Hydrolytic stability of 32 synthetic cannabinoids with valine- and tert-leucine methyl ester or amide as linked groups in blood serum and cardiac blood samples

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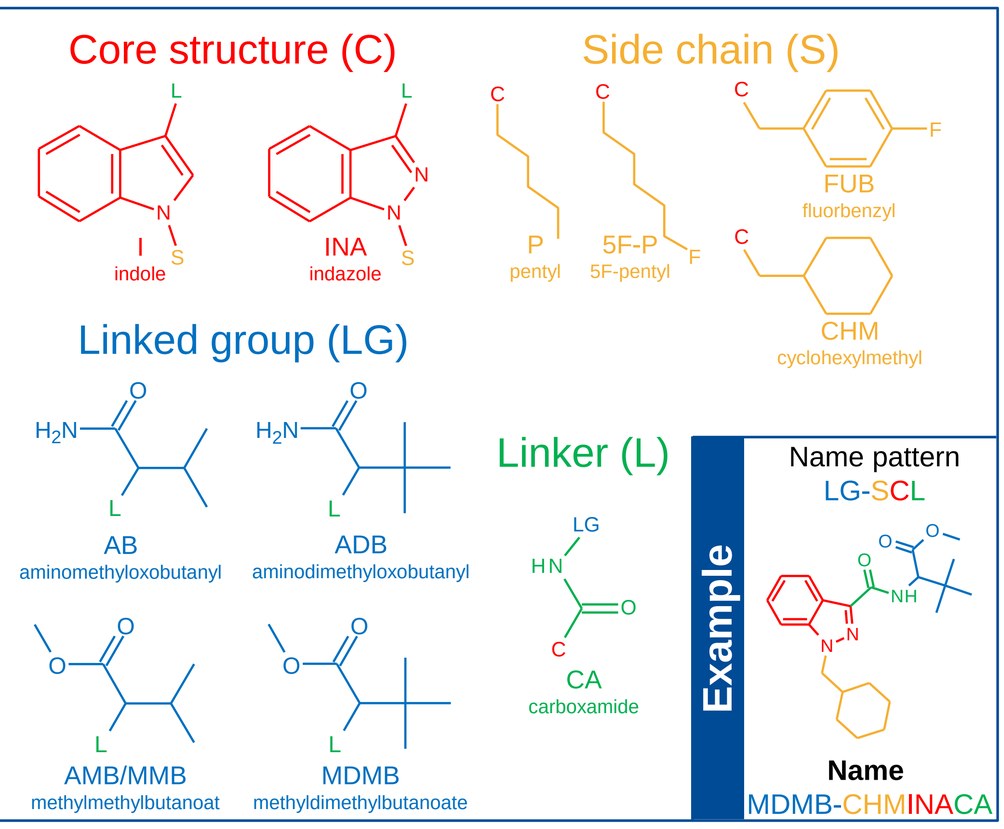
Background

Although the number of newly appeared synthetic cannabinoid receptor agonists (SCRAs) decreased in the last three years, SCRAs remain to be the largest group of „new psycho-active substances“ (NPS) monitored by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). Especially the number of synthetic cannabinoid related emergencies and death cases increased significantly in the last four years in the EU [1]. Besides the classical SCRAs synthesized by John W. Huffman (JWH-series) and Alexandros Makriyannis (AM-series) another generation of SCRAs with valine- and tert-leucine methyl ester or amide as linked groups occurred. These contain an indole or indazole core structure and one of the four side chain elements depicted to the right (Nomenclature).
When analyzing blood samples for SCRAs, the most common targets are the parent compounds. In case of the mentioned generation of SCRAs the detection of hydrolysis products could also provide a proof of consumption. The aim of this study was to determine the extent of formation of hydrolysis products of 32 different synthetic cannabinoids carrying amide or methyl ester functions during storage of spiked samples under different storage conditions.

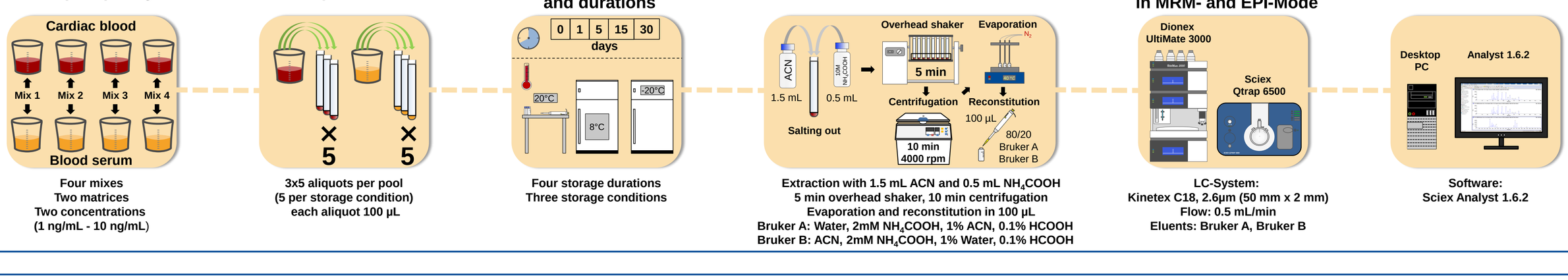
Nomenclature



Mixes

Mix 1		Mix 2		Mix 3		Mix 4	
AB-CHMICA	AMB-CHMINACA	AMB-CHMICA	AB-CHMINACA	ADB-CHMICA	MDMB-CHMINACA	MDMB-CHMICA	ADB-CHMINACA
AB-FUBICA	AMB-FUBINACA	AMB-FUBICA	AB-FUBINACA	ADB-FUBICA	MDMB-FUBINACA	MDMB-FUBICA	ADB-FUBINACA
AB-PICA	AMB-PINACA	AMB-PICA	AB-PINACA	ADB-PICA	MDMB-PINACA	MDMB-PICA	ADB-PINACA
5F-AB-PICA	5F-AMB-PINACA	5F-AMB-PICA	5F-AB-PINACA	5F-ADB-PICA	5F-MDMB-PINACA	5F-MDMB-PICA	5F-ADB-PINACA

Methods



Results & Discussion

Figure 1 shows the theoretically possible hydrolytic cleavage reactions regarding the compounds analyzed during the study. As shown with the green mark, the hydrolysis of the methyl esters, most likely caused by enzymatic cleavage, is the only hydrolytic reaction that takes place in both matrices. Hydrolysis of the carboxamide linker could not be observed at any time during the study by any storage condition.
Table 1 shows the detection of the parent compound (1 ng/mL) and of the hydrolysis product 1 during the study. The synthetic cannabinoids were subdivided into four different classes on the basis of their linked group. It is conspicuous that compounds with an amide as functional group were not affected of hydrolysis, neither in blood serum nor in cardiac blood, whereas, methyl esters in particular valine methyl esters were hydrolyzed. While in blood serum the parent compounds of the methyl esters were detectable throughout the entire study, for valine methyl esters this is not the case in cardiac blood. Already after 5 days at storage at room temperature, six of eight synthetic cannabinoids of the mentioned subclass could not be detected anymore, whereas their hydrolysis products could be detected for all of the eight. Roughly the same could be observed at 8°C in the refrigerator. Even at freezer conditions hydrolysis products of most of the valine methyl esters could be detected, while the corresponding parent compounds were still detectable, although the intensities of the detected signals were much lower than at the beginning of the study. The parent compounds of the tert-leucine methyl esters could be found throughout the entire study, but hydrolysis products were also mentioned in blood serum samples and in cardiac blood samples at room temperature as well as under refrigerator conditions in the case of cardiac blood. When compared to the isopropyl group the additional methyl group of the tert-butyl group led to a lower rate of hydrolysis. Most likely this fact could be explained by a higher steric hindrance induced by the additional methyl group. The absence of enzymes like amidases in blood and the ineffectiveness of esterases for amide hydrolysis could explain the amides were resistant against hydrolysis. Nevertheless, hydrolysis of the compounds could still occur in post mortem samples due to autolysis.

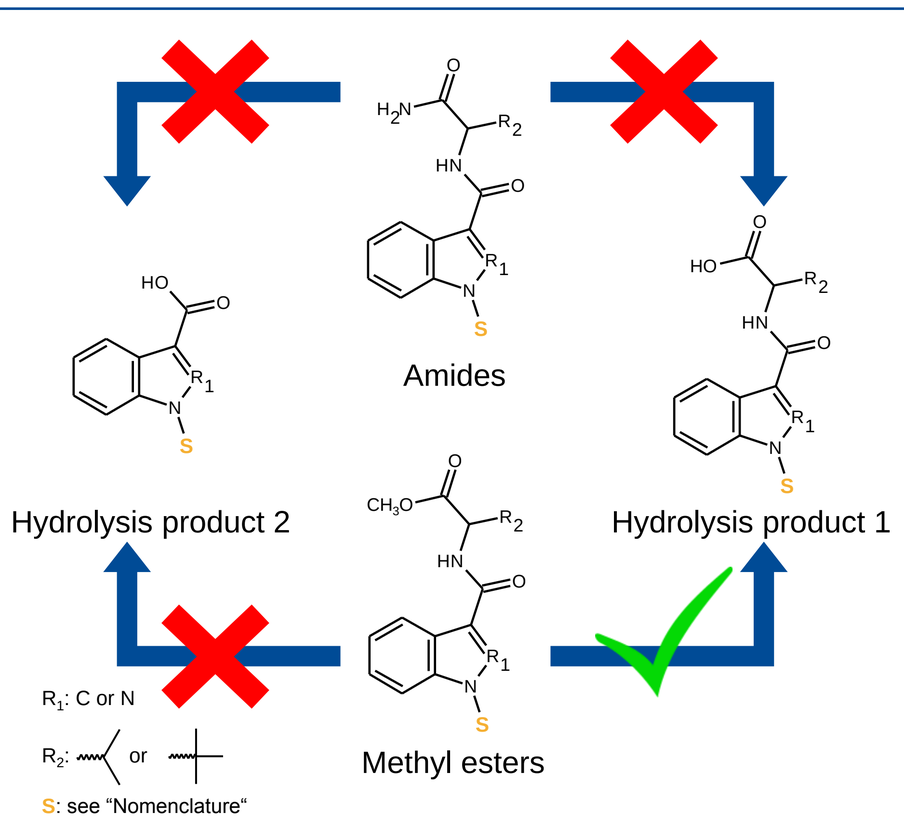


Figure 1: Overview of analyzed parent compounds and their hydrolytic stability

Table 1: Overview of the detected parent compounds (1 ng/mL) and their hydrolysis products in two different matrices by three storage conditions and during five different dates

Synthetic cannabinoid subclass	Blood serum																								Cardiac blood																							
	Room temperature (20 °C)										Refrigerator (8°C)								Freezer (-20°)								Room temperature (20 °C)										Refrigerator (8°C)								Freezer (-20°)					
	Parent compounds					Hydrolysis products 1					Parent compounds				Hydrolysis product 1				Parent compounds				Hydrolytic product 1				Parent compounds					Hydrolysis products 1					Parent compounds				Hydrolysis products 1				Parent compounds				Hydrolysis products 1			
	0	1	5	15	30	0	1	5	15	30	1	5	15	30	1	5	15	30	1	5	15	30	1	5	15	30	0	1	5	15	30	0	1	5	15	30	1	5	15	30	1	5	15	30	1	5	15	30	1	5	15	30
Valine amides																																																		
Valine methyl esters																																																		
Tert-leucine amides																																																	
Tert-leucine methyl esters																																																	

Parent compound/hydrolysis product was found in all samples
Parent compound/hydrolysis product was found in at least one sample
Parent compound/hydrolysis product was not found in any sample

Conclusion

The study investigated the hydrolytic instability for two subclasses of synthetic cannabinoids during storage at 20°C and 8°C. As might be expected, by storing at lower temperatures stability increases. Synthetic cannabinoids with amides as the linked group were resistant against hydrolysis, while the corresponding methyl esters are clearly more susceptible to hydrolytic cleavage. It is noticeable that hydrolysis in cardiac blood samples seems to be more pronounced than in serum samples. Due to the complete hydrolysis of the parent compound, these were partially not detectable anymore. To prevent false negative results, the monitoring of hydrolysis products can be helpful. On the basis of the results of the study the detection of the hydrolysis product should only occur after the uptake of methyl ester compounds, although hydrolysis of the amides would theoretically lead to the identical products. Hydrolysis of the carboxamide linker was not observed. The monitoring of the corresponding products does not seem to provide additional information.

Ref.

[1] European Drug Report
Trends and Developments 2018, EMCDDA
URL:
http://www.emcdda.europa.eu/publications/edr/trends-developments/2019

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