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Identification and quantitation of 3,4-methylenedioxy-*N*-methylamphetamine (MDMA, ecstasy) in human urine by ^{1}H NMR spectroscopy. Application to five cases of intoxication

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ABSTRACT

Identification of 3,4-methylenedioxy-*N*-methylamphetamine (MDMA, ecstasy) in five cases of intoxication using nuclear magnetic resonance (NMR) spectroscopy of human urine is reported. A new water suppression technique PURGE (Presaturation Utilizing Relaxation Gradients and Echoes) was used. A calibration curve was obtained using spiked samples. The method gave a linear response (correlation coefficient of 0.992) over the range 0.01–1 mg/mL. Subsequently, quantitation of the amount of MDMA present in the samples was performed. The benefit and reliability of NMR investigations of human urine for cases of intoxication with MDMA are discussed.

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1. Introduction

3,4-Methylenedioxy-*N*-methylamphetamine, more commonly called MDMA or "ecstasy", is a synthetic drug similar in structure to methamphetamine (Fig. 1). It is an illegal drug with stimulatory and psychedelic effects, and is commonly associated with rave and trance clubs. It is believed that MDMA inhibits serotonin reuptake in synapses, and also causes release of more serotonin into the synapse. In addition, dopamine, acetylcholine, and norepinephrine release is induced, along with the release of several other hormones [1]. Its effects cause it to behave as an entactogen, making it popular among all age groups. Illicit use of MDMA became popular in the 1980s, resulting in its assignment as a Schedule I controlled substance. Although there are many metabolites formed from MDMA, roughly 65% of the dose is eliminated from the body unchanged in urine [2].

There has been a considerable amount of research done on MDMA found in urine. Current analytical techniques for detection of MDMA and its metabolites in urine and other bodily fluids include HPLC, GC–MS, LC–MS, capillary electrophoresis (CE), and immunoassays. In a review by Butler and Guilbault [3], sample preparation plays an important role for the identification of MDMA. Methods such as cleavage of conjugates, liquid–liquid extraction, solid phase extraction, and derivatization procedures including UV spectroscopy and using fluorogenic reagents, although very time consuming, have facilitated the identification of MDMA in biological matrices. MDMA concentrations in fatal and non-fatal cases do vary. Plasma concentrations of 0.424 mg/L from a 150 mg dose of MDMA resulted in severe hyperthermia and death, whereas another patient ingested 42 pills and registered a plasma concentration of 7.72 mg/L and was asymptomatic [4]. A more recent publication by Garcia-Repetto et al. [2] also reported finding cases where consumers have survived a 40–50-tablet overdose without any symptoms, whereas other users have died after 1–3 tablets with MDMA serum concentrations between 0.1 and 0.4 mg/L.

NMR spectroscopy is a powerful tool to analyze chemical structures and can be used to analyze biological fluids for the diagnosis of acute poisoning and drug overdoses [5] without the need for separation and/or derivatization steps. Studies by Imbenotte et al. [6] used NMR to identify and quantitate levels of various xenobiotics causing poisoning, such as salicylate, valproic acid, paraquat, and tetrahydrofuran. Work done by Wahl et al. [7] investigated ^{1}H NMR as a tool for diagnosis and quantification of poisoning with methanol and ethylene glycol. Not only did the NMR technique make a rapid diagnosis possible, requiring only a small sample without derivatization or extraction required, but it also allowed for qualitative analysis as well as reliable quantification. Another example is the work on GHB

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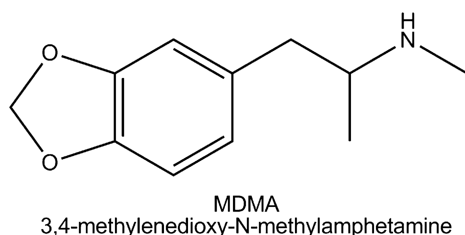

Fig. 1. Structure of 3,4-methylenedioxy-*N*-methylamphetamine.

(gamma hydroxybutyric acid) and GBL (gamma hydroxybutyrolactone), increasingly popular drugs of abuse, which were analyzed with ^{1}H NMR in a study by Del Signore et al. [8].

However, there has not been any work conducted using NMR to detect MDMA in urine samples. In this paper, we propose an NMR procedure for the analysis of urine from 5 cases of MDMA intoxication using ^{1}H NMR and a new water suppression technique PURGE (Presaturation Utilizing Relaxation Gradients and Echoes).

2. Materials and methods

2.1. Biological samples

Samples of urine from MDMA users were collected in this project. In accordance with an IRB-approved protocol, the urine was donated purely on a voluntary basis. Additionally, donors were asked to record the estimated time between MDMA dosage and urine collection. Since MDMA is classified as a schedule 1 controlled substance by the DEA under the Controlled Substances Act, volunteers were completely anonymous with no physical evidence linking them to the project. Urine sample were collected and immediately frozen at −20 °C until required for NMR measurements.

2.2. Proton NMR spectroscopy

All data (^{1}H NMR) were collected on a Bruker 500 MHz NMR spectrometer (Bruker BioSpin, Billerica, MA) at the Chemistry Department of Columbia University. Depending on the estimated concentration of the analytes observed, data collection differed, because better signal to noise ratios are obtained by an increasing number of scans. A trial-and-error approach was used to determine the amount of scans needed for different samples.

One-dimensional spectra were obtained using the water suppression technique PURGE (Presaturation Utilizing Relaxation Gradients and Echoes) described by Simpson et al. to suppress the signal from water [9]. The experiment produces flat baselines, excellent phase properties, and highly selective suppression that is equal or superior to that produced by commonly used sequences such as WATERGATE, WET, or excitation sculpting. Except for the 4.5–4.7 ppm range of the water signal, the excitation profile of PURGE is uniform over the full proton spectrum and does not suffer from the periodic null "sidebands" that plague some techniques. The uniform excitation allows the use of quantitative calibration standards with chemical shifts that are well separated from the MDMA peaks. PURGE has the further significant advantage that the only parameter that needs adjustment is the presaturation power, making it easy to implement even for non-spectroscopists and, thus, it could be easily adapted to a clinical setting. In practice, little or no adjustment of the presaturation power is necessary once the experiment has been optimized.

Five hundred microlitres of each sample was introduced into a 5 mm tube with a coaxial capillary tube containing a solution of 3-trimetyhylsilyl 2,2′,3,3′-tetradeuteropropionic acid (TSP-d_4) providing an internal field frequency lock and reference for proton chemical shifts ($\delta = 0$ ppm).

2.3. Spectrum of MDMA in D_2O

A ^{1}H NMR Spectrum of MDMA in D_2O was recorded and is shown in Fig. 2. Chemical shifts, coupling constants are listed in Table 1. Diastereotopic protons C are designated as C1 and C2. MDMA is supplied as a racemic mixture, however, it is not possible to resolve the two enantiomers using NMR spectroscopy as both enantiomers give identical spectra.

2.4. Quantification by proton NMR spectroscopy

A concentration gradient consisting of nine solutions in control urine each with a volume of 500 μL were prepared. Concentrations of MDMA reported are expressed in mg/mL of the free base. Serial dilutions of 1.00, 0.8, 0.5, 0.2, 0.1, 0.08, 0.05, 0.02, and 0.01 mg/mL of MDMA, in the blank urine were analyzed using ^{1}H NMR. Concentrations of 1.00, 0.8, 0.5, 0.2, 0.1, and 0.08 mg/mL were scanned 128 times. Concentrations of 0.05 mg/mL were scanned 512 times. Concentrations of 0.02 mg/mL were scanned 2048 times. Concentrations of 0.01 mg/mL were scanned in overnight experiments, allowing for a range from 10,000 to 12,000 scans. Fig. 3 is a representative spectrum of a sample with a 0.1 mg/mL concentration of MDMA. Boxed regions are caused by MDMA

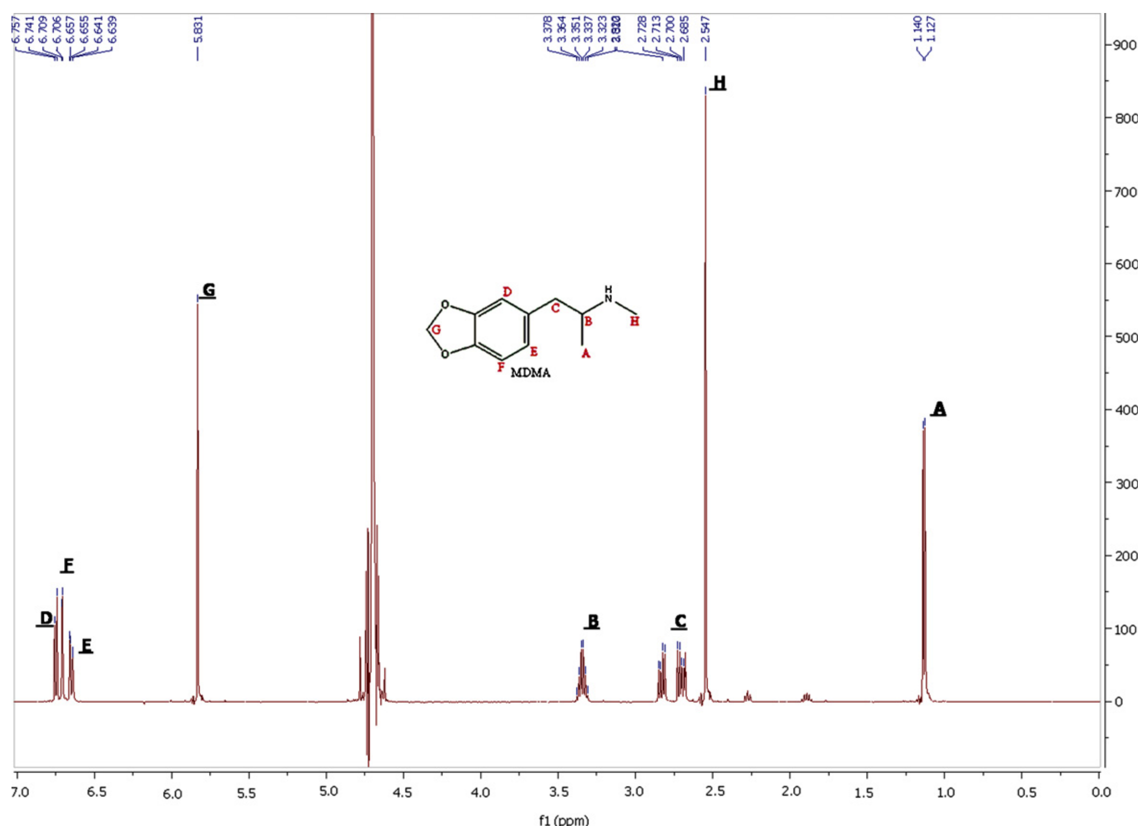

Fig. 2. Spectrum of 1 mg/mL MDMA in D_2O, 16 scans.

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Table 1
Chemical shifts, coupling constants, number of protons, and peak multiplicity of MDMA in D_2O.

Proton	Chemical shift (ppm)	Chemical shift (Hz)	*J* coupling constant (Hz)	# protons	Peak multiplicity
A	1.13	566.94	6.6	3	d
H	2.55	1273.64	–	3	s
C_1	2.71	1353.25	$J_1 = 14$ $J_2 = 7.6$	2	dd
C_2	2.83	1414.75	$J_1 = 14$ $J_2 = 6.6$		dd
B	3.34	1672.35	7	1	Apparent sextet
E	6.65	3324.90	$J_1 = 7.9$ $J_2 = 1.3$	1	dd
F	6.71	3354.70	1.4	1	d
D	6.75	3375.42	7.9	1	d

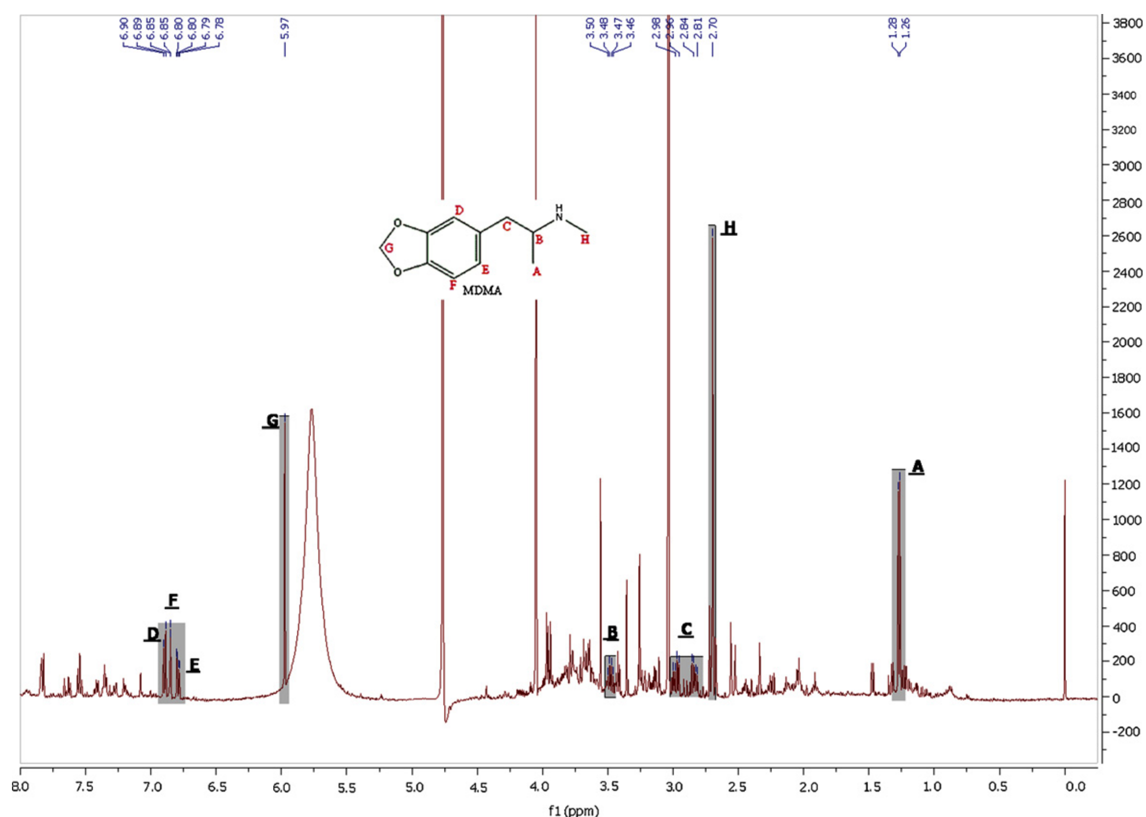

Fig. 3. 0.1 mg/mL MDMA in spiked control urine, 128 scans.

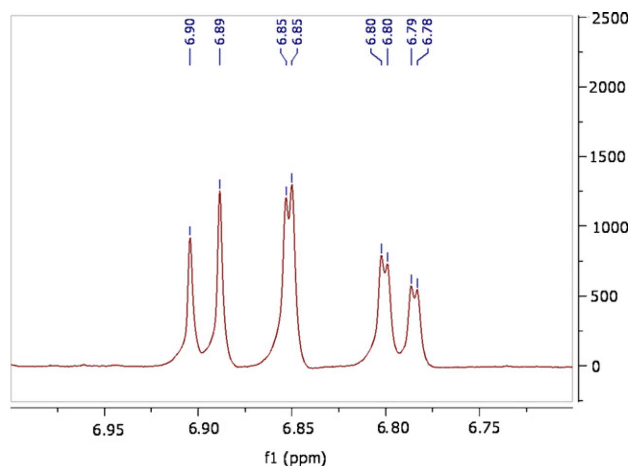

Fig. 4. 0.1 mg/mL MDMA in spiked control urine. Aromatic region.

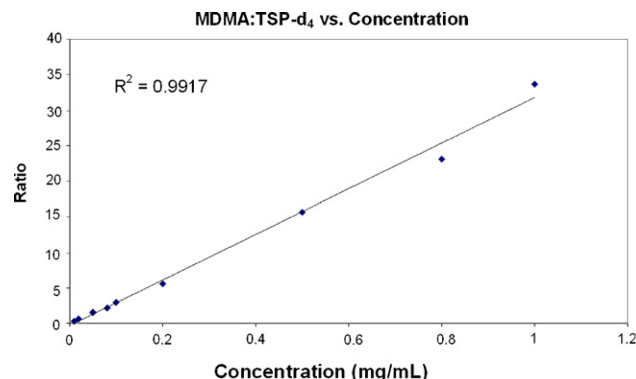

Fig. 5. Aromatic MDMA peaks area to TSP-d_4 peak area ratio vs. concentration of MDMA in urine samples.

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peaks, and the regions outside the boxes are from the urinary metabolites. As expected, the 1.0–4.0 ppm region is difficult to integrate accurately due to interference by urinary metabolites. In this region and at lower concentrations some of the drug peaks are completely masked by metabolite peaks. Aromatic peaks (Fig. 4, protons D, F, and E) in the 6.6–7.0 ppm range were chosen for integration. Fig. 5 shows the calibration curve generated from the following plotting: [area from the MDMA aromatic peaks/area from the internal standard peak] vs. drug concentration.

2.5. Standard deviation, limit of detection and limit of quantitation

In any analytical method, it is important to determine the limit of detection and limit of quantitation. These two limits, along with variance and standard deviation calculations can be used to define the sensitivity of a test. To determine the standard deviation, seven independent urine samples spiked with MDMA at a concentration of 0.05 mg/mL were run. Statistical analysis of the seven different spiked urine samples of MDMA was performed based on the results of integrating the area of the aromatic peaks vs. the area of the internal standard peak in their NMR spectra. For the MDMA samples, relative standard deviation was calculated to be 0.56%.

As for the LOD, the very nature of NMR makes it impossible to determine as it depends on the amount of scans used for the experiment. However, in this study we have limited the experimental time to overnight experiments, allowing for a range from 10,000 to 12,000 scans. This experimental time allows for a limit of quantification in the 0.01 mg/mL concentrations range.

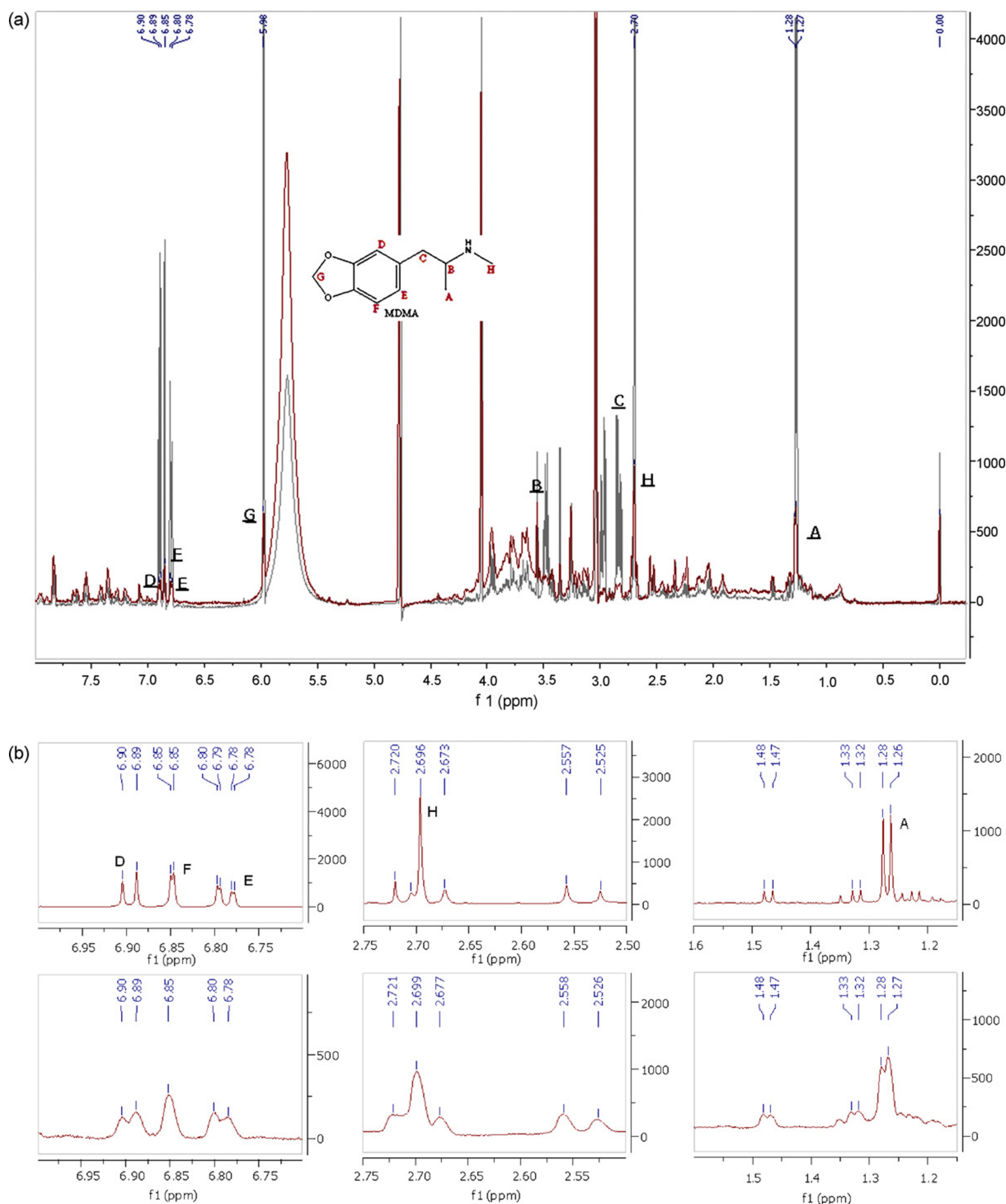

Fig. 6. (a) Sample 1 256 scans. Superimposed in gray is the spectrum of MDMA spiked urine (0.50 mg/mL). (b) Top: blow up of different regions (6.7–6.9; 2.75–2.5; 1.6–1.15 ppm) of the MDMA spiked urine spectrum, bottom: blow up of the same regions (6.7–6.9; 2.75–2.5; 1.6–1.15 ppm) of the spectrum from sample 1.

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Table 2
Summary of the five intoxication cases.

Sample	Time after dose	Suspected contents	Total concentration (mg/mL)	Number of scans
1	6 h	MDMA	0.082	256
2	8 h	MDMA	0.029	579
3	8 h	MDMA	0.078	128
4	7 h	MDMA	0.042	400
5	18 h	MDMA	0.045	526

3. Results and discussion

The five samples of urine donated by volunteers were analyzed with different numbers of scans based on a trial-and-error method. The number of scans necessary for each sample is indicated in Table 2. Presence of MDMA was based on the observed peak pattern in the 6.7–7.0 ppm region along with the presence of the peak caused by the methylenic protons G which appear on the trailing edge of the urea peak. The presence of the peaks from the methyl protons (A and H) of MDMA in the spectra helped confirm the results. Fig. 6a shows the spectrum of sample 1. Superimposed in gray is the spectrum of MDMA spiked urine (0.50 mg/mL). Spectra for the other samples can be seen in the supportive information documents. In all spectra, we consistently observed the peak for proton G as well as all the peaks in the aromatic region for protons D, E and F. However, for samples 2, 3 and 5 a broadening of the peak responsible for the signal of proton F was observed as well as a small extra peak around 6.83 ppm probably resulting from the presence of metabolites. The signals for the diastereotopic protons C were only observed distinctively in the case of sample 1. Proton B was masked by endogenous compounds in all cases. The peak at $\delta = 2.69$ from proton H could be observed in all cases. However, because it partially overlaps with the citrate peaks it is not a reliable indication of the presence of MDMA in the sample. Finally, a doublet at $\delta = 1.27$ ppm from protons A was observed in all spectra but because of the crowded nature of this region of the spectrum, this doublet cannot be considered for positive identification either.

For all samples, a baseline correction was performed. Quantitative analysis was performed by calculating the ration between the area of the aromatic peaks (D, F, and E) and the area of the TSP-d_4 peak and using the calibration curve previously established. Table 2 summarizes the results of the sample analysis.

In all cases, NMR analysis of the samples confirmed the presence of MDMA. The concentration of the drug in urine varied from 0.029 to 0.082 mg/mL. There was no observed correlation between the time elapsed between the ingestion of MDMA and the collection of the sample. In Table 2, even samples collected in the same 8 h timeframe after dosage had a wide range of concentrations. Sample 2 had an estimated concentration of 0.029 mg/mL whereas sample 3, had a far higher concentration at 0.078 mg/mL. This is not surprising as the subject's metabolic rates, along with the amount of drug and fluids consumed would affect amounts excreted. In addition, it is difficult to confirm how much of the drug was ingested due to the clandestine process used to manufacture MDMA. As a result, the pills may not always be pure, as they are commonly cut with other substances. Furthermore, each volunteer may not have consumed the same brand of pill or may have ingested a different number of pills.

These results suggest the ^{1}H NMR spectroscopy could provide a convenient tool for the rapid detection of MDMA in human urine. This method presents the advantage of a rapid diagnosis with little urine needed and no sample preparation. Furthermore, in the concentration range studied, quantitative data can be collected and samples were analyzed within 20–30 min. In an emergency clinical context, the diagnosis problem could be at least partially solved if a rapid identification procedure for MDMA was available even is the dose is not related to toxicity. The NMR method should be useful for such a rapid identification. Furthermore, the NMR method could also be useful in a forensic context for the following reasons: the magnitude of the exposure to MDMA could be assessed *via* quantification by the NMR technique and it is also a non-destructive method.

The limitation of using NMR for the identification of MDMA is that at lower concentrations, the presence of small amounts of metabolites or other therapeutic agents can interfere. In that case, the quantification procedure can be difficult. Two-dimensional sequences may be used in that case. When signal overlap in the 1H dimension precludes quantitation, 2D carbon-proton spectra, such as HSQC, can resolve the overlap due to the greater dispersion available in the carbon dimension. Often analytes with overlapped proton spectra will have distinguishable cross peaks in an HSQC spectra. The principles of quantitative HSQC spectra have been demonstrated [10] and its application to MDMA is the subject of future work.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.forsciint.2009.10.022.

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