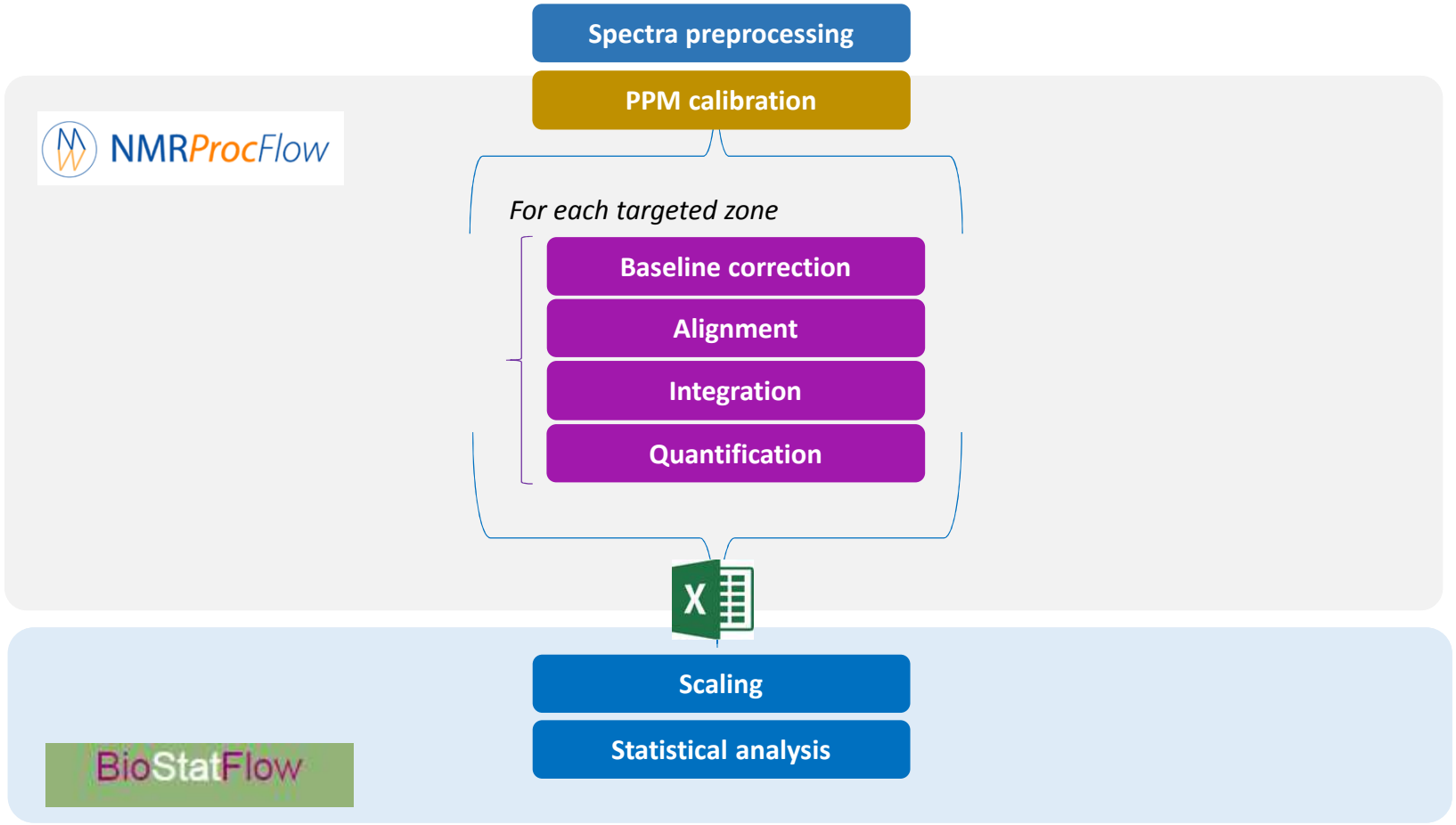
Guideline Overview



NMRProcFlow

Targeted Metabolomics

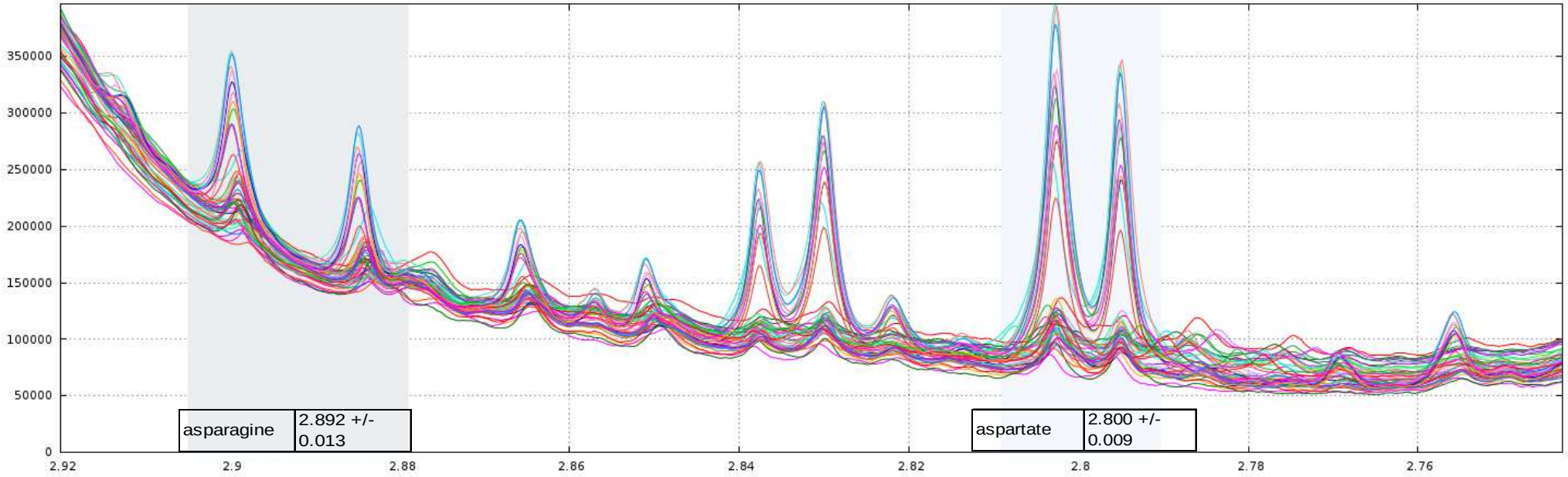
Quantitative approach wherein a set of known metabolites are quantitated.

The identified metabolite peaks are then quantified based on internal or external reference compounds.

Explanation are based on a study realized within the Eranet EraSysBio+ FRIM project (2010-2013)
(See http://nmrprocflow.org/ex1 for more details)

48 ^{1}H-NMR spectra – Solvent: D2O – Pulse: ZG – 64K – TSP / ERETIC

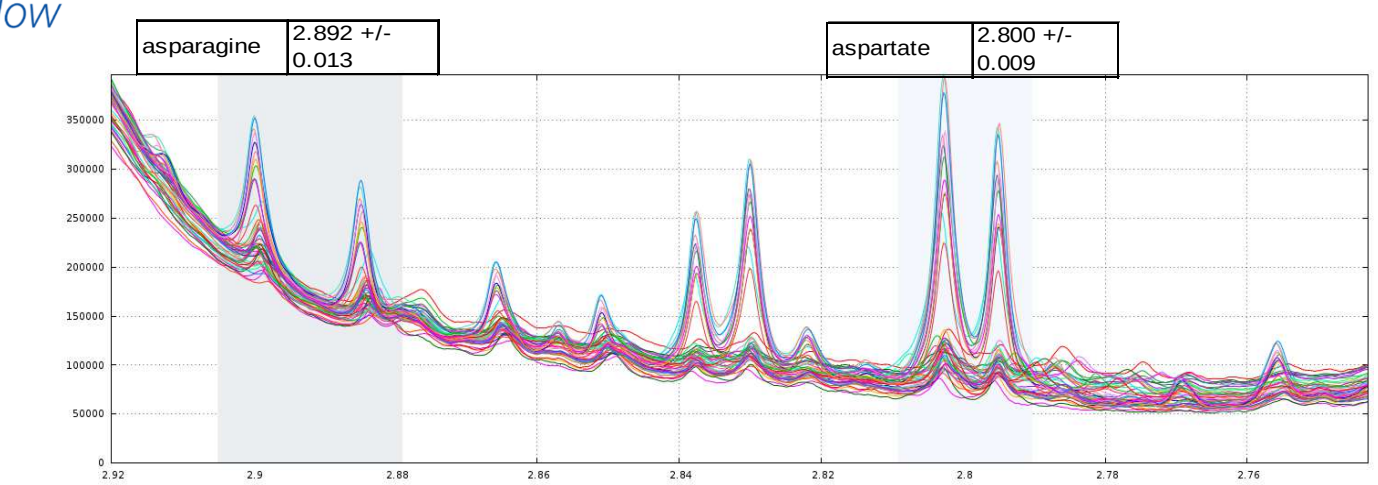
Pericarp of Tomato Fruit



Targeted Metabolomics

NMRProcFlow

STEP 1



For each targeted zones

Baseline correction

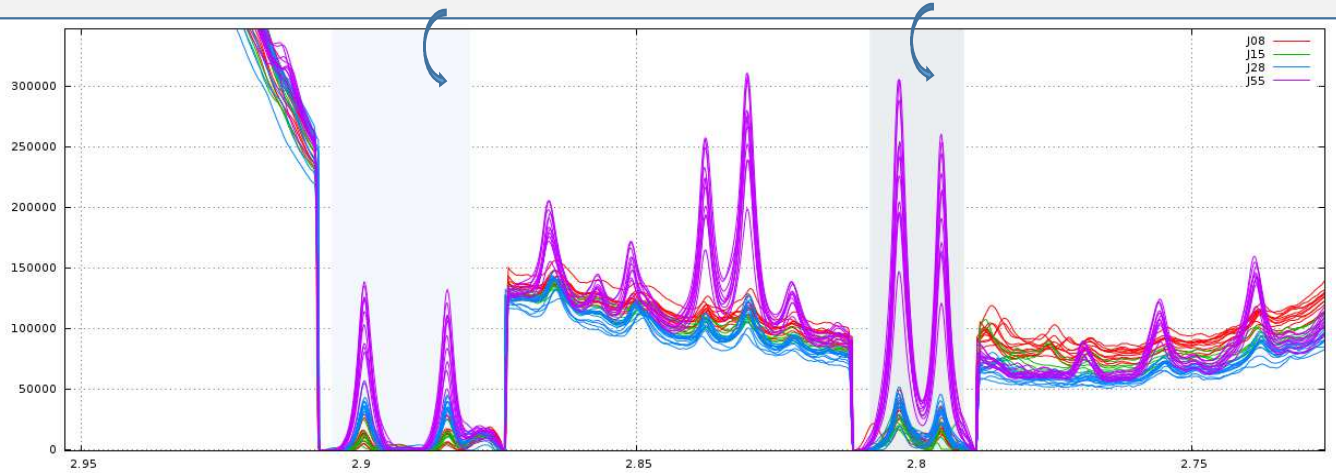
Alignment

```
# Baseline Correction: PPM Range = (  2.874  ,  2.907  )
qnmrbline 10.2 10.5 2.874 2.907

# Alignment of the selected zones ...
align 2.874 2.907 0.06 0
```

```
# Baseline Correction: PPM Range = (  2.789  ,  2.811  )
qnmrbline 10.2 10.5 2.789 2.811

# Alignment of the selected zones ...
align 2.789 2.811 0.06 0
```



INRA SCIENCE & IMPACT

METABOHUB

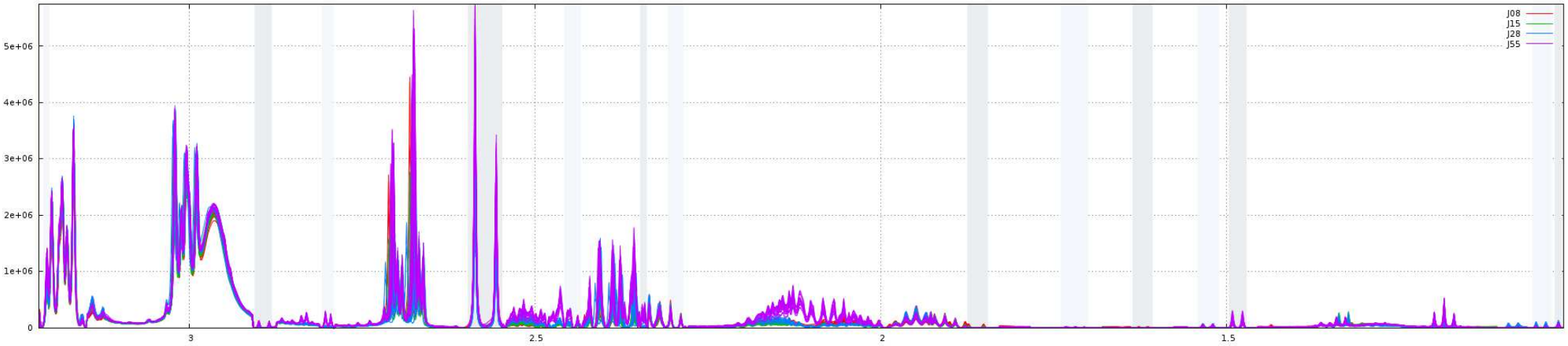
NMRProcFlow

Targeted Metabolomics

qHNMR Template

STEP 2

Integration



Processing | Bucketing | Data Export

Data Type to Export:
- Data matrix
- Buckets table
- SNR matrix
- XLSX Workbook
- Spectral data

Template Format: qHNMR template

SNR threshold: 3

noisy PPM range: 10.5 10.2

Normalization Method: None

PPM range of the Reference: 10.095 9.894

ERETIC (optional)

Export Workbook

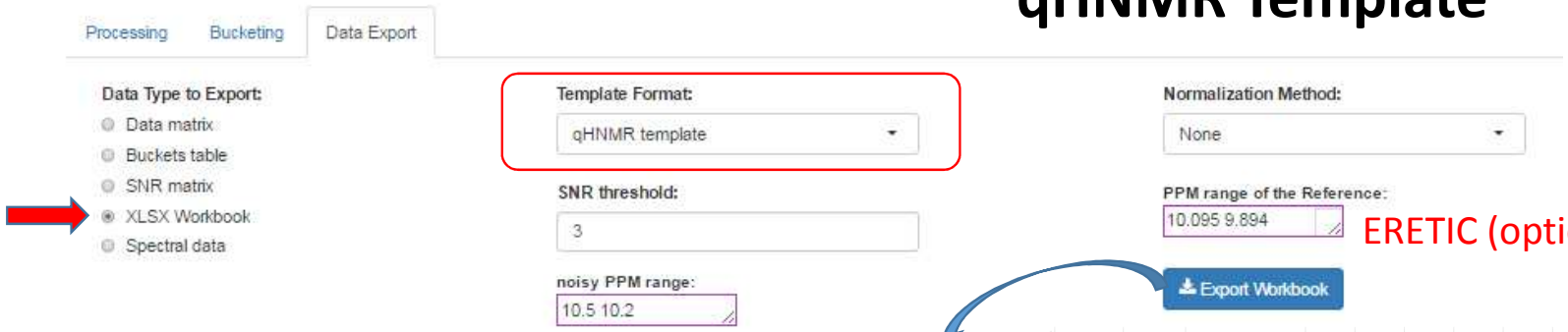
NMRProcFlow

Targeted Metabolomics

qHNMR Template

STEP 2

Integration



ERETIC (optional)

"All in one !"

Rawnames	Samplecode	expno	procno	Condition	Stage	Volume (10^-6 L)	ngDM	Dilution
MMBBI_15P07-F3-00	F3-001	1	1	Control	J08	500	20	1
MMBBI_15P07-F3-04	F3-049	1	1	Control	J08	500	20	1
MMBBI_15P07-F3-09	F3-097	1	1	Control	J08	500	20	1
MMBBI_15P07-F3-00	F3-002	1	1	Shadow	J08	500	20	1
MMBBI_15P07-F3-05	F3-050	1	1	Shadow	J08	500	20	1
MMBBI_15P07-F3-09	F3-098	1	1	Shadow	J08	500	20	1
MMBBI_15P07-F3-01	F3-013	1	1	Control	J15	500	20	1
MMBBI_15P07-F3-06	F3-061	1	1	Control	J15	500	20	1
MMBBI_15P07-F3-10	F3-109	1	1	Control	J15	500	20	1
MMBBI_15P07-F3-06	F3-062	1	1	Shadow	J15	500	20	1
MMBBI_15P07-F3-11	F3-110	1	1	Shadow	J15	500	20	1
MMBBI_15P07-F3-02	F3-025	1	1	Control	J28	500	20	1
MMBBI_15P07-F3-07	F3-073	1	1	Control	J28	500	20	1
MMBBI_15P07-F3-12	F3-121	1	1	Control	J28	500	20	1
MMBBI_15P07-F3-02	F3-026	1	1	Shadow	J28	500	20	1
MMBBI_15P07-F3-07	F3-074	1	1	Shadow	J28	500	20	1
MMBBI_15P07-F3-12	F3-122	1	1	Shadow	J28	500	20	1
MMBBI_15P07-F3-08	F3-085	1	1	Control	J55	500	20	1
MMBBI_15P07-F3-13	F3-133	1	1	Control	J55	500	20	1
MMBBI_15P07-F3-03	F3-038	1	1	Shadow	J55	500	20	1
MMBBI_15P07-F3-08	F3-086	1	1	Shadow	J55	500	20	1
MMBBI_15P07-F3-13	F3-134	1	1	Shadow	J55	500	20	1
MMBBI_15P07-F4-00	F4-001	1	1	Control	J08	500	20	1
MMBBI_15P07-F4-00	F4-009	1	1	Control	J08	500	20	1
MMBBI_15P07-F4-06	F4-065	1	1	Control	J08	500	20	1
MMBBI_15P07-F4-00	F4-005	1	1	Shadow	J08	500	20	1
MMBBI_15P07-F4-06	F4-069	1	1	Shadow	J08	500	20	1
MMBBI_15P07-F4-03	F4-037	1	1	Shadow	J08	500	20	1
MMBBI_15P07-F4-01	F4-017	1	1	Shadow	J15	500	20	1
MMBBI_15P07-F4-04	F4-045	1	1	Shadow	J15	500	20	1
MMBBI_15P07-F4-02	F4-021	1	1	Control	J28	500	20	1
MMBBI_15P07-F4-04	F4-049	1	1	Control	J28	500	20	1
MMBBI_15P07-F4-08	F4-081	1	1	Control	J28	500	20	1
MMBBI_15P07-F4-02	F4-025	1	1	Shadow	J28	500	20	1
MMBBI_15P07-F4-05	F4-053	1	1	Shadow	J28	500	20	1

Samples | Buckets | Data | SNR | Quantification

name	center	min	max	width	Compound	Number of H	Signal Proportion	Molar Mass	Calibration Factor
B0_9509	0.9509	0.9212	0.9806	0.0594	C1	1	1	100	1
B1_0206	1.02055	1.0148	1.0263	0.0115	C2	1	1	100	1
B1_0442	1.04425	1.0304	1.0581	0.0277	C3	1	1	100	1
B1_4839	1.4839	1.4708	1.497	0.0262	C4	1	1	100	1
B1_5269	1.52685	1.5114	1.5423	0.0309	C5	1	1	100	1
B1_6215	1.6215	1.607	1.636	0.029	C6	1	1	100	1
B1_7200	1.72	1.7	1.74	0.04	C7	1	1	100	1
B1_8600	1.86	1.845	1.875	0.03	C8	1	1	100	1
B2_2965	2.2965	2.285	2.308	0.023	C9	1	1	100	1
B2_3430	2.343	2.338	2.348	0.01	C10	1	1	100	1
B2_4450	2.445	2.433	2.457	0.024	C11	1	1	100	1
B2_5720	2.572	2.547	2.597	0.05	C12	1	1	100	1
B2_7995	2.7995	2.791	2.808	0.017	C13	1	1	100	1
B2_8925	2.8925	2.88	2.905	0.025	C14	1	1	100	1
B3_2066	3.20655	3.2021	3.211	0.0089	C15	1	1	100	1
B3_6060	3.606	3.603	3.609	0.006	C16	1	1	100	1
B4_1155	4.1155	4.105	4.126	0.021	C17	1	1	100	1
B4_1645	4.1645	4.16	4.169	0.009	C18	1	1	100	1
B4_3025	4.3025	4.284	4.321	0.037	C19	1	1	100	1
B4_6508	4.6508	4.6272	4.6744	0.0472	C20	1	1	100	1
B5_1040	5.104	5.096	5.112	0.016	C21	1	1	100	1
B5_1870	5.187	5.182	5.192	0.01	C22	1	1	100	1
B5_2385	5.2385	5.229	5.248	0.019	C23	1	1	100	1
B5_2710	5.271	5.264	5.278	0.014	C24	1	1	100	1
B5_3085	5.3085	5.3	5.317	0.017	C25	1	1	100	1
B5_3925	5.3925	5.387	5.398	0.011	C26	1	1	100	1
B5_4175	5.4175	5.408	5.427	0.019	C27	1	1	100	1
B5_8275	5.8275	5.817	5.838	0.021	C28	1	1	100	1
B6_1525	6.1525	6.14	6.165	0.025	C29	1	1	100	1
B6_4235	6.4235	6.399	6.448	0.049	C30	1	1	100	1
B6_5231	6.5231	6.5185	6.5277	0.0092	C31	1	1	100	1
B6_9035	6.9035	6.886	6.921	0.035	C32	1	1	100	1
B7_4290	7.429	7.415	7.443	0.028	C33	1	1	100	1
B7_5585	7.5585	7.543	7.574	0.031	C34	1	1	100	1
B7_6185	7.6185	7.604	7.633	0.029	C35	1	1	100	1

Samples | Buckets | Data | SNR | Quantification

			C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13
		Number of H	1	1	1	1	1	1	1	1	1	1	1	1	1
		Signal Proportion	1	1	1	1	1	1	1	1	1	1	1	1	1
		Molar Mass	100	100	100	100	100	100	100	100	100	100	100	100	100
		Calibration Factor	1	1	1	1	1	1	1	1	1	1	1	1	1
Samplecode	Condition	Stage	B0_9509	B1_0206	B1_0442	B1_4839	B1_5269	B1_6215	B1_7200	B1_8600	B2_2965	B2_3430	B2_4450	B2_5720	B2_7995
F3-001	Control	J08	2.94347	0.29308	0.5207	0.88419	0.38641	0.12332	0.39461	0.43648	2.09922	0.17842	1.08928	30.8965	0.30171
F3-049	Control	J08	2.28359	0.14289	0.2742	0.69361	0.25601	0.20965	0.2779	0.46919	1.3442	0.11053	0.8055	31.1965	0.32219
F3-097	Control	J08	2.9401	0.19715	0.34815	0.89682	0.38976	0.24768	0.36083	0.53295	1.58259	0.12337	0.71943	31.2566	0.19623
F3-002	Shadow	J08	3.8762	0.47487	0.79138	1.24715	0.63934	0.15109	0.46165	0.43079	2.72106	0.27916	0.841	28.6079	0.26459
F3-050	Shadow	J08	2.55652	0.13723	0.27775	0.75288	0.29996	0.36925	0.36918	0.51671	1.89065	0.13125	0.85406	33.1719	0.22461
F3-098	Shadow	J08	2.97264	0.16905	0.35786	1.00888	0.35079	0.42493	0.47198	0.56719	2.03515	0.06599	1.11453	35.4409	0.261
F3-013	Control	J15	3.05023	0.37199	0.54586	0.90506	0.44505	0.0448	0.2703	0.2997	1.6761	0.29468	0.64992	23.6867	0.1488
F3-061	Control	J15	3.24461	0.4027	0.58213	1.27332	0.6996	0.03524	0.33422	0.18903	1.27377	0.13534	1.49031	21.1426	0.25691
F3-109	Control	J15	3.62542	0.44268	0.6808	1.95032	1.03312	0.06158	0.34838	0.22838	1.50218	0.18577	1.27691	21.3943	0.18683
F3-062	Shadow	J15	2.95932	0.366	0.53313	0.98181	0.6	0.04606	0.20901	0.22846	1.49513	0.1541	0.97858	22.4673	0.18713
F3-110	Shadow	J15	3.06642	0.33149	0.51419	1.07999	0.59254	0.07862	0.35158	0.28538	1.7256	0.26909	0.79308	25.127	0.33286
F3-025	Control	J28	2.46032	0.3819	0.59847	1.02962	0.54566	0.02984	0.24152	0.11603	1.56742	0.16829	0.90246	22.1112	0.27783
F3-073	Control	J28	2.96893	0.45487	0.69999	1.02822	0.50832	0.05975	0.29929	0.10559	2.06015	0.20836	1.35564	22.2527	0.41205
F3-121	Control	J28	3.5063	0.57841	0.82177	1.065	0.49699	0.07503	0.34439	0.06865	2.79035	0.2459	1.52141	22.1607	0.41872
F3-026	Shadow	J28	2.57793	0.43984	0.69591	1.24137	0.54994	0.03296	0.28932	0.10594	3.14302	0.77536	1.1458	14.3057	0.15749
F3-074	Shadow	J28	3.75182	0.61668	0.99617	1.66627	0.80547	0.05199	0.42082	0.10643	2.17131	0.19106	1.85947	21.0282	0.33042
F3-122	Shadow	J28	3.43968	0.51756	0.74739	1.10021	0.52693	0.06269	0.33198	0.11517	2.64783	0.25249	1.50503	21.1284	0.36068
F3-085	Control	J55	3.61817	0.37787	0.43556	0.65634	0.21127	0.05621	0.57684	0.1404	2.55922	2.38563	3.99649	35.351	2.11201
F3-133	Control	J55	3.49662	0.37999	0.36364	1.79093	0.62101	0.13041	0.52536	0.10847	2.25366	2.04288	3.16685	42.6551	1.85528
F3-038	Shadow	J55	4.31829	0.45762	0.50826	3.20786	1.00194	0.04461	0.68401	0.14962	2.39428	2.56497	4.05297	46.228	2.55532
F3-086	Shadow	J55	4.05544	0.39734	0.29775	0.92315	0.29073	0.0724	0.64948	0.14156	2.49815	3.29281	4.43528	45.2228	2.63418
F3-134	Shadow	J55	4.61482	0.49626	0.44283	2.88951	0.91663	0.06507	0.62075	0.13742	2.36269	3.10309	4.56086	35.4538	2.45658
F4-001	Control	J08	3.07221	0.2584	0.53404	1.34929	0.50214	0.23077	0.43646	0.6284	2.94116	0.21893	1.30583	36.5649	0.28632
F4-009	Control	J08	1.97225	0.09442	0.24376	0.64389	0.24247	0.34852	0.23681	0.64245	1.7592	0.14157	0.70305	33.3308	0.19881
F4-065	Control	J08	3.11684	0.19362	0.43459	1.18224	0.45381	0.28908	0.4462	0.65959	1.96602	0.1339	1.28126	32.8772	0.23279
F4-005	Shadow	J08	2.65968	0.17687	0.41484	0.80262	0.32226	0.35411	0.44223	0.71512	3.46266	0.24666	0.95568	39.4408	0.32703
F4-069	Shadow	J08	2.51704	0.14666	0.36372	1.06176	0.34704	0.27117	0.3175	0.71497	2.13036	0.11619	0.85913	36.3927	0.29818
F4-037	Shadow	J08	2.39414	0.14275	0.35963	0.76393	0.26	0.35506	0.33128	0.84612	1.91434	0.11429	1.06374	34.2292	0.23618
F4-017	Shadow	J15	1.84725	0.17387	0.31129	0.76692	0.35111	0.14247	0.0806	0.41038	1.36949	0.12552	0.67194	26.5745	0.16364
F4-045	Shadow	J15	2.49432	0.30439	0.50093	1.84186	0.93827	0.07097	0.2934	0.41642	1.41425	0.17997	0.7748	25.2747	0.16893

Samples | Buckets | Data | SNR | Quantification

Samples | Buckets | **data_NMRFRIM3-4** | snr_NMRFRIM3-4 | data_GlucGln | data_FruGlu | Quantif

STEP 2

Integration

Data matrix

Samplecode	Condition	Stage	B0_9509	B1_0206	B1_0442	B1_4839	B1_5269	B1_6215	B1_7200	B1_8600	B2_2965	B2_3430
F3-001	Control	J08	1.197219917	0.11920527	0.2117872	0.359632854	0.157167137	0.05015902	0.160503996	0.177534661	0.853829515	0.072569616
F3-049	Control	J08	0.924675782	0.057859812	0.111029016	0.280858892	0.103663735	0.084892166	0.112526937	0.189986646	0.544297043	0.04475733
F3-097	Control	J08	1.189932036	0.079790696	0.140904923	0.362965256	0.157745771	0.100240783	0.146035722	0.215697044	0.640511817	0.04992885
F3-002	Shadow	J08	1.562665595	0.191439372	0.319040281	0.502781217	0.257744716	0.06091033	0.18611254	0.173671735	1.096977261	0.112541814
F3-050	Shadow	J08	1.049527262	0.056337073	0.114026268	0.309080287	0.123141416	0.151588195	0.151560436	0.212124384	0.776167973	0.053883556
F3-098	Shadow	J08	1.2147108	0.069079124	0.146232894	0.412259683	0.143342919	0.173638568	0.192866244	0.231771975	0.831622487	0.026965075
F3-013	Control	J15	1.230249243	0.150036456	0.220160476	0.365036244	0.179500156	0.018069171	0.109019589	0.120879878	0.67602002	0.118853645
F3-061	Control	J15	1.315441699	0.163266044	0.236011184	0.516234488	0.283632725	0.014287287	0.135501366	0.076636391	0.51641915	0.054868788
F3-109	Control	J15	1.455521949	0.177726307	0.273326662	0.783008806	0.414775131	0.024724258	0.139867876	0.091691053	0.60308891	0.074583399
F3-062	Shadow	J15	1.199053209	0.148293862	0.216013646	0.397807574	0.243109345	0.018661245	0.084687269	0.092568151	0.605795009	0.062439579
F3-110	Shadow	J15	1.238750361	0.133912552	0.207718047	0.436287704	0.239369047	0.031758704	0.142027392	0.115287408	0.69709523	0.108704623
F3-025	Control	J28	0.997455155	0.154826918	0.24263087	0.417426987	0.221219606	0.012098044	0.097915988	0.047040385	0.63545924	0.06822784
F3-073	Control	J28	1.210740956	0.185499293	0.285459491	0.419312993	0.207293477	0.024365789	0.122052583	0.043059982	0.84013724	0.084969811
F3-121	Control	J28	1.416305079	0.233637849	0.331938863	0.430187547	0.200749872	0.030308306	0.13910798	0.027728982	1.127111323	0.099325543
F3-026	Shadow	J28	1.061406682	0.181095714	0.286524633	0.511107219	0.226427303	0.013570912	0.119122818	0.043617397	1.294068891	0.319238688
F3-074	Shadow	J28	1.524294254	0.250545419	0.404725006	0.676973622	0.327245516	0.021121932	0.170971458	0.043242006	0.882162649	0.077625512
F3-122	Shadow	J28	1.388375062	0.208906639	0.301672852	0.444083463	0.212689173	0.025305086	0.133998658	0.046486906	1.068754647	0.101913921
F3-085	Control	J55	1.483960462	0.154978219	0.178640806	0.269190406	0.086650538	0.023053824	0.236587427	0.057584323	1.049641099	0.97844667
F3-133	Control	J55	1.43946186	0.156429634	0.149701721	0.737277535	0.25565374	0.053685673	0.216276457	0.044655033	0.927770076	0.840996125
F3-038	Shadow	J55	1.740518112	0.184446031	0.204858909	1.292949511	0.40383945	0.017978665	0.27569556	0.060306864	0.965029952	1.033831262
F3-086	Shadow	J55	1.63868709	0.160552595	0.12031416	0.373019116	0.117474094	0.029256057	0.262435486	0.05720183	1.00943081	1.330530755
F3-134	Shadow	J55	1.86712884	0.200782078	0.179166512	1.169079619	0.370861878	0.026325021	0.251152082	0.055598126	0.955928493	1.255492902
F4-001	Control	J08	1.258949103	0.105888885	0.218840237	0.552921194	0.205770356	0.094566156	0.178853911	0.257511222	1.205245553	0.089714285
F4-009	Control	J08	0.806972389	0.03863403	0.099737387	0.263456623	0.099210454	0.142600631	0.096893676	0.26286479	0.719801612	0.057925787
F4-065	Control	J08	1.262186477	0.078409287	0.175992037	0.478757145	0.183774169	0.117063485	0.180691929	0.267104586	0.796154097	0.054224632
F4-005	Shadow	J08	1.08534168	0.072174999	0.169283749	0.327528727	0.131503341	0.144502762	0.180461883	0.291821724	1.413014357	0.100654376
F4-069	Shadow	J08	1.022104358	0.05955417	0.147699021	0.431154636	0.140921917	0.110114805	0.128927617	0.290329317	0.865085583	0.04718058

Integral $_{\text{Bucket}}$ (ua)

STEP 3

Quantification

Review

Reference and normalization methods: Essential tools for the intercomparison of NMR spectra

Patrick Giraudeau, Illa Tea, Gérald S. Remaud, Serge Akoka
EBSI Team, Chimie et Interdisciplinarité: Synthèse, Analyse, Modélisation (CEISAM), Université de Nantes, CNRS, UMR 6230, LUNAM Université, B.P. 92208, 2 rue de la Houssinière, F-44322 Nantes Cedex 03, France

Journal of Pharmaceutical and Biomedical Analysis
Volume 93, May 2014, Pages 3–16
NMR Spectroscopy in Pharmaceutical and Biomedical Analysis

Calculate Quantifications

NMRProcFlow

$$\frac{\frac{Volume_{tube}\ (\mu L) \times Rate_{dilution}}{MasseSampleDW_{tube}\ (mg)} \times \frac{Integral_{Bucket}\ (ua)}{Calib_{Ref} \times N_{1H} \times \%\ Signal\ (ua \cdot mM^{-1})}}{mM_{tube}} \times \frac{MM_{cmpd}\ (g/mole)}{} \times 10^{-3} \Rightarrow (mg / gDW)$$

NMRProcFlow

Targeted Metabolomics

STEP 3

Quantification

qHNMR Template

Rawnames	Samplecode	expno	procno	Condition	Stage	Volume (10^-6.L)	ngDW	Dilution
MMBBI_15P07-F3-001	F3-001	1	1	Control	J08	500	20	1
MMBBI_15P07-F3-049	F3-049	1	1	Control	J08	500	20	1
MMBBI_15P07-F3-097	F3-097	1	1	Control	J08	500	20	1
MMBBI_15P07-F3-002	F3-002	1	1	Shadow	J08	500	20	1
MMBBI_15P07-F3-050	F3-050	1	1	Shadow	J08	500	20	1
MMBBI_15P07-F3-098	F3-098	1	1	Shadow	J08	500	20	1
MMBBI_15P07-F3-013	F3-					500	20	1
MMBBI_15P07-F3-061	F3-					500	20	1
MMBBI_15P07-F3-109	F3-					500	20	1
MMBBI_15P07-F3-062	F3-					500	20	1
MMBBI_15P07-F3-110	F3-					500	20	1
MMBBI_15P07-F3-025	F3-025	1	1	Control	J28	500	20	1
MMBBI_15P07-F3-073	F3-073	1	1	Control	J28	500	20	1
MMBBI_15P07-F3-121	F3-121	1	1	Control	J28	500	20	1
MMBBI_15P07-F3-026	F3-026	1	1	Shadow	J28	500	20	1
MMBBI_15P07-F3-074	F3-074	1	1	Shadow	J28	500	20	1
MMBBI_15P07-F3-122	F3-122	1	1	Shadow	J28	500	20	1
MMBBI_15P07-F3-085	F3-085	1	1	Control	J55	500	20	1
MMBBI_15P07-F3-133	F3-133	1	1	Control	J55	500	20	1
MMBBI_15P07-F3-038	F3-038	1	1	Shadow	J55	500	20	1
MMBBI_15P07-F3-086	F3-086	1	1	Shadow	J55	500	20	1
MMBBI_15P07-F3-134	F3-134	1	1	Shadow	J55	500	20	1
MMBBI_15P07-F4-001	F4-001	1	1	Control	J08	500	20	1
MMBBI_15P07-F4-009	F4-009	1	1	Control	J08	500	20	1
MMBBI_15P07-F4-065	F4-065	1	1	Control	J08	500	20	1
MMBBI_15P07-F4-005	F4-005	1	1	Shadow	J08	500	20	1
MMBBI_15P07-F4-069	F4-069	1	1	Shadow	J08	500	20	1
MMBBI_15P07-F4-037	F4-037	1	1	Shadow	J08	500	20	1
MMBBI_15P07-F4-017	F4-017	1	1	Shadow	J15	500	20	1
MMBBI_15P07-F4-045	F4-045	1	1	Shadow	J15	500	20	1
MMBBI_15P07-F4-021	F4-021	1	1	Control	J28	500	20	1
MMBBI_15P07-F4-049	F4-049	1	1	Control	J28	500	20	1
MMBBI_15P07-F4-081	F4-081	1	1	Control	J28	500	20	1
MMBBI_15P07-F4-025	F4-025	1	1	Shadow	J28	500	20	1
MMBBI_15P07-F4-053	F4-053	1	1	Shadow	J28	500	20	1

Samples

name	center	min	max	width	Compound	Number of H	Signal Proportion	Molar Mass	Calibration Factor
B0_9509	0.9509	0.9212	0.9806	0.0594	C1	1	1	100	1
B1_0206	1.02055	1.0148	1.0263	0.0115	C2	1	1	100	1
B1_0442	1.04425	1.0304	1.0581	0.0277	C3	1	1	100	1
B1_4839	1.4839	1.4708	1.497	0.0262	C4	1	1	100	1
B1_5269	1.52685	1.5114	1.5423	0.0309	C5	1	1	100	1
B1_6215	1.6215	1.607	1.636	0.029	C6	1	1	100	1
B1_7200	1.72	1.7	1.74	0.04	C7	1	1	100	1
B1_8600	1.86	1.845	1.875	0.03	C8	1	1	100	1
B2_2965	2.2965	2.285	2.308				1	100	1
B2_3430	2.343	2.338	2.348				1	100	1
B2_4450	2.445	2.433	2.457				1	100	1
B2_5720	2.572	2.547	2.597				1	100	1
B2_7995	2.7995	2.791	2.808				1	100	1
B2_8925	2.8925	2.88	2.905	0.025	C14	1	1	100	1
B3_2066	3.20655	3.2021	3.211	0.0089	C15	1	1	100	1
B3_6060	3.606	3.603	3.609	0.006	C16	1	1	100	1
B4_1155	4.1155	4.105	4.126	0.021	C17	1	1	100	1
B4_1645	4.1645	4.16	4.169	0.009	C18	1	1	100	1
B4_3025	4.3025	4.284	4.321	0.037	C19	1	1	100	1
B4_6508	4.6508	4.6272	4.6744	0.0472	C20	1	1	100	1
B5_1040	5.104	5.096	5.112	0.016	C21	1	1	100	1
B5_1870	5.187	5.182	5.192	0.01	C22	1	1	100	1
B5_2385	5.2385	5.229	5.248	0.019	C23	1	1	100	1
B5_2710	5.271	5.264	5.278	0.014	C24	1	1	100	1
B5_3085	5.3085	5.3	5.317	0.017	C25	1	1	100	1
B5_3925	5.3925	5.387	5.398	0.011	C26	1	1	100	1
B5_4175	5.4175	5.408	5.427	0.019	C27	1	1	100	1
B6_5231	6.5231	6.5185	6.5277	0.0092	C31	1	1	100	1
B6_9035	6.9035	6.886	6.921	0.035	C32	1	1	100	1
B7_4290	7.429	7.415	7.443	0.028	C33	1	1	100	1
B7_5585	7.5585	7.543	7.574	0.031	C34	1	1	100	1
B7_6185	7.6185	7.604	7.633	0.029	C35	1	1	100	1

Buckets

Change with the appropriate values …

		C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13
	Number of H	1	1	1	1	1	1	1	1	1	1	1	1	1
	Signal Proportion	1	1	1	1	1	1	1	1	1	1	1	1	1
	Molar Mass	100	100	100	100	100	100	100	100	100	100	100	100	100
	Calibration Factor	1	1	1	1	1	1	1	1	1	1	1	1	1
Samplecode	Condition	Stage	B0_9509	B1_0206	B1_0442	B1_4839	B1_5269	B1_6215	B1_7200	B1_8600	B2_2965	B2_3430	B2_4450	B2_5720	B2_7995
F3-001	Control	J08	2.94347	0.29308	0.5207	0.88419	0.38641	0.12332	0.39461	0.43648	2.09922	0.17842	1.08928	30.8965	0.30171
F3-049	Control	J08	2.28359	0.14289	0.2742	0.69361	0.25601	0.20965	0.2779	0.46919	1.3442	0.11053	0.8055	31.1965	0.32219
F3-097	Control	J08	2.9401	0.19715	0.34815	0.89682	0.38976	0.24768	0.36083	0.53295	1.58259	0.12337	0.71943	31.2566	0.19623
F3-002	Shadow	J08	3.8762	0.47487	0.79138	1.24715	0.63934	0.15109	0.46165	0.43079	2.72106	0.27916	0.841	28.6079	0.26459
F3-050	Shadow	J08	2.55652	0.13723	0.27775	0.75288	0.29996	0.36925	0.36918	0.51671	1.89065	0.13125	0.85406	33.1719	0.22461
F3-098	Shadow	J08	2.97264	0.16905	0.35786	1.00888	0.35079	0.42493	0.47198	0.56719	2.03515	0.06599	1.11453	35.4409	0.261
F3-013	Control	J15	3.05023	0.37199	0.5						761	0.29468	0.64992	23.6867	0.1488
F3-061	Control	J15	3.24461	0.4027	0.5						377	0.13534	1.49031	21.1426	0.25691
F3-109	Control	J15	3.62542	0.44268	0.						218	0.18577	1.27691	21.3943	0.18683
F3-062	Shadow	J15	2.95932	0.366	0.53313	0.98181	0.6	0.04606	0.20901	0.22846	1.49513	0.1541	0.97858	22.4673	0.18713
F3-110	Shadow	J15	3.06642	0.33149	0.51459	1.07999	0.59254	0.07862	0.35158	0.28538	1.7256	0.26909	0.79308	25.127	0.33286
F3-025	Control	J28	2.46032	0.3819	0.59847	1.02962	0.54566	0.02984	0.24152	0.11603	1.56742	0.16829	0.90246	22.1112	0.27783
F3-073	Control	J28	2.96893	0.45487	0.69999	1.02822	0.50832	0.05975	0.29929	0.10559	2.06015	0.20836	1.35564	22.2527	0.41205
F3-121	Control	J28	3.5063	0.57841	0.82177	1.065	0.49699	0.07503	0.34439	0.06865	2.79035	0.2459	1.52141	22.1607	0.41872
F3-026	Shadow	J28	2.57793	0.43984	0.69591	1.24137	0.54994	0.03296	0.28932	0.10594	3.14302	0.77536	1.1458	14.3057	0.15749
F3-074	Shadow	J28	3.75182	0.61668	0.99617	1.66627	0.80547	0.05199	0.42082	0.10643	2.17131	0.19106	1.85947	21.0282	0.33042
F3-122	Shadow	J28	3.43968	0.51756	0.74739	1.10021	0.52693	0.06269	0.33198	0.11517	2.64783	0.25249	1.50503	21.1284	0.36068
F3-085	Control	J55	3.61817	0.37787	0.43556	0.65634	0.21127	0.05621	0.57684	0.1404	2.55922	2.38563	3.99649	35.351	2.11201
F3-133	Control	J55	3.49662	0.37999	0.36364	1.79093	0.62101	0.13041	0.52536	0.10847	2.25366	2.04288	3.16685	42.6551	1.85528
F3-038	Shadow	J55	4.31829	0.45762	0.50826	3.20786	1.00194	0.04461	0.68401	0.14962	2.39428	2.56497	4.05297	46.228	2.55532
F3-086	Shadow	J55	4.05544	0.39734	0.29775	0.92315	0.29073	0.0724	0.64948	0.14156	2.49815	3.29281	4.43528	45.2228	2.63418
F3-134	Shadow	J55	4.61482	0.49626	0.44283	2.88951	0.91663	0.06507	0.62075	0.13742	2.36269	3.10309	4.56086	35.4538	2.45658
F4-001	Control	J08	3.07221	0.2584	0.53404	1.34929	0.50214	0.23077	0.43646	0.6284	2.94116	0.21893	1.30583	36.5649	0.28632
F4-009	Control	J08	1.97225	0.09442	0.24376	0.6	0.24247	0.34852	0.23681	0.64245	1.7592	0.14157	0.70305	33.3308	0.19881
F4-065	Control	J08	3.11684	0.19362	0.43459	1.	0.45381	0.28908	0.4462	0.65959	1.96602	0.1339	1.28126	32.8772	0.23279
F4-005	Shadow	J08	2.65968	0.17687	0.41484	80262	0.32226	0.35411	0.44223	0.71512	3.46266	0.24666	0.95568	39.4408	0.32703
F4-069	Shadow	J08	2.51704	0.14666	0.3	1.06176	0.34704	0.27117	0.3175	0.71497	2.13036	0.11619	0.85913	36.3927	0.29818
F4-037	Shadow	J08	2.39414	0.14275	0.35963	0.76393	0.26	0.35506	0.33128	0.84612	1.91434	0.11429	1.06374	34.2292	0.23618
F4-017	Shadow	J15	1.84725	0.17387	0.31129	0.76692	0.35111	0.14247	0.0806	0.41038	1.36949	0.12552	0.67194	26.5745	0.16364
F4-045	Shadow	J15	2.48332	0.30439	0.50093	1.84186	0.93827	0.07097	0.2934	0.41642	1.41425	0.17997	0.7748	25.2747	0.16893

Quantification

… then the 'Quantification' tab will be automatically updated

$$\frac{\text{Volume}_{tube} \times \text{Rate}_{dilution}}{\text{MasseSampleDW}_{tube}} \times \frac{\text{Integral}_{Bucket}}{\text{Calib}_{Ref} \times N_{1H} \times \%\,\text{Signal}} \times \frac{\text{MM}_{cmpd}}{10^{3}}$$

(µL) (mg) (ua) (ua .mM^{-1}) (g / mole)

$$\text{mM}_{tube} \quad \text{(mg / gDW)}$$

Samples | Buckets | data_NMRFRIM3-4 | snr_NMRFRIM3-4 | data_GlucGln | data_FruGlu | Quantif

Rawnames	Samplecode	expno	procno	Condition	Stage	Volume (10^-6.L)	mgDW	Dilution
MMBBI_15P07-F3-001	F3-001	1	1	Control	J08	505	20.5	1
MMBBI_15P07-F3-049	F3-049	1	1	Control	J08	505	20	1
MMBBI_15P07-F3-097	F3-097	1	1	Control	J08	505	19.8	1
MMBBI_15P07-F3-002	F3-002	1	1	Shadow	J08	510	20.2	1
MMBBI_15P07-F3-050	F3-050	1	1	Shadow	J08	510	20	1
MMBBI_15P07-F3-098	F3-098	1	1	Shadow	J08	510	20.3	1
MMBBI_15P07-F3-013	F3-013	1	1	Control	J15	510	20.3	1
MMBBI_15P07-F3-061	F3-061	1	1	Control	J15	510	19.7	1
MMBBI_15P07-F3-109	F3-109	1	1	Control	J15	510	19.9	1
MMBBI_15P07-F3-062	F3-062	1	1	Shadow	J15	510	19.8	1
MMBBI_15P07-F3-110	F3-110	1	1	Shadow	J15	510	20	1
MMBBI_15P07-F3-025	F3-025	1	1	Control	J28	510	19.6	1
MMBBI_15P07-F3-073	F3-073	1	1	Control	J28	510	20.1	1
MMBBI_15P07-F3-121	F3-121	1	1	Control	J28	510	19.6	1
MMBBI_15P07-F3-026	F3-026	1	1	Shadow	J28	510	19.9	1
MMBBI_15P07-F3-074	F3-074	1	1	Shadow	J28	510	20	1
MMBBI_15P07-F3-122	F3-122	1	1	Shadow	J28	510	19.9	1
MMBBI_15P07-F3-085	F3-085	1	1	Control	J55	510	20.4	1
MMBBI_15P07-F3-133	F3-133	1	1	Control	J55	510	19.9	1
MMBBI_15P07-F3-038	F3-038	1	1	Shadow	J55	510	19.7	1
MMBBI_15P07-F3-086	F3-086	1	1	Shadow	J55	510	19.9	1
MMBBI_15P07-F3-134	F3-134	1	1	Shadow	J55	510	19.9	1
MMBBI_15P07-F4-001	F4-001	1	1	Control	J08	510	20.5	1
MMBBI_15P07-F4-009	F4-009	1	1	Control	J08	510	20.3	1
MMBBI_15P07-F4-065	F4-065	1	1	Control	J08	510	20.1	1
MMBBI_15P07-F4-005	F4-005	1	1	Shadow	J08	510	20	1
MMBBI_15P07-F4-069	F4-069	1	1	Shadow	J08	510	20.5	1
MMBBI_15P07-F4-037	F4-037	1	1	Shadow	J08	510	19.7	1
MMBBI_15P07-F4-017	F4-017	1	1	Shadow	J15	505	20	1
MMBBI_15P07-F4-045	F4-045	1	1	Shadow	J15	505	20.1	1
MMBBI_15P07-F4-021	F4-021	1	1	Control	J28	505	20.3	1
MMBBI_15P07-F4-049	F4-049	1	1	Control	J28	505	20.4	1

STEP 3a

$$\frac{Volume_{tube}\ (\mu L) \times Rate_{dilution}}{MasseSampleDW_{tube}\ (mg)}$$

Samples | **Buckets** | data_NMRFRIM3-4 | snr_NMRFRIM3-4 | data_GlucGln | data_FruGlu | Quantif

STEP 3b

name	center	min	max	width	Compound	Number of H	Signal Proportion	Molar Mass	Calibration Factor
B0_9509	0.9509	0.9212	0.9806	0.0594	isoleucine + leucine	1	1	100	0.9657
B1_0206	1.02055	1.0148	1.0263	0.0115	isoleucine	3	0.5	180.6	0.9657
B1_0442	1.04425	1.0304	1.0581	0.0277	valine	3	1	117.15	0.9657
B1_4839	1.4839	1.4708	1.497	0.0262	alanine	3	1	89.09	0.9657
B1_5269	1.52685	1.5114	1.5423	0.0309	unkm1.53	1	1	100	0.9657
B1_6215	1.6215	1.607	1.636	0.029	unkm1.62	1	1	100	0.9657
B1_7200	1.72	1.7	1.74	0.04	lysine	2	0.684	146.1876	
B1_8600	1.86	1.845	1.875	0.03	quinate	1	0.72	192.1666	0.9657
B2_2965	2.2965	2.285	2.308	0.023	GABA	2	0.746	103.12	0.9657
B2_3430	2.343	2.338	2.348	0.01	glutamate	1	1	147.13	0.4831
B2_4450	2.445	2.433	2.457	0.024	glutamine	1	1	146.1	0.9168
B2_5720	2.572	2.547	2.597	0.05	citrate	2	1		
B2_7995	2.7995	2.791	2.808	0.017	aspartate	2	0.315		
B2_8925	2.8925	2.88	2.905	0.025	asparagine	2	0.297		
B3_2066	3.20655	3.2021	3.211	0.0089	choline	9	1		
B3_6060	3.606	3.603	3.609	0.006	inositol	2	0.308		
B4_1155	4.1155	4.105	4.126	0.021	fructose	1	1		
B4_1645	4.1645	4.16	4.169	0.009	pyroglutamate	1	0.251		
B4_3025	4.3025	4.284	4.321	0.037	malate	1	1		
B4_6508	4.6508	4.6272	4.6744	0.0472	glucose_C1_beta	1	1		
B5_1040	5.104	5.096	5.112	0.016	Unkm5.10	1	1		
B5_1870	5.187	5.182	5.192	0.01	mannose	1	1		
B5_2385	5.2385	5.229	5.248	0.019	glucose_C1_alpha	1	1		
B5_2710	5.271	5.264	5.278	0.014	galactose	1	0.634		
B5_3085	5.3085	5.3	5.317	0.017	galacturonate	1	1	194.14	0.9657
B5_3925	5.3925	5.387	5.398	0.011	unkS5.39	1	1	100	0.9657
B5_4175	5.4175	5.408	5.427	0.019	saccharose	1	1	342.3	0.9657
B5_8275	5.8275	5.817	5.838	0.021	unkM5.83	1	1	100	0.9657
B6_1525	6.1525	6.14	6.165	0.025	adenosine_like	1	1	267.24	0.9657
B6_4235	6.4235	6.399	6.448	0.049	chlorogenate	1	0.5	354.3	0.9657

$$mM_{tube} = \frac{Integral_{Bucket}\ (ua)}{Calib_{Ref} \times N_{1H} \times \%\ Signal\ (ua \cdot mM^{-1})} \times MM_{cmpd}\ (g / mole) \times 10^{-3}$$

Calibration-curve method (1/2)

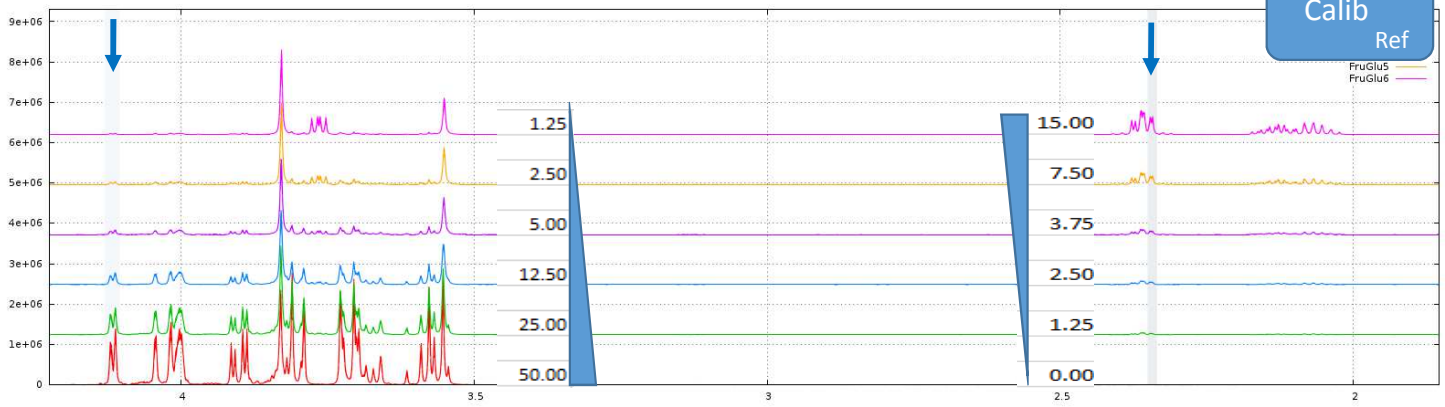
NMRProcFlow

STEP 3b

Gammes
- FruGlu.zip
  - FruGlu
    - 1
      - pdata
        - 1
        - 2
        - 700
    - 2
    - 3
    - 4
    - 5
    - 6
- GlucGln.zip
  - GlucGln
    - 1
      - pdata
        - 1
        - 2
        - 700
    - 2
    - 3
    - 4
    - 5
    - 6

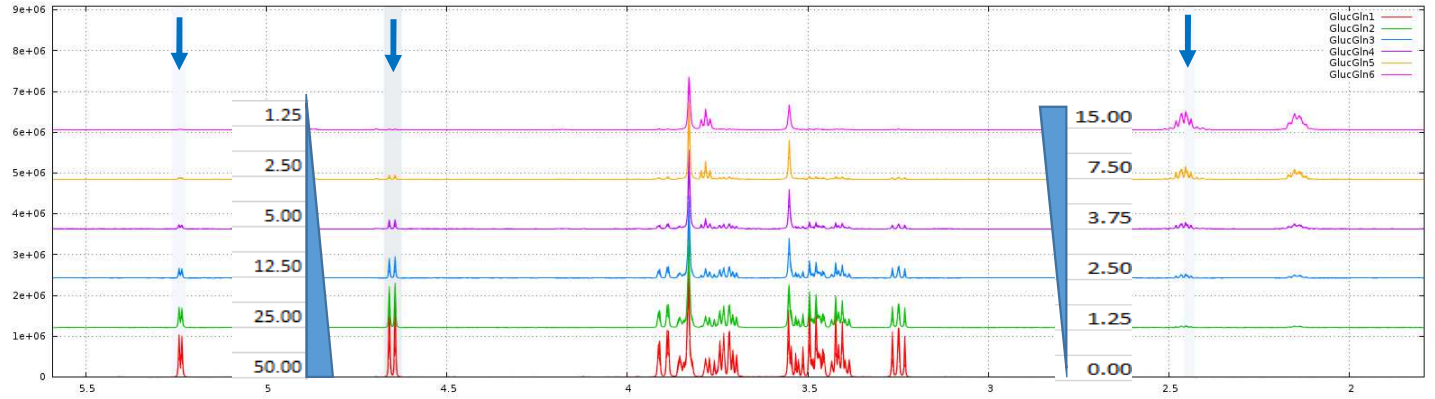
	A	B	C	D
1	Spectrum	Samplecode	EXPNO	PROCNO
2	FruGlu	FruGlu1	1	2
3	FruGlu	FruGlu2	2	2
4	FruGlu	FruGlu3	3	2
5	FruGlu	FruGlu4	4	2
6	FruGlu	FruGlu5	5	2
7	FruGlu	FruGlu6	6	2

	A	B	C	D
1	Spectrum	Samplecode	EXPNO	PROCNO
2	GlucGln	GlucGln1	1	2
3	GlucGln	GlucGln2	2	2
4	GlucGln	GlucGln3	3	2
5	GlucGln	GlucGln4	4	2
6	GlucGln	GlucGln5	5	2
7	GlucGln	GlucGln6	6	2



Fructose [] mM

Glutamate [] mM



Glucose [] mM

Glutamine [] mM

Samples | Buckets | data_NMRFRIM3-4 | snr_NMRFRIM3-4 | **data_GlucGln** | data_FruGlu | Quantif

STEP 3b

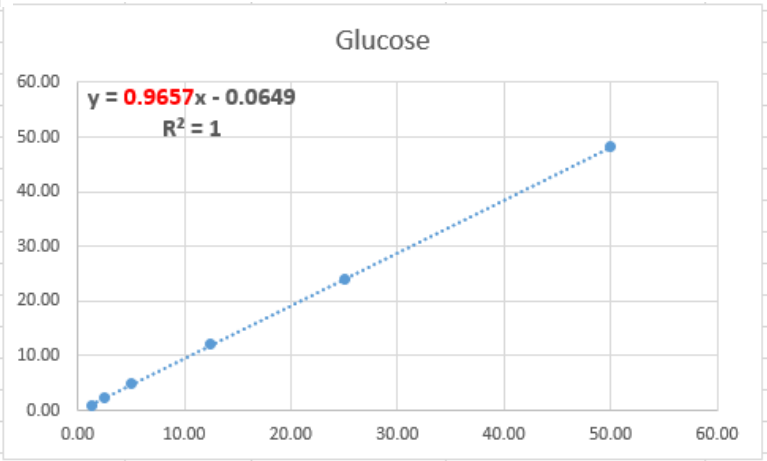
Calib
Ref

Samplecode	B5_2420	B4_6510	B2_4450
GlucGln1	17.8207	30.3884	0.0124
GlucGln2	8.9029	15.1691	1.1437
GlucGln3	4.4622	7.5522	2.3060
GlucGln4	1.8468	3.0388	3.3920
GlucGln5	0.9009	1.4756	6.8828
GlucGln6	0.4250	0.5739	13.7435

		Max	Min
ERETIC	9.996 +/- 0.024	10.02	9.972
alpha_glucose	5.242 +/- 0.020	5.262	5.222
glucose_beta	4.651 +/- 0.024	4.675	4.627
glutamine	2.445 +/- 0.012	2.457	2.433

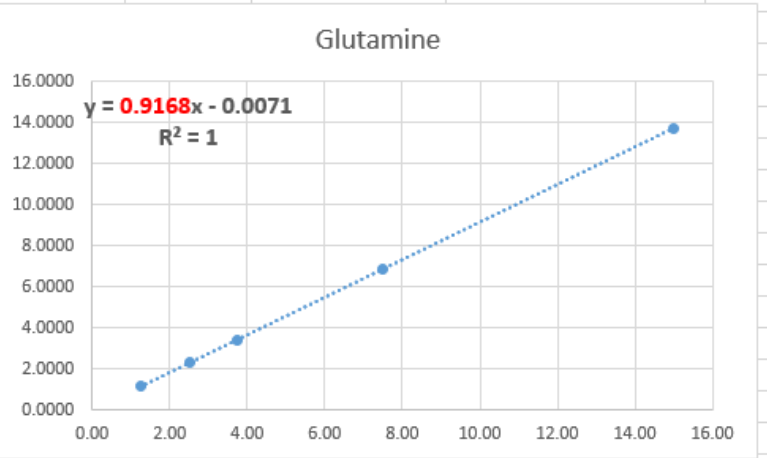
Samplecode	glucose ([] mM)	Sum Alpha+Beta
GlucGln1	50.00	48.21
GlucGln2	25.00	24.07
GlucGln3	12.50	12.01
GlucGln4	5.00	4.89
GlucGln5	2.50	2.38
GlucGln6	1.25	1.00

Glucose



Samplecode	Glutamine [] mM	B2_4450
GlucGln2	1.25	1.1437
GlucGln3	2.50	2.3060
GlucGln4	3.75	3.3920
GlucGln5	7.50	6.8828
GlucGln6	15.00	13.7435

Glutamine



Calibration-curve method (2/2)

NMRProcFlow

METABOHUB

Asparagine - https://peakforest.org/PFs000474

peak index	ν (F1) [ppm]	intensity [rel]	half width [Hz]
1	4.03	0.87	0.69
2	4.02	0.95	0.73
3	4.01	0.99	0.64
4	4.0	0.9	0.6
5	2.98	0.53	0.73
6	2.97	0.54	0.69
7	2.95	1.17	0.73
8	2.94	1.11	0.69
9	2.89	1.13	0.73
10	2.88	1.1	0.69
11	2.86	0.52	0.69
12	2.85	0.52	0.69

Sm = 4.681
i ∈ {5, ..., 12}

Sp = 1.584
i ∈ {9, 10}

Aspartate - https://peakforest.org/PFs000475

peak index	ν (F1) [ppm]	intensity [rel]	half width [Hz]
1	3.92	0.66	1.6
2	3.91	0.71	1.37
3	3.9	0.74	1.69
4	3.89	0.69	1.19
5	2.84	0.48	1.69
6	2.83	0.49	1.24
7	2.8	0.84	1.69
8	2.8	0.81	1.24
9	2.71	0.78	1.14
10	2.7	0.76	1.19
11	2.68	0.48	1.24
12	2.66	0.46	1.14

STEP 3b

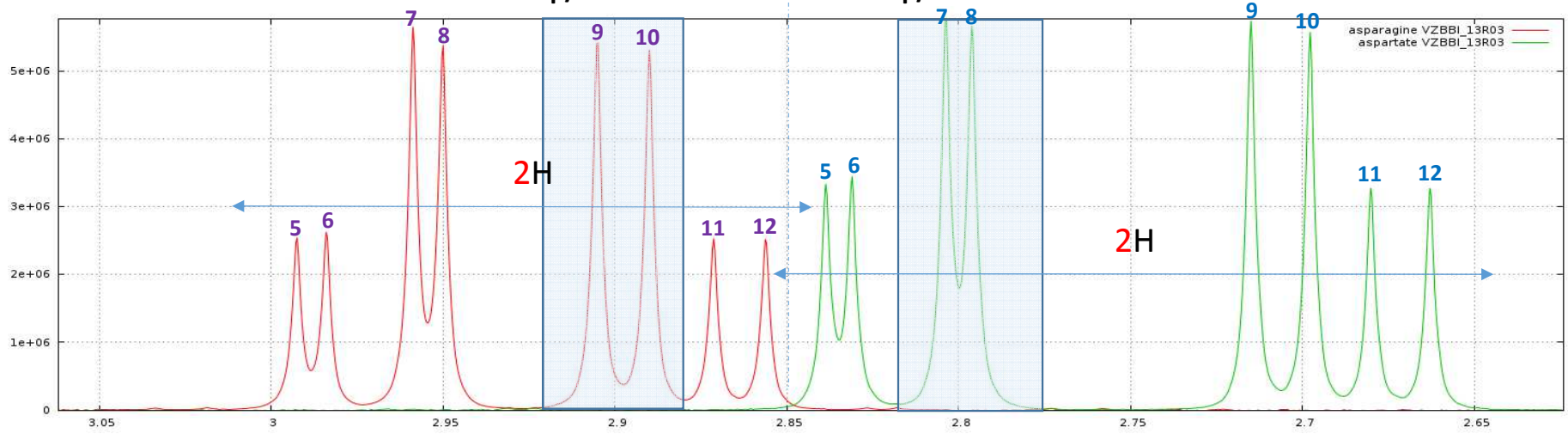
N 1H | % Signal

$Sm = \sum_{i \in \{m\}} Int._i \times Half\text{-}width_i$

Sp = 2.424 / i ∈ {7, 8}

Sm = 6.779 / i ∈ {5, ..., 12}

Sp/Sm = 33.8% **Sp/Sm = 35.76%**



https://peakforest.org/

Correction factor *due to the fact that only the central part is integrated.*

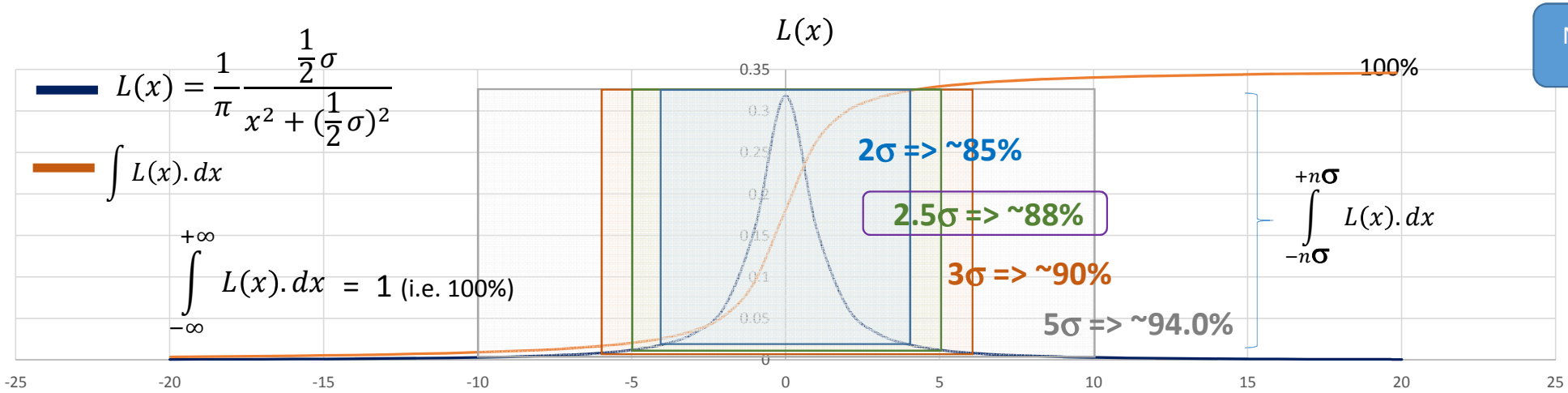
STEP 3b

N 1H | % Signal

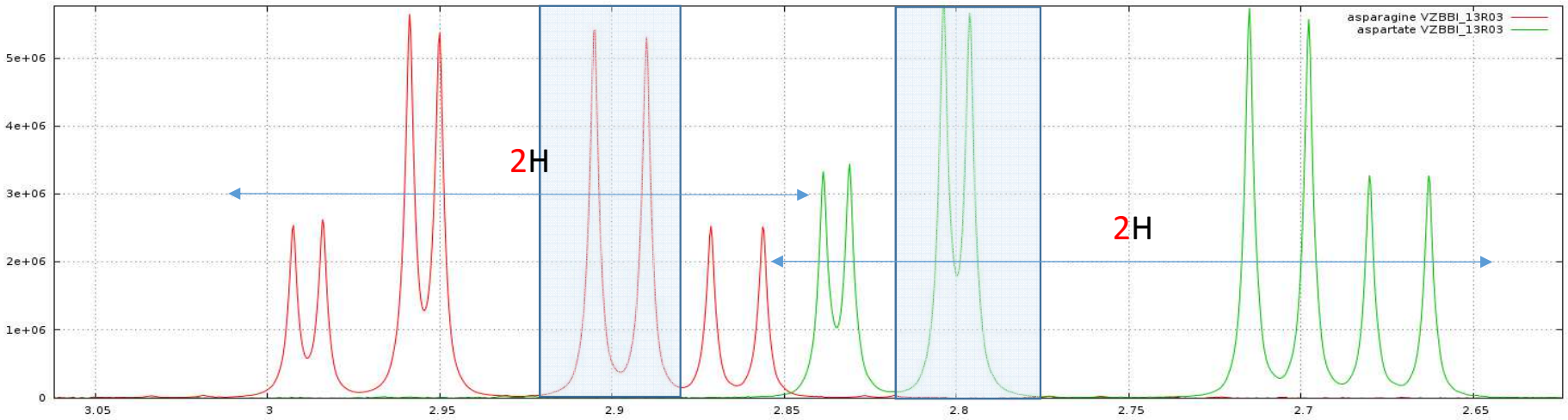
$$L(x) = \frac{1}{\pi}\frac{\frac{1}{2}\sigma}{x^2 + (\frac{1}{2}\sigma)^2}$$

$$\int L(x).dx$$

$$\int_{-\infty}^{+\infty} L(x).dx = 1 \text{ (i.e. 100\%)}$$



Sp/Sm = 0.338*0.88 => 29.7% **Sp/Sm** = 0.3576*0.88 => 31.5%



Summary

STEP 3

Quantification

$$\frac{Volume_{tube}\ (\mu L) \times Rate_{dilution}}{MasseSampleDW_{tube}\ (mg)} \times \frac{Integral_{Bucket}\ (ua)}{Calib_{Ref} \times N_{1H} \times \%\ Signal\ (ua\ .mM^{-1})} \times MM_{cmpd}\ (g / mole) \times 10^{-3} = mM_{tube}$$

(mg / gDW)

	aspartate	asparagine
Number of H	2	2
Signal Proportion	0.315	0.297
Molar Mass	133.1	132.1
Calibration Factor	0.9657	0.9657
Quantification Unit	mg/gDW	mg/gDW

Samplecode	Condition	Stage	Volume (10^-6.L)	mgDW	Dilution	B2_7995	B2_8925
F3-001	Control	J08	505	20.5	1	0.661364516	0.212810673
F3-049	Control	J08	505	20	1	0.72066603	0.242929712
F3-097	Control	J08	505	19.8	1	0.443156239	0.2683103
F3-002	Shadow	J08	510	20.2	1	0.589172929	0.304296199
F3-050	Shadow	J08	510	20	1	0.514406795	0.271083658
F3-098	Shadow	J08	510	20.3	1	0.586196427	0.216657164
F3-013	Control	J15	510	20.3	1	0.329863411	0.225329721
F3-061	Control	J15	510	19.7	1	0.589920237	0.532354354
F3-109	Control	J15	510	19.9	1	0.420560526	0.205775089
F3-062	Shadow	J15	510	19.8	1	0.42726105	0.266898284

Samples | Buckets | data_NMRFRIM3-4 | snr_NMRFRIM3-4 | data_GlucGln | data_FruGlu | **Quantif**

NMRProcFlow

Targeted Metabolomics

STEP 3

Quantification

qHNMR Template

Rawnames	amplecode	dexp	no proc	no	Condition	Stage	Volume (10^-6.L)	mgDW	Dilution
MMBBI_15P07-F3-001	F3-001	1	1		Control	J08	505	20.5	1
MMBBI_15P07-F3-049	F3-049	1	1		Control	J08	505	20	1
MMBBI_15P07-F3-097	F3-097	1	1		Control	J08	505	19.8	1
MMBBI_15P07-F3-002	F3-002	1	1		Shadow	J08	510	20.2	1
MMBBI_15P07-F3-050	F3-050	1	1		Shadow	J08	510	20	1
MMBBI_15P07-F3-098	F3-098	1	1		Shadow	J08	510	20.3	1
MMBBI_15P07-F3-013	F						510	20.3	1
MMBBI_15P07-F3-061	F						510	19.7	1
MMBBI_15P07-F3-109	F						510	19.9	1
MMBBI_15P07-F3-062	F						510	19.8	1
MMBBI_15P07-F3-110							510	20	1
MMBBI_15P07-F3-025	F3-025	1	1		Control	J28	510	19.6	1
MMBBI_15P07-F3-073	F3-073	1	1		Control	J28	510	20.1	1
MMBBI_15P07-F3-121	F3-121	1	1		Control	J28	510	19.6	1
MMBBI_15P07-F3-026	F3-026	1	1		Shadow	J28	510	19.9	1
MMBBI_15P07-F3-074	F3-074	1	1		Shadow	J28	510	20	1
MMBBI_15P07-F3-122	F3-122	1	1		Shadow	J28	510	19.9	1
MMBBI_15P07-F3-085	F3-085	1	1		Control	J55	510	20.4	1
MMBBI_15P07-F3-133	F3-133	1	1		Control	J55	510	19.9	1
MMBBI_15P07-F3-038	F3-038	1	1		Shadow	J55	510	19.7	1
MMBBI_15P07-F3-086	F3-086	1	1		Shadow	J55	510	19.9	1
MMBBI_15P07-F3-134	F3-134	1	1		Shadow	J55	510	19.9	1
MMBBI_15P07-F4-001	F4-001	1	1		Control	J08	510	20.5	1
MMBBI_15P07-F4-009	F4-009	1	1		Control	J08	51	20.3	1
MMBBI_15P07-F4-065	F4-065	1	1		Control	J08	51	20.1	1
MMBBI_15P07-F4-005	F4-005	1	1		Shadow	J08	51	20	1
MMBBI_15P07-F4-069	F4-069	1	1		Shadow	J08			
MMBBI_15P07-F4-037	F4-037	1	1		Shadow	J08			
MMBBI_15P07-F4-017	F4-017	1	1		Shadow	J15			
MMBBI_15P07-F4-045	F4-045	1	1		Shadow	J15			
MMBBI_15P07-F4-021	F4-021	1	1		Control	J28	50	20.3	1
MMBBI_15P07-F4-049	F4-049	1	1		Control	J28	50	20.4	1
MMBBI_15P07-F4-081	F4-081	1	1		Control	J28	505	20.2	1
MMBBI_15P07-F4-025	F4-025	1	1		Shadow	J28	505	20.5	1
MMBBI_15P07-F4-053	F4-053	1	1		Shadow	J28	505	20.4	1

Samples | Buckets | Data | SNR | Quantification

name	center	min	max	width	Compound	# 1H	% Signal	Molar Mass	Calibration
B0_9509	0.9509	0.9212	0.9806	0.0594	isoleucine+leucine	1	1	100	0.9657
B1_0206	1.02055	1.0148	1.0263	0.0115	isoleucine	3	0.5	180.6	0.9657
B1_0442	1.04425	1.0304	1.0581	0.0277	valine	3	1	117.15	0.9657
B1_4839	1.4839	1.4708	1.497	0.0262	alanine	3	1	89.09	0.9657
B1_5269	1.52685	1.5114	1.5423	0.0309	unkm1.53	1	1	100	0.9657
B1_6215	1.6215	1.607	1.636	0.029	unkm1.62	1	1	100	0.9657
B1_7200	1.72	1.7	1.74	0.04	lysine	2	0.684	146.1876	0.9657
B1_8600	1.86	1.845				1	0.72	192.1666	0.9657
B2_2965	2.2965	2.285				2	0.746	103.12	0.9657
B2_3430	2.343	2.338				1	1	147.13	0.4831
B2_4450	2.445	2.433				1	1	146.1	0.9168
B2_5720	2.572	2.547				2	1	192.1	0.9657
B2_7995	2.7995	2.791	2.808	0.017	aspartate	2	0.608	133.1	0.9657
B2_8925	2.8925	2.88	2.905	0.025	asparagine	2	0.318	132.1	0.9657
B3_2066	3.20655	3.2021	3.211	0.0089	choline	9	1	104.17	0.9657
B3_6060	3.606	3.603	3.609	0.006	inositol	2	0.308	180.16	0.9657
B4_1155	4.1155	4.105	4.126	0.021	fructose	1	1	180.2	0.4931
B4_1645	4.1645	4.16	4.169	0.009	pyroglutamate	1	0.251	129.11	0.9657
B4_3025	4.3025	4.284	4.321	0.037	malate	1	1	134.1	0.9657
B4_6508	4.6508	4.6272	4.6744	0.0472	glucose_C1_beta	1	1	180.2	0.9657
B5_1040	5.104	5.096	5.112	0.016	Unkm5.10	1	1	100	0.9657
B5_1870	5.187	5.182	5.192	0.01	mannose	1	1	1810.2	0.9657
B5_2385	5.2385	5.229	5.248	0.019	glucose_C1_alpha	1	1	180.2	0.9657
B5_2710	5.271	5.264	5.278	0.014	galactose	1	0.634	180.2	0.9657
B5_3085	5.3085	5.3	5.317	0.017	galacturonate	1	1	194.14	0.9657
B5_3925	5.3925	5.387	5.398	0.011	unkS5.39	1	1	100	0.9657
						1		342.3	0.9657
						1		100	0.9657
						1		267.24	0.9657
						1	.5	354.3	0.9657
B6_5231	6.5231	6.5185	6.5277	0.0092	fumarate	1		116.1	0.9657
B6_9035	6.9035	6.886	6.921	0.035	tyrosine	1		181.19	0.9657
B7_4290	7.429	7.415	7.443	0.028	phenylalanine	2	0.632	165.19	0.9657
B7_5585	7.5585	7.543	7.574	0.031	tryptophane	1	1	204.22	0.9657
B7_6185	7.6185	7.604	7.633	0.029	unkM7.62	1	1	100	0.9657

Samples | Buckets | Data | SNR | Quantification

Changes have be made ...

		isoleucine+leucine	isoleucine	valine	alanine	unkm1.53	unkm1.62	lysine
	Number of H	1	3	3	3	1	1	2
	Signal Proprotion	1	0.5	1	1	1	1	0.684
	Molar Mass	100	180.6	117.15	89.09	100	100	146.1876
	Calibration	0.9657	0.9657	0.9657	0.9657	0.9657	0.9657	0.9657
Samplecode	Condition	Stage	B0_9509	B1_0206	B1_0442	B1_4839	B1_5269	B1_6215	B1_7200
F3-001	Control	J08	3.000122242	0.089913927	0.207245812	0.267628328	0.393846291	0.125693858	0.201088628
F3-049	Control	J08	2.386656197	0.04495147	0.111907169	0.21527632	0.267563723	0.219112925	0.145208995
F3-097	Control	J08	3.108342499	0.062737247	0.143732148	0.281565519	0.412063772	0.261849145	0.19072273
F3-002	Shadow	J08	4.0523762	0.149431262	0.323080141	0.387195637	0.668395437	0.157955466	0.241299257
F3-050	Shadow	J08	2.69776623	0.043588444	0.114455466	0.235933495	0.316529894	0.389651159	0.19477494
F3-098	Shadow	J08	3.093200824	0.052947876	0.145412365	0.311755263	0.365015636	0.442162003	0.245543301
F3-013	Control	J15	3.169644246	0.116353836	0.22159208	0.279293678	0.467468594	0.046553853	0.14042958
F3-061	Control	J15	3.478950385	0.129			012384	0.037785607	0.179166556
F3-109	Control	J15	3.849696749	0.141			034967	0.065392965	0.184953428
F3-062	Shadow	J15	3.158037159	0.117			295476	0.049149532	0.111515068
F3-110	Shadow	J15	3.245321404	0.109			107378	0.08320256	0.186029866
F3-025	Control	J28	2.647722674	0.123706374	0.25150476	0.329054127	0.587222557	0.03211399	0.129947931
F3-073	Control	J28	3.124926855	0.144111082	0.287709464	0.321391693	0.535025224	0.062888191	0.157496984
F3-121	Control	J28	3.777189092	0.187551961	0.345693374	0.340704173	0.535386223	0.080830186	0.185481501
F3-026	Shadow	J28	2.734160783	0.140416098	0.288220744	0.39098667	0.583271862	0.034958377	0.153417123
F3-074	Shadow	J28	3.97063954	0.196446643	0.411692284	0.523686166	0.852443013	0.055020596	0.222664975
F3-122	Shadow	J28	3.656918283	0.165625495	0.310288528	0.34736076	0.560213839	0.066652472	0.176459702
F3-085	Control	J55	3.75750532	0.118117551	0.176635903	0.202415868	0.21940602	0.058374105	0.299506036
F3-133	Control	J55	3.717218627	0.12159157	0.150961351	0.565401098	0.660191751	0.138636104	0.279231129
F3-038	Shadow	J55	4.625790823	0.147551372	0.212609829	1.02046171	1.07328778	0.047782062	0.366331156
F3-086	Shadow	J55	4.307108567	0.12702044	0.1234888	0.291157901	0.308767719	0.076896326	0.344864895
F3-134	Shadow	J55	4.902640373	0.158689134	0.183710352	0.911606774	0.973795903	0.069123302	0.329707765
F4-001	Control	J08	3.163491407	0.080089459	0.214736911	0.412600194	0.517060421	0.237626146	0.224695084
F4-009	Control	J08	2.050369109	0.029546761	0.09895834	0.198788016	0.252075602	0.362322097	0.123085091
F4-065	Control	J08	3.27756956	0.061286158	0.178460642	0.369191223	0.477213654	0.303983382	0.234586919
F4-005	Shadow	J08	2.81104918	0.056267192	0.171213604	0.251917921	0.340595377	0.374264048	0.233681369
F4-069	Shadow	J08	2.596089316	0.045530567	0.146495082	0.325210945	0.357933983	0.279685597	0.163721979
F4-037	Shadow	J08	2.572827053	0.046173599	0.150917812	0.243792469	0.27940151	0.381558284	0.177987022
F4-017	Shadow	J15	1.934229398	0.054798	0.127283831	0.238472209	0.367643755	0.149173029	0.042191959
F4-045	Shadow	J15	2.600365814	0.08	0.20392849	0.570226067	0.978159139	0.073992316	0.152926884

Samples | Buckets | Data | SNR | Quantification

Quantification

$$mM_{tube} = \frac{Volume_{tube} \times Rate_{dilution}}{MasseSampleDW_{tube}} \times \frac{Integral_{Bucket}}{Calib_{Ref} \times N_{1H} \times \%\ Signal} \times MM_{cmpd} \times 10^{-3}$$

(µL) (ua) (g / mole)

(mg) (ua .mM^{-1})

(mg / gDW)

… then the 'Quantification' tab has been automatically updated

Samples | Buckets | data_NMRFRIM3-4 | **snr_NMRFRIM3-4** | data_GlucGln | data_FruGlu | Quantif

STEP 4

Check SNR

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
1					isoleucine+	isoleucine	valine	alanine	unkm1.53	unkm1.62	lysine	quinate	GABA	glutamate	glutamine	citrate	aspartate	asparagine	cholin
2	Samplecode	Condition	Stage		B0_9509	B1_0206	B1_0442	B1_4839	B1_5269	B1_6215	B1_7200	B1_8600	B2_2965	B2_3430	B2_4450	B2_5720	B2_79		
3	F3-001	Control	J08		90	68	64	112	33	8	14	54	363	36	99	4721			
4	F3-049	Control	J08		61	28	32	83	21	14	9	59	216	19	68	4442			
5	F3-097	Control	J08		74	38	37	97	27	14	10	57	237	20	56	4097	32	11	
6	F3-002	Shadow	J08		104	93	82	129	46	8	15	45	384	47	62	3602	34	19	
7	F3-050	Shadow	J08		77	33	38	103	23	24	13	66	351	23	87	5370	32	19	
8	F3-098	Shadow	J08		90	35	40	115	24	27	16	63	325	12	113	4737	42	18	
9	F3-013	Control	J15		93	81	66	110	39	3	12	35	269	51	64	3394	21	14	
10	F3-061	Control	J15		101	89	72	159	62	3	12	20	202	23	142	3220	34	30	
11	F3-109	Control	J15		116	103	86	249	93	5	14	29	250	35	131	3266	22	16	
12	F3-062	Shadow	J15		91	77	63	119	50	4	9	24	238	31	92	3221	24	18	
13	F3-110	Shadow	J15		80	67	58	126	44	5	11	30	263	40	71	3463	41	17	
14	F3-025	Control	J28		90	95	80	143	54	3	12	11	287	35	98	3665	52	41	
15	F3-073	Control	J28		109	112	90	136	50	4	16	15	369	41	150	3638	56	44	
16	F3-121	Control	J28		111	120	93	119	42	4	15	15	414	44	139	3006	52	45	
17	F3-026	Shadow	J28		100	119	103	181	59	3	15	20	606	168	125	2490	31	60	
18	F3-074	Shadow	J28		133	146	123	212	77	4	19	13	363	36	184	3211	53	54	
19	F3-122	Shadow	J28		120	127	97	144	50	4	16	13	456	50	154	3348	65	69	
20	F3-085	Control	J55		117	58	31	154	39	2	19	15	225	280	258	3610	194	71	
21	F3-133	Control	J55																
22	F3-038	Shadow	J55																
23	F3-086	Shadow	J55																
24	F3-134	Shadow	J55																
25	F4-001	Control	J08																
26	F4-009	Control	J08																
27	F4-065	Control	J08																
28	F4-005	Shadow	J08																
29	F4-069	Shadow	J08																
30	F4-037	Shadow	J08																
31	F4-017	Shadow	J15																
32	F4-045	Shadow	J15																
33	F4-021	Control	J28																
34	F4-049	Control	J28																
35	F4-081	Control	J28																
36	F4-025	Shadow	J28																
37	F4-053	Shadow	J28																
38	F4-085	Shadow	J28		120	138	118	251	97	2	14	19	383	56	167	3567	37	45	
39	F4-029	Control	J55		89	64	22	85	21	3	16	10	303	265	214	5162	177	73	
40	F4-057	Control	J55		148	121	42	261	66	3	24	14	456	465	341	6114	303	101	
41	F4-089	Control	J55		150	111	52	74	20	7	26	13	437	285	340	5329	224	117	
42	F4-033	Shadow	J55		124	88	43	125	33	4	23	11	371	277	302	5738	216	126	
43	F4-061	Shadow	J55		161	112	57	289	73	5	29	15	464	355	365	6449	313	161	
44	F4-093	Shadow	J55		94	60	24	114	32	3	17	8	257	329	296	6728	256	101	

Calculate Signal to Noise Ratio

(same as the Bruker TopSpin 'sino' command)

$$SNR\,(S_i, b_j) = \frac{maxval\,(S_i, b_j)}{2.\ noise\,(S_i)}$$

Where:

- ***maxval*** is the highest intensity in the spectral region defined by the bucket ***j*** on the spectrum ***i***
- ***noise*** is the estimated noise in the given spectral region for each spectrum ***i***

NMRProcFlow

http://nmrprocflow.org/