Supporting Information

In vitro metabolism of the synthetic cannabinoid 3,5-AB-CHMFUPPYCA and its 5,3-regioisomer and investigation of their thermal stability

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Abbreviations:

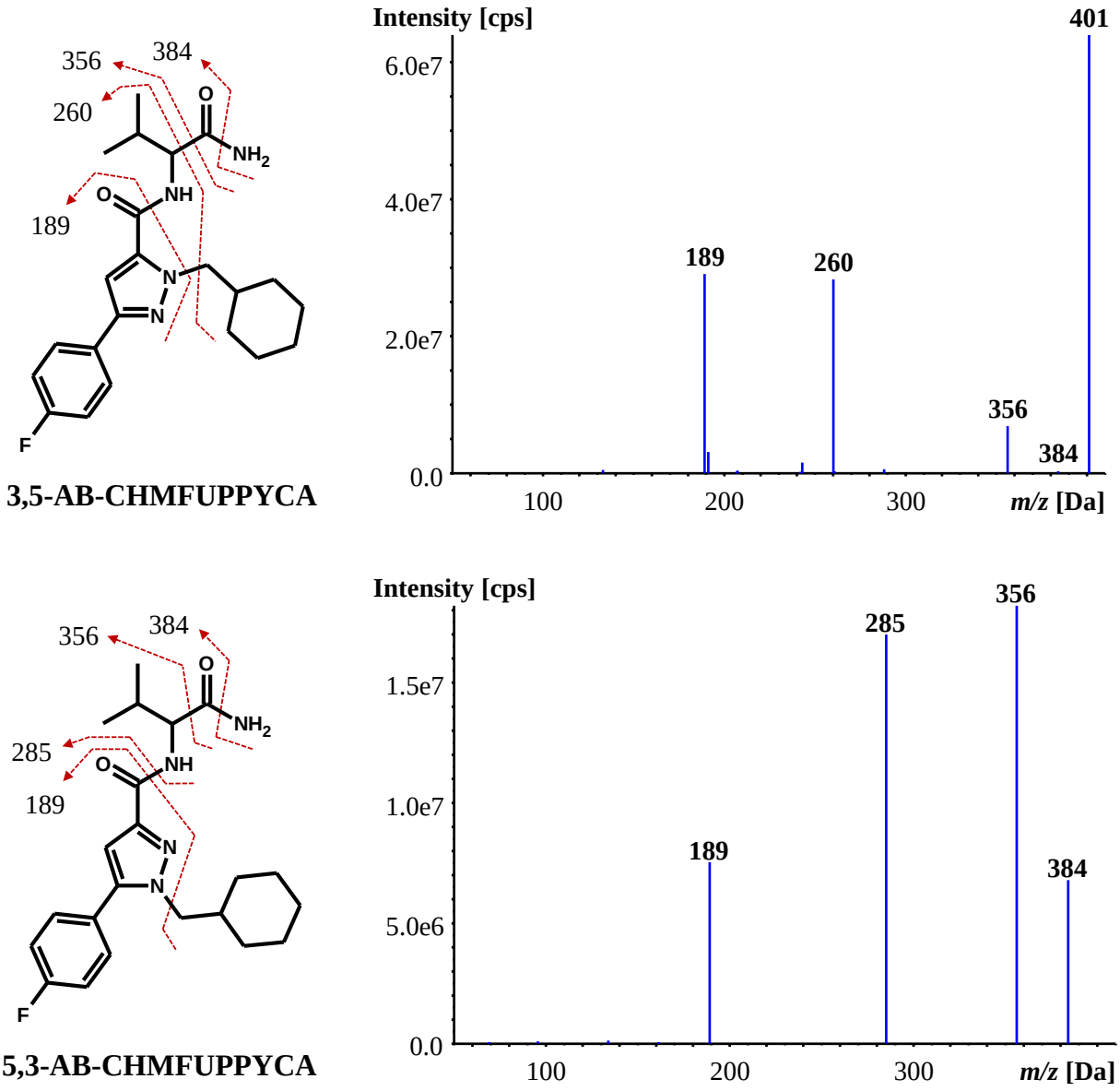
Q1	*m/z* of the precursor ion
Q3	*m/z* of the fragment ion
DP	Declustering potential
EP	Entrance potential
CE	Collision energy
CXP	Cell exit potential

Supplemental Table S1. Specific mass transitions of the identified metabolites included in the MRM method used for the analysis of the metabolite spectrum of 3,5-AB-CHMFUPPYCA.

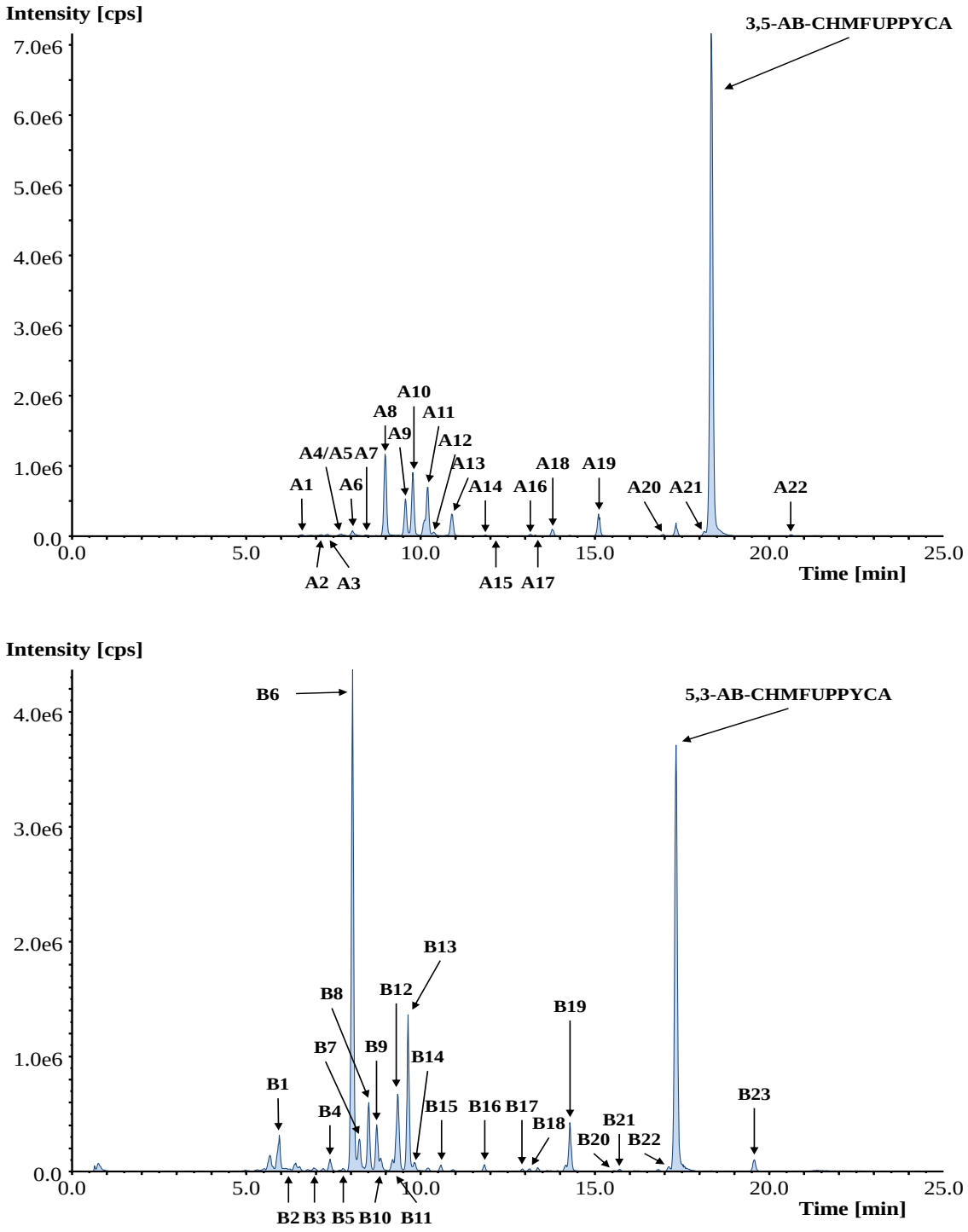
Q1 [amu]	Q3 [amu]	Dwell time [msec]	ID	DP [V]	EP [V]	CE [V]	CXP [V]
401	356	10	3,5-AB-CHMFUPPYCA 1	65	6	25	13
401	260	10	3,5-AB-CHMFUPPYCA 2	65	6	33	14
401	189	10	3,5-AB-CHMFUPPYCA 3	65	6	52	24
402	356	10	primary amid hydrolysis 1	65	6	25	13
402	260	10	primary amid hydrolysis 2	65	6	33	14
402	189	10	primary amid hydrolysis 3	65	6	52	24
303	285	10	secondary amide hydrolysis 1	60	10	38	10
303	189	10	secondary amide hydrolysis 2	60	10	54	10
399	354	10	dehydrogenation 1	65	6	25	13
399	260	10	dehydrogenation 2	65	6	33	14
399	189	10	dehydrogenation 3	65	6	52	24
399	283	10	dehydrogenation 4	65	6	33	14
305	260	10	dealkylation 1	65	6	33	14
305	189	10	dealkylation 2	65	6	52	24
417	356	10	hydroxylation 1	65	6	25	13
417	260	10	hydroxylation 2	65	6	33	14
417	189	10	hydroxylation 3	65	6	52	24
417	372	10	hydroxylation 4	65	6	25	13
417	276	10	hydroxylation 5	65	6	33	14
433	356	10	dihydroxylation 1	65	6	25	13
433	260	10	dihydroxylation 2	65	6	33	14
433	189	10	dihydroxylation 3	65	6	52	24
433	372	10	dihydroxylation 4	65	6	25	13
433	276	10	dihydroxylation 5	65	6	33	14
433	388	10	dihydroxylation 6	65	6	25	13
433	292	10	dihydroxylation 7	65	6	33	14
418	356	10	hydrolysis + hydroxylation 1	65	6	25	13
418	260	10	hydrolysis + hydroxylation 2	65	6	33	14
418	189	10	hydrolysis + hydroxylation 3	65	6	52	24
418	372	10	hydrolysis + hydroxylation 4	65	6	25	13
418	276	10	hydrolysis + hydroxylation 5	65	6	33	14
415	356	10	ketone 1	65	6	25	13
415	260	10	ketone 2	65	6	33	14
415	189	10	ketone 3	65	6	52	24
415	370	10	ketone 4	65	6	25	13
415	274	10	ketone 5	65	6	33	14
431	356	10	ketone + hydroxylation 1	65	6	25	13
431	260	10	ketone + hydroxylation 2	65	6	33	14
431	189	10	ketone + hydroxylation 3	65	6	52	24
431	370	10	ketone + hydroxylation 4	65	6	25	13
431	274	10	ketone + hydroxylation 5	65	6	33	14
431	372	10	ketone + hydroxylation 6	65	6	25	13
431	276	10	ketone + hydroxylation 7	65	6	33	14
431	386	10	ketone + hydroxylation 8	65	6	25	13
431	290	10	ketone + hydroxylation 9	65	6	33	14

Supplemental Table S2. Specific mass transitions of the identified metabolites included in the MRM method used for the analysis of the metabolite spectrum of 5,3-AB-CHMFUPPYCA.

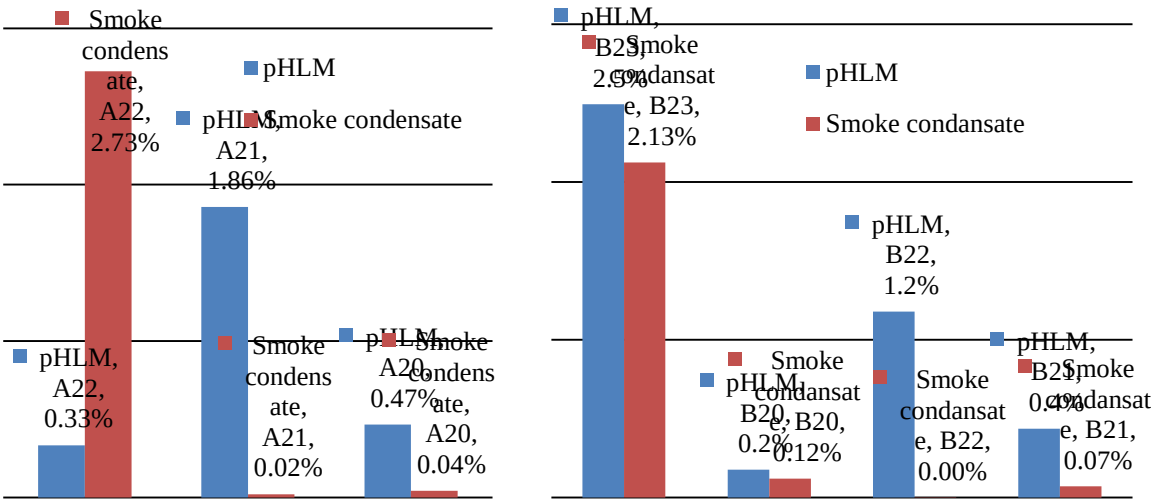
Q1 [amu]	Q3 [amu]	Dwell time [msec]	ID	DP [V]	EP [V]	CE [V]	CXP [V]
401	356	10	5,3-AB-CHMFUPPYCA 1	60	10	23	13
401	285	10	5,3-AB-CHMFUPPYCA 2	60	10	38	10
401	189	10	5,3-AB-CHMFUPPYCA 3	60	10	54	10
402	356	10	primary amid hydrolysis 1	60	10	23	13
402	285	10	primary amid hydrolysis 2	60	10	38	10
402	189	10	primary amid hydrolysis 3	60	10	54	10
303	285	10	secondary amide hydrolysis 1	60	10	38	10
303	189	10	secondary amide hydrolysis 2	60	10	54	10
399	354	10	dehydrogenation 1	60	10	23	13
399	285	10	dehydrogenation 2	60	10	38	10
399	189	10	dehydrogenation 3	60	10	54	10
399	283	10	dehydrogenation 4	60	10	38	10
305	260	10	dealkylation 1	65	6	33	14
305	189	10	dealkylation 2	65	6	52	24
417	356	10	hydroxylation 1	60	10	23	13
417	285	10	hydroxylation 2	60	10	38	10
417	189	10	hydroxylation 3	60	10	54	10
417	372	10	hydroxylation 4	60	10	23	13
417	301	10	hydroxylation 5	60	10	38	10
433	356	10	dihydroxylation 1	60	10	23	13
433	285	10	dihydroxylation 2	60	10	38	10
433	189	10	dihydroxylation 3	60	10	54	10
433	372	10	dihydroxylation 4	60	10	23	13
433	301	10	dihydroxylation 5	60	10	38	10
433	388	10	dihydroxylation 6	60	10	23	13
433	317	10	dihydroxylation 7	60	10	38	10
418	356	10	hydrolysis + hydroxylation 1	60	10	23	13
418	285	10	hydrolysis + hydroxylation 2	60	10	38	10
418	189	10	hydrolysis + hydroxylation 3	60	10	54	10
418	372	10	hydrolysis + hydroxylation 4	60	10	23	13
418	301	10	hydrolysis + hydroxylation 5	60	10	38	10
415	356	10	ketone 1	60	10	23	13
415	285	10	ketone 2	60	10	38	10
415	189	10	ketone 3	60	10	54	10
415	370	10	ketone 4	60	10	23	13
415	299	10	ketone 5	60	10	38	10
431	356	10	ketone + hydroxylation 1	60	10	23	13
431	285	10	ketone + hydroxylation 2	60	10	38	10
431	189	10	ketone + hydroxylation 3	60	10	54	10
431	370	10	ketone + hydroxylation 4	60	10	23	13
431	299	10	ketone + hydroxylation 5	60	10	38	10
431	372	10	ketone + hydroxylation 6	60	10	23	13
431	301	10	ketone + hydroxylation 7	60	10	38	10
431	386	10	ketone + hydroxylation 8	60	10	23	13
431	315	10	ketone + hydroxylation 9	60	10	38	10



Supplemental Figure S1. Enhanced product ion scans of 3,5-AB-CHMFUPPYCA (top) and 5,3-AB-CHMFUPPYCA (bottom) recorded using a collision energy of 35 V with a spread of 15 V and proposed fragmentation. The main fragment ions *m/z* 260 and *m/z* 285 are suitable for a differentiation of the two isomers.



Supplemental Figure S2. Comparison of the metabolic profiles of 3,5-AB-CHMFUPPYCA (top) and 5,3-AB-CHMFUPPYCA (bottom) after recording a multiple reaction monitoring scan using the most abundant ion transitions for each identified metabolite (see Supplemental Table S1 and S2 for a list of the metabolites and the respective ion transitions). pHLM assays were performed with the same starting concentration for both substrates.



Supplemental Figure S3. Investigation of the thermal stability of 3,5-AB-CHMFUPPYCA (left) and 5,3-AB-CHMFUPPYCA (right). For the 3,5-isomer the metabolites A20, A21 and A22 were also formed artificially by amide cleavage and dehydrogenation under smoking conditions. For 5,3-AB-CHMFUPPYCA, the metabolites B20, B21, B22 and B23 were identified in the smoke condensate. The area ratios (degradation product/parent compound) are shown in comparison of the pHLM sample for each isomer.