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Straightforward single-calibrant quantification of seized designer drugs by liquid chromatography–chemiluminescence nitrogen detection

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ABSTRACT

Sixty-one different psychoactive substances were quantified by liquid chromatography–chemiluminescence nitrogen detection (LC–CLND) in 177 samples, using a single secondary standard (caffeine), in a trial concerning the quantitative purity assessment of drug-related material seized by the police in 2011–2012 and customs in 2011–2013 in Finland. The substances found were predominantly substituted phenethylamines, cathinones, tryptamines and synthetic cannabinoids, which were identified by appropriate methods prior to submitting the samples for quantification by LC–CLND. The equimolarity and expanded uncertainty of measurement by LC–CLND were on average 95% and 13%, respectively, based on 16 different substances. The median (mean) purity of stimulant/hallucinogenic drug samples seized at the border was 92.9% (87.6%) and in the street 82.0% (64.5%). The corresponding figures for powdery synthetic cannabinoid samples seized at the border and in the street were 99.0% (96.8%) and 90.0% (92.2%), respectively. There was generally only one active drug to be quantified in each sample. Seized herbal samples contained 0.15–9.2% of between one and three components. LC–CLND was found to be suitable for quantification of the nitrogen-containing drugs encountered in the study, showing sufficient *N*-equimolarity for both stimulant/hallucinogenic drugs and synthetic cannabinoids. The technique possesses great potential as a standard technique in forensic laboratories.

1. Introduction

The term designer drug came into public awareness three decades ago along with the appearance of some fentanyl derivatives on the illicit drug market in the USA. After a fairly steady period, the last few years have shown a sharp increase in the emergence of designer drugs – today more appropriately called new psychoactive substances (NPS) [1]. The annual number of NPS formally notified for the first time through the Early Warning System of the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) was 24, 41, 49 and 73 in 2009–2012. Of the substances reported in 2012, 30 compounds were synthetic cannabinoid receptor agonists, 19 compounds did not conform to any readily recognized chemical group, 14 compounds were phenethylamines, while the rest were cathinones, tryptamines and piperazines [2]. The rapid appearance of non-controlled alternatives to controlled drugs is a typical feature of today.

Archer et al. [3] have recently discussed the challenges of acquiring analytical reference standards for the chemical analysis of NPS. The traditional way of purchasing standards from commercial producers with a timescale of months or years is no longer appropriate, as the sudden appearance and sometimes brief lifetime of NPS on the illicit market present difficulties for reference material producers. Obtaining materials from unregulated sources and converting them into reference standards has other problems. A complete range of purification and analysis techniques, such as preparative chromatography, high resolution mass spectrometry (HR-MS), infrared (IR) and nuclear magnetic resonance (NMR), are required to produce in-house reference material with proven quality from law enforcement seizures. However, neither the commercial nor the in-house approach to reference material production is likely to fully satisfy the requirements of all forensic laboratories [3].

In the absence of an appropriate reference standard, one option is to rely first on identification by molecular properties and, for quantification, on detectors with a universal or equimolar response. In an earlier study, we suggested a simple solution for the analysis of scheduled and designer drugs in seized material,

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independent of the availability of primary reference standards [4]. Identification was by liquid chromatography/time-of-flight mass spectrometry (LC–TOFMS), essentially based on accurate mass determination. Quantification was by liquid chromatography/chemiluminescence nitrogen detection (LC–CLND) with a single secondary standard (caffeine), utilizing the detector's equimolar response to nitrogen. Using current instrumentation, substance identification by HR-MS accompanied by occasional NMR analysis is even more feasible than ten years ago [5,6]. As >90% of drugs contain nitrogen, LC–CLND is a prominent technique for rapid single-calibrant quantification of NPS [7,8].

In this study, our objective is first to validate the performance of single-calibrant LC–CLND for several NPS, and second to use LC–CLND to assess the purity of NPS in various materials seized by the police and customs in Finland during 2011–2013.

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 Customs Laboratory for quantitative analysis by LC–CLND. The number of powdery samples was 159, the number of herbal products was 14, and the number of ground plant material was 4.

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. NPS standards were selected according to their availability to the laboratory, and also classical drugs of abuse were included. Amphetamine (purity 99.8%) and methamphetamine (99.6%) solutions (1 mg/mL) in methanol were from Cerilliant (Round Rock, TX, USA). Butylone HCl (99.6%), MDAI (99.3%), methedrone HCl (99.5%) and methylone HCl (99.7%) were from LGC GmbH (Luckenwalde, Germany). 2-CB HCl (>98.5%), dimethyltryptamine (>98.5%), mescaline HCl (>98.5%), MDMA HCl (>98.5%) and methcathinone HCl (>98.5%) were from Lipomed AG (Arlesheim, Switzerland). Ethyl phenidate HCl (98%), JWH-147 (97%), MDPV HCl (98%) and mephedrone (98%) were form Toronto Research Chemicals Inc. (North York, ON, Canada). JWH-019 (≥98%) was from Cayman Chemical (Ann Arbor, MI, USA).

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 × 2 mm (3 μm) column and a 4 × 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 for phenethylamines, cathinones and tryptamines: a quantity of 2–4 mg of seized material was weighed and dissolved in methanol to obtain a solution of 1.0 mg/mL. This solution was diluted with 0.1% formic acid (FA) to obtain a solution of 0.10 mg/mL of seized material for LC–CLND analysis. For some low-content samples, a solution of 10 mg/mL was first prepared and correspondingly diluted to 1.0 mg/mL of seized material.

Synthetic cannabinoids: a quantity of 2–4 mg of seized material was weighed and dissolved in methanol to obtain a solution of 1.0 mg/mL. Subsequently, 20 μL of this solution was diluted with 20 μL of 0.1% FA and 160 μL of methanol to obtain a solution of 0.10 mg/mL of seized material for LC–CLND analysis.

Herbal products: a quantity of 20–40 mg of seized material was weighed and dissolved in methanol to obtain a solution of 10 mg/mL. This solution was sonicated for 10 min and allowed to stand at room temperature for 24 h. Subsequently, 20 μL of the supernatant was diluted with 20 μL of 0.1% FA and 160 μL of methanol to obtain a solution of 1.0 mg/mL of seized material for LC–CLND analysis. For some low-content samples, a solution of 10 mg/mL of seized material was analyzed by LC–CLND following sonication.

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 4 min. Post-time was 9 min. The DAD signal was generally

Table 1
Equimolarity of nitrogen detection and uncertainty of measurement for new psychoactive substances by LC–CLND.

Compound	Equimolarity (%)	Systematic error U_1 (%)	Random error U_2 (%)	Expanded uncertainty of measurement $2 \times U$ (%)
Amphetamine	100.4	0.4	2.0	4.1
Butylone	95.2	−4.8	2.6	10.9
2-CB	93.0	−7.0	2.3	14.7
Dimethyltryptamine	103.1	3.1	1.5	6.9
Ethyl phenidate	85.9	−14.1	1.7	28.4
JWH-019	94.3	−5.7	2.0	12.1
JWH-147	105.4	5.4	2.0	11.5
MDAI	83.1	−16.9	2.3	34.1
MDMA	97.1	−2.9	2.3	7.4
MDPV	100.4	0.4	2.2	4.5
Mephedrone	91.6	−8.4	2.1	17.3
Mescaline	95.6	−4.4	2.6	10.2
Methamphetamine	94.8	−5.2	2.1	11.2
Methcathinone	93.0	−7.0	2.6	14.9
Methedrone	97.7	−2.3	2.4	6.6
Methylone	90.7	−9.3	2.3	19.2
Mean	94.5			13.4
Median	94.8			11.4