N. Suryaprakash,[1] *Ph.D.; Myriam Azoury,*[2] *M.Sc.; Zafrir Goren,*[3] *Ph.D.; and Raz Jelinek,*[4] *Ph.D*

Identification of Heroin in Street Doses Using 1D-TOCSY Nuclear Magnetic Resonance

REFERENCE: Suryaprakash N, Azoury M, Goren Z, Jelinek R. Identification of heroin in street doses using 1D-TOCSY nuclear magnetic resonance. J Forensic Sci 2000;45(5):963–967.

ABSTRACT: Heroin street doses are complex mixtures commonly analyzed in forensic laboratories. Identification of the illicit substance in these street doses is among the primary analytical tasks of a forensic laboratory. We demonstrate that the one-dimensional 1D-TOCSY NMR experiment permits identification of heroin in standard mixtures containing up to ten or more different components. This method produces an easily-identified and effective "fingerprint" for heroin within a mixture of other substances. The method has been successfully tested as a tool for identification of heroin in street doses from police casework in Israel. This NMR technique is robust and quick (a measurement can be carried out in 10–15 min), and it does not require any preliminary physical or chemical treatments of the sample to be examined, due to the effective spectroscopic "filtering" of the interfering components. The 1D-TOCSY NMR method can potentially be used in combination with additional analytical methods as a routine tool in forensic laboratories to positively identify heroin for court purposes.

KEYWORDS: forensic science, heroin, nuclear magnetic resonance, TOCSY, identification, street doses

Unambiguous and rapid identification of illicit substances in street doses is among the primary analytical tasks of a forensic laboratory. The legal requirements for unequivocal determination of the presence of illicit drugs in given samples pose continuously evolving scientific and technical challenges.

Drug exhibits containing street doses of heroin are commonly analyzed in the Analytical Chemistry Laboratory in the Division of Identification and Forensic Sciences (DIFS) at the Israel National Police Headquarters. Generally, these street doses contain a variety of substances in addition to heroin. The additional substances include naturally occurring opiate alkaloids originating from opium (e.g., narcotine, papaverine, morphine, and codeine), impurities that arise from acylation of opiate alkaloids during heroin production from crude morphine (e.g., acetylcodeine, O^3-acetylmorphine), degradation products of heroin and other opiate alkaloids (e.g., O^6-acetylmorphine), and various adulterants (e.g., caffeine, paracetamol, and procaine) (1).

Routine identification of heroin in street doses in analytical forensic laboratories generally includes gas chromatography (GC), gas chromatography/mass spectrometry (GC/MS), and infrared (IR) spectroscopy (2–4). In many instances, the presence of several substances in a mixture requires specific sample preparation and/or purification of the desired substance prior to the analysis. This procedure is often time consuming and may also increase the cost of the analysis.

In our laboratory, positive identification of heroin for court purposes is carried out by a combination of analytical methods. All samples are initially screened in thin layer chromatography (TLC) in comparison to heroin standard to obtain an indication for heroin presence. Consequently, infrared (IR) tests are performed. Heroin extraction and standard spectral subtractions are employed to unambiguously identify heroin in the IR test. In cases where the IR results are insufficient to positively identify heroin, i.e., low heroin concentration, or when extraction fails to isolate heroin, a gas chromatography/mass spectrometry (GC/MS) test is also performed to verify heroin presence in the sample.

NMR spectroscopy is a powerful tool for identification and analysis of organic compounds (5), and has been used for this purpose in forensic laboratories (6,7). NMR analysis allows structure elucidation of complex compounds, based on the different magnetic environments of the nuclei and the variation of their spin interactions. The significant sensitivity of the NMR signals to the structural properties of organic compounds usually allows unambiguous identification of these molecules in solution. In particular, the NMR spectrum of a molecule within a mixture would not be affected, in general, by the presence of other species in solution (unless interactions among different species exist). Various NMR techniques have been developed and applied towards analysis of drugs and drug mixtures (6–9). However, NMR methods have not yet been fully adopted by forensic chemists as a method of choice in routine forensic analysis, probably due to low sensitivity, relatively high instrument cost and expensive maintenance. In this study, a simple and elegant NMR experiment, namely one-dimensional 1D-TOCSY (1D-TOCSY), is described and evaluated for heroin identification in complex mixtures. The 1D-TOCSY technique could be used in combination with additional methods (TLC, GC, and IR) to positively identify heroin for court purposes.

Materials and Methods

Materials—All standard compounds were provided by the Analytical Chemistry Laboratory of the DIFS. The samples included heroin (as both a free base and hydrochloride salt), morphine, O^3-

[1] Research fellow, Department of Chemistry, Ben Gurion University of the Negev, Beer-Sheva, Israel. On leave from Sophisticated Instruments Facility, Indian Institute of Science, Bangalore 560 012, India.

[2] Forensic chemist, Deputy Head, Analytical Chemistry Laboratory, Jerusalem, Israel.

[3] Forensic chemist, Head, Analytical Chemistry Laboratory, Division of Identification and Forensic Sciences (DIFS), Israel Police, National Headquarters, Jerusalem, Israel.

[4] Lecturer, Department of Chemistry, Ben Gurion University of the Negev, Beer-Sheva, Israel.

Received 18 May 1999; and in revised form 1 Nov. 1999; accepted 2 Nov. 1999.

acetylmorphine, O^6-acetylmorphine, codeine, acetylcodeine, narcotine, papaverine, caffeine, paracetamol, and procaine.

Multi-component standard mixtures were prepared by mixing equal weights of the above-mentioned compounds. For NMR experiments, approximately 5 mg of each compound and compound-mixtures were dissolved in chloroform-d. Heroin street doses in powder form were randomly collected from routine police casework in Israel.

Methods—All street doses were analyzed by FTIR and/or GC/MS prior to the NMR analysis. Quantitative analyses of heroin content were carried out using HPLC.

FTIR—Infrared spectra were recorded between 400 cm^{-1} to 4000 cm^{-1} in KBr pellets using a Nicolet Protege-IR model 460 FT-IR spectrometer.

GC/MS—The analyses were performed using a Hewlett-Packard (HP) 5890 gas chromatograph equipped with a DB-5, 15 m × 0.25 mm ID × 0.25 μm film coupled to an HP 5970B Mass Selective Detector (MSD) using helium as the carrier gas at 1 mL/min. Injection port temperature of the GC was set at 220°C, with column temperature programmed from 50°C to 290°C at 25°C/min. The retention time of heroin was 4.5 min. The GC is run in the split mode at a ratio of 9:1. One microliter of a 0.5 mg/mL to 1 mg/mL sample solution was injected.

HPLC—Analyses were performed using a Waters HPLC system consisted of a Waters model 616 pump, a Waters model 717 plus autosampler with temperature controller at 4°C, a Waters model 996 UV-VIS photodiodearray detector (PDA), and Millennium v 2.1 software. Tests were performed on a 125 × 4 mm Lichrospher 60 Select B column (5 μm) at 27°C, detection at 230 nm. Linear gradient elusion at a flow rate of 1.8 mL/min was performed as follows: 0 to 4 min − 96% A, 4% C; 4 to 19 min − 85% A, 13% B, 2% C; 19 to 25 min − 85% A, 13% B, 2% C; 25 to 25.5 min − 85% A, 11% B, 4% C; 25.5 to 29 min − 85% A, 11% B, 4% C; 29 to 32 min. (A) water, (B) acetonitrile, and (C) methanol. Each solvent contained 2 mM sulfuric acid.

NMR—Measurements were carried out at 27°C on a Bruker DMX500 spectrometer operating at a magnetic field of 11.7 Tesla (500 MHz proton) equipped with an inverse detection proton Txi probe. The proton 90° pulsewidth was 8.3 μsec. For the 1D-TOCSY experiment (10), excitation of the desired resonance was achieved using a soft Gaussian-shaped pulse of 1024 points with duration of 80 msec. A mixing period of 50 ms was used in the experiments, with the MLEV-17 sequence (11) applied during the mixing. One hundred free induction decays (FID's) were accumulated in each experiment, with relaxation delay between FID's of 5 sec. TMS was used as the reference.

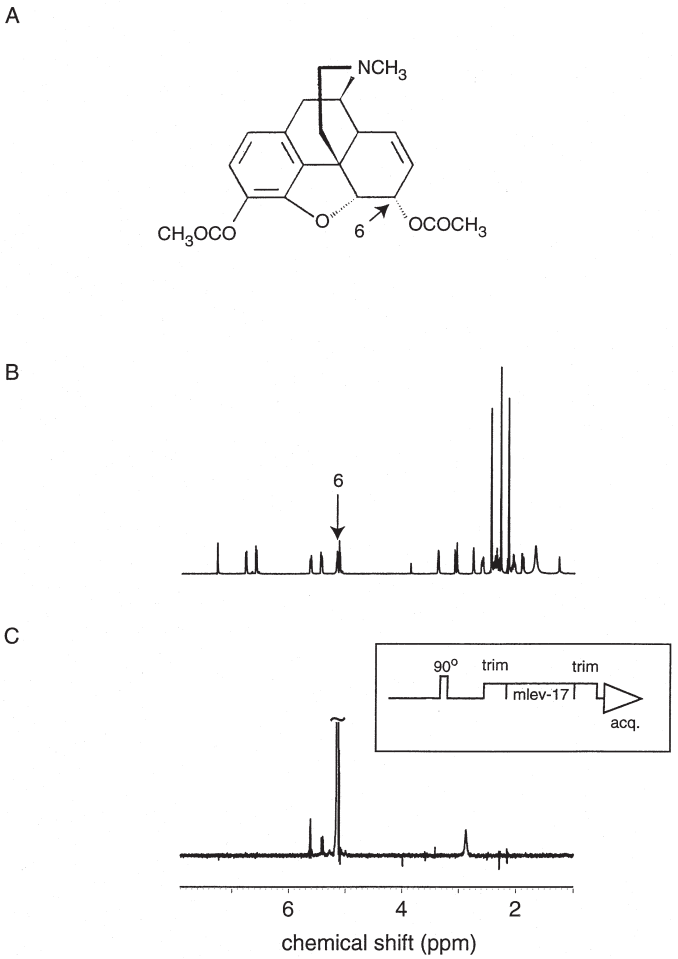


FIG. 1—A. *Molecular structure of heroin indicating selectively excited proton at position 6 (H-6).* B. *One-dimensional proton NMR spectrum of heroin.* C. *One-dimensional 1D-TOCSY spectrum of heroin by selective excitation of H-6. The 1D-TOCSY pulse-sequence (10) is shown in the insert.*

Results and Discussion

Figure 1 shows the structure of heroin (1A), and the corresponding proton NMR Block-decay spectrum (1B) and 1D-TOCSY spectrum (1C). The 1D-TOCSY pulse-sequence, shown in Fig. 1(*C*), allows excitation of a specific nuclear spin in the molecule, and detection of associated resonances that correspond to a smaller subset of nuclei attached to the nucleus being excited through a *covalent bond* network (12–14). Thus, the 1D-TOCSY experiment can significantly simplify a "crowded" NMR spectrum by selecting only nuclei that are connected to the excited nucleus through covalent bonds. The 1D-TOCSY experiment is carried out by placing the excitation resonance on the selected signal. Soft pulse is then applied on resonance (exciting only the selected resonance), and a mixing time of between 50–100 msec is used to allow magnetization transfer to occur to adjacent protons. The 1D-TOCSY spectrum is usually observed after acquiring several scans, and a satisfying result can be usually obtained after a few minutes (and as short as a minute, depending on the concentration of the desired compound).

The selectively excited resonance in the 1D-TOCSY experiment shown in Fig. 1(*C*) corresponds to H-6 of heroin (Fig. 1*A*). Consequently, the spectrum in Fig. 1(*C*) features only about five peaks ascribed to the proton nuclei belonging to the network of the excited resonance. The assignment of the observed proton resonances has been independently carried out through application of a two-dimensional TOCSY experiment (15), and confirmed by previous studies (16–18). The number of coupled nuclear spins observed in 1D-TOCSY experiments is determined, in general, by various structural and spectroscopic parameters, such as the mixing time during which the magnetic coherence can be transferred between the nuclei, the magnetic relaxation, and the mobility of the

molecule (12–14). The 1D-TOCSY experiment, however, will almost always display a much smaller number of proton resonances of a particular molecule, compared with a conventional one dimensional proton spectrum, thus achieving spectra that are much easier to interpret.

The 1D-TOCSY experiment is a particularly powerful technique for identification of individual components in a mixture. Due to observation of only covalently-bonded nuclei using 1D-TOCSY, the technique can effectively filter out all resonances from other molecules present in a mixture. This is clearly demonstrated in Figs. 2 and 3. Figure 2 features proton one-dimensional and 1D-TOCSY spectra of a binary mixture of heroin and morphine dissolved in 80% chloroform-d_1/20% DMSO-d_6. Figure 2(*A*) is the conventional one-dimensional proton spectrum. Figure 2(*B*) shows

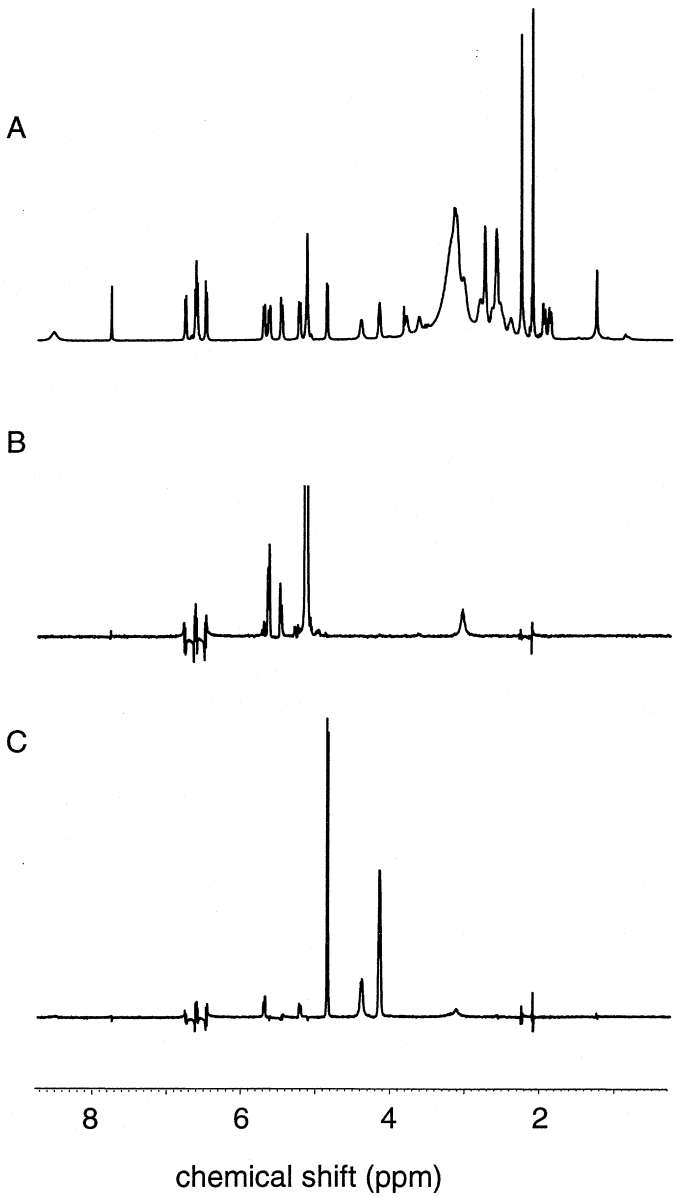


FIG. 2—A. *Proton spectrum of a binary mixture of heroin and morphine dissolved in 80% chloroform-d_1/20% DMSO-d_6.* B. *1D-TOCSY spectrum of the binary heroin-morphine mixture in which H-6 of heroin has been selectively excited.* C. *1D-TOCSY spectrum in which a H-5 of morphine has been selectively excited.*

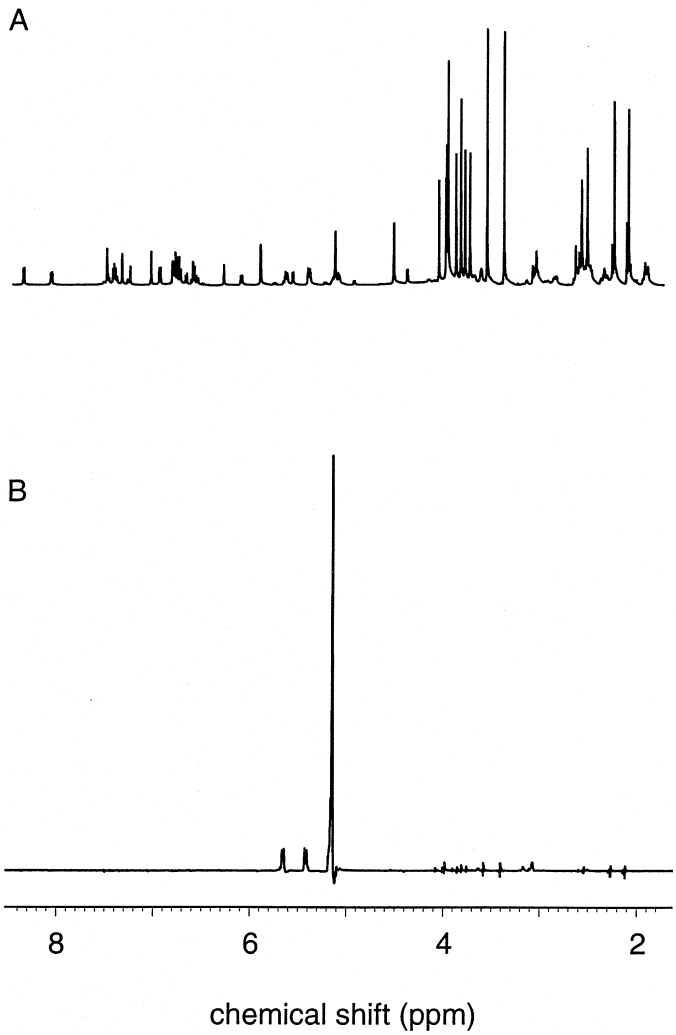
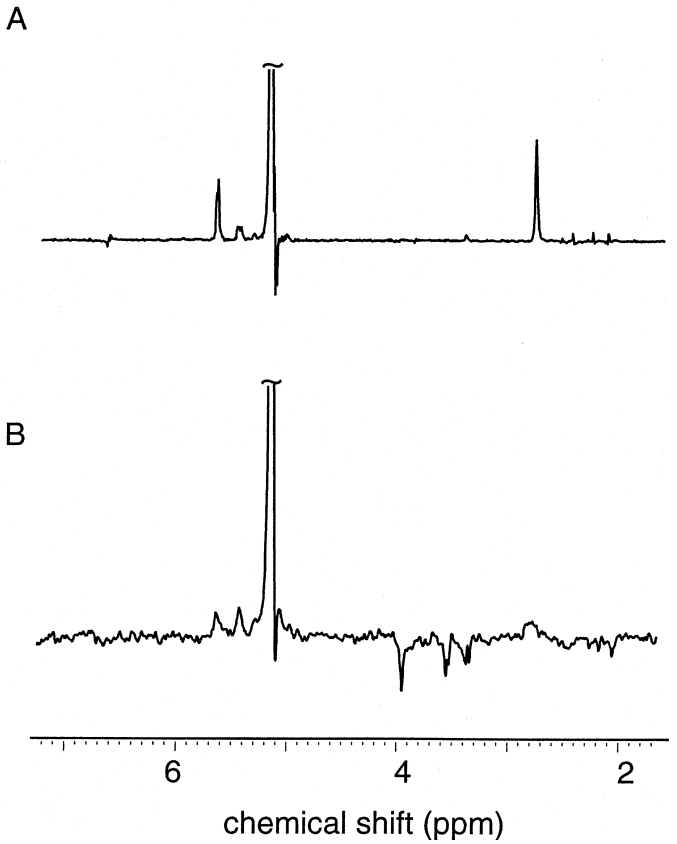


FIG. 3—A. *Proton NMR spectrum of a 10-component mixture in chloroform-d, prepared by mixing standard samples of heroin-HCl, heroin base, narcotine, acetyl-codeine, paracetamol, procaine, caffeine, papaverine, O^6-monoacetylmorphine benzoate, O^3-monoacetylmorphine benzoate, and morphine.* B. *1D-TOCSY spectrum of the 10-component mixture in which H-6 of heroin has been selectively excited.*

the 1D-TOCSY spectrum in which H-6 of heroin has been excited. The spectrum shown in Fig. 2(*B*) clearly shows that the selective 1D-TOCSY experiment completely filters out all resonances from morphine, displaying only the proton resonances that are covalently-bonded and within close proximity to H-6 of heroin. Similarly, Fig. 2(*C*) depicts only morphine resonances when a 1D-TOCSY experiment is carried out using selective irradiation of hydrogen-5 of morphine (16).

An important issue that should be emphasized is the very high reliability of the 1D-TOCSY technique in unambiguously identifying a compound such as heroin in a mixture. The extreme sensitivity of NMR phenomena to the chemical environments within a molecule means that an organic molecule will give rise to distinct peak positions in the 1D-TOCSY spectrum, due to its unique molecular structure and the different environments of the nuclei. Even though the structures of heroin and morphine are similar, their 1D-TOCSY spectra are distinctly different. The spectra shown in Figs. 2(*B*) and (*C*) indicate that the 1D-TOCSY method



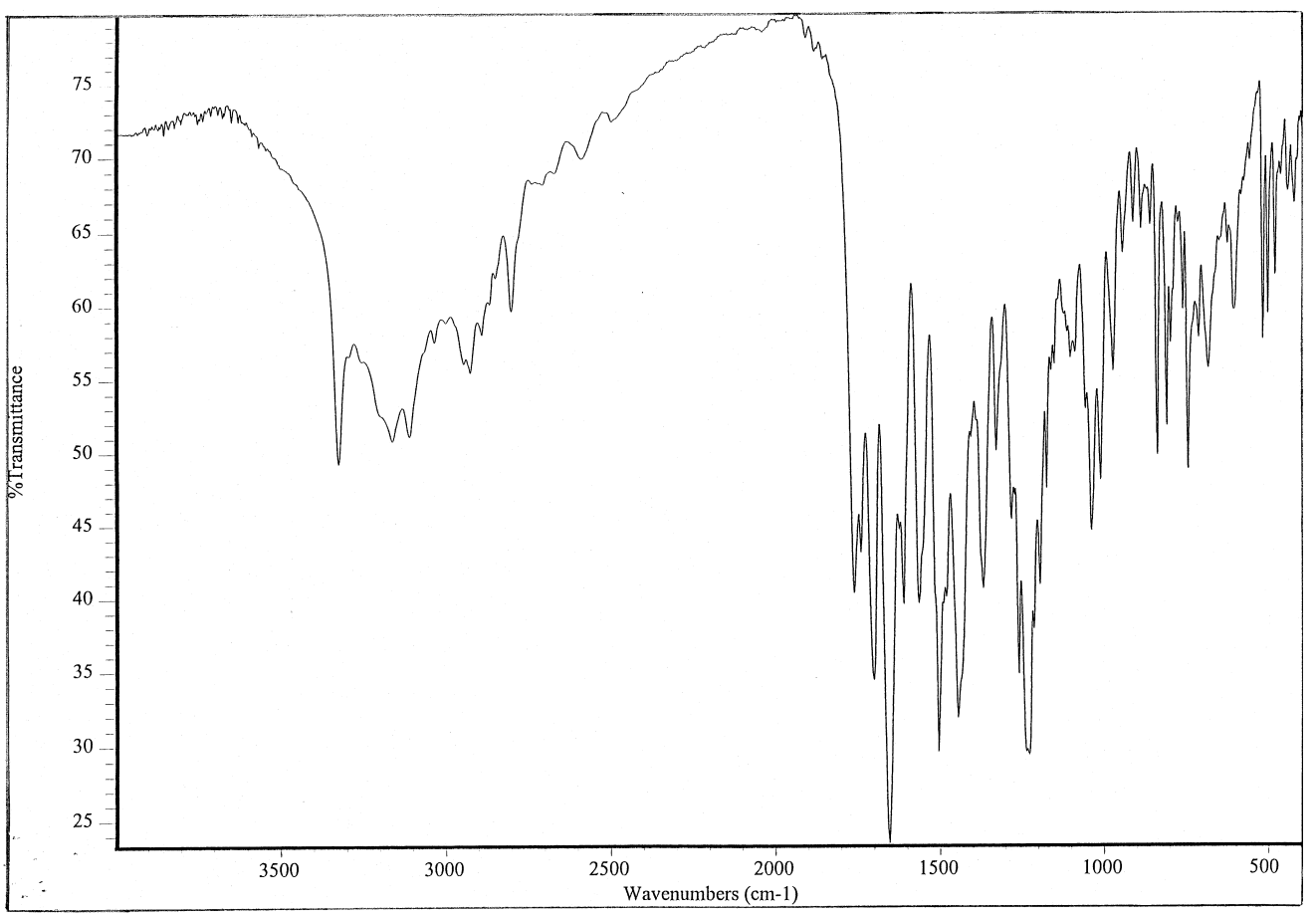


FIG. 4—A–B: *1D-TOCSY spectra of two street doses confiscated by the Israel Police in chloroform-d. The samples contained approximately A. 30%; and B.4% heroin, as determined by HPLC.* C. *IR spectrum of a street sample containing approximately 15% heroin. Other major components in the sample include narcotine, papaverine, caffeine, paracetamol, and* O^6*-monoacetylmorphine.*

can be employed to determine the presence of various components in a mixture, using the same sample and experimental apparatus, by essentially providing a spectral "fingerprint" for each component in the mixture.

Figure 3(*A*) depicts proton spectra of a 10-component mixture prepared in the laboratory using standard substances including heroin. The 1D-TOCSY experiment in Fig. 3(*B*) shows the dramatic improvement of resolution and ease of identification of heroin in the mixture, since the technique successfully filters out the large majority of proton resonances observed in the conventional one-dimensional NMR experiment. The spectrum shown in Fig. 3(*B*) demonstrates the power of 1D-TOCSY to identify a single component in a seemingly complex mixture.

The 1D-TOCSY experiments have been successfully applied in our laboratories to more than 20 street doses (spectra attached as supplementary material). Figure 4(*A,B*) display the NMR data obtained for two heroin street doses in Israel, in which the heroin content is around 30% and 4%, respectively. The presence of heroin in the samples have been independently confirmed using GC/MS and IR spectroscopy, and heroin concentration has been determined using HPLC. In both samples, the identification of heroin using 1D-TOCSY is unambiguous, even though the concentration of heroin in each sample is significantly different. Moreover, sample purification prior to the NMR analysis, can be avoided due to the effective "filtering" of all other interfering components.

Figure 4*C* depicts the infrared spectrum of a street sample containing 15% heroin. Reliable identification of heroin is clearly more difficult in this mixture using infrared spectroscopy, compared to the 1D-TOCSY experiment. The infrared spectrum shown in Fig. 4*C* is a superimposition of the infrared spectra of various components. Mixture components are unidentifiable from the infrared spectrum without proper sample treatment prior to infrared analysis. In particular, the two carbonyl bands of heroin at around 1750 cm^{-1} overlap in this street sample with the narcotine carbonyl band.

The one-dimensional 1D-TOCSY NMR technique is fast—an experiment can be carried out in 10 to 15 min. The method is also extremely robust—even inhomogeneous samples in which dissolution is not complete do not affect the quality and reliability of the data, because the only requirement is dissolution of heroin and identification of a single heroin proton resonance within the spectrum. The 1D-TOCSY NMR measurement can be used, in combination with additional analytical techniques, as an important method for identification of heroin in the forensic laboratory. The results obtained in the 1D-TOCSY experiments are reproducible, and we are working towards making the technique also quantitative.

Conclusions

We have demonstrated that one-dimensional 1D-TOCSY NMR can be successfully applied to detect heroin in multi-component mixtures. The method allows heroin identification through its unique "spectral fingerprint," i.e., the positions of the proton resonances in the spectrum. The experiment is fast, robust, and relatively easy to perform even for personnel with limited familiarity with NMR spectroscopy. The experiment shows that NMR spectroscopy could be an alternative, routine, and highly useful technique in forensic laboratories for heroin street doses analysis without resorting to physical separation of the components. A research plan to expand the scope of this method to other illicit drugs is currently underway in our laboratories.

Additional Information

Supplemental material includes Bloch-decay and 1D-TOCSY spectra of 20 street doses provided by the Analytical Laboratory of DIFS.

Acknowledgments

The authors gratefully acknowledge the financial support of this work by the Anti Drug Authority of Israel. The authors also acknowledge Mrs. R. Levy and Mr. Y. Avissar from DIFS for their assistance in HPLC analyses.

References

1. Levy R, Raverby M, Meirovich L, Shapira-Heiman O. A survey and comparison of heroin seizures in Israel during 1992 by FTIR spectrometry. J Forensic Sci 1996;41(1):6–11.
2. Saferstein R. Criminalistics: an introduction to forensic science, 6th edition, Engelwood Cliffs, New Jersey, Princeton Hall, 1997.
3. Adamovics JA. Analysis of addictive and misused drugs. New York: Marcel Dekker, Inc. 1995.
4. Groombridge CJ. NMR spectroscopy in forensic science. Annual Reports on NMR Spectroscopy. 1996:215–97.
5. Ernst RR, Bodenhausen G, Wokaun A. Principles of nuclear magnetic resonance in one and two dimensions. Clarendon, Oxford, 1987.
6. Dal Cason TA, Meyers JA, Lankin DC. Proton and carbon-13 NMR assignments of 3,4-methylenedioxyamphetamine (MDA) and some analogues of MDA. Forensic Sci Int 1997;86:15–24.
7. Burns DJ, Lewis RJ, Stevenson P. Determination of 3,4-methylenedioxyamphetamine analogues ("ecstasy") by proton nuclear magnetic resonance spectrometry. Anal Chim Acta 1997;339:259–63.
8. Nair MM, Jackson GE, Campbell WE. Structural assignment of the opium alkaloid codeine via 2D NMR techniques. Spectroscopy Letters 1997;30:497–505.
9. Chazin WJ, Colebrook LD. Use of proton spin-lattice relaxation and nuclear overhauser effect data in structure analysis of alkaloids. J Org Chem 1986;51:1243–53.
10. Davis DG, Bax A. Simplification of ^{1}H NMR spectra by selective excitation of experimental subspectra. J Am Chem Soc 1985;107:7197–8.
11. Bax A, Davis DG. MLEV-17 based two-dimensional homonuclear magnetization transfer spectroscopy. J Magn Reson 1985;65:355–60.
12. Kanthimathi T, Moorthy YVSN, Pillai CN, Subramaniam S. Spectral separation of two very closely-related organic compounds by 1d TOCSY. Magn Reson Chem 1994;32(8):452–7.
13. Subramanian S, Bax A. Generation of pure phase NMR subspectra for measurement of homonuclear coupling constants. J Magn Reson 1987;71:325–30.
14. Davis DG, Bax A. Assignment of complex 1H NMR spectra via two-dimensional homonuclear Hartmann-Hahn spectroscopy. J Am Chem Soc 1985;107:2820–1.
15. Braunschweiler L, Ernst RR. Coherence transfer by isotropic mixing: application to proton correlation spectroscopy. J Magn Reson 1983; 53:521–8.
16. Neville GA, Ekiel I, Smith ICP. High-resolution proton magnetic resonance spectra of morphine and its three o-acetyl derivatives. Magn Res Chem 1987;25:31–5.
17. Allen AC, Moore JM, Cooper DA. 16,17-dehydroheroinium chloride: synthesis and characterization of a novel impurity detected in illicit heroin. J Org Chem 1983;48:3951–4.
18. Carroll FI, Moreland CG, Brine GA, Kepler JA. Carbon-13 nuclear magnetic resonance spectra of morphine alkaloids. J Org Chem 1976;41: 996–1001.

Additional information and reprint requests:
Dr. Raz Jelinek
Department of Chemistry
Ben Gurion University of the Negev
Beersheva 84105
Israel
Fax: 972-7-6472943
E-mail: razj@bgumail.bgu.ac.il