Analytical challenges in the forensic toxicological analysis of novel synthetic opioids from the class of ‘U-drugs’

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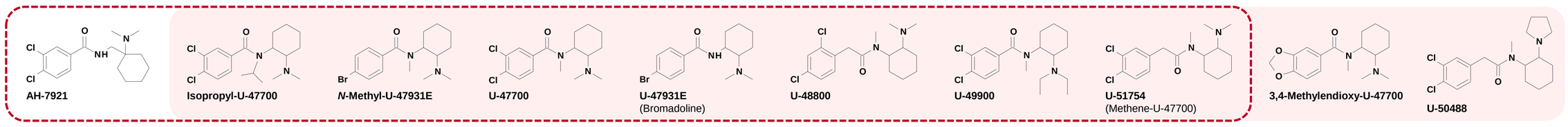
AIMS

In total, nine novel synthetic opioids from the class of so-called ‘U-drugs’ (developed by Upjohn), another class of new opioids besides fentanyl analogues, have been reported to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) until the end of 2018. The unambiguous identification of these compounds or their metabolites is often hampered by structural similarities (e.g. isomeric metabolites). The aim of this study was to investigate the phase I *in vitro* metabolism of eight compounds using pooled human liver microsomes (pHLM) and to identify isomeric and specific metabolites.

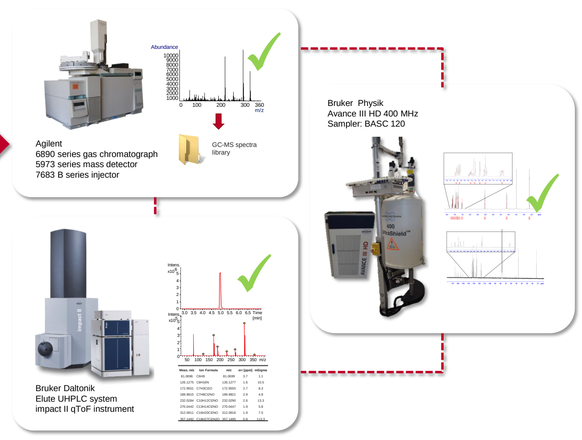
METHODS

Substance acquisition (online monitoring) and analyses (structure verification)

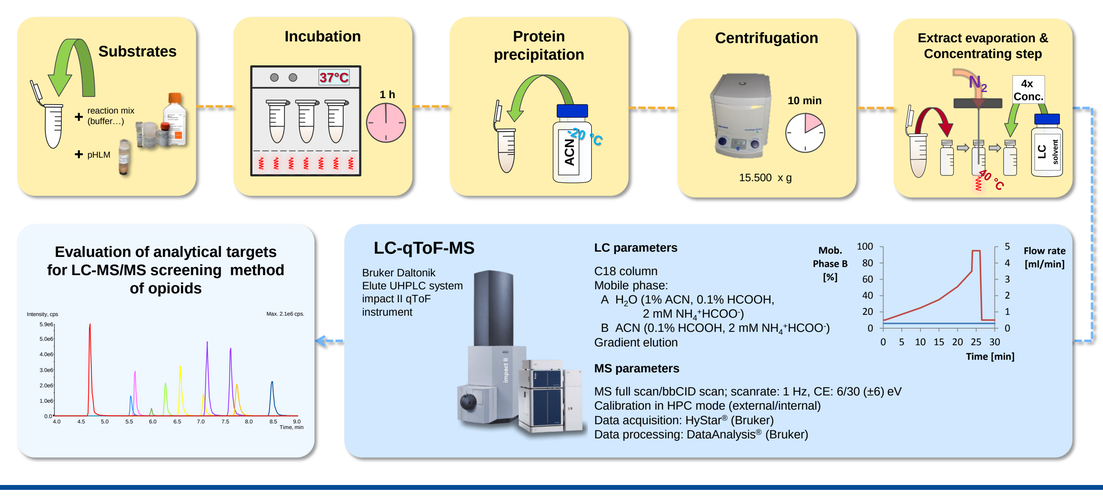


Compounds – used for pHLM assay to investigate (*in vitro*) phase I metabolites

Compounds – reported to the EMCDDA until the end of 2018



In vitro phase I metabolism studies using pooled human liver microsomes (pHLM)



RESULTS

Structural isomeric compounds (parent compounds)

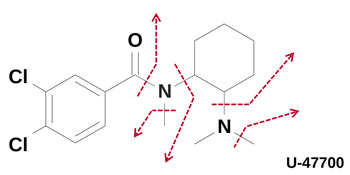


Fig. 1: Fragmentation reactions commonly observed for the class of ‘U-drugs’, examplified for U-47700.

Six of the investigated ‘U-drugs’ form pairs of constitutional isomers showing the same exact masses and partly forming identical fragment ions in HRMS-bbCID analysis.

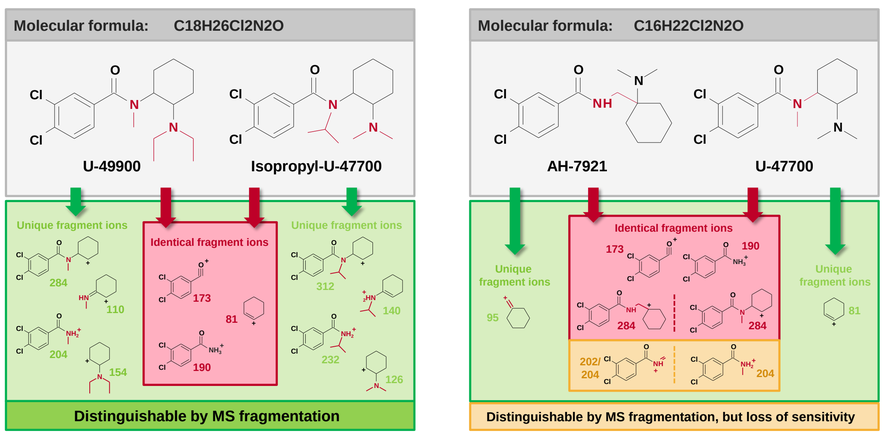


Fig. 2: Pairs of isomeric compounds. Unique fragment ions for each compound are shown within the green box; the fragment ions in the yellow box are unique by monoisotopic mass, but analytical interference with a heavier chlorine-isotopolgue occurs; fragment ions highlighted by the red boxes are identical by constitution and/or by exact mass.

While U-49900 and Isopropyl-U-47700 only showed few identical fragment ions, AH-7921 and U-47700 exhibited a very similar spectrum of fragment ions, and were distinguishable only by low abundant fragment ions. Furthermore some of the ‘U-drugs’ are regio-isomers, e.g. U-48800 and U-51754, differing in the substitution pattern of the two chlorine substituents (2,4- vs. 3,4-position), which are not distinguishable by the MS-fragment ions (Fig. 2).

In vitro phase I metabolites – complexity of analytical findings driven by ‘second level‘ isomerism

The compounds showed extensive metabolism in the pHLM assay. The main phase I metabolic reaction observed was *N*-dealkylation resulting in several isomeric metabolites (Fig. 3).

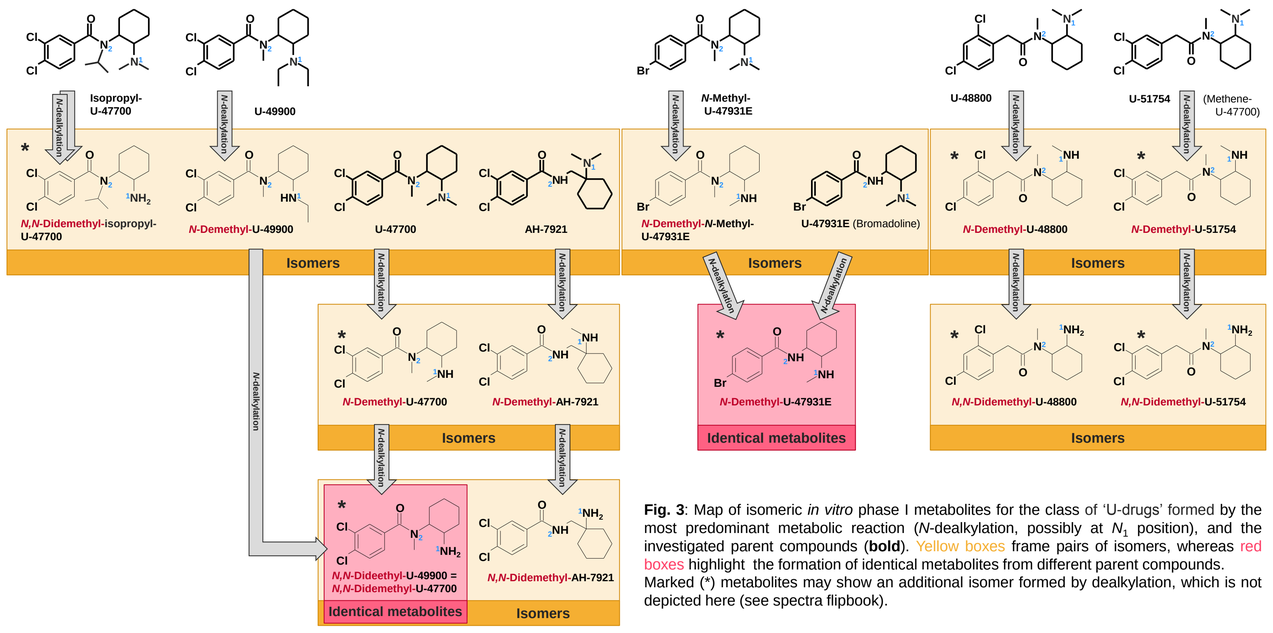


Fig. 3: Map of isomeric *in vitro* phase I metabolites for the class of ‘U-drugs’ formed by the most predominant metabolic reaction (*N*-dealkylation, possibly at N_1 position), and the investigated parent compounds (**bold**). Yellow boxes frame pairs of isomers, whereas red boxes highlight the formation of identical metabolites from different parent compounds. Marked (*) metabolites may show an additional isomer formed by dealkylation, which is not depicted here (see spectra flipbook).

More than a hundred *in vitro* phase I metabolites and potential degradation products were identified for the eight investigated compounds. Besides *N*-dealkylation, several hydroxylated metabolites either of the parent compound or the *N*-dealkylated metabolites were detected. Hydrolysis of the amide function seems to play a minor role for this class of opioids considering the *in vitro* data. Dehalogenation was detected only for the bromine-substituted analoguess U-47931E & *N*-methyl-U-47931E, though the debrominated metabolites showed relatively low abundances. Moreover, further oxidation products such as ketones, diketones, *N*-oxides or carboxylic acids – possibly formed by ring opening – were also detected for most of the parent compounds or their corresponding *N*-dealkylated metabolites.

e-poster version:

Please find spectra flipchart in print version or download from our homepage via QR-code

Flipbook: HRMS (fullscan/bbCID) spectra of the investigated parent compounds and their 5 most abundant metabolites (and suggestions of the structutal formulae) identified for each of the investigated compounds.

CONCLUSION

Sufficient chromatographic separation is essential for valid identification of ‘U-drugs’. Furthermore, the extensive metabolism of these compounds may cause interferences by isomers which need to be considered when interpreting analytical findings, especially when analysing biological samples.

Literature

[1] European database on new drugs (EDND) provided by the EMCDDA https://ednd.emcdda.europa.eu/html.cfm/index7246EN.html

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