



Toxic lifespan of the synthetic opioid U-47,700 in Finland verified by re-analysis of UPLC-TOF-MS data

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ABSTRACT

U-47,700 is a synthetic opioid that emerged on the novel psychoactive substance market a few years ago. After incorporating the substance into the urine UPLC-TOF-MS screening used in post-mortem toxicology, the drug was detected in 10 autopsy cases within routine case work. In all cases, the cause of death was accidental poisoning by U-47,700 alone or in combination with other psychoactive substances. The concentration of U-47,700 in the blood samples ranged between 0.15–2.0 mg/L with a median of 0.30 mg/L. In one of the cases with a U-47,700 concentration of 0.27 mg/L, no other psychoactive substances were detected.

The stored TOF-MS analytical data from the year preceding the incorporation of U-47,700 into the screening was reprocessed in order to search for more positive cases. The data-independent acquisition of the original screening allowed for retrospective re-analysis of the full-scan data without additional experiments on the actual sample. The retrospective data-analysis revealed two additional cases positive for U-47,700.

The first mention of U-47,700 on a Finnish internet discussion forum was in March 2015. After having been detected in several death cases, the drug was put under national control in November 2016 and the last fatality occurred in 2017. The toxic lifespan of U-47,700 thus lasted for approximately 2 years in Finland.

Forensic and clinical laboratories need to rapidly adjust their screening procedures in order to adapt to the continuously expanding field of novel psychoactive substances. Retrospective data-analysis is a practical tool for monitoring the emergence of new substances onto the market.

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1. Introduction

Opioids are commonly abused by users of illegal drugs, and they account for the majority of fatal poisonings worldwide [1,2]. In Finland, the opioid most commonly implicated as the cause of death is buprenorphine, a semi-synthetic partial agonist at the μ -opioid receptor, whereas heroin abuse and deaths are rare. Also other medicinal opioids used in the treatment of pain are frequently detected in fatal poisoning cases, often together with other psychoactive substances.

In addition to medicinal opioids, recent years have brought new opioids onto the illicit drug market; opioids that have never been approved for medical use. One of these is U-47,700 (3,4-dichloro-N-[2-(dimethylamino)cyclohexyl]-N-methylbenzamide) (Fig. 1), a highly selective μ -receptor agonist originally developed by the

company Upjohn in 1970s [3]. Since 2016, several reports have been published on serious adverse effects associated with U-47,700, including death cases [4–15].

The emergence of new psychoactive substances (NPS) is a challenge for forensic and clinical laboratories since new substances enter the drug market in an ever escalating pace. Acquiring reference material for each NPS is hardly sensible due to financial reasons or due to the fact that these substances are not readily available. In traditional toxicological screening it is only possible to find those substances that are looked for. It is complicated to estimate whether a certain NPS is of relevance in one's own setting and should be incorporated into the screening. Using time-of-flight mass spectrometry (TOF) with data-independent acquisition, the full precursor ion data set as well as the full fragment ion data set are acquired in a single run. Thus it is possible to revisit previously analysed raw data and look for new information without the need to run the samples again.

LC-TOFMS has gained popularity in the forensic field due to the accurate mass and high mass resolution capabilities combined with easily expandable reference databases but relatively few

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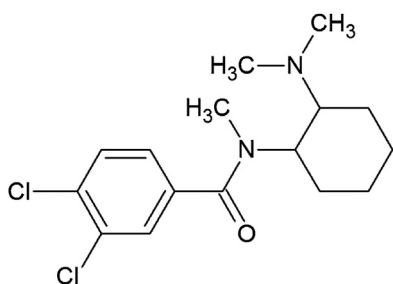


Fig. 1. Structure of U-47,700.

reports are available of realizing the possibility to retrospectively reprocess the historic data [13,16–18]. In this project we aimed to study the lifespan of U-47,700 in Finland, taking advantage of the possibility to retrospectively reanalyse the stored data.

2. Material and methods

Urine and femoral blood samples from unexpected death cases were collected at autopsies and stored refrigerated until analysis.

2.1. Adding U-47,700 to the TOF-MS database

A sample of seized U-47,700 was generously provided by the Finnish Customs Laboratory in March 2016. A standard solution with the approximate concentration of 1 µg/mL of U-47,700 was injected into the system twice (two parallel injections). The precursor exact mass, retention time and assigned fragment ions information of the substance was added to the TOF-MS analyte database. The following criteria were met for the two injections: $\Delta R_t < 0.3$ min, mass precision of the precursor ion < 3 mDa, and mass precision of the qualifier ion < 5 mDa. As customary for updating the analyte database, the fragmentation pattern of U-47,700 was further confirmed by the ACD/MS Fragmenter software (Advanced Chemistry Development, Toronto, Canada) and the Smart Formula 3D feature of Bruker Data Analysis software (Bruker Daltonics GmbH, Bremen, Germany).

The exact mass of the precursor protonated molecule added to the database was m/z 329.1182, and the exact masses of the characteristic fragment ions were m/z 284.0603 and m/z 203.9977. The primary fragment (m/z 284.0603) used as qualifier ion for U-47,700 is also a fragment of the structural isomer AH-7921 – another abused opioid. However, in the current procedure, these two analytes are chromatographically separated (retention time difference > 0.35 min). Ion ratios of the two substances are different and the fragment 284.0603 is not the most abundant fragment of AH-7921. Consequently, the qualifier ions assigned for AH-7921 (m/z 172.9552 and 189.9821) are different from those of U-47,700. In addition, m/z 189.9821 is unique for AH-7921, which further minimizes the risk of false identification.

After the first detection of the substance in biological samples, an official reference standard was purchased from Chiron (Trondheim, Norway) enabling appropriate quantitative analysis.

2.2. Sample preparation and qualitative screening with UPLC-TOFMS

Urine samples were screened for U-47,700 by ultra-high performance liquid chromatography coupled with high-resolution time-of-flight mass spectrometry (UPLC-TOFMS). The screening method is described in detail elsewhere [19]. In short, after hydrolysis and solid phase extraction, the extracts were dissolved in a mixture of methanol and 0.1% formic acid (45:55) without concentrating the samples. The extracts were injected into the

UPLC-TOFMS system consisting of a Waters Acquity UPLC coupled with a Bruker MicroTOF Q II. Data were acquired in full-scan MS and bbCID (broad-band collision-induced dissociation) MS modes by switching the collision energy between 8 eV (precursor MS data acquisition) and 30 eV (fragment bbCID MS data acquisition).

The UPLC-TOFMS screening provided full-scan data that was subsequently processed against the in-house analyte database consisting of hundreds of medicinal drugs, drugs of abuse, NPS and metabolites. The principles of the database search have been published elsewhere [19].

2.3. Quantitative analysis with GC-MS

Quantification of U-47,700 in femoral blood and urine was performed by gas chromatography–mass spectrometry (GC-MS), using a method originally developed for the determination of MDPV [20]. The GC-MS analysis was performed by selected ion monitoring, using four ions (m/z 125 (target), 84, 145, and 173) for U-47,700. The heptafluorobutyric acid derivative of MDMA-d5 (m/z 254, 210) was used as an internal standard.

Linearity of the method was studied by constructing calibration curves in blood and urine, consisting of seven concentration points over the range of 0.02–2.5 mg/L with five parallel measurements in each point. The correlation coefficient (r^2) was ≥ 0.991 . Intra-day accuracy (bias) and precision was $< 11\%$ and $< 6\%$ CV (at 0.02–2.5 mg/L), respectively. Between-day accuracy and precision were measured on seven separate days over a period of four weeks. Between-day accuracy (bias) at 0.1 and 1.0 mg/L was 10% and 16%, respectively, and between-day precision 12% and 16% CV, respectively. The limits of detection (LOD) and quantification (LOQ) were 0.01 and 0.02 mg/L, respectively.

The GC-MS-method was able to chromatographically separate U-47,700 from the structural isomer AH-7921 (retention time difference 0.35 min).

2.4. Retrospective data-analysis

Retrospective analysis of the stored TOF-MS data for the one-year period prior to inclusion of U-47,700 in the database was conducted in the same way as the original data analysis, but the analyte database had in the meantime been updated with new analytes, including U-47,700.

Reanalysis of the raw data was rather time-consuming due to the high number of cases and, consequently, the volume of data to be reanalysed. The software was let to reprocess the data overnight, one month at the time.

3. Results and discussion

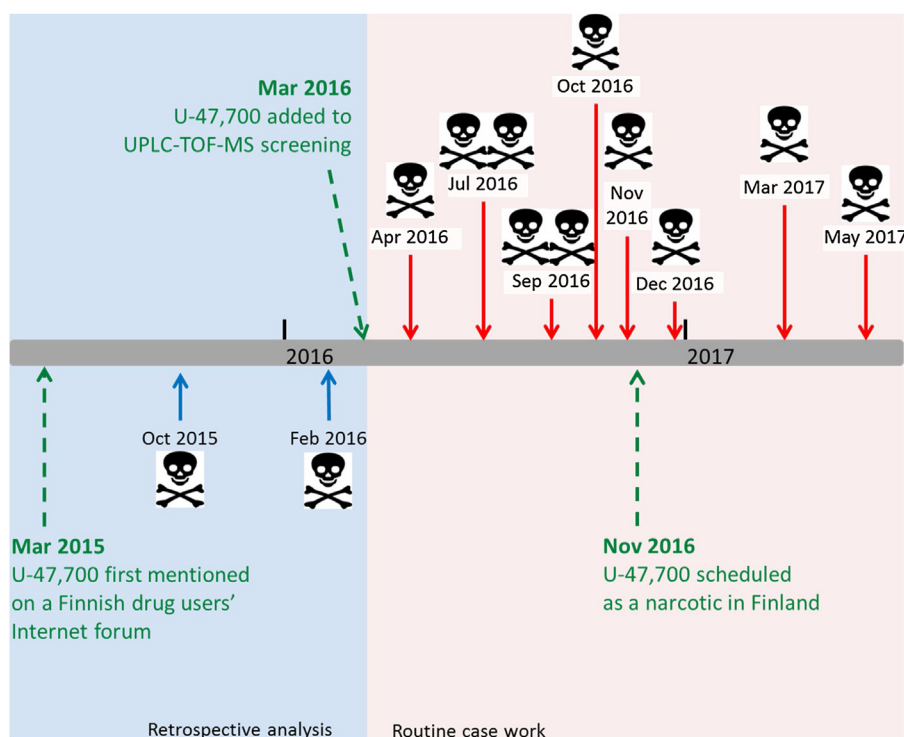
U-47,700 was first detected in a post-mortem sample in April 2016; one month after the drug had been included in the screening. U-47,700 was subsequently detected in seven other post-mortem cases in 2016. Consequently, U-47,700 was controlled under the Narcotics Act in Finland in November 2016. In 2017, the drug was detected twice but there were no positive autopsy cases in 2018. Details of the ten cases positive for U-47,700 are given in Table 1. In all of the 10 cases, the cause of death was accidental poisoning by U-47,700 alone or in combination with other psychoactive substances.

All of the deceased positive for U-47,700 were male, and their mean age (range) was 26 (23–31) years. The median (range) blood concentration of U-47,700 was 0.30 (0.15–2.0) mg/L. In most cases other psychoactive drugs were detected along with U-47,700. According to the background information, in most cases the deceased had a history of drug abuse. The concentrations detected in these 10 cases were similar to those reported by Mohr et al. [6], Rojek et al. [14] and Smith et al. [15] who found median U-47,700

Table 1

Details of U-47,700-positive cases in 2016 and 2017.

Case	Age	Gender	BAC g/kg	Concentration of U-47,700		Other drugs detected in blood (mg/L)
				Blood (mg/L)	Urine (mg/L)	
1	23	Male	3.00	0.22	0.02	–
2	27	Male	–	0.32	0.03	MDMA 2,4; amphetamine 0,67; pregabalin 40; alprazolam 0,006
3	28	Male	–	0.19	posit	N-MBZP 0,092; BZP 0,0081; m-CPP 0,020; phenazepam 0,26; alprazolam 0,034;
4	24	Male	–	0.27	0.40	–
5	29	Male	2.00	0.19	0.06	Buprenorphine 0.53 µg/L; 7-aminoclonazepam 0.18; pregabalin 3.9; cannabis positive
6	23	Male	0.75	0.37	0.10	7-aminoclonazepam 0.16
7	25	Male	–	0.15	0.23	Pregabalin 19; alprazolam 0.013; lorazepam 0.011; THC 6.4 µg/L
8	25	Male	–	2.00	0.89	Buprenorphine 1 µg/L; 7-aminoclonazepam 0.14
9	31	Male	–	0.64	0.57	α-PVP 0.34; THC 1.1 µg/L; pregabalin 11; alprazolam 0.030; tramadol 0.47; diazepam 0.059; olanzapine 0.42
10	23	Male	–	0.96	2.20	Amphetamine 0.17; 7-aminoclonazepam 0.24; alprazolam 0.009; buprenorphine <0.5 µg/L
R1	27	Male	–	–	Posit	Etizolam 0.040; 7-aminoclonazepam 0.083; THC-COOH 20 µg/l
R2	32	Male	–	–	Posit	Amphetamine 0.32; etizolam <0.025

**Fig. 2.** U-47,700 in Finland.

PM blood concentrations of 0.247 mg/L (N = 16), 0.306 mg/L (N = 12), and 0.126 mg/L (N = 15) respectively.

Among the studied cases, there was one case in which no other psychoactive substances, besides U-47,700, were detected (Case 4, Table 1). The blood concentration of U-47,700 in this case (0.27 mg/L) did not significantly differ from the median U-47,700 concentration. However, regardless of the blood concentration, the toxicological significance of detecting U-47,700 or any other opioid is profoundly connected to the state of opioid tolerance the victim may have possessed, as well as the route of administration and other findings. Thus, defining a fatal concentration for a strong opioid, such as U-47,700, is considered somewhat meaningless.

Retrospective re-analysis of the acquired TOF-MS data was performed for all samples screened for illegal drugs within a one-year period before inclusion of U-47,700 in the screening method. A total of 1836 samples were reprocessed. The procedure resulted in two additional positive findings of U-47,700. Details on these two cases are presented in Table 1 (R1 & R2) and the toxic lifespan of U-47,700 in

post-mortem investigations in Finland is illustrated on a timeline in Fig. 2. Unfortunately, at the time of the reprocessing, the original sample material was no longer available and thus no quantitative results are available. Interestingly, in both of the retrospectively detected cases, etizolam was detected together with U-47,700.

Retrospective re-analysis is a tool often mentioned as a useful option but relatively seldom used in forensic laboratories. To our knowledge, only in a handful of cases retrospective re-analysis has been successfully used to detect drugs in previously analysed biological samples. Partridge et al. was able to detect several NPS in a death case after having updated the in-house database [13]. Noble et al. screened thousands of blood samples for fentanyl derivatives using retrospective re-analysis of previously acquired data [18]. Retrospective data processing has also been used to screen for metabolites in samples in which the parent compound has already been identified [17,21,22].

There are several factors affecting the lifespan of an NPS. Considering the total number of all different NPS reported, only a

few of them have gained considerable prevalence or media attention. It has been shown that the legal status of a substance in the end-users' country has a considerable influence on the interest in the drug and on the corresponding harms [23,24].

Drug marketplaces on the hidden web are increasingly used for anonymous sale of drugs [25], and the earliest indication of the possible appearance of a new substance might indeed be evidenced on the hidden web [26]. One study found that vendors selling NPS had short lifespans, and while individual NPS had longer lifespans, only a quarter of the studied substances were generally available over a period of one year [25]. Post-mortem toxicology usually contributes to the monitoring of emerging NPS only when their use has become prevalent in the society. However, potent opioids are exceptional in this respect, as has been evidenced with the case of 3-methylfentanyl in Finland, where all the findings of the drug were from fatalities that occurred within a short period of time [27].

Another important factor affecting the lifespan of a particular NPS is the drug supply in the country of origin. Concerning U-47,700, the substance was added to China's list of controlled substances as of July 1, 2017, which coincides with the end of the toxic epidemic in Finland.

In addition to laboratory measures, a number of other methods for monitoring the appearance and use of NPS are being used. These include e.g. monitoring the online user forums, user questionnaires, information from poisons information services, and international early warning systems [28]. As demonstrated in the case of U-47,700 in Finland and in the few studies published before, retrospective data-analyses can be a valuable tool in assessing the time point at which the drug has entered the market [13,16].

4. Conclusion

A series of cases positive for an NPS opioid U-47,700 was detected in post-mortem investigations within a relatively short time period prompting the idea of retrospectively re-analysing the data before the first detection. The re-analysis resulted in two additional cases. The post-targeted screening approach enabled relatively easy access to the full-scan data acquired before incorporating the substance into the screening. Retrospective data analysis can be especially useful when monitoring the lifespan of NPS in clinical and forensic settings.

Author contribution statement

Pirkko Kriikku conceived of the presented idea, performed the retrospective analyses and wrote the manuscript with input from all other authors. Anna Pelander contributed with in-depth knowledge of the instrumentation and data analysis procedures. Ilpo Rasanen performed the quantitative analyses. Ilkka Ojanperä aided in interpreting the results especially with regards to the concept of NPS lifespan. All authors discussed the results and contributed to the final manuscript.

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