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Gas chromatographic detection of methadone and morphine in the urine of drug addicts

Methadone is an addictive drug used by opiate addicts. During the so-called methadone maintenance therapy¹ it is administered to out-patients over a long period of time. Before beginning such a therapy, it is necessary to be able to detect morphine and methadone in the urine of patients. Moreover, during treatment a strict control on the use of methadone and other drugs is desirable.

Although methadone can be detected with many of the thin-layer chromatographic systems² that are in use for the detection of morphine, these methods proved to be insufficiently dependable in an out-patient department where methadone doses are kept low (< 50 mg/day) and the patients often use a multiplicity of other drugs besides opium and morphine.

Using the gas chromatographic method described here, it is possible to detect methadone in the presence of morphine in the urine of patients using or abusing a variety of other drugs.

Reagents

The following reagents were used:

Glucuronidase solution: β -glucuronidase/aryl sulphatase (Boehringer Mannheim GmbH—activity of glucuronidase approximately 5.2 U/ml and of aryl sulphatase 2.6 U/ml).

Borate buffer, pH 9.3: 950 ml of saturated disodium tetraborate and 50 ml of 0.3 N sodium hydroxide.

Chloroform-isopropanol solvent: 3 vols. of chloroform and 1 vol. of isopropanol.

BBG solvent: *n*-butanol-di-*n*-butyl ether-glacial acetic acid (4:5:1).

Nalorphine solution: 1 mg of nalorphine dissolved in 10 ml of methanol.

Gas chromatograph

A Hewlett Packard F&M 402 gas chromatograph with flame ionisation detector was used. Moseley recorder, 1 mV full-scale deflection. Glass column 3 mm I.D., length 120 cm. 3.5% UCW 98 on Chromosorb W-AW-DMCS, 80–100 mesh. Flash heater, 270°. Column oven, 235°. Flame ionisation detector, 290°. Carrier gas: nitrogen at 35 ml/min.

Procedure

15 ml of urine are pipetted into a 50-ml glass-stoppered tube and the pH is adjusted to 4.5–5.0. After addition of 0.05 ml of glucuronidase, the mixture is left overnight at 37°. Next the pH is adjusted to 9.3, 10 ml of borate buffer and 15 ml of chloroform-isopropanol solvent are added and the tube is shaken for 10 min on a mechanical shaker. The phases are separated by centrifuging, and the organic (lower) layer is transferred to a conical centrifuge tube and evaporated in a 50° water bath under a stream of nitrogen. The residue is spotted on a thin-layer silica gel plate (F₂₅₄ Merck) and developed in the BBG solvent for 1.5 h (the solvent front has run about 15 cm). The plate is air-dried and, as both methadone and morphine

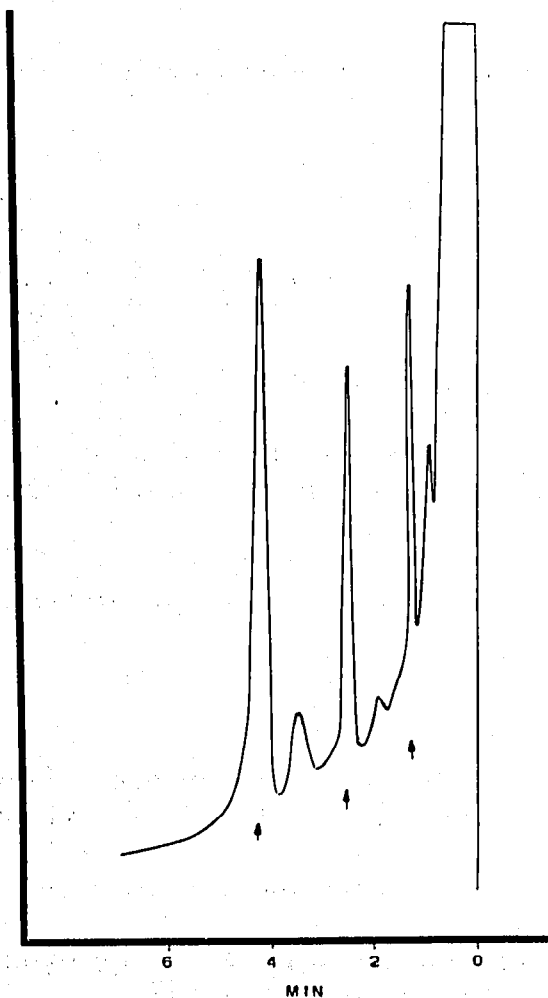


Fig. 1. Urine extract of patient using both methadone and morphine. The arrows indicate, from right to left, the position of methadone, morphine and nalorphine, respectively. Amount injected, $1.2 \mu\text{l}$; attenuation, 10×8 .

have an R_F value of about zero in the BBG solvent, an area of about 1.5 cm^2 around the origin is scraped out and transferred to a conical centrifuge tube.

The powder is extracted twice with 2-ml portions of chloroform-isopropanol solvent, and the extracts are combined and evaporated just to dryness in a 50° water bath under a stream of nitrogen.

The residue is dissolved in 0.05 ml of nalorphine solution and $1.0 \mu\text{l}$ are injected into the gas chromatograph.

Discussion

The method described is intended for the qualitative detection of morphine and methadone. Nalorphine is added only to check possible variations in the performance of the gas chromatograph. However, if quantitative analysis is desirable, the method can be modified very easily by adding an internal standard (*e.g.* a known amount of nalorphine) to the urine sample before extraction. A large group of drugs that are used in psychiatric therapy, including hypnotics, tranquilisers and anti-depressants, do not interfere with the analysis. Only in the urine of patients using

disipal (orphenadrine hydrochloride) a peak is found in the gas chromatogram that coincides with the methadone peak.

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