

# Journal Pre-proof

Technical Note: Single-calibrant quantification of seized synthetic opioids by liquid chromatography-chemiluminescence nitrogen detection

Ilpo Rasanen (Methodology) (Validation) (Formal analysis) (Writing - original draft), Marianne Kyber (Resources), Ilmari Szilvay (Resources) (Investigation), Janne Rintatalo (Resources), Ilkka Ojanperä (Conceptualization) (Methodology) (Writing - review and editing) (Supervision)



PII: S0379-0738(19)30413-X

DOI: <https://doi.org/10.1016/j.forsciint.2019.110001>

Reference: FSI 110001

To appear in: *Forensic Science International*

Received Date: 12 August 2019

Revised Date: 6 October 2019

Accepted Date: 20 October 2019

Please cite this article as: Rasanen I, Kyber M, Szilvay I, Rintatalo J, Ojanperä I, Technical Note: Single-calibrant quantification of seized synthetic opioids by liquid chromatography-chemiluminescence nitrogen detection, *Forensic Science International* (2019), doi: <https://doi.org/10.1016/j.forsciint.2019.110001>

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2019 Published by Elsevier.

# Technical Note: Single-calibrant quantification of seized synthetic opioids by liquid chromatography-chemiluminescence nitrogen detection

Ilpo Rasanen<sup>a</sup>, Marianne Kyber<sup>b</sup>, Ilmari Szilvay<sup>b</sup>, Janne Rintatalo<sup>c</sup>, Ilkka Ojanperä<sup>a,d,\*</sup>

<sup>a</sup>National Institute for Health and Welfare, Forensic Toxicology Unit, PO Box 30, FI-00271 Helsinki, Finland

<sup>b</sup>Finnish Customs Laboratory, PO Box 53, FI-02151 Espoo, Finland

<sup>c</sup>National Bureau of Investigation, PO Box 285, FI-01370 Vantaa, Finland

<sup>d</sup>University of Helsinki, Department of Forensic Medicine, PO Box 40, FI-00014 University of Helsinki, Finland; e-mail: ilkka.ojanpera@helsinki.fi

\*Corresponding author

## Highlights

- synthetic opioids play an increasing role in the drug problem
- wide variety of illicit fentanyl derivatives have been seized
- chemiluminescence nitrogen detector (CLND) possesses equimolar response to nitrogen
- LC-CLND enables quantification of seized samples without actual reference standards

## Abstract

Drug seizures involving a wide variety of emerging new psychoactive substances (NPS) call for new approaches for instant quantification and valuation. Liquid chromatography–chemiluminescence nitrogen detection (LC–CLND) was used in the quantification of opioids with a single secondary standard (caffeine), utilizing the detector's equimolar response to nitrogen. The mean *N*-equimolarity of CLND for ten fentanyl derivatives and U-47700 by the present LC–CLND method was 96.4% (range 91 – 101%). The furanylfentanyl samples consisted of 112 powdery samples with a mean (median, range) hydrochloride purity of 13% (4.9%, 0.08 – 100%). The purity distribution of the furanylfentanyl samples was distinctly bipartite, showing only lower than 9% (*N* = 98) and higher than 60% (*N* = 14) purities. The carfentanil samples consisted of eight brownish sticky samples with a mean (median, range) hydrochloride purity of 0.064% (0.063%, 0.052 –

0.092%). The U-47700 samples consisted of seven powdery samples with a mean (median, range) hydrochloride purity of 89.0% (100%, 51 – 100%). The present application to synthetic opioid analysis widens the scope of the established LC-CLND method, previously found useful for single-calibrant quantification of stimulant/hallucinogenic and cannabinoid type of NPS.

## Keywords

- New psychoactive substances
- Drug seizure
- Fentanyl derivative
- Furanylfentanyl
- Chemiluminescence nitrogen detection
- Reference standard

## 1. Introduction

Opioids are essential in the medical treatment of severe pain but they are also largely abused by drug addicts. During the recent years, the illicit drugs scene has been shaken by the emergence of various fentanyl derivatives and other potent opioids onto the stage of new psychoactive substances (NPS) [1,2]. As the fentanyl structure is exceptionally prone to be modified, the number of different derivatives with pharmacological activity is high. It has been estimated that out of approximately 1400 fentanyl analogues, more than 200 compounds have been described in the literature [3].

The diversity and rapid turnover of NPS makes their analysis challenging, as high-quality reference standards are not readily accessible. While unequivocal identification of seized samples can be carried out by a combination of high resolution mass spectrometry and NMR spectroscopy, the purity estimation in the absence of reference standards requires a technique with universal or predictable response as opposed to mass spectrometry. In our earlier papers, we have studied liquid chromatography–chemiluminescence nitrogen detection (LC–CLND) in the quantification of NPS with a single secondary standard (caffeine), utilizing the detector's equimolar response to nitrogen [4,5]. The developed method enabled analysis of phenethylamines, cathinones and tryptamines, as well as to nitrogen containing synthetic cannabinoids, at a level of uncertainty of measurement (<20%) comparable to conventional quantification methods [5].

Here, using essentially the same conditions for sample preparation and LC-CLND analysis as in the previous paper [5], we focus on the quantification of fentanyl derivatives and report purity values for seized samples of furanylfentanyl, carfentanil and U-47700.

## 2. Materials and methods

### 2.1. Materials

Seized material with previously positively identified main components was obtained through the National Bureau of Investigation and from the Finnish Customs Laboratory for quantitative analysis by LC–CLND.

The secondary reference standard used for quantification by LC–CLND was caffeine (Sigma–Aldrich C1778, purity 98.9%).

Certified reference standards were used to determine the equimolarity of the CLND detector. Standards were selected according to their availability to the laboratory. Fentanyl solution (99.9%) 1.0 mg/mL in methanol was from LGC GmbH (Luckenwalde, Germany), para-fluorofentanyl HCl (> 95%) and furanylfentanyl HCl (98.9 %) were from Cayman Chemical (Ann Arbor, MI, USA), U-47700 HCl ( $92.8 \pm 0.5\%$ ) was from Chiron (Trondheim, Norway), alpha-methylfentanyl HCl (98%) was from Toronto Research Chemical Inc (North York, ON, Canada), cis-3-methylfentanyl HCl (99.6%), trans-3-methylfentanyl HCl (98.7%) and norfentanyl HCl hydrate (99.9%) were from Lipomed (Arlesheim, Switzerland), carfentanil citrate was from United Nations Narcotics Lab (Vienna, Austria), remifentanil HCl (99.5%) was from GlaxoSmithKline (Durham, UK) and sufentanil citrate (99.6%) was from Janssen Pharmaceutica N.V. (Beerse, Belgium).

### 2.2. Apparatus

LC–CLND analysis was performed with an Agilent Technologies (Santa Clara, CA, USA) 1100 series liquid chromatograph equipped with an autosampler, binary pump, column oven, 1260 Infinity degasser, and 1260 Infinity UV diode array detector (DAD). Chromatographic separation was performed with a Phenomenex (Torrance, CA, USA) Luna PFP(2) 100 x 2mm (3  $\mu$ m) column and a 4 x 2 mm PFP precolumn. The nitrogen-specific detector was an Antek (PAC, Houston, TX, USA) 8060 CLND, coupled online after the DAD. The detector was interfaced with the computer using an Agilent 35900E analog-to-digital converter.

### 2.3. Sample preparation

General procedure: a quantity of 10–20 mg of the seized material was dissolved in 0.1% formic acid (FA): methanol (MeOH) 9:1 v/v to obtain a solution of 10 mg/mL of seized material. This solution was analyzed directly in case of low-content samples or diluted proportionally to obtain a solution of 1.0 mg/mL of seized material for LC–CLND analysis.

For some high-content samples, a solution of 1.0 mg/mL was prepared in MeOH and diluted with 0.1% FA to obtain a solution of 0.10 mg/mL of seized material. In all cases, sample preparation was carried out to obtain an analyte concentration corresponding to approximately 20 – 80 ng of nitrogen per injection.

### 2.4. LC-CLND analysis

LC separation was performed in gradient mode at 40 °C. The mobile phase components were 0.1% FA and methanol. Flow rate was 0.25 mL/min and injection volume 10 µL. The proportion of methanol was increased from 10% to 90% over 15 min and held at 90% for 5 min. Post-time was 10 min. The DAD signal was recorded at 230 nm and peak controlled spectra were recorded at 210–400 nm. Although the compounds were pre-identified by other methods, the DAD signal was used to confirm the peak purity by means of the Agilent ChemStation similarity factor algorithm.

For CLND analysis, the inlet oxygen flow was 250 mL/min, helium flow 50 mL/min, and make-up helium flow 50 mL/min. The ozone generator oxygen flow was 25 mL/min, and furnace temperature 1050 °C. The photomultiplier tube voltage was set at 750 V, and the amplification factor was 1. UV and CLND data were processed using Agilent ChemStation. External calibration was performed at the beginning of each data acquisition sequence with caffeine as standard. Caffeine (2–4 mg) was weighed and dissolved in 0.1% FA: methanol 90:10 (v/v) to obtain a solution of 1 mg/mL. Subsequently, 20 mL of this solution was diluted with 180 mL of 0.1% FA: methanol 90:10 (v/v) to obtain a solution of 0.10 mg/mL, and this solution was further diluted to 0.01 mg/mL, respectively. Caffeine calibration standards were prepared from these standards by diluting with 0.1% FA: methanol 90:10 (v/v) to obtain caffeine concentrations of 1.0, 2.0, 3.0, 4.0, 5.0, 10, 20, 30, 40 and 50 mg/mL which were equivalent to the calibration points of 2.9, 5.8, 8.7, 11.5, 14.4, 28.9, 57.7, 86.6, 115 and 144 ng of nitrogen per injection. The caffeine curve fit was found to be linear with  $R^2 > 0.999$ .

### 2.5. Equimolarity

The equimolarity of nitrogen detection was investigated by analyzing different concentration levels (three levels per compound) of certified reference standard solutions of selected compounds using the caffeine calibration. All analyses were performed in duplicate. Equimolarity was calculated in the concentration level of approximately 20 – 80 ng of nitrogen per injection, depending on the nitrogen content of the compound. Equimolarity was reported as an average of calculated equimolarities at the different concentration levels.

## 3. Results and discussion

Table 1 shows the *N*-equimolarity of chemiluminescence nitrogen detection for ten fentanyl derivatives and U-47700 by the present LC–CLND method. The grand mean (range) equimolarity of the means of the eleven substances was 96.4% (91 – 101%). Within the nitrogen range indicated in the table, the equimolarity showed no concentration dependence.

Seized samples containing furanylfentanyl, carfentanil and U-47700 were quantified by the caffeine-calibrated LC–CLND method described above. These drugs were identified by high resolution mass spectrometry and, initially by NMR spectroscopy, without other illicit drug findings prior to submitting the samples for quantification by LC–CLND. However, the DAD signal was used to assess the peak purity for CLND calculations, applying a DAD peak purity criterion of 99% as well as visual inspection. The expanded uncertainty of measurement, calculated as shown before [5], was always < 20% as the systematic error component was low.

The furanylfentanyl samples, seized between December 2016 and November 2018 by the customs or police, consisted of 112 powdery samples with a mean (median, range) hydrochloride purity of 13% (4.9%, 0.08 – 100%). Table 2 describes the purity distribution of the furanylfentanyl samples showing a distinctly bipartite nature of low (lower than 9%) and high (higher than 60%) purity samples. The majority of the samples were of low purity, and they originated from small 1 – 10 g batches that obviously were intended for personal use on the Finnish market with limited demand for the product.

The carfentanil samples, seized in August and December 2017 by the police, consisted of eight brownish sticky samples with a mean (median, range) hydrochloride purity of 0.064% (0.063%, 0.052 – 0.092%).

The U-47700 samples, seized between December 2016 and August 2017 by the police and customs, consisted of seven powdery samples with a mean (median, range) hydrochloride purity of 89.0% (100%, 51 – 100%).

The limitations of the LC-CLND method include only moderate chromatographic resolution and the requirement of a nitrogen atom in the analyte molecule. It is acknowledged that any DAD peak purity algorithm can only confirm the presence of impurities and never prove absolutely that the peak is pure. The CLND response is sensitive to the actual nitrogen configuration for compounds containing adjacent nitrogen atoms [6]. However, these limitations did not negatively affect the presented analysis, because all the studied drugs were nitrogenous and there were no interfering signals in the relevant chromatographic region. The common adulterants caffeine and paracetamol, for instance, eluted much earlier than the opioids investigated. Clearly, the method is amenable to even more general quantification of synthetic opioids.

As stated in the European Drug Report 2019 by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), new synthetic opioids pose a serious threat to individual and public health, while fentanyl derivatives make up the majority of the 49 new opioids monitored by the EMCDDA [7]. The present analytical approach meets the requirements posed by the rapidly changing illicit drug scene for instant quantification and valuation of drug seizures. Our future studies will focus on the development of parallel drug analysis by CLND and high resolution mass spectrometry.

### **Credit author statement**

Ilpo Rasanen: Methodology, Validation, Formal Analysis, Writing – Original Draft

Marianne Kyber: Resources

Ilmari Szilvay: Resources, Investigation

Janne Rintatalo: Resources

Ilkka Ojanperä: Conceptualization, Methodology, Writing – Review & Editing, Supervision

## References

- [1] European Monitoring Centre for Drugs and Drug Addiction (2018), Fentanils and synthetic cannabinoids: driving greater complexity into the drug situation. An update from the EU Early Warning System (June 2018), Publications Office of the European Union, Luxembourg.  
[http://www.emcdda.europa.eu/publications/rapid-communications/fentanils-and-synthetic-cannabinoids-ews-update\\_en](http://www.emcdda.europa.eu/publications/rapid-communications/fentanils-and-synthetic-cannabinoids-ews-update_en) (accessed 3 October 2019)
- [2] P.J. Jannetto, A. Helander, U. Garg, G.C. Janis, B. Goldberger, H. Ketha, The fentanyl epidemic and evolution of fentanyl analogs in the United States and the European Union, *Clin. Chem.* 65 (2019) 242-253.
- [3] A. Skulska, M. Kała, A. Parczewski, Fentanyl and its analogs in the forensic laboratory. Medical and analytical problems, *Probl. Forensic Sci.* 59 (2004) 127-142.
- [4] S. Laks, A. Pelander, E. Vuori, E. Ali-Tolppa, E. Sippola, I. Ojanperä, Analysis of street drugs in seized material without primary reference standards, *Anal. Chem.* 76 (2004) 7375-7379.
- [5] I. Rasanen, M. Kyber, I. Szilvay, J. Rintatalo, I. Ojanperä, Straightforward single-calibrant quantification of seized designer drugs by liquid chromatography-chemiluminescence nitrogen detection, *Forensic Sci. Int.* 237 (2014) 119-125.
- [6] B. Yan, J. Zhao, K. Leopold, B. Zhang, G. Jiang, Structure-dependent response of a chemiluminescence nitrogen detector for organic compounds with adjacent nitrogen atoms connected by a single bond, *Anal. Chem.* 79 (2007) 718-726.
- [7] European Monitoring Centre for Drugs and Drug Addiction (2019), European Drug Report 2019: Trends and Developments, Publications Office of the European Union, Luxembourg.  
<http://www.emcdda.europa.eu/publications/edr/trends-developments/2019> (accessed 3 October 2019)

**Table 1**

Equimolarity of nitrogen detection for fentanyl derivatives and U-47700 by LC–CLND.

<b>Compound</b>	<b>Retention time (min)</b>	<b>Mean equimolarity (%)</b>	<b>Range ng N/inj.</b>	<b>Equimolarity range (%)<sup>a</sup></b>
Norfentanyl	8.86	96	24 – 104	95.0 – 96.5
Remifentanil	10.95	95	15 – 71	94.7 – 95.7
U-47700	12.35	97	18 – 84	96.5 – 99.2
Fentanyl	12.51	96	16 – 79	94.8 – 98.7
Furanylfentanyl	12.74	98	15 – 70	94.1 – 99.2
$\alpha$ -Methylfentanyl	12.79	98	14 – 72	89.0 – 104.0
<i>para</i> -Fluorofentanyl	12.89	101	15 – 77	97.2 – 103.7
<i>trans</i> -3-Methylfentanyl	13.04	91	16 – 90	83.4 – 95.4
<i>cis</i> -3-Methylfentanyl	13.07	93	16 – 73	91.9 – 99.0
Carfentanil	13.11	98	15 – 70	93.1 – 100.5
Sufentanil	13.50	97	13 – 65	95.4– 98.4

<sup>a</sup> three concentration levels within the ng/N range indicated were analysed in duplicate

**Table 2**

Purity distribution of 112 seized furanylfentanyl samples

<b>Purity as HCl %</b>	<b>N</b>
0 – 0.99	1
1.0 – 1.9	1
2.0 – 2.9	32
3.0 – 3.9	17
4.0 – 4.9	7
5.0 – 5.9	25
6.0 – 6.9	10
7.0 – 7.9	4
8.0 – 8.9	1
9.0 – 59	0
60 – 69	5
70 – 79	6
80 – 89	1
90 – 100	2