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Combination of Screening Procedures - Mesocarb Detection as an Example
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Mesocarb detection as an example

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Introduction:

Depending on the chemistry and/or biochemistry dope agents are detected in different screening procedures\(^1\). Polar substances and their conjugates present problems when an integration in existing screening procedures is intended. A typical example is mesocarb (3-(1-methyl-2-phenylethyl)-N-(phenylaminocarbonyl)-sydnon imine, "SYDNOCARB\(^\circledR\)"), a psycho stimulator and therefore a potential doping agent in sports\(^2\). The main metabolite excreted in human urine is a conjugated hydroxy-mesocarb (3-(1-methyl-2-phenylethyl)-N-(4-hydroxy-phenylaminocarbonyl)-sydnon imine). It should be possible to include hydroxy-mesocarb in the screening procedure for conjugated phenolalkylamines. But difficulties occurred detecting it after selective derivatization using trifluoroacetic acid as a solvent.

Mesocarb is thermolabile and undergoes pyrolysis in the injector block of a gas-chromatograph, and so does its metabolite. The pyrolysis product in both cases is N-nitroso-N-cyanomethyl-amphetamine which can be detected very sensitively with an appropriate GC/NPD/ or GC/MS/system.

This paper presents a method for the detection of mesocarb by combining two already existing screening procedures.
*Experimental:*

Instrumentation:

**GC 1:**
- HP 5880 with a NPD;
- column: SE 54; 0.35 μm film thickness; 0.25 i.D.;
- head pressure: 15 psi;
- TInjector: 280°C;
- TDetector: 300°C;
- Temp.program: 100°C; 25°C/min.; 300°C; 3 min.

**GC 2:**
- HP 5890 with MSD 5971A;
- column: SE 54; 0.35 μm film thickness; 0.25 i.D.;
- head pressure: 15 psi;
- TInjector: 280°C;
- TTransfer line: 300°C;
- Temp.program: 100°C; 23°C/min.; 310°C; 2 min.

Standards and chemicals:

N,N-Diisopropyl-dodecylamine (DIPA12) was synthesised in our laboratory;
β-glucuronidase from E. coli was purchased from BOEHRINGER-Mannheim;
XAD 2-resin was purchased from SERVA-Heidelberg;
all other chemicals used were of analytical grade.

Hydrolysis and sample preparation:

2 ml aliquots of a Mesocarb excretion urine, obtained after application of 10 mg of
mesocarb (24 h urine) were prepared in the following ways:

1. without hydrolysis
2. acidic hydrolysis
3. enzym. hydrolysis of the concentrated XAD 2 extract using β-glucuronidase
   from E. coli

Sample 2 was adjusted to pH 1-2 by adding 1 ml 6 M hydrochloric acid and then
heated for 1 h at 100°C. Sample 3 was adjusted to pH 7 with 1 ml 0.2 mol/l
phosphate buffer before adding 50 μl of the enzyme and then heated for 1 h at 50°C.
Sample 1 and Samples 2,3 after hydrolysis were adjusted to pH 9.6 by adding ca. 300 mg of solid carbonate/bicarbonate buffer (1:2/w:w). Then 2 μg of ISTD (DIPA 12) and 5 ml tert.-butyl-methylether were added, the samples were shaken for 20 min. and centrifuged. The organic layer was injected without further preparation.

**Chromatography:**

All samples were injected into a HP 5880 with NPD. For determination of the retention times of mesocarb and DIPA 12 (ISTD) a standard solution consisting of 50 μg mesocarb/ ml and 50 μg ISTD/ ml in methanol was used.

The retention time for the mesocarb was 4.19 min., for the ISTD 4.56 min..

The same solution was injected to a HP 5890 with a MSD 5971A to obtain the mass spectrum of mesocarb. The mass spectrum showed a base peak with m/z 91 and two small peaks with an intensity of about 5 % of the base peak with m/z 118 and 173.

**Results:**

The results of the different sample preparations are summarised in the following table:

<table>
<thead>
<tr>
<th>Type</th>
<th>height[cnt]</th>
<th>ratio</th>
<th>conc.[μg/ml]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mesocarb/ OH-</td>
<td>ISTD</td>
<td>(M/ISTD)</td>
</tr>
<tr>
<td></td>
<td>mesocarb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 no hydrol.</td>
<td>15,54</td>
<td>0,00</td>
<td>0,0</td>
</tr>
<tr>
<td>2 acidic hydrol.</td>
<td>71,2</td>
<td>3,03</td>
<td>1,5</td>
</tr>
<tr>
<td>3 enzym. hydrol.(E.c.)</td>
<td>13,3</td>
<td>0,35</td>
<td>0,2</td>
</tr>
<tr>
<td>STD mesocarb</td>
<td>84,74</td>
<td>2,08</td>
<td>1,0</td>
</tr>
</tbody>
</table>

Table 1: Yields of the different hydrolysates

The excretion study demonstrates that without any hydrolysis no mesocarb is extracted with tert.-butyl-methylether. The highest yield is obtained after acidic hydrolysis. After enzymatic hydrolysis using β-glucuronidase from E. coli mesocarb was detected with a concentration of about one eighth of the concentration of mesocarb after acidic hydrolysis.
The mass spectrum of the mesocarb peak is identical with the mass spectrum of a N-nitroso-N-cyanomethyl-amphetamine 3.

Mesocarb standard and the mesocarb metabolite give a signal at the same retention time and show mass spectra identical with N-nitroso-N-cyanomethyl-amphetamine.

**Conclusions:**

The expected concentrations of mesocarb after a 10 mg dose can be detected in an aliquot of the ether extract of the screening procedure for conjugated anabolic steroids. The procedure presented consists of a solid phase extraction using XAD 2 resin, enzymatic hydrolysis with β-glucuronidase from E. coli and finally a basic extraction with 5 ml tert.-butyl-methylether at pH 9.6. A 200 μl aliquot of the organic layer, which in our procedure is left in the centrifuge tube after decantation, is evaporated to dryness and reconstituted with 50 μl of methanol. 4 μl of this solution are then injected into a GC equipped with a NPD or a MSD.

Using this method even a ten fold dilution of the mesocarb excretion urine used in this study, which means a concentration of 0.2 mg/ml, gives a clear positive signal for mesocarb.

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1 M. Donike et al; Dope Analysis; in P. Belotti et al; 1st International Athletic Foundation World Symposium on Doping in Sport, Florence 1987; International Athletic Foundation ; Florence (1988); 53-80

2 M. Polgár et al; Metabolism of mesocarb in rat; Xenobiotica; 9; 1979; 8:511-520

3 Communication to the IOC accredited laboratories; Nov. 1991