Challenges Related to Three Cases of Fatal Intoxication to Multiple Novel Synthetic Opioids

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Abstract

In the last two decades, a large increase in opioid overdose death rates has been recorded in North America. This phenomenon, related to the misuse of prescription opioids, has been dubbed an "opioids crisis". Recent years have seen the entrance of novel synthetic opioids (NSO) on the market, compounding the fatal intoxications issue. This brings several challenges for forensic toxicology laboratories: an increased number of cases, a large number of novel structurally similar compounds to include in screening analytical methods, the low concentration of drugs in biological fluids, and the challenging interpretation in the absence of sufficient literature. Three cases of fatal intoxication highlighting those challenges are presented, complete with post-mortem concentrations in cardiac blood, femoral blood and urine. Toxicological screening and quantitative analyses were performed on the biological specimens. In the first and second cases, furanylfentanyl, U-47700 and 4-anilino-N-phenethylpiperidine (4-ANPP) were detected at similar concentrations in cardiac blood. In the third case, a total of seventeen different NSO were detected. All intoxications showed a combination of NSO and other drugs. These three cases appear to be the harbinger of an increased NSO prevalence in the province of Québec, Canada.

1. Introduction

In recent years, North America has experienced a considerable growth in mortality related to misuse of prescribed opioids [1, 2, 3, 4] that has been dubbed an "opioids crisis". However, the number of deaths resulting from commonly prescribed opioids has been stable since 2012 in the United States and Canada [4, 5, 6]. The increase of fatal opioids overdoses observed since then can be tied to the appearance of novel synthetic

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opioids (NSO) on the market, alone or in combination with other drugs, mainly heroin [4]. These new psychoactive substances (NPS) are derivatives of well-known prescribed opioids, failed pharmaceuticals or medicinal products [7]. Amongst the reasons for NPS' production is the will to generate substances that are not controlled by national or international regulations, but still generate psychoactive effects for the user [7].

NSO are generally categorized as fentanyl analogs (e.g., acetylfentanyl, butyrylfentanyl, furanylfentanyl) or as non-fentanyl compounds (e.g., U-47700, AH-7921, mitragynine) [3]. Even though some non-pharmaceutical fentanyls (e.g., alpha-methylfentanyl, 3-methylfentanyl) were seen on the market as early as 1979 [8, 9], the recent diversification of NSO intertwined with the opioids epidemic has resulted in a major public health concern in North America. In Canada, 2,978 deaths were related to opioids during 2016. Of these, 55% were related to fentanyl and NSO specifically. One year later, in 2017, the number of deaths had increased by 34% to 3,987, and NSO and fentanyl were involved in 72% of cases [2]. The crisis shows no sign of slowing down in the United States either. Between 2015 and 2016, the death rate from synthetic opioids and fentanyl (excluding methadone) doubled, bringing the percentage of drug overdose deaths involving opioids to 66% [4].

For forensic toxicology laboratories, NPS and NSO bring major challenges. NSO's structural similarity demands highly specific analytical methods, and their low biological concentrations call for high sensitivity [10, 11, 12]. In addition, new NSO compounds regularly appear on the market [10], requiring an adjustment in analytical methods and rapid production of new certified reference materials (CRMs) [7].

Three cases of intoxication highlighting those challenges are presented therein. These fatal overdoses occurred between January 2018 and February 2018 in the province of Québec, Canada. Detailed results of quantitative analysis of various post-mortem matrices and qualitative analysis of physical evidence collected at the scene are also included.

2. Methods

2.1. Sample collection and storage

A complete autopsy was performed on each victim. Peripheral blood from the femoral vein, cardiac blood pooled from both ventricles, urine, gastric contents and vitreous humor were collected for toxicological analyses. Femoral blood and vitreous humor were stored in a BD Vacutainer storage tube (part number 367001, Franklin Lakes, USA) containing 100 mg of sodium fluoride and 20 mg of potassium oxalate as a preservative. Cardiac blood and urine were stored in 50 mL polypropylene tubes (Sarstedt, part number 62.559.001, Newton, NC, USA). 500 mg of a mixture of sodium fluoride and phosphate oxalate (5:1, wt:wt) was added to cardiac blood as preservative. All samples were stored at 4 °C until completion of the toxicological analyses. Since very little is known about the stability of NSO in biological matrices, occurrence of degradation is not precluded and this should be kept in mind when interpreting concentrations.

2.2. Toxicological analysis

Blood alcohol content was determined in biological matrices via gas chromatography coupled to a flame ionization detector (GC-FID) (Agilent 6890 A and 6890 N) method. Briefly, 100 μL of biological matrix is mixed with 1 mL of a tert-butanol solution (5 mg/mL) and heated to equilibrium in a sealed vial; 1 mL of the headspace is injected. Other common volatile substances (isopropanol, acetone, methanol and n-propanol) were also screened for.

Femoral blood, cardiac blood and urine samples extracts were submitted to a high throughput liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) screening and quantification assay covering 144 xenobiotics [13] including the NSO butyrylfentanyl, furanylfentanyl and carfentanil. A 100 μL volume of biological matrix was mixed with an internal standard solution comprising 36 deuterated compounds and precipitated with 400 μL of a 7:3 (v:v) acetonitrile:acetone mixture. A 25 μL volume of supernatant was diluted in 180 μL of 0.2% formic acid prior to the injection of a 5 μL aliquot on an Agilent Zorbax Eclipse Plus C18 column (2.1 $mm \times 100\, mm$, 3.5 μm). This method was carried out on an Agilent 1200 HPLC coupled to a Sciex 5500 QTrap operated in positive electrospray ionization mode with multiple reaction monitoring (MRM).

General unknown screening was carried out using a solid phase extraction preparation (Oasis HLB cartridges, Waters, part number WAT094226, Mississauga, ON, Canada) followed by a gas chromatography coupled with mass spectrometry and nitrogen-phosphorus detectors (GC-MS/NPD) analysis (Agilent 7890/5975 MSD). National Institute of Standards and Technology (NIST), Cayman Chemical and local spectrum libraries were mined for possible matches.

All quantitative methods were validated according to the Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines [14], and were accredited under ISO 17025:2005 and CAN-P 1578 standards.

2.3. Quantification of NSO

Confirmation and quantification of NSO were performed via LC-MS/MS by NMS Labs (Willow Grove, PA, USA) [15], with an extended method heavily based on [16].

3. Results

3.1. Case 1

A 46-year-old woman, known alcoholic, was found dead in her unsanitary apartment. Putrefactive changes were apparent on the body, such as a large green stain across her abdomen.

The woman was 158 cm tall and weighed 55 kg. A complete autopsy and histological examination of the heart, liver, kidneys and endometrium tissues were performed.

Overall, no traumatic or pre-existing lesions could be considered as the cause of death.

Toxicological analysis was performed on urine as well as cardiac and femoral blood. Results, shown in Table 1, highlight the consumption of methamphetamine, cocaine, methadone at recreational levels and the NSO furanylfentanyl and U-47700. 4-ANPP, a metabolite and precursor of fentanyl related NSO [17, 18], was also detected.

The cause of death was determined to be drug polyintoxication.

3.2. Case 2

A 30-year-old man was found in cardiorespiratory arrest in his father's kitchen. Resuscitation attempts were unsuccessful. The victim had a history of drug and alcohol addiction. He had been in jail a few weeks before the incident, and had reported being dependent to oxycodone since. The man had a record of active prescriptions for pregabalin, methylphenidate and clonazepam. At the time of the incident, about 10 clonazepam tablets were reported missing.

The man was 172 cm tall and weighed 84 kg. Six broken ribs observed in autopsy were attributed to the reanimation procedures. Furthermore, the coronary artery presented moderate to severe atherosclerosis which was confirmed by histological examinations of the tissue. No other traumatic lesions were found.

Toxicological analysis was performed on urine as well as cardiac and femoral blood. Results (Table 1) reveal the presence of several prescription drugs and drugs of abuse, including the NSO furanylfentanyl, 4-ANPP and U-47700. Δ9-tetrahydrocannabinol (THC), clonazepam, pregabalin and methylphenidate were found at therapeutic/recreational levels, whereas diazepam, naproxen and nortriptyline were present in trace amounts.

A program for free access to naloxone in drugstores was in place at the time of the events. Nevertheless, there is no toxicological evidence of naloxone administration in this case.

The cause of death was declared to be a polyintoxication with the likely contribution of coronary atherosclerosis.

Interestingly, deceased individuals in cases 1 and 2 were both found in the same city, and had similar blood concentrations of furanylfentanyl and U-47700. This suggests the two cases might be related (e.g., victims knew each other or had the same drug dealer), but this possibility was not further investigated by law enforcement.

3.3. Case 3

A 29-year-old man was found dead with a syringe in his hand and empty bottles of steroid nearby. Large putrefactive changes were apparent on the body. The victim had a history of drug consumption, but had undergone a drug addiction rehabilitation

Table 1: Analytical findings in each fatality

Case	Analyte	Cardiac blood (ng/mL)	Femoral blood (ng/mL)	UM* (± %)	Urine		
1	Methamphetamine	664	198	15	+		
	Amphetamine	103	31	14	+		
	Cocaine	12	15	15	+		
	Benzoylecgonine	840	725	15	+		
	Methadone	154	75	17	+		
	EDDP**	27	10	22	+		
	4-ANPP**	33	NA***	NA	+		
	Furanylfentanyl	14	NA***	NA	+		
	U-47700	54	NA***	NA	+		
Case	Analyte	Cardiac blood (ng/mL)	Femoral blood (ng/mL)	UM* (± %)	Urine		
2	Clonazepam	126	94	18	+		
	Methylphenidate	19	15	14	+		
	Pregabalin	5,201	5,571	10	+		
	THC**	17	18	16	–		
	THC-OH**	7.7	3.3	14	–		
	THC-COOH**	199	32	14	+		
	Naproxen	3,633	2,118	12	+		
	Nortriptyline	42	14	16	+		
	Diazepam	14	11	13	–		
	Oxazepam-glucuronide	-	-	-	+		
	Temazepam-glucuronide	-	-	-	+		
	Benzoylecgonine	-	-	-	+		
	Naproxen O-desmethyl	-	-	-	+		
	4-ANPP**	32	18	NA	+		
	Furanylfentanyl	2.4	0.89	NA	+		
	U-47700	45	26	NA	+		
Case	Analyte	Cardiac blood (ng/mL)	Femoral blood (ng/mL)	UM* (± %)	Urine	Syringe	White powder
3	Alprazolam	20 (B)	12 (B)	17	+ (A, B)	-	+ (B)
	Quetiapine	940 (B)	58 (B)	15	+ (A, B)	-	-
	Gabapentin	+ (B)	15,000 (A, B)	11	+ (A, B)	-	-
	Duloxetine	+ (B)	+ (B)	-	+ (B)	-	-
	4-ANPP**	5.1 (C, D)	9.7 (A, C, D)	NA	+ (C, D)	-	+ (D)
	p-fluoro(iso)butyrylfentanyl	31 (C, D)	27 (A, C, D)	NA	+ (A, C, D)	+ (A)	+ (A, C, D)
	Methoxyacetylfentanyl	70 (C)	14 (A, C)	NA	+ (A, C)	+ (A)	+ (A, C)
	Cyclopropylfentanyl/ crotonylfentanyl	0.15 (C, D)	0.10 (C, D)	NA	+ (C, D)	-	+ (D)
	Cyclopropyl norfentanyl/ crotonyl norfentanyl	-	-	-	+ (D)	-	-
	U-47700	+ (D)	+ (D)	-	+ (C, D)	-	+ (A, D)
	Acetylfentanyl	+ (D)	+ (D)	-	+ (D)	-	+ (B, D)
	Despropionyl fluorofentanyl	+ (D)	+ (D)	-	+ (D)	-	+ (A, D)
	N-methyl U-47931 E	+ (D)	-	-	+ (D)	-	+ (D)
	U-47931 E	-	-	-	+ (D)	-	+ (A, D)
	Isobutyrylfentanyl	-	-	-	+ (C, D)	+ (B)	+ (A, B, D)
	p-fluorofentanyl	-	-	-	+ (C)	-	+ (D)
	Valerylfentanyl	-	-	-	+ (C)	-	-
	Furanylfentanyl	-	-	-	-	-	+ (B, D)
	U-49900	-	-	-	-	-	+ (A, D)
	U-48800	-	-	-	-	-	+ (A, D)

*UM: Uncertainty of measurement.

**EDDP: 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine, 4-ANPP: 4-anilino-N-phenethylpiperidine, THC: Δ 9-tetrahydrocannabinol, THC-OH: 11-hydroxy-Δ9-tetrahydrocannabinol, THC-COOH: 11-nor-9-carboxy-Δ9-tetrahydrocannabinol.

***Not enough sample to conduct analysis.

A: Detected by a general unknown GC-MS screening.

B: Detected by a LC-MS/MS screening and quantification method.

C: Detected by LC-MS/MS by NMS Labs.

D: Detected by a LC-MS/MS NSO screening method in development.

program. The man was 172 *cm* tall and weighed 89 *kg*. No traumatic or pre-existing lesions were noted during autopsy.

In the initial toxicological analysis, femoral and cardiac blood as well as urine were analyzed using the high throughput LC-MS/MS screening and quantification method. General unknown GC-MS screening was also performed on femoral blood and urine. These analyses revealed alprazolam, quetiapine and gabapentin at therapeutic levels (Table 1), as well as the presence of duloxetine. Three weeks later, evidence collected on the scene was sent to the forensic laboratory by police investigators for chemical analysis. The general unknown GC-MS assay of this white powder and syringe using the newly updated Cayman Chemical GC-MS Library revealed the presence of several NSO, all listed in Table 1.

These new results changed the outlook on the case, and prompted a retrospective analysis of femoral blood GC-MS chromatograms with the newly updated Cayman Chemical GC-MS library. This new data mining revealed the presence of para-fluoro(iso)butyrylfentanyl, methoxyacetylfentanyl, and 4-ANPP. The two first were also identified in urine, along with p-fluorofentanyl, U-47700, isobutyrylfentanyl, butyrylfentanyl and valerylfentanyl. These biological matrices were therefore sent for confirmation and quantitative analysis at NMS Labs. Complete results are reported in Table 1.

A later analysis with an LC-MS/MS NSO screening method under development revealed several additional NSO both in the white powder and the biological matrices (Table 1). Although butyrylfentanyl was initially identified in the white powder by the high throughput LC-MS/MS assay, further investigation using an LCMS/MS method in development revealed that isobutyrylfentanyl was actually present in the sample (Figure 1). Similarly, U-51754 identified by GC-MS was revealed to actually be U-48800.

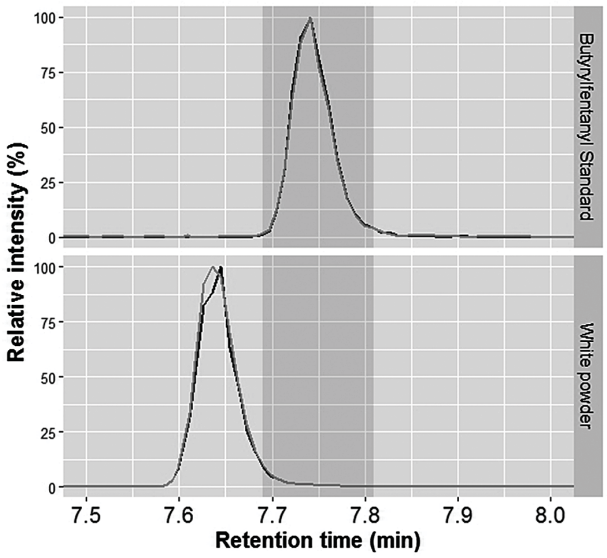
Para-fluoro(iso)butyrylfentanyl, cyclopropylfentanyl/crotonylfentanyl, despropionyl para-fluorofentanyl and N-methyl U-47931 E were identified as the main components of the white powder, with a proportion exceeding 10% (w:w). All other NSO can be considered to be present at trace levels in the powder, although it should be noted that a quantitative evaluation was not possible for methoxyacetylfentanyl due to the lack of available standard at the moment of analysis.

Cause of death was determined to be a polyintoxication.

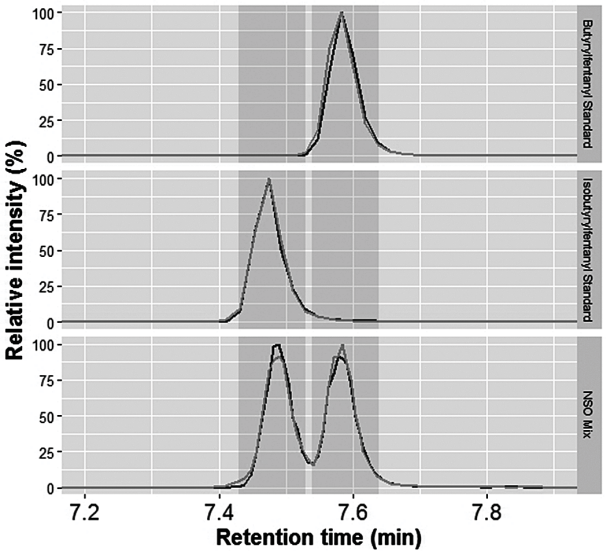
4. Discussion

The arrival of NSO on the recreational market is an unprecedented phenomenon that generates several challenges for forensic laboratories. Forensic toxicologists have to deal with an increasing number of cases, often presenting several analytical and interpretation challenges.

In the United States, the misuse and addiction to opioids has spread throughout the country, although certain regions are much more impacted than others [19]. In Canada,



(a) LC-MS/MS Chromatograms of the selected MRM transitions (m/z 351.2 − > 188.0 and m/z 351.2 − > 105.0) for the butyrylfentanyl reference standard and the white powder exhibit under the high throughput LC-MS/MS screening.



(b) LC-MS/MS Chromatograms of the selected MRM transitions for the butyrylfentanyl (m/z 351.2 − > 188.0 and m/z 351.2 − > 105.0) and isobutyrylfentanyl (m/z 351.2 − > 188.0 and m/z 351.2 − > 105.0) reference standards analyzed individually, and together as part of an NSO mix under the targeted NSO LC-MS/MS analysis method currently in development.

Figure 1: LC-MS/MS chromatograms

the opioids crisis unfurled west to east, beginning its route in British Columbia and then the prairies (Alberta, Saskatchewan, Manitoba) [20]. The province of Ontario reported in 2017 a sudden increase in opioid related deaths [20]. Until now, it seemed like the province of Québec was spared. Opioid related death rates were relatively stable [2], while the prevalence of opioids in driving under the influence cases was significantly lower than in other provinces [21]. Furthermore, opioid related hospitalizations rate is still the lowest in the country [6]. Even though the number of opioids prescription per capita remains similar to other provinces, the quantity prescribed is lower, yielding the lowest country-wide defined daily doses [6]. NSO have been detected in several cases (drug seizures, driving under the influence of drugs and overdoses) in Canada since 2011 [20]. However, their appearance has mostly mirrored the development of the opioids epidemic. In that sense, the three cases presented therein might be a harbinger of the crisis to come in the province of Québec.

In addition to an increasing number of cases, the diversification of NSO can also bring supplemental analytical challenges due to their structural similarity. The third case presented here is a good example of this problematic aspect. Two misidentification occurred in the first analysis of the white powder: isobutyrylfentanyl, which was not initially in the LC-MS/MS method scope, was mistaken for the targeted butyrylfentanyl (Figure 1) and U-48800 was erroneously identified as U-51754 by GC-MS spectrum database comparison. Analysis by an LC-MS/MS NSO screening method under development allowed discrimination of the compounds. This type of confusion is likely to be observed with many pairs of NSO isomers, such as para-fluorobutyrylfentanyl and para-fluoroisobutyrylfentanyl, or cyclopropylfentanyl and crotonylfentanyl. This can potentially lead to misidentification of NSO, with serious issues in terms of interpretation and accuracy of published data.

The third case also emphasizes the importance of maintaining and updating GC-MS spectra libraries to keep up with the pace of NSO apparition on the market. Initially, GC-MS appears like a method of choice for screening illicit drugs in this constantly evolving market, due to the ability to compare results to worldwide shared spectra libraries. However, GC-MS limits of detection for analysis targeting NSO are typically between 1 and 10 ng/mL [22]. A similar situation arises with high resolution mass spectrometers (HRMS) (e.g., time of flight mass spectrometers (Q-TOF-MS)) which proved their reliability in doing non-targeted and retrospective analysis. Despite that, like GC-MS, HRMS instruments are known to be less sensitive than triple quadrupole systems in target mode analysis [23]. As numerous sources reported concentrations under 2 ng/mL for both postmortem [23, 24, 25, 26] or antemortem [27, 28] cases, LC-MS/MS analyses remain relevant to detect biological concentrations of most potent NSO like carfentanil.

Even when laboratories do have the technology and the capability to monitor NSO in circulation, interpretation challenges still need to be addressed. Biological concentrations reported here are similar to those found in other publications. For example, Mohr et al. reported on five polyintoxication cases positive for both furanylfentanyl (2.5–76 ng/mL, mean 26 ng/mL) and U-47700 (17–490 ng/mL, mean 253 ng/mL) [29], akin to what was found in cases 1 and 2 here. Martucci et al. also reported a case with furanylfentanyl at concentrations of 1.9 ng/mL in peripheral blood and 2.8 ng/mL in cardiac blood [30]. But the contribution of an NSO in an intoxication fatality is dif-

ficult to assess and could not reliably be done here, mainly because only a handful of studies containing post-mortem concentrations have been reported yet for human subjects [3, 31, 32]. Therefore, suspected effects and concentrations have to be inferred from animal studies. Nonetheless, these substances are reputed to easily cross the blood brain barrier and interact with opioid receptors. Like traditionally prescribed opioids, they can cause the classical opioid toxidrome including respiratory depression, miosis and altered mental state [22, 31, 32, 33].

Beyond their individual psychoactive effects, the combined action of NSO between themselves or with other drugs remains to elucidate. This is an important topic to consider since NSO can be used as adulterant in counterfeit opioids [32]. Furthermore, the mixture of several NSO is a common theme in the three cases presented here. In the third case, an impressive number of 17 different NSO were detected. To the best knowledge of the authors, this is the first case reported in literature involving as many different NSO. Since the beginning of 2018, analysis of pieces of evidence and biological specimens in our laboratory routinely reveal NSO combinations.

5. Conclusions

This paper presents the concentration values in postmortem cases (femoral blood, cardiac blood and urine) for the NSO 4-ANPP, furanylfentanyl, U-47700, p-fluoro(iso)butyrylfentanyl, methoxyacetylfentanyl and cyclopropylfentanyl (Table 1), which will contribute to build a comprehensive database for toxicological interpretation.

The unprecedented spread of NPS and NSO across the world brings several challenges to the forensic toxicology community. The increasing number of cases, potential for misidentification and low biological concentrations require sensitive, specific, extensive and high throughput LC-MS/MS methods. These reliable analyses will be crucial in providing relevant toxicological results to help sort out forensic cases involving NSO. Additionally, they can be used as an investigation tool to better understand the trends of the recreational markets.

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