Electronica Supplementary Material (ESI) for Analytical Methods

Evaluation of pure shift NMR methods for the analysis of complex metabolite mixtures with a benchtop NMR spectrometer

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1. **Spectral simplification in the aromatic region**

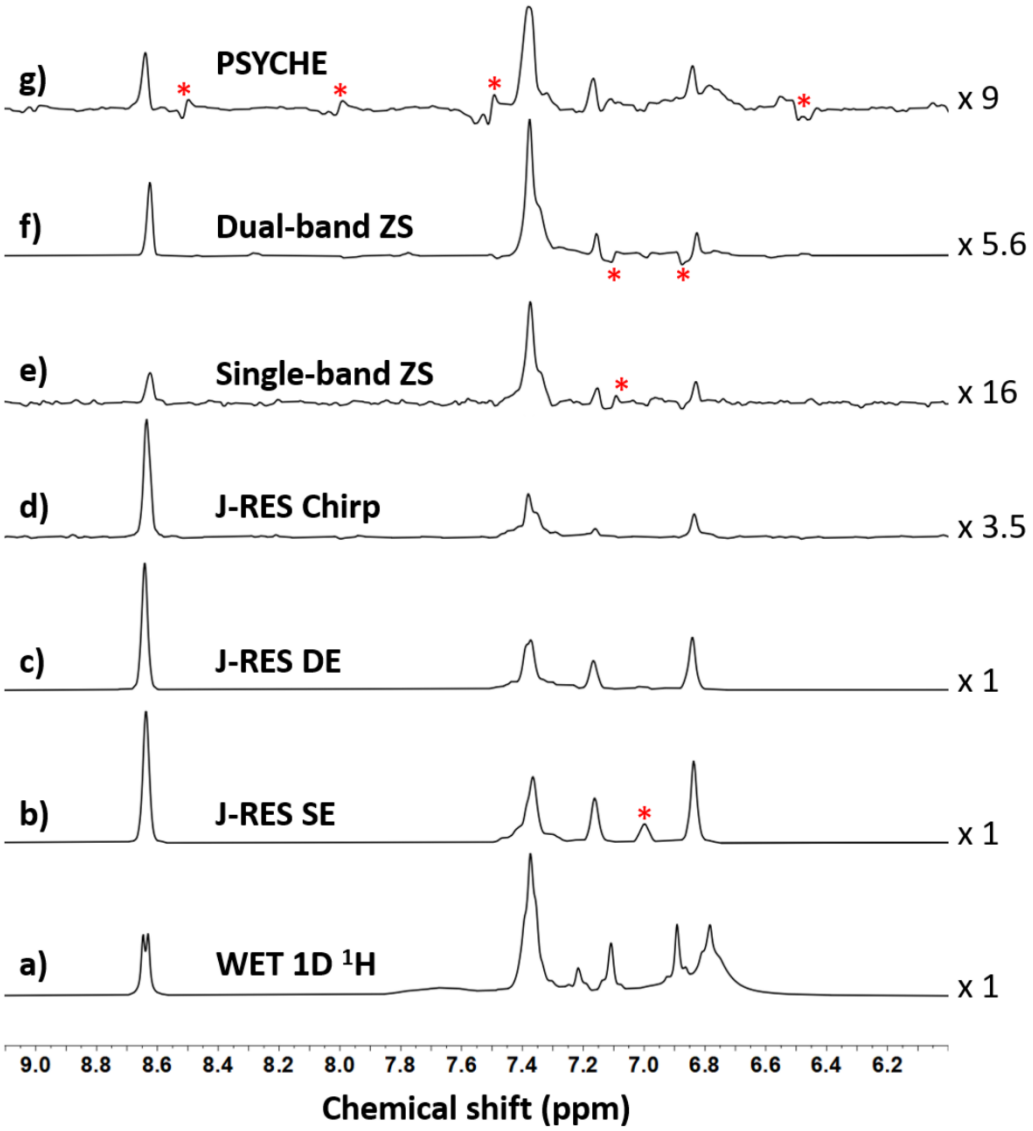

Fig.S1: Spectra acquired on the model mixture of seventeen metabolites in the aromatic spectral regions. WET based: (a) 1D ^{1}H spectrum. (b) J-RES SE PS spectrum. (c) J-RES DE PS spectrum. (d) J-RES Chirp PS spectrum. (e) Single-band ZS PS spectrum. (f) Dual-band ZS PS spectrum. (g) PSYCHE PS spectrum. Strong coupling artefact(s)/baseline distortion(s) are symbolized by the red stars.

2. Annotation of the model mixture

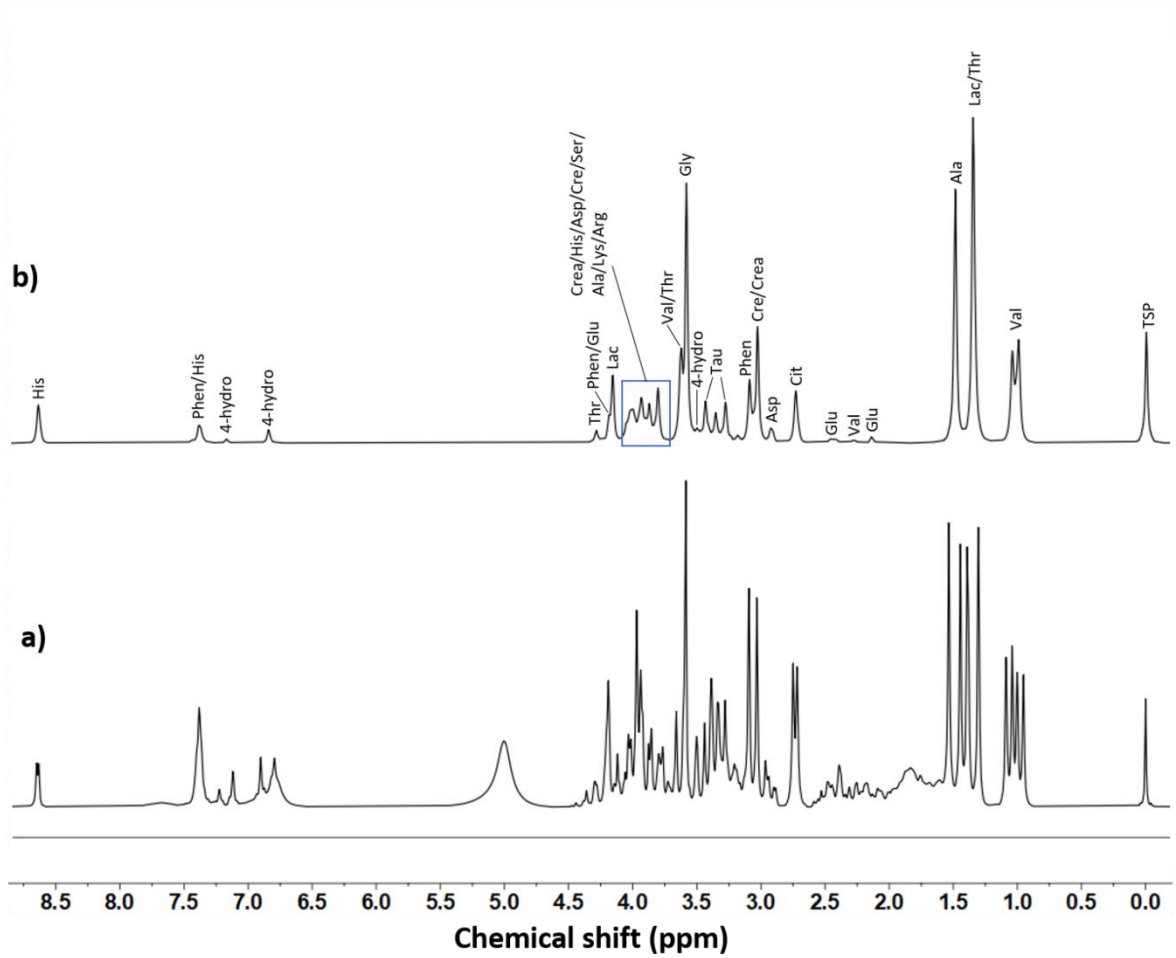

Fig.S2: Stack representation of the full WET based: (a) 1D ^{1}H spectrum. (b) J-RES DE PS spectrum. Both spectra intensities were normalized for a clear visualization of the spectral information. The J-RES DE PS spectrum was fully annotated. Signal annotation was made with the help of Human Metabolome Database (HMDB).

In order to validate the elucidation of the model mixture of seventeen metabolites, additional 2D NMR acquisitions were performed on the 80 MHz benchtop spectrometer.

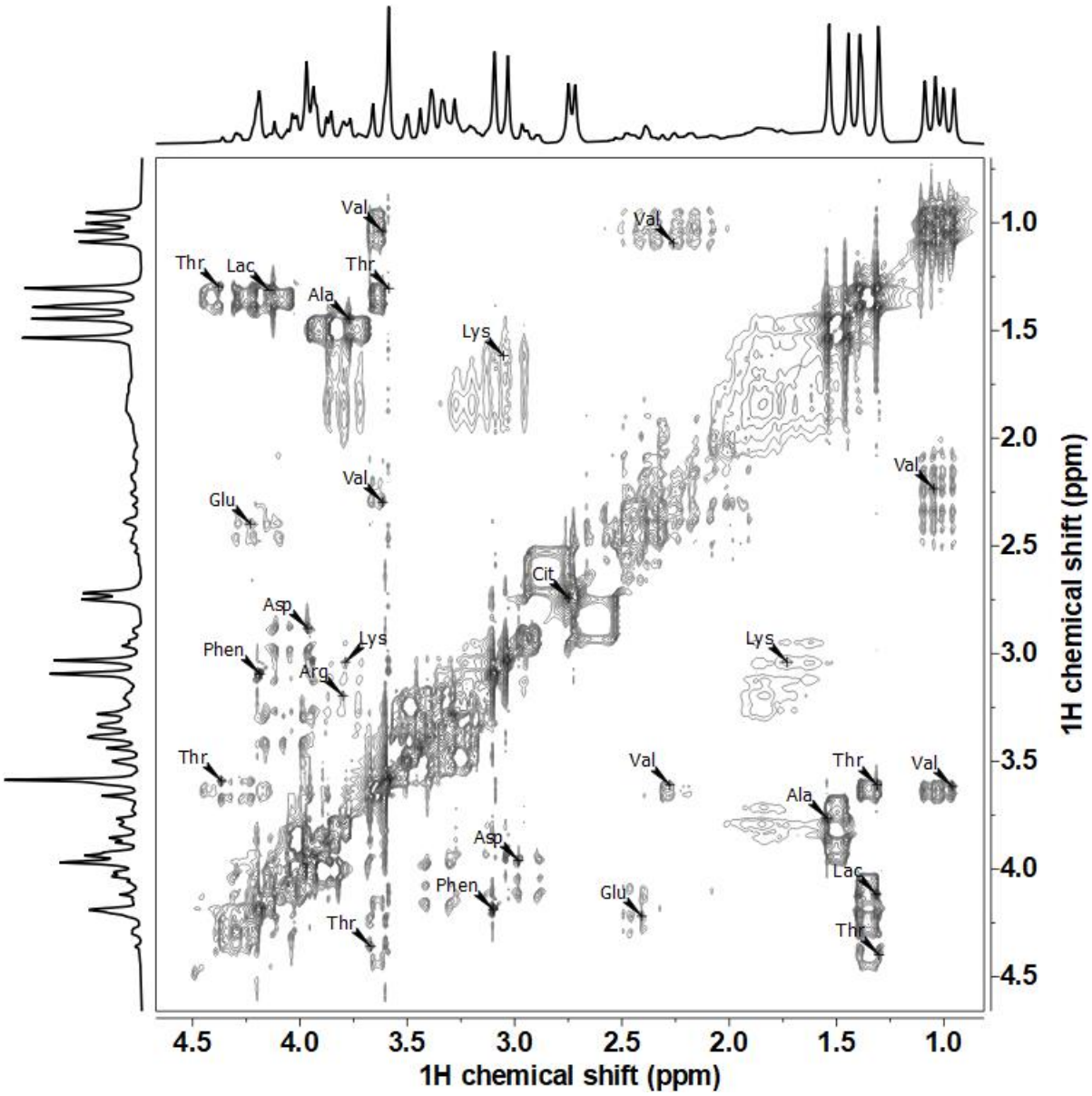

Fig.S3: WET TOtal Correlation SpectroscopY (WET TOCSY) spectrum at 80 MHz of the model mixture of seventeen metabolites in the aliphatic region. Signal annotation was made with the help of Human Metabolome Database (HMDB).

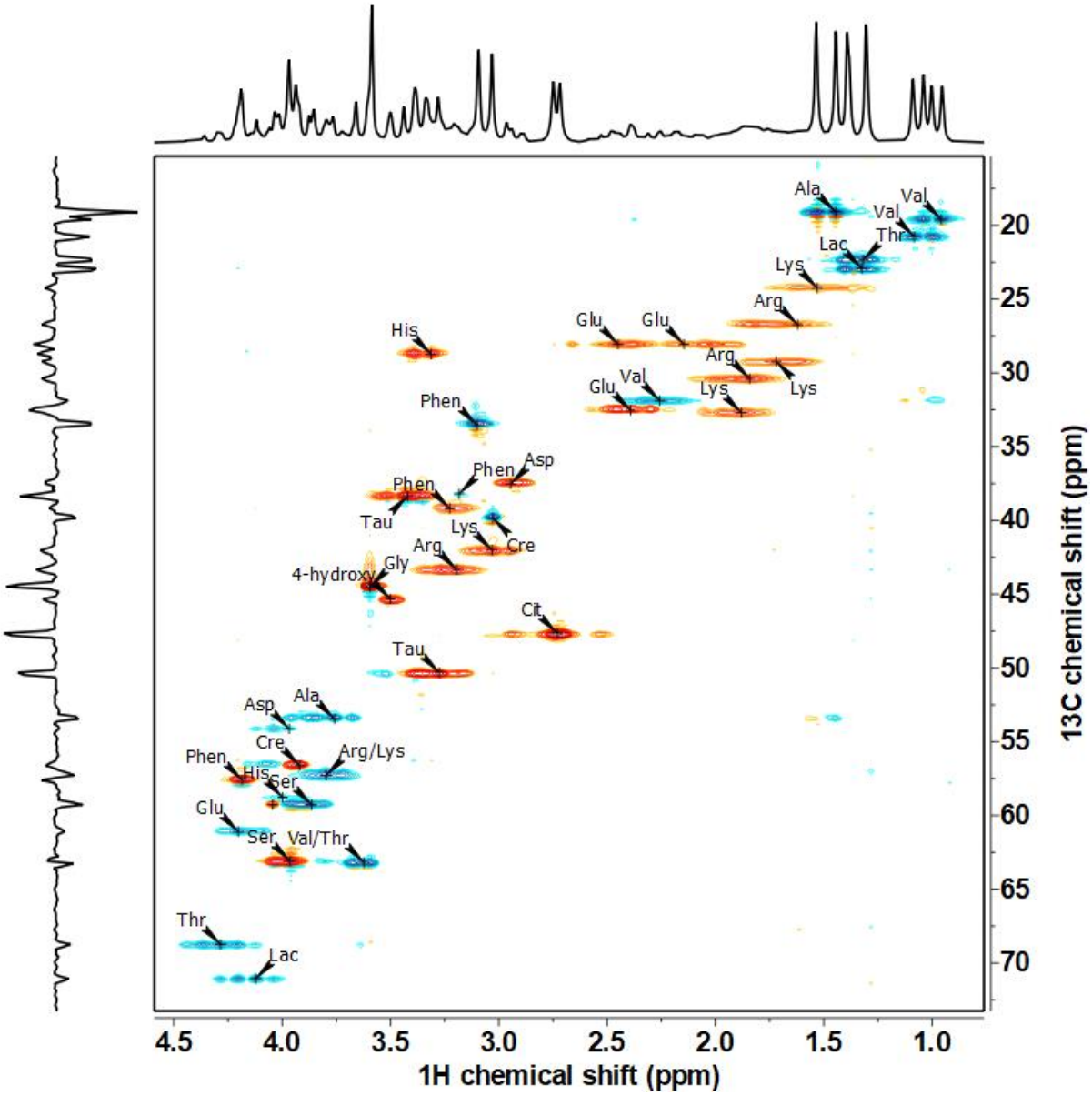

Fig.S4: Heteronuclear Single Quantum Correlation Multiplicity-Edited (HSQC-ME) spectrum at 80 MHz of the model mixture of seventeen metabolites in the aliphatic region. Full chemical shift annotations are summarized in **Table S1**. Signal annotation was made with the help of Human Metabolome Database (HMDB) and the WET TOCSY spectrum presented in Fig. S2.

3. Chemical shifts assignment

N°	^{1}H (ppm)	^{13}C (ppm)	Annotation	N°	^{1}H (ppm)	^{13}C (ppm)	Annotation
1	0	0	TSP	24	3.44	38.35	Tau
2	1	19.59	Val	25	3.5	45.33	4-hydro
3	1.04	20.75	Val	26	3.59	44.5	Gly
4	1.35	22.34	Thr	27	3.63	63.17	Val/ Thr
5	1.35	22.91	Lac	28	3.82	57.28	Arg/ Lys
6	1.49	19.09	Ala	29	3.82	53.46	Ala
7	1.53	24.24	Lys	30	3.88	59.28	Ser
8	1.62	26.73	Arg	31	3.94	56.62	Cre
9	1.72	29.22	Lys	32	3.95	63.09	Ser
10	1.84	30.38	Arg	33	3.99	54.13	Asp
11	1.88	32.71	Lys	34	4	58.77	His
12	2.03	28.06	Glu	35	4.04	59.27	Crea
13	2.26	31.88	Val	36	4.12	71.06	Lac
14	2.39	32.54	Glu	37	4.16	57.61	Phen
15	2.73	47.65	Cit	38	4.19	61.1	Glu
16	2.95	37.53	Asp	39	4.29	68.74	Thr
17	3.03	39.85	Cre	40	6.85	118.21	4-hydro
18	3.03	42.01	Lys	41	7.17	133.32	4-hydro
19	3.09	33.54	Phen	42	7.37	132.16	Phen
20	3.2	43.33	Arg	43	7.37	130.33	Phen
21	3.23	39.18	Phen	44	7.37	131.83	Phen
22	3.28	50.31	Tau	45	7.4	120.37	His
23	3.31	28.72	His	46	8.64	137.14	His

Table S1: Full ^{1}H and ^{13}C annotations of the model mixture of seventeen metabolites for the HSQC-ME presented in Fig.S4. Annotations in bold correspond to the signals appearing in the aromatic region.

4. Fitting of the PS spectra

The removal of multiplicity provided by PS strategies divides complex spectral regions into narrower and less complex spectral regions. For very complex mixtures (like the model of seventeen metabolites), it remains difficult to fully annotate PS spectra. The following Fig.S4 and Fig.S5 show how the use of the 2D J-resolved data and deconvolution algorithms can facilitate the annotation/processing of complex regions inside PS spectra.

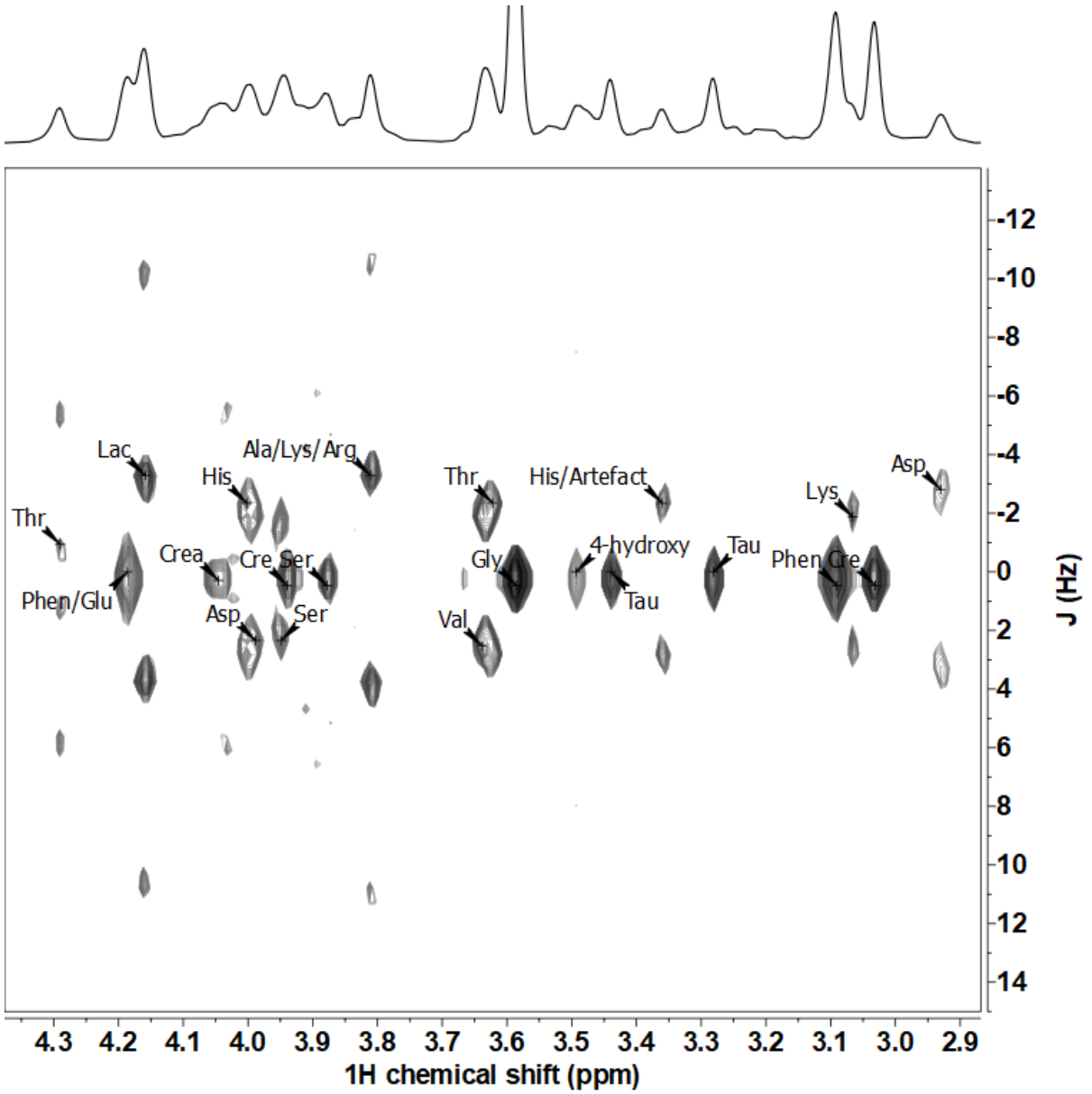

Fig.S5: Annotated 2D J-resolved spectrum (J-RES DE) after a 45° tilt and symmetrization on the spectral region ranging from 2.9 to 4.35 ppm. The most complex spectral region observed in PS spectra is highlighted in the blue square.

As illustrated in Fig.S5, the more in depth 2D data provided by the acquisition of J-resolved spectra facilitates the recovering of sometimes hidden chemical shifts in the PS spectra. These chemical shifts can then be used to perform spectral deconvolution of the singlets. PS spectroscopy is easy to combine with deconvolution since it only requires the knowledge of the chemical shifts. An example of a deconvoluted PS spectrum by GSD is illustrated in Fig.S6.

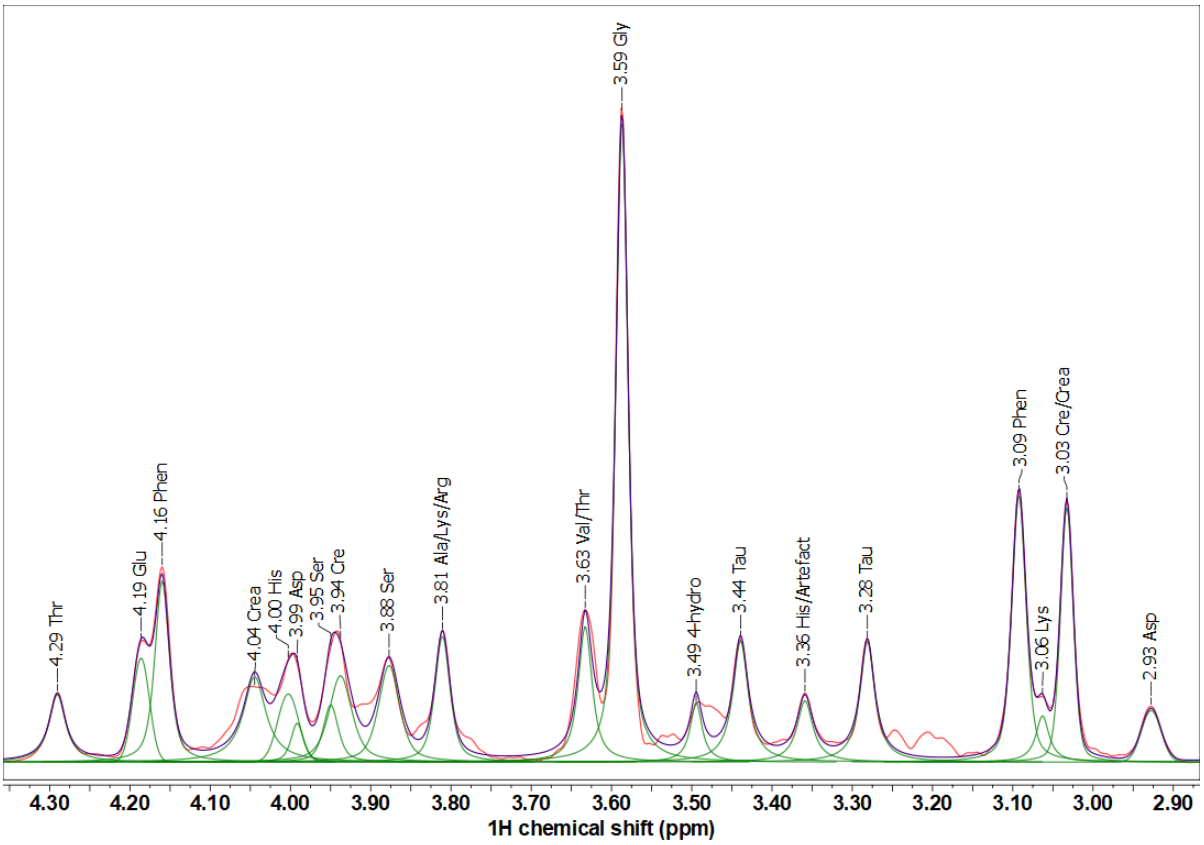

Fig.S6: Fitting of a 1D projection of a 2D J-RES DE acquisition. Fitted singlets were annotated according to Table S1 and with the help of Human Metabolome Database (HMDB). The original PS spectrum (from J-RES DE) is in red. The deconvoluted singlets are in green. The sum of the deconvoluted singlets is in indigo.

5. Site-by-site performances of the J-RES SE experiment

Analytical performance of the J-RES SE experiment

Chemical shift (ppm)	Average SNR	CV of relative areas (%)	LW_{10} (Hz)
8.64	257	4	3.8
7.17	90	13	4.6
6.84	156	9	4.2
4.29	194	9	4.6
4.16	427	7	4.2
3.63	364	6	4.4
3.59	1216	6	3.5
3.44	292	11	5.4
3.28	296	13	4.4
3.09	588	9	4.9
3.03	519	5	4.0
2.93	130	11	3.9
2.73	428	12	5.1
1.49	1462	2	3.6
1.35	1332	-	4.5
1.04	670	7	5.4
1.00	699	5	4.8
Average	**536**	**8**	**4.4**

Table S2**:** Average site-by-site SNR, CV of relative areas and LW_{10} of the J-RES SE pure shift strategy over five repetitions.

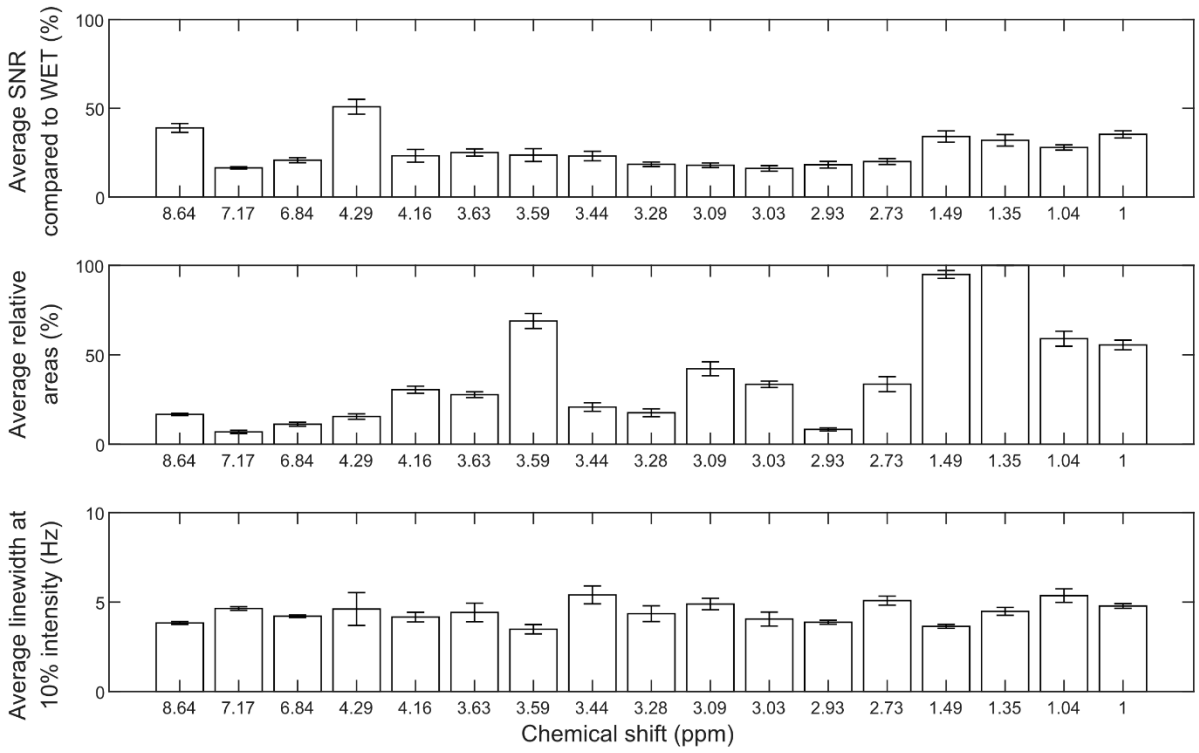

Fig.S7**:** Average site-by-site characterization of the analytical performance of the J-RES SE pure shift strategy over five repetitions. Top panel: average SNR compared to WET 1D ^{1}H. Middle panel: average relative areas values normalized with the signal of lactate at 1.35 ppm. Bottom panel: average linewidth at 10% of the maximum intensity of the signal.

6. Site-by-site performances of the J-RES DE experiment

Analytical performance of the J-RES DE experiment

Chemical shift (ppm)	Average SNR	CV of relative areas (%)	LW_{10} (Hz)
8.64	297	6	3.6
7.17	70	4	4.7
6.84	125	4	4.6
4.29	177	2	4.8
4.16	484	4	4.0
3.63	363	6	4.2
3.59	1548	6	2.9
3.44	318	4	4.6
3.28	304	2	4.1
3.09	624	6	4.5
3.03	627	6	3.4
2.93	141	5	4.1
2.73	383	3	4.8
1.49	1688	3	3.3
1.35	1598	-	3.8
1.04	675	4	4.6
1.00	735	5	4.3
Average	**597**	**4**	**4.1**

Table S3**:** Average site-by-site SNR, CV of relative areas and LW_{10} of the J-RES DE pure shift strategy over five repetitions.

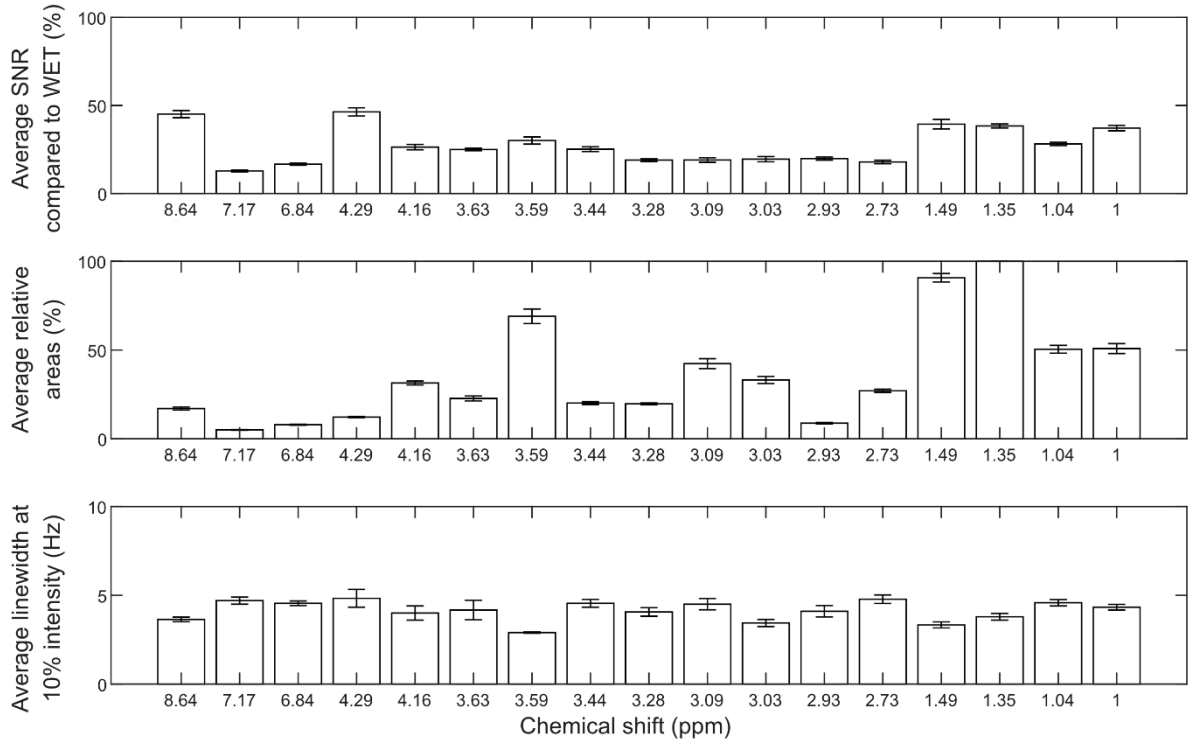

Fig.S8: Average site-by-site characterization of the analytical performance of the J-RES DE pure shift strategy over five repetitions. Top panel: average SNR compared to WET 1D ^{1}H. Middle panel: average relative areas values normalized with the signal of lactate at 1.35 ppm. Bottom panel: average linewidth at 10% of the maximum intensity of the signal.

7. Site-by-site performances of the J-RES Chirp experiment

Analytical performance of the J-RES Chirp experiment

Chemical shift (ppm)	Average SNR	CV of relative areas (%)	LW_{10} (Hz)
8.64	85	3	3.7
7.17	6	8	4.8
6.84	19	15	4.4
4.29	45	4	4.8
4.16	118	5	4.3
3.63	121	3	4.5
3.59	320	1	3.4
3.44	71	7	4.2
3.28	57	4	4.3
3.09	117	2	4.7
3.03	166	1	3.8
2.93	28	8	3.9
2.73	152	2	4.9
1.49	348	1	3.4
1.35	367	-	4.0
1.04	134	4	4.7
1.00	153	2	4.5
Average	**136**	**4**	**4.2**

Table S3**:** Average site-by-site SNR, CV of relative areas and LW_{10} of the J-RES Chirp pure shift strategy over five repetitions.

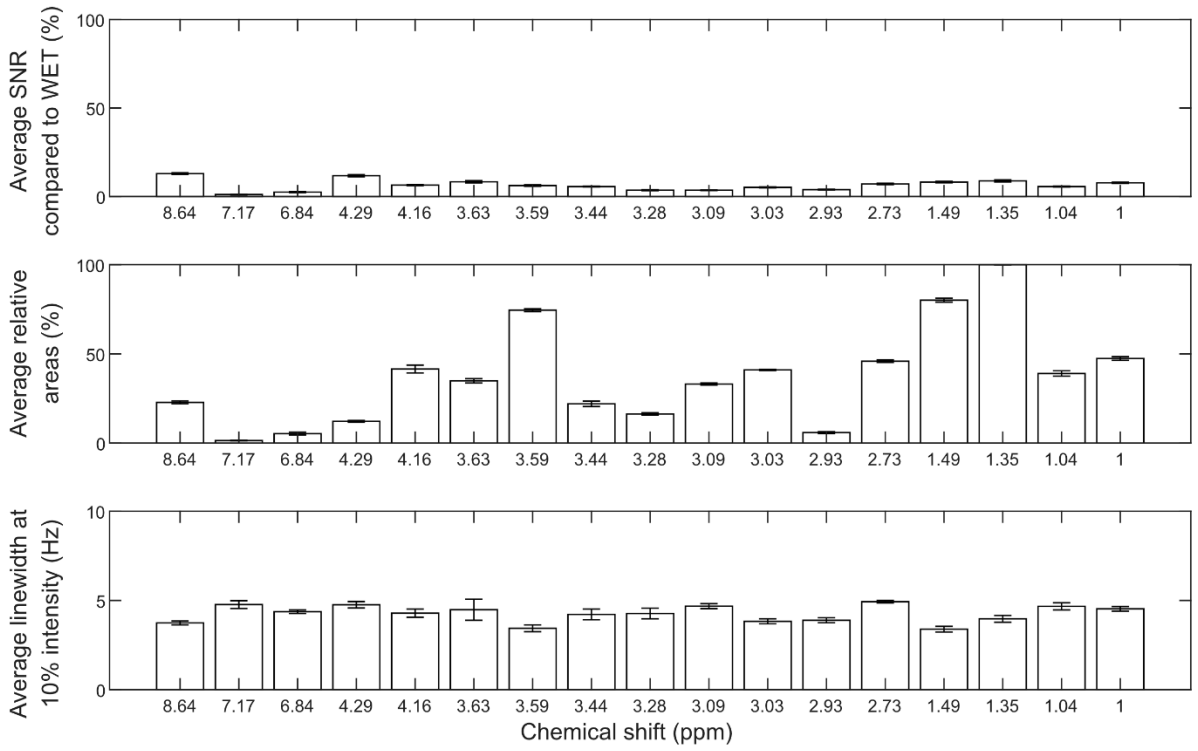

Fig.S9: Average site-by-site characterization of the analytical performance of the J-RES CHIRP pure shift strategy over five repetitions. Top panel: average SNR compared to WET 1D ^{1}H. Middle panel: average relative areas values normalized with the signal of lactate at 1.35 ppm. Bottom panel: average linewidth at 10% of the maximum intensity of the signal.

8. Site-by-site performances of the single-band ZS experiment

Analytical performance of the single-band ZS experiment

Chemical shift (ppm)	Average SNR	CV of relative areas (%)	LW_{10} (Hz)
8.64	11	7	3.9
7.17	6	3	2.6
6.84	8	5	2.9
4.29	32	5	4.2
4.16	89	5	4.2
3.63	74	8	4.2
3.59	147	7	2.5
3.44	32	8	4.2
3.28	36	8	4.2
3.09	134	1	3.1
3.03	91	4	3.3
2.93	20	11	5.5
2.73	0	0	-
1.49	151	1	4.2
1.35	165	-	4.7
1.04	69	7	4.4
1.00	71	6	5.9
Average	**67**	**5**	**4.0**

Table S4**:** Average site-by-site SNR, CV of relative areas and LW_{10} of the single-band ZS pure shift strategy over five repetitions.

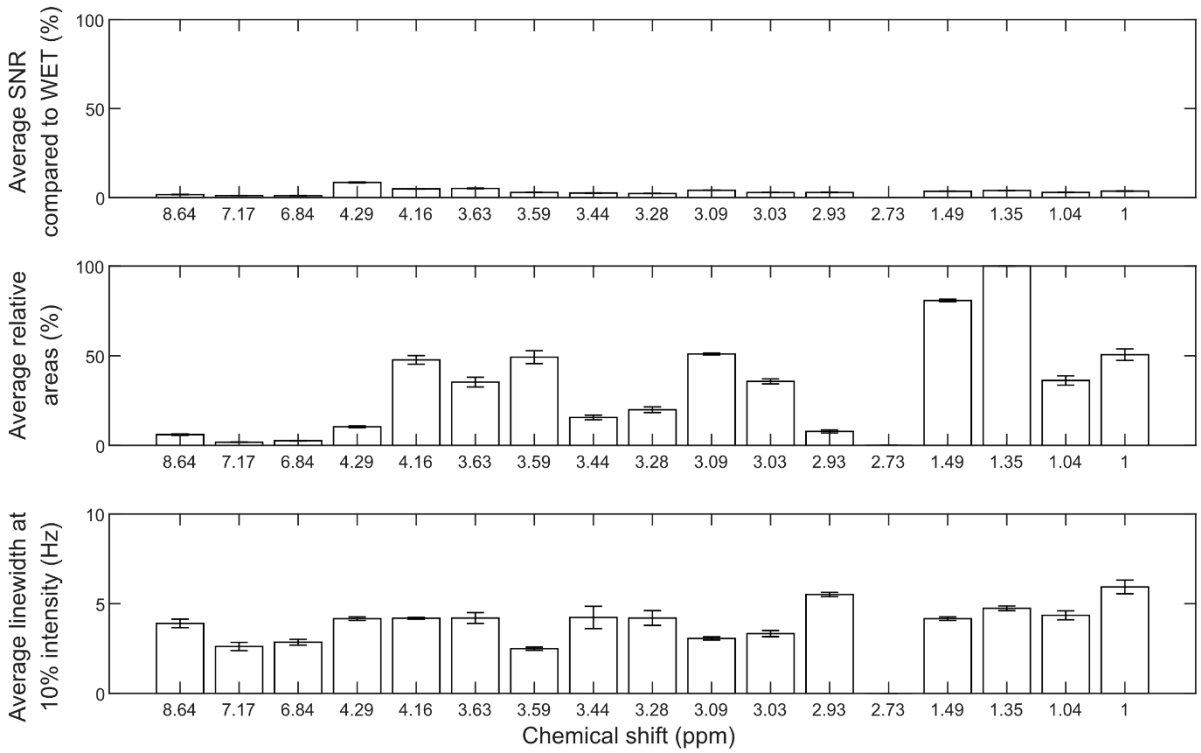

Fig.S10: Average site-by-site characterization of the analytical performance of the single-band ZS pure shift strategy over five repetitions. Top panel: average SNR compared to WET 1D ^{1}H. Middle panel: average relative areas values normalized with the signal of lactate at 1.35 ppm. Bottom panel: average linewidth at 10% of the maximum intensity of the signal.

9. Site-by-site performances of the dual-band ZS experiment

Analytical performance of the dual-band ZS experiment

Chemical shift (ppm)	Average SNR	CV of relative areas (%)	LW_{10} (Hz)
8.64	122	3	3.0
7.17	39	7	2.2
6.84	36	6	2.6
4.29	46	24	4.9
4.16	185	10	4.1
3.63	292	8	4.6
3.59	567	3	2.8
3.44	137	5	4.2
3.28	162	25	4.5
3.09	596	6	3.5
3.03	414	5	3.5
2.93	103	7	5.7
2.73	0	0	-
1.49	752	1	4.2
1.35	753	0	4.8
1.04	231	11	5.2
1.00	219	8	6.6
Average	**274**	**8**	**4.1**

Table S5**:** Average site-by-site SNR, CV of relative areas and LW_{10} of the dual-band ZS pure shift strategy over five repetitions.

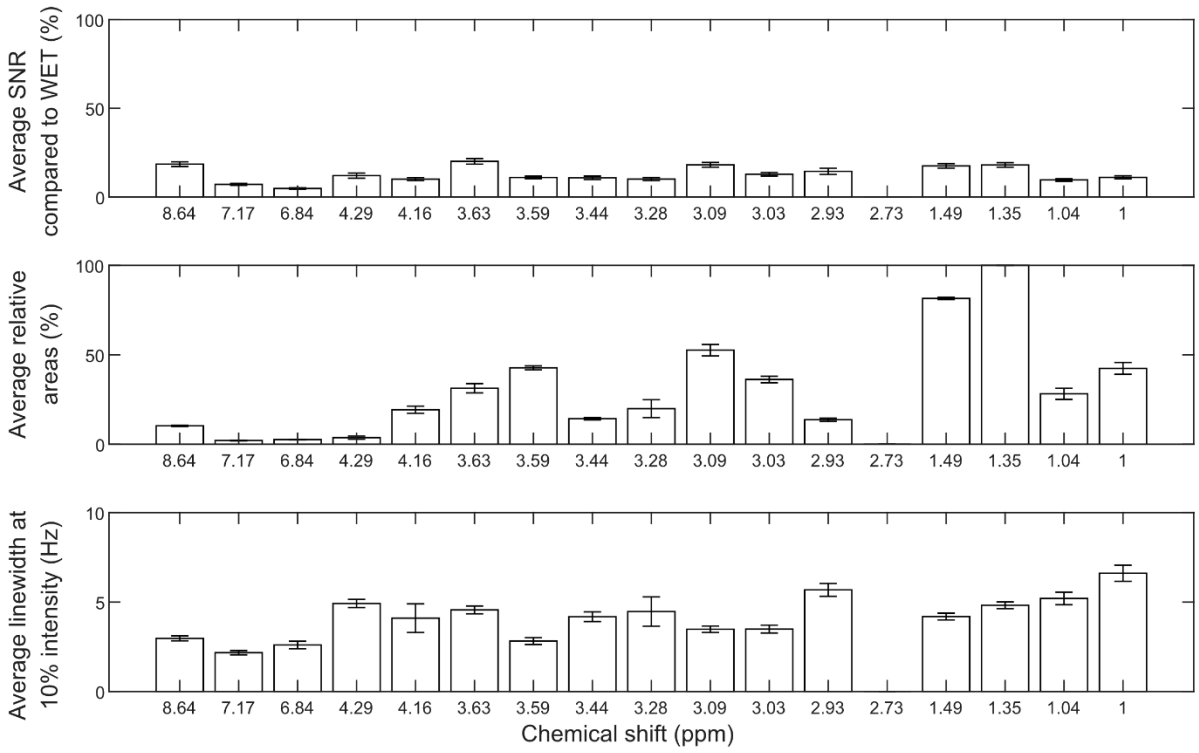

Fig.S11**:** Average site-by-site characterization of the analytical performance of the dual-band pure shift strategy over five repetitions. Top panel: average SNR compared to WET 1D ^{1}H. Middle panel: average relative areas values normalized with the signal of lactate at 1.35 ppm. Bottom panel: average linewidth at 10% of the maximum intensity of the signal.

10. Site-by-site performances of the PSYCHE experiment

Analytical performance of the PSYCHE experiment

Chemical shift (ppm)	Average SNR	CV of relative areas (%)	LW_{10} (Hz)
8.64	37	20	4.1
7.17	21	12	3.7
6.84	32	19	6.1
4.29	0	0	-
4.16	114	13	5.9
3.63	90	18	6.1
3.59	160	11	4.0
3.44	41	30	5.3
3.28	50	34	5.0
3.09	168	7	4.8
3.03	124	5	4.6
2.93	29	16	3.5
2.73	218	11	4.5
1.49	262	3	5.9
1.35	313	-	6.9
1.04	159	14	4.6
1.00	166	19	5.4
Average	**117**	**14**	**5.0**

Table S6**:** Average site-by-site SNR, CV of relative areas and LW_{10} of the PSYCHE pure shift strategy over five repetitions.

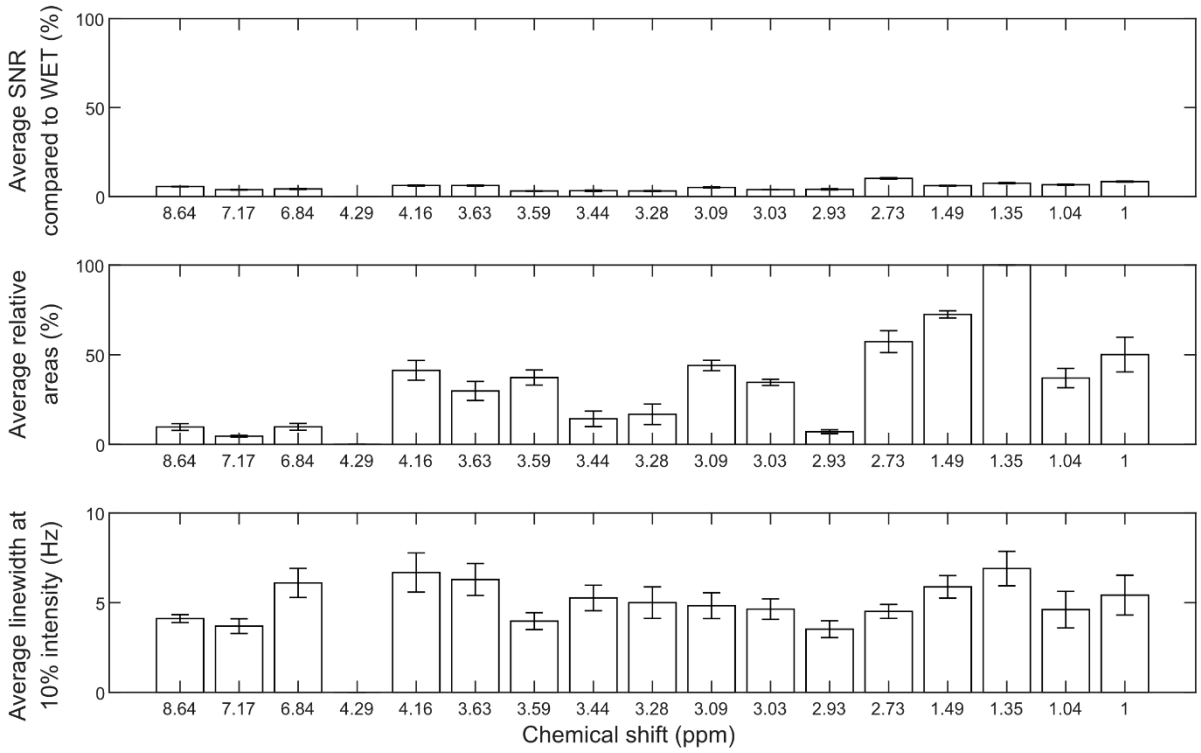

Fig.S12: Average site-by-site characterization of the analytical performance of the PSYCHE pure shift strategy over five repetitions. Top panel: average SNR compared to WET 1D ^{1}H. Middle panel: average relative areas values normalized with the signal of lactate at 1.35 ppm. Bottom panel: average linewidth at 10% of the maximum intensity of the signal.

11. Additional spectra of the fish feed of commercial formulation

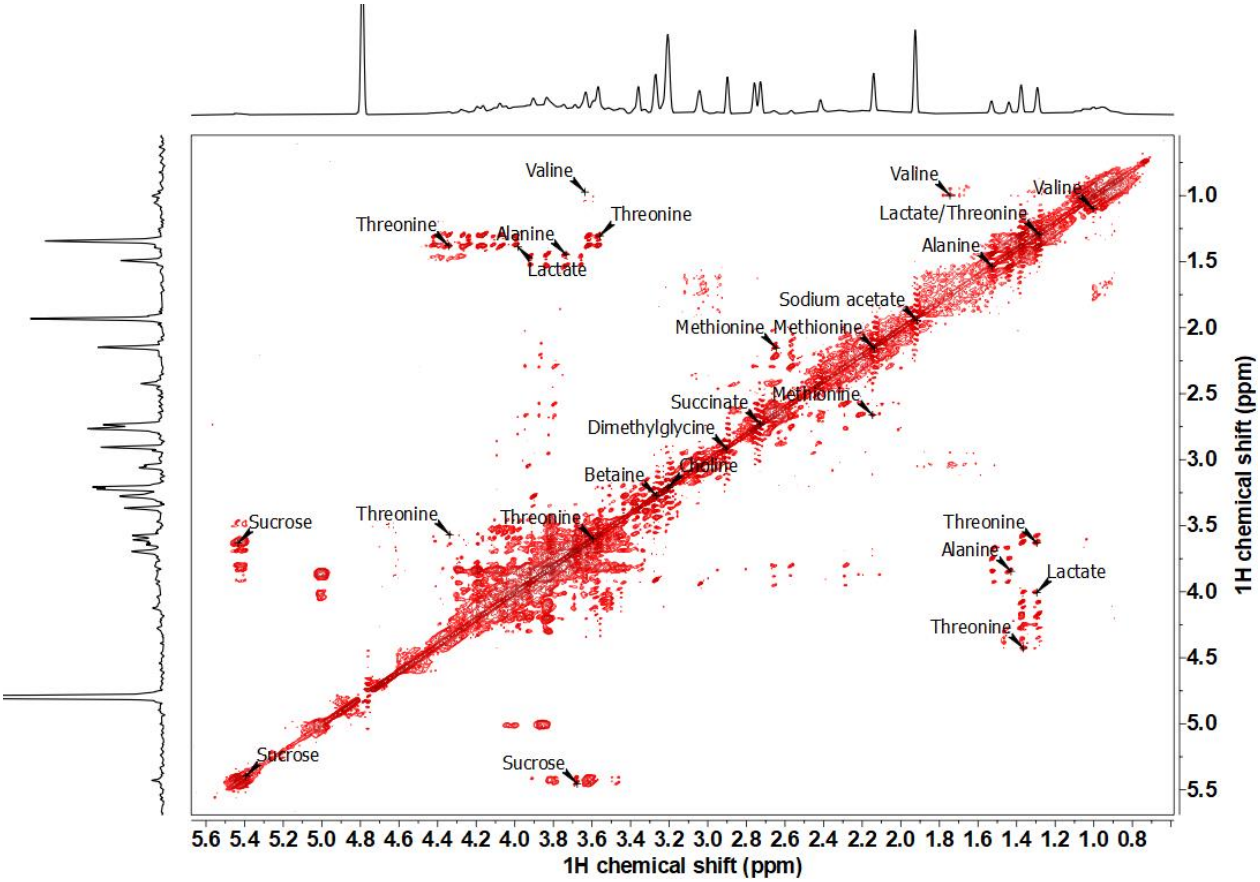

Fig.S13: WET TOCSY spectrum the fish feed of commercial formulation acquired on an 80 MHz NMR spectrometer. Signal annotation was made with the help of Human Metabolome Database (HMDB).

^{1}H (ppm)	^{13}C (ppm)	Annotation
5.42	94.76	Sucrose
4.18	79.15	Sucrose
3.81	62.88	Sucrose
3.81	65.04	Sucrose
3.69	64.04	Sucrose
3.57	73.79	Sucrose
3.49	71.85	Sucrose
3.28	56.04	Betaine
3.2	56.57	Choline
3.04	41.8	Lysine
2.92	47.27	Dimethylglycine
2.73	37.14	Succinate
2.16	16.57	Methionine
1.95	25.86	Sodium acetate
1.46	18.88	Alanine
1.3	22.87	Lactate
1.3	22.04	Threonine
0	0	TSP

Table S7**:** Full ^{1}H and ^{13}C annotations of the fish feed of commercial formulation for the HSQC-ME presented in Fig.S14. Signal annotation was made with the help of Human Metabolome Database (HMDB).

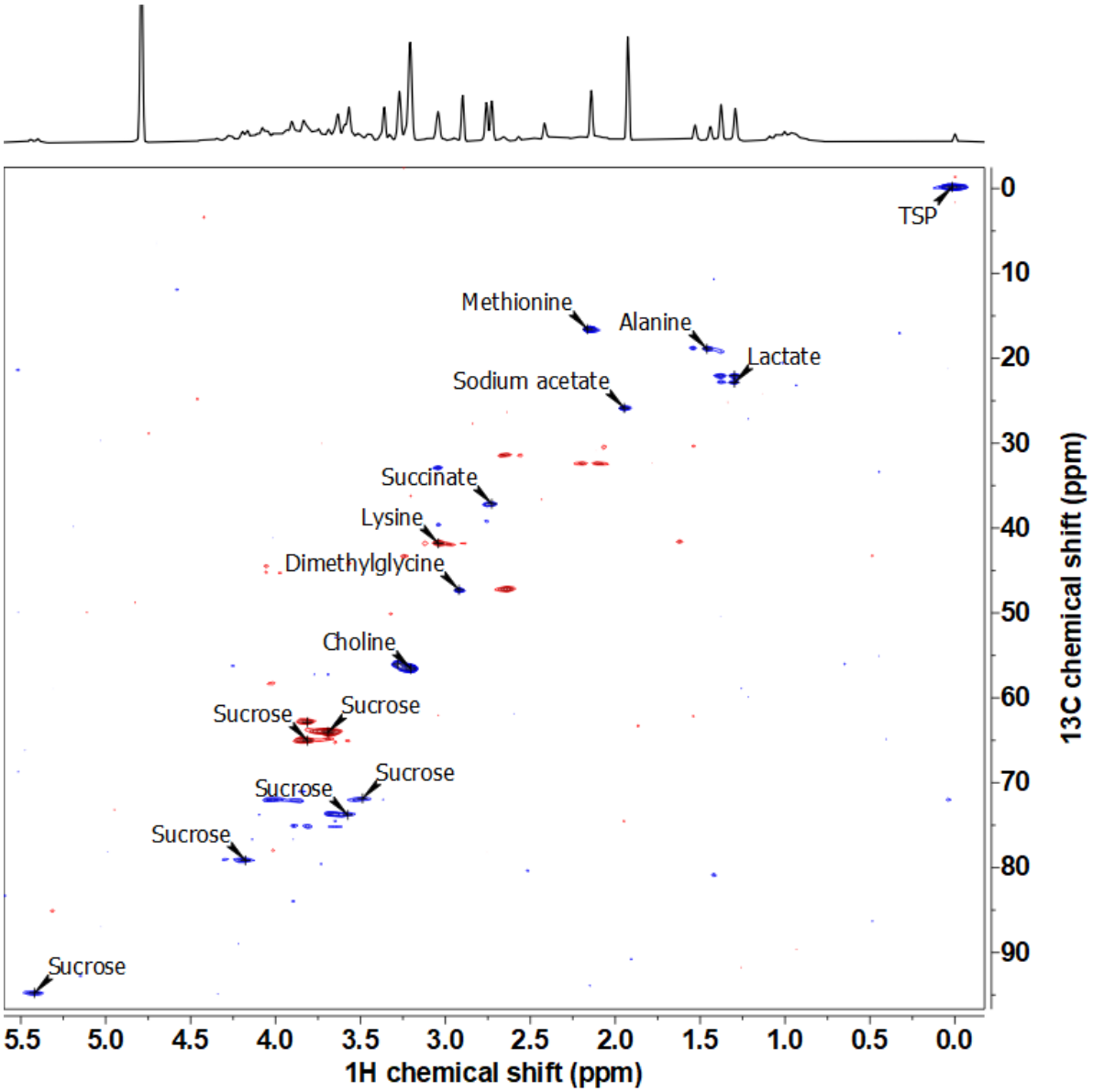

Fig.S14: Heteronuclear Single Quantum Correlation Multiplicity-Edited (HSQC-ME) spectrum of fish feed of commercial formulation acquired on an 80 MHz NMR spectrometer. Signal annotation was made with the help of Human Metabolome Database (HMDB).

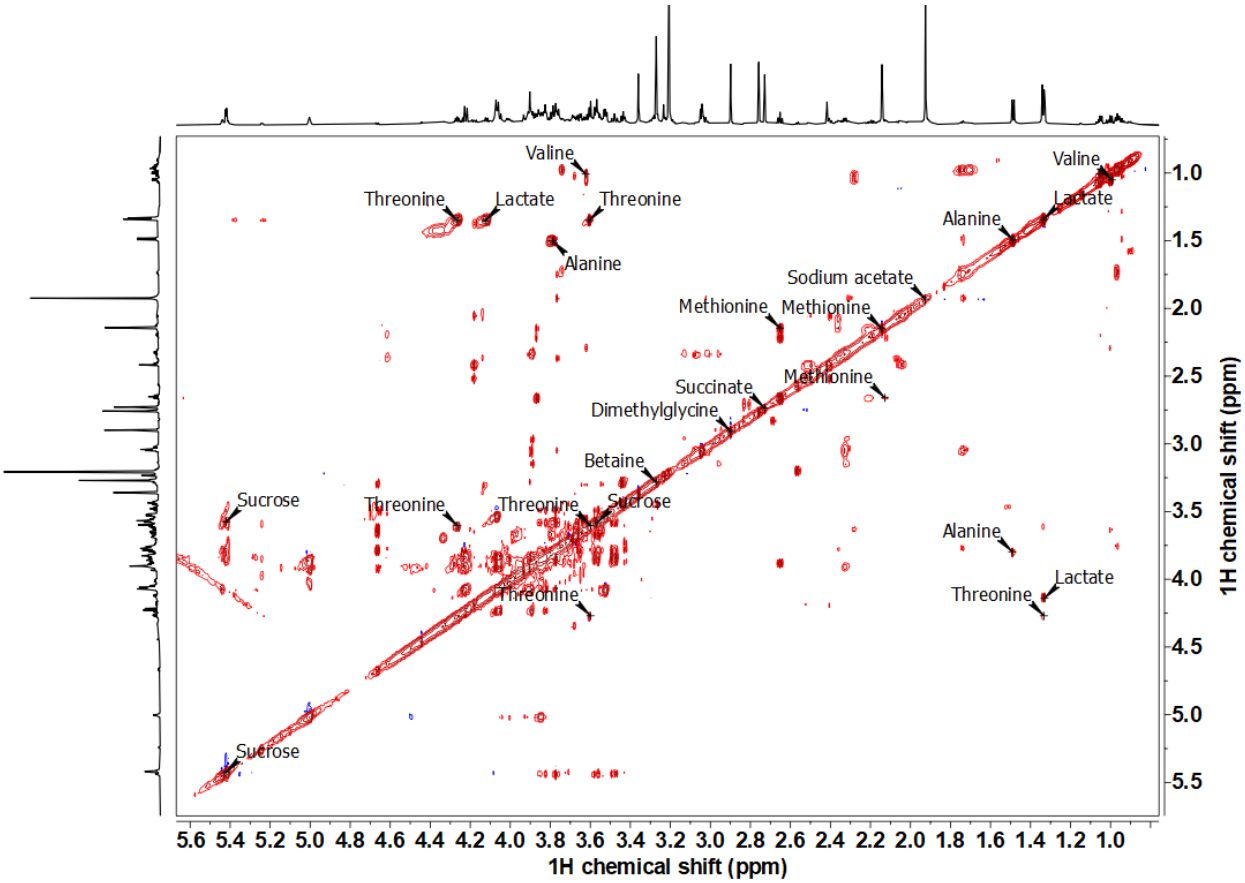

Fig.S15: T2 project zTOCSY spectrum the fish feed of commercial formulation acquired on a 700 MHz NMR spectrometer. Signal annotation was made with the help of Human Metabolome Database (HMDB).

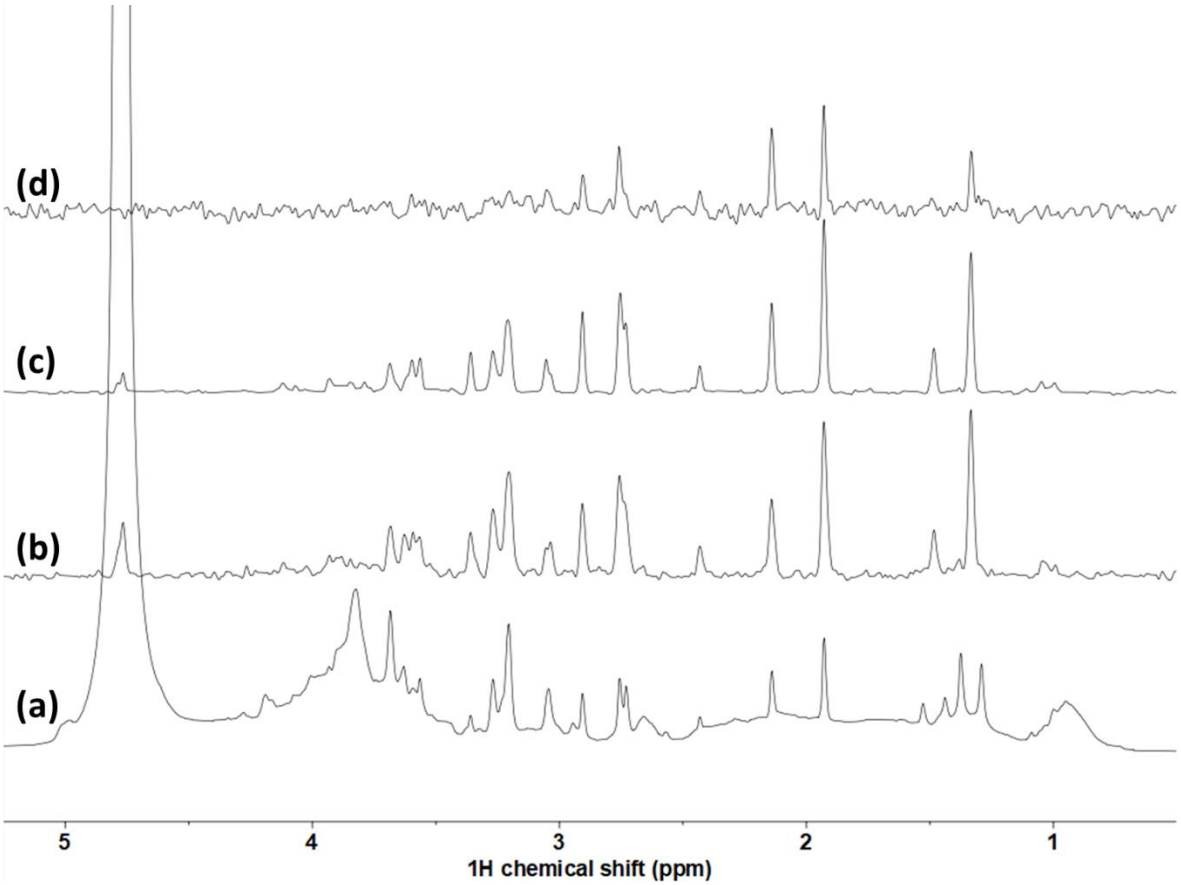

Fig.S16: Additional PS NMR spectra of the commercial fish feed extract (COM). **(a)** 1D ^{1}H NMR spectrum **(b)** 1D projection of a WET 2D J-resolved single echo spectrum. **(c)** 1D projection of a WET 2D J-resolved double echo spectrum. **(d)** 1D projection of a WET 2D J-resolved chirp spectrum. Acquisition and processing parameters are similar to those used for the analytical comparison of the model mixture.

The additional PS spectra shown in Figure S16 clearly shows the lower sensitivity of the J-RES Chirp strategy, with missing singlets and a noisier baseline. The more sensitive J-RES SE and J-RES DE spectra show similar information, but the SNR of the J-RES DE approach are higher in average, which is consistent with the observations made on the model mixture of metabolites. SNRs of the J-RES SE approach range from 7 to 38 while SNRs of the J-RES DE approach range from 13 to 86.

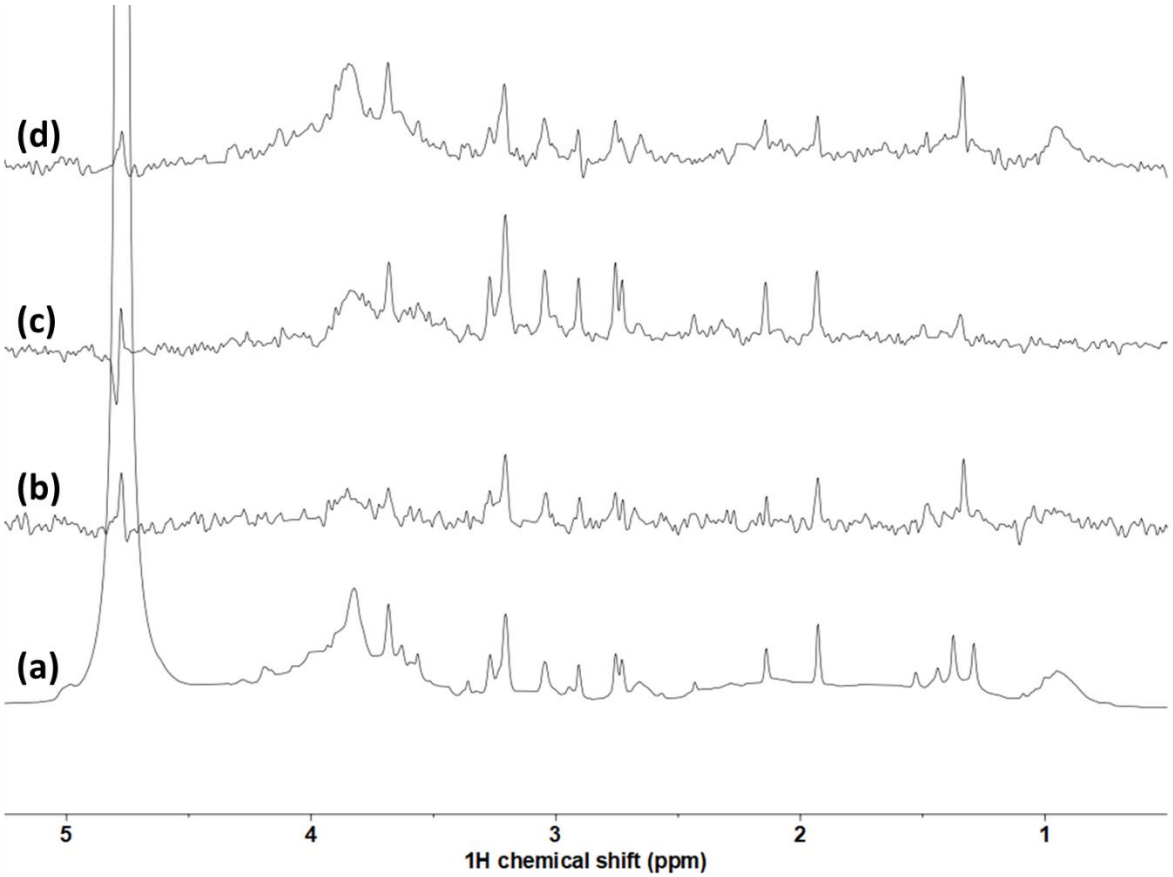

Fig.S17: Additional PS NMR spectra of the commercial fish feed extract (COM). **(a)** 1D ^{1}H NMR spectrum **(b)** WET single-band Zangger-Sterk spectrum. **(c)** WET double-band Zangger-Sterk spectrum. **(d)** WET PSYCHE spectrum. Acquisition and processing parameters are similar to those used for the analytical comparison of the model mixture.

The additional PS spectra shown in Figure S17 show the lack of sensitivity of the gradient-encoded PS strategies: especially for the single-band ZS and PSYCHE strategies. SNR on the detected singlets are very low and there is much more noise.