APPLICATION NOTE

Differentiating Constitutional Isomers of Synthetic Cathinone NPS Drugs with Benchtop NMR

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Introduction

Cathinone is a natural alkaloid occurring in the khat plant[1], known for its mild stimulating effects when chewing the leaves which is of cultural importance in East Africa and the Middle East.[2] Synthetic cathinone derivatives played a big role in the emergence of the new psychoactive substances (NPS) grey market in the 2000s and were sold online mislabeled as "bath salts" or "research chemicals" trying to circumvent potential control mechanisms.[3] Cathinones show strong similarities in its molecular structure with other phenylethylamine based drugs as shown in comparison with amphetamine and methamphetamine in **Figure 1**. Cathinones share the β-keto amphetamine (cathinone) core structure.

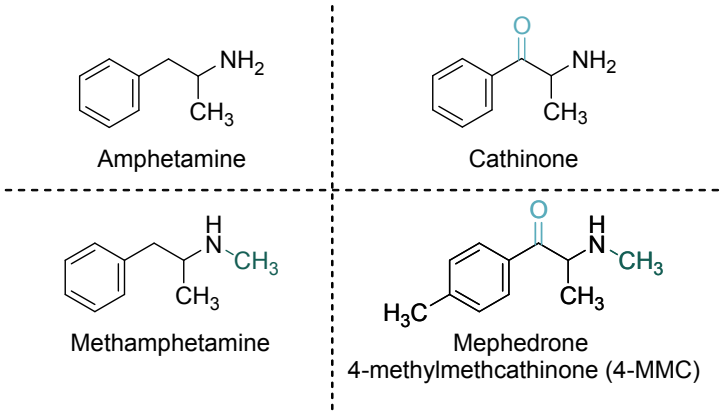

Figure 1. *Molecular structures of some phenylethylamine based drugs, cathinone, and the synthetic derivative 4-MMC.*

The use of synthetic cathinones leads to stimulations similar to those of amphetamines, such as increased energy, concentration, and euphoria, through interaction with dopamine, serotonin, and norepinephrine transporters in the brain.[4,5] Since 2009, synthetic cathinones and phenethylamines were the most frequently reported NPS class. Between 2016 and 2020, most of the NPS identified were stimulants, followed by synthetic cannabinoids.[6] More recently, synthetic opioids started to play a bigger role as well. In 2020, 25% of the NPS that were reported for the first time, were stimulants (mostly cathinones and phenethylamines, **Figure 2**, left), but there is a reason why so many synthetic cathinones appear on the drug market each year. The molecular structure of cathinone bears many chemical functionalities which allows to design novel NPS by derivatization through relatively easy modification of the aromatic substituents, the N-alkylation, or variation of the alkyl substituents. [7] However, NPS are not necessarily always novel substances, they only recently emerged on the drug market.[4] Mephedrone or 4-methylmethcathinone (4-MMC, **Figure 1** bottom right) was originally synthesized in 1929 but was reported to be used as a recreational drug as late as 2007.[8]

Methylmethcathinones (MMC) are one subgroup of synthetic cathinones and were mainly traded over the internet as 'legal highs' and alternatives for amphetamine ('speed'), cocaine, or 3,4-methylenedioxymethamphetamine (MDMA). 4-MMC, appeared early on the market and used to be very popular. While this substance became a scheduled drug, in some countries at a certain period of time, the *ortho-* and *meta-*substituted isomers, 2-MMC and 3-MMC, were still legal. During 2020, a strong increase of cathinone seizures in Europe was reported by the European Monitoring Center for Drugs and Drug Addiction (EMCDDA). Interestingly, 4-MMC and the recently re-emerging 3-MMC, along with their respective chloromethcathinone (CMC) analogs made up for almost 75% of the overall number of cathinone seizures (**Figure 2**, right).[6, 9, 10]

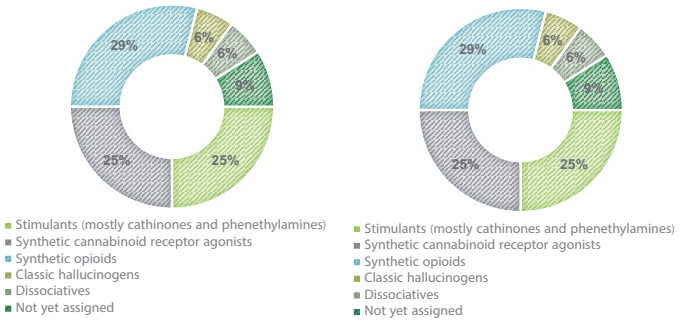

Figure 2. *Distribution of NPS reported in 2020 for the first time at the global level (left).* [6] *Quantity of cathinone powders seized by substance in 2020 in the European Union (right).* [10]

Thus, it is very important to be able to differentiate between constitutional isomers (compounds with the same molecular weight) of such NPS. Mass spectrometry is one of the most used detection techniques in forensics labs, but it shows the same fragmentation pattern and cannot differentiate these isomers without addition of another analytical layer like liquid or gas chromatography (*e.g.*, LC-MS or GC-MS)[6]. However, for chromatographic methods, certified reference materials are required, which are usually very expensive or simply not available for brand new NPS. Nuclear magnetic resonance (NMR) instruments are not commonly found in forensic laboratories but are essential for structural elucidation of new synthetic cathinones[4] because they are capable to differentiate constitutional isomers. More importantly, NMR spectroscopy allows the detection of similar fragments in the analyte molecular structures, thus, unknown novel compounds that could potentially be used as a recreational drug can be detected based on their similar ^{1}H NMR spectrum of a known illicit drug. Despite the game-changing advantages of this powerful analytical technique, the high purchase costs and required maintenance of superconducting high-field NMR instruments make this technique prohibitive for most forensic chemistry labs. However, benchtop NMR instruments are offered for a fraction of the cost of traditional high-field NMR spectrometers, which do not use cryogens and are easy to operate, offering an interesting alternative for these underserved users. In this application note, we will discuss the 60 MHz ^{1}H NMR spectra of the *ortho-*, *meta-*, and *para-*substituted MMC isomers, and their potentially automated differentiation.

Procedure

Highly pure street drug samples of the solid hydrochloric salt forms of 2-MMC, 3-MMC, and 4-MMC, were dissolved in DMSO-d_6 to obtain 100 mM solutions. Approximately 0.6 mL of these solutions were transferred into a regular 5 mm NMR tube and placed into the Nanalysis 60 MHz benchtop NMR instrument. Qualitative data was acquired, but quantification via qNMR can easily be done by employing an internal calibrant.[11]

Results and Discussion

In **Figure 3**, the 60 MHz ^{1}H NMR spectra of the synthetic cathinones 2-MMC, 3-MMC, and 4-MMC are shown. The three compounds are constitutional isomers and their molecular structures only vary in the position of the methyl substituent in the aromatic ring.

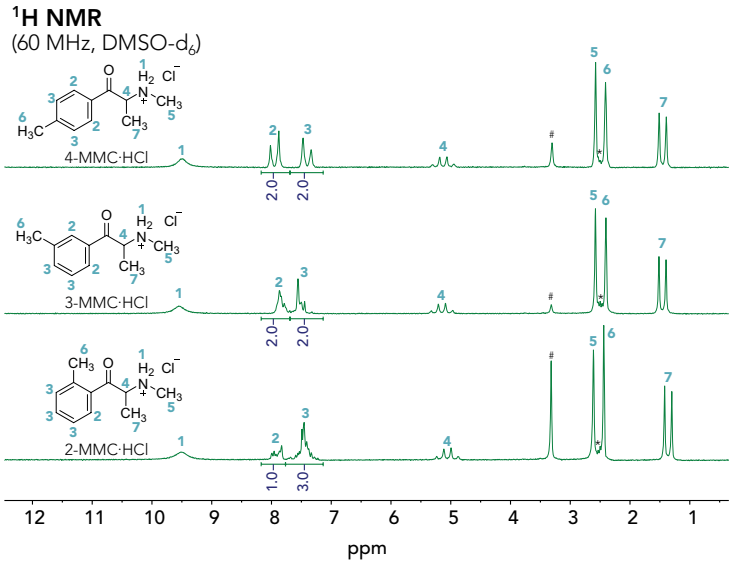

Figure 3. *Stacked 60 MHz ^{1}H NMR spectra of the three methylmethcathinone (MMC) isomers. The residual solvent signal is indicated by an asterisk (*) and the water signal by a hash mark (#).*

The chemical shifts of the NMR signals of alkyl group hydrogen atoms 4 – 7 are very similar and are not suitable for unambiguous differentiation of the isomers. The electronic environment of these hydrogen atoms are effectively the same for the three isomers. Despite their similarity, the isomers do show differences especially in the aromatic areas of their NMR spectra. The *para*-substitution pattern of the aromatic signals 2 and 3 between 7 ppm to 8 ppm appear as two doublets which clearly identifies 4-MMC. Thus, visually clearly but also numerically by the reduced number of peaks, 4-MMC is different to 2-MMC and 3-MMC. These show stronger second order effects in this range and the substitution pattern cannot be as easily extracted. However, the integration ratio of the signals 2 and 3 allows for differentiation between the *ortho*- and *meta*-substituted methylmethcathinones. For 2-MMC we find an integration ratio of 1:3, while in 3-MMC it reads 2:2.

These criteria allow for both, manual and automated differentiation between the conformational isomers 2-MMC, 3-MMC, and 4-MMC. Thus, 60 MHz benchtop NMR is sufficient for differentiating the three isomers via the relative integration area in conjunction with the number of peaks of the aromatic proton signals corresponding to hydrogen atoms 2 and 3. Interestingly, this information can be extracted from the spectra in an automated fashion. A script can test the number of peaks and the relative integration area for distinguishing between the three MMC isomers.

Conclusions

To date, NMR instrumentation is still underrepresented in forensic analysis laboratories, most likely due to the prohibitive cost of high-field instruments. The fact that a) NPS detection can be automated, b) isomers can be differentiated, and c) multiple components of a mixture can simultaneously be quantified without the need of reference materials, makes benchtop NMR an invaluable tool for any forensic or customs laboratory. While chromatographic methods and mass spectrometry still provide the necessary sensitivity for blood or urine analysis, portable benchtop NMR systems will be more and more employed for quick and easy identification, discrimination, and quantification measurements of seized materials in forensic analytical laboratories.

If you have any questions about incorporating benchtop NMR into your (mobile) forensic laboratory, please don't hesitate to contact us via sales@nanalysis.com.

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