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CASE REPORT

Determination of ocfentanil and W-18 in a suspicious heroin-like powder in Belgium

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Abstract

Purpose Since the early 2010s, synthetic opioids have been on the rise in the illicit drug markets of North America and Europe, often as adulterants or substitutes for heroin. Ocfentanil, an early-onset fentanyl analogue, has been implicated in several fatalities, predominantly in Europe. W-18 is more prevalent in Canada and the United States. We present the findings for an unknown brown powder, advertised and purchased as heroin, which was sent to our laboratory for analysis in the framework of the Belgian Early Warning System on Drugs.

Methods The sample was screened using liquid chromatograph coupled to a diode array detector and gas chromatograph coupled to a single quadrupole mass spectrometer. The findings were confirmed by liquid chromatography—triple quadrupole mass spectrometry.

Results No heroin, 6-monoacetylmorphine or morphine was detected. Our analysis detected two new psychoactive substances: the synthetic opioid ocfentanil (1.6% m/m), and W-18 (0.3% m/m). The ocfentanil concentration was similar to those found in previous reports. No reference concentrations have been published for W-18. The presence of these two new psychoactive substances together in the same powder is unprecedented and prompted a warning by the Belgian Early Warning System on Drugs.

Conclusion The unknown powder tested positive for ocfentanil and W-18. To the authors' best knowledge this is the first case of the combined detection of these two new psychoactive substances in the same powder. We additionally demonstrate the advantage of building an in-house reference library beyond retention time.

Keywords New psychoactive substances · Synthetic opioids · Fentanyl analogues · Ocfentanil · W-18

Abbreviations

CSL	Cayman Spectral Library
dMRM	Dynamic multiple reaction monitoring
DAD	Diode array detector
GC	Gas chromatography
LC	Liquid chromatography
MS	Mass spectrometry
QQQ	Triple quadrupole mass spectrometer
RT	Retention time

Introduction

Synthetic opioids, most of them fentanyl analogues, made their first appearance on the illicit drug market in the 1970s–1980s. Governmental control measures, such as the introduction of a broad fentanyl analogue legislation in the United States, prevented the widespread emergence of these substances. However, since 2014 an increasing number of novel fentanyl derivatives have been sold online. Most of these analogues were produced in China, where legislation on fentanyl analogues was inadequate or non-existent at the time. A 72% increase in synthetic opioid deaths has been reported in the United States from 2014 to 2015 [1–3]. Although their share on the European drug market

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is reportedly minor (around 2% of the seized new psychoactive substances reported), 38 new synthetic opioids have been detected since 2009, 13 in 2017 alone [4]. Estimated 1400 opioid analogues have been synthesised to date; most are sold as powders or tablets, however increasing number are available in liquid formulations (e.g. nasal sprays or solutions intended for e-cigarettes) [1, 3]. The inherent high potency of most fentanyl analogues and associated low active dose pose a particular risk for recreational opioid users, especially when the user is unaware of the identity of the substance (since they are often sold as heroin). Unexpected or severe side effects can also occur, as most of the compounds have never been pharmacologically evaluated [5, 6].

One such fentanyl analogue, ocfentanil, was first developed in 1986, however it was never approved for medical use. Its potency is estimated to be 100 to 200 times that of morphine (twice that of fentanyl) [5, 7, 8]. Users describe experiencing typical opiate-like effects such, as euphoria and relaxation, but with a more rapid onset (after 3 min) and a shorter duration of action (around 3 h) compared to heroin. Withdrawal symptoms occur earlier on as well [9]. The majority of intoxications have occurred in Europe where it is used as an adulterant or as a substitute for heroin. Some cases have also been reported in Canada and the United States [1, 9]. Ocfentanil is not included in the United Nations drug conventions and is therefore not subject to international control, unlike most other opioids. In response, several European countries, as well as China and Japan, have scheduled it as an illicit drug. The United States recently listed ocfentanil as a Schedule I drug because of its imminent hazard to public safety [9, 10].

W-18 belongs to the W-series of synthetic opioids and as such cannot be classified as a fentanyl analogue. It was developed by the University of Alberta (Canada) in 1981, who patented the drug in 1984 (US Patent 4468403). It was never commercialised as a pharmaceutical analgesic and scarce data exist regarding its pharmacokinetic and pharmacodynamic properties. Initial reports characterised W-18 as having a potency of up to 10,000 times that of morphine. However, these reports were based solely on results from a mouse tail flick test, which indicates analgesic properties of a substance but does not provide further information on its receptor binding properties [11, 12]. A recent in vitro and in vivo laboratory animal study by Huang et al. found no noteworthy opioid receptor activity for W-18 or any of its metabolites, which they attributed to the significant differences from the core fentanyl structure [13]. Similarly, the Laboratory of Toxicology at Ghent University (Belgium) concluded W-18 to be devoid of any μ-opioid receptor activation potential using their in vitro bioassay, even at a concentration as high as 1 μM [14, 15]. However, other receptor activity cannot be excluded, neither can an added or synergistic—potentially toxic—effect in combination with opioids. Its continued presence in seized drugs gives further cause for concern. The Canadian government has ruled to classify this drug as a Schedule I compound, with the United States government considering temporarily scheduling it [11, 12]. Only two sightings of W-18 in Europe have been reported to the European Monitoring Centre for Drugs and Drug Addiction.

We present the case of an unknown brown powder, sold as "heroin #3", containing both ocfentanil and W-18. As far as the authors are aware this is the first reported detection of these two new psychoactive substances combined in a powder sold as heroin.

Materials and methods

Unknown powder

An unknown brown powder (Fig. 1) was acquired by the Antwerp Free Clinic and sent to our laboratory for analysis in the framework of the functioning of the Belgian Early Warning System on Drugs. The drug user in question admitted to buying the substance from an online darkweb marketplace where it was sold as "heroin #3".

Toxicological investigations

Following arrival in our laboratory, 10 mg of the unknown powder was dissolved in 10 mL methanol (Fisher Chemical, Loughborough, UK) and stored at − 20 °C until further analysis. As for the in-house systematic toxicological analysis, the sample was screened using a liquid chromatograph coupled to a diode array detector (DAD) and a

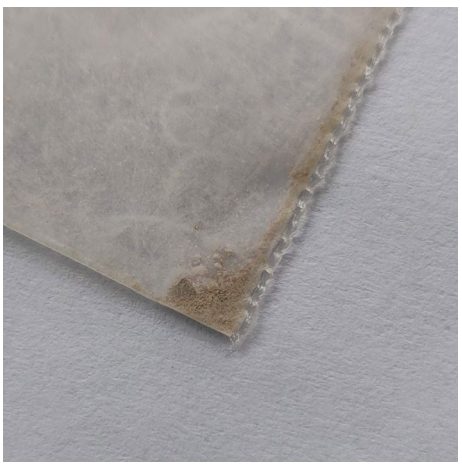

Fig. 1 Remnants of the unknown powder, thought to be heroin. Further analysis by our laboratory revealed the presence of ocfentanil and W-18 as well as some common cutting agents

gas chromatograph coupled to a single quadrupole mass spectrometer. Initial results were confirmed using a liquid chromatograph coupled to a triple quadrupole mass spectrometer (QQQ).

LC-DAD screening

1.0 µL of the dissolved powder was injected on an Agilent 1200 series liquid chromatograph, coupled to an Agilent G1315C DAD (Agilent Technologies, Santa Clara, California, US). Chromatographic separation was performed using a Zorbax Eclipse Plus C8 column (3.0 × 150 mm, 3.5 µm; Agilent Technologies), column temperature 40 °C. The mobile phase consisted of (A) 10 mM phosphate buffer (pH 2.3) and (B) acetonitrile:10 mM phosphate buffer (pH 2.3; 80:20). The flow rate was 0.615 mL/min; the gradient profile was as follows: initial conditions 5% B, from 0.0 to 19.0 min a linear increase to 100% B, from 19.0 to 23.0 min held at 100% B, from 23.0 to 24.0 min return to 5% B, and from 24.0 to 30.0 min re-equilibration at 5% B. Spectra were acquired at wavelengths between 200 and 380 nm. The Agilent ChemStation software (Agilent Technologies) was used for both data acquisition and data analysis.

GC–MS screening

In addition to the LC-DAD analysis, 1.0 µL of the sample was injected on an Agilent 6890N Network GC system, coupled to an Agilent 5973N mass spectrometer (Agilent Technologies), using helium as carrier gas. The injector was set in splitless mode at 300 °C. Analytes were separated on an Agilent DB-5ms column (30 m, 250 µm internal diameter, 0.25 µm film thickness). The initial column temperature of 70 °C was held for 2 min, then increased to 250 °C at 15 °C/min and subsequently to 315 °C at 5 °C/min (held for another 15 min). Mass spectra were acquired in continuous mode over a 50–660 *m*/*z* range with a 4.5 min solvent delay. Results were analysed using the MSD ChemStation software (Agilent Technologies).

LC-QQQ confirmation

Confirmation of the screening results was obtained by injecting 0.5 µL of the dissolved powder (diluted to 1 ng/µL) on an Agilent 1200 series liquid chromatograph, coupled to an Agilent 6410 QQQ (Agilent Technologies). The mobile phase consisted of (A) water + 0.1% formic acid (V/V), and (B) acetonitrile:water (90:10) + 0.1% formic acid (V/V). Gradient elution was applied going from 18% B to 40% B in 7.0 min, then to 95% B at 10.0 min. This was kept for 0.5 min before returning to starting conditions and allowing time for re-equilibration. The total run time was 15.0 min per sample. The analytical method used a Zorbax Eclipse Plus C8 column (2.1 × 150 mm, 3.5 µm; Agilent Technologies) to separate 34 fentanyl analogues and synthetic opioids, which were subsequently detected in dynamic multiple reaction monitoring mode (dMRM, Table 1). Results were interpreted using the Agilent MassHunter software (Agilent Technologies).

Databases

The in-house databases used for comparison have been acquired under the same conditions. Certified reference standards for both ocfentanil and W-18 were purchased from Chiron AS (Trondheim, Norway). For the GC–MS screening the freely online available Cayman Spectral Library (CSL; [16]) was used in addition to the in-house one.

Results

LC-DAD screening

The chromatogram in Fig. 2 shows the presence of six compounds following LC-DAD screening. A positive hit was defined as a match score ≥ 999 (as determined by the Agilent ChemStation software) as well as a maximum retention time (RT) difference of 0.2 min compared to the in-house library. Ocfentanil (RT 9.2 min, Figure S.1B) and W-18 (RT 16.4 min, Figure S.1C) were the opioids present. Semi-quantitative analysis based upon a comparison with the signal of pure standards indicated that they made up 1.6% (m/m) and 0.3% (m/m) of

Table 1 Dynamic multiple reaction monitoring settings for the LC-QQQ confirmation

Compound	Precursor ion (*m*/*z*)	Fragmentor voltage (V)	Product ions (*m*/*z*)	Ratio (%)*	Collision energy (eV)	Retention time (min)
Ocfentanil	371.2	125	<u>188.2</u>	100	20	4.3
			105.1	98	45	
W-18	422.1	150	<u>111.1</u>	100	50	10.5
			273.1	45	20	
			174.9	26	30	

*Theoretical ion ratios are proportional to that of the most abundant product ion (underlined)

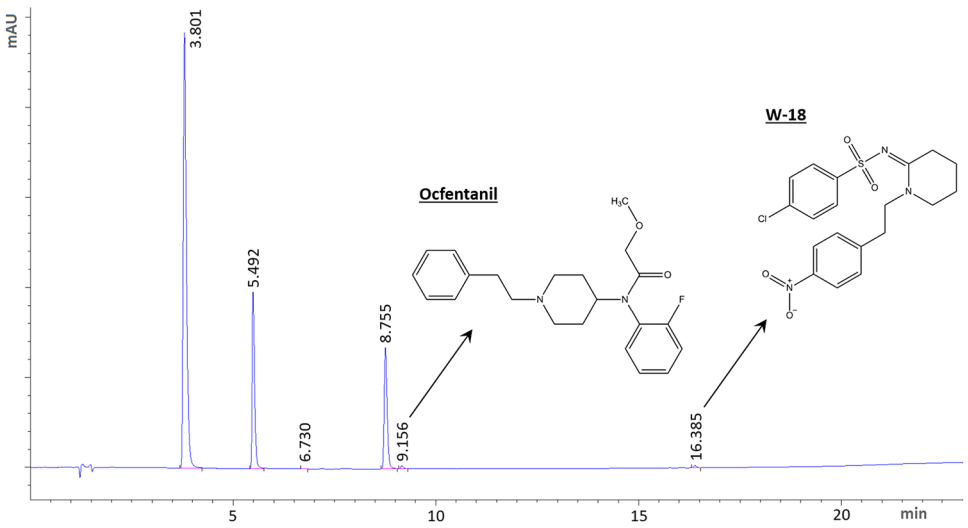

Fig. 2 Results from the LC-DAD analysis (screening). Obtained chromatogram (at a wavelength of 225 nm with 10 nm bandwidth) showing the presence of six compounds in the powder. Ocfentanil and W-18 elute at 9.2 min and 16.4 min respectively

the powder, respectively. Other substances identified were paracetamol (RT 3.8 min, 56% m/m), caffeine (RT 5.5 min, 24% m/m), quinine (RT 6.7 min, trace amounts) and benzoic acid (RT 8.8 min, 9.5% m/m), which were used as cutting agents.

GC–MS screening

All six compounds could be identified by GC–MS (Figure S.2A–C). The spectrum of ocfentanil matched with both that of the in-house database and the CSL. W-18 had a lower match factor with the CSL but was positively identified based on the mass spectrum and RT in the in-house database. Quinine was present in trace amounts only.

LC-QQQ confirmation

The results of the LC-QQQ analysis confirmed those of the two screening methods (Figure S.3A–C). A positive hit was defined as a peak with (1) all MRM transitions present, (2) ion ratios within ± 20% of those determined during method optimisation (Table 1) and (3) a retention time match with a subsequently injected reference standard. For ocfentanil the monitored transitions (with their actual ion ratios) were *m/z* 371.2 → 105.1 (100%)/188.2 (90%), for W-18 *m/z* 422.1 → 111.1 (100%)/273.1 (45%)/174.9 (25%). Retention times were identical to those of the reference standards, with ocfentanil eluting at 4.4 min and W-18 at 10.4 min. A blank methanol sample was run to verify the absence of any interfering transitions.

Discussion

The case sample went through our systematic toxicological analysis, consisting of LC-DAD and GC–MS screening, and LC-QQQ confirmation. Such an approach has previously been reported to be highly successful in the detection of synthetic opioids in seized materials [9]. A combination of (sensitive) detection methods is recommended for the low concentrations typically observed for these compounds and their often complex compositions. For the current case the results from all three analytical techniques were in agreement. Despite the powder being sold as heroin and its striking resemblance to heroin in both consistency and colour, no heroin, 6-monoacetylmorphine or morphine was detected. However all analyses agreed on the presence of the new psychoactive substances ocfentanil and W-18. The LC-QQQ method was selective for the detection of fentanyl, 31 of its analogues, W-15 and W-18. Apart from ocfentanil and W-18, no other synthetic opioids were detected. The other compounds present were the commonly used cutting agents paracetamol, caffeine and benzoic acid. Quinine was detected in trace amounts only. For the GC–MS screening, no match was found between the W-18 spectra of the sample and in the CSL, in part due to the absence of the molecular ion at *m/z* 421 (Fig. 3). A positive identification was based on the in-house library's spectrum and corresponding retention time. The mismatch between the spectra in the CSL and in our in-house library is of particular importance as it may have resulted in underreporting of cases. Although we were unable to determine the exact origin of this difference, it may in part be related to the (non-subtraction of) background signals in the CSL. We were also unable to verify the purity of the standards used in the CSL (the in-house database was created using certified reference standards). However, neither of these unequivocally explain the absence of the molecular ion in the sample. This highlights the advantage of creating an in-house reference database, besides the benefit of retention time, to ensure the highest level of confidence and accuracy in the findings. Despite there being the need to carefully interpret the results from

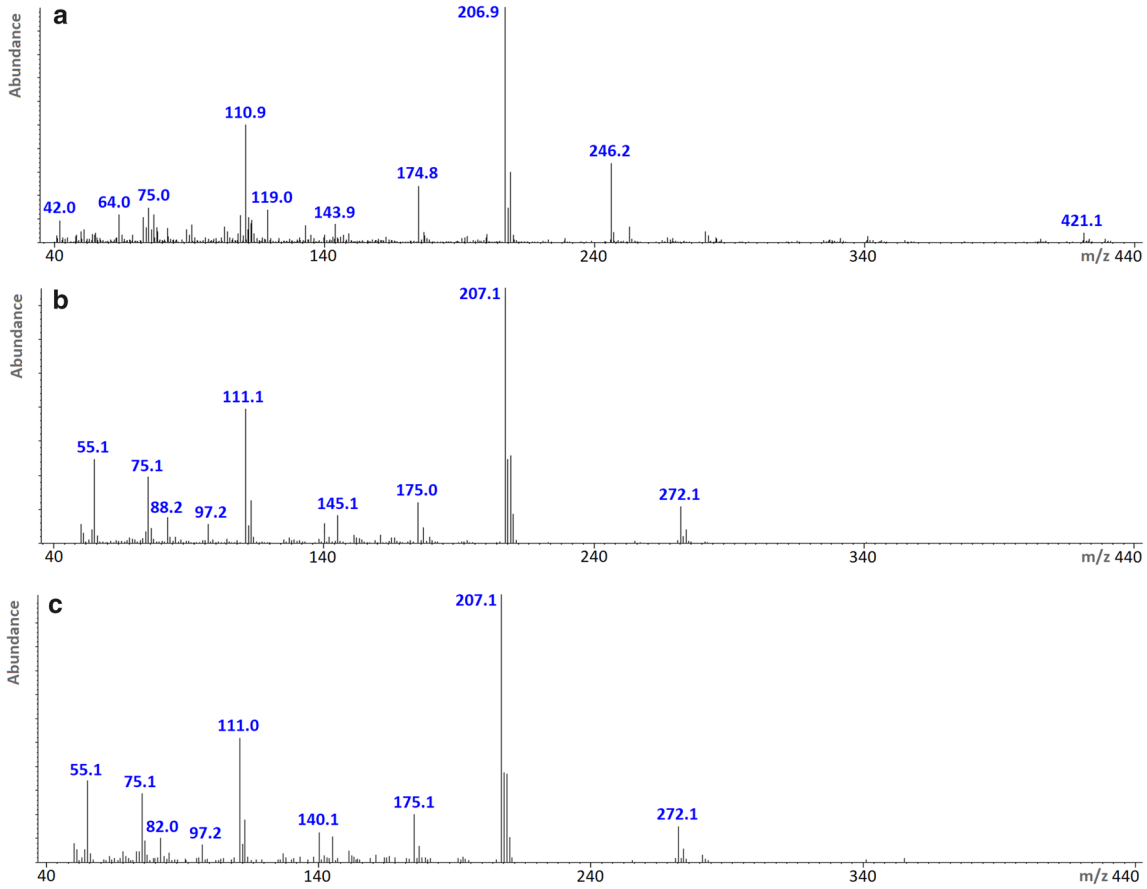

Fig. 3 W-18 reference spectra from the Cayman Spectral Library (**a**) and our in-house database (**b**), and the spectrum as acquired for the seized powder (**c**). The molecular ion is present in the CSL at *m/z* 421 but absent in the spectra of both the in-house database and the sample itself. The origin of the spectral mismatch could not be determined. A combination of detection methods minimised the possibility of a false positive hit

non-peer reviewed databases, they enable users to quickly and freely share information on old and new compounds identified. For reference purposes the W-18 spectra in the CSL, in our in-house library and of the sample are shown in Fig. 3.

Ocfentanil has previously been found in samples sold as heroin. Coopman et al. reported the first case of an ocfentanil-related overdose: a 17-year-old Belgian man found dead in his parents' house. Analysis of a post-mortem, femoral blood sample showed the presence of ocfentanil at a concentration of 15.3 µg/L. A brown powder found next to the body contained 2.54% (m/m) ocfentanil [7]. A second ocfentanil-related death was reported to the Belgian Early Warning System on Drugs in 2017 (personal communication). In this case a 24-year-old man was found dead in his home in Switzerland with an ocfentanil femoral blood concentration of 9.1 µg/L. A brown powder of Belgian origin was discovered close to the body and it contained 0.91% (m/m) ocfentanil. The current case shows a similar concentration of ocfentanil in the unknown powder (1.6% m/m) [17].

Contrary to the previous reports, the user who submitted this sample had not suffered a fatal intoxication, neither did he claim to have needed medical treatment. Marchard et al. reported a case from France where a powder meant for sniffing was found to contain 17% (m/m) ocfentanil. Users survived the drug intake albeit needing urgent medical treatment [18]. In this case we did not receive any biological samples from the drug user, thus we were unable to determine whether the drug had been ingested or not, nor could we compare ocfentanil blood levels with those previously reported. Ocfentanil is not inherently more toxic compared to the classic opioids (such as heroin) when taking the potency and associated lower active dose into account. However, opioid users may lack knowledge of the aforementioned pharmacology or be unaware of the presence of synthetic opioids, leading to toxicity and resulting in deaths [19, 20].

W-18 made up 0.3% (m/m) of the powder. To our knowledge, no publications are available that report the concentrations of this synthetic opioid in seized powders. The

current combination with ocfentanil is unprecedented and has prompted an alert warning by the Belgian Early Warning System on Drugs, which was sent out in June 2018.

Conclusion

The current case reports the analysis of a brown powder purchased as heroin, containing both ocfentanil (1.6% m/m) and W-18 (0.3% m/m). The observed ocfentanil concentrations are consistent with previously reported samples seized on the Belgian drug market. To the authors' best knowledge this is the first case of a combined occurrence of ocfentanil and W-18 sold online as "heroin".

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Compliance with ethical standards

Conflict of interest Alexander van Nuijs received a postdoctoral scholarship from the Research Foundation Flanders (FWO, Grant no. 1285216N).

Ethical standards This article does not contain any studies involving living human participants or animals, performed by any of the authors.

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