Supporting Information for: Improved Differentiation of NPS analogs through the application of chemometric methods to GC-solid deposition-FTIR spectra

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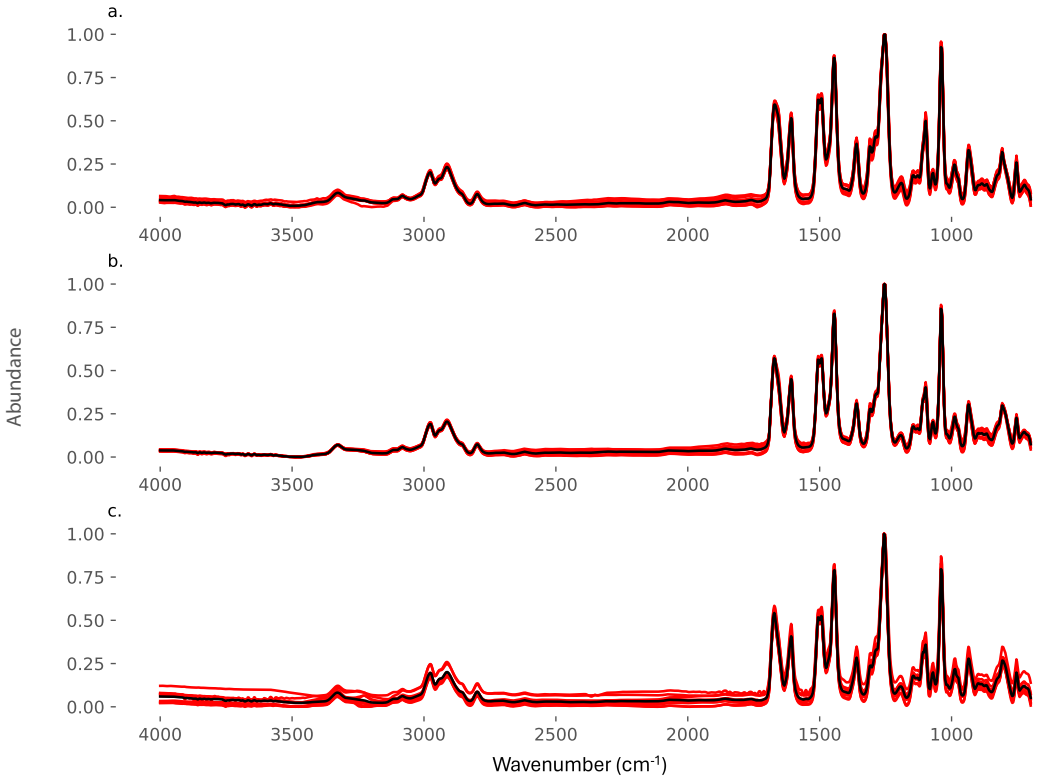

Figure S1: APL absorbance spectra for base extracted methylone samples in the primary dataset, separated by week. a. Week 1 (n=9), b. Week 2 (n=10), c. Week 3 (n=10). The average spectrum for each week is shown in black.

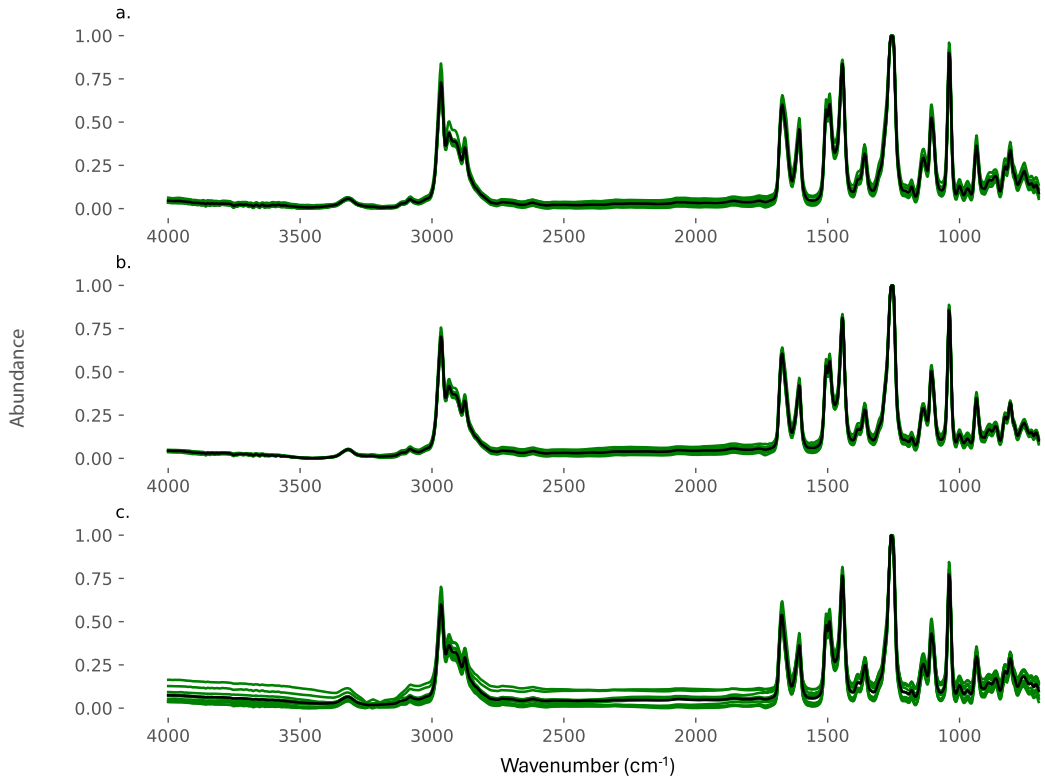

Figure S2: APL absorbance spectra for base extracted N-ethylpentylone samples in the primary dataset, separated by week. a. Week 1 (n=8), b. Week 2 (n=9), c. Week 3(n=10). The average spectrum for each week is shown in black.

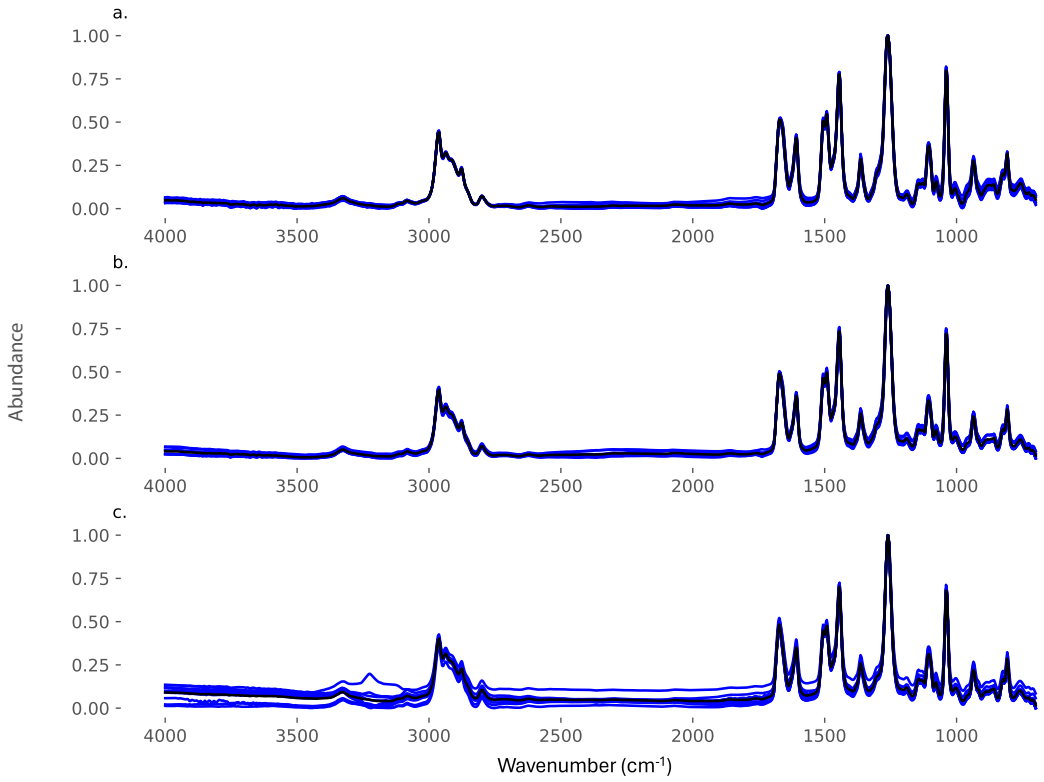

Figure S3: APL absorbance spectra for base extracted pentylone samples in the primary dataset, separated by week. a. Week 1 (n=8), b. Week 2 (n=9), c. Week 3 (n=10). The average spectrum for each week is shown in black.

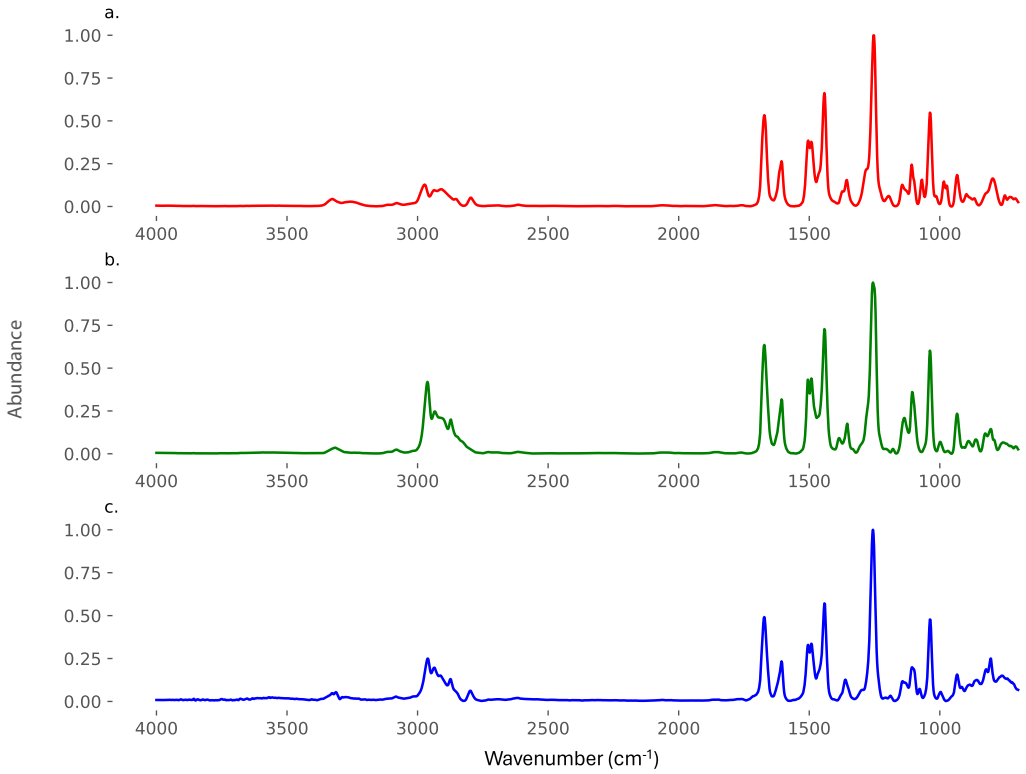

Figure S4: Full (4000cm^{-1} - 700cm^{-1}) DFS average base extracted absorbance spectra for a. methylone, b. N-ethylpentylone, c. pentylone.

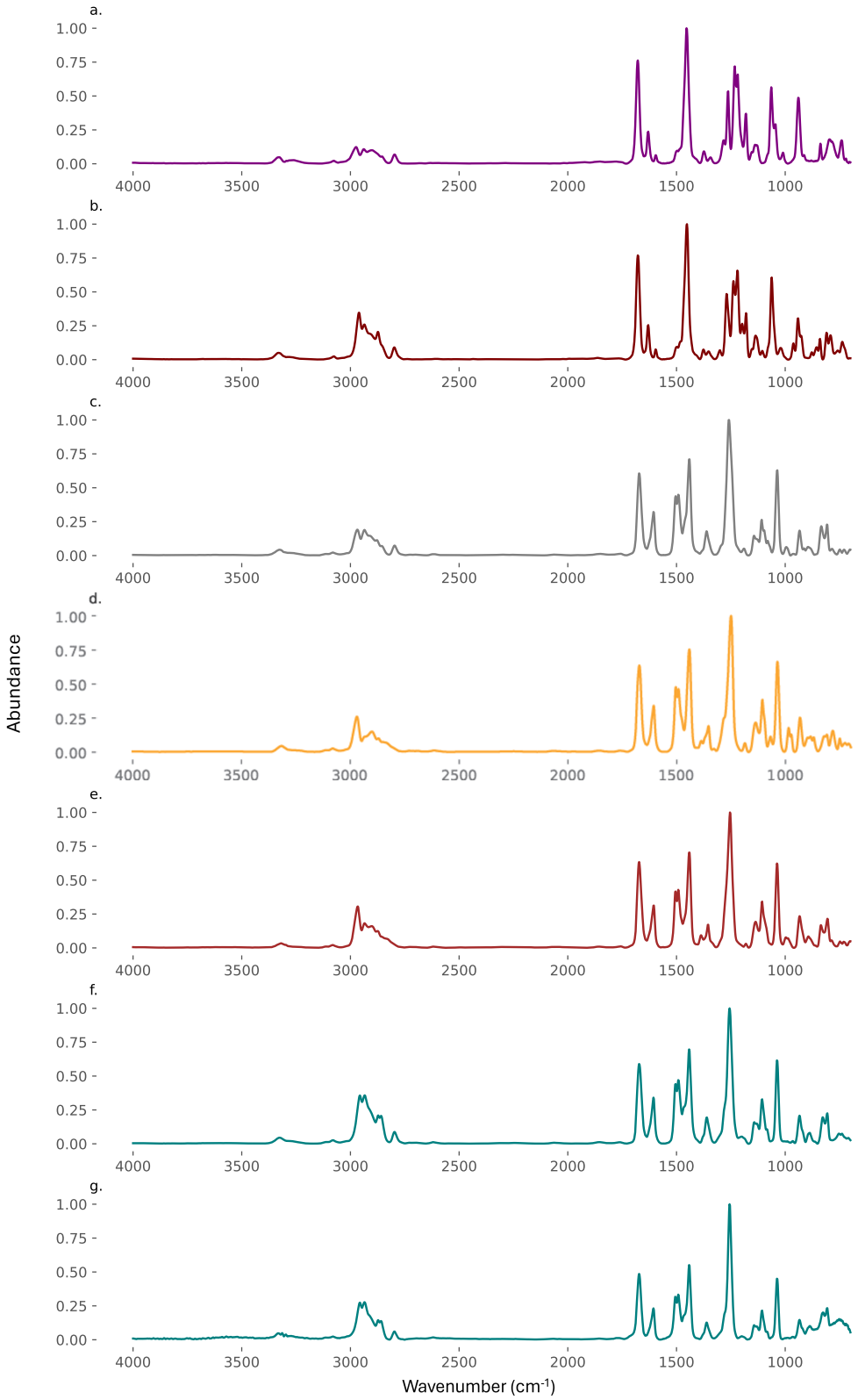

Figure S5: Full (4000cm^{-1} - 700cm^{-1}) DFS average absorbance spectra for compounds not included in the primary dataset. a. 2,3-methylone, b. 2,3-pentylone, c. butylone, d. ethylone, e. eutylone, f. N-methylhexylone (methanolic), g. N-methylhexylone (base extraction).

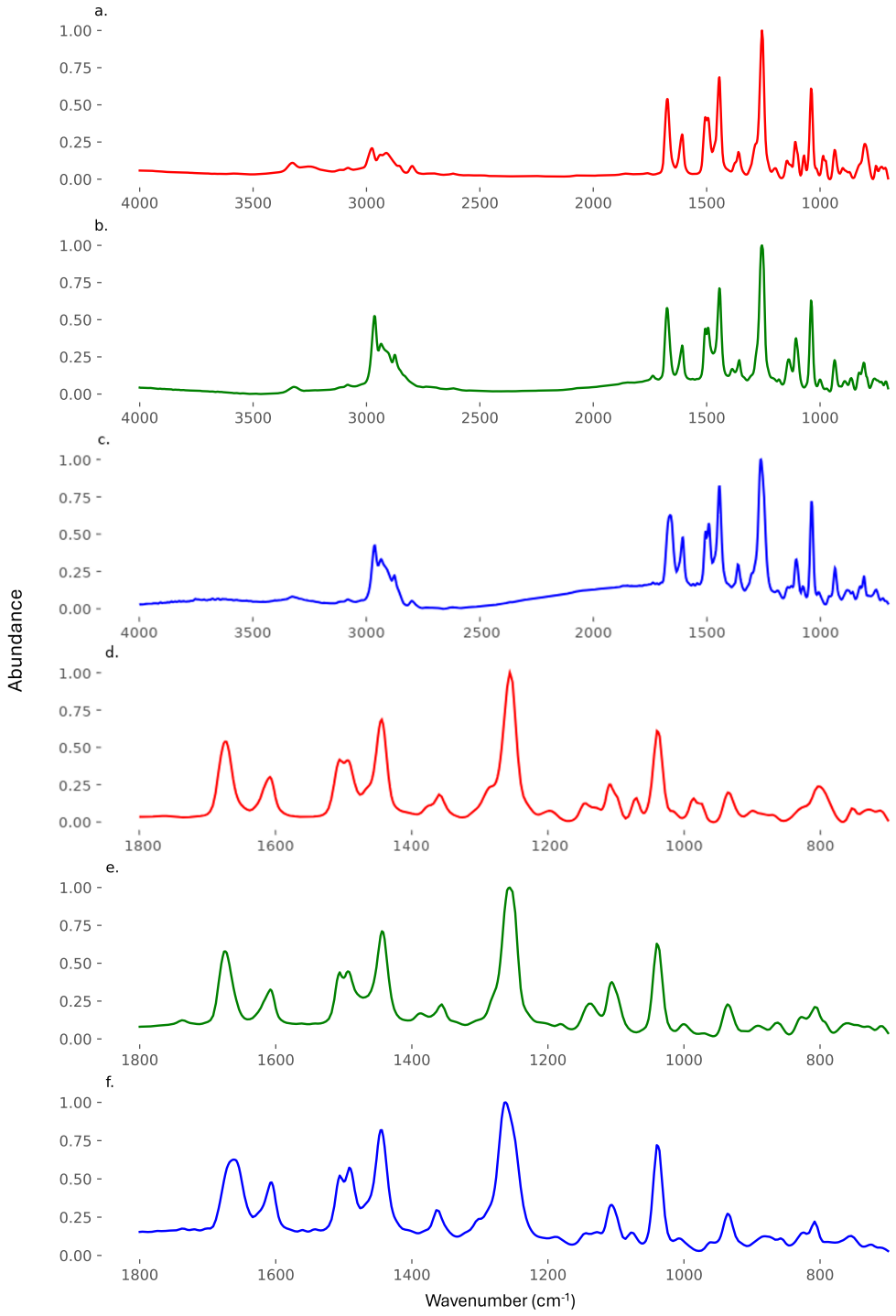

Figure S6: APL Methanolic Library absorbance spectra for a. methylone, b N-ethylpentylone, c. pentylone, d. methylone, e. N-ethylpentylone, f. pentylone (d-f: Truncated to 1800cm^{-1} - 700cm^{-1}).

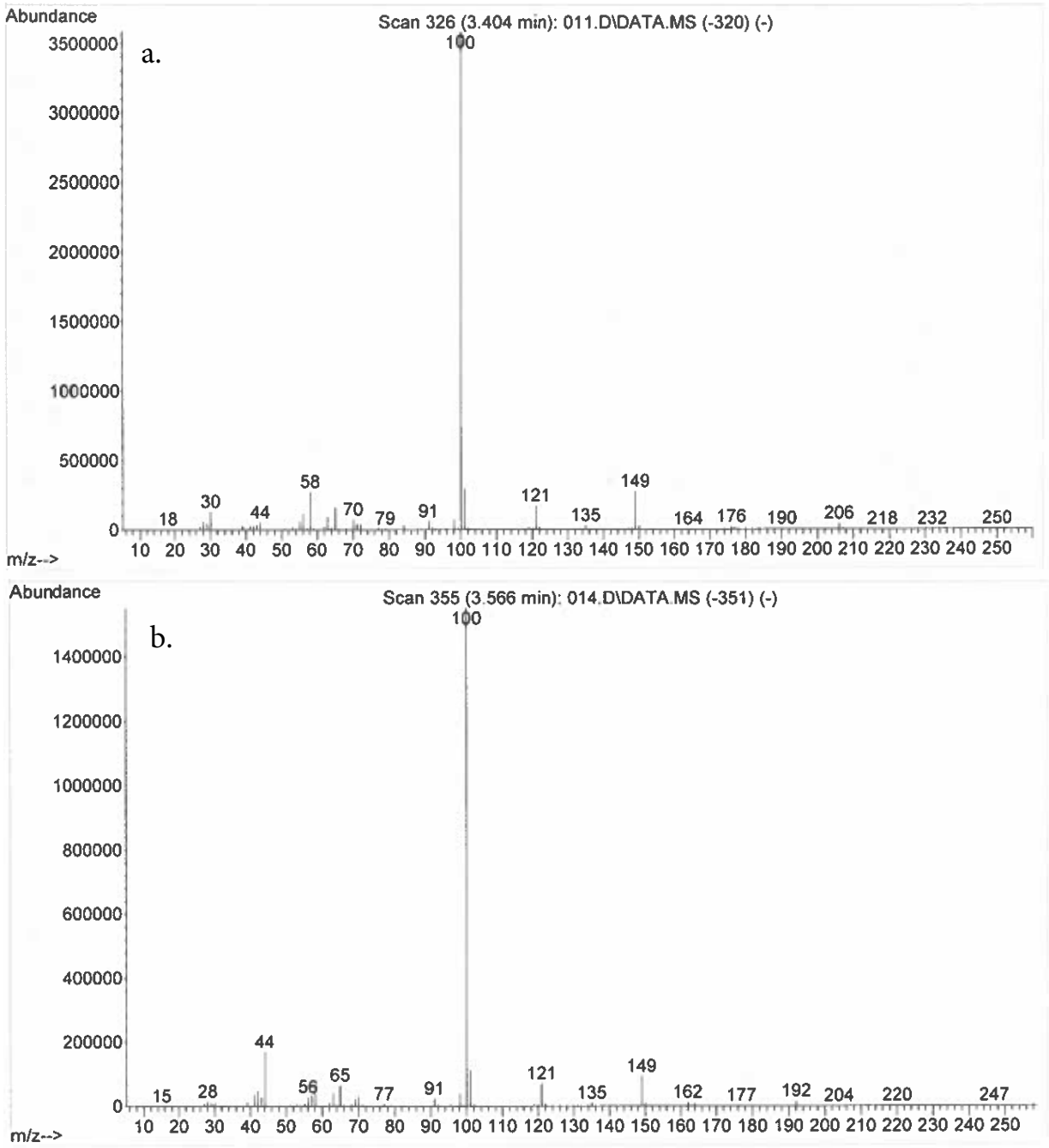

Figure S7: Electron ionization mass spectra for a. N-ethylpentylone, b. N-methylhexylone, obtained at DFS.

Table S1: Match scores for each methylone sample in the primary data set compared to library spectra for methanolic and base-extracted methylone, N-ethylpentylone, and pentylone. The lowest score (highest similarity) for each sample is shown in bold. Samples which would have been misidentified if only the methanolic library was used are shown in red.

	methylone		N-ethylpentylone		pentylone	
Week - replicate	*base*	*MeOH*	*base*	*MeOH*	*base*	*MeOH*
1 - 1	**0.003**	0.300	0.250	0.363	0.211	0.389
1 - 2	**0.006**	0.301	0.254	0.364	0.211	0.241
1 - 3	**0.007**	0.292	0.252	0.360	0.216	0.246
1 - 4	**0.009**	0.277	0.251	0.348	0.207	0.237
1 - 5	**0.011**	0.261	0.248	0.337	0.201	0.232
1 - 6	**0.012**	0.247	0.242	0.330	0.201	0.233
1 - 7	**0.015**	0.235	0.242	0.332	0.199	0.231
1 - 8	**0.017**	0.228	0.241	0.317	0.197	0.229
1 - 9	**0.021**	0.222	0.247	0.314	0.196	0.228
2 - 1	**0.034**	0.199	0.243	0.290	0.181	0.211
2 - 2	**0.042**	0.177	0.239	0.274	0.178	0.213
2 - 3	**0.042**	0.174	0.240	0.273	0.180	0.215
2 - 4	**0.045**	0.164	0.265	0.265	0.184	0.219
2 - 5	**0.038**	0.174	0.231	0.273	0.185	0.221
2 - 6	**0.055**	0.148	0.238	0.255	0.181	0.218
2 - 7	**0.051**	0.152	0.232	0.259	0.189	0.225
2 - 8	**0.059**	0.139	0.237	0.252	0.187	0.223
2 - 9	**0.058**	0.139	0.235	0.252	0.187	0.224
2 - 10	**0.060**	0.138	0.235	0.253	0.164	0.229
3 - 1	**0.067**	0.134	0.241	0.246	0.185	0.220
3 - 2	**0.073**	0.155	0.244	0.246	0.199	0.245
3 - 3	**0.090**	0.109	0.252	0.232	0.186	0.224
3 - 4	**0.089**	0.113	0.255	0.233	0.188	0.228
3 - 5	0.102	**0.099**	0.260	0.226	0.192	0.232
3 - 6	0.118	**0.084**	0.268	0.220	0.201	0.240
3 - 7	0.103	**0.092**	0.257	0.224	0.194	0.234
3 - 8	0.102	**0.092**	0.254	0.223	0.194	0.233
3 - 9	0.101	**0.095**	0.255	0.223	0.192	0.232
3- 10	**0.097**	0.101	0.251	0.227	0.194	0.234

Table S2: Match scores for each N-ethylpentylone sample in the primary data set compared to library spectra for methanolic and base-extracted methylone, N-ethylpentylone, and pentylone. The lowest score (highest similarity) for each sample is shown in bold.

	methylone		**N-ethylpentylone**		**pentylone**	
Week - replicate	*base*	*MeOH*	*base*	*MeOH*	*base*	*MeOH*
1 - 1	0.245	0.353	**0.005**	0.155	0.125	0.160
1 - 2	0.234	0.307	**0.013**	0.124	0.101	0.135
1 - 3	0.237	0.289	**0.020**	0.111	0.093	0.125
1 - 4	0.232	0.293	**0.021**	0.114	0.099	0.133
1 - 5	0.239	0.287	**0.018**	0.104	0.099	0.131
1 - 6	0.244	0.287	**0.017**	0.100	0.103	0.136
1 - 7	0.238	0.275	**0.027**	0.096	0.098	0.131
1 - 8	0.235	0.280	**0.025**	0.100	0.102	0.136
2 - 1	0.266	0.292	**0.017**	0.092	0.112	0.145
2 - 2	0.258	0.269	**0.027**	0.081	0.105	0.140
2 - 3	0.269	0.268	**0.027**	0.072	0.112	0.148
2 - 4	0.267	0.266	**0.030**	0.073	0.111	0.147
2 - 5	0.274	0.245	**0.042**	0.058	0.111	0.145
2 - 6	0.281	0.271	**0.029**	0.063	0.123	0.160
2 - 7	0.277	0.247	**0.041**	0.057	0.115	0.151
2 - 8	0.276	0.262	**0.034**	0.063	0.121	0.158
2 - 9	0.278	0.251	**0.041**	0.057	0.120	0.157
3 - 1	0.275	0.261	**0.033**	0.063	0.118	0.152
3 - 2	0.288	0.232	0.054	**0.046**	0.119	0.154
3 - 3	0.283	0.211	0.075	**0.044**	0.117	0.150
3 - 4	0.290	0.201	0.096	**0.042**	0.120	0.154
3 - 5	0.297	0.198	0.090	**0.036**	0.124	0.159
3 - 6	0.296	0.198	0.091	**0.036**	0.124	0.158
3 - 7	0.295	0.198	0.100	**0.038**	0.125	0.159
3 - 8	0.289	0.209	0.090	**0.042**	0.127	0.162
3 - 9	0.296	0.205	0.084	**0.035**	0.129	0.165
3 - 10	0.294	0.207	0.080	**0.034**	0.128	0.165

Table S3: Match scores for each pentylone sample in the primary data set compared to library spectra for methanolic and base-extracted methylone, N-ethylpentylone, and pentylone. The lowest score (highest similarity) for each sample is shown in bold.

	methylone		**N-ethylpentylone**		**pentylone**	
Week - replicate	*base*	*MeOH*	*base*	*MeOH*	*base*	*MeOH*
1 - 1	0.222	0.311	0.140	0.231	**0.004**	0.027
1 - 2	0.223	0.307	0.136	0.225	**0.004**	0.027
1 - 3	0.220	0.316	0.134	0.229	**0.009**	0.036
1 - 4	0.225	0.300	0.135	0.216	**0.005**	0.030
1 - 5	0.226	0.293	0.136	0.211	**0.006**	0.030
1 - 6	0.223	0.304	0.140	0.219	**0.011**	0.038
1 - 7	0.226	0.298	0.139	0.214	**0.010**	0.037
1 - 8	0.229	0.286	0.141	0.204	**0.010**	0.035
2 - 1	0.246	0.274	0.158	0.199	**0.014**	0.033
2 - 2	0.253	0.264	0.161	0.188	**0.018**	0.039
2 - 3	0.261	0.260	0.170	0.186	**0.023**	0.042
2 - 4	0.261	0.261	0.178	0.191	**0.026**	0.047
2 - 5	0.255	0.252	0.159	0.174	**0.021**	0.047
2 - 6	0.267	0.247	0.172	0.172	**0.029**	0.052
2 - 7	0.262	0.248	0.170	0.173	**0.027**	0.052
2 - 8	0.269	0.247	0.180	0.175	**0.033**	0.057
2 - 9	0.275	0.239	0.172	0.159	**0.036**	0.060
3 - 1	0.279	0.238	0.170	0.156	**0.038**	0.061
3 - 2	0.295	0.242	0.201	0.173	**0.053**	0.082
3 - 3	0.291	0.258	0.206	0.184	**0.054**	0.079
3 - 4	0.301	0.240	0.206	0.169	**0.058**	0.082
3 - 5	0.304	0.243	0.211	0.172	**0.061**	0.084
3 - 6	0.310	0.260	0.223	0.186	**0.070**	0.092
3 - 7	0.301	0.241	0.209	0.169	**0.062**	0.090
3 - 8	0.305	0.244	0.212	0.171	**0.064**	0.090
3 - 9	0.308	0.239	0.216	0.166	**0.069**	0.098
3 - 10	0.301	0.234	0.207	0.162	**0.061**	0.089

Table S4: Average absolute difference between match scores obtained when comparing the samples in the primary dataset to the two different solvent libraries.

		Library		
		methylone	N-ethylpentylone	pentylone
Primary Dataset	methylone	0.126	0.046	0.042
	N-ethylpentylone	0.052	0.056	0.035
	pentylone	0.049	0.042	0.024

Table S5: Match scores for samples analyzed at DFS compared to library spectra for methanolic and base-extracted methylone, N-ethylpentylone, and pentylone. The lowest value for each sample is shown in bold. Samples which would result in false positive associations is identifying a sample based solely on the lowest match score are shown in red. *These spectra were generated utilizing a different pentylone solution.

Sequence - replicate	Sample Preparation	Substance	methylone		N-ethylpentylone		pentylone	
			base	*MeOH*	*base*	*MeOH*	*base*	*MeOH*
1 - 1	Methanol	methylone	0.360	**0.115**	0.361	0.225	0.394	0.434
1 - 2	Methanol		0.375	**0.111**	0.378	0.223	0.404	0.444
1 - 3	Methanol		0.359	**0.111**	0.361	0.222	0.391	0.430
2 - 1	Methanol		0.374	**0.119**	0.381	0.228	0.409	0.448
2 - 2	Methanol		0.357	**0.119**	0.363	0.226	0.395	0.436
2 - 3	Methanol		0.379	**0.114**	0.386	0.226	0.410	0.449
3 - 1	Base Extraction		0.410	**0.125**	0.412	0.238	0.440	0.480
3 - 2	Base Extraction		0.412	**0.123**	0.413	0.237	0.439	0.479
3 - 3	Base Extraction		0.411	**0.121**	0.413	0.236	0.437	0.477
1 - 1	Methanol	N-ethylpentylone	0.352	0.326	0.180	**0.125**	0.291	0.320
1 - 2	Methanol		0.368	0.303	0.203	**0.113**	0.301	0.331
1 - 3	Methanol		0.355	0.328	0.183	**0.126**	0.295	0.325
2 - 1	Methanol		0.323	0.322	0.159	**0.129**	0.257	0.279
2 - 2	Methanol		0.341	0.324	0.174	**0.128**	0.278	0.303
2 - 3	Methanol		0.348	0.308	0.183	**0.117**	0.279	0.306
3 - 1	Base Extraction		0.392	0.279	0.239	**0.110**	0.317	0.347
3 - 2	Base Extraction		0.400	0.271	0.251	**0.109**	0.324	0.355
3 - 3	Base Extraction		0.394	0.275	0.241	**0.108**	0.318	0.348
1 - 1*	Methanol	pentylone	0.325	0.291	0.236	**0.185**	0.205	0.242
1 - 2*	Methanol		0.337	0.262	0.250	**0.167**	0.206	0.243
1 - 3*	Methanol		0.324	0.287	0.235	**0.183**	0.202	0.239
1 - 4	Methanol		0.345	0.252	0.259	**0.163**	0.212	0.248
1 - 5	Methanol		0.343	0.254	0.257	**0.163**	0.209	0.245
1 - 6	Methanol		0.348	0.248	0.262	**0.161**	0.212	0.248
2 - 1	Methanol		0.312	0.275	0.229	**0.177**	0.183	0.214
2 - 2	Methanol		0.312	0.286	0.226	**0.183**	0.188	0.221
2 - 3	Methanol		0.331	0.253	0.247	**0.164**	0.195	0.228
3 - 1	Base Extraction		0.486	0.208	0.412	**0.189**	0.349	0.388
3 - 2	Base Extraction		0.485	0.203	0.415	**0.190**	0.349	0.388
3 - 3	Base Extraction		0.483	0.204	0.408	**0.183**	0.343	0.384

Table S6: Match scores with standard deviation for DFS spectra for the six additional cathinones compared to methanolic and base extracted methylone, N-ethylpentylone, and pentylone library spectra. The lowest average is shown in bold. Match scores that are of a similar scale to those obtained by the false positive DFS results are shown in red.

			methylone		**N-ethylpentylone**		**pentylone**	
Sample	Sample Preparation	n	*base*	*MeOH*	*base*	*MeOH*	*base*	*MeOH*
2,3-methylone	Methanol	2	>0.900	>0.900	>0.900	>0.900	>0.900	>0.900
2,3-pentylone	Methanol	2	>0.900	>0.900	>0.900	>0.900	>0.900	>0.900
butylone	Methanol	2	0.368 ± 0.005	0.287 ± 0.005	0.326 ± 0.006	0.261 ± 0.001	**0.249 ± 0.001**	0.272 ± 0.001
ethylone	Methanol	2	0.323 ± 0.011	0.260 ± 0.007	0.290 ± 0.010	**0.251 ± 0.004**	0.376 ± 0.007	0.411 ± 0.008
eutylone	Methanol	2	0.384 ± 0.009	0.262 ± 0.002	0.270 ± 0.008	**0.189 ± 0.001**	0.320 ± 0.013	0.360 ± 0.015
N-methylhexylone	Methanol	6	0.378 ± 0.012	0.233 ± 0.004	0.297 ± 0.012	**0.179 ± 0.001**	0.270 ± 0.014	0.309 ± 0.016
	Base extraction	3	0.505 ± 0.004	**0.213 ± 0.003**	0.441 ± 0.003	0.222 ± 0.004	0.405 ± 0.006	0.450 ± 0.005

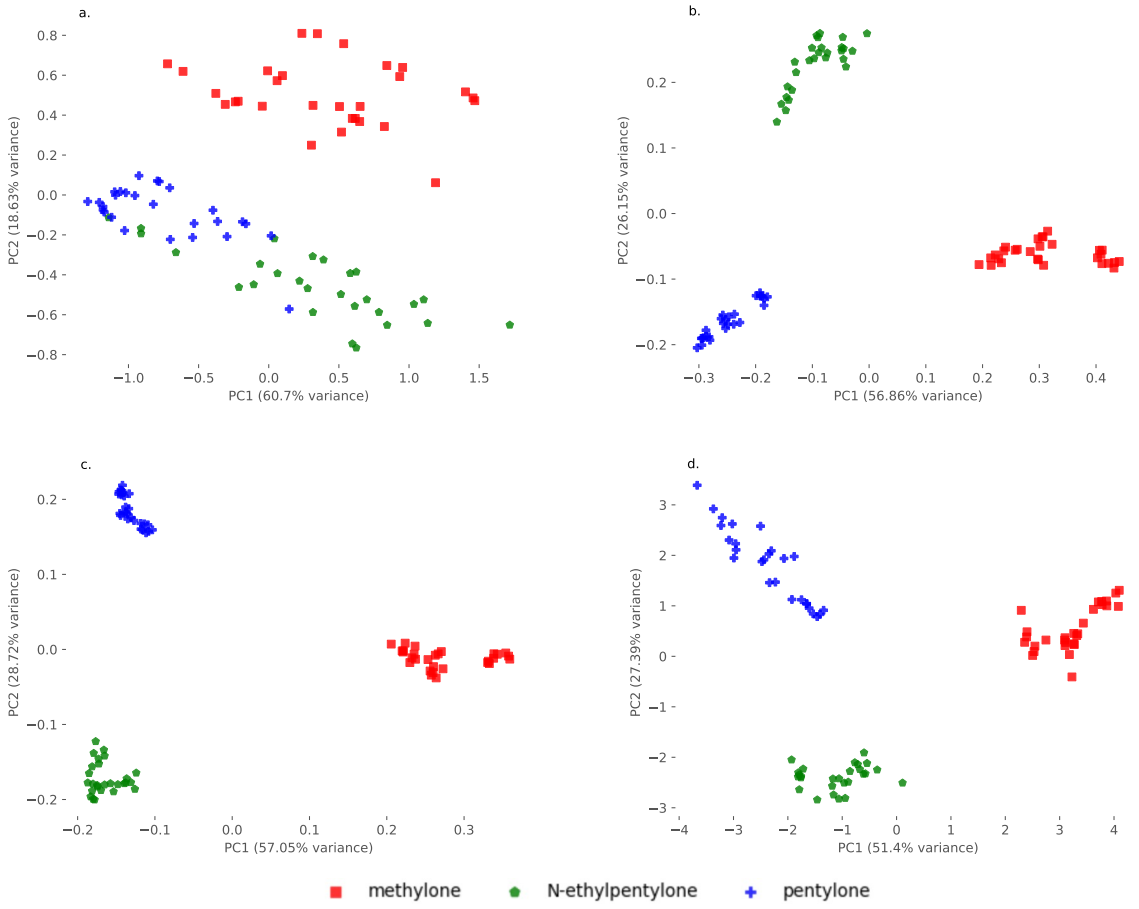

Figure S8: PCA Score plots of 0th (original) (a.), 1st (b.), 2nd (c.) derivative and SNV (d) spectra using the primary dataset. Truncated spectra (1800cm^{-1} - 700cm^{-1}) used. The proportion of variance described by each principal component is shown on the respective axis.

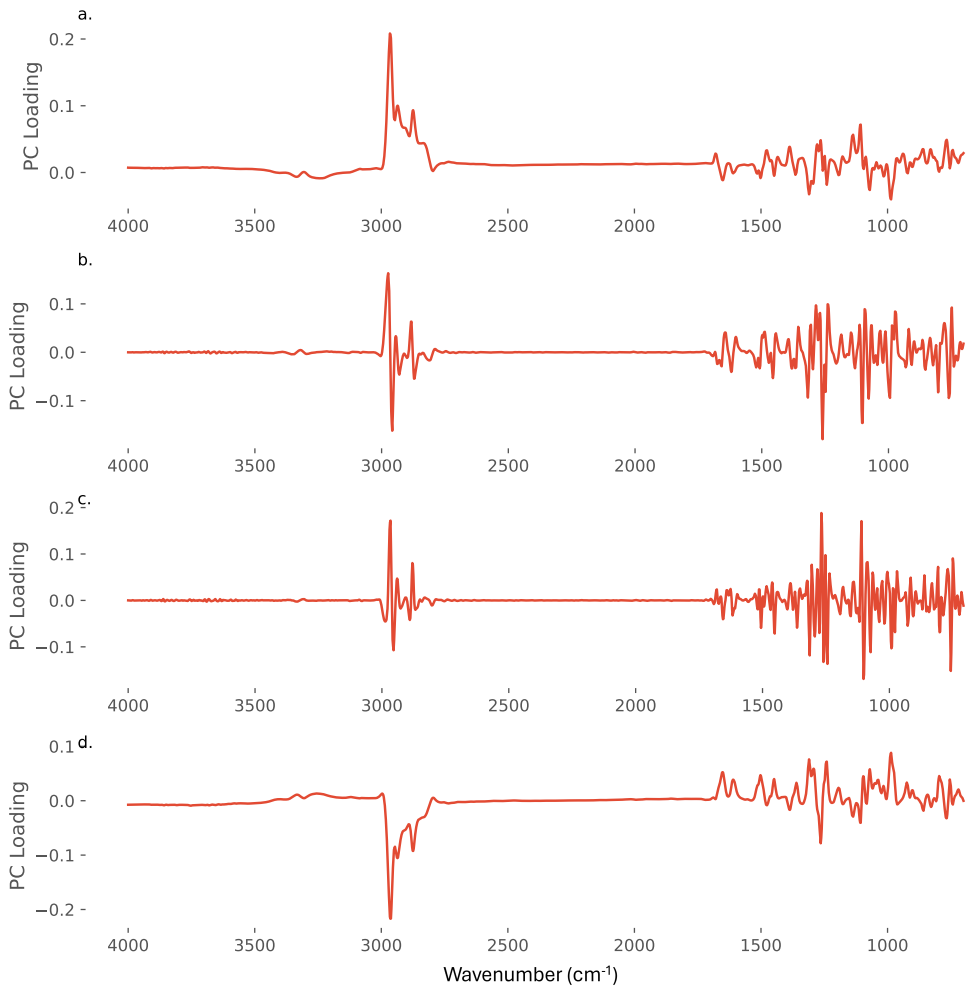

Figure S9: PC1 loadings for 0th (original) (a.), 1st (b.), 2nd (c.) derivative and SNV (d) spectra using the primary dataset.

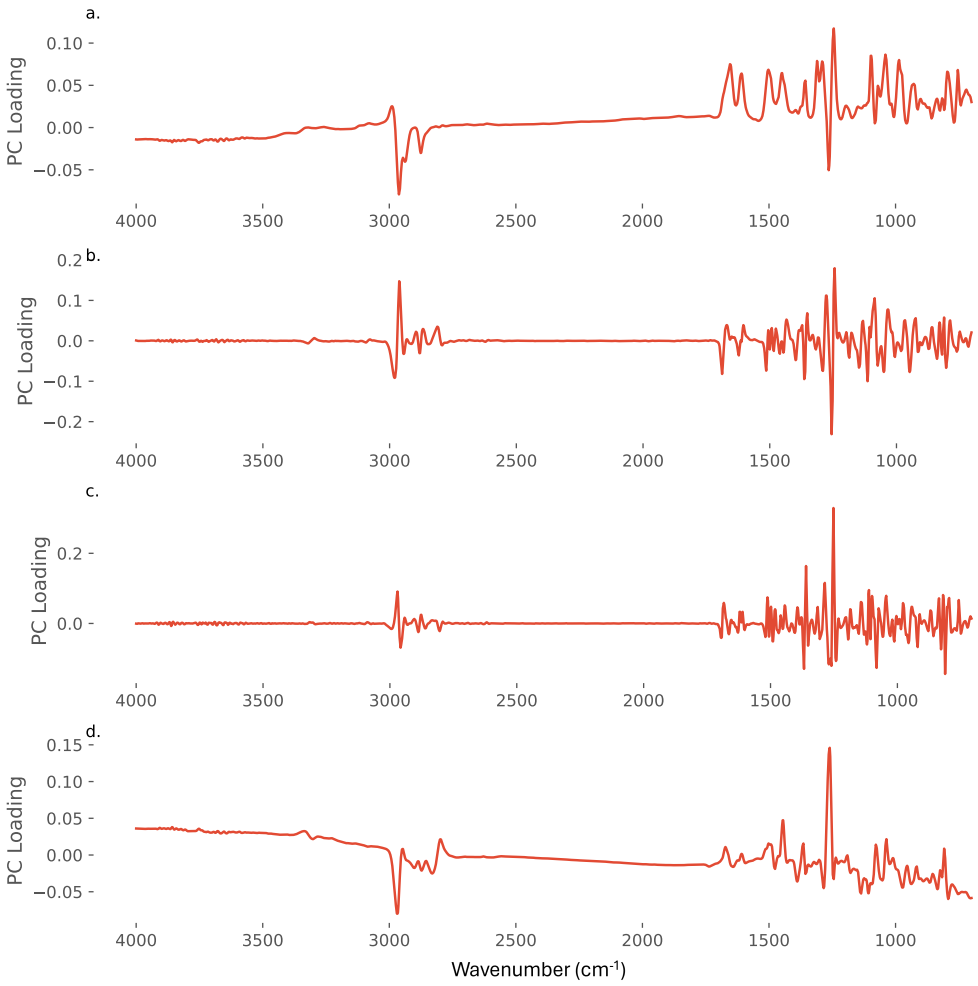

Figure S10: PC2 loadings for 0th (original) (a.), 1st (b.), 2nd (c.) derivative and SNV (d) spectra using the primary dataset.

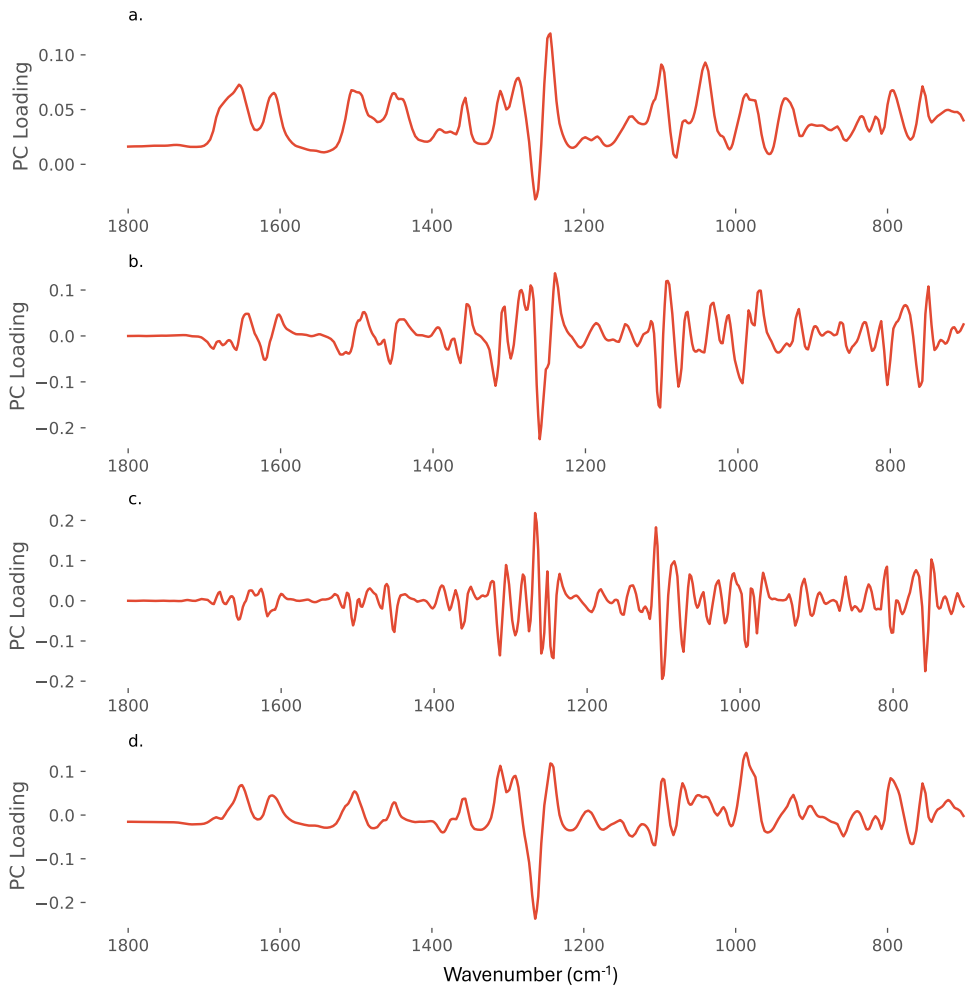

Figure S11: PC1 loadings for truncated 0th (original) (a.), 1st (b.), 2nd (c.) derivative and SNV (d) spectra using the primary dataset.

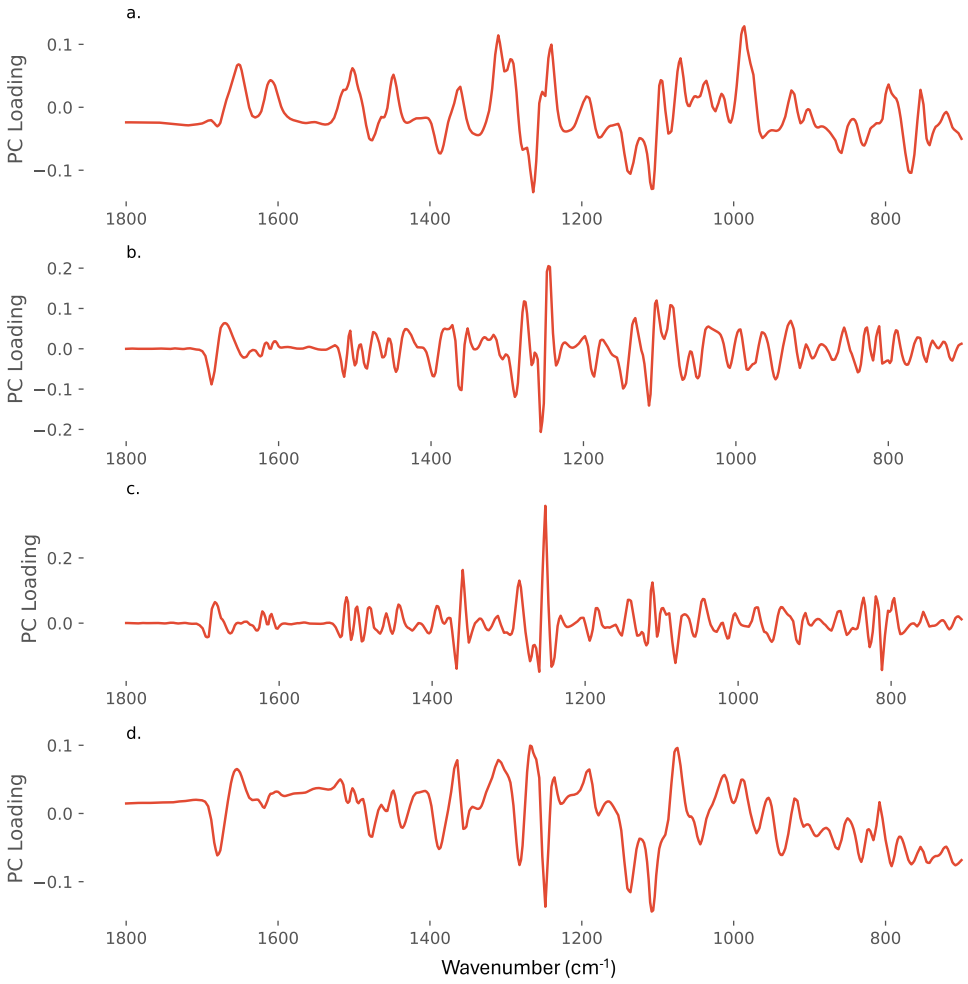

Figure S12: PC2 loadings for truncated 0th (original) (a.), 1st (b.), 2nd (c.) derivative and SNV (d) spectra using the primary dataset.

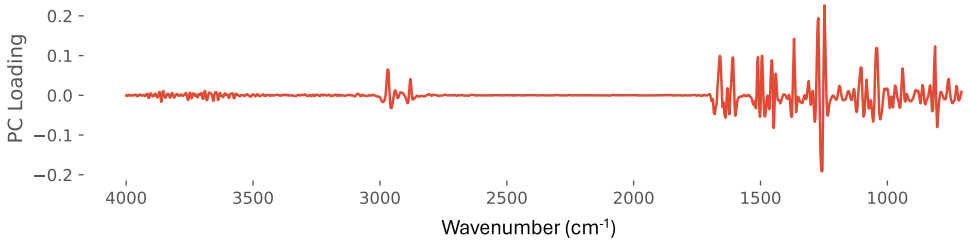

Figure S13: PC3 loadings for 2nd derivative spectra using the primary dataset.

Table S7: Mahalanobis distances (D_m) between test samples and the compounds in the primary dataset using two principal components. When n>1, the average and standard deviation are provided. The distances representing the correct identity are highlighted in bold. When the smallest distances are encountered for the correct compound cluster, the result in shown in green.

Samples	Lab	Sample Preparation	n	methylone	N-ethylpentylone	pentylone
methylone	DFS	Base extraction	3	**290 ± 8**	360 ± 3	703 ± 5
		Methanol	6	**361 ± 23**	386 ± 10	730 ± 14
	APL	Base extraction (library)	1	**2.4**	1313	5360
		Methanol (library)	1	**17**	398	1200
N-ethylpentylone	DFS	Base extraction	3	791 ± 4	**0.9 ± 0.1**	917 ± 8
		Methanol	6	1008 ± 66	**5.1 ± 1.7**	1175 ± 86
	APL	Base extraction (case)	3	717 ± 84	**1.0 ± 1.1**	944 ± 173
		Base extraction (library)	1	841	**1.4**	983
		Methanol (library)	1	566	**10**	1026
pentylone	DFS	Base extraction	3	124 ± 4	127 ± 4	**13 ± 4**
		Methanol	9	104 ± 2	91 ± 4	**45 ± 6**
	APL	Base extraction (case)	3	228 ± 46	173 ± 21	**2.6 ± 1.2**
		Base extraction (library)	1	136	127	**3.2**
		Methanol (library)	1	147	132	**3.8**

Table S8: Mahalanobis distances (D_m) between compounds whose identity is not reflected in the primary dataset and the compounds in the primary dataset, using two principal components. The average and standard deviation are provided.

Sample	Sample Preparation	n	methylone	N-ethylpentylone	pentylone
2,3-methylone	Methanol	2	302 ± 2	363 ± 2	817 ± 2
2,3-pentylone	Methanol	2	614 ± 4	307 ± 3	78 ± 13
butylone	Methanol	2	160 ± 14	147 ± 6	19 ± 1
ethylone	Methanol	2	590 ± 8	325 ± 9	508 ± 15
eutylone	Methanol	2	206 ± 11	55 ± 0.2	110 ± 5
N-methylhexylone	Methanol	6	96 ± 2	75 ± 3	50 ± 9
	Base extraction	3	87 ± 4	122 ± 0.4	55 ± 8

Table S9: Mahalanobis distances (D_m) between compounds whose identity is not reflected in the primary dataset and the compounds in the primary dataset, using three principal components. The average and standard deviation are provided.

Sample	Sample Preparation	n	methylone	N-ethylpentylone	pentylone
2,3-methylone	Methanol	2	3210 ± 17	3819 ± 33	1512 ± 19
2,3-pentylone	Methanol	2	3123 ± 76	4154 ± 141	218 ± 30
butylone	Methanol	2	1937 ± 44	1266 ± 33	342 ± 1
ethylone	Methanol	2	877 ± 35	2389 ± 29	12527 ± 94
eutylone	Methanol	2	1026 ± 15	141 ± 21	3953 ± 157
N-methylhexylone	Methanol	6	1529 ± 55	400 ± 28	975 ± 51
	Base extraction	3	607 ± 30	156 ± 13	2867 ± 179