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Nuclear Magnetic Resonance (NMR), a powerful tool in the field of illicit drug analyses

V. Gilard[1], S. Balayssac[1], G. Bertrand[2], M.P. Escot[2], M. Malet-Martino[1]

[1]Groupe de RMN Biomédicale, Laboratoire SPCMIB (UMR CNRS 5068), University of Toulouse 3, 118 route de Narbonne, 31062 Toulouse cedex 9, France
[2] Institut National de Police Scientifique-Laboratoire de Police Scientifique de Toulouse, 23 Boulevard de l'Embouchure, BP 92162, 31021 Toulouse cedex 02, France

Introduction

The objective of this study is to propose Nuclear Magnetic Resonance (NMR) approaches for the analysis of illicit drugs. Even if chromatographic methods with various detection modes are the most currently used for detection and characterization of these chemicals and remain gold standards, we show here that NMR is a powerful technique in the field of illicit drug analyses.
Firstly, 24 heroin samples coming from different seizures were analyzed. Spectral signatures of the samples were obtained using ^{1}H NMR and two-dimensional Diffusion Ordered SpectroscopY (2D DOSY) ^{1}H NMR, thus allowing the whole characterization of each sample.
Secondly, the structural characterization of recent psychoactive drugs was achieved using ^{1}H, ^{13}C, and two-dimensional NMR experiments.

DOSY NMR analysis of heroin samples

The ^{1}H NMR profile represents a spectral signature typical of each mixture but a more acute view of this spectral fingerprint can be obtained using 2D DOSY ^{1}H NMR.

- DOSY relies on differences in translation diffusion as a mean to separate components in a solution mixture. All the peaks of a same compound are lined up.
- The diffusion coefficient generally decreases with increasing molecular weight.
- The three samples shown in figure 1 have different spectral signatures. The main advantage of DOSY NMR is to provide spectral patterns that reveal significantly different chemical signatures.

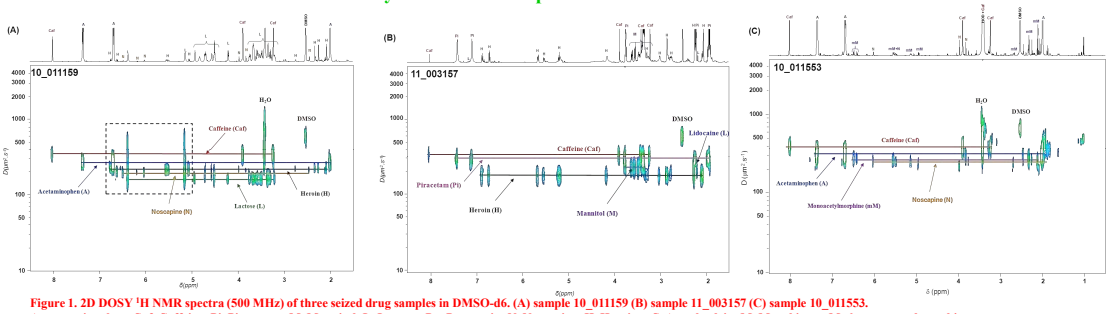

Figure 1. 2D DOSY ^{1}H NMR spectra (500 MHz) of three seized drug samples in DMSO-d6. (A) sample 10_011159 (B) sample 11_003157 (C) sample 10_011553. A, acetaminophen; Caf, Caffeine; Pi, Piracetam; M, Mannitol; L, Lactose; Pp, Papaverin; N, Noscapine; H, Heroin; aC, Acetylcodein; M, Morphine; mM, 6-monoacetylmorphine.

Quantitative NMR analysis of heroin samples

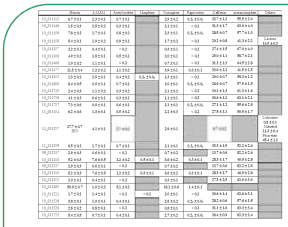

Table 1. Amounts of heroin, impurities and cutting agents detected using ^{1}H NMR. Results are expressed as percentages (% ± SD). All experiments were performed in triplicate (n=3).

NMR quantification was performed by comparison of the integrated peak areas of the analyte to that of the internal reference TSP.

- ^{1}H NMR analysis of heroin samples in DMSO allows the accurate quantification of heroin and its main impurities (6-monoacetylmorphine, acetylcodeine, morphine, noscapine and papaverine) but also cutting agents (caffeine, acetaminophen, lactose, lidocaine, mannitol, piracetam).
- The limit of detection (LOD) with the spectrometer employed after 40 min recording is 3µmol.L^{-1} (corresponding to ≈ 0.02% for heroin impurities) at an S/N ratio of 3. The limit of quantification (LOQ) after 40 min recording is 20 µmol.L^{-1} (≈0.15%) at a S/N ratio of 10.

Figure 2 shows the comparison of NMR and GC assays for heroin, noscapine, acetaminophen and caffeine. Results in percentage are in good agreement with only slight variations.

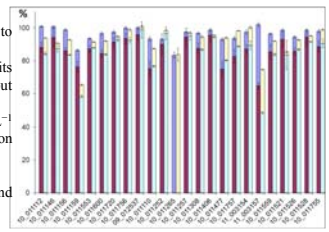

Figure 2. Comparison of GC and NMR quantifications

Assignment of signals in heroin samples using S-TOCSY

S-TOCSY analysis is useful for determining relationships between NMR signals as well as for structural assignment of individual molecules in complex mixtures. It shows the modeled correlation as NMR lines, enabling straightforward interpretation of the variable contributions.
In figure 3, the S-TOCSY plots are correlated to the resonance of heroin at 6.6 ppm (d) (Fig. 3A), 6-monoacetylmorphine at 4.9 ppm (d) (Fig. 3B); acetylcodeine at 5.0 ppm (d) (Fig. 3C); noscapine at 6.2 ppm (d) (Fig. 3D).

- High correlations (red lines; r >0.95) are observed and correspond to intramolecular or structural correlations with other resonances from the same compound. Indeed, in each spectrum signals in red belong to the targeted compound.
- Lower correlations (orange to light green, r = 0.85-0.95) are measured for areas of the spectrum where signals of targeted compounds are overlapped with resonances from other compounds.

This statistical tool is a powerful additional aid for assignment of signals in these complex mixtures as signals of the targeted molecule will appear in colors from red to light green depending on the overlapping.

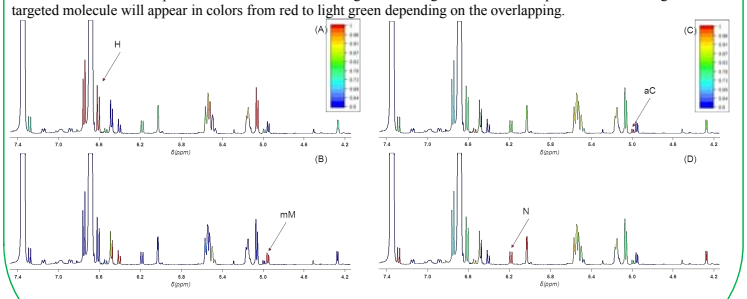

Figure 3. One-dimensional S-TOCSY analysis of (A) the doublet at δ 6.6 ppm (heroin, H), (B) the doublet at δ 4.9 ppm (6-monoacetylmorphine, mM), (C) the doublet at δ 5.0 ppm (acetylcodein, aC), (D) the doublet at δ 6.2 ppm (noscapine, N).

Structural characterization of new designer drugs

The structural characterization of recent psychoactive drugs was achieved using ^{1}H, ^{13}C, and two-dimensional NMR experiments. Such compounds have flooded the illicit drug market under the terms "designer drugs" or "research chemicals". These structurally related drugs often carry unknown safety profiles with serious potential health consequences. In this study, different designer drugs were analyzed: butylone and 5-APB, two substituted phenethylamines; methoxetamine and 2-meoketamine, two ketamine derivatives; JWH-081, an analgesic which acts as a cannabinoid receptor agonist; 5F-AKB-48, a new fluorinated derivative of AKB-48, a potent agonist for the cannabinoid receptors. In Figure 4 are presented ^{1}H, ^{13}C and HSQC NMR spectra for this fluorinated derivative. This research chemical was sold as AKB-48 but the presence of a fluorine atom with characteristic coupling is easily detected using both ^{1}H and ^{13}C NMR.

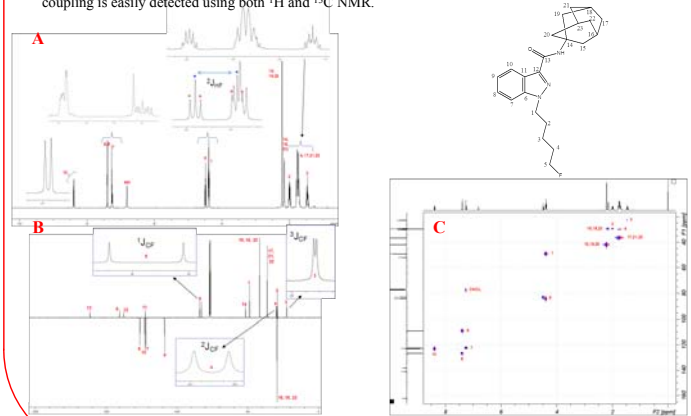

Figure 4. ^{1}H (A), ^{13}C (B) and HSQC (C) NMR spectra of 5F-AKB-48 (Bruker Avance 500, $CDCl_3$)

Conclusion

We demonstrate by these approaches that NMR has several assets for illicit drug analyses. Indeed, it provides structural information on the chemicals contained in the sample as well as its comprehensive analysis, thus allowing direct spectral profiling. In the case of complex mixtures, the main advantage of NMR is its holistic nature that does not require prior information on the chemical content for the detection of all proton-containing compounds. Also, DOSY NMR is particularly powerful as the diffusion dimension enables a rapid visual overview of the information and thus permits rapid comparison between samples.
Recent technical improvements of NMR over the years with higher magnetic field strengths and cryogenically cooled NMR probes combined with the widespread use of sample changers led nowadays the NMR methods to be considered for high throughput analyses.