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METHOD FOR THE DETECTION OF DIETHYLAMINE, A METABOLITE OF DISULFIRAM, IN URINE

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SUMMARY

Disulfiram is a drug used in the treatment of chronic alcoholism in man. Accurate assessment of patient compliance is important in this treatment. This paper describes a method for the detection and quantitative analysis of diethylamine, a metabolite of disulfiram, in urine. The method involves conversion of the water-soluble diethylamine in the urine to a derivative, N,N-diethyl-3,5-dinitrobenzamide, that is soluble in an organic solvent. This derivative is extracted from urine with diethyl ether and then subjected to thin-layer chromatography. A spectrophotometric procedure is used for quantification. This method provides a means of determining whether or not a patient is taking his prescribed disulfiram.

INTRODUCTION

Disulfiram (tetraethylthiuramdisulfide, Antabuse®) is given for the treatment of chronic alcoholism. This drug acts as a deterrent to drinking because a patient will develop nausea, vomiting, abdominal pain and decreased arterial blood pressure if he drinks within three to seven days after taking this drug[1]. Because alcoholic patients are not always reliable in reporting whether they are taking the drug or not, it is important for physicians taking care of such patients to have a method for determining if the disulfiram has been taken. After oral administration of disulfiram, large quantities of diethyldithiocarbamate, a metabolite, are excreted in the urine[2]. Tests for disulfiram have depended upon the detection of this metabolite in the urine[3,4]. However, diethyldithiocarbamate rapidly disintegrates in urine, particularly when the urine is acid, and thus these tests have not proven satisfactory for clinical purposes[4]. The breakdown products of diethyldithiocarbamate are diethylamine and carbon disulfide[4]. Diethylamine is not normally present in urine[5]. Therefore, a method for the determination of the presence of diethylamine in urine could provide a means of determining whether a patient has been taking his prescribed disulfiram. Methods for the determination of the presence of a secondary amine such as diethylamine are available[6]. However, most methods require that the diethylamine be in relatively pure form

prior to analysis and thus these methods cannot be directly applied to urine. Further, diethylamine is water soluble and cannot be quantitatively extracted from urine.

The present paper reports a new method for the detection of diethylamine in urine. This method involves conversion of the diethylamine in the urine to a derivative, N,N-diethyl-3,5-dinitrobenzamide, that is soluble in an organic solvent. This derivative is readily extracted with diethyl ether and is then specifically identified by thin-layer chromatography (TLC).

EXPERIMENTAL

Standard 3,5-dinitrobenzamides

Standard 3,5-dinitrobenzamides were prepared by a standard Schotten–Baumann technique. Two milliliters of the amine were mixed with 0.5 g 3,5-dinitrobenzoyl chloride (Matheson, Coleman and Bell, Norwood, Ohio, U.S.A.). The mixture was heated gently for 5 min. Ten milliliters of water were then added and the precipitate collected on a filter, washed with 2% sodium carbonate and then washed with water. The derivative was then recrystallized twice from aqueous ethanol. The standard N,N-diethyl-3,5-dinitrobenzamide had a melting point of 91–93°.

Samples of urine for analysis of diethylamine

Samples of urine were collected and made acid with acetic acid to a pH of less than pH 4.0. The acidified urine was stored at 4° overnight. To analyze for diethylamine, 2 ml of urine were mixed in a 125-ml separatory funnel with 15 ml diethyl ether (No. 0804, Mallinckrodt, St. Louis, Mo., U.S.A.), 0.2 ml of pyridine, 2 ml of 5% potassium carbonate, and 0.5 ml of 3,5-dinitrobenzoyl chloride in diethyl ether (0.25 g/ml). The mixture was shaken at intervals for 20 min.

The aqueous layer was then removed and discarded. The ether layer, which contained the 3,5-dinitrobenzamide, was then washed twice with 5 ml of 5% sodium carbonate, twice with 5 ml of 5% hydrochloric acid, and then twice with 5 ml of distilled water. The ether layer was dried over anhydrous sodium sulfate and then the ether was removed by evaporation with a stream of air and with warming.

Thin-layer chromatography

The residue from benzoylation of standard or unknown amines in urine was dissolved in 0.5 ml of chloroform–methanol (1:1). An aliquot, 0.1 ml, was spotted on a precoated silica gel G TLC plate (Analtech, Wilmington, Del., U.S.A.). The TLC plate was then developed with chloroform–methanol (99:1) for 1 to 1½ h.

After developing, the plate was air dried and the spots were visualized by exposing the plate overnight to iodine vapor.

Quantitative determination of diethylamine in urine

After exposing the TLC plate to iodine vapor for 5–10 min, the N,N-diethyl-3,5-dinitrobenzamide spots were identified and outlined. The iodine was then removed by allowing the plate to remain at room temperature and atmosphere overnight. The next day, the spots containing the diethylamine derivative and a corresponding blank silica gel spot and a blank urine spot were scraped into 5-ml centrifuge tubes. Three milliliters of methanol were added and the tubes were heated at 40° for 5 min with

mixing to dissolve the N,N-diethyl-3,5-dinitrobenzamide. The silica gel was then removed by centrifugation at 500 *g* for 20 min. The clear supernates were assayed in a Beckman DU spectrophotometer at 300 nm. The molar absorptivity of standard N,N-diethyl-3,5-dinitrobenzamide at 300 nm in methanol was found to be 1256.

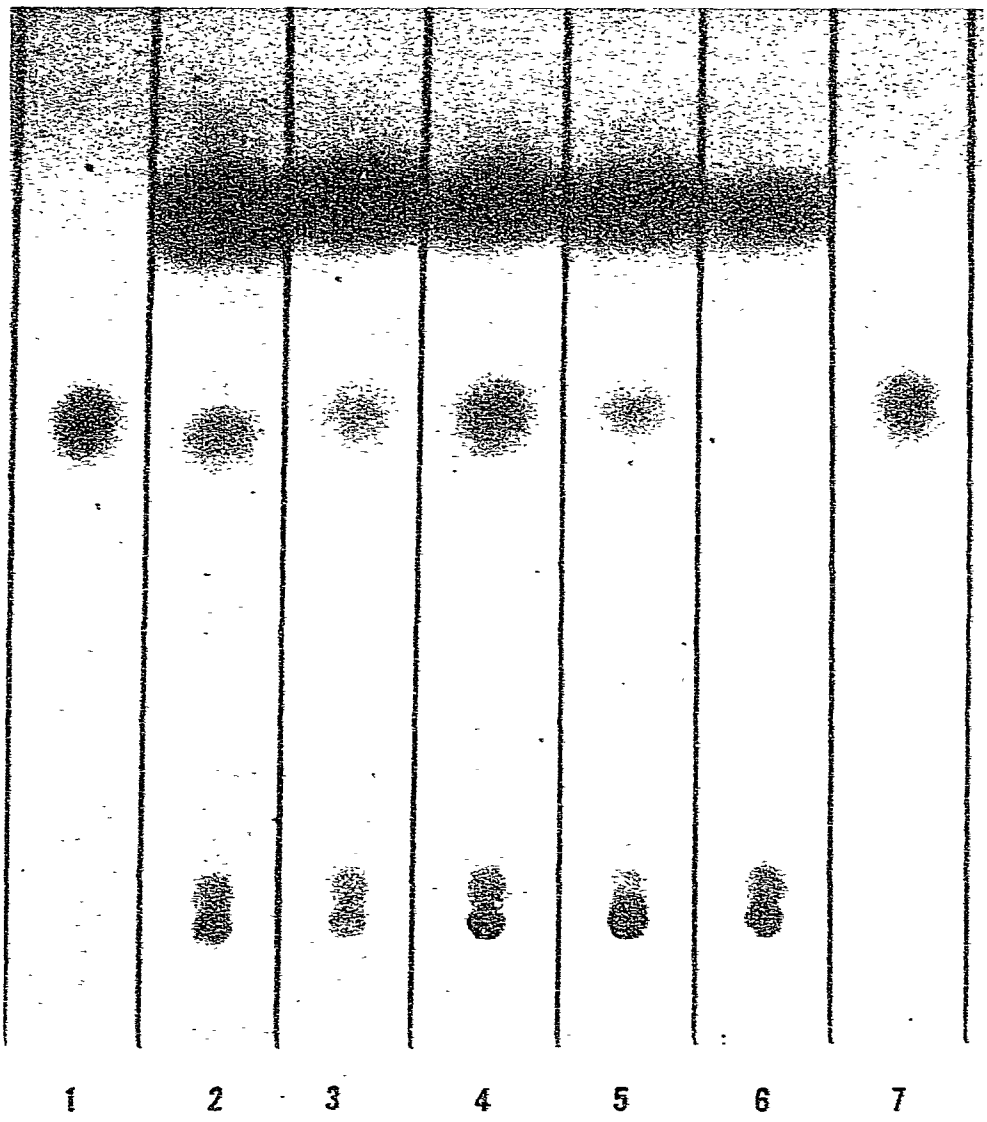

Fig. 1. TLC pattern of patient urines. On lanes 1 and 7, standard N,N-diethyl-3,5-dinitrobenzamide, 50 μg, was chromatographed. On lanes 2 through 6, aliquots of the extract from benzoylation of urines were chromatographed as described in Methods. For lanes 2 and 3, 120 and 160 μg, respectively, of diethyldithiocarbamate were added to 2 ml of urine and for lane 4, 71 μg of diethylamine were added to 2 ml of urine. Lane 5 is from urine of a patient known to be taking disulfiram and lane 6 is from a control urine of a patient not taking disulfiram.

RESULTS AND DISCUSSION

The TLC pattern observed in urine containing diethylamine and related substances is shown in Fig. 1. Chloroform–methanol (99:1) was used as the solvent for developing the chromatogram. The diethylamine and diethyldithiocarbamate were added to urine and the mixtures were allowed to remain overnight in acid pH prior to this TLC study. On lanes 1 and 7, 50 μg of standard N,N-diethyl-3,5-dinitrobenzamide were chromatographed. After benzoylation, the derivative was present in the urines to which diethyldithiocarbamate (lanes 2 and 3) and diethylamine (lane 4) had

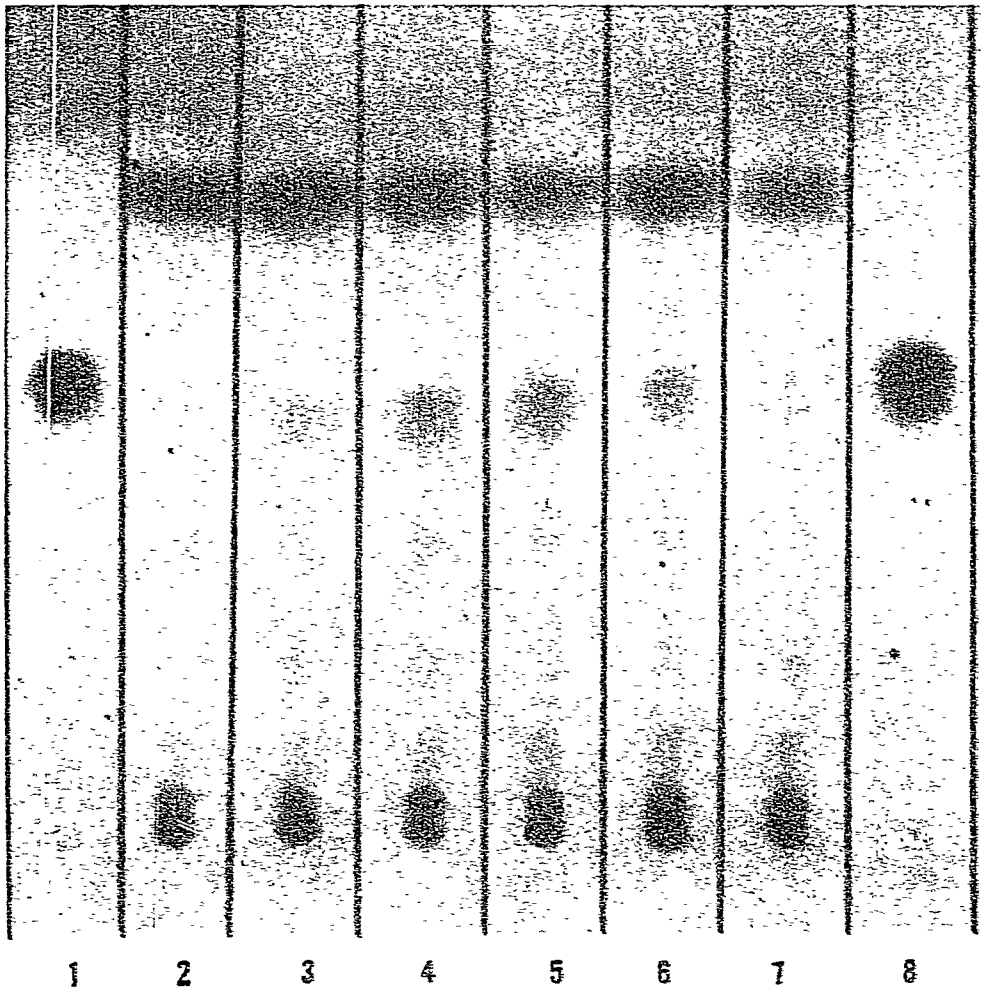

Fig. 2. Excretion pattern of diethylamine in the urine from a patient given 500 mg disulfiram. On lanes 1 and 8, standard N,N-diethyl-3,5-dinitrobenzamide, 50 μg, was chromatographed. Lane 2 is from a urine sample collected at 9:00 a.m., when the drug was prescribed. Lanes 3, 4, 5, and 6 are from urine samples collected 2, 4, 12, and 20 h after administration of the drug, and lane 7 is from a urine sample collected 24 h after administration of the drug.

TABLE I

QUANTITATIVE DETERMINATION OF DIETHYLAMINE IN URINE

Compound added to urine	*N,N-diethyl-3,5-dinitrobenzamide*	
	*Theory**	*Found***
None	0.00	0.02–0.19
Diethylamine	0.41	0.48
	0.82	0.99
	1.64	1.36
	2.06	2.00
	4.11	4.11
Diethyldithiocarbamate***	2.96	2.66
	5.91	5.10

* Quantity in μmoles of compound added to urine.

** Quantity in μmole of N,N-diethyl-3,5-dinitrobenzamide found after benzoylation and quantitative analysis at 300 nm.

*** The diethyldithiocarbamate was added to acid urine and allowed to remain at 4° overnight prior to benzoylation. Such conditions convert diethyldithiocarbamate to diethylamine and carbon disulfide.

been added. For lanes 2 and 3, 120 and 160 μg, respectively, of diethyldithiocarbamate were added to 2 ml of urine and for lane 4, 71 μg of diethylamine were added to 2 ml of urine. Urine taken from a patient known to have been taking disulfiram was also tested and diethylamine was found (lane 5). A control urine taken from a patient not taking disulfiram showed no spot for diethylamine (lane 6) after benzoylation.

To test the excretion pattern of diethylamine after administration of disulfiram, volunteers were given a single dose of 500 mg disulfiram. The urines were then collected at intervals for 48 h. A representative excretion pattern of diethylamine is given in Fig. 2. The standard N,N-diethyl-3,5-dinitrobenzamide, 50 μg, was spotted on lanes 1 and 8. The patient was given the disulfiram at 9:00 a.m., at which time a urine was collected. Diethylamine was not present (Fig. 2, lane 2). Two hours after administration of the disulfiram (lane 3) a positive spot for diethylamine was obtained. Positive spots were observed at 4 h (lane 4), 12 h (lane 5), and 20 h (lane 6). A slight positive spot was observed at 24 h (lane 7) and the urine was negative 48 h later.

This report describes a method for the detection of the secondary amine, diethylamine, a metabolite of disulfiram in the urine of patients. Tertiary amines do not react with 3,5-dinitrobenzoylchloride and thus, trimethylamine which is present in normal human urine[5] would not cause problems by producing a false positive reaction. Primary amines are also present in human urine and they do react with 3,5-dinitrobenzoyl chloride, however, their benzamide derivatives are water soluble and are removed in the washing procedure used in the method reported here. Thus, only secondary amines will be isolated by this method. Further, derivatives of secondary amines other than diethylamine (R_F value of 0.55) have different R_F values[7]. Thus, this method will specifically identify the presence of diethylamine in urine.

The N,N-diethyl-3,5-dinitrobenzamide in urine can readily be quantitated, as shown in Table I. With our method, more than 90% of diethylamine added to urine is recovered as the derivative. The recoveries of diethyldithiocarbamate as the derivative were somewhat lower but were greater than 80%. Analysis of representative

urine samples from patients known to be receiving disulfiram showed 0.9 and 2.0 μmoles of diethylamine per 2 ml of urine.

This test offers a means of determining whether patients who have been prescribed disulfiram are taking their medication. During the course of our experiments, Kraml[8] described a test for disulfiram ingestion which utilized the detection of carbon disulfide, the other metabolite of this drug[4] in the breath. This method, like ours, offers a rapid test for disulfiram ingestion, however, sources of false positive results remain to be determined.

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