

## Isolation and Identification of Methaqualone in Urine

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**PURPOSE:** An accurate test suitable for rapid testing of large numbers of urine samples for the presence of methaqualone or its metabolites was required.

**BACKGROUND:** Methaqualone (Mandrax, Parest, Quaalude, Sopor, Tuazole) abuse is a serious public health problem in the United States and abroad. Information that this drug was being abused by American teenaged children in Bangkok stimulated an attempt to develop a method for detection of this drug or its metabolites in urine.

**PROGRESS:** A thin layer chromatography (TLC) screening method was developed which readily detects methaqualone metabolites in urine. Urine must be subjected to hydrolysis before the metabolites can be extracted.<sup>1</sup> Positive identification of these metabolites can then be made by gas-liquid chromatography (GLC).

### METHOD:

*Screening procedure with GLC confirmation:* Urine (15 ml) is placed in a capped glass tube with concentrated HCL (5 ml) and autoclaved (110°C, 15 min, 15 psi). The hydrolyzed urine is transferred to cylindrical extraction tubes and adjusted to pH 8.5 with KOH (= 4 ml, 6 N), then chloroform-methanol (9:1, 25 ml) is added. After agitation the aqueous layer is removed, the organic layer is dried (anhydrous sodium sulfate, 3 gms), filtered and evaporated to dryness. The residue is spotted on TLC plates (Merck, Silica gel G). Separation is accomplished using ethylacetate-methanol-ammonium hydroxide (85:10:5) and the metabolites are located with acidified iodoplatinate reagent (0.5 g chloroplatinic acid, 9.0 g potassium iodide and 200 ml water; add equal volume 2 N HCL before use).

The metabolites (Rf 0.80, 0.75 and 0.70) from positive samples can be scraped from the TLC plate and extracted from the silica gel with methanol (3 ml). After centrifugation, the methanol is carefully transferred to narrow bottom centrifuge tubes and evaporated to dryness. The residue is reconstituted in 20  $\mu$ l methanol and examined by glc. The instrument used is a Varian 2700 Gas Chromatograph with hydrogen flame ionization detector using a 6 ft glass coil-shaped column containing 3% OV-1 on Gas-Chrom Q (100-120 mesh). The column temperature is 235°C using Nitrogen (25 ml/min) as the carrier gas. The retention times and Rf values of the three major metabolites are contained in Table 1.

*Direct extraction of urine for GLC:* Urine (15 ml) is hydrolyzed as before and extracted at pH 9.0 (sat. KOH) with chloroform-methanol (9:1, 25 ml). The organic layer is separated and extracted with H<sub>2</sub>SO<sub>4</sub> (3 ml, 0.5 N). To the separated aqueous layer saturated KOH is added until pH 9.0 is reached, then chloroform-methanol (9:1, 3 ml) is added. The organic layer is separated and evaporated in narrow bottom tubes under nitrogen. The residue is reconstituted in 20  $\mu$ l methanol and examined by glc. All three of the major metabolites can be observed with a single injection.

**RESULTS:** These procedures have been used on 650 urine samples from teenaged children in Bangkok. Of these, 2.5% were found to contain methaqualone metabolites.

Scrutiny of urine samples from the military drug urinalysis program which are positive for morphine showed that about 0.8 % also contained methaqualone metabolites.

A study is now underway to determine the prevalence of methaqualone abuse in U.S. military personnel in Thailand by randomly sampling and testing urine samples received through the drug urinalysis program.

REFERENCE :

1. Burnett, D., Goudie, J.H., and Sherriff, J.M. : Detection of methaqualone and its metabolites in urine., J. Clin. Path. 22 : 602 - 604, 1969.

Table 1.  
TLC and GLC mobility of methaqualone metabolites

	Rf ( A )	Rf ( B )	GLC*
Methaqualone	0.88	0.70	--
Codeine	0.52	--	1.0
Metabolite 1	0.80	0.50	1.0
Metabolite 2	0.75	0.55	1.32
Metabolite 3	0.70	0.58	1.43

A. Ethylacetate - methanol - NH<sub>4</sub>OH ( 85 - 10 - 5 )

B. Ether

\* Retention time, relative to codeine