Rapid TLC Determination of Methadyl Acetate and Some *In Vitro* Metabolites

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Abstract □ Methadyl acetate was metabolized by microsomal preparations of rat liver to yield nor-methadyl acetate and 6-(dimethylamino)-4,4-diphenyl-3-heptanol. The identification and separation of these three compounds was established by TLC, using iodoplatinate spray as a visualizing agent.

Keyphrases □ Methadyl acetate—TLC analysis, metabolism by rat liver microsomal preparations □ TLC—analysis and separation of methadyl acetate from metabolites, rat liver microsomes □ Analgesics—methadyl acetate, TLC analysis and separation from metabolites, rat liver microsomes

Methadyl acetate, a potent, long-acting, narcotic analgesic (1), is currently under clinical evaluation for the treatment of opiate addiction (2, 3). Its usefulness as a substitute for methadone prompted the development of a simple, accurate, and sensitive method for its identification using TLC.

Few methods to identify methadyl acetate and its metabolites have been reported (4, 5). Sung and Way (6) reported a colorimetric determination from tissue homogenates. Kaiko and Inturrisi (7, 8) reported the detection of methadyl acetate [6-(dimethylamino)-4,4-diphenyl-3-heptanol acetate], 6-(methylamino)-4,4-diphenyl-3-heptanol acetate (nor-methadyl acetate), 6-(dimethylamino)-4,4-diphenyl-3-heptanol, and 6-(methylamino)-4,4-diphenyl-3-heptanol by GLC using 3% SE-30 on Gas Chrom Q, 3.8% W-98 on Diatoport S, 5% OV-17 on Gas Chrom Q, 4% XF-1112 on Chromosorb W, 3% XE-60 on Gas Chrom Q, 3% 8BP on Gas Chrom Q, and 3% OV-225 on Gas Chrom Q. Billings and McMahon (1) identified the trichloroacetamide derivative of nor-methadyl acetate using GLC with an electron-capture detector.

This paper describes a highly sensitive TLC method for the detection of methadyl acetate, 6-(dimethylamino)-4,4-diphenyl-3-heptanol, and nor-methadyl acetate.

EXPERIMENTAL[1]

Male Sprague-Dawley rats were decapitated, and the livers were removed and immediately homogenized in two volumes of a cold tromethamine–hydrochloric acid–potassium chloride solution (0.05 *M* tromethamine buffer, pH 7.4, and 1.15% KCl) in a Teflon glass homogenizer. The homogenate was centrifuged for 20 min at 9000×*g* in a refrigerated centrifuge[2]. The supernate was then centrifuged for 1 hr at 78,000×*g* in a ultracentrifuge[3]. The microsomal pellet was resuspended in the same buffer to a volume of 10 ml.

Protein was determined by the biuret method described by Gornall *et al.* (9), and the microsomes were diluted to a concentration of 10 mg/ml. Metabolism of methadyl acetate was determined in a 3.0-ml incubation mixture consisting of 5 m*M* magnesium chloride, 12 m*M* glucose-6-phosphate dehydrogenase, 0.33 m*M* nicotinamide adenine dinucleotide phosphate (NADP), 50 m*M* tromethamine buffer (pH 7.4), 10 mg of microsomal protein, and 5 m*M* methadyl acetate. The mixture was incubated at 37° for 90 min. *N*-Demethylation of methadyl acetate was estimated by measuring the amount of formaldehyde formed by the method of Nash (10). After 90 min, the reaction was quenched by adding 1 *N* HCl. The mixture was centrifuged, and the supernate was removed.

The acidic supernate containing metabolites and unreacted methadyl acetate was adjusted to pH 11.5 with 1 *N* NaOH. The alkaline solution was extracted five times with 25-ml portions of ether. The ether layers were combined, dried over anhydrous magnesium sulfate, filtered, and evaporated under reduced pressure to 10 ml.

Three different types of precoated plates, instant thin-layer chromatography (ITLC) SG[4], LQD[5], and LQ6D[5], were used. The plate was spotted with approximately 5 μl of the ether solution concentrate previously prepared. The ITLC SG plates were then developed in a developing tank[4] containing ethyl acetate–cyclohexane–ammonia (50:40:0.1) (Solvent System A). The LQD and LQ6D plates were developed in ethyl acetate–methanol–water–ammonia (85:13:1:1) (Solvent System B).

The development requires 15 min for the ITLC plates and 45 min for the LQD and LQ6D plates. To visualize the spot, the LQD and LQ6D plates were dried and sprayed with iodoplatinate reagent (11) and the ITLC SG plates were dried and sprayed with iodine.

Table I—R_f Values and Color Reactions for Methadyl Acetate, Nor-methadyl Acetate, 6-(Dimethylamino)-4,4-diphenyl-3-heptanol, and Methadone Using ITLC SG Plates[a]

Compound	R_f	Color
Methadyl acetate	0.90	Brown
Nor-methadyl acetate	0.53	Light brown
6-(Dimethylamino)-4,4-diphenyl-3-heptanol	0.92	Dark brown
Methadone	0.72	Light yellowish brown

[a] Solvent System A was used, the spray reagent was iodine, and the sensitivity was 0.1 μg.

RESULTS AND DISCUSSION

In vitro metabolic studies indicated that approximately 0.05 μmole of formaldehyde was produced during the 90 min of incubation. A total of 15 μmoles of methadyl acetate was present, so approximately 0.3% was demethylated to form nor-methadyl acetate. However, three spots were observed on each of the three precoated plates (Tables I–III), indicating the presence of three compounds. These compounds were identified as nor-methadyl acetate, 6-(dimethylamino)-4,4-diphenyl-3-heptanol, and methadyl acetate by comparisons with authentic samples. These results were confirmed by GLC.

The R_f values and color reactions of each spot were compared with the pure compounds methadyl acetate, nor-methadyl acetate, 6-(dimethylamino)-4,4-diphenyl-3-heptanol, and methadone. The pure compounds were spotted on the same TLC plates along with *in vitro* metabolites. The nor-methadyl acetate and 6-(dimethyl-

[1] Methadyl acetate was obtained from the National Institute on Drug Abuse, Rockville, Md. Nor-methadyl acetate was supplied by Eli Lilly and Co., Indianapolis, Ind.

[2] Model RC-2, Ivan Sorvall Inc., Norfolk, CT 06470

[3] Model L3-50, Beckman Inc., Scientific Instrument Division, Fullerton, CA 92634

[4] Gelman Instrument Co., Ann Arbor, Mich.

[5] Quatum Assays Corp., Fairfield, NJ 07006

Table II—R_f Values and Color Reactions for Methadyl Acetate, Nor-methadyl Acetate, 6-(Dimethylamino)-4,4-diphenyl-3-heptanol, and Methadone Using LQD Plates[a]

Compound	R_f	Color
Methadyl acetate	0.75	Pinkish purple
Nor-methadyl acetate	0.64	Pinkish purple
6-(Dimethylamino)-4,4-diphenyl-3-heptanol	0.72	Purple
Methadone	0.68	Pinkish purple

[a] Solvent System B was used, the spray reagent was iodoplatinate, and the sensitivity was 0.1 μg.

Table III—R_f Values and Color Reactions for Methadyl Acetate, Nor-methadyl Acetate, 6-(Dimethylamino)-4,4-diphenyl-3-heptanol, and Methadone Using LQ6D Plates[a]

Compound	R_f	Color
Methadyl acetate	0.75	Pinkish purple
Nor-methadyl acetate	0.40	Pinkish purple
6-(Dimethylamino)-4,4-diphenyl-3-heptanol	0.68	Purple
Methadone	0.62	Pinkish purple[b]

[a] Solvent System B was used, the spray reagent was iodoplatinate, and the sensitivity was 0.1 μg. [b] Pinkish purple changed to brownish purple after a few minutes.

amino)-4,4-diphenyl-3-heptanol are the same metabolites found in human urine (12).

The identity of methadyl acetate, nor-methadyl acetate, and 6-(dimethylamino)-4,4-diphenyl-3-heptanol was confirmed by GLC, using 3% OV-1 on Shimalite W, 80–100 mesh[6]. The column temperature was 230°, and helium was the carrier gas (60-ml/min flow rate). The retention times for methadyl acetate, nor-methadyl acetate, 6-(dimethylamino)-4,4-diphenyl-3-heptanol, and methadone were 5.0, 4.8, 5.8, and 4.4 min, respectively. Methadone, a structurally related compound, was included for comparison because it is used along with methadyl acetate in opiate addicts.

Of the three different TLC plates used, LQ6D was the best in separating the methadyl acetate, nor-methadyl acetate, 6-(dimethylamino)-4,4-diphenyl-3-heptanol, and methadone. Iodoplatinate proved to be a better spraying reagent than iodine.

[6] American Instrument Co., Atlanta, Ga.

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High-Speed Liquid Chromatographic Determination of Canrenone in Pharmaceutical Dosage Forms

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Abstract □ Canrenone can be determined by high-speed liquid chromatography in pharmaceutical dosage forms without interference from common excipients or degradation products. This stability-indicating assay, using *o*-nitroaniline as the internal standard, is rapid and accurate.

Keyphrases □ Canrenone—analysis, high-speed liquid chromatography, pharmaceutical dosage forms ■ High-speed liquid chromatography—analysis, canrenone, pharmaceutical dosage forms

Canrenone (I), 17-hydroxy-3-oxo-17α-pregna-4,6-diene-21-carboxylic acid γ-lactone, is a steroid that is an aldosterone antagonist and diuretic (1). Its synthesis was reported previously (2, 3).

Previous methods of analysis in biological fluids involved GC and fluorometric analyses (4–6). The fluorometric analysis involved the conversion of canrenone, a dienone, to a trienone using sulfuric acid (62% v/v). Since the major acid degradation product of canrenone is a trienone (II), this procedure could not be used as a stability-indicating assay for pharmaceutical dosage forms. Attempts by this author to use GC were not successful.

Recently, high-speed liquid chromatography

Table I—Standard Additions

Milligrams of Canrenone Added beyond 50 mg/25 μl	Found, mg/25 ml	Total Recovery, %
0.00	97.3	100.0
31.02	128.5	100.1
54.34	151.2	99.7