Identification of Solvents of Abuse Using Gas Chromatography/Fourier Transform Infrared Spectrometry After Headspace Sampling

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Key Words
Gas chromatography
Fourier transform IR detection
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Solvents of abuse

Summary
After experimental intoxication of rats, gas chromatography — Fourier transform infrared spectrometry with headspace sampling (HS/GC/FT-IR) was used to identify solvents of abuse in biological material (blood, liver, lungs and brain). Limits of detection were measured for acetone, 2-butanone, ether, toluene and trichloroethylene with standard solutions. All the solvents have been identified in the organs of the intoxicated rats. For blood samples a salting-out effect was obtained with potassium carbonate. HS/GC/FT-IR allowed the identification of metabolites of acetone (isopropanol) and of 2-butanone (2-butanol) in blood and organs.

Introduction
Identification of solvents of abuse in biological fluids using gas chromatography-Fourier transform infrared spectrometry (GC/FT-IR) has been investigated by classic methodology-extraction and direct liquid injection [1–3]. The limitations of this method have been previously discussed [2, 3]. Since headspace sampling is well suited to the gas chromatographic analysis of volatile compounds [4, 5], the aim of this work was to investigate the capability of gas chromatography-Fourier transform infrared spectrometry after headspace sampling (HS/GC/FT-IR) which, to our knowledge, has never been published. We present here the limits of concentration for identifying some solvents commonly used by sniffers and the application of this methodology to the analysis of biological samples after the experimental intoxication of rats.

Experimental
Reagents
Five solvents were studied: acetone, 2-butanone, ether, toluene and trichloroethylene. They were all of analytical grade (Merck, Darmstadt, FRG). Potassium carbonate, ammonium sulphate and sodium chloride were obtained from Prolabo (Paris, France).

Material and Procedures
Five millilitres headspace vessels, rubber septa covered with Teflon film, and aluminium caps were provided by Brosse (Paris, France).

Standard solutions of acetone, 2-butanone and ether were prepared in ultra-pure water. For non-water soluble solvents (trichloroethylene, toluene), we used suspensions in water obtained by sonication for 10 min at 48 kHz [6]. The sealed vessels containing 2 ml of mixture were kept for 30 min at 80 °C [7, 8]. The salting-out effect was tested with 2 g of salt (K2CO3, (NH4)2SO4 or NaCl) for aqueous solutions. 250 μl of headspace were withdrawn using a gastight syringe and injected into the chromatograph. Experimental intoxications were made on Wistar male rats in a four litres glass tank. Twenty millilitres of solvent were poured in the tank and the animal was put down on a grid. This simple device avoids two major causes of mistakes:

– No contact was possible between the rat and the solvent. This prevents any ingestion and any pollution during the autopsy.
– The lid of the tank was not completely closed in order to induce death by subacute intoxication and not by anoxia.

Biological sampling was made at 4 °C. Two ml of cardiac blood were divided between two vessels, one of them containing 1 g of potassium carbonate. Lungs, liver and brain were separately homogenized in a Potter crusher. Two grams of each organ were introduced in a vessel which was sealed and stored at –20 °C. Just before the analysis
the vessels were incubated in an identical manner to the standards.

The GC/FT-IR system was a Nicolet 7199B (170 SX) infrared spectrometer linked to a Varian 3700 gas chromatograph, as previously described [9]. The column was 10 ft x 1/8 in. o.d., stainless steel, packed with 3% SP-1500 on 80–120 mesh Carbopack B (Supelco). Carrier gas was nitrogen at flowrate of 15 ml/min. Temperatures were: injection port 150°C, column programmed from 70°C to 240°C at 4°C/min, transfer line and IR cell 160°C.

Eluted compounds were detected by absorbance measurement in the following spectral windows: 810–850 cm⁻¹, 1100–1150 cm⁻¹, 1700–1750 cm⁻¹, 2900–2950 cm⁻¹, 3000–3100 cm⁻¹.

Results and Discussion

Determination of Detection Limits

Table I gives for the five solvents the concentration limits which allow both detection and identification by comparison of their IR spectra (4000–800 cm⁻¹) to the “EPA Vapor Phase” spectra library [10]. An accurate diagnosis needs an absorbance level at least equal to 0.015 absorbance unit. Below this limit, a chromatographic peak may be observed, but the signal-to-noise ratio is often too low to identify the eluted compound with certainty and reproducibility.

Great differences in the detection limits appear between aqueous solutions of acetone, 2-butanone and ether, and aqueous suspensions of trichloroethylene and toluene. We believe that the way of preparing the samples, and more generally the thermodynamic parameters (volatility, activity coefficients) of the mixtures solvent-water, are not the only reason of these results. The IR molecular absorbance must be taken into account:

- strong and characteristic absorbance of acetone and 2-butanone in the 1700–1750 cm⁻¹ spectral window;
- sharp and intense band of the C–O–C stretching vibration of ether at 1134 cm⁻¹;
- lower IR absorbance of toluene;
- the IR absorbance of trichlorethylene appears only under 1000 cm⁻¹, where the signal-to-noise ratio is low.

Moreover, the salting-out effect which increases sensitivity for aqueous solutions (Fig. 1) may not be used with suspensions.

Table I. Detection limits for identifying five solvents in HS/GC/FT-IR

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Sample</th>
<th>Detection limit (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone</td>
<td>Aqueous solution</td>
<td>8</td>
</tr>
<tr>
<td>2-Butanone</td>
<td>Aqueous solution</td>
<td>6</td>
</tr>
<tr>
<td>Ether</td>
<td>Aqueous solution</td>
<td>5</td>
</tr>
<tr>
<td>Toluene</td>
<td>Aqueous suspension</td>
<td>30</td>
</tr>
<tr>
<td>Trichloroethylene</td>
<td>Aqueous suspension</td>
<td>20</td>
</tr>
</tbody>
</table>

We have compared these HS/GC/FT-IR results with detection limits previously obtained after extraction of standard solutions and direct injection of the organic liquid phase [2]. An enhancement of about 3 is obtained for acetone, 2-butanone and ether, but no enhancement is obtained for toluene and trichlorethylene. The main advantage of HS/GC/FT-IR lies in the amount of sample which can be injected, 250 μl of headspace gas versus 10 μl of liquid sample. Headspace injections lead to sharp and symmetrical GC peaks suitable for FT-IR interpretation.

Minimal detectable concentrations are significantly less than fatal blood levels for each solvent [3]. This fact will be confirmed by experimental intoxications results.