



[biblio.ugent.be](https://biblio.ugent.be)

The UGent Institutional Repository is the electronic archiving and dissemination platform for all UGent research publications. Ghent University has implemented a mandate stipulating that all academic publications of UGent researchers should be deposited and archived in this repository. Except for items where current copyright restrictions apply, these papers are available in Open Access.

This item is the archived peer-reviewed author-version of:

Title:

A case of acute intoxication due to combined use of fentanyl and 3,4-dichloro-N-[2-(dimethylamino)cyclohexyl]-N-methylbenzamide (U-47700)

Coopman V, Blanckaert P, Van Parys G, Van Calenbergh S, Cordonnier J.

*Forensic Sci.Int.* **2016**, *266*, 68-72.

**Author(s):** Coopman V, Blanckaert P, Van Parys G, Van Calenbergh S, Cordonnier J.

Source: *Forensic Sci.Int.* 2016, 266, 68-72.

Manuscript Number:

Title: A case of acute intoxication due to combined abuse of fentanyl and 3,4-dichloro-N-[2-(dimethylamino)cyclohexyl]-N-methylbenzamide (U-47700)

Article Type: Case Report

Keywords: U-47700, fentanyl, inhalation, new psychotropic substance (NPS), ultra performance liquid chromatography mass spectrometry (UPLC-MS)

Corresponding Author: Miss. Vera Anna Esther Coopman, Pharm D.

Corresponding Author's Institution: Eurofins Forensics Belgium

First Author: Vera Anna Esther Coopman, Pharm D.

Order of Authors: Vera Anna Esther Coopman, Pharm D.; Peter Blanckaert, PhD.; Geert Van Parys, MD; Serge Van Calenbergh, Prof, PhD; Jan Cordonnier, PhD., PharmD.

Abstract: A 30-year old man was found dead in his home after inhaling fumes of a powder burned on aluminium foil. Blood and urine were taken by the medical examiner during the external body examination and submitted to the laboratory for a comprehensive systematic toxicological analysis. A toxic fentanyl level of 10.9 µg/L was measured in the subclavian blood. Police investigation revealed that the man searched the internet for information on new psychotropic substances, among others about U-47700. A powder found in the victims home was transferred to the laboratory for analysis, in which trace amounts of fentanyl (35 mg/kg) and U-47700 (12 mg/kg) were identified by gas chromatography mass spectrometry. U-47700 is an opioid analgesic drug, considered to have a potency of approximately 7.5 times that of morphine. A target analysis on U-47700 was performed using liquid-liquid extraction and ultra performance liquid chromatography tandem mass spectrometry operating in multiple reaction monitoring mode. The method validation was based on the Scientific Working Group of Forensic Toxicology document 'Standard Practices of Method Validation in Forensic Toxicology'. In blood and urine the U-47700 concentration was 13.8 µg/L and 71.0 µg/L, respectively. To our knowledge, this is the first case report of an intoxication involving U-47700 abused as a new psychotropic substance.

Suggested Reviewers:

**A case of acute intoxication due to combined abuse of fentanyl and 3,4-dichloro-N-[2-(dimethylamino)cyclohexyl]-N-methylbenzamide (U-47700)**

Vera Coopman<sup>1</sup>, Peter Blanckaert<sup>2</sup>, Geert Van Parys<sup>3</sup>, Serge Van Calenbergh<sup>4</sup> and Jan Cordonnier<sup>1</sup>

<sup>1</sup> Eurofins Forensics Belgium, Lieven Bauwensstraat 6, 8200 Brugge, Belgium

<sup>2</sup> Belgian Early Warning System on Drugs, Juliette Wytsmanstraat 14, 1050 Brussel, Belgium

<sup>3</sup> Department of Pathology and Forensic Medicine, AZ Damiaan, Gouwelozestraat 100, 8400 Oostende, Belgium

<sup>4</sup> Laboratory for Medicinal Chemistry, Ghent University, Ottergemsesteenweg 460, 9000 Gent, Belgium

\* Corresponding author. Tel.: +32 50 31 02 52; fax. + 32 50 31 02 54

E-mail address : [jancordonnier@eurofins.be](mailto:jancordonnier@eurofins.be) (J. Cordonnier)

## 1. Introduction

The intoxication appeared in January 2016. The following situation was found at the scene: a 30-year old man was found dead in his home, on the ground of a small enclosed storage room. He was dressed, lying on his left side with raised knees, the left arm in extension and his right arm in flexion. The right hand was lying on the left elbow and was holding a lighter. Next to the right leg, a piece of kitchen foil was present; the bottom blackened (burned) and a brown residu on the upper side. The victim was a well-muscled man of normal build. He weighted approximately 70 kg and was 179 cm tall. On careful examination, no injection sites or traumatic injuries were found. Death appeared at least 48 h before and pulmonary oedema was observed.

Drug paraphernalia were present on the table in the living room: a recent delivered envelope from China, a white powder (36 gram), a digital scale and spoon. The police investigation revealed that, among his friends, the man was know to abuse illegal drugs and experiment with substances purchased over the internet. On his mobile device a search history for the purchase of carfentanil and U-47700 over the internet was found.

## 2. Materials and Methods

### 2.1 Materials

Reference U-47700 (molecular formula:  $C_{16}H_{22}C_{12}N_2O$ ; molecular weight: 329.26 g/mol; chemical structure shown in Figure1.) was kindly provided by the Belgium Early Warning System on Drugs. The reference material of the internal standard fentanyl-d<sub>5</sub> (100 µg/mL in methanol) was obtained from Cerilliant. Standard compounds were diluted in methanol and stored at – 18 °C. All other chemicals were analytical grade.

## 2.2 Apparatus

The target analysis on the presence of U-47700 was performed by UPLC-MS/MS, using a Acquity separations module coupled to the Acquity TQD mass detector equipped with ES interface (Waters Milford, MA, USA). Chromatographic separation was achieved using a Acquity UPLC HSS C18 column (150-mm length x 2.1 mm i.d., 1.8  $\mu\text{m}$  particle size) with a HSS C18 Vanguard column (5-mm length x 2.1 mm i.d., 1.8  $\mu\text{m}$  particle size) as guard column at 50 °C. The mobile phases consisted of 0.15% formic acid (A) and 0.15% formic acid in acetonitrile (B). The following gradient elution was used (runtime 15.00 min): at time 0 min. 13% B held for 0.50 min., changed to 50 % B in 9.50 min., changed to 95 % B in 0.75 min and held for 1.50 min., changed back to the initial conditions in 0.25 min and held for 2.50 min. The flow rate was 0.400 mL/min. The electrospray source was operated in the positive ionisation mode. Product ions were obtained by collision-induced dissociation allowed the MS/MS to be operated in the multiple reaction monitoring (MRM) mode. The MRM transitions and conditions for the measurement of U-47700 (retention time: 6.55 min.); 330.20/285.20 (qualifier) and 330.20/173.20; cone voltage 28 V; collision energy 15 V and 28 V respectively; fentanyl-d5 (retention time: 6.51 min.): 345.42 /188.25 (qualifier) and 342.42/105.10; cone voltage 40 V; collision energy 25 V and 28 V respectively. Quantitations were carried out using the first transition (qualifier). For confirmation, the percent ratio of the second transition to the qualifier was calculated and monitored. The source temperature and desolvation gas (nitrogen) temperature were set at 150 °C and 400 °C, respectively. The gas flow was delivered at a rate of 800 L/h. The capillary voltage was 3.00 kV. Waters Mass-lynx system software Version 4.1 was used for instrument control and quantitation. The method validation was based on the document 'Standard Practices of

Method Validation in Forensic Toxicology' published by the Scientific Working Group of Forensic Toxicology [1].

### 2.3 Samples

Only an external body examination was ordered by the magistrate during which subclavian blood (left) and urine were sampled by the medical examiner. Approximately 2 g of the powder was submitted to the laboratory for analysis. The tissue samples were stored at -18 °C for 2 weeks before quantitation was performed.

### 3. Methods

#### *Systematic toxicological analysis*

The blood and urine were submitted to a comprehensive systematic analysis for the detection of alcohol and volatiles, CO and CN, medical and illegal drugs and new psychotropic substances (previously detected in 'regional' seizures) using different extraction procedures and analytical techniques including headspace gas chromatography with flame ionization detector, liquid chromatography with diode array detector (HPLC/PDA), gas chromatography mass spectrometry in full scan mode (GC/MS), presumptive colour test and several methods applying ultra performance liquid chromatography mass spectrometry in multiple reaction monitoring mode (on blood and enzyme hydrolyzed urine). The method for the quantitation of fentanyl and norfentanyl was previously described [2]. The white powder was subjected to the authors identification scheme (ISO/IEC 17025:2005 accredited) based on HPLC/PDA and GC/MS.

## Sample preparation and extraction

For the quantitation of U-47700 in blood and urine, the liquid-liquid extraction was applied as described for fentanyl and norfentanyl [2]: to a 0.5 mL aliquot of sample (blood or enzyme hydrolyzed urine), 5  $\mu$ L of internal standard solution (fentanyl-d5 0.5  $\mu$ g/mL in methanol) was added. After addition of the internal standard solution, the samples were vortex mixed and allowed to equilibrate 30 min. prior to extraction. Alkalinization was obtained by addition of 1.0 mL 1 M potassium carbonate solution followed by agitation on a vortex mixer. Extraction was performed with 5 mL of a mixture of n-hexane: ethyl acetate (7:3, v/v). After vortex mixing during 2 min. and centrifugation at 3000 rpm for 5 min., the upper organic layer was evaporated to dryness under a slow stream of nitrogen at 40 °C. The dried extracts were reconstituted in 0.25 mL of initial mobile phase. The reconstituted tissue extracts were centrifuged at 14000 rpm for 5 min. A 10  $\mu$ L aliquot was injected into the UPLC-MS/MS system. A blank was injected before every sample. Calibrators and quality controls were prepared by addition of standard solution to blank whole blood prior to extraction. Calibrators and controls were different preparations of the same drug standard lot. Samples were analyzed in triplicate and mean values are given. Two aliquots of the powder (approximately 100 mg weighted in a 10 ml volumetric flask) were extracted with methanol. Three volumes of each flask were quantitated by UPLC/MS-MS.



## Toxicological results

The comprehensive systematic toxicological analysis showed the presence of 10.9  $\mu$ g/L fentanyl and a therapeutic level of 0.18 mg/L sertraline (HPLC/PDA) in the subclavian blood. Fentanyl and sertraline were identified in the alkaline extract of the urine analyzed on GC/MS. The target analysis performed by UPLC-MS/MS revealed the presence of U-47700

at a concentration of 13.8 µg/L in blood and 71.0 µg/L in urine. An overview of the assessed validation parameters and validation data are shown in Table 1. No other compounds were detected which contributed to the death of the man. Fentanyl and U-47700 were identified in the alkaline extract of the powder analyzed by GC/MS, based on retention time and spectrum match with the reference. Quantitative results were obtained with UPLC-MS/MS: 35 mg/kg for fentanyl and 12 mg/kg for U-47700 by analysis of methanolic sample extracts ( $y = 1730.15x + 1807.62$ ,  $r^2 = 0.999046$ ).

## Discussion

Fentanyl and U-47700 are µ-opioid receptor agonists. Fentanyl (**N-phenyl-N-(1-2-phenylethyl-4-piperidyl)propanamide**) is a synthetic narcotic analgesic which was first synthesized by Janssen in 1959 and is widely used for the management of chronic pain. In literature, a great number of fentanyl intoxications are reported due to improper use of transdermal therapeutic patches such as overuse, inhalation, intravenous or buccal abuse [2-9]. U-47700 (3,4-dichloro-N-[2-(dimethylamino)cyclohexyl]-N-methylbenzamide) is a opioid developed by Upjohn in the 1970s, which has never been studied in humans and **its** not approved for medical use. Very little, if any, information is available from scientific literature [10]. The substance is openly sold on the internet. Anecdotal evidence can be derived from user reports found on the internet. From these 'trip reports', it is obvious that this compound is being used as a 'legal' substitute for other strong opioids, such as morphine, heroin or even fentanyl [11]. U-47700 is only controlled in Finland and Sweden (the latter since 26/01/2016). Fentanyl is 75 to 100 times more potent and U-47700 is approximately 7.5 times more potent than morphine, making the abuse in the recreational drug scene very dangerous due to the narrow range between its effective dose and lethal dose, in particular in opioid intolerant users. In opioid naive patients, the minimum effective analgesic serum concentrations of



fentanyl range from 0.2 to 1.2 µg/L. With serum concentration greater than 2 µg/L, the risk of hypoventilation increases [12]. It is thought that the same health risks apply for U-47700 as for other strong opioids such as fentanyl and heroin, including respiratory arrest/depression, miosis, constipation, coma. Very high dosages might result in death, probably due to respiratory arrest and pulmonary oedema. In the reported case, the latter was observed at the external body examination.

Based on the crime scene findings, it was concluded that the route of administration was inhalation of the vaporized substances. The aluminium foil with brown residu from the scene was not available for analysis. No other packages or powders were found in the victims home. Due to the low toxic concentration of fentanyl and U-47700 in blood, target analysis and hyphenated techniques needed to be applied for the identification and quantitation [13]. The recognition and analysis of highly potent, new psychotropic substances (NPS) in tissues are challenging in postmortem toxicology. A multidisciplinary approach with exchange of circumstantial evidence is vital, as experienced in this and previous cases [14]. The powder was found to be insoluble in methanol and ultrapure water (native and alkaline). The powder needed to be extracted under alkaline conditions (pH 9) with diethylether and the extract concentrated to obtain an identification by GC/MS and HPLC/PDA. Fentanyl was initially identified on GC/MS by means of computer based library search of the SWGDRUG Mass Spectral Library (Version 2.4) installed on the Agilent Chemstation. Neither a library hit, nor a reference mass spectrum of U-47700 was found in literature. The chromatogram of the alkaline extract of the powder, with mass spectra of U-47700 from the powder and reference is shown in Figure 2. The UV-spectrum of U-47700 is given in Figure 3. Fentanyl and U-47700 in the powder were quantitated by UPLC/MS-MS and were 35 mg/kg and 12 mg/kg, respectively.

Based on the circumstantial evidence (police investigation, crime scene) and the results of the toxicological analysis, we concluded that the man's death was caused by acute intoxication with fentanyl and U-47700 immediately after inhaling the fumes of the powder. The manner of death was presumed to be accidental due to excessive combined abuse of fentanyl and U-47700. The substances were purchased over the internet as NPS.

## References

- [1] Scientific Working Group for Forensic Toxicology (SWGTOX) standard practices for method validation in forensic toxicology. *J Anal Toxicol* 37 (7) (2013) 452-474.
- [2] V. Coopman, J. Cordonnier, K. Pien, D. Van Varenbergh. LC–MS/MS analysis of fentanyl and norfentanyl in a fatality due to application of multiple Durogesic®transdermal therapeutic systems, *Forensic Sci. Int.* 169 (2007) 223–227.
- [3] M. Arvantis, R. Satonik. Transdermal fentanyl abuse and misuse. *Am J Emerg Med.* 2002;20(1) 58–59.
- [4] A. Gibson, B. Larance, A. Roxburgh, L. Degenhardt, E. Black. The Extent of Diversion of Fentanyl for Non-Medical Purposes in Australia: What Do We Know? National Drug and Alcohol Research Centre, Technical Report No 265 (2007), ISBN 978 0 7334 2420 5.
- [5] KA Marquardt, RS Tharratt. Inhalation abuse of fentanyl patch. *J Toxicol Clin Toxicol.* 1994;32(1):75–8.
- [6] J. DeSio, D. Bacon, G. Peer, M. Lema. Intravenous abuse of transdermal fentanyl therapy in a chronic pain patient, *Anesthesiology*, 79(5) (1193) 1139–1141.
- [7] M. Purucker, W. Swann W. Potential for duragesic patch abuse. *Ann Emerg Med.* 35(3) (2000) 314.
- [8] K. Woodall, T. Martin, B. McLellan. Oral abuse of fentanyl patches (Duragesic): seven case reports, *J Forensic Sci*, 53(1) (2008) 222-225.
- [9] G.L. Henderson, Fentanyl-related deaths: demographics, circumstances, and toxicology of 112 cases, *J. Forensic Sci.*, 36 (2) (1991) 422–433.

- [10] B. Cheney, J. Szmuszkowicz., R. Lahti, D. Zichi. Factors affecting binding of trans-N-[2-(methylamino) cyclohexyl] benzamides at the primary morphine receptor. *J Med Chem.*, 28(12) (1985) 1853-1864.
- [11] <http://www.bluelight.org/vb/threads/739960-Novel-opioid-U-47700> (accessed 21/02/2016).
- [12] Janssen Pharmaceutica. Durogesic (Fentanyl transdermal system). Package insert<sup>®</sup>J-C, 1998.
- [13] A. Skulska, M. Kala, A. Parczewski. Fentanyl and its analogues in the forensic laboratory. Medical and analytical problems. *Problems of Forensic Sciences*, vol. 59, 2004, 127–142
- [14] V. Coopman, J. Cordonnier, M. De Leeuw, V. Cirimele. Ocfentanil overdose fatality in the recreational drug scene (submitted for publication).

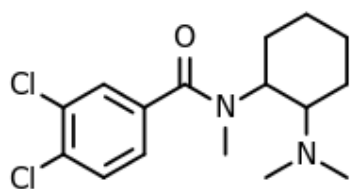


Figure 1. Chemical structure of U-47700

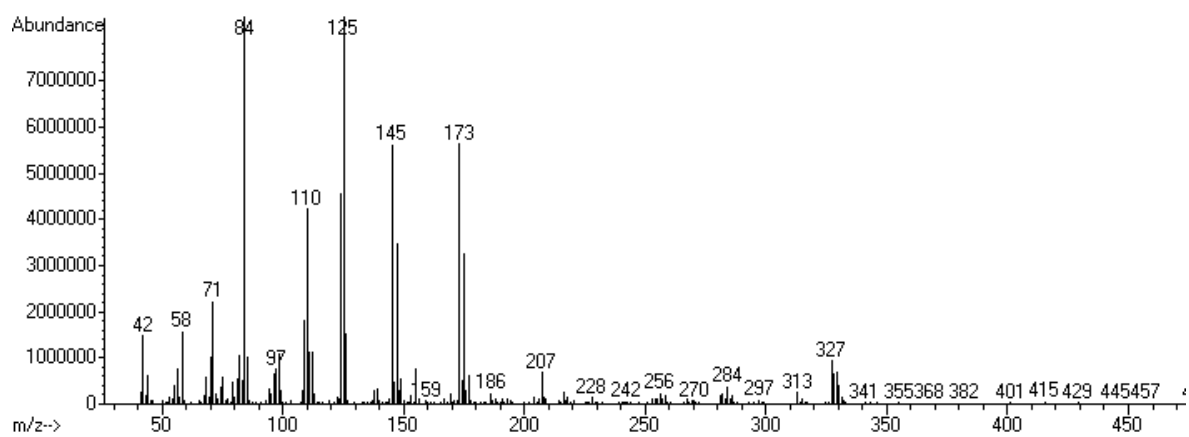
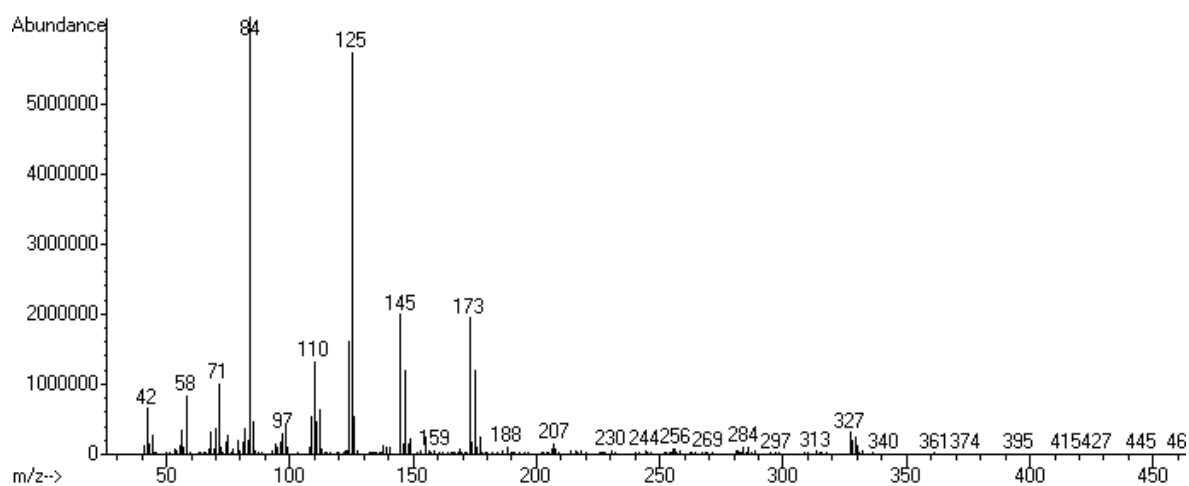
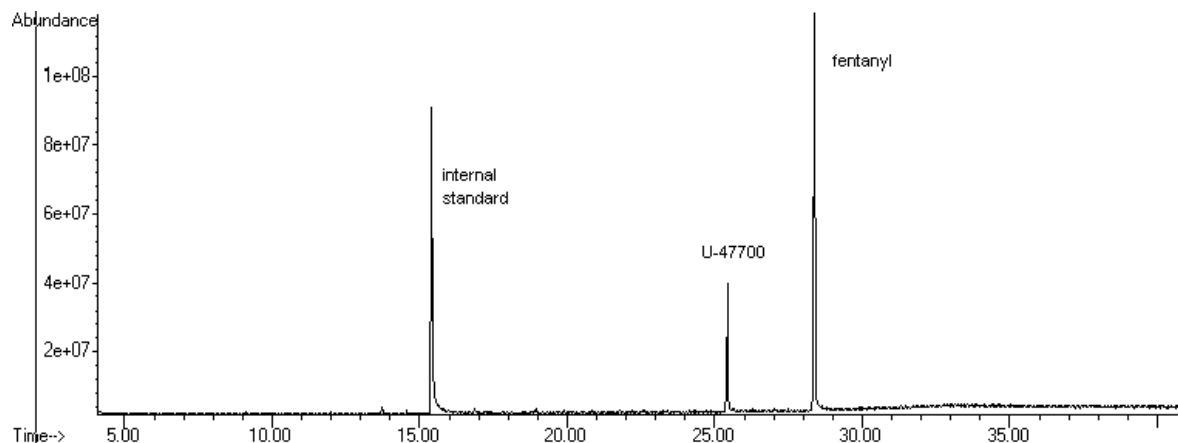


Figure 2. Chromatogram of the alkaline extract of the powder with internal standard diphenylamine (upper), mass spectra of U-47700 from the powder (middle) and reference (below)

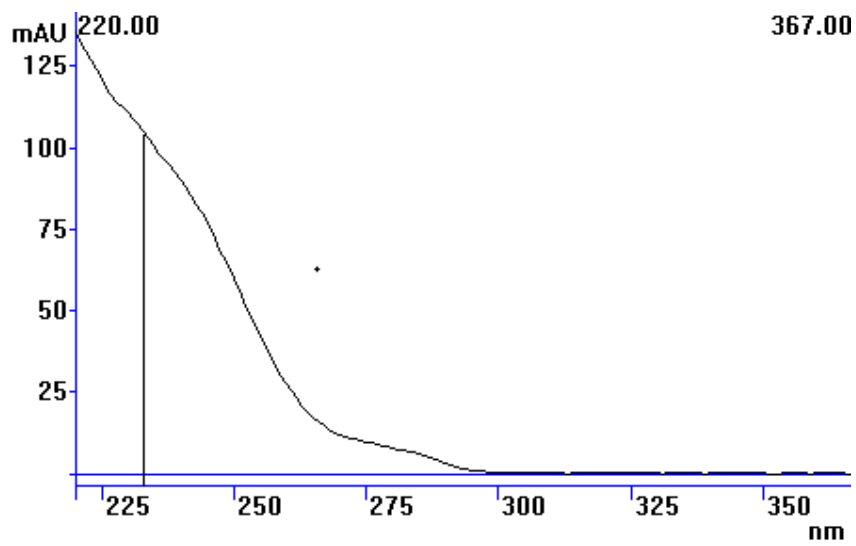


Figure 3. UV spectrum of U-47700

Table 1. Validation parameters and Validation data

Validation parameter	Validation data
Calibration model	<p>unweighted linear curve fit</p> <p>mean correlation coefficient (r): 0.9997</p> <p>residual plots were evaluated, confirming that the used calibration model was appropriate</p> <p>calibration range: 1.6 µg/L – 63.5 µg/L (six point calibration curves)</p>
Bias	<p>at concentration low (6.4 µg/L): 1.1 %</p> <p>at concentration high (47.6 µg/L): 0.9 %</p>
Precision	<p>within-run CV: 5.6 % (low), 3.8 % (high)</p> <p>between-run CV: 3.7 % (low), 2.9 % (high)</p>
Carryover	no carryover was observed after highest calibrator
Interference studies	no interfering signal from matrix, internal standard, common drugs abuse (including other common opiates/metabolites) and prescription medications
Ionization suppression/enhancement	<p>post-extraction addition approach:</p> <p>+ 2.5 % (1.9 % CV) at concentration low</p> <p>+ 1.4 % (4.5 % CV) at concentration high</p>



Limit of detection (LOD) / limit of quantitation (LOQ)	value of lowest non-zero calibrator (1.6 µg/L)
--	--