APPLICATION NOTE

Wiley KnowItAll and Nanalysis Benchtop NMR for Identifying Street Drug Mixtures

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Forensics

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Introduction

Fast and accurate untargeted identification of street drug mixtures remains a challenging task for border control and forensic laboratories. Seized samples often contain multiple compounds blending the controlled active substance with adulterants and cutting agents in mixtures of varying complexity. Conventional analytical instrumentation in forensic science laboratories, such as chromatographic methods, IR and UV spectroscopy, typically rely on lengthy workflows as well as reference materials limiting their effectiveness when composition is uncertain or standards are not available for new designer drugs.

Nuclear magnetic resonance (NMR) spectroscopy undoubtedly provides the highest wealth of structural information compared to other analytical techniques and can be used for untargeted analysis. It is a non-destructive spectroscopic method, and typical workflows can deliver results very quickly. However, due to capital costs, maintenance requirements and expert staff needed to run classical liquid cryogen cooled superconducting magnet systems, NMR spectrometers have found limited application outside of academia and larger R&D laboratories.

Highly accessible, powerful, and portable benchtop NMR spectrometers requiring almost no maintenance allow forensic analysts to rapidly measure seized illicit drug mixtures even outside traditional laboratory environments. Analysts can then analyze, identify, and manage the NMR data in modern analytical software tools like Wiley's KnowItAll.

This study demonstrates a practical and efficient method for identifying illicit street drug samples by pairing a Nanalysis[1] system with the analytical tools available in KnowItAll[2], including the creation of an representative benchtop NMR illicit drugs user database of relevant reference spectra. Three case studies, amphetamine, cocaine, and heroin street samples, highlight the fast identification of mixtures with varying spectral complexity. The combination of accessible benchtop NMR technology with Wiley's KnowItAll platform creates a strong tandem, enabling forensic analysts to streamline compound identification and allowing for automated workflows, significantly reducing the need for specialized NMR expertise.

Straightforward Spectral User Database Building and Management

To perform drug mixture identification runs, we created an representative user database including 31 relevant single components using a 60 MHz benchtop ^{1}H NMR data (Figure 1). While Wiley offers an extensive collection of reference libraries, users can also build custom databases using KnowItAll's comprehensive tools. These user-generated databases can be added to the search workflow and enhance identification within KnowItAll's SearchIt.

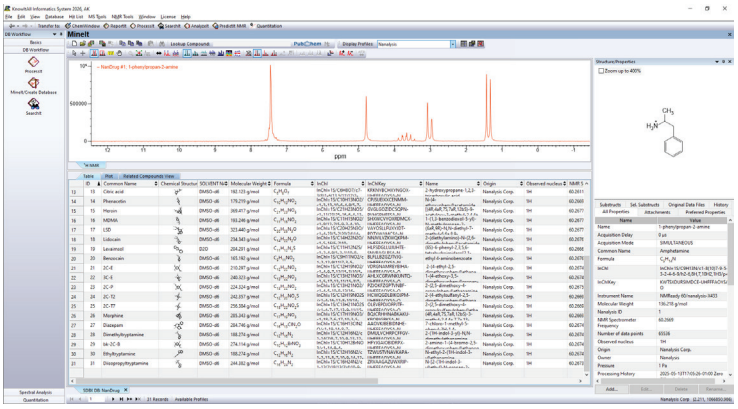

Figure 1. Screenshot of an illicit drugs user database in MineIt (excerpt shown).

The smooth workflow in KnowItAll allows for a very efficient and speedy population of the user database. After creation of an empty user database, the Nanalysis raw data JCAMP-dx file was opened in KnowItAll's ProcessIt tool and processed by running a macro which replaces manual 2x zero filling, 0.1 Hz exponential line broadening window function, Fourier Transformation, Auto Phase correction with Wiley's proprietary "GoodLook" method, and polynomial baseline correction. The resulting spectrum was referenced to the internal Tetramethylsilane (TMS) signal or the residual protonated solvent signal and transferred to the KnowItAll MineIt tool creating a new database entry.

From there, a molecular structure can be generated from the substance name using the OPSIN Name2Structure feature in the included ChemWindow application. This will automatically populate fields such as molecular weight, chemical formula, name, InChI and InChIKey, which is a very helpful automation step. Spectral metadata like the solvent, NMR spectrometer frequency, observed nucleus, or origin of the data is automatically extracted from the file header. Thus, only the common name for the drugs was needed to complete the entry.

For the purpose of the mixture spectrum search, this was repeated to add more datasets to the database. However, MineIt offers additional robust features such as the ability to add multiple analytical datasets or techniques to one entry or to categorize entries according to DEA regulations. These databases can be managed and shared within an organization to extend the reach of these resources by using cloud storage services.

Spectrum Search for Street Sample Identification

For each seized drug sample, the ^{1}H NMR spectrum was processed in ProcessIt using the same macro and then transferred to SearchIt. Here, the user database was selected as the Search Database and a Spectrum Search was performed (Figure 2). As a general guideline, we observed the best identification results using the search methods: Correlation, Correlation (classic), or 2nd Derivative Euclidean Distance.

While the number of components can be selected, this is usually unknown for street drug samples, but the algorithm will include Composite Results with different numbers of detected analytes, and we found best results starting with two or three components selected and to iterate further if needed. Optimized Corrections were enabled as per default settings, including baseline, horizontal and vertical offset corrections, duplicate and replicate removal. Setting the Hit List Size Limit to 10 (50 per default) can significantly speed up the search. Finally, in the loaded spectrum the residual solvent signals, water impurity and internal reference signal ranges should be excluded, which work seamlessly using the Exclude Range Bar at top of the spectrum.

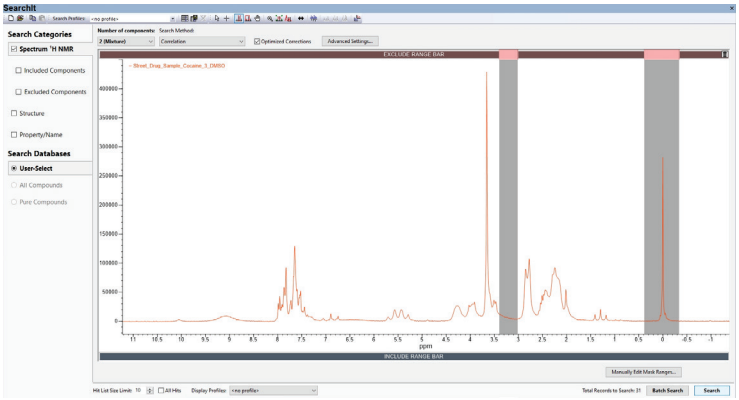

Figure 2. *SearchIt in KnowItAll allows for spectral comparison of single compound or mixture spectra with Reference and/or User Databases.*

Case 1: Amphetamine

As an initial test, a relatively simple amphetamine street sample ^{1}H NMR spectrum was investigated. The Correlation search mode was used and the first result with the highest hit quality index (HQI) of 65.13 clearly matched all signals and the sample is identified as a mixture containing caffeine and amphetamine. Important to note, KnowItAll does not just list single components found in the mixture but also automatically generates Composite Spectra, combining the individual components into a sum spectrum as a very user-friendly way to visually confirm the best fit for the found Composite Spectrum (red in Figure 3) with the experimental data (blue in Figure 3).

As a second confirmation method, the Residual Spectrum as the difference between experimental and Composite Spectrum can be visually inspected. Despite some peak shifts in the amphetamine signals, potentially due to different matrix effects like concentrations or through the presence of the basic caffeine in the mixture, all mixture signals are clearly identified.

Figure 3. *Search results for a relatively clear amphetamine street sample spectrum (^{1}H NMR, 60 MHz, DMSO-d_6). Composite Spectrum (red) superimposed with the experimental mixture data (blue).*

Case 2: Cocaine

A cocaine street drug sample was chosen as a second, more complex example. Once again, the Correlation search mode was employed. Although the Mixture Search was configured for two components, the highest ranked hit found was cocaine as a single compound. From visual inspection, some low intensity signals, notably at 1.3 ppm and 6.9 ppm, were not explained by the cocaine spectrum alone, where the second hit with an HQI of 85 listing cocaine and phenacetin did show great agreement between the mixture spectrum and the Composite Spectrum.

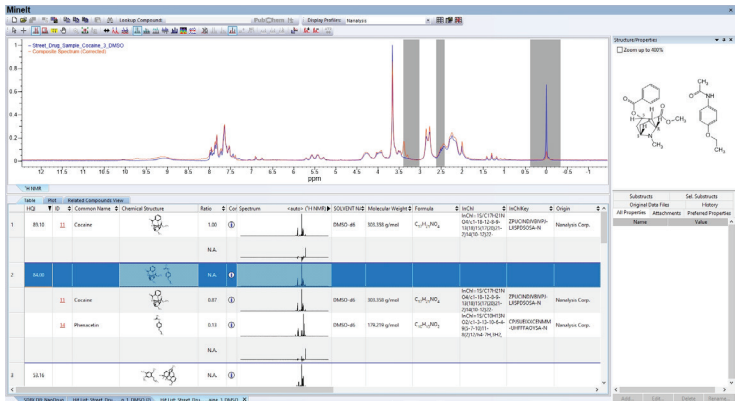

Figure 4. *Search results for more crowded cocaine street sample spectrum (^{1}H NMR, 60 MHz, DMSO-d_6). Composite Spectrum (red) superimposed with the experimental mixture data (blue).*

Case 3: Heroin

In the third case, a heroin street sample was investigated. Due to substantial signal overlap and molecular complexity, this proved to be the most challenging example to evaluate the SearchIt algorithm. During the testing series, we found that the 2nd Derivative Euclidean Distance search mode can particularly be helpful for identification of more complex mixture spectra. In a first search run for the heroin spectrum using this search mode and having selected two compounds, the highest ranked hit with an HQI of 58.56 revealed paracetamol and heroin as components of the mixture. However, clearly the spectrum was not fully matched as multiple signals have not been identified (blue signals without corresponding red Composite Spectrum signals). A second search with three components selected was performed, where Correlation search mode gave the best result, identifying caffeine as the third component in the heroin street drug mixture.

Figure 5. Search results for a heroin street sample spectrum (^{1}H NMR, 60 MHz, DMSO-d_6). Composite Spectrum (red) superimposed with the experimental mixture data (blue).

Conclusion

Pairing software that includes database building and search features with NMR is a frequently requested combination by benchtop NMR users especially in fields where quick and easy but also reliable identification is required. Through this series of examples, we have demonstrated a practical workflow to create a benchtop NMR user database in Wiley KnowItAll and perform mixture analyses of unknown illicit drug mixtures for qualitative determination of its composition. The herein presented method is not limited to illicit drugs. Databases can be constructed to contain the components to meet your specific application needs.

The KnowItAll software features an integrated, modular architecture comprising integrated tools to make this NMR workflow in these analyses simple that work logically as you move from task to task: NMR data processing (ProcessIt), custom database creation (MineIt), and spectral identification through database matching (SearchIt). This design enables an efficient, streamlined analytical environment from initial data processing through final identification. For support on and more information, please contact us under sales@nanalysis.com or visit https://sciencesolutions.wiley.com/

References

(1) Nanalysis benchtop NMR spectrometer; Nanalysis Scientific Corporation: Calgary, AB, Canada; https://www.nanalysis.com/products-overview/

(2) KnowItAll Analytical Edition, version 2026.0: John Wiley and Sons, Inc.: Hoboken, NJ, 2025; https://sciencesolutions.wiley.com/knowitall-analytical-edition-software/

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