Supplemental Information for

Benefits of Derivatization in GC-MS-based Identification of New Psychoactive Substances

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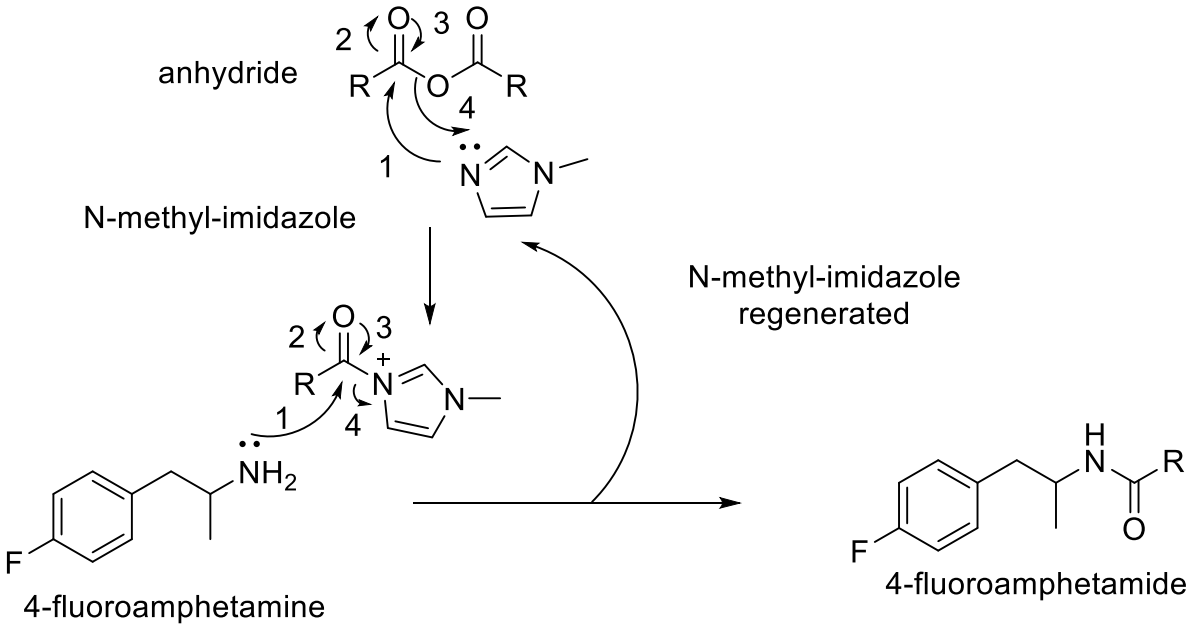

Fig. S1. Reaction mechanism of the N-methyl-imidazole catalyzed acylation of 4-FA with acid anhydride.

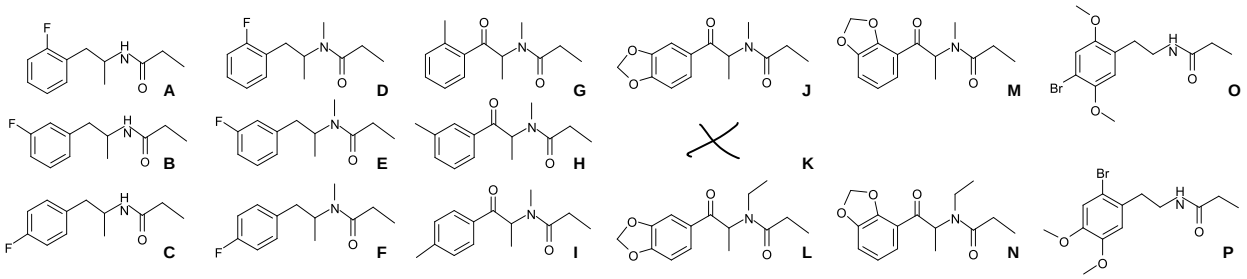

Fig. S2. Molecular structures of the Fig. 1 substances after derivatization with propionic anhydride: 2-FA-propionyl (A), 2-FA-propionyl (B), 4-FA-propionyl (C), 2-FMA-propionyl (D), 2-FMA-propionyl (E), 4-FMA-propionyl (F), 2-MMC-propionyl (G), 3-MMC-propionyl (H), 4-MMC-propionyl (I), methylone-propionyl (J), ethylone-propionyl (L), 2,3-methylone-propionyl (M), 2,3-ethylone-propionyl (N), 2C-B-propionyl (O), 2-Br-4,5-DMPEA-propionyl (P). Dimethylone (Fig. 1K) does not form a propionyl-derivate due to a lacking active hydrogen in the tertiary amine.

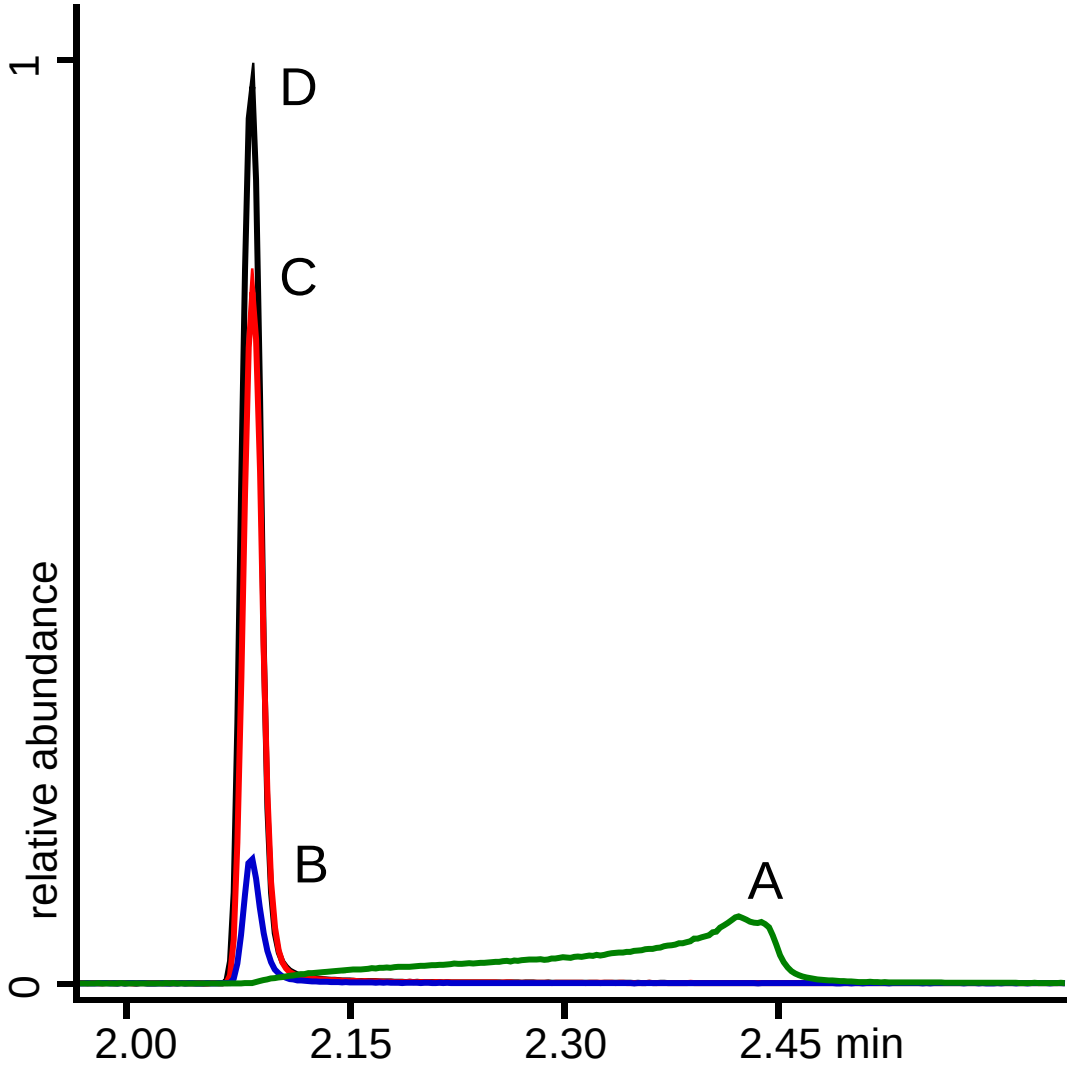

Fig. S3. GC-MS total ion chromatograms of a 4-FA case sample following direct methanolic extraction (A), dichloromethane extraction (B), methanolic extraction after $NaHCO_3$ treatment (C) and acid/base extraction (D).

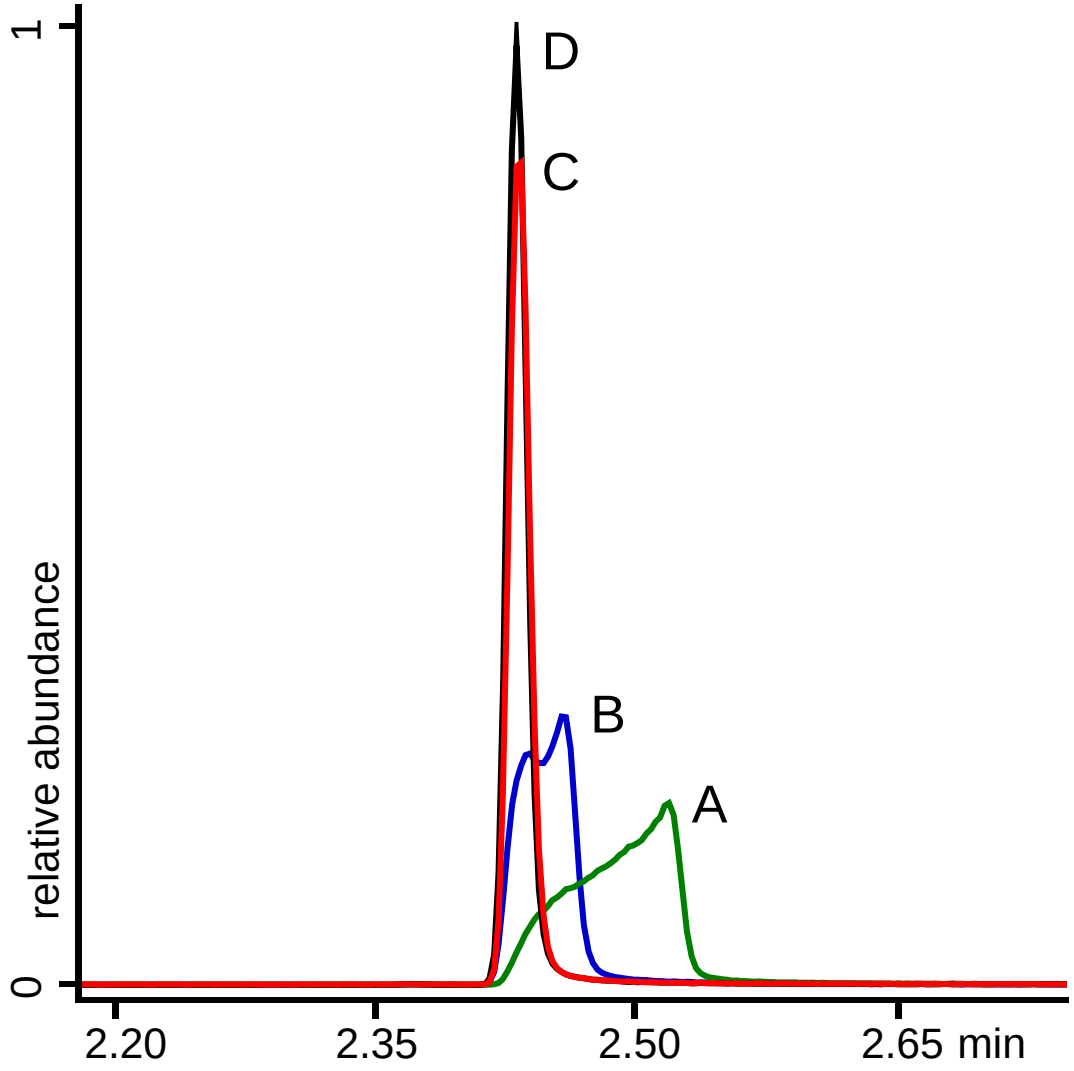

Fig. S4. GC-MS total ion chromatograms of a 4-FMA case sample following direct methanolic extraction (A), dichloromethane extraction (B), methanolic extraction after $NaHCO_3$ treatment (C) and acid/base extraction (D).

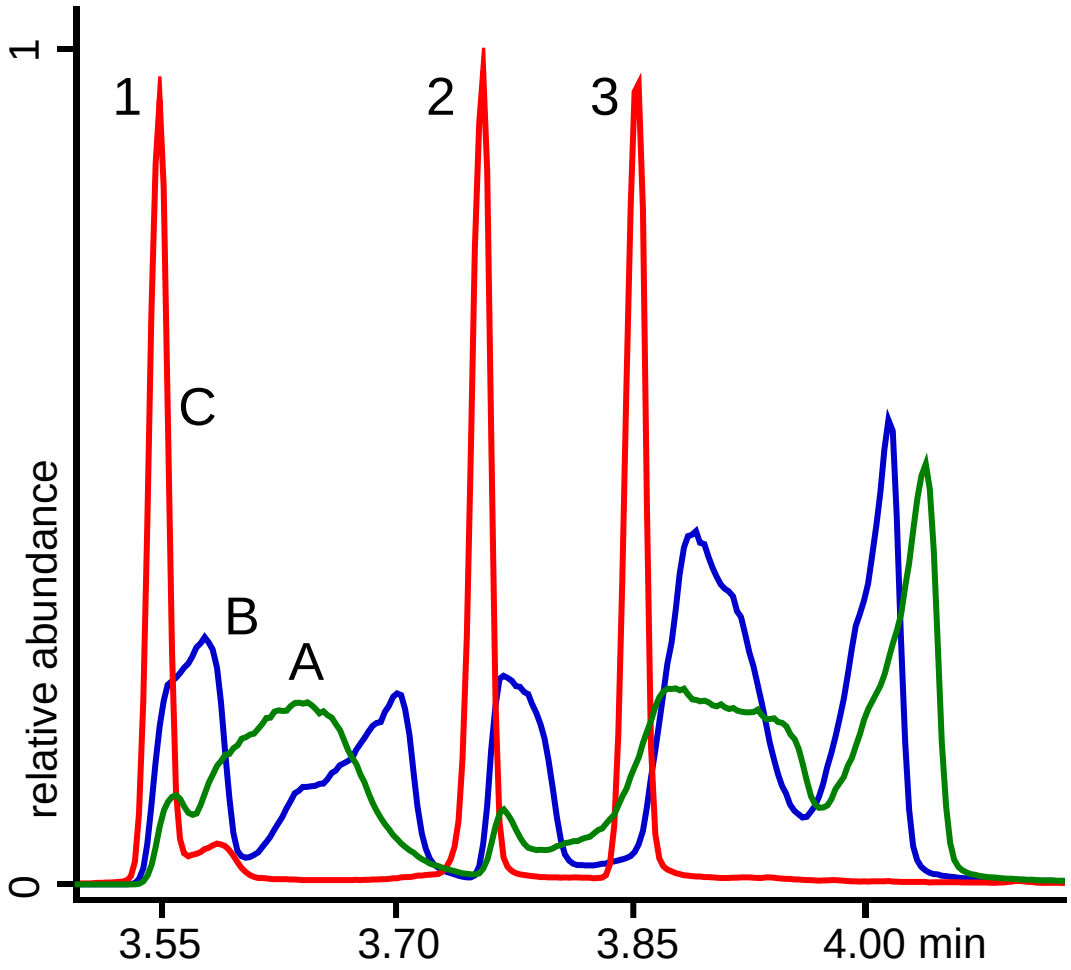

Fig. S5. GC-MS total ion chromatograms of a 2-MMC (1), 3-MMC (2) and 4-MMC (3) mixture; 3 extraction methods are shown: analysis directly following methanolic extraction (A, green plot); methanol after 10 min neutralization with $NaHCO_3$ (B, red plot) and after 20 minute neutralization (C, black plot).

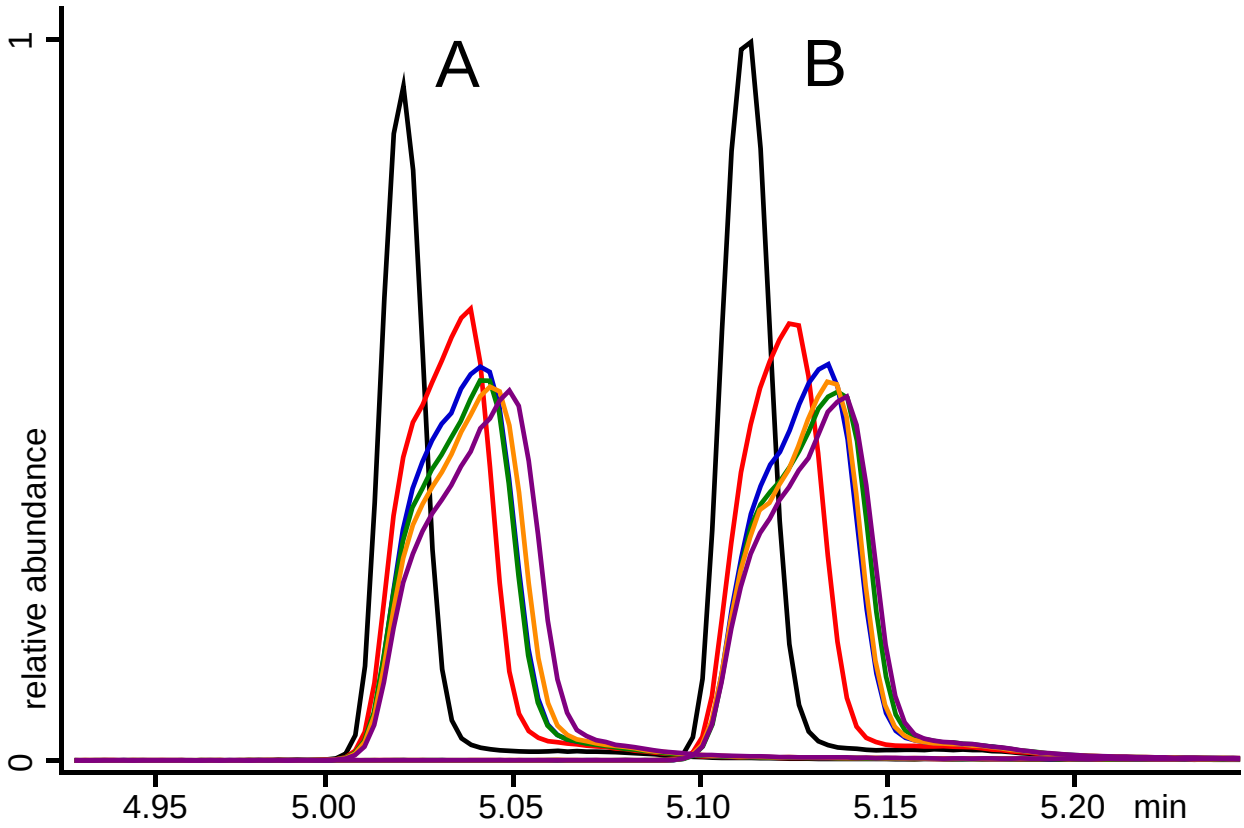

Fig. S6. GC-MS total ion chromatograms of 3-MMC and 4-MMC methanolic solution replicate analysis, freshly prepared (black), after 3,5 hours (red), 7 hours (blue), 10 hours (green), 14 hours (orange) and 17 hours (purple).

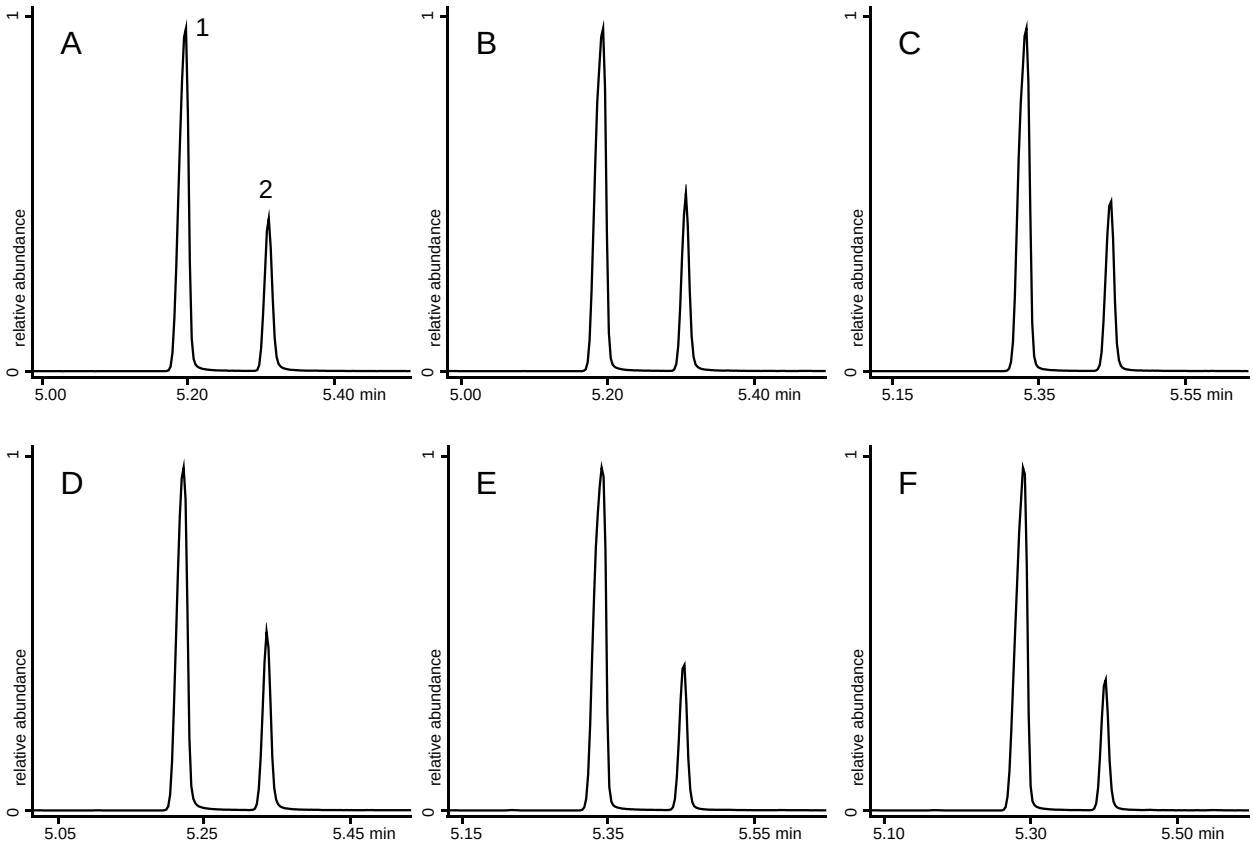

Fig. S7. GC-MS total ion chromatograms of a derivatized 3-MMC and 4-MMC mixture showing peaks of 3-MMC-propionyl (1) and 4-MMC-propionyl (2) after 2 weeks (A), 1 month (B), 2 months (C), 1 year (D), 2 years (E) and 3 years (F) after preparation. Extracts were stored in closed vials in a refrigerator at 4 ^{o}C.

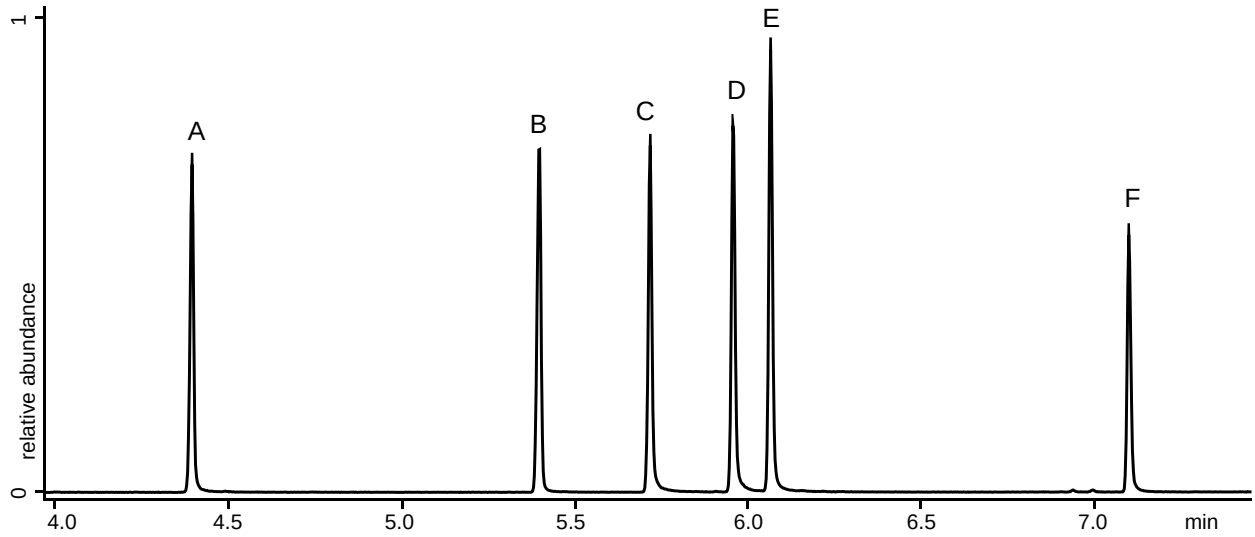

Fig. S8. GC-MS total ion chromatogram of a 6.5 years old extract of a derivatized reference standard containing amphetamine-propionyl (A), caffeine (B), MDA-propionyl (C), MDMA-propionyl (D), MDEA-propionyl (E) and internal standard tetracosane (F), individual concentration without derivatization: 2 mg/mL, solution was stored in closed amber glass at ambient temperature.

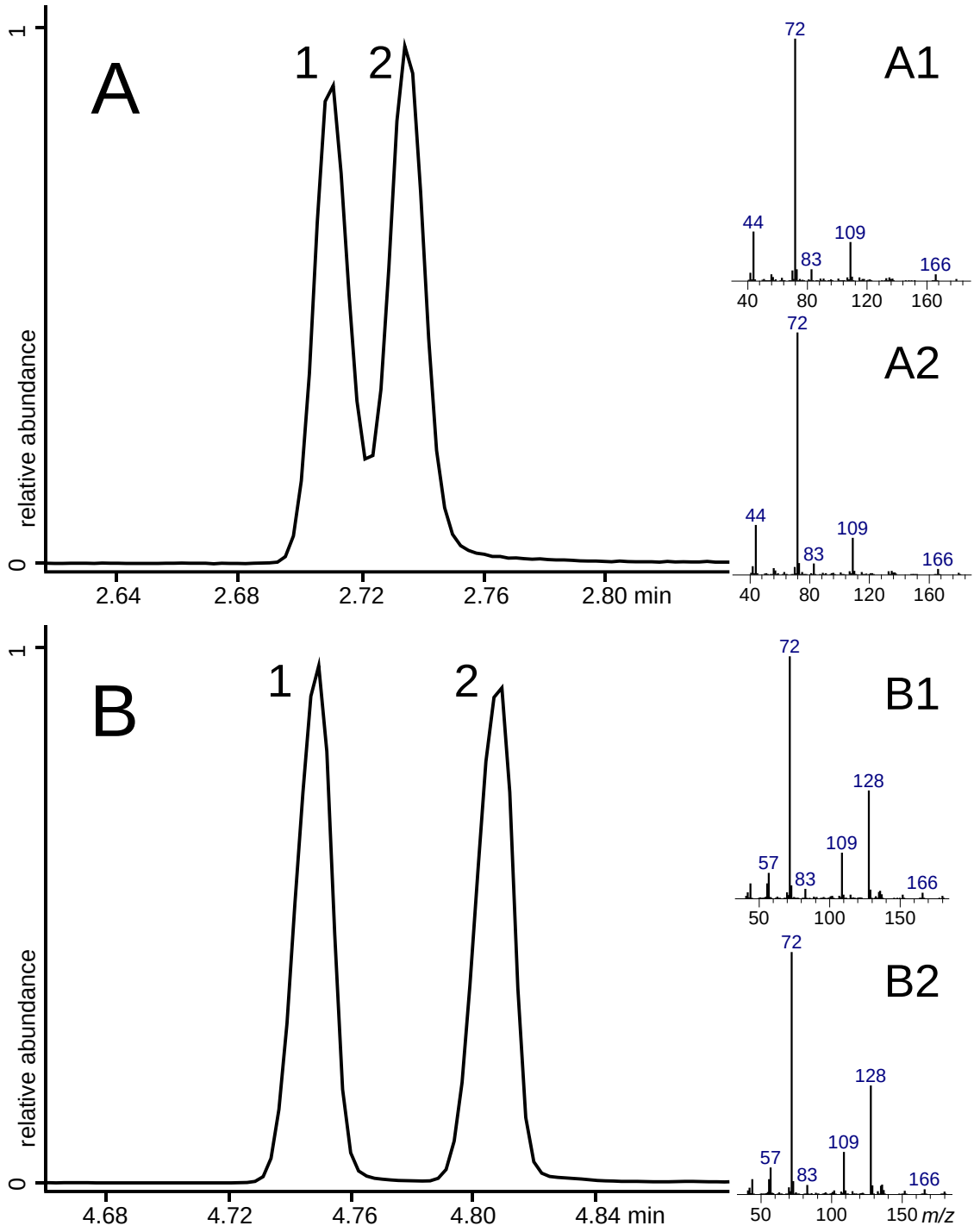

Fig. S9. Underivatized (A) and PA-derivatized (B) GC-MS chromatograms of the same case sample containing 2 different FEA-isomers with EI-mass spectra of the peaks shown as insets.

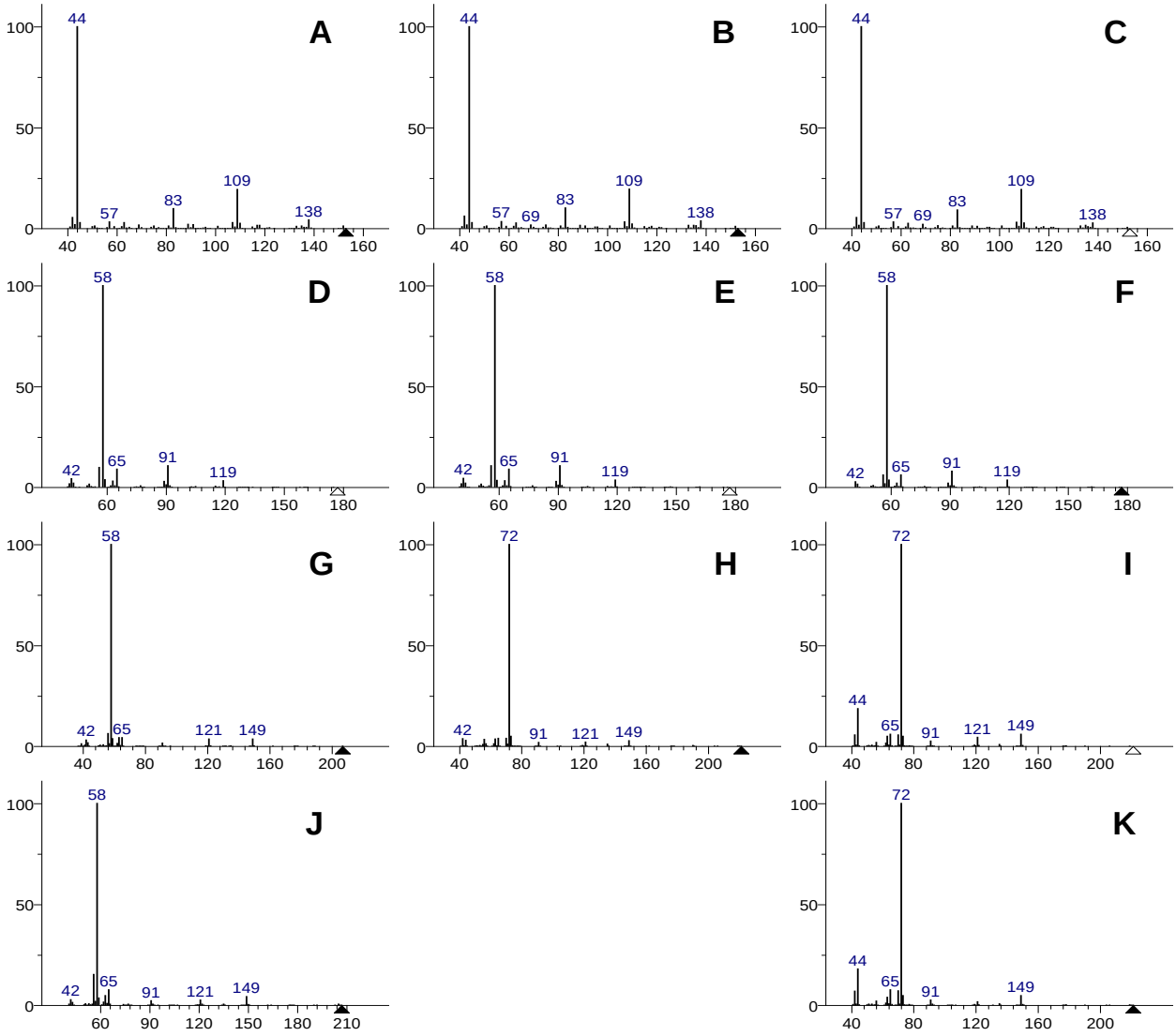

Fig. S10. Mass spectra of the underivatized compounds 2-FA (A), 3-FA (B), 4-FA (C), 2-MMC (D), 3-MMC (E), 4-MMC (F), methylone (G), dimethylone (H), ethylone (I), 2,3-methylone (J), 2,3-ethylone (K)

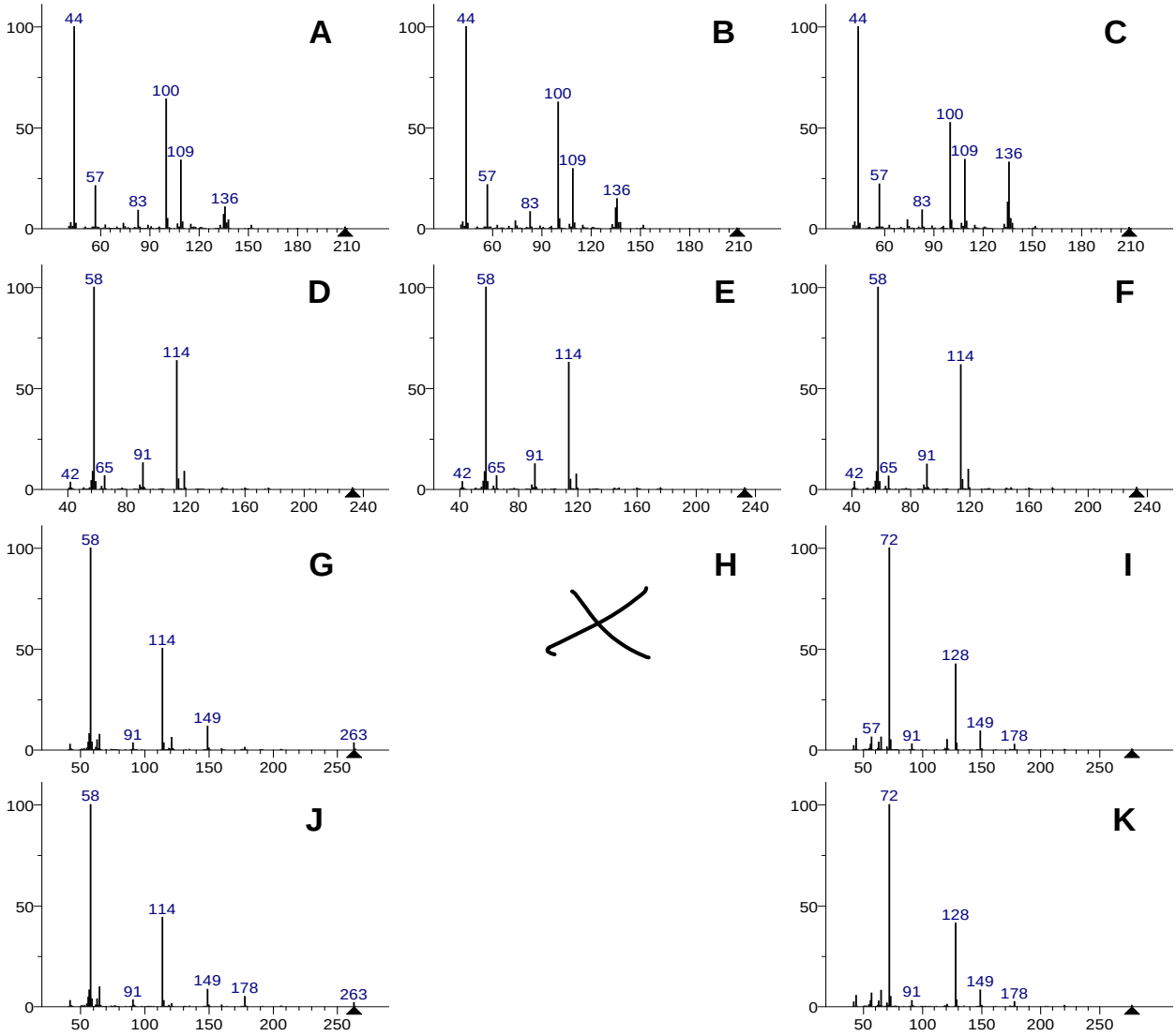

Fig. S11. Mass spectra of the compounds 2-FA-propionyl (A), 2-FA-propionyl (B), 4-FA-propionyl (C), 2-MMC-propionyl (D), 3-MMC-propionyl (E), 4-MMC-propionyl (F), methylone-propionyl (G), ethylone-propionyl (I), 2,3-methylone-propionyl (J), 2,3-ethylone-propionyl (K), 2C-B-propionyl (L), 2-Br-4,5-DMPEA-propionyl (M). Dimethylone (H) does not form a propionyl-derivate due to a lacking active hydrogen in the tertiary amine.

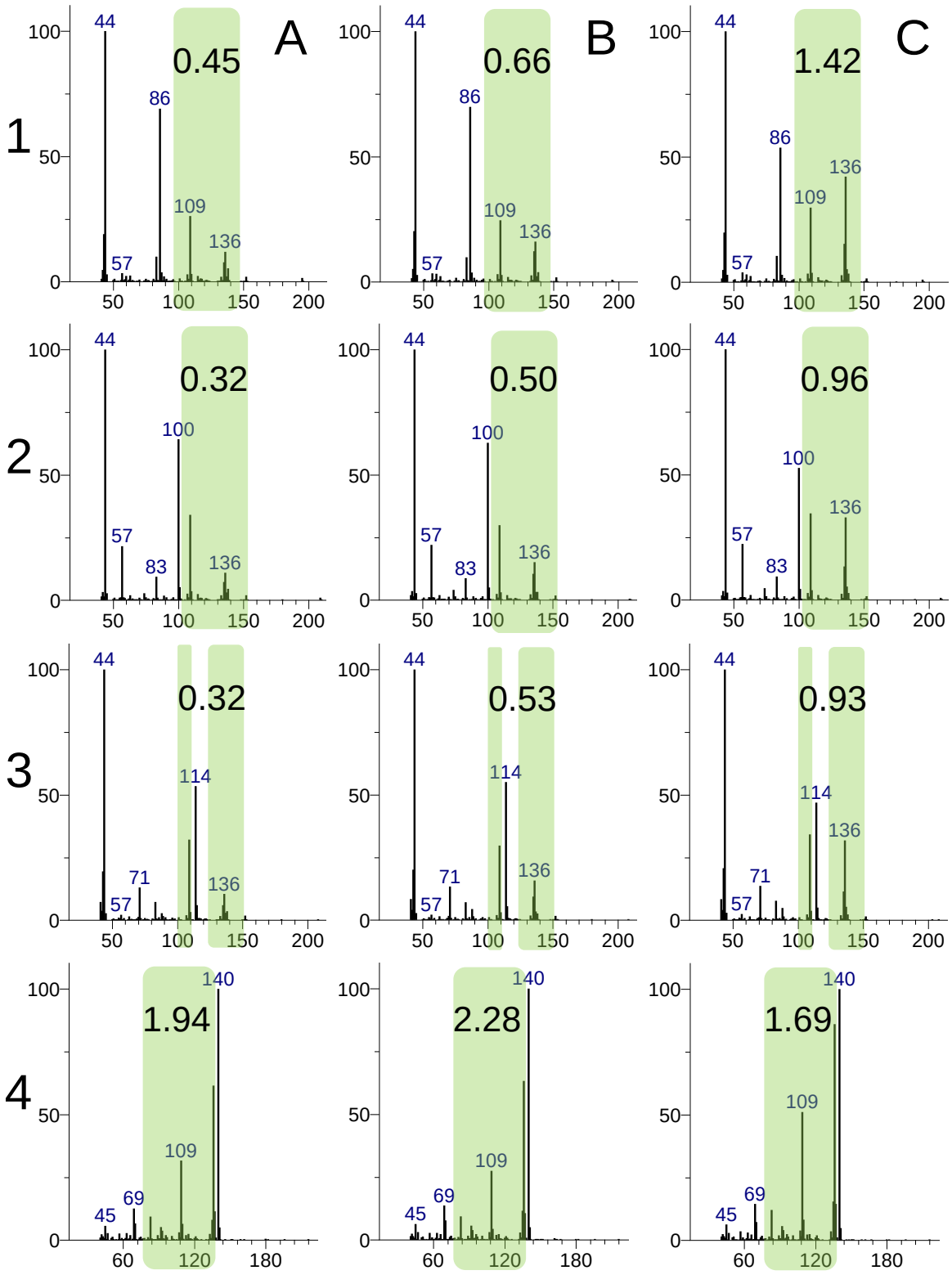

Fig. S12. Mass spectra of 2-FA (A), 3-FA (B) and 4-FA (C) as acetyl- (1), propionyl- (2), butyryl- (3) and trifluoroacetyl- (4) derivatives, with the ratio of diagnostic ions *m/z* 136 : *m/z* 109 shown in the green shade.

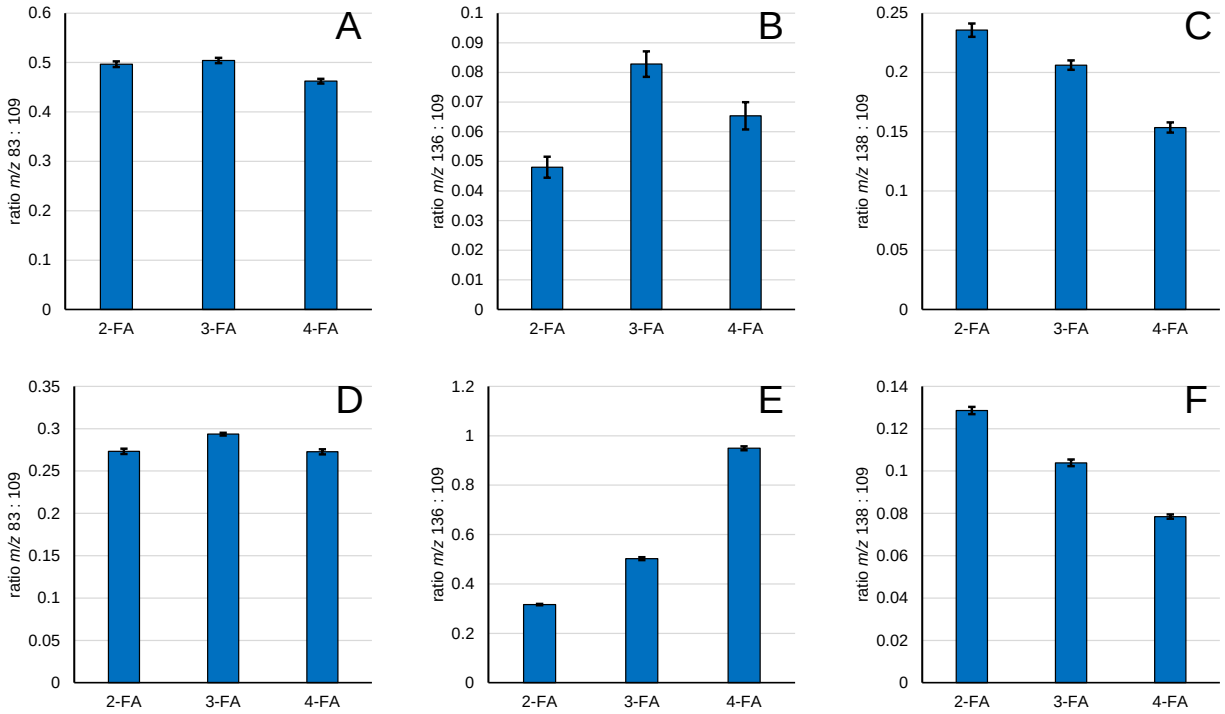

Fig. S13. The *m/z* 83:109*; m/z* 136:109 and *m/z* 138:109 ion abundance ratios in 10 mass spectra of 2-FA, 3-FA and 4-FA (A,B,C) and 2-FA-propionyl, 3-FA-propionyl and 4-FA-propionyl (D,E,F)

origin			PCA-LDA predictions				
type	year	GC result	2-MMC	3-MMC	4-MMC	Class	log LR
case 01	2019	2-MMC	-17.331	-692.004	-450.596	2-MMC	188
case 02	2016	3-MMC	-479.636	-13.6031	-377.025	3-MMC	158
case 03	2016	3-MMC	-558.745	-12.9298	-427.724	3-MMC	180
case 04	2016	3-MMC	-448.597	-14.5723	-352.802	3-MMC	147
case 05	2018	3-MMC	-620.283	-22.8841	-290.16	3-MMC	116
case 06	2018	3-MMC	-616.517	-19.3789	-298.1	3-MMC	121
case 07	2019	3-MMC	-588.548	-14.6249	-294.107	3-MMC	121
case 08	2019	3-MMC	-533.701	-4.37247	-313.437	3-MMC	134
case 09	2019	3-MMC	-322.335	-41.4287	-178.712	3-MMC	60
case 10	2019	3-MMC	-721.309	-17.9722	-441.787	3-MMC	184
case 11	2019	3-MMC	-680.36	-11.7294	-440.842	3-MMC	186
case 12	2019	3-MMC	-502.438	-1.72113	-329.796	3-MMC	142
case 13	2019	3-MMC	-545.682	-3.23789	-342.644	3-MMC	147
case 14	2019	3-MMC	-469.402	-9.99397	-389.923	3-MMC	165
case 15	2019	3-MMC	-456.073	-10.3581	-381.522	3-MMC	161
case 16	2019	3-MMC	-421.652	-14.879	-368.745	3-MMC	154
case 17	2019	3-MMC	-456.426	-9.23369	-367.931	3-MMC	156
case 18	2019	3-MMC	-411.707	-12.8774	-348.522	3-MMC	146
case 19	2019	3-MMC	-436.439	-8.85041	-352.426	3-MMC	149
case 20	2019	3-MMC	-439.018	-6.65233	-330.891	3-MMC	141
case 21	2019	3-MMC	-399.02	-11.7446	-311.173	3-MMC	130
case 22	2019	3-MMC	-419.339	-9.1713	-326.45	3-MMC	138
case 23	2019	3-MMC	-408.711	-9.92522	-314.457	3-MMC	132
case 24	2019	3-MMC	-391.876	-12.099	-291.615	3-MMC	121
case 25	2019	3-MMC	-599.562	-4.96735	-367.106	3-MMC	157
case 26	2019	3-MMC	-610.402	-5.15502	-386.681	3-MMC	166
case 27	2019	3-MMC	-626.873	-7.09721	-380.154	3-MMC	162
case 28	2019	3-MMC	-530.014	-2.14557	-381.953	3-MMC	165
case 29	2020	3-MMC	-520.677	-4.75523	-299.236	3-MMC	128
case 30	2020	3-MMC	-519.572	-4.01739	-302.681	3-MMC	130
case 31	2020	3-MMC	-520.702	-2.667	-315.418	3-MMC	136
case 32	2020	3-MMC	-527.294	-4.0857	-306.174	3-MMC	131
case 33	2020	3-MMC	-528.752	-5.37047	-297.947	3-MMC	127

Table S1. part 1 of 4

origin			PCA-LDA predictions				
type	year	GC result	2-MMC	3-MMC	4-MMC	Class	log LR
case 34	2020	3-MMC	-555.998	-3.23177	-331.987	3-MMC	143
case 35	2020	3-MMC	-565.918	-2.22694	-355.518	3-MMC	153
case 36	2020	3-MMC	-601.345	-4.41661	-368.172	3-MMC	158
case 37	2020	3-MMC	-536.625	-1.29697	-347.368	3-MMC	150
case 38	2015	4-MMC	-527.391	-521.571	-37.4639	4-MMC	210
case 39	2015	4-MMC	-456.806	-480.586	-21.0691	4-MMC	189
case 40	2015	4-MMC	-420.329	-516.493	-20.7987	4-MMC	174
case 41	2015	4-MMC	-425.662	-424.793	-12.7569	4-MMC	179
case 42	2015	4-MMC	-436.448	-426.39	-14.3231	4-MMC	179
case 43	2016	4-MMC	-453.057	-389.395	-16.879	4-MMC	162
case 44	2016	4-MMC	-478.416	-426.13	-22.1969	4-MMC	175
case 45	2016	4-MMC	-427.18	-361.848	-12.8391	4-MMC	152
case 46	2016	4-MMC	-347.094	-426.055	-8.62657	4-MMC	147
case 47	2016	4-MMC	-354.215	-419.2	-8.53493	4-MMC	150
case 48	2018	4-MMC	-292.302	-482.232	-13.0846	4-MMC	121
case 49	2018	4-MMC	-471.555	-436.825	-22.3081	4-MMC	180
case 50	2019	4-MMC	-324.151	-408.25	-4.6619	4-MMC	139
QC 01	2020	2-MMC	-1.35445	-547.484	-316.85	2-MMC	137
QC 02	2020	2-MMC	-3.92833	-590.262	-361.143	2-MMC	155
QC 03	2015	3-MMC	-581.8	-16.3451	-282.603	3-MMC	116
QC 04	2015	3-MMC	-510.886	-11.8686	-257.903	3-MMC	107
QC 05	2015	3-MMC	-627.838	-14.6736	-326.546	3-MMC	135
QC 06	2015	3-MMC	-626.305	-15.3221	-322.405	3-MMC	133
QC 07	2016	3-MMC	-686.402	-20.2787	-363.478	3-MMC	149
QC 08	2016	3-MMC	-683.881	-16.7946	-379.433	3-MMC	157
QC 09	2016	3-MMC	-600.718	-7.52255	-384.347	3-MMC	164
QC 10	2016	3-MMC	-517.68	-12.5673	-399.712	3-MMC	168
QC 11	2016	3-MMC	-551.504	-13.6087	-430.1	3-MMC	181
QC 12	2017	3-MMC	-620.206	-10.8559	-337.306	3-MMC	142
QC 13	2017	3-MMC	-584.991	-11.2591	-304.74	3-MMC	127
QC 14	2017	3-MMC	-558.214	-19.5144	-257.926	3-MMC	104
QC 15	2018	3-MMC	-563.584	-3.07842	-341.718	3-MMC	147
QC 16	2018	3-MMC	-382.238	-16.1864	-240.198	3-MMC	97

Table S1. part 2 of 4

origin			PCA-LDA predictions				
type	year	GC result	2-MMC	3-MMC	4-MMC	Class	log LR
QC 17	2018	3-MMC	-687.732	-17.1728	-389.348	3-MMC	162
QC 18	2019	3-MMC	-723.669	-18.2608	-443.773	3-MMC	185
QC 19	2019	3-MMC	-720.077	-16.9719	-459.59	3-MMC	192
QC 20	2019	3-MMC	-476.906	-2.99513	-346.118	3-MMC	149
QC 21	2019	3-MMC	-520.996	-2.73743	-339.575	3-MMC	146
QC 22	2019	3-MMC	-509.067	-1.8205	-347.217	3-MMC	150
QC 23	2019	3-MMC	-448.196	-15.503	-402.389	3-MMC	168
QC 24	2019	3-MMC	-431.849	-17.5191	-395.993	3-MMC	164
QC 25	2019	3-MMC	-447.942	-18.7132	-415.49	3-MMC	172
QC 26	2019	3-MMC	-458.691	-9.81574	-378.589	3-MMC	160
QC 27	2019	3-MMC	-416.809	-13.6875	-361.403	3-MMC	151
QC 28	2019	3-MMC	-407.481	-10.8568	-327.378	3-MMC	137
QC 29	2019	3-MMC	-429.505	-8.77708	-344.252	3-MMC	146
QC 30	2019	3-MMC	-436.79	-6.55321	-333.443	3-MMC	142
QC 31	2019	3-MMC	-449.856	-5.05703	-336.283	3-MMC	144
QC 32	2019	3-MMC	-451.472	-5.42071	-343.507	3-MMC	147
QC 33	2019	3-MMC	-473.947	-4.00929	-355.79	3-MMC	153
QC 34	2019	3-MMC	-609.498	-5.44919	-380.555	3-MMC	163
QC 35	2019	3-MMC	-528.651	-1.30361	-364.432	3-MMC	158
QC 36	2019	3-MMC	-544.498	-1.33449	-355.218	3-MMC	154
QC 37	2020	3-MMC	-568.923	-2.10998	-362.119	3-MMC	156
QC 38	2020	3-MMC	-539.456	-1.43641	-344.184	3-MMC	149
QC 39	2020	3-MMC	-500.774	-1.49967	-344.31	3-MMC	149
QC 40	2020	3-MMC	-531.038	-1.20815	-344.926	3-MMC	149
QC 41	2020	3-MMC	-543.541	-1.23489	-358.572	3-MMC	155
QC 42	2020	3-MMC	-534.525	-1.15211	-352.768	3-MMC	153
QC 43	2020	3-MMC	-496.495	-2.57882	-363.121	3-MMC	157
QC 44	2015	4-MMC	-395.262	-378.994	-7.59182	4-MMC	161
QC 45	2015	4-MMC	-307.761	-324.058	-1.96814	4-MMC	133
QC 46	2015	4-MMC	-376.029	-388.846	-5.44525	4-MMC	161
QC 47	2015	4-MMC	-362.337	-409.333	-5.11542	4-MMC	155
QC 48	2016	4-MMC	-339.446	-355.984	-2.37797	4-MMC	146
QC 49	2016	4-MMC	-369.99	-334.309	-5.57395	4-MMC	143

Table S1. part 3 of 4

origin			PCA-LDA predictions				
type	year	GC result	2-MMC	3-MMC	4-MMC	Class	log LR
QC 50	2016	4-MMC	-312.335	-410.834	-6.66189	4-MMC	133
QC 51	2016	4-MMC	-235.965	-425.412	-17.8905	4-MMC	95
QC 52	2016	4-MMC	-220.401	-468.492	-26.8766	4-MMC	84
QC 53	2017	4-MMC	-362.372	-324.986	-5.38304	4-MMC	139
QC 54	2017	4-MMC	-376.701	-357.735	-5.65347	4-MMC	153
QC 55	2017	4-MMC	-402.434	-475.471	-14.0466	4-MMC	169
QC 56	2018	4-MMC	-341.526	-369.504	-2.37248	4-MMC	147
QC 57	2018	4-MMC	-253.204	-310.457	-4.15665	4-MMC	108
QC 58	2018	4-MMC	-386.207	-390.308	-7.29801	4-MMC	165
QC 59	2019	4-MMC	-377.028	-297.207	-10.1568	4-MMC	125
QC 60	2019	4-MMC	-377.771	-281.047	-12.1103	4-MMC	117
QC 61	2019	4-MMC	-239.721	-356.178	-4.89018	4-MMC	102
QC 62	2019	4-MMC	-308.888	-388.721	-3.09524	4-MMC	133
QC 63	2019	4-MMC	-235.934	-368.51	-6.13754	4-MMC	100
QC 64	2019	4-MMC	-166.496	-309.221	-22.3322	4-MMC	63
QC 65	2019	4-MMC	-156.289	-322.77	-25.3911	4-MMC	57
QC 66	2019	4-MMC	-157.937	-293.319	-27.087	4-MMC	57
QC 67	2019	4-MMC	-174.52	-381.312	-20.981	4-MMC	67
QC 68	2019	4-MMC	-215.134	-312.496	-9.14819	4-MMC	89
QC 69	2019	4-MMC	-207.562	-391.796	-12.3269	4-MMC	85
QC 70	2019	4-MMC	-199.243	-380.25	-13.3139	4-MMC	81
QC 71	2019	4-MMC	-220.695	-323.592	-7.75964	4-MMC	92
QC 72	2019	4-MMC	-265.126	-355.31	-2.40372	4-MMC	114
QC 73	2019	4-MMC	-227.615	-367.552	-6.99704	4-MMC	96
QC 74	2019	4-MMC	-223.311	-365.351	-7.63332	4-MMC	94
QC 75	2019	4-MMC	-377.561	-388.936	-6.46447	4-MMC	161
QC 76	2019	4-MMC	-233.085	-326.347	-5.76156	4-MMC	99
QC 77	2019	4-MMC	-243.718	-334.332	-4.29318	4-MMC	104
QC 78	2020	4-MMC	-260.198	-308.169	-3.75999	4-MMC	111
QC 79	2020	4-MMC	-234.141	-343.641	-5.47774	4-MMC	99
QC 80	2020	4-MMC	-256.121	-329.172	-3.21389	4-MMC	110
QC 81	2020	4-MMC	-270.179	-336.458	-2.1053	4-MMC	116
QC 82	2020	4-MMC	-285.494	-312.891	-2.36187	4-MMC	123

Table S1. Part 4 of 4. PCA-LDA results of case samples and QC samples retrospectively classified on a PCA-LDA model of 261 MMC-propionyl mass spectra analyzed in 2020. PCA-LDA prediction scores are natural logarithms of the posterior probabilities. Log LRs are calculated towards the closest other isomer than the predicted one.

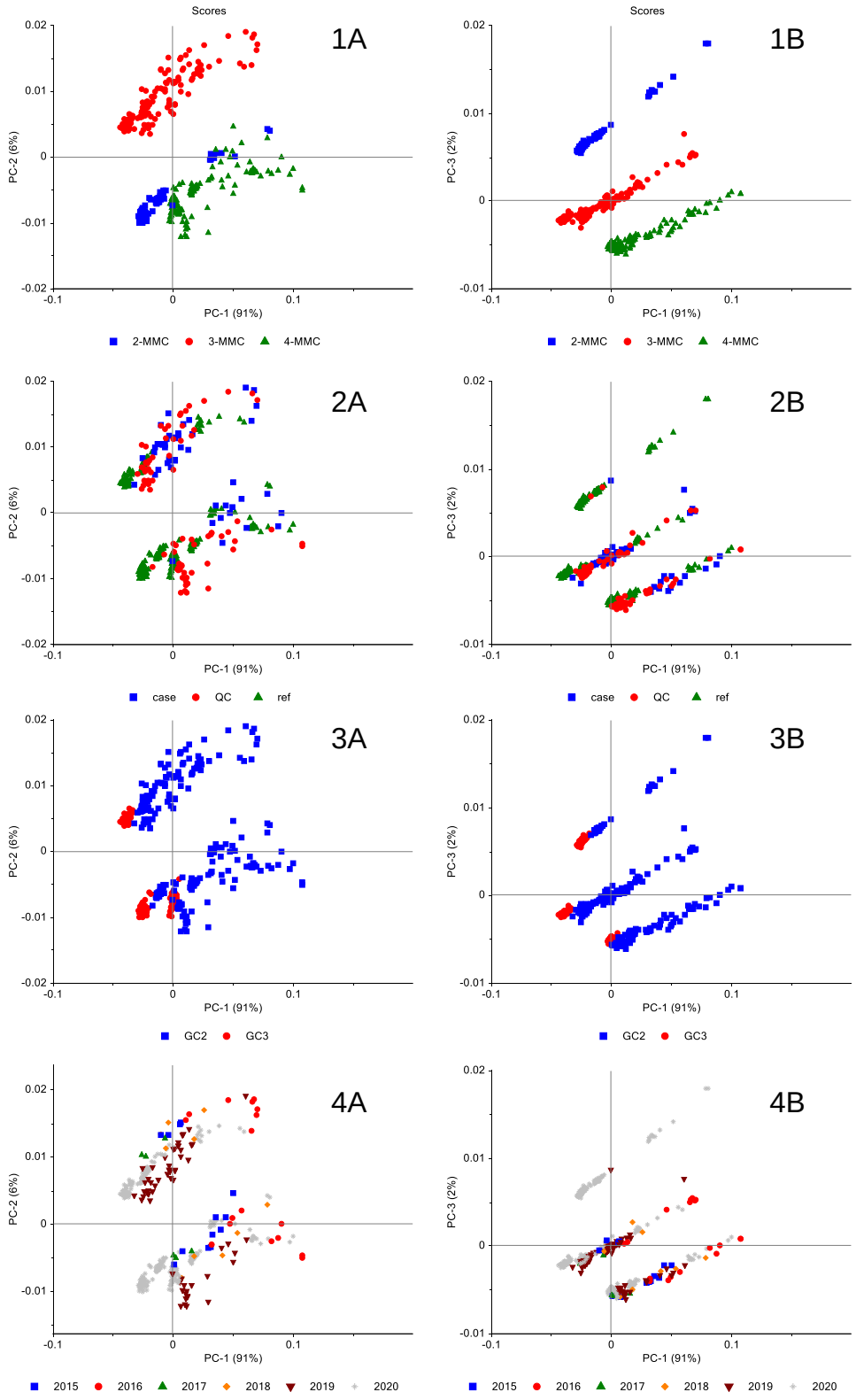

Fig. S14. PCA-plots showing PC1 *vs.* PC2 (A) and PC1 *vs.* PC3 (B) following *m/z* 100 – 240 data selection and area normalization. Data grouped by known identity (1), sample type (2), instrument used (3) and year of analysis (4)

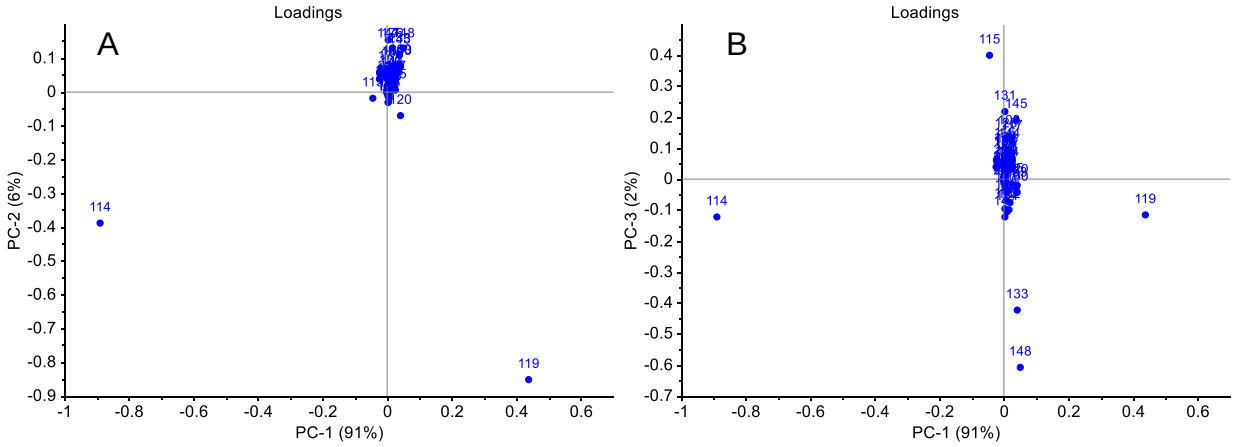

Fig. S15. PCA loading plots showing PC1 *vs.* PC2 (A) and PC1 *vs*. PC3 (B) following *m/z* 100 – 240 data selection and area normalization.