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

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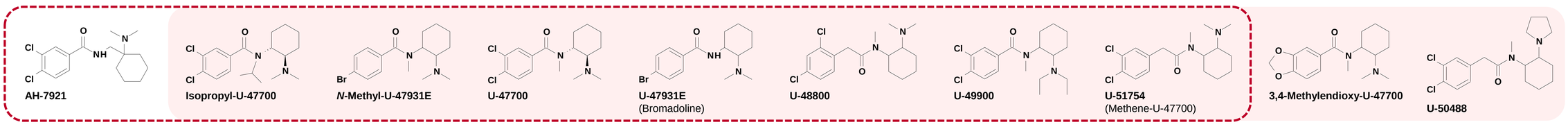
AIMS

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 Abuse (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

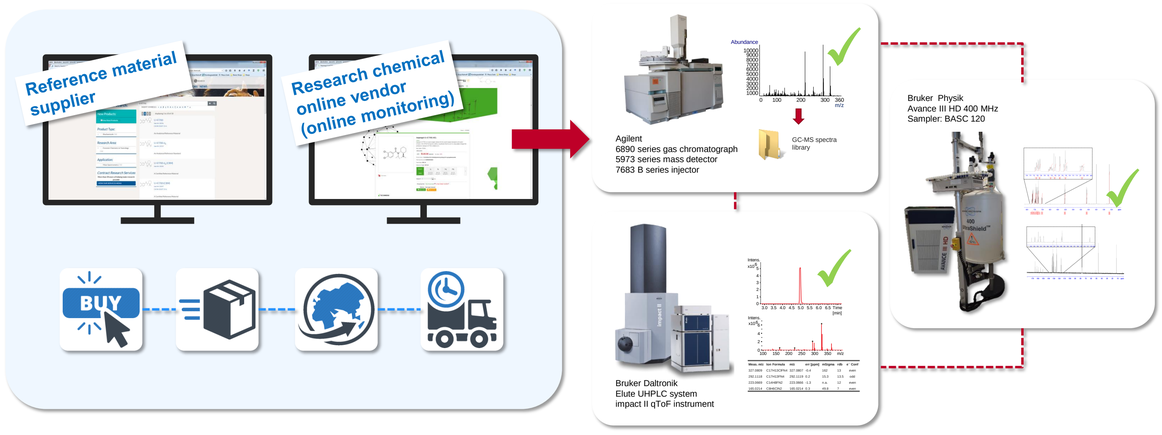
Substance acquisition (online monitoring) and analyses (structure verification)

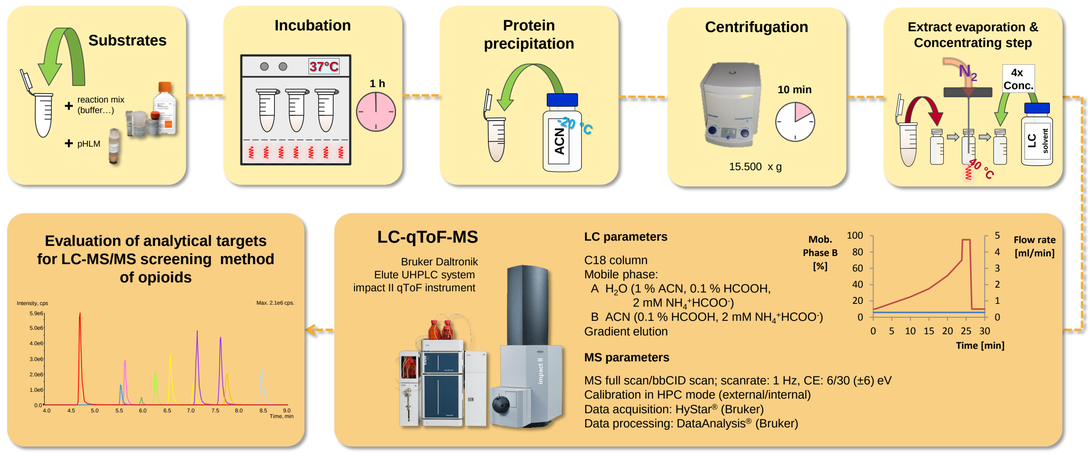
In vitro phase I metabolism studies using pooled human liver microsomes (pHLM)



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

Compounds – reported to the EMCDDA until the end of 2018





RESULTS

Structural isomeric (parent) compounds

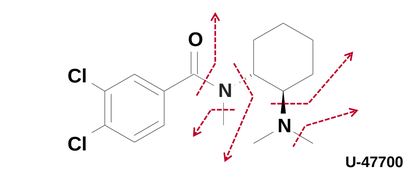


Fig. 1: Fragmentation reactions commonly seen for the class of “U-drugs”, shown for U-47700.

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.

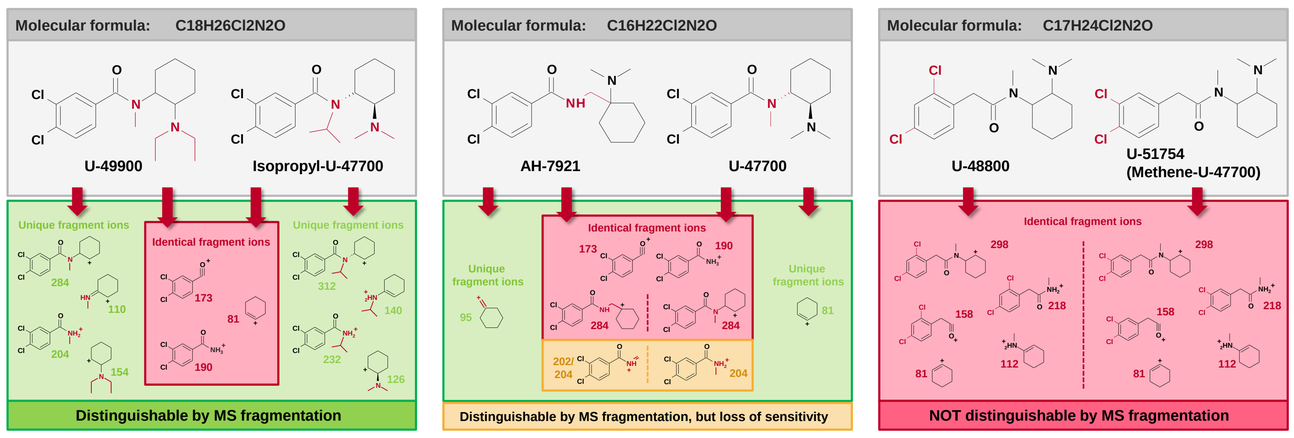


Fig. 2: Pairs of isomeric compounds. Unique fragment ions for each compound are shown within the green box; fragment ions surrounded by the yellow box are unique by monoisotopic mass, but interference with a heavier chlorine-isotopolgue occurs; fragment ions highlighted by the red boxes are identical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

For instance, 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-isomer, 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 by the next level of 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).

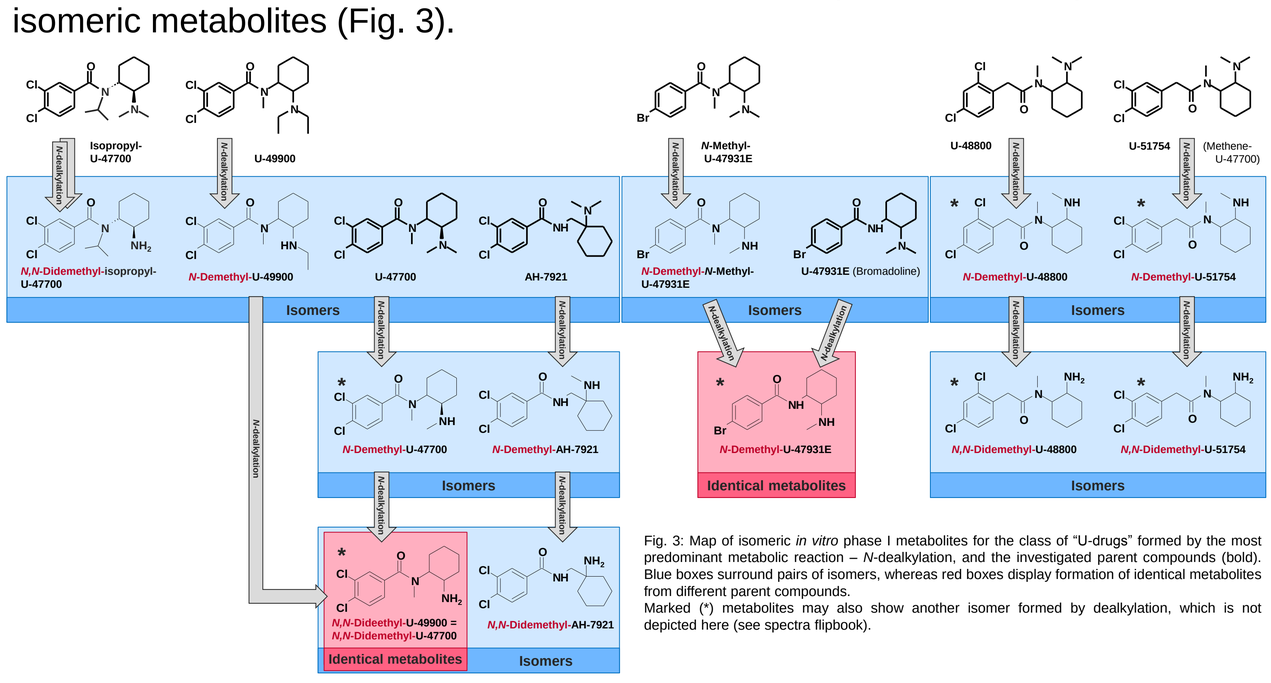


Fig. 3: Map of isomeric *in vitro* phase I metabolites for the class of “U-drugs” formed by the most predominant metabolic reaction – *N*-dealkylation, and the investigated parent compounds (bold). Blue boxes surround pairs of isomers, whereas red boxes display formation of identical metabolites from different parent compounds.
Marked (*) metabolites may also show another isomer formed by dealkylation, which is not depicted here (see spectra flipbook).

Up to over a hundred *in vitro* phase I metabolites and potential degradation products were identified for the 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 analogs U-47931E and *N*-methyl-U-47931E, the resulting metabolites showed relatively low abundances.
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 the corresponding *N*-dealkylated metabolites.

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

CONCLUSION

Sufficient chromatographic separation is essential for proper 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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