Analysis of Ingested Material and Urine by GC–MS and 1H NMR Spectroscopy: Poisoning of an Adult with Adulterated Soda

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Abstract

The purpose of this work is to characterize chemical compounds added to an ingested soda by 1H nuclear magnetic resonance (1H NMR) spectroscopy and by gas chromatography–mass spectrometry in the electron impact mode. A second point was to highlight possible metabolic disturbances by considering urinary profile. Without any pretreatment, dimethylphthalate, 2-butanone, and 2,2,4-trimethylpentanediol diisobutyrate were found in the adulterated soda. Quantitative analysis was performed by relative integration of peak areas. Huge quantities of 2,2,4-trimethylpentanediol diisobutyrate and dimethylphthalate were found in the oily layer. 2-Butanone, which is miscible in water, was found in the two phases as well as small quantities of dimethylphthalate. The urine sample was collected on hospital admission and was also analyzed by 1H NMR spectroscopy. The major abnormal compound found was 1,2-propanediol. Other disturbances concerned endogenous metabolites such as 2-ketoglutaric acid, lactic acid, and betaine.

Introduction

Observations of intentional poisonings at the workplace are relatively uncommon, and previously published papers on the subject are scarce. For instance, a product such as tricresylphosphate was previously involved in a few cases (1), and a suicide by ingestion of aluminum phosphide, available at the victim's workplace, was reported (2). The chemicals implicated can potentially be very different (e.g., drugs, alcohols, or solvents). The toxicological and analytical approach in this type of poisoning is quite different from that used in classical clinical investigations because industrial chemicals or mixtures can be used. Toxicokinetical and toxicodynamical interactions are possible and can cloud the issue and give a complex clinical picture.

Although inhalation is generally the major route of absorp-

tion at the workplace, owing to the volatility of concerned chemicals, Woolf and Flynn (3) analyzed adolescent occupational toxic exposures and established that the most common location for them was food services. Unnoticed administration is generally performed via foodstuffs and/or beverages. In this case, care is necessary when concentrations of xenobiotics in aqueous media are interpreted because the absorption process at the gastrointestinal interface can also proceed from possible lipidic suspension present in the aqueous matrix.

The aim of this study was to investigate the composition of the two phases of an adulterated soda ingested by a woman and to determine, by urine analysis, the consequences of the ingestion of xenobiotics on its own metabolism and the metabolism of endogenous compounds. This can constitute a way to find markers of target organ toxicity. To identify the chemical structures of implicated compounds, both separative and non-separative techniques can be used because no a priori has to be established relative to the composition of heterogeneous mixtures. Classical chromatographic procedures coupled to mass spectrometry (MS) were used and the potential of nuclear magnetic resonance (NMR) spectroscopy investigated. Recently, Ogrine et al. (4) used both techniques to take advantage of the fact that GC–MS and NMR spectroscopy are complementary to detect possible adulteration of beverages. Moreover, NMR spectroscopy has already proven to be a useful tool in different fields for a large variety of compounds, and applications have been done for different biological fluids: analysis of volatile compounds (5,6), poisoning with solvents (7), analyses of herbicides (8) or rodenticides (9), and analysis of urines for doping practices with creatine (10).

Experimental

Reagents

For 1H NMR spectroscopy, internal standard, 3-(trimethylsilyl)-2,2,3,3-tetradeteropropionic acid (TSP-d4), was purchased from Eurisotop (Saint Aubin, France). Other standard
compounds, 2-butanone and 2,2,4-trimethylpentanediol diisobutyrate, were obtained from Sigma Aldrich (Saint Quentin Fallavier, France).

**Case history**

A 45-year-old woman presented herself at the hospital without any visible clinical symptoms. She complained of having ingested, 2 h earlier at her workplace, three mouthfuls of a bad-tasting soda. The drink was a 1.5-L plastic bottle of a first price soda, sold in a supermarket. The poisoned woman worked in a dye plant and was admitted to a reanimation unit. On admission, her blood pressure was 181/90 mm Hg with a heart rate of 106 bpm. Epigastralgia (i.e., pain and burning sensation in the upper abdomen) was noted during the abdominal examination.

Laboratory findings were as follows: glucose, 1.19 g/L; urea, 250 mg/L; and creatinine, 8 mg/L. Biological analyses only showed hepatic disturbances [aspartate aminotransferase (AST) and alanine aminotransferase (ALT)] values: 134 and 133 IU/L, respectively. Standard toxicological screening revealed the presence of fluoxetine and hydroxyzine, corresponding to her medication. As the poisoned woman presented with hepatic disturbances, acetaminophen was unsuccessfully looked for first.

The adulterated soda consisted of two phases, which could be analyzed separately. The upper layer was aqueous, and the lower one was oily. Both of them were transparent. Because the woman worked in a chemical plant specializing in dyes, the investigation first turned towards some chemicals currently used in her workplace: chlorinated solvents, aromatic compounds, and glycol ethers. Classical GC–MS analysis revealed the presence of dimethylphthalate with characteristic fragments at m/z 194, 163, 135, 92, and 77 and the presence of 2-butanone with fragments at m/z 72, 57, and 43. GC–MS results were relatively poor in a routine analysis, and some other peaks could not be assigned to specific structures. NMR analyses were then run on the adulterated soda.

**Sample collection**

In the emergency toxicological context of this study, no specimens were specifically collected, and procedures were therefore in accordance with the revised Helsinki declaration of 1983.

The adulterated soda was obtained. It had the aspect of normal, transparent soda with about 10 mL of another phase at the bottom of the bottle.

Only one urine sample was collected on hospital admission. The urine was immediately frozen and stored at −20°C until analysis.

**NMR and GC–MS analyses**

**Apparatus.** 1H NMR spectra were recorded on a Bruker Avance 300 MHz spectrometer (Bruker S.A., Wissembourg, France) at ambient probe temperature.

GC–MS analyses were performed on an Agilent 6890 GC (Agilent, Massy, France) equipped with an SGE BPX5 (25 m × 0.25 mm) capillary column. The GC was interfaced with an Agilent 5973 quadrupole MS, and spectra were recorded in electron impact mode at 10 or 70 eV.

**Sample preparation.** A 500-μL sample (urine, genuine or adulterated soda, or standard solution) was introduced into a 5-mm diameter NMR tube. A capillary tube containing a titrated solution of TSP-d4 in deuterium oxide was coaxially inserted into the NMR tube. TSP-d4 solution was used as reference for chemical shift (6 1H = 0.00 ppm) and quantitation by relative surface integration. A presaturation sequence was used to suppress the intense water signal. Depending on the sample concentration, 128 to 512 transients were collected into a 16 K data point computer, with a spectral width of 3200 Hz and a 30° pulse. Prior to Fourier transform, an exponential apodization function was applied, corresponding to a 0.3 Hz broadening of the line. Data processing was carried out using the 1D WIN NMR program from Bruker.

**Quantitative analysis.** The NMR tube contains the liquid to be analyzed as well as a sealed capillary tube, which contains a titrated solution of TSP-d4. Quantitation of compounds is readily done by integration of each characteristic signal area. In our experimental conditions, signal areas are proportional to the number of protons in this signal. Calibrations for each chemical were performed from aqueous and organic standards and from spiked control urine samples.

**Results and Discussion**

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**1H NMR analysis of the upper layer**

From the analysis of the aqueous layer, the 1H NMR spectrum showed some signals which could be assigned to sucrose (58.2 g/L) and citric acid (2.0 g/L), the main expected components (Figure 1A). Indeed, these compounds were also found in the spectrum from an original soda of the same trademark and corresponded to the soda composition described on the label (Figure 1B).

Supplementary and unusual signals were also detected. The singlet at 2.18 ppm and the quadruplet at 2.55 ppm belonging to the ethyl group was overlapped with other signals. Other resonances in the aromatic part of the spectrum (multiplets at 7.60 and 7.83 ppm, data not shown) could be related by relative integration to a singlet at 3.9 ppm, and the whole was assigned to dimethylphthalate. These assignments were confirmed by adding commercial compounds to the analyzed phase. The concentration of dimethylphthalate in the aqueous phase was 2.8 g/L.

2-Butanone appears to be the most potentially hepatotoxic compound. The liver is known to be a target organ for this chemical, as assessed by ultrastructural and functional studies (11,12), and the concentration of 2-butanone in the upper phase was 7.5 g/L.
The oily layer was analyzed by $^1$H NMR spectroscopy and produced a complex spectrum (Figure 2). At first, chlorinated solvents and glycolethers, which were currently used in this plant, were investigated without positive result. Secondly, a group of chemicals already present in the aqueous layer was determined: dimethylphthalate and 2-butanone. The latter was as high as 18.5 g/L in the oily phase. If a part of the oily phase is ingested, 2-butanone, even in an oily medium, can constitute a reserve of xenobiotics for membrane transport. Further absorption can occur in the gastrointestinal tract, thus increasing the uptake of this hepatotoxic compound. Xenobiotics with lipophilic character may increase the bioavailability of other ingested xenobiotics by raising their dissolution in the gastric content.

Numerous supplementary resonances could also be observed at 0.9–1.3, 2.11, 2.63, 3.7–4.0, and 4.84 ppm for instance. Considering the complex pattern of these signals, $^{13}$C NMR analysis was also carried out, and to simplify overlapping resonances, two-dimensional (2D) NMR experiments had to be performed. $^1$H–$^1$H total correlation spectroscopy could specify the different proton families in the spectrum (data not shown): the two CH$_2$ protons of propanediol at 3.90 and 4.00 ppm are strongly correlated to each other but also to signals from 0.90 to 1.10 ppm assigned to the four methyl groups of pentanediol. The two CH from the isobutyrate groups gives a multiplet at 2.63 ppm, which is correlated to the signals between 1.20 and 1.30 ppm, assigned to the four methyl groups from isobutyrate. 2D NMR spectroscopy of $^1$H–$^1$H heteronuclear correlation optimized on a long range coupling (HMBC) was also used, and a spectrum is presented in Figure 3. For instance, the multiplet at 2.63 ppm is, in this case, correlated to the two signals at 178.2 and 178.3 ppm, assigned to quaternary carbon from carbonyl groups, and also to carbon signals between 19 and 20 ppm, assigned to the four methyl groups from isobutyrate groups. Besides, the only methylene group of the molecule gives an AB-type signal at 3.90 and 4.00 ppm and is correlated to four types of carbon: (i) at 178.2 and 178.3 ppm (C from carbonyl groups), (ii) at 80 ppm (CH from C(3) propanediol), (iii) at 39.8 ppm (quaternary carbon from C(2) propanediol), and (iv) to the group of carbon around 22.4 ppm, assigned to the four methyl groups of propanediol. The whole assignment was then completed, and the new chemical compound was found to be 2,2,4-trimethylpentanediol disisobutyrate (Figure 4). This assignment could be confirmed by adding the standard compound. It is currently used as a plastifier agent, especially in paints (13) and rubber gloves (14). These findings fit with the occupational environment of the poisoned woman.

To verify NMR results, an aliquot of the oily phase was diluted with methanol, and 2 µL was directly injected into the GC–MS apparatus at 10 eV without any derivatization. The major ions were found to be m/z 243, 173, 159, 143, 111, 98, 83, 71, and 43. The m/z 71 corresponded to the isopropyl-carbonyl group and was the base peak of the spectrum. The molecular ion of the ester was not detectable, and the highest mass-to-charge ratio value was 243, which corresponded to the loss of an isopropyl group from the molecular ion. This confirmed that the major peak in the chromatogram was 2,2,4-trimethylpentanediol diisobutyrate.

So, apart from dimethylphthalate, which has been identified simultaneously by both techniques, the chemical structure of the other compounds have been elucidated only by $^1$H NMR spectroscopy and further confirmed by GC–MS. In the first analyses done by GC–MS, ethylmethylketone was missed because it was eluted too close to the solvent peak. Its structure...
was confirmed by GC–MS only by modifying the routine temperature program. On the other hand, numerous compounds are generally included in the databases of GC–MS devices. In this particular case, the 2,2,4-trimethylpentanediol diisobutyrate, which was added to the adulterated soda, is a very unusual compound and it was not in the GC–MS library. Its structure would have been very difficult to elucidate with only the GC–MS procedure.

1H NMR analysis of urine

1H NMR urinalysis from the poisoned patient (Figure 5) revealed the following findings. The presence of endogenous compounds such as dimethylglycine (62 mg/L), citric acid (866 mg/L), creatinine (1821 mg/L), trimethylamine oxide (84 mg/L), glycine (114 mg/L), and hippuric acid (770 mg/L) were within the normal range (15,16).

The presence of other endogenous compounds, quite uncommon in urines, such as 2-ketoglutaric acid (203 mg/L) and betaine (477 mg/L) were of a significant level compared to controls. This is in agreement with the observation published by Sequeira et al. (17) in their study concerning hepatotoxic xenobiotics. These authors effectively stated that the most significant biochemical change was an increase in betaine concentrations, and they related this disturbance in the hepatic methylation pathways to the occurrence of a hepatotoxic episode assessed by the development of ALT and AST values during hospitalization.

The presence of xenobiotics [a doublet at 1.11 ppm (J = 5.5 Hz)] was assigned to 1,2-propanediol (377 mg/L). The presence of propylene glycol in this urine is quite amazing because it cannot originate from the chemicals identified in the two phases of the ingested adulterated soda. This compound could come from prescribed medication because it is widely used to dissolve drugs (18).

Conclusions

The present study exemplifies two major benefits of NMR. First, any chemical species of xenobiotic compounds concerned can be detected. Structures of dimethylphthalate and 2-butanone can be determined, as well as 2,2,4-trimethyl-1,3-pentanediol diisobutyrate. Second, without being limited to a particular class of biological markers, NMR spectroscopy may reveal metabolic disturbances consecutive to poisoning.

Moreover, this analytical technique could give precious information on the metabolic state of a patient. Indeed, in the case of the poisoned woman, xenobiotics with medium lipophilic character, such as dimethylphthalate and 2,4,6-trimethyl-1,3-pentanediol diisobutyrate, may raise the bioavailability of other orally ingested products by increasing their dissolution in gastric contents. Metabolic interferences could also occur.
The present study illustrates the usefulness of NMR spectroscopy associated with GC-MS in the investigation of adulterated beverages and of the consequences to the endogenous metabolism of exposure to xenobiotics.

Acknowledgments

The authors are grateful to Mrs. Alexandra Tavernier, M.A. (University of Glasgow), and Professeur Agrégé (English), for her expert advice in the revision of the English manuscript.

References


Manuscript accepted February 9, 2005; revision received June 6, 2005.