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## Note

# Estimation of *l*-α-[2-$^3$H]acetylmethadol in biological materials and its separation from some metabolites and congeners on glass fibre sheets

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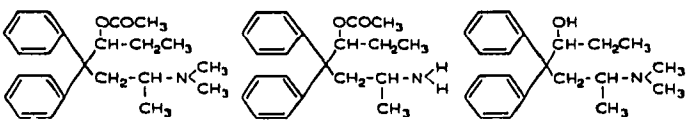
(—)-*l*-α-Acetylmethadol (LAAM), (I), a synthetic congener of methadone, possesses a long duration of action[1–3] and can effectively suppress opiate withdrawal symptoms for periods up to 72 h[4,5]. This property of LAAM has practical therapeutic advantages over methadone in the treatment and rehabilitation programs for chronic heroin users[6,7].

Previous[3,8] and recent[9–11] studies on the disposition and metabolism of LAAM have shown that some of its pharmacological activity and long duration of action may be due to its metabolites (—)-*l*-α-acetylnormethadol (V) and (—)-*l*-α-acetylbisnormethadol (II).



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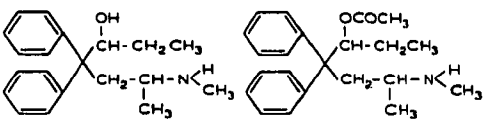

IV V

This communication describes a sensitive method for the estimation of *l*-α-[2-$^3$H]acetylmethadol in biological materials and the application of glass-fibre silica gel impregnated sheets (ITLC) to the separation of LAAM, its metabolites and congeners. This technique possesses the advantage of speed, convenience and ease of radioscanning by direct transfer of sectioned planimetric strips of ITLC sheets to vials, the addition of eluent, toluene–phosphor[12] and subsequent assay of the radioactivity in a liquid scintillation spectrometer.

## MATERIALS AND METHODS

*Samples*

*l*-α-[2-$^3$H]Acetylmethadol and non-labeled compound, its metabolites and congeners used in this study were obtained from the Research Triangle Institute (Chemistry and Life Sciences Div.), Research Triangle Park, N.C., U.S.A., through the courtesy of the National Institute of Drug Abuse (NIDA), Rockville, Md., U.S.A.

*Estimation of l-α-[2-$^3$H]acetylmethadol in biological materials*

Two-millilitre aliquots of diluted urine or plasma (1:5) or tissue homogenate (10% w/v in 0.9% saline or 0.5 *N* HCl) in duplicate were transferred to 40-ml centrifuge tubes containing 1 ml non-radioactive LAAM as carrier (500 μg/ml as free base), the pH of the solution was adjusted to 8 by addition of 10% $NH_4OH$ and the solution was buffered with 2 ml of 1 *M* phosphate buffer pH 8. Fifteen millilitres of ethyl acetate were added and the mixture shaken in an Eberbach shaker for 15 min. After centrifugation at 1500 rpm for 10 min, the aqueous phase was aspirated and the organic phase was shaken for 1 min with 4 ml of 0.1 *M* phosphate buffer pH 8. The solution was centrifuged for 10 min, the aqueous phase was aspirated and 10 ml of the organic phase were transferred to counting vials and evaporated at 45–50° on a Fisher slide warmer. The residue was dissolved in 0.5 ml methanol, 10 ml toluene–phosphor[12] were added and radioactivity determined in a liquid scintillation spectrometer using standard techniques described previously[12].

*Estimation of non-labeled metabolites and congeners by gas chromatography*

Known concentrations of metabolites in the range 100–1000 ng were carried through the above extraction procedure and the final residue in counting vials dissolved in a suitable volume of methanol; an aliquot was injected into a Varian Aerograph Model 1400 gas chromatograph equipped with glass columns 6 ft. long packed with 3% SE-30 on Gas-Chrom Q and a flame ionisation detector. The oven temperature was 200°, the injector and detector temperatures were 240°, the carrier gas (helium) flow-rate was 40 ml/min, the hydrogen flow-rate 40 ml/min, and the air flow-rate 300 ml/min. The uncorrected retention times of these metabolites and congeners were approximately 4 min.

*Thin-layer chromatography*

ITLC sheets, obtained from Gelman Instrument Co. (Ann Arbor, Mich., U.S.A.), were used for TLC with the application of standard techniques. The compounds were located after development by spraying with iodoplatinate reagent.

## RESULTS

The results obtained on the *in vitro* recovery of *l*-α-[2-$^3$H]acetylmethadol, its metabolites and congeners in the concentration range 10–1000 ng are given in Table I. Quantitative recoveries of $^3$H-labeled LAAM were obtained by extraction with ethyl acetate at pH 8. Lowering of the pH to 6 or washing of the organic phase with 0.01 *N* HCl led to much lower recoveries. Various other solvents, such as cyclohexane, benzene, and ethylene dichloride, were tried to determine if greater specificity

TABLE I

*IN VITRO* MEAN PERCENTAGE RECOVERIES OF *l*-α-[2-$^3$H]ACETYLMETHADOL AND ITS NON-LABELED METABOLITES AND CONGENERS FROM BIOLOGICAL MATERIALS

The mean percentage recoveries were obtained in the concentration range 10–1000 ng using a liquid scintillation counting technique for *l*-α-[2-$^3$H]acetylmethadol and a gas chromatographic technique for the estimation of non-labeled metabolites and congeners (100–1000 ng).

| *Compounds* | *Mean percentage recoveries with different solvents at pH 8* | | | |
|---|---|---|---|---|
| | *Ethyl acetate* | *Ethylene dichloride* | *Cyclohexane* | *Benzene* |
| *l*-α-[2-$^3$H]Acetylmethadol | 100 | 100 | 98 | 96 |
| (−)-*l*-α-Acetylbisnormethadol | 4 | 9 | 8 | 5 |
| (−)-*l*-α-Methadol | 100 | 43 | 57 | 26 |
| (−)-*l*-α-Normethadol | 65 | 49 | 70 | 44 |
| (−)-*l*-α-Acetylnormethadol | 95 | 73 | 79 | 62 |

could be achieved. However, these solvents also extracted, at pH 8, varying amounts of metabolites and congeners along with quantitative recovery of $^3$H-labeled LAAM.

Thin-layer chromatographic experiments (on ITLC sheets) were undertaken to effect separation of these metabolites from LAAM and the results are given in Table II. Solvent systems $S_1$ and $S_2$ separated all these compounds from slower-moving glucuronide conjugated *in vivo* metabolites. Solvent system $S_3$ separated LAAM from (−)-*l*-α-acetylbisnormethadol (II), (−)-*l*-α-acetylnormethadol (V) and (−)-*l*-α-normethadol (IV). Solvent systems $S_4$, $S_5$ and $S_6$ effected good separation of these three compounds. Solvent systems $S_7$ and $S_8$ gave good separation of LAAM and (−)-*l*-α-methadol (III), which were difficult to separate in other systems. Combinations of these solvent systems, therefore, would adequately separate LAAM, its metabolites and congeners.

TABLE II

CHROMATOGRAPHIC MOBILITIES ON GELMAN ITLC (SHEETS) OF *l*-α-ACETYLMETHADOL, ITS METABOLITES AND CONGENERS IN DIFFERENT SOLVENT SYSTEMS

$S_1$ = *n*-butanol–acetic acid–water (35:3:10); $S_2$ = ethyl acetate–methanol–conc. ammonia (17:2:1); $S_3$ = *n*-hexane–ethyl acetate–conc. ammonia (85:15:0.1); $S_4$ = benzene–ethyl acetate–diethylamine (40:60:0.05); $S_5$ = *n*-hexane–ethyl acetate–ether–conc. ammonia (70:25:5:0.1); $S_6$ = benzene–ethyl acetate–methanol–conc. ammonia (80:20:1.2:0.1); $S_7$ = benzene–methanol–conc. ammonia (100:0.5:0.1); $S_8$ = benzene–ethyl acetate–methanol–conc. ammonia (80:20:0.5:0.1).

| *Compound* | $R_F \times 100$ | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | $S_1$ | $S_2$ | $S_3$ | $S_4$ | $S_5$ | $S_6$ | $S_7$ | $S_8$ |
| (−)-*l*-α-Acetylmethadol | Solvent front | Solvent front | 80 | 80 | 97 | Solvent front | 42 | 75 |
| (−)-*l*-α-Acetylbisnormethadol | Solvent front | Solvent front | 19 | 54 | 55 | 64 | 11 | 34 |
| (−)-*l*-α-Methadol | Solvent front | Solvent front | 64 | 70 | 84 | 98 | 27 | 62 |
| (−)-*l*-α-Normethadol | Solvent front | Solvent front | 7 | 29 | 15 | 41 | 8 | 16 |
| (−)-*l*-α-Acetylnormethadol | Solvent front | Solvent front | 11 | 40 | 25 | 54 | 10 | 22 |

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REFERENCES

1 K. K. Chen, *Ann. N.Y. Acad. Sci.*, 51 (1948) 83.
2 N. B. Eddy, E. L. May and E. Mosettig, *J. Org. Chem.*, 17 (1952) 320.
3 C. Y. Sung and E. L. Way, *J. Pharmacol. Exp. Ther.*, 110 (1954) 260.
4 H. F. Fraser and H. Isbell, *J. Pharmacol. Exp. Ther.*, 105 (1952) 458.
5 A. S. Keats and H. K. Beecher, *J. Pharmacol. Exp. Ther.*, 105 (1952) 210.
6 J. H. Jaffe, C. R. Schuster, B. B. Smith and P. H. Blachley, *J. Amer. Med. Ass.*, 211 (1970) 1834.
7 R. Levine, A. Zaks, M. Fink and A. M. Freedman, *J. Amer. Med. Ass.*, 226 (1973) 316.
8 R. E. McMahon, H. W. Culp and F. J. Marshall, *J. Pharmacol. Exp. Ther.*, 149 (1965) 436.
9 R. E. Billings, R. Booher, S. Smits, A. Pohland and R. E. McMahon, *J. Med. Chem.*, 16 (1973) 305.
10 R. E. Billings, R. E. McMahon and D. A. Blake, *Life Sci.*, 14 (1974) 1437.
11 R. Nickander, R. Booher and H. Miles, *Life Sci.*, 14 (1974) 2011.
12 A. L. Misra, S. J. Mulé, R. Bloch and N. L. Vadlamani, *J. Pharmacol. Exp. Ther.*, 185 (1973) 287.