15. TOXICOLOGICAL DETERMINATION OF HEROIN AND MORPHINE IN URINE OF MAN
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INTRODUCTION
For many years there have been difficulties in the determination of Heroin and its metabolites in drug addicts. For this purpose, different extraction procedures and determinations, such as Thin layer chromatography and Gas chromatography were studied. The results of this research are discussed.

EXPERIMENTAL
1. Thin Layer Chromatographic analysis

A large number of urine samples from cancer patients, who were treated with Heroin were analysed using different extraction procedures. The following procedures gave the best results.

Determination of Heroin

Heroin can be extracted from spiked urine samples with high efficiency as described by S.Y. Yeh and R.L. McQuinn (1975). A 5 ml aliquot of urine is adjusted to about pH 4.5 with acetic acid, buffered with 1 ml of 1 M sodium acetate buffer to pH 4.5; salted out with 1.6 g sodium chloride, shaken with 15 ml of chloroform and centrifuged.

The organic phase is transferred to a conical centrifuge tube, and evaporated to dryness under a stream of nitrogen in a water bath at 60-70°C.

Determination of Morphine

3 ml of concentrated hydrochloric acid is added to 15 ml of urine. By autoclaving the urine sample at 120°C for 15 minutes the conjugates can be efficiently hydrolyzed (Sunshine 1975). Alternatively, conjugated morphine can be efficiently hydrolyzed enzymatically with glucuronidase-arylsulfatase, in a buffered medium of pH 5.5 (sodium acetate buffer) at 40-45°C overnight. Autoclaving is preferred to enzymatic hydrolysis, because of the time saved.

After cooling, the urine sample is extracted with 25 ml of chloroform containing 20% propanol. After removal of the organic phase, the
aqueous layer is adjusted to about pH 8.4, buffered with 6 ml of \( \text{NH}_4\text{Cl}/\text{NH}_4\text{OH} \) at pH 8.4, and extracted with 25 ml of chloroform containing 10% methanol.

6-monoacetylmorphine, acetylcodeine, codeine and free morphine are extracted without autoclaving the urine sample.

The organic phase is dried over anhydrous sodium sulphate, and evaporated to dryness in a rotating Büchi apparatus.

The obtained residue is dissolved in 3 x 1 ml of methanol, and transferred quantitatively into a conical centrifuge tube. Final evaporation occurs in a waterbath at 60°C, under a stream of nitrogen. The extract is dissolved in methanol, and spotted on a TLC-plate (Merck R 60F254).

Different developing solvents were used in order to obtain a sufficient separation of: heroin, morphine, 6-monoacetylmorphine, acetylcodeine and codeine.

**Developing solvents and \( R_F \)-values**

<table>
<thead>
<tr>
<th></th>
<th>Heroin</th>
<th>Morphine</th>
<th>6-Monoacetylmorphine</th>
<th>Acetylcodeine</th>
<th>Codeine</th>
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</thead>
<tbody>
<tr>
<td>( \text{CHCl}_3 ) 90</td>
<td>0.67</td>
<td>0.09</td>
<td>0.32</td>
<td>0.56</td>
<td>0.26</td>
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<td>Ethanol 10</td>
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<tr>
<td>( \text{CHCl}_3 ) 80</td>
<td>0.89</td>
<td>0.21</td>
<td>0.60</td>
<td>0.87</td>
<td>0.48</td>
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<tr>
<td>Ethanol 20</td>
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</tr>
<tr>
<td>( \text{CHCl}_3 ) 90</td>
<td>0.87</td>
<td>0.17</td>
<td>0.48</td>
<td>0.80</td>
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<tr>
<td>( \text{CH}_3\text{OH} ) 10</td>
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