



# Quantification of U-47700 and its metabolites: *N*-desmethyl-U-47700 and *N,N*-didesmethyl-U-47700 in 12 autopsy blood samples employing SPE/LC–ESI–MS–MS

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## Abstract

**Purpose** The paper presents a report on the comprehensive assessment of a novel synthetic opioid (NSO) termed U-47700, and its two metabolites: *N*-desmethyl-U-47700 and *N,N*-didesmethyl-U-47700 in autopsy blood samples taken from 12 cases of fatal poisonings.

**Methods** The analysis of the examined samples was based on the solid-phase extraction/liquid chromatography–electrospray ionization–tandem mass spectrometry method, which was developed in the validation process. In the quantitative analytical studies, deuterium analytes analogs were used, namely U-47700-*d*<sub>6</sub> and *N*-desmethyl-U-47700-*d*<sub>3</sub>.

**Results** The validation parameters of the method were determined, including the limits of quantification on the 1 ng/mL level, calibration curves range of 1–1000 ng/mL, intra-assay precisions and accuracies of 1.1–20.2% and –18.9–9%, respectively, and inter-assay precisions and accuracies of 2.9–13.0% and –11.4–3.3%, respectively. The matrix effects and the extraction efficiencies were formed at the levels of 54.0–119% and 53.0–118%, respectively. The parent substance and its metabolites in blood samples have been shown to be relatively stable under various conditions within the 21-day study. The concentration levels demonstrated in the analyzed blood samples were: U-47700 in the range of 83–24,000 ng/mL, *N*-desmethyl-U-47700 in the range of 2.0–7520 ng/mL and *N,N*-didesmethyl-U-47700 in the range of 18–1947 ng/mL.

**Conclusions** The simultaneous quantification of U-47700, *N*-desmethyl-U-47700 and *N,N*-didesmethyl-U-47700 seems very useful to confirm the cause of death and to estimate antemortem interval. To our knowledge this is the first trial to measure phase I metabolites of U-47700 in authentic human blood samples.

**Keywords** Novel synthetic opioids · U-47700 · *N*-Desmethyl-U-47700 · *N,N*-Didesmethyl-U-47700 · Fatal intoxication · LC–MS/MS

## Introduction

New psychoactive substances (NPS) are synthetic alternatives to more traditional drugs of abuse, specifically engineered to circumvent the existing drug control laws. The emergence of NPS is a global phenomenon fuelled by the growth of Internet commerce and manufacturing capacity of Asian countries [1]. As the recreational consumption of NPS has been increasing in Europe and in the world for the

past decade, such compounds have proven to be quite a challenge for public health and drug policies globally [2]. By the end of 2017, the EMCDDA was monitoring more than 670 NPS that had been identified in Europe [3]. While synthetic cathinones and cannabinoids continue to predominate, an increasing number of synthetic opioids including fentanyl derivatives and non-fentanyl analogs are more commonly encountered in the recreational (i.e., non-medical) drug market in Europe and elsewhere [3, 4].

Novel synthetic opioids (NSOs) are progressively more often encountered in illicit heroin and counterfeit pain pills. Many NSOs are resurrected from older biomedical literature or patent applications, but limited information is available about their biological effects [1].

Recently, U-47700 [3,4-dichloro-*N*-[(1*R*,2*R*)-2-(dimethylamino)-cyclohexyl]-*N*-methylbenzamide] has

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been noted to appear on the drug of abuse market; its synthesis was patented by Szmuszkovicz in 1978 [5] as a result of studies that he carried out for the Upjohn Company. Preclinical studies determined that U-47700 was approximately 7.5 times more potent than morphine, but about 10 times less potent than fentanyl. The compound has not been studied in humans and no pharmacokinetic data are available [6, 7].

In 2016, Elliot et al. [8] were the first to describe a U-47700-associated death and to attempt probing into the problem of analytic differentiation between synthetic opioids U-47700 and AH-7921 that were mutual isobaric compounds. Since that time, the number of cases of intoxication being reported in the scientific literature concerning morbidity and mortality associated with U-47700 use has been consistently increasing [9].

In the qualitative and quantitative analysis of NSOs, including U-47700 and its metabolites, the method of liquid chromatography–(quadrupole) time-of-flight-mass spectrometry (LC–(Q)TOF-MS) and LC–ion trap MS<sup>n</sup> technology were used [4, 10, 11]. According to Mohr et al. [12] and Gerace et al. [13], however, the LC–MS/MS method provides a “golden standard” for quantitative analysis of NSOs in biological samples.

The present paper constitutes a report on the comprehensive assessment of a novel synthetic opioid U-47700 and its two metabolites *N*-desmethyl-U-47700 and *N,N*-didesmethyl-U-47700 in autopsy blood samples collected from 12 cases of poisonings involving U-47700. The evaluation of these compounds was preceded by development of the solid-phase extraction (SPE)/LC–electrospray ionization (ESI)-MS/MS method dedicated especially to such analysis, which had been subjected to a detailed validation process. The method included determination of significant validation parameters and the application of internal isotopically labeled standards in the form of U-47700-*d*<sub>6</sub> and *N*-desmethyl-U-47700-*d*<sub>3</sub>.

## Materials and methods

### Biological materials

Samples of peripheral blood were collected from 12 autopsy cases. The autopsies were carried out within 24 h after death. The samples were kept refrigerated (+4 °C) until the analyses were performed. Blank whole blood samples for development and validation of the analytical method were purchased from the Regional Blood Centre Donation in Kraków, Poland. Blank autopsy blood samples were also collected at the time of autopsy from non-poisoned subjects.

### Standards and chemicals

The standard solution of U-47700 (CRM) at the concentration of 1 mg/mL was obtained from Chiron (Trondheim, Norway); the standard solutions of *N*-desmethyl-U-47700 (purity ≥98%), *N,N*-didesmethyl-U-47700 (purity ≥98), and U-47700-*d*<sub>6</sub> (CRM) at the concentration of 1 mg/mL from Cayman Chemical (Ann Arbor, MI, USA); the standard solution of *N*-desmethyl-U-47700-*d*<sub>3</sub> (CRM) at the concentration of 0.1 mg/mL from Cerrilant (Round Rock, TX, USA). Other common chemicals and solvents were purchased from Sigma-Aldrich (Poznań, Poland).

### Validation samples

For the calibrator samples, three working solutions in methanol were prepared for constructing nine plots using the following concentrations: 0.1, 1 and 10 ng/μL of U-47700, *N*-desmethyl-U-47700 and *N,N*-didesmethyl-U-47700. Additionally, methanolic solutions were prepared for quality control (QC) samples at the concentrations of 1 and 10 ng/μL for U-47700 and its metabolites. Calibrator and QC working solutions were made from different source lots. All the working solutions were stored at –20 °C when not in use. Daily calibration samples were prepared by fortifying 1 mL of blank blood with known amounts of U-47700 and its metabolites at the concentrations ranging from 1 to 1000 ng/mL.

Two QC specimens were also prepared daily at the concentrations of 25 and 250 ng/mL for U-47700 and its metabolites. For the deuterated internal standards (ISs), a working solution of 1 ng of U-47700-*d*<sub>6</sub> and *N*-desmethyl-U-47700-*d*<sub>3</sub>/μL in methanol was prepared and stored at –20 °C, when not in use. Blank blood samples with and without ISs were prepared for evaluating peak purity.

### Extraction

Each 1 mL sample was put into a clean 15 mL tube and diluted with 0.01 M carbonate buffer to pH 9.3 (1:5, v/v). One hundred microliters of IS working solution was added to each sample prior to extraction, producing a final deuterated IS concentration at 100 ng/mL. Subsequently, the samples were vortexed and centrifuged for 7.5 min at 4400 × *g*. Bond Elut cartridge, filled with a non-polar bed of silica gel modified by octadecyl (C<sub>18</sub>) reversed phase, with a weight of 500 mg was used (Agilent, Santa Clara, CA, USA). The SPE cartridge was firstly equilibrated with 1 mL each of methanol, distilled water and the above carbonate buffer (pH 9.3), and then the supernatant of each blood sample was applied onto

the cartridge and slowly passed through it. In the next step, matrix impurities were cleaned with 2 mL of the carbonate buffer (pH 9.3), and then vacuum was applied to each cartridge for 30 min to remove residual moisture. The analytes were eluted with 2 mL of 1 M acetic acid in methanol (1:9, v/v). The eluate was evaporated to dryness in a vacuum concentrator at 40 °C, and the residue was dissolved in 1 mL of the mixture of mobile phases (60% phase A + 40% phase B), and 10 µL was injected into the LC–MS system. The phase A was water, which contained 0.2% formic acid and 2 mM of ammonium formate, and the phase B was acetonitrile with 0.2% formic acid and 2 mM of ammonium formate. In the case of forensic blood samples, in which the concentrations of analytes exceeded the calibration ranges, the samples were adequately diluted with blank blood.

### LC–ESI–MS/MS method

An Agilent 1200 liquid chromatograph (Agilent) equipped with a binary pump (G1312 A) and an autosampler (G1329 A) was used. The chromatographic separation was performed with a Poroshell 120 C18 column (100 × 3 mm i.d., particle size 2.7 µm, Agilent). Chromatographic separation of U-47700 and its metabolites was performed under isocratic conditions at the following composition of mobile phases: 60% A and 40% B at a flow rate of 0.5 mL/min. The total time of acquisition was 3 min. A 6410 triple quadrupole mass spectrometer (Agilent) with an ESI source, operated under a positive mode was used. The multiple reaction monitoring (MRM) detection was employed. The operational parameters of the ESI source were as follows: vaporizing temperature 350 °C; pressure of the nebulizing gas 40 psi; flow of the drying gas 9 L/min; capillary potential 3.5 kV. The fragmentation parameters of the analyzed compounds are listed in Table 1.

### Validation of the SPE/LC–ESI–MS/MS method

#### Selectivity and specificity

To evaluate the peak purity and selectivity, blank blood samples (no analyte or IS added) were analyzed with each batch to check for peaks that might interfere with the detection of U-47700 and its metabolites *N*-desmethyl-U-47700 and *N,N*-didesmethyl-U-47700. To assess possible interferences of other analytes (specificity), QC samples were spiked individually to contain 1000 ng/mL of a mixture of about 400 substances, including medicines, drugs of abuse, and NPS together with NSOs.

#### Linearity and limits of detection and quantification

The calibration curves were constructed after the analysis of drug-free blood containing known amounts of U-47700 and its metabolites. To prepare these standards, blank blood samples were spiked with the compounds to the following concentrations: 1, 5, 10, 20, 50, 100, 200, 500 and 1000 ng/mL for blood. Each level was prepared three times. The samples were extracted according to the procedure described above. The calibration curves were constructed by plotting the peak area ratios of the analyte U-47700 and *N,N*-didesmethyl-U-47700/IS (U-47700-*d*<sub>6</sub>), *N*-desmethyl-U-47700/IS (*N*-desmethyl-U-47700-*d*<sub>3</sub>). Negative QC samples were analyzed after each calibration sample to evaluate the potential carry-over. The limit of detection (LOD) of the method was determined by analyzing validation samples (*n* = 5) to determine if the acceptance criteria were met for each analyte. LOD was defined as the lowest concentration at which the analyte ion signal-to-noise ratio (determined by peak height) was ≥ 10, and chromatography (peak shape and resolution) and relative retention time (± 2% of target retention time) were acceptable. The limit of quantification (LOQ) was defined as the lowest concentration that met the

**Table 1** Fragmentation parameters for analytes and internal standards

Compound	Precursor ion ( <i>m/z</i> )	Production ( <i>m/z</i> )	Fragmentor voltage (V)	Collision energy (V)	Retention time (min)
U-47700- <i>d</i> <sub>6</sub>	335.2	<b>284.0</b>	75	17	1.6
		172.9	75	37	
U-47700	329.1	<b>284.1</b>	60	13	1.6
		173.0	60	37	
<i>N</i> -Desmethyl-U-47700- <i>d</i> <sub>3</sub>	318.1	<b>175.9</b>	69	37	1.5
		147.9	69	57	
<i>N</i> -Desmethyl-U-47700	315.1	<b>172.9</b>	64	33	1.5
		144.9	64	57	
<i>N,N</i> -Didesmethyl-U-47700	301.1	270.0	65	13	1.4
		<b>172.9</b>	65	33	

Bold indicates quantitative ions

LOD criteria and had analyte quantification within  $\pm 20\%$  of the target value.

### Accuracy and precision

Inter- and intra-assay accuracy and precision data for U-4700 and its metabolites were determined for each QC sample. Intra-assay data were obtained from one run ( $n=3$ ), and inter-assay data were determined among three separate runs ( $n=9$ ). Accuracy, expressed as a percentage, was calculated by taking the difference between the mean measured concentration and reference concentration, dividing it by the reference concentration and multiplying by 100 for two QC concentrations (25 and 250 ng/mL). Precision, expressed as a percent relative standard deviation, was determined by calculating the percent ratio of the standard deviation divided by the mean measured concentration  $\times 100$ .

### Extraction efficiency, matrix effect and process efficiency

Extraction efficiencies, matrix effects and process efficiencies were evaluated via three sets of samples ( $n=5$  for each set). Extraction efficiency for each analyte was measured at each QC concentration. Blank blood (five different lots) was fortified with reference standard solution before and after SPE. Percent extraction efficiency from blood was expressed as the mean analyte area of samples ( $n=5$ ) fortified with reference standard solution before extraction divided by the mean area of samples ( $n=5$ ) with reference standard solution added after SPE. Matrix effect was assessed by comparing the analyte peak areas in blank extracted blood specimens fortified with reference standard solutions after SPE with the peak areas of samples at the same nominal concentrations prepared in a 60:40 mixture of the mobile phase A and mobile phase B (neat sample). Matrix suppression or enhancement was calculated as follows:  $[100 \times (\text{mean peak area of fortified blood after SPE} / \text{mean peak area of neat sample})]$ . Process efficiency was examined for the overall effect of SPE extraction efficiency and matrix effect on the quantification of the analytes of interest. It was determined by comparing the mean analyte peak area of five samples fortified before SPE with the mean peak area of five neat samples prepared in the mobile phase at the same concentration.

### Stability

The stability of U-47700 and its metabolites in the system of low QC blood samples was checked under the following conditions:  $-30\text{ }^\circ\text{C}$ ,  $+3\text{ }^\circ\text{C}$  and ambient temperature ( $+20\text{ }^\circ\text{C}$ ) in the 21-day study. The determinations of the analytes were carried out after 1, 7, 14 and 21 days of storage.

## Results

In the first part of the work, to accurately quantify U-47700 and its metabolites, a detailed procedure by SPE/LC–ESI–MS/MS with the validation of the method was established.

At first, the specificity of the above method was tested by the use of more than about 400 compounds, such as medicines, drugs of abuse and NPS including NSOs. None of them interfered with the peak of U-47700, its metabolites or IS. Even AH-7921, the isobaric compound of U-47700, did elute at another retention time. The selectivity experiment showed that all the blank blood samples were free of coeluting peaks at the retention times of U-47700, its metabolites and their respective deuterated ISs. The analysis of the blank blood samples in each assay also demonstrated that the ISs did not contain relevant amounts of native U-47700 and its metabolites (Fig. 1a).

An overview of characteristic calibration data over a dynamic range from LOQs to 1000 ng/mL for U-47700 and its metabolites was done. A linear relationship between the concentration and peak area was obtained only for *N,N*-desmethyl-U-47700. In case of U-47700 and *N*-desmethyl-U-47700, the best fit of the trend line was obtained for the exponential and logarithmic function, respectively. All of the three curves were characterized by the coefficient of determination  $\geq 0.999$ . The values of LODs, LOQs and the characteristics of the calibration curves are shown in Table 2.

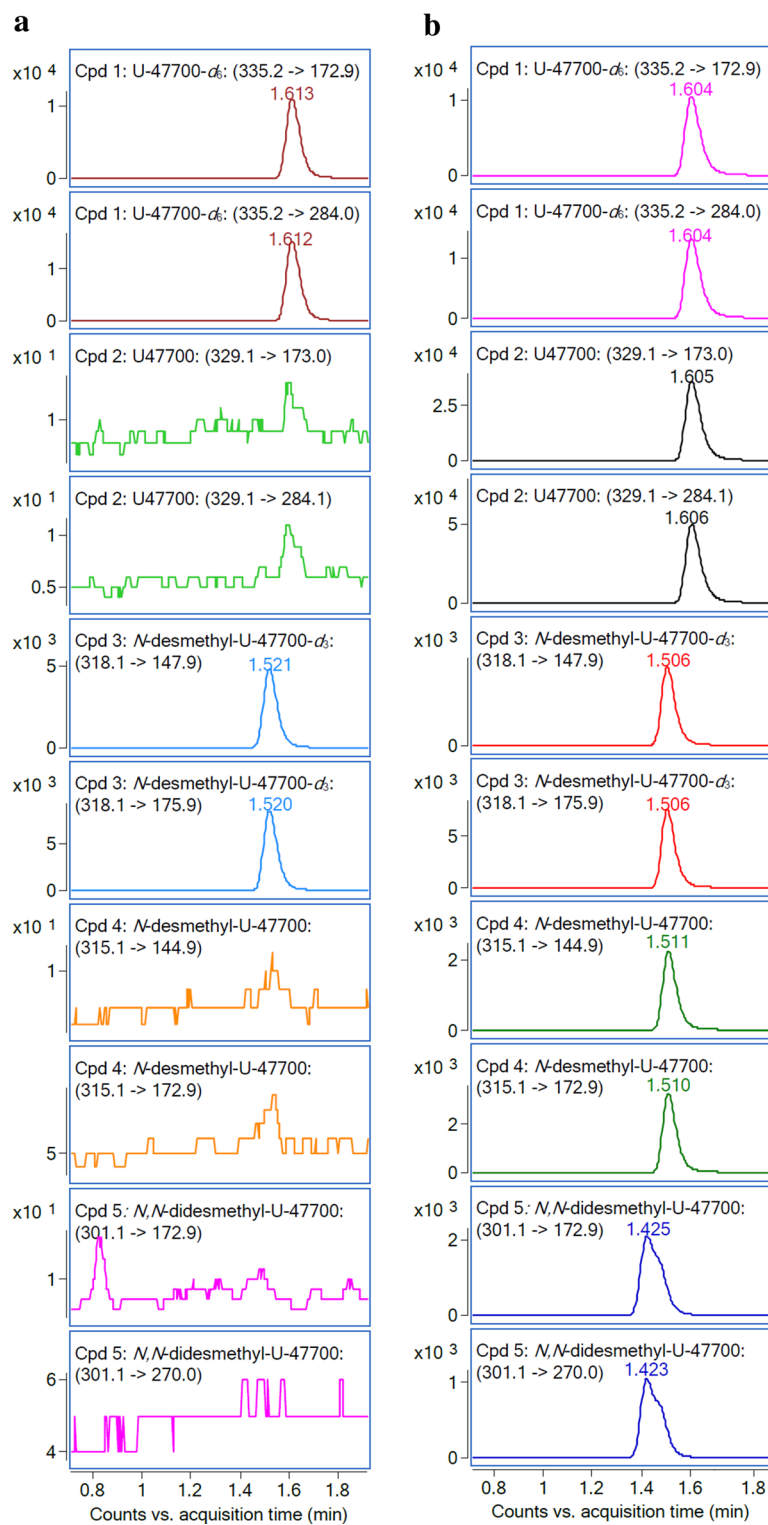
No detectable carry-over occurred following the 1000 ng/mL U-47700 and its metabolites sample. Precision and accuracy of the method were evaluated at two concentrations within the dynamic range (25 and 250 ng/mL). The data for both intra-assay ( $n=3$ ) and inter-assay ( $n=9$ ) are presented in Table 3.

The matrix effects for MRM quantification of the analytes and deuterated counterparts of ISs were practically on the identical levels. A similar relationship was observed for MRM qualification. The result of this phenomenon was a “mathematical abolition” of the matrix effects, which improved the precision and accuracy of the analytical methods. In the process of calibration, no commercially available deuterated IS of *N,N*-didesmethyl-U-47700 was used, which affected the deterioration of intra- and inter-assay precision and accuracy. All the above data are shown in Table 4.

U-47700 and its metabolites in the QC blood samples were tested for the following conditions:  $-30\text{ }^\circ\text{C}$ ,  $+3\text{ }^\circ\text{C}$  and  $+20\text{ }^\circ\text{C}$  in the 21-day study. All of the analytes were characterized by high stability for 21 days even at  $20\text{ }^\circ\text{C}$ .

The results of toxicological findings of the examined samples are presented in Table 5. In Fig. 1b, the analytical data taken from Case no. 11 were selected to document the analytical procedure.

**Fig. 1** Multiple reaction monitoring chromatograms obtained from the analysis of blank autopsy samples spiked with ISs (a) and Case no. 11 (b)



## Discussion

U-47700 as a synthetic opioid is a  $\mu$ -receptor agonist, the action of which is manifested by a reduction in the sensation of pain and a reduction of emotions associated with pain, such as anxiety, fear and a sense of danger. The particularly

dangerous side effects of opioid receptor agonists include the development of tolerance and addiction, and respiratory depression that can lead to death. Although U-47700 was synthesized at the end of the last century as an opioid analgesic drug class in an attempt to develop a non-addicting analgesic, it has appeared only in recent years on the drug

**Table 2** Calibration parameters

Compound	Calibration curve	Coefficient of determination ( $R^2$ )	Limit of detection (LOD) [ng/ml]	Limit of quantification (LOQ) [ng/ml]
U-47700	$y = 0.90077x^{1.05569}$	0.9997	0.5	1.0
<i>N</i> -Desmethyl-U-47700	$y = -0.02757x^2 + 0.91125x + 0.00011$	0.9998	0.5	1.0
<i>N,N</i> -Didesmethyl-U-47700	$y = 0.24458x - 0.00004$	0.9994	0.75	1.0

**Table 3** Precision and accuracy data

Compound	Concentration in blood [ng/mL]	Intra-assay precision [%RSD] ( $n = 3$ )	Intra-assay accuracy (%) ( $n = 3$ )	Inter-assay precision [%RSD] ( $n = 9$ )	Inter-assay accuracy (%) ( $n = 9$ )
U-47700	25	1.6	- 3.9	2.9	- 0.9
	250	1.1	7.3	4.7	1.7
<i>N</i> -Desmethyl-U-47700	25	1.4	- 4.7	3.3	- 1.1
	250	1.2	9.0	4.3	3.3
<i>N,N</i> -Didesmethyl-U-47700	25	13.6	- 18.9	10.9	- 11.4
	250	20.2	- 10.9	13.0	- 4.7

RSD relative standars deviation

market, gaining popularity among drug users as a legal alternative to heroin. U-47700 is available on the Internet in a powdered form which is usually taken through nasal inhalation or ingested in a capsule form. In 2016, U-47700 was sought with a very high interest and was highly visible on the Internet, suggesting a new trend on the horizon for synthetic opioid abuse [14].

Toxicological findings of deaths associated with U-47700, which were the subject of the present study, originating from expert's medico-legal opinions issued in the years 2016–2017, are shown in Table 5. The concentrations of the drug in the samples of blood were in a wide range of 83–24,000 ng/mL. In all the examined cases, apart from U-47700, other xenobiotics were detected, including alcohol in four instances, synthetic cannabinoids in four cases, medications in one case, phytocannabinoid, synthetic cannabinoids, synthetic cathinone and medications in one case, and synthetic cathinone and medications in two cases.

According to the published sources, the toxic effects of U-47700 in humans were demonstrated as overdosing [15–17] and fatalities [12, 18–20]. In the cases of U-47700-related deaths, the concentration of the drug in autopsy blood samples was in the range of 59–525 ng/mL [12, 18, 19], and in the cases of fatal poisonings in multiple drug use, U-47700 concentration values in autopsy blood samples were in the range of 13.8–1460 ng/mL [8, 9].

As it follows from the analysis of fatal poisoning cases originating from the present study and other publications, the unpredictability of the toxic effect in cases of poisoning with one compound is possible; the said unpredictability

may be the more strongly manifested in complex cases. Although no quantitative assessment of mutual relations based on casuistry is possible in cases of NPS taking, the governing principle is implying an intensified risk of their toxic effects [21, 22].

In the presently discussed cases, owing to employing the highly selective and sensitive method, apart from U-47700, the investigated blood samples showed the presence of *N*-desmethyl-U-47700 in the concentration range of 2–7520 ng/mL and *N,N*-didesmethyl-U-47700 in the concentration range of 18–1947 ng/mL. Their concentration values in the blood samples as compared to those of the precursor were variable, without showing a discernible trend. It could be also hypothetically assumed that they can demonstrate biological activities, similarly as desmethyl derivatives of numerous xenobiotics, which may significantly affect the toxicity of U-47700.

The U-47700 metabolism in the human body has never been the subject of detailed investigations; however, Krotulski et al. [23] performed identification in vitro studies of U-47700 metabolites by LC–Q(TOF)–MS using human liver microsomes and confirmed the presence of four metabolites. Tracing the metabolic pathways of U-47700 indicated that *N*-desmethyl-U-47700 as the primary metabolite and *N,N*-didesmethyl-U-47700 constitute products of demethylation of the parent substance at the nitrogen atom of the dimethylamino group of the cyclohexane ring, probably followed by forming hydroxylation products *N*-desmethyl-hydroxyl-U-47700 and *N,N*-didesmethyl-hydroxyl-U-47700, respectively (Fig. 2). Due to the fact that hydroxylation occurs

**Table 4** Matrix effects (MEs), extraction efficiencies (EEs) and process efficiencies (PEs) for U-47700, its metabolites and internal standards as a function of blank whole blood matrices

Analyte	U-47700		U-47700- <i>d6</i>		N-Desmethyl-U-47700		N-Desmethyl-U-47700- <i>d3</i>		N,N-Didesmethyl-U-47700	
	329.1 → 173.0	329.1 → 284.1	335.2 → 172.9	335.2 → 284.0	315.1 → 144.9	315.1 → 172.9	318.1 → 147.9	318.1 → 175.9	301.1 → 172.9	301.1 → 270.0
<b>Blank 1<sup>a</sup></b>										
25 ng/mL										
EE (%)	105	109	107	111	98	106	101	102	101	107
ME (%)	80	79	76	78	110	110	110	114	63	63
PE (%)	84	87	81	86	109	116	110	117	64	67
250 ng/mL										
EE (%)	77	77	83	83	83	87	86	88	87	89
ME (%)	76	76	72	73	88	89	85	88	81	82
PE (%)	58	58	60	61	72	77	73	77	71	73
<b>Blank 2 (postmortem)</b>										
25 ng/mL										
EE (%)	98	100	100	103	96	103	100	103	109	112
ME (%)	72	71	68	69	101	102	102	106	54	54
PE (%)	70	71	69	71	98	104	102	108	59	60
250 ng/mL										
EE (%)	88	88	95	95	86	89	91	91	87	89
ME (%)	72	70	67	66	85	85	81	84	80	80
PE (%)	63	62	63	63	73	76	74	77	69	72
<b>Blank 3 (postmortem)</b>										
25 ng/mL										
EE (%)	97	101	101	104	95	99	96	99	101	102
ME (%)	82	80	78	79	113	114	116	119	62	62
PE (%)	80	81	79	82	108	114	111	118	62	63
250 ng/mL										
EE (%)	83	84	91	93	86	88	90	92	84	85
ME (%)	79	76	72	71	89	89	85	88	84	84
PE (%)	65	64	66	65	76	78	77	81	71	71
<b>Blank 4 (postmortem)</b>										
25 ng/mL										
EE (%)	85	85	88	87	87	91	89	91	89	95
ME (%)	76	74	73	74	99	98	100	104	60	57
PE (%)	64	63	64	65	86	90	89	94	54	54
250 ng/mL										
EE (%)	78	79	87	87	85	87	91	91	82	84
ME (%)	80	77	71	71	88	88	82	85	88	88
PE (%)	62	61	62	62	75	77	74	77	72	74

Table 4 (continued)

Analyte	U-47700		U-47700- <sub>d6</sub>		N-Desmethyl-U-47700		N-Desmethyl-U-47700- <sub>d3</sub>		N,N-Didesmethyl-U-47700	
	329.1 → 173.0	329.1 → 284.1	335.2 → 172.9	335.2 → 284.0	315.1 → 144.9	315.1 → 172.9	318.1 → 147.9	318.1 → 175.9	301.1 → 172.9	301.1 → 270.0
Blank 5 (postmortem)										
25 ng/mL										
EE (%)	88	90	90	92	89	94	90	91	87	88
ME (%)	73	71	71	71	100	99	102	106	60	60
PE (%)	64	63	64	65	89	92	92	96	53	53
250 ng/mL										
EE (%)	76	75	86	86	86	87	92	93	88	88
ME (%)	72	71	66	66	82	82	79	81	77	78
PE (%)	55	53	56	57	70	72	72	75	67	68

<sup>a</sup>Blank whole blood purchased from the Regional Blood Centre Donation

within the cyclic ring and may happen in various positions of the ring, several positional isomers may be generated. The analysis of urine autopsy samples taken from five subjects confirmed the hypothetical metabolic profile with *N*-desmethyl-U-47700 predominating. Unfortunately, the previous analyses of the metabolites in urine samples were qualitative and did not include blood samples [17, 23, 24].

Thus, in analyzes of blood autopsy samples collected from the victims of fatal poisonings presented in this article, one can note confirmation of the metabolic profile of U-47700. The hypothetical metabolic profile of U-47700 is presented in Fig. 2.

Various investigators, including Kubo et al. [25] and Chung et al. [26], proved that fatal cases are an extremely valuable source of knowledge about trends in the use of various drugs in individual countries around the world. Analyzing the subject literature, the similarity in cross-section in NPS-related deaths discussed in this paper can be seen in the work of Kubo et al. [25], who presented the problem in a large research material covering 94 deaths. In most of reports published at the beginning of 2017, however, there were no deaths caused by synthetic opioids, which appeared in the world and also in Poland in the first half of the same year, and could be a novelty on the drug market.

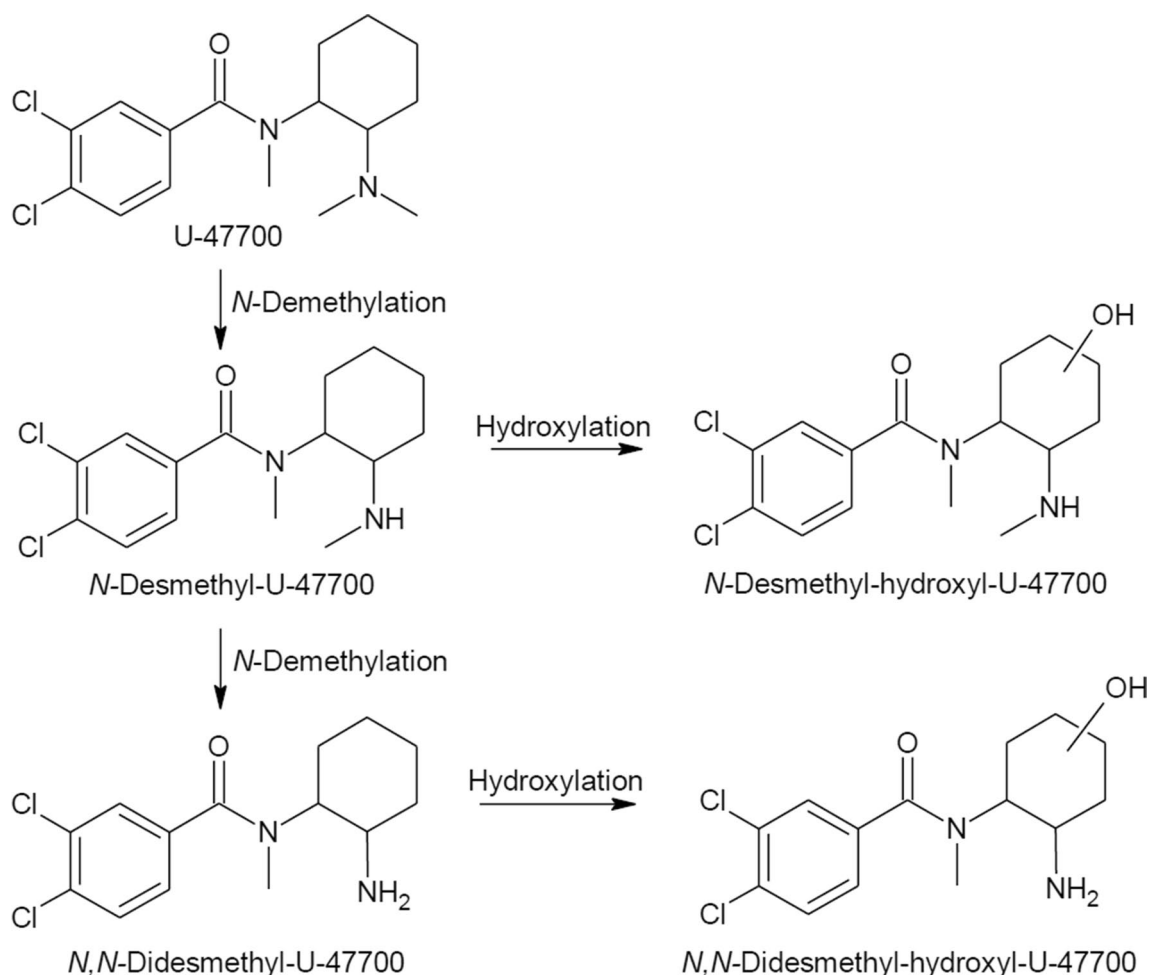
In this article, we have quantified U-47700 and its two metabolites *N*-desmethyl-U-47700 and *N,N*-didesmethyl-U-47700 in blood samples collected from 12 fatal poisoning victims. Being different from the concentrations of a xenobiotic and its metabolites, their concentrations seem to reflect toxicities being present at the time of cardiac arrest. This means that the values are very useful to confirm the cause of death and to estimate the antemortem-to-death interval. To our knowledge, this is the first trial to quantify phase I metabolites of U-47700 in authentic human blood samples.

## Conclusions

We have presented, for the first time, a report on the comprehensive assessment of an NSO termed U-47700, and its two metabolites *N*-desmethyl-U-47700 and *N,N*-didesmethyl-U-47700 in autopsy blood samples collected from 12 cases of fatal poisonings. For this purpose, we developed a sensitive and high-selective SPE/LC-ESI-MS/MS method in the validation process. The values quantified by the present method for U-47700 and its two metabolites in human blood samples seem very useful to confirm the cause of death and also to estimate the antemortem-to-death interval after consumption of U-47700.

**Table 5** Results of toxicological analyses of U-47700, *N*-desmethyl-U-47700 and *N,N*-didesmethyl-U-47700 in authentic whole blood samples collected at autopsies

No.	Case	Analyte	Concentration (ng/mL)	Other (ng/mL)
1	Male, 24 years old Found dead in his apartment	U-47700	208	AB-CHMINACA (0.15)
		<i>N</i> -Desmethyl-U-47700	398	
		<i>N,N</i> -Didesmethyl-U-47700	132	
2	Male, 26 years old Found unconscious in his apartment, dead after ineffective resuscitation in hospital	U-47700	1140	Bromazepam (100), trazodone (230), hydroxyzine (10), cetirizine (80), chlorprotixen (12), phenobarbital (1400)
		<i>N</i> -Desmethyl-U-47700	248	
		<i>N,N</i> -Didesmethyl-U-47700	178	
3	Male, 16 years old Admitted to hospital after school activities, dead after ineffective resuscitation	U-47700	854	Hydroxyzine (20), cetirizine (60), 4-CMC (20)
		<i>N</i> -Desmethyl-U-47700	342	
		<i>N,N</i> -Didesmethyl-U-47700	418	
4	Male, 24 years old Found dead in his apartment	U-47700	254	0.6 % of ethyl alcohol
		<i>N</i> -Desmethyl-U-47700	34	
		<i>N,N</i> -Didesmethyl-U-47700	–	
5	Male, 37 years old Found dead near the shopping mall	U-47700	779	0.7 % of ethyl alcohol
		<i>N</i> -Desmethyl-U-47700	184	
		<i>N,N</i> -Didesmethyl-U-47700	317	
6	Male, 25 years old Found dead in his apartment	U-47700	166	$\Delta^9$ -THC (1.6), THC-COOH (8), alprazolam (92), 7-aminoclonazepam (22), nordiazepam (16), lamotrigine (2600), quetiapine (88), mianserin (180), paroxetine (230), <i>N</i> -ethylhexedrone (53), 5F-MDMB-PINACA <i>O</i> -desmethyl acid metabolite (0.40), AB-FUBINACA <i>O</i> -desmethyl acid metabolite (2.30)
		<i>N</i> -Desmethyl-U-47700	38	
		<i>N,N</i> -Didesmethyl-U-47700	18	
7	Male, 25 years old Found dead on the sidewalk	U-47700	24,000	1.8 % of ethyl alcohol
		<i>N</i> -Desmethyl-U-47700	7520	
		<i>N,N</i> -Didesmethyl-U-47700	1250	
8	Male, 58 years old Admitted to hospital because of respiratory depression and cardiological problems, drugs abuse, dead after ineffective resuscitation	U-47700	83	Tramadol (370), diazepam (37), nordiazepam (25), 7-aminoclonazepam (10), haloperidol (14), sertraline (27), propranolol (10), paracetamol (2800), PV8 (100)
		<i>N</i> -Desmethyl-U-47700	150	
		<i>N,N</i> -Didesmethyl-U-47700	45	
9	Male, 35 years old Found dead in the toilet at the gas station	U-47700	204	2.9 % of ethyl alcohol
		<i>N</i> -Desmethyl-U-47700	2	
		<i>N,N</i> -Didesmethyl-U-47700	–	
10	Male, 39 years old Drug dealer. Found dead in a bathtub in his apartment	U-47700	83	AB-FUBINACA <i>O</i> -desmethyl acid metabolite (2.44)
		<i>N</i> -Desmethyl-U-47700	260	
		<i>N,N</i> -Didesmethyl-U-47700	1947	
11	Male, 26 years old He murdered his girlfriend with a knife and then committed suicide by drug overdose	U-47700	1935	AB-FUBINACA <i>O</i> -desmethyl acid metabolite (110)
		<i>N</i> -Desmethyl-U-47700	250	
		<i>N,N</i> -Didesmethyl-U-47700	410	
12	Female, 26 years old She was murdered by her boyfriend with a knife	U-47700	357	AB-FUBINACA <i>O</i> -desmethyl acid metabolite (196)
		<i>N</i> -Desmethyl-U-47700	200	
		<i>N,N</i> -Didesmethyl-U-47700	200	



**Fig. 2** Probable metabolic pathways for U-47700

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### Compliance with ethical standards

**Conflict of interest:** The authors have no conflicts of interest to declare.

**Ethical approval:** This article does not contain any studies with living human participants or animals performed by any of the authors.

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