APPLICATION NOTE

Benchtop NMR Spectroscopy for Quick and Easy Identification of Illicit Street Drugs with Database Software

nanalysis™

Forensic

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Introduction

The identification of illicit drugs and new psychoactive substances (NPS) in forensic laboratories has become an increasingly critical and time-sensitive challenge for both law enforcement and public health agencies. The rapid evolution and proliferation of NPS - many of which are unlisted or novel - frequently leave forensic analysts without the tools needed for targeted identification. Traditional chromatographic and optical methods often require time-consuming preparation and rely heavily on reference materials, which need to be developed and thus often are not available for emerging NPS. Additionally, most street drugs are adulterated, where substances such as heroin or cocaine can show a much higher concentration of the cutting agents than the illicit drug, making it even harder to identify both the drug itself and the other components in a seized sample. Accurately detecting and measuring such complicated mixtures requires advanced analytical tools capable of providing as much non-targeted information as possible. NMR spectroscopy is one of the most information-rich analytical techniques, but the size, capital costs, maintenance and other aspects have largely prevented the use of superconducting high-field NMR spectrometers in forensic analysis. However, benchtop NMR systems - a new class of permanent magnet based low maintenance spectrometers - are portable, robust, affordable, highly accessible and allow for a wide range of applications including the identification of illicit drugs.

We composed a specialized illicit drugs database using Nanalysis benchtop NMR spectrometer and the ACD/Labs' NMR Workbook Suite software[1]. Herein we present examples of use cases for which such an illicit drug database in NMR Workbook Suite allows forensic analysts to automate illicit drug identification even without any NMR expertise.

The Nanalysis Illicit Drugs Database

A database can easily be created with any substance class of interest. In this case the presented illicit drugs database contains more than 230 reference ^{1}H NMR spectra, covering a wide range of illicit drugs, new psychoactive substances, and commonly used relevant cutting agents. For each database entry essential information, including molecular structures, identifiers such as CAS numbers, InChI keys, and SMILES, as well as chemical formulas, and molecular weights can be added to the respective unique ^{1}H NMR spectrum (**Figure 1**).

ACD/NMR Workbook Suite facilitates the storage and management of compiled information together with the NMR spectra, but most importantly, it lets users perform detailed spectral searches in peak search or similarity search mode, for effortless identification of street drug samples in law enforcement or harm reduction applications.

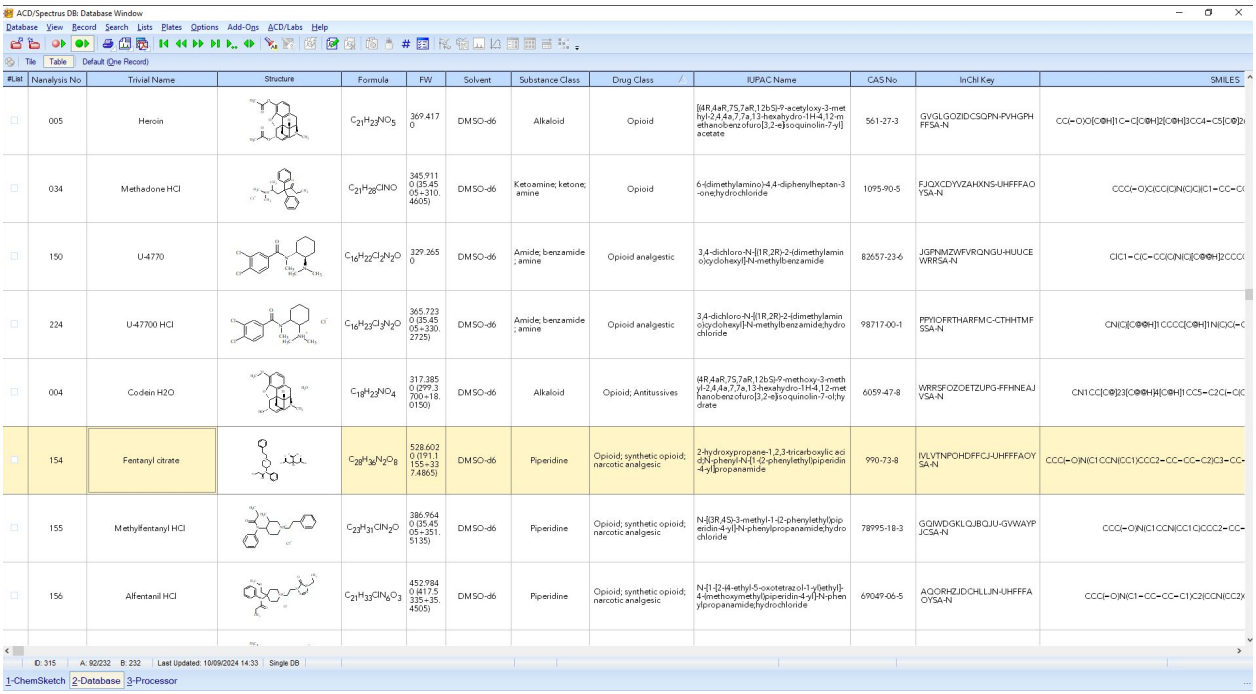

Figure 1. *Screenshot of an illicit drugs database in NMR Workbook Suite (excerpt shown).*

Peak Search and Similarity Search Mode for Different Tasks

Peak Search – the mixture analysis tool

Street drug samples are often distributed as mixtures of the actual illicit drug and adulterants, contaminants, or diluents. The presence of multiple analytes in a "cut" illicit street drug may be due to several reasons. It is possible that the product mixture contains contaminants from the synthesis, extraction, formulation or degradation products from storage leading to a lower purity, or some combination of the aforementioned. Also, agents can be added to facilitate the preferred way of administration of the drug, for example, caffeine is added to heroin or cocaine to facilitate smoking the drug.[2] Similarly, binding agents can be added to allow a powder to be pressed into pills. Additionally, the inclusion of different stimulating or psychoactive compounds can increase or change the effects experienced during recreational use. There are many commonly used cutting agents for bulking or diluting a product that are often combined with a specific drug, due to their ability to mimic certain properties of the original pure drug substance, like the fluffiness of a powder, the solubility in water for injection, or the melting/sublimation points for smoking drugs, or other ways to disguise the drug being cut. Benzocaine, a local anesthetic used in dental care, is used as a common cutting agent in cocaine due to it exhibiting a similar numbing effect.[3] NMR spectra of real, seized street drug samples were recorded and tested against this database to demonstrate the accuracy and effectiveness of the identification capabilities of Nanalysis benchtop NMR combined with NMR Workbook Suite. As a first example we examined a 60 MHz ^{1}H NMR spectrum of an unknown street sample recorded in D_2O (**Figure 2**). From the different signal intensities, it is obvious that the investigated sample is a mixture of at least two compounds. While we could try to "manually" identify the analytes present in the spectrum by looking at the chemical shifts, multiplicity, and integration of the individual signals, this is not trivial, and it would be too time-consuming and prone to errors. Instead, we want to automatically identify the different components in the mixture with the Nanalysis illicit drugs database in NMR Workbook Suite.

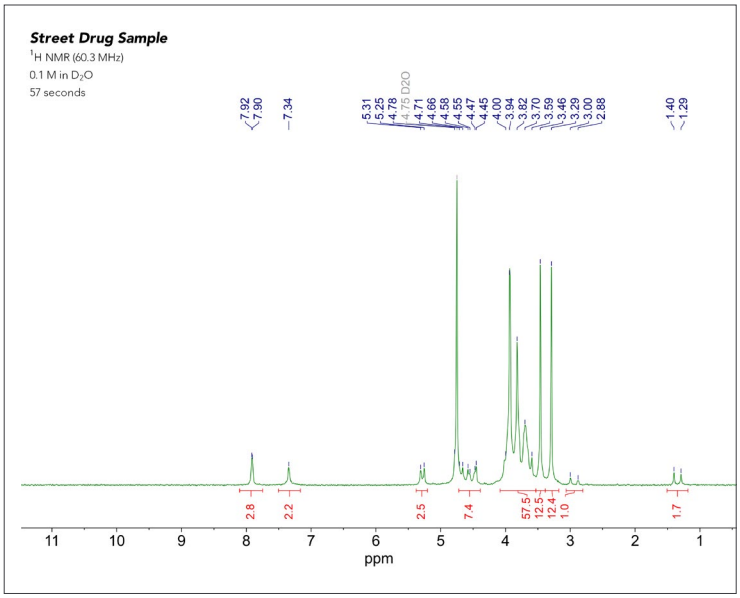

Figure 2. ^{1}H NMR (60.3 MHz) spectrum of a street drug mixture in D_2O.

For the identification of the individual moieties in the mixture, the raw data in JCAMP-DX file format from the Nanalysis benchtop NMR is loaded in NMR Workbook Suite, with the preprocessing options enabled, the software automatically processes the spectrum and picks the peaks. With this spectrum a peak search can be performed and the results will be displayed within a few seconds. In this case the list of hits contains only 7 potential candidates from a total of >230 database reference spectra. These seven hits can now be compared with the search query spectrum one by one to confirm or ignore the individual hit spectra. In this example, the seven potential candidates ranked by their Hit Quality Index (HQI) score rating are: amphetamine sulfate; lactose; caffeine (in D_2O); caffeine (in DMSO-d_6); citric acid; sorbitol; and D-mannitol. The selected hits can be optimized to consider slight shifts and the sum spectrum (yellow) of the selected compound spectra can be projected onto the Query (green) to confirm all signals are identified. In this case, the projected sum spectrum of the selected reference spectra aligns perfectly with the search query, so the mixture is fully identified, and the analyzed mixture is confirmed to be a "speed" street drug containing amphetamine,[4] lactose, and caffeine (**Figure 3**). While detailed information on cutting agents in amphetamine is rarely available, lactose is a known bulking agent,[5] and a recent study from Poland reported the stimulant caffeine to be present in 94% of amphetamine samples studied between 2016 and 2020.[6]

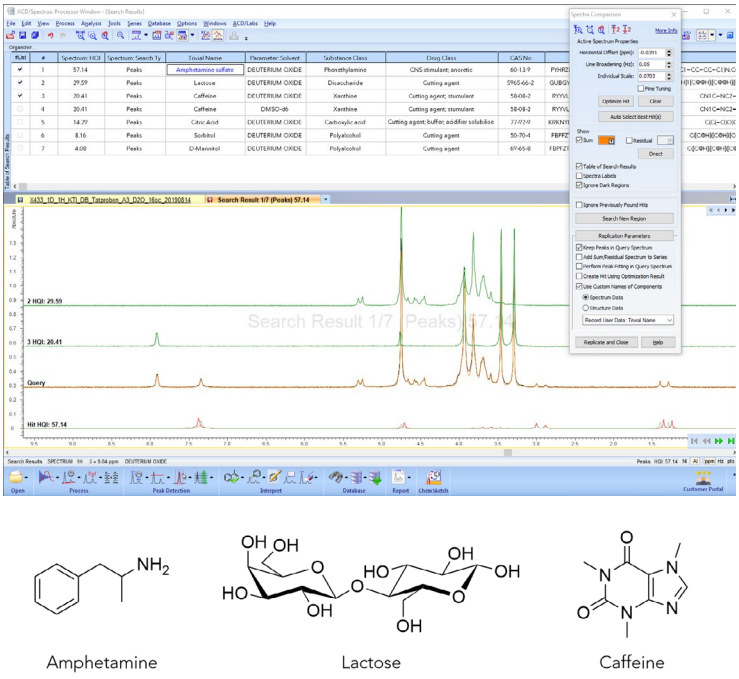

Figure 3. Stacked selected results (top) of amphetamine, lactose, and caffeine with sum spectrum (yellow) projected onto the Query spectrum (green). The respective molecular structures of the identified components are shown at the bottom.

Similarity Search – for recognizing fragment patterns in derivatives

In contrast to peak search, the *similarity* search in NMR Workbook Suite finds *similar* spectra based on the spectrum shape, not based on (picked) peaks. This search mode has limited applicability for mixture analysis. However, an interesting potential use of this feature would be searching for a specific range of the spectrum containing signals that could not be identified via peak search

when the specific compound was not available in the illicit drugs database. In this case the similarity search can suggest spectra that look similar and help to identify the substance class or provide a hint for the potential presence of an illicit drug in a sample. In general, the similarity search can find similar substances to the ones you have listed in your database, so this can be an interesting tool for NPS detection. As an example, a street drug sample of a synthetic cannabinoid is loaded in ACD/Spectrus Processor (**Figure 4**).

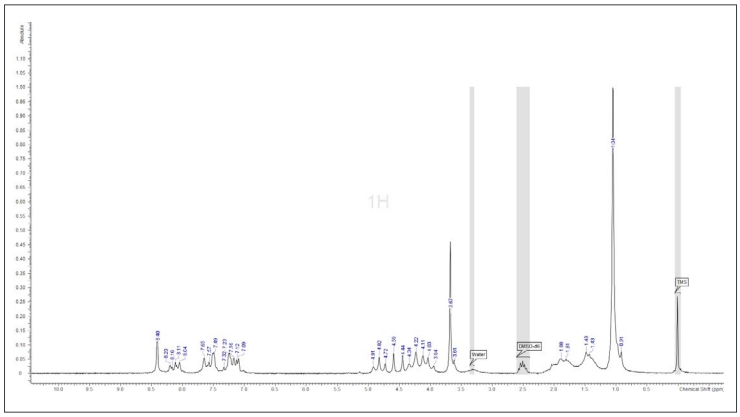

Figure 4. *^{1}H NMR (60.3 MHz) spectrum of a synthetic cannabinoid street drug in ACD/Spectrus Processor with automated preprocessing and peak picking*

A similarity search over the full spectral range containing analyte signals between −0.5 ppm to 9.0 ppm is performed with a maximum of 5 hits selected to limit the results list. Obviously, the first hit, 5F-MDMB-PICA is the compound in question and the identification is accomplished with a high degree of certainty, but for explaining the capabilities of this search mode, the three first results are displayed in comparison with the search query (**Figure 5**, top). The only notable difference between the query (black, second from bottom) and the 5F-MDMB-PICA reference (red, bottom) spectrum is the broadness of the water signal at 3.3 ppm and a few impurity signals, which can be ignored. Interestingly, the second and third highest rated hits, 5F-ADBICA and 4F-MDMB-BICA are very similar in their molecular structures compared to 5F-MDMB-PICA (**Figure 5**, bottom). This highlights the unique capabilities of NMR analysis for NPS detection. NMR spectra have a direct correlation with molecular fragments such that they can easily reveal similarities ranging from slightly different structures to major differences, which are required by forensic analysts to be able to detect NPS as potential harmful or illegal substances.

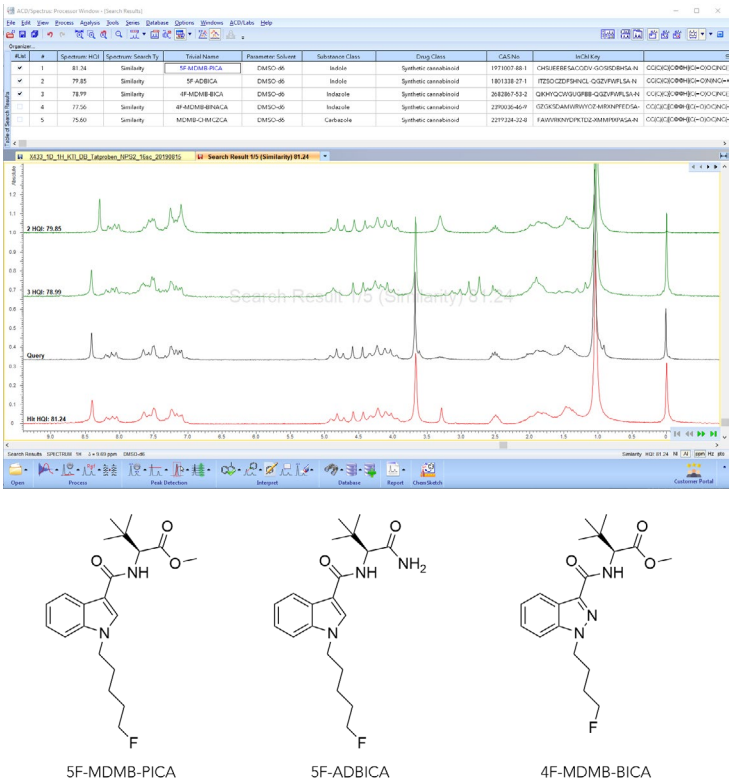

Figure 5. *Similarity search results for a synthetic cannabinoid sample. 5F-MDMB-PICA reference spectrum (red, bottom), Query spectrum (black, second from bottom), two structural very similar synthetic cannabinoids, 5F-ADBICA (green second from top), and 4F-MDMA-BICA (green, top) for comparison highlight the capabilities of the similarity search (top). Molecular structures of three synthetic cannabinoids with very similar molecular structures are shown at the bottom.*

Conclusion

The combination of Nanalysis' powerful small footprint benchtop NMR spectrometer and ACD/Labs' NMR Workbook Suite software offers a fast, accurate, and accessible solution for forensic labs challenged with analyzing illicit drugs including NPS. With the addition of automated match indication and mixture analysis capabilities, this tool is invaluable for law enforcement and harm reduction in the fight against illicit drugs emergence. Forensic analysts can now take advantage of NMR technology without needing to be experts, empowering them to make quicker, more reliable decisions in the field of forensic drug analysis.

For more information on how to work with NMR databases, please contact us under sales@nanalysis.com and sales@acdlabs.com.

References

[1] *NMR Software for Data Analysis and Structure Verification*, https://www.acdlabs.com/products/spectrus-platform/nmr-workbook-suite/ (accessed 2024-11-22).

[2] Cole, C.; Jones, L.; McVeigh, J.; Kicman, A.; Syed, Q.; Bellis, M. A., Cut – *A Guide to Adulterants, Bulking Agents and other Contaminants found in Illicit Drugs*; https://www.drugsandalcohol.ie/13119/1/Cut_a_guide_to_adulterants.pdf (accessed 2024-11-20).

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[6] Żubrycka, A.; Kwaśnica, A.; Haczkiewicz, M.; Sipa, K.; Rudnicki, K.; Skrzypek, S.; Poltorak. L. *Talanta* **2022**, *237*, 122904.