NMR

Nuclear magnetic resonance methodology for new psychoactive substances analysis

New work on synthetic cathinones and a new model of distributed analysis

Innovation with Integrity

Introduction

Important research has been published in recent years where scientists have specifically assessed nuclear magnetic resonance (NMR) methodology for the analysis of new psychoactive substances (NPS). All are based on the central analytical challenge, namely, that other techniques (chromatography, mass spectrometry etc.) require compound-specific reference material in order to both identify and/or quantify an unknown. With the diversity and rate of change of NPS compounds, such materials are simply not available to enable the timely analysis that the police, customs, border control and front-line law enforcement agencies, for example, require.

In this app note, we describe patterns of usage, clinical implications and the analytical challenges surrounding NPS. We also highlight new experimental methodology for synthetic cathinones that has been established on Bruker NMR systems and consider how this fits in to the company's overarching concept of distributed laboratory topology (DLT).

The NPS problem

Published in the 2022 report[2], Alexis Goosdeel, Director of The European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), said: *"An overarching conclusion I draw from this year's report is that we are now facing a more complex drug situation, characterized by high availability and greater diversity in patterns of drug consumption. We see from our reporting on the NPS phenomenon that almost anything that has psychoactive potential is now at risk of appearing on the market, often mislabeled, meaning that those consuming these substances may be unaware of what they are actually using."*

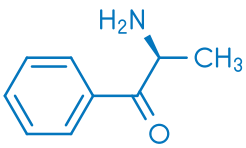
This latest report appears to confirm recent trends, reported elsewhere, that despite a general decrease in the use of internationally controlled drugs, the impact of NPS continues to grow. Importantly, given their complex pharmacodynamics, there are increasing levels of concern about the onset of acute/chronic psychopathological associated with NPS intake.

A useful review[1], provides an overview of a range of NPS-related issues: preclinical, epidemiological, and clinical pharmacological; the medical and psychopathological consequences associated with their intake. It also summarizes the analytical chemistry and forensic analysis challenges associated with the NPS phenomenon.

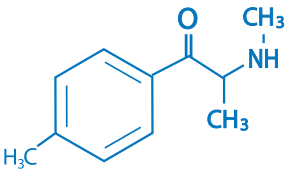
To assess the prevalence of these new compounds, a crawling/navigating software (NPS.Finder®) has been used since 2017 to automatically scan the open web for new, novel, and emerging NPS. After around 18 months of operation, and having eliminated false positives and duplicates, a total of 4,204 unique NPS molecules had been recorded. 3

Amongst these, 171 were cathinones, in four categories – those possessing:

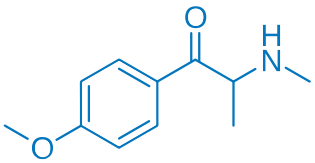
- methamphetamine-like effects
- cocaine/3,4-methylenedioxymethamphetamine-related effects
- 3,4-methylenedioxymethamphetamine-like effects
- pyrovalerone-like effects



Cathione



Mephedrone



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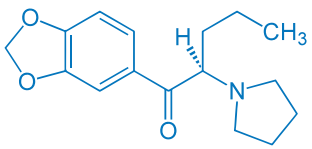

MDPV

Figure 1 Example chemical structures of cathinone and synthetic derivatives.
Source: EMCDDA website4 (accessed 6 July 2022).

Most of the NPS cathinone derivatives that have been marketed in the past few years are ring-substituted. Figure 1 shows some example structures of synthetic cathinones, with the EMCDDA reporting on more than 25 substitution patterns for cathinone derivatives that have been used as an active pharmaceutical ingredient (API), found in drug seizures, samples collected for monitoring purposes or offered for sale on internet sites.[4]

In their 'natural' state, i.e. in the absence of ring-substitution, cathinones behave as central nervous system (CNS) stimulants. The chemical substitutions made to produce synthetic cathinones may have some effect on the stimulant behavior, but more importantly, they are easily manufactured, and render each a 'novel molecule' as far as law enforcement and legal process is concerned. Judicial practice in drug-related crime requires clear information on the type (identification) and quantity (quantification) of the illicit substance to support conviction of any defendant.

Analytical challenges solved by NMR

The variation of chemical structure, the fact that NPS products on the street may contain mixtures of ring-substituted molecules in unknown proportions, and the fact that new drug products are appearing regularly on the market, combine to pose a significant analytical challenge.

Chromatographic techniques, such as high-performance liquid chromatography (HPLC), ultra HPLC, and liquid chromatography-mass spectrometry, for example – whilst rapid, robust and reproducible, all require suitable compound-specific reference materials to facilitate qualitative and quantitative measurements. NMR analysis does not, needing simply a generic internal standard to discriminate between almost identical molecules and enable quantitative analysis (figure 2).

In addition, NMR offers a series of other advantages, namely:

- Non-destructive 'primary analysis' technique
- Simple sample prep and handling requirements
- Targeted and untargeted identification
- Known molecular structures are verified and new molecular structures are elucidated
- Quantification of known and unknown substances
- Quantification of substances in a mixture

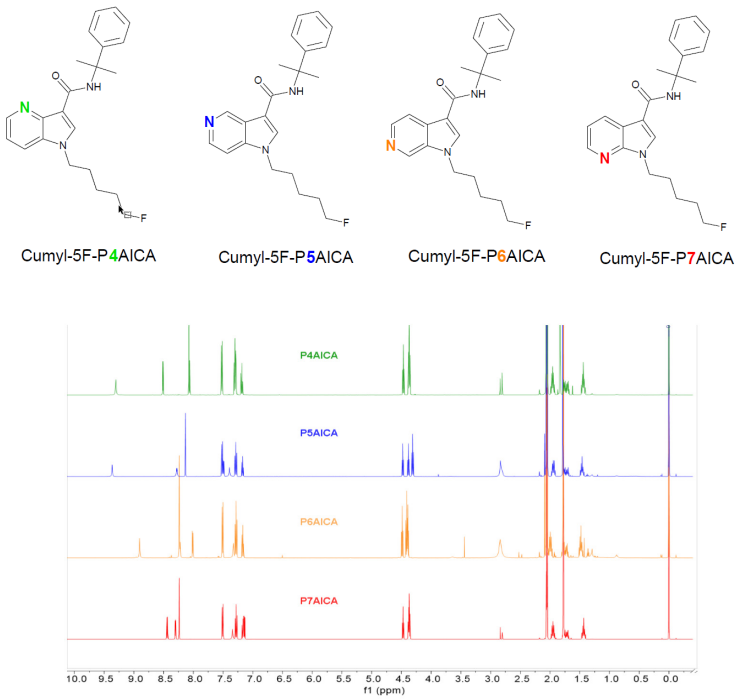

Figure 2 Nuclear magnetic resonance can discriminate between almost identical molecules.

Trailblazing NMR methodology for NPS

A research study jointly published in late-2021 by Professor Mengxiang Su's team at China Pharmaceutical University, in collaboration with the National Narcotics Control Commission of China and the Narcotics Information Technology Center of the Ministry of Public Security, and with graduate student Yuxin Zhao as first author, gave details of how the team developed, for the first time, a quantitative NMR (qNMR) method for cathinone analogues.

Experimental
All experiments were carried out using a Bruker 500Mz spectrometer equipped with a Quattro Nucleus Probe (QNP) that was maintained at a temperature of 303 K. Data processing was performed with Bruker TopSpin 2.0 software with manual baseline, phase correction, and integration.

Initial developments used four samples, seized by the Public Security Bureau, for structure verification and identification. The ^{1}H NMR signals for the synthetic cathinones are in three parts: a benzine ring, a methylidyne group, and alkyl chain. NMR signals from the benzine ring and the methylidyne group can be considered as specific features of the synthetic cathinones.

In subsequent work, a ^{1}H qNMR method was established using maleic acid as the internal standard and the shared signal (i.e., the methylidyne hydrogen) on the parent synthetic cathinones structure as the quantitative peak. Maleic acid was chosen because its unique chemical shift does not interfere with the shared signal of the cathinones, and its solubility in the chosen buffer system produced a single sharp singlet.

Using 3-methoxy-2-(methylamino)-1-(4-methylphenyl)propan-1-one (mexedrone) as the test compound, the group optimized the acquisition parameters and conducted method validation, including an evaluation of the specificity, linearity, accuracy, precision, and robustness.

Results and discussion
In summary, all four sized samples were clearly identified and characterized by the NMR method.

In addition, using the optimized 1H qNMR method, mexedrone and its analogues, including 1-(3-chlorophenyl)-2-(ethylamino)-propan-1-one (3-CEC), 4-chloro-α-pyrroli-dinopropiophenone (4-Cl-α-PVP), 1-(3,4-methylenedioxy-phenyl)-2-propylamino-propan-1-one (propylone), and methcathinone, were accurately quantified.

Data for specificity, linearity, accuracy, precision, and stability of the qNMR method were all in line with expectations for a routine method. To view the complete dataset for the experiments performed, please refer to the original paper.[5] The authors concluded that this new methodology can be used to address qualitative and quantitative analysis of not only synthetic cathinones, but other similar substances too.

Front-line analysis as part of a distributed topology

Against this background of a fast-moving market in NPS, Bruker is the only vendor to offer both floor standing instruments, and a powerful, easy to use benchtop NMR system. The company's unique approach, known as DLT (distributed laboratory topology), allows government and commercial forensic services to plan and implement a network of instruments, appropriate for the work that will be performed at each location (figure 3).

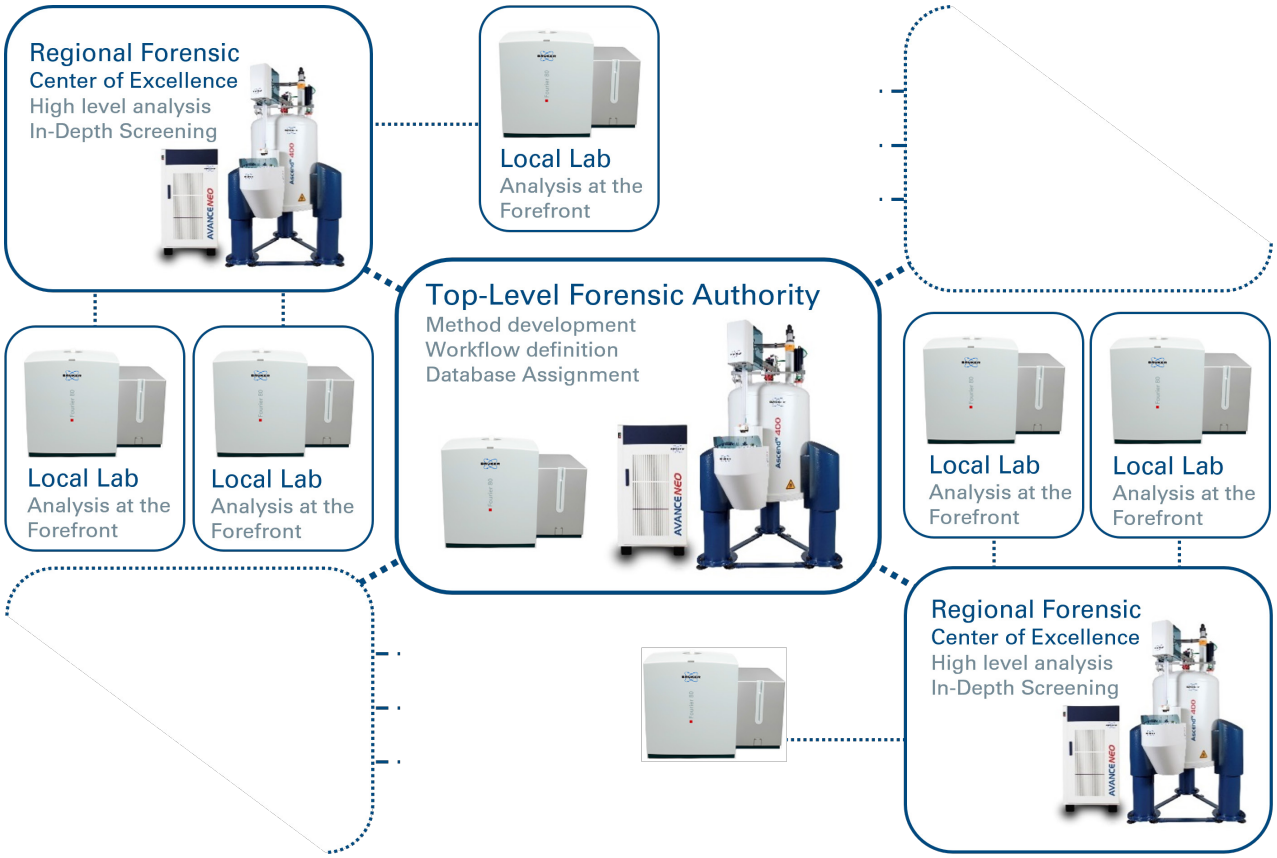

Figure 3 Bruker's distributed laboratory topology approach to narcotics analysis.

A growing body of literature is highlighting the potential application for benchtop NMR in forensic, synthetic chemistry and natural products analysis. A recent review6 offers an overview of these systems in forensics applications. In addition, several groups are utilizing a benchtop system with experimental samples to investigate usefulness. In one such publication7 seven psychedelic 2,5-Dimethoxy-Phenylethylamine-based 'designer drugs' were analyzed. The 1H-NMR data generated with the benchtop system was found to be in good agreement with the chemical shifts and signal pattern reported in previous literature.

Conclusion

Building on the use of its floor standing NMR equipment – exemplified in this app note by the ground-breaking qualitative and quantitative NMR method development work for synthetic cathinones – Bruker supports customers working in police, customs, federal, state, and border control laboratories in over 40 countries across the world.

Translating this expertise into a benchtop system has led to the company's unique DLT approach, where central (reference) authority labs use traditional high-power NMR instruments for method development, workflow definition and database management. An example of this is regional centers of excellence also employ floor standing NMR systems for high level analysis and in-depth screening where it is needed, and local labs run approved methods on benchtop NMR systems, delivering analysis at the frontline.

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