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Analytical Tools for Instant Determination of New Psychoactive Substances in Biological Samples and Drug Seizures

Ilkka Ojanperä*

Abstract: Identification and quantification of new drugs and metabolites without possessing the required reference standards is a challenge. Although reference standards may be available from commercial, governmental and international sources, their delivery time is lengthy, ranging from several weeks to several months. In this paper, some analytical tools are described that are probably helpful in the combat against NPS on an international level.

Key words: New Psychoactive Substances, Synthetic Opioids, Synthetic Stimulants, High-Resolution Mass Spectrometry, Chemiluminescence Nitrogen Detection

* PhD, professor, University of Helsinki and Finnish Institute for Health and Welfare, P.O. Box 40, FI-00014 Helsinki, Finland

Introduction

According to the United Nations Office on Drugs and Crime (UNODC) the severity and complexity of world drug situation is increasing. Opioids continue to cause the most harm, accounting for two-thirds of the deaths attributed to drug use disorders. In recent years, hundreds of new psychoactive substances (NPS) have been synthesized. Synthetic opioids, especially fentanyl analogues have become the second most important NPS group after stimulants reported for the first time (United Nations, 2019).

Limited access to reference standards for NPS delays the analysis of these compounds by conventional methods that rely on the use of certified reference standards. However, there has been an increasing interest for fast determination of NPS in biological samples and in seized material. NPS are of interest in cause-of-death determination due to the potential toxicity of the drugs. Analyses of new illicit drugs are also often asked by prosecutors, since possession of a small amount of these substances can be sufficient for a prosecution for a serious narcotics offence.

Identification

Today, the tools for routine drug identification include Fourier transform infrared and Raman spectroscopy for seized samples, while gas chromatography – mass spectrometry (GC-MS) and liquid chromatography – triple quadrupole mass spectrometry (LC-MS/MS) are feasible for both seized materials and biological samples. Increased identification power can be obtained from high resolution – high mass accuracy techniques, such as quadrupole time-of-flight (QTOFMS) or Orbitrap MS. Efficient data-independent acquisition methods can now be exploited together with extensive inter-laboratory spectral libraries. Nuclear magnetic resonance (NMR) spectrometry is the ultimate tool for structure elucidation of previously unknown chemicals.

The performance of QTOFMS is usually similar or better than those of the nominal mass-based methods that rely on selected reaction monitoring or product ion spectrum library search. In our laboratory, we have found LC-QTOFMS an especially attractive technique for comprehensive drug screening in terms of scope, sensitivity and reliability of identification. The procedure for biological samples involves solid-phase extraction, including both acidic/neutral and basic fractions, followed by LC separation on a reversed phase column, positive ion electron spray ionization, and data-independent acquisition. The instrumentation is based on Bruker Daltonics maXis Impact HR-QTOFMS technology with simultaneous acquisition of MS and broad band collision induced fragmentation (bbCID). Compound identification is based on post-targeted database search with

preset reporting criteria for mass accuracy, isotopic pattern match, retention time and abundance criteria for qualifier ions (Sundström vd., 2017: 41, 623-630.). The scope of the method comprises conventional drugs of abuse, various classes of NPS, such as synthetic cannabinoids, opioids and cathinones, and commonly abused prescription drugs such as tramadol and pregabalin. The in-house database now consists of more than 1000 entries, while international databases are also available for searching.

An example of the capabilities of LC-QTOFMS is the emergence of the new synthetic opioid U-47.700 on the illicit drug market a few years ago. After incorporating the substance into the urine LC-QTOFMS screening used in post-mortem toxicology, U-47.700 was detected in 10 autopsy cases within routine case work. The data-independent acquisition of the original screening allowed for retrospective re-analysis of the full-scan data without re-running any samples, and consequently two more U-47.700 cases were revealed from the preceding year (Kriikku, vd., 2019, ss. 85-88).

Quantification

Quantification without reference standards remains mostly unfeasible by the conventional analytical armoury. However, LC coupled to nitrogen chemiluminescence detection (CLND) can be used with a single external nitrogenous calibrant to quantify nitrogen-containing compounds of known molecular formula, based on the detector's equimolar response to nitrogen. As approximately 90% of drugs contain nitrogen, the N-equimolar response of this detector enables facile quantification of both traditional illicit drugs and NPS using a single secondary standard, such as caffeine, for calibration. The LC-NCD approach is feasible with seized drugs but it is less applicable to bioanalysis due to the limited sensitivity and rather slow data acquisition capacity of the detector, requiring high sample volumes and sufficiently broad LC peaks, respectively.

LC-CLND (PAC Antek 8060) is especially useful in the analysis of solid and liquid samples of seized drug material using a very simple sample preparation procedure (Rasanen, 2019, s. 305). As an example, seized samples previously shown to contain ocfentanil, furanylfentanyl, carfentanil, 4-fluorobutyrylfentanyl or 2-fluorofentanyl were quantified. For sample preparation, 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. The method's expanded uncertainty of measurement was <20%. The purity of seized samples ranged 1.4 – 2.6% for ocfentanil, 0.08 – 100% for furanylfentanyl, and 0.052 – 0.092% for carfentanil. Some samples were mixtures of two different

synthetic opioid derivatives or contained an opioid derivative together with other drugs. Concentrations of the synthetic opioid derivatives in the liquid samples ranged 0.057 – 16 mg/ml.

Simultaneous Identification and Quantification

Due to the challenges of using LC-CLND in bioanalysis, an integrated platform for simultaneous identification and quantification of drugs has been developed in our laboratory. The concept takes advantage of the recently introduced GC-atmospheric pressure chemical ionization (APCI) - interfacing to QTOFMS. In this approach, the GC flow is divided in appropriate proportions between Agilent 6540 UHD QTOFMS analyzer and Agilent 255 Nitrogen Chemiluminescence Detector (NCD). Identification is based on high-resolution accurate-mass spectra and quantification relies on the N-equimolar response by NCD. The GC-APCI ion source allows soft ionization following the choice of highly abundant protonated molecules ($[M+H]^+$) as precursor ions, in contrast to the extensive fragmentation often found in traditional electron ionization GC-MS spectra. The Sievers-type of NCD detector, on the other hand, allows higher sensitivity and acquisition frequency than what could be obtained by the LC-CLND.

As an example of this approach, a method was developed for quantitative estimation of 38 illicit psychostimulants in blood (Mesihää, vd., 2019). Quantification relied on the NCD's N-equimolar response to nitrogen, using amphetamine, 3,4-methylenedioxymethamphetamine (MDMA) and methylenedioxypyrovalerone as external calibrators for prim-, sec- and tert- amines, respectively. The mean between-day accuracy at the limit of quantification was as high as 93.5%, as compared with theoretical values.

Conclusions

This work shows how high resolution mass spectrometric and chemiluminescence detection methods can be applied to the rapid analysis of biological samples and seized materials without an immediate access to actual reference substances. Dissemination of new methodology in drug analysis as well as broader international co-operation are key factors in uncovering illicit drugs within forensic sciences.

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