

Illicit Drug Analysis Using Benchtop NMR: Amphetamines

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Key Words

Amphetamine, Designer Drugs, Illicit Drugs, NMR Reference Library, Presumptive Testing, Rapid Screening

Introduction

Customs and postal seizures of illicit drugs expose a wide variety of illegal and “legal or not” synthetic designer drugs. There has been an increase in the variety of these drugs from several classes, most notably analogues of phenethylamine and cathinone. The result is a dizzying array of structurally similar drugs that challenge existing rapid screening and presumptive testing methodologies. Current methods can lack specificity for preliminary identification of illicit drugs.

Current presumptive test methods include color tests, infrared spectroscopy (IR) and thin layer chromatography (TLC). Color tests are the most widely used presumptive testing method because they are simple to apply in the field or lab. Color testing can be prone to false positive and false negative results, and lacks the discriminating power or structural sensitivity needed to combat designer drugs. Infrared spectroscopy is a very rapid screening technique that provides more information on chemical composition, but which must be matched exactly against a spectral library. In the face of continual designer drug development, spectral libraries need frequent updates. TLC is a chromatographic separation and color-matching technique that is best applied to pure and cut samples.

There is a need for time-sensitive, rapid screening methods for initial drug identification with increased structure selectivity and high discriminating power to help identify the assortment of designer drugs. Nuclear Magnetic Resonance (NMR) spectroscopy offers a solution to this challenge by providing structure sensitivity and high discriminating power. It is often one of several confirmatory testing techniques, along with GC-MS and GC-FTIR, for conclusive qualitative and quantitative analysis. High-field proton (^1H) NMR can be used but is an expensive, overburdened, often centralized and resource-limited technology reserved for confirmatory analysis, making it cost prohibitive for rapid sample analysis applications in the field.



The Thermo Scientific™ picoSpin™ 80 NMR spectrometer provides affordability and convenience in a compact benchtop instrument. NMR spectroscopy produces spectral data that is straightforward to evaluate and is sensitive to slight modifications of chemical structure. The advantage is that key functional groups defining a given drug class, such as amphetamine-type substances, retain their signature NMR profile which allows for drug class discrimination. Whereas, changing a functional group or its position in the molecule causes distinctive changes in NMR spectra, providing the necessary sensitivity to characterize the specific drug.

NMR spectroscopy offers a solution to the initial identification of an assortment of designer drugs with increased structure selectivity and high discriminating power.

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Experimental

A ^1H NMR spectral library of reference drug samples was developed for a series of amphetamine-type substances (ATS). Samples tested (Figure 1) included amphetamine (1), 4-(2-Aminopropyl)phenol (PHA; 1a), 1-(2,5-Dimethoxyphenyl)propan-2-amine (2,5-DMA; 1b), 1-(4-Bromo-2,5-dimethoxyphenyl)propan-2-amine (DOB; 1c), 1-(1,3-Benzodioxol-5-yl)propan-2-amine (MDA; 1d), 1-(2-Fluorophenyl)propan-2-amine (2-FA; 1e), 1-(4-Fluorophenyl)propan-2-amine (4-FA; 1f).

All samples tested were either hydrochloride or sulfate salts, as is often the case with many drugs, making them readily soluble to the desired concentration in aqueous solution. Data acquisition time was constrained to 6 min (25 co-added scans), a time determined acceptable for the depth of qualitative and quantitative information attained from NMR spectra.

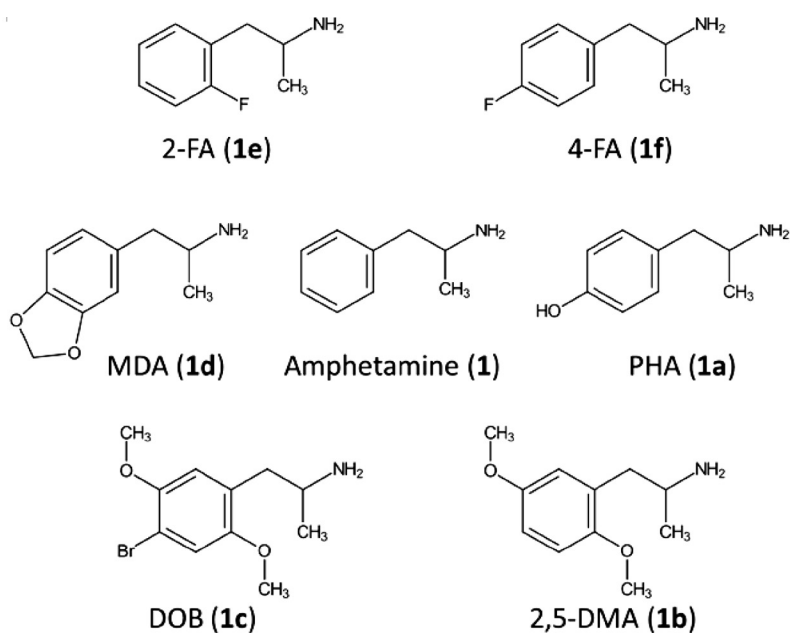


Figure 1: ATS reference sample structures

Solutions of sample drug materials were prepared by dissolving 5–12 mg (depending on sample) in 200 μL of deuterated aqueous solvent to produce a target concentration of 250 mM. Deuterium oxide solvent (D_2O) was prepared with 0.35% (w/v) TMSP- d_4 as an internal chemical shift reference. Liquid samples were injected manually into the capillary cartridge using an inexpensive disposable 1 mL slip-tip polypropylene syringe and 22-gauge blunt-tip needle.

Spectra were acquired using a Thermo Scientific picoSpin 80 NMR spectrometer. The spectrometer is an 82 MHz pulsed, Fourier transform ^1H NMR permanent magnet instrument equipped with a capillary cartridge probe. The fluid path is 400 μm ID Teflon/Quartz capillary tubing with a total flow path volume of 40 microliters (μL), and an active volume within the RF coil of approximately 70 nL.

Results

The chemical structure and numbering scheme for amphetamine is given in Figure 2. Derivatives of amphetamine retain the α -methyl-ethylamine sidechain ($-\text{CH}_2\text{CH}(\text{CH}_3)\text{NH}_2$) while functionalization occurs on the phenyl ring (Figure 1). Sidechain substitution converts ATS into a drug of a different class with its own signature NMR profile. For instance, substituting a methyl for a proton on the amine converts amphetamine into methamphetamine, whereas converting the β -methylene into a carbonyl yields cathinone (β -ketoamphetamine), the parent structure of synthetic designer “bath salts”. In both examples, the characteristic spectral signature seen in the NMR spectrum of an ATS is altered, providing additional discriminating power for drug class identification.

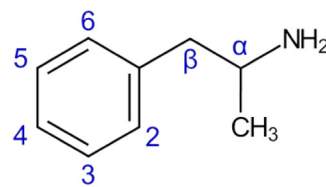


Figure 2: General chemical structure of amphetamine

A library of ATS reference samples is shown in Figure 3; it contains ^1H NMR spectra of amphetamine (1) and a series of analogues (1b–1f).

The proton spectrum of amphetamine is simple and provides the key to identifying a compound as belonging to a particular drug subclass. The α -methyl-ethylamine sidechain produces a characteristic doublet pair (highlighted in red boxes in Figure 3) separated by nearly a 2 ppm chemical shift, with no intervening signals. The signal at 1.25 ppm belongs to the α -methyl group ($-\text{CH}_3$), where coupling to the α -methine proton ($-\text{CH}$) produces a doublet structure. At 3 ppm, a β -methylene doublet ($-\text{CH}_2-$) appears, also coupled to the α -methine proton. The methine proton is coupled to both β -methylene and α -methyl protons causing it to appear as a multiplet, and attachment to the amine shifts its signal to the 3.5–3.75 ppm range. Its signal, however, is not used for profiling because it is weak. The α -methyl-ethylamine doublet pair indicates an ATS as belonging to the amphetamine drug subclass.

Looking at the aromatic region (6.5–8.0 ppm) will help identify the specific analogue. An unsubstituted phenyl group produces a singlet at 7.4 ppm in the parent amphetamine structure. Substitution anywhere around the ring disrupts this symmetry, causing the singlet to shift position and, depending on the type of substituent, to appear with new multiplicity.

Compounds 1a, 1b and 1c, for example, show a distinctive doublet of doublets and doublet pair pattern, respectively, shifted upfield; compound 1d produces an upfield singlet, and the -F substituent on positional isomers 1e and 1f shifts the aromatic signal downfield and additional coupling to -F yields distinctive multiplicity. Aromatic splitting patterns aid in narrowing down structure identification by indicating substitution patterns. Aromatic proton signals used to characterize the amphetamine derivative are highlighted by a blue box in Figure 3.

Other substituents, if protonated, likewise reveal information regarding the specific derivative by generating new signals in the spectrum. The methoxy functional group (-OCH₃) in compounds 1b and 1c, for instance, each produce a signal near 3.8 ppm (highlighted by a green box in Figure 3), giving the appearance of a closely spaced doublet. Disubstituted 3,4-methylenedioxy groups (-OCO-) seen in 1d and homologues, generate a singlet near 6 ppm, a region of the NMR spectrum most frequently populated by alkene protons, making it an isolated signature for these substituents. This region is highlighted by a purple box in Figure 3.

Conclusion

The benchtop picoSpin 80 ¹H NMR solution from Thermo Scientific provides structure selectivity and high discriminating power needed for screening the increasing numbers of synthetic drug analogues penetrating national borders. The compact instrument is conveniently placed in workspace-limited labs and testing areas, while enhancing the presumptive testing capabilities of illicit drug screening facilities. NMR signal profiling is made possible by structural similarities of drugs within a given class, and developing an NMR reference spectral library of key drug class species helps expedite case sample identification.

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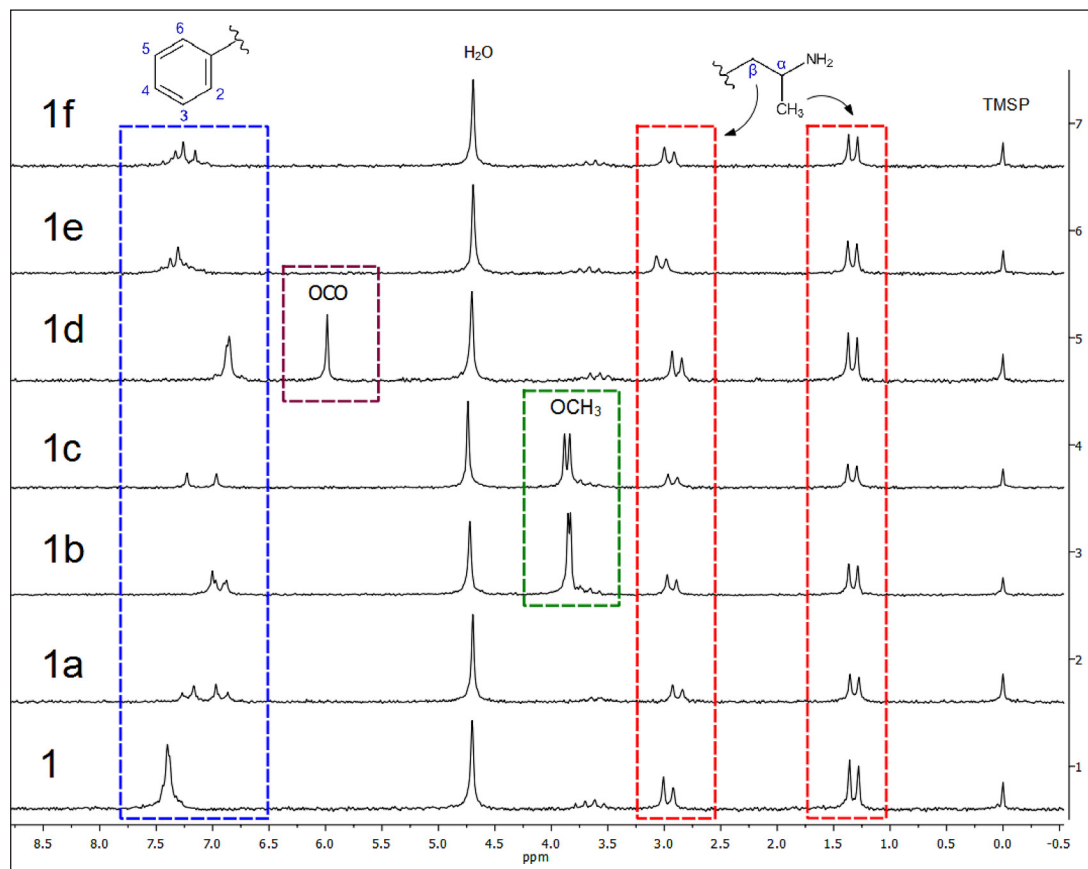


Figure 3:
Experimental ¹H NMR
82 MHz spectra
of amphetamine
reference samples

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