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Note

Simultaneous determination of acetylmethadol and its major metabolites by gas–liquid chromatography

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Kaiko *et al.*[1] recently described a method utilizing solvent extraction and gas–liquid chromatography for the quantitative determination of acetylmethadol simultaneously with its two major metabolites, noracetylmethadol and dinoracetylmethadol, in human biofluids. The metabolites were measured as their corresponding amides, which were formed by adding four drops of 50% sodium hydroxide solution to the final extract (resultant pH = 13) and incubating at 70° for 30 min. These authors reported that acetylmethadol itself was unaffected by this procedure.

We have adapted this method to determine acetylmethadol, noracetylmethadol, dinoracetylmethadol, as well as another major metabolite, methadol, quantitatively in biological fluids. During the course of generating standard curves for these four compounds, however, it was repeatedly observed that the detector response to acetylmethadol was low while that to methadol was high. It was therefore decided to investigate the possibility of loss of acetylmethadol via alkaline hydrolysis, hence the *in situ* synthesis of methadol during the assay procedure.

MATERIALS AND METHODS

α-*l*-Acetylmethadol hydrochloride, α-*l*-noracetylmethadol hydrochloride, α-*l*-dinoracetylmethadol hydrochloride and α-*l*-methadol hydrochloride were provided by the National Institutes of Health (Bethesda, Md., U.S.A.).

A Perkin-Elmer Model 3920B gas chromatograph equipped with a flame-ionization detector was used. The column was a 6-ft. helical glass column with an I.D. of 2 mm and an O.D. of 6 mm, packed with 3% XE-60 on 80–100-mesh Gas-Chrom Q. The electrometer was set at an amplifier range of 1 and an attenuation of 8. The carrier gas was nitrogen with a flow-rate of 30 ml·min^{-1} while the flame gases were hydrogen and compressed air at flow-rates of 30 and 360 ml·min^{-1} respectively. The temperatures of the detector and injector port were 300° and 275° respectively, and a column temperature of 200° was used for analysis.

Acetylmethadol hydrochloride (0.4 μg) was placed in each of two 15-ml siliconized centrifuge tubes with Teflon-lined screw-caps. Five milliliters of 0.2 *N* hydrochloric acid were added to each tube, followed by four drops of 50% sodium

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hydroxide solution to yield a pH of approximately 13. After thorough mixing, the contents of one tube were immediately extracted into 8 ml of chloroform by shaking for 10 min followed by centrifuging at 1000 *g* for 5 min. The second tube was incubated in a heating block at 70° for 30 min prior to extraction with chloroform. The upper, aqueous phase was removed by aspiration and discarded, while the organic phase was concentrated by evaporation under nitrogen, transferred into a 1-ml Reacti-vial and subsequently evaporated to dryness. The residue was dissolved in 20 μl of carbon disulfide which contained 0.8 μg of triacontane as external standard. One microliter of the sample was injected into the gas chromatograph. The drug: triacontane peak-height ratios were calculated and compared. The experiment was repeated four times.

RESULTS AND DISCUSSION

Under the aforementioned operating conditions, the retention times were 190 sec for acetylmethadol, 213 sec for methadol and 549 sec for triacontane.

The samples which were not incubated yielded only the acetylmethadol and the triacontane peaks. Incubation at pH 13 resulted in a significantly lower acetylmethadol peak and the appearance of a sizable peak at 213 sec, the retention time of methadol. Fig. 1 shows the relative peak heights of acetylmethadol and methadol from acetylmethadol samples with and without incubation.

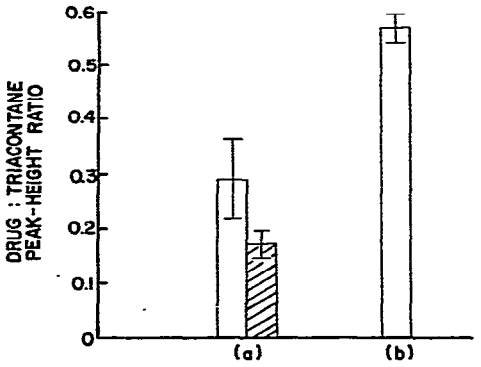

Fig. 1. Relative peak heights of acetylmethadol (□) and methadol (▨) from acetylmethadol samples (a) with and (b) without incubation at 70° for 30 min. Values represent the average from four experiments. The vertical bars indicate 1 standard deviation.

The results show that part of the acetylmethadol was hydrolyzed to form methadol during incubation at pH 13, a step which is necessary in order to convert noracetylmethadol and dinoracetylmethadol into their corresponding amides for better peak resolution. Any standard calibration graphs generated from such data will inevitably lead to incorrect calculation of acetylmethadol and methadol concentrations in biofluids where acetylmethadol and methadol may be present in variable relative amounts.

Hence, in order to measure simultaneously acetylmethadol, noracetylmetha-

dol, dinoracetylmethadol and methadol, it seems necessary, after the addition of strong base in the last step in the extraction procedure[1], to divide the sample extract into two equal portions. One portion is immediately extracted into chloroform and subsequently analyzed for acetylmethadol and methadol, while the other is incubated and then analyzed for noracetylmethadol and dinoracetylmethadol as their corresponding amides.

REFERENCES

1 R. F. Kaiko, N. Chatterjie and C. E. Inturrisi, *J. Chromatogr.*, 109 (1975) 247.