Journal of Chromatography, 229 (1982) 470—474
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 1215

Note

High-performance liquid chromatographic method for the determination of diethylamine, a metabolite of disulfiram, in urine*

DEWEY H. NEIDERHISER* and RICHARD K. FULLER

Medical Research Service, Cleveland VA Medical Center and the Department of Medicine, Case Western Reserve University School of Medicine, 10701 East Boulevard, Cleveland, OH 44106 (U.S.A.)

(First received October 20th, 1981; revised manuscript received December 21st, 1981)

Disulfiram (tetraethylthiuram disulfide, Antabuse®, Ayerst Labs., New York, NY, U.S.A.) is a drug used in the treatment of alcoholism in man [1]. Methods to measure compliance to a disulfiram regimen are important for two reasons: (1) in clinical practice to know if patients prescribed the drug are actually taking it, and (2) to determine the extent of compliance in clinical trials evaluating the efficacy of disulfiram. Disulfiram, after ingestion and absorption, is rapidly metabolized and excreted in the form of carbon disulfide, diethylamine and esters of diethyldithiocarbamate [2, 3]. The former is excreted in expired air and the latter two are excreted in urine [3, 4]. In previous studies, we found that after ingestion of disulfiram labelled with [1-^{14}C]diethylamine, 87% of the radioactivity was excreted in the urine and that a major portion of this radioactivity was in the form of [^{14}C]diethylamine [2]. We have been interested in the determination of diethylamine as a measure of disulfiram intake because diethylamine is not normally present in urine [5] but appears in increased concentrations after ingestion of disulfiram [2]. A thin-layer chromatographic (TLC) method was developed to measure diethylamine in urine [6] and this method has been used to measure compliance to a disulfiram regimen [7].

Although the TLC screening procedure proved to be a rapid and useful method for testing whether a patient was taking disulfiram regularly, we had some urine specimens which gave faint or questionable positive values for diethyl-

*This work was presented in part at the Medical-Scientific Conference of the National Alcoholism Forum, New Orleans, LA, April 12—15, 1981. An abstract appeared in Alcoholism: Clin. Exp. Res., 5 (1981) 163.

0378-4347/82/0000—0000/$02.75

amine. It was not possible to be certain whether these specimens were from patients who were still taking disulfiram but analyzed at a time when excretion of the drug was at a minimum or were from patients who were no longer taking the drug. We also, on occasion, found a few specimens from hospitalized patients who were known not to be taking disulfiram that gave faintly positive results. This necessitated that a reliable confirmational procedure be developed that could be used in combination with the TLC screening procedure to determine diethylamine in urine. This report presents a new high-performance liquid chromatographic (HPLC) method for the detection and quantitative analysis of diethylamine in urine.

MATERIALS AND METHODS

Derivatization of diethylamine

Diethylamine was purchased from Fisher Scientific (Pittsburgh, PA, U.S.A.) and ethylpropylamine, the internal standard, from The Ames Laboratory (Milford, CT, U.S.A.). The standard 3,5-dinitrobenzamide derivatives were prepared and purified as previously described [6]. Diethylamine and/or ethylpropylamine added to urine were converted to the benzamide derivative in the urine and extracted with diethyl ether [6, 7]. As previously reported, the efficiency of this derivatization is greater than 90% [6].

Processing of urine specimens

For analysis of unknown concentrations of diethylamine, 530 nmol of ethylpropylamine, the internal standard, were added per ml of urine prior to processing. The recovery experiments were performed with urine specimens from volunteers known not to be taking disulfiram. The urine specimens from a volunteer given disulfiram (250 mg) orally were collected and made acid with glacial acetic acid to a pH of less than pH 4.0. They were stored frozen (−10°C) until analysis. After thawing, the specimens were adjusted to pH 7.0 with 10 *N* sodium hydroxide. In all studies, 1 ml of urine was used for preparation of the benzamide derivative.

HPLC procedure

The HPLC separations were carried out on a Waters liquid chromatograph equipped with a Model 440 absorbance detector and a Model 6000A solvent delivery system. The residue from the benzoylation of urine was dissolved in ethanol (usually 0.5 ml) and a 15-μl aliquot was analyzed on a μBondapak C_{18} column (10 μm, 30 cm × 3.9 mm I.D.) with a mobile phase containing methanol—water (55:45, v/v) at a flow-rate of 2 ml/min. All chromatograms were run at ambient temperature. The column effluent was monitored by absorbance at 254 nm at a sensitivity of 0.5 a.u.f.s. Calibration curves and quantitation were determined using peak areas.

TLC procedure

The residue from the benzoylation of urine with no added internal standard was dissolved in chloroform (usually 0.5 ml). A 50-μl aliquot was applied to a TLC plate precoated with silica gel G, and containing a 254-nm phosphor

(Kontes, Vineland, NJ, U.S.A.). The plates were developed with chloroform—ethanol (99:1, v/v) and the resulting spots were visualized under short-wave ultraviolet light [6].

RESULTS

A chromatogram illustrating the separation of the benzamide derivatives of diethylamine and ethylpropylamine, the internal standard, is given in Fig. 1A. The benzamide derivatives of these amines and other secondary amines tested

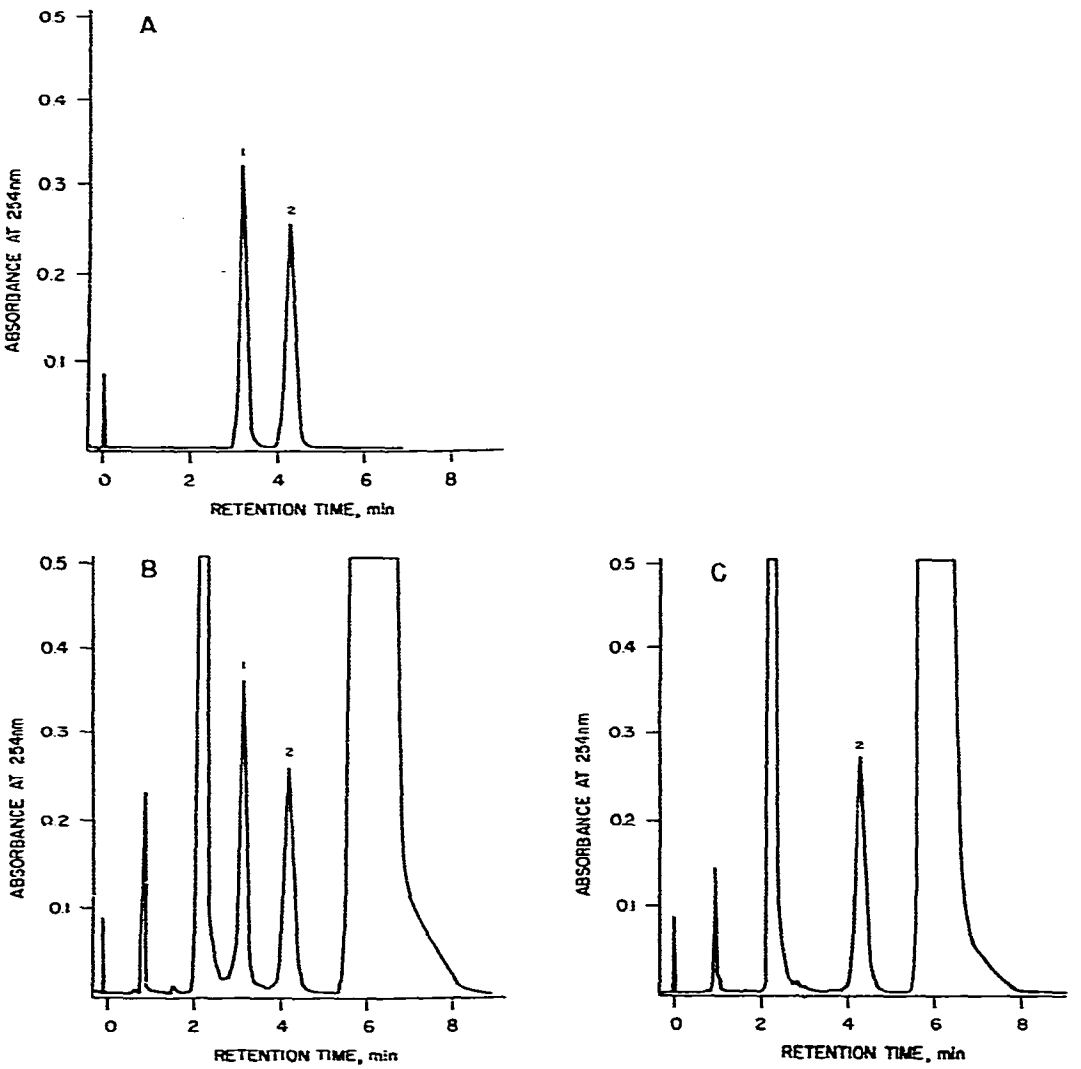

Fig. 1. Chromatograms of (A) standard N,N-diethyl-3,5-dinitrobenzamide (1) and the internal standard, N,N-ethylpropyl-3,5-dinitrobenzamide (2), (B) representative urine from a patient prescribed disulfiram to which the internal standard had been added, and (C) representative urine from a control patient not prescribed disulfiram to which the internal standard had been added.

but not present in the figure had the following retention times: N,N-dimethyl 2.3 min, N,N-diethyl 3.2 min, N,N-ethylpropyl 4.3 min, N,N-dipropyl 5.8 min, and N,N-dibutyl 16.0 min. The benzamide derivative of norpropoxyphene, the metabolite of Darvon which has previously been reported to give a spot by the TLC screening procedure that was near that of diethylamine [7], had a retention time of 2.6 min. Our derivatization is selective for secondary amines such as diethylamine; primary amines are removed by a washing procedure while tertiary amines do not react with the reagent [6].

In Fig. 1B, a representative chromatogram from urine of a patient known to be taking disulfiram is given. The peaks from the point of injection to 2.4 min and the one at 6.4 min are by-products of the benzoylation reaction and are found in all samples. The peak area for diethylamine was equivalent to 620 nmol of diethylamine per ml of urine. Control urine from a patient not taking disulfiram (Fig. 1C) does not give a response at the retention time for the derivative of diethylamine.

The calibration curves for the diethylamine and ethylpropylamine derivatives were determined. Linearity was observed between 1 and 20 nmol (r = 0.998 and 0.999, respectively). This response would be observed for concentrations of diethylamine ranging from 22 to 670 nmol per ml of urine. The limit of detection for diethylamine was 10 nmol/ml urine. We analyzed urine to which known quantities of diethylamine and the internal standard were added and we observed quantitative recovery of the diethylamine as the benzamide derivative (Table I).

TABLE I

THE RECOVERY OF DIETHYLAMINE ADDED TO URINE

Mean ± S.D., n = 4.

Added (nmol/ml)	Measured (nmol/ml)	Recovery from urine (%)
72.3	70.9 ± 7.3	98.0 ± 10.1
144.6	142.3 ± 26.0	98.4 ± 18.0
361.5	356.1 ± 56.5	98.5 ± 15.6
723.1	721.5 ± 9.3	99.8 ± 1.3

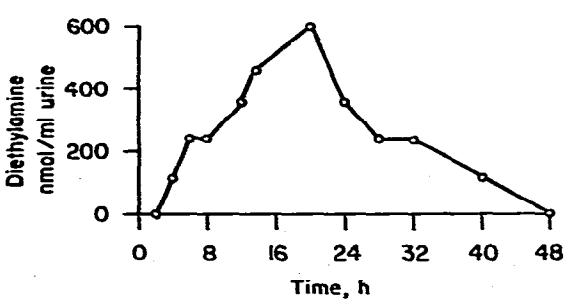

Fig. 2. Time course of urinary excretion of diethylamine in a human subject following oral administration of 250 mg disulfiram.

We also tested urine from a patient given a single dose of 250 mg disulfiram. Urine specimens were collected for 48 h after dosage. The diethylamine content of this urine is given in Fig. 2. No diethylamine was found 2 h after dosage. Values ranging from 40 to 600 nmol/ml urine were found 4 to 32 h after dosage and the urine was again negative at 48 h. When these same specimens were analyzed by our TLC screening procedure, the 6- through 24-h specimens were judged positive, the 4-, 28- and 32-h specimens were faintly positive or questionable, and the 2- and 48-h specimens were negative. When the same patient was given 250 mg disulfiram daily for 4 days and the urine was then analyzed by HPLC for diethylamine after the last dose, the 0- and 2-h specimens were also positive as well as the other specimens up to 96 h after dosage.

DISCUSSION

The present HPLC procedure provides an improved reliable method for the detection of diethylamine, a metabolite of disulfiram, in urine. This method allows both the detection and the quantitation of diethylamine in the urine of patients receiving disulfiram. The urinary concentration of diethylamine depends on the time of collection of the specimen after ingestion of disulfiram. Our method can reliably detect concentrations of diethylamine as low as 22 nmol/ml urine. This enables the detection of diethylamine up to 48 h after a single dose of disulfiram and 96 h after multiple doses. Because specimens that are collected from patients during a period when diethylamine excretion is minimal will be correctly identified as containing diethylamine by HPLC, this method can separate urine specimens which appear questionably or faintly positive by TLC into true positives or true negatives. The procedure when combined with the TLC screening assay provides an accurate method for determining compliance to a disulfiram regimen.

ACKNOWLEDGEMENTS

This research was supported by funds from the Medical Research Service of the Veterans Administration.

REFERENCES

1 R. Fox, Alcoholism: Behavioral Research, Therapeutic Approaches, Springer, New York, 1967, p. 242.
2 D.H. Neiderhiser and R.K. Fuller, Alcohol. Clin. Exp. Res., 4 (1980) 277.
3 D.I. Eneanya, J.R. Bianchine, D.O. Duran and B.D. Andresen, Ann. Rev. Pharmacol. Toxicol., 21 (1981) 575.
4 H. Linderholm and K. Berg, Scan. J. Clin. Lab. Invest., 3 (1951) 96.
5 C. Long (Editor), Biochemists Handbook, Van Nostrand, Princeton, NJ, 1961, p. 926.
6 D.H. Neiderhiser, R.K. Fuller, L.J. Hejduk and H.P. Roth, J. Chromatogr., 117 (1976) 187.
7 R.K. Fuller and D.H. Neiderhiser, J. Stud. Alcohol, 42 (1981) 202.