GLC Determination of Methadone in Human Urine

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Abstract □ Human urine is buffered with an equal volume of pH 9.3 borate buffer and extracted with a chloroform–isopropanol (3:1) solution. After the extract is concentrated to dryness, the resultant residue is dissolved in methanol containing 4-benzylbiphenyl as an internal standard. The solution is assayed for methadone content by GLC. This method was evaluated for concentrations of methadone ranging from 3 to 30 mcg./ml. in human urine and gave an overall precision and accuracy of ±7% (*RSD* and *RE*).

Keyphrases □ Methadone in human urine—quantitative GLC determination □ GLC—quantitative determination of methadone in human urine

Methadone is now widely used in rehabilitation programs for heroin addicts. The need for a simple assay method suitable for the determination of methadone levels in urines of patients receiving a new liquid methadone formulation prompted this investigation. Previous assay procedures (1–5) for methadone in animal or human urine primarily utilized the formation of a dye complex and subsequent spectrophotometric determination. These methods lacked specificity for methadone and were general assays which did not distinguish between methadone, its metabolites, and other basic compounds in urine.

Methods have appeared (6, 7) which are suitable for qualitative GLC determination of methadone in urine. Beckett *et al.* (8) used GLC to identify methadone and a metabolite[1] in human urine. Pohland *et al.* (9) reported the results of a GLC urine assay for methadone and its metabolites in a 24-hr. human urine sample, but they gave no indication of how the method was evaluated. In this laboratory, extraction of urine by this method produced emulsions in the organic phase which dissipated only after standing for an extended time. While this work was in progress, Beckett *et al.* (10) reported a quantitative assay method for methadone and its metabolites in microsomal homogenates, although no adaptation to urine was given.

In the present method, equal volumes of urine and pH 9.3 borate buffer are mixed together and extracted with chloroform–isopropanol (3:1). [A report appeared (11) after this work was completed which used the same buffer and extraction procedure for the qualitative determination of methadone and morphine in human urine.] The extract is concentrated to dryness, the resultant residue is dissolved in a small amount of methanol containing 4-benzylbiphenyl (the internal standard), and the solution is analyzed by GLC. Emulsions were frequently encountered in the organic phase during the extraction of urine samples but were easily broken by filtering the organic phase through a fine sintered-glass funnel.

From previously reported studies (12, 13) with ^{14}C-methadone, the level of methadone expected in the urine was in the range of 5–30 mcg./ml. Thus, the present assay method was evaluated in that range. Furthermore, two known metabolites of methadone[2] in human urine were evaluated using the experimental GLC conditions and were found not to interfere with the determination of methadone.

Table I—Precision and Accuracy in Recovery of Methadone Added to Human Urine

Added, mcg./ml.	Found[a], mcg./ml.	*n*	*RSD*, % ±	*RE*, %
3.0	2.95 (2.7–3.2)	2	11.99	−1.67
4.0	3.66 (3.0–4.0)	7	10.17	−8.50
5.0	4.55 (4.1–4.7)	2	4.66	−9.00
6.0	5.26 (5.12–5.4)	2	3.76	−12.33
7.0	6.10 (6.10)	2	0.00	−12.86
8.0	7.78 (7.2–8.3)	4	7.85	−2.75
10.0	9.40 (8.4–10.8)	4	11.34	−6.00
12.0	11.30 (10.2–12.0)	6	7.36	−5.83
15.0	14.25 (14.1–14.4)	2	1.49	−5.00
16.0	13.93 (13.1–14.4)	3	5.19	−12.94
20.0	18.95 (18.0–19.9)	2	7.09	−5.25
30.0	30.5 (30.0–31.0)	2	2.32	+1.67
Average percent recovered	93.2			

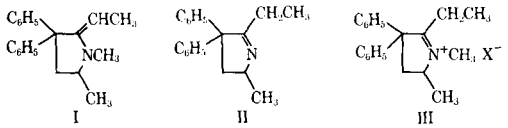
[a] Average of *n* different samples at each added concentration level (range).

EXPERIMENTAL

Extraction Procedure—A stock solution of pH 9.3 borate buffer was prepared according to the method of Dole *et al.* (14). Urine (100 ml.) was diluted with 100 ml. of the pH 9.3 borate buffer and mixed thoroughly by shaking. The buffered urine was extracted four times with 200 ml. of chloroform–isopropanol (3:1). Each extract was filtered through a fine sintered-glass funnel to break any emulsions which might form in the organic phase. The small amount of aqueous solution present in the pooled organic extracts was separated and extracted with 100 ml. of the chloroform–isopropanol solution which was used to wash the transferring flask. The pooled extracts were concentrated to dryness under reduced pressure at 70°, and the resultant residue was analyzed by GLC.

Gas Chromatographic Determination—All experiments were performed using a gas chromatograph[3] equipped with a hydrogen flame-ionization detector. The column employed was a 1.22-m. ×

[1] Two metabolites of methadone, I and II, were identified in human urine by Pohland *et al.* (9). Using GLC–mass spectroscopy, these authors demonstrated that I was a major metabolite. They also found that treatment of I with acid produced the endocyclic form III, which was reported by Beckett *et al.* (8).



I II III

[2] The authors thank Dr. Albert Pohland, The Lilly Research Laboratories, for making the metabolites available.

[3] Barber-Colman series 5000.

Table II—Assay Method Evaluation on Urines of Patients in a Methadone Maintenance Program

Patient Number	Methadone·HCl[a], mg.	Day	24-hr. Urine Volume, ml.	Methadone Found, mcg./ml.
1	35	1	405	3.0
		2	1100	4.3
2	70	1	540	10.0
		2	830	6.7
3[b]	90	1	1300	8.0
		2	620	15.0

[a] Dissolved in 90 ml. of Tang in water and given orally each day. [b] Also received 10 mg. of chlordiazepoxide hydrochloride.

5.5-mm. (i.d.) glass U-tube packed at the injector zone with siliconized glass wool. Column packing was 3% OV-17 on 100–200-mesh Gas Chrom Q. The column operating condition was at 240° with a nitrogen flow rate of 50 ml./min. The injector was operated at 250° and the detector at 250° with 3.5 kg./cm.^{2} air and 1.4 kg./cm.^{2} hydrogen. Chromatograms were recorded on a 5-mv. recorder, and peak areas were determined using the method of triangulation. With these conditions, the retention times for 4-benzylbiphenyl, methadone, and two known human metabolites (III and II[4]) were 11.1, 7.7, 5.9 and 4.1 min., respectively.

Standards—Six standard solutions containing methadone hydrochloride and 4-benzylbiphenyl were prepared from two stock solutions. The methadone stock solution contained 1 mg./ml. of methadone hydrochloride in methanol, whereas the 4-benzylbiphenyl solution contained 0.5 mg./ml. of 4-benzylbiphenyl in methanol. Aliquots of 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 ml. of the methadone stock solution were transferred to suitable containers, each containing 2 ml. of the 4-benzylbiphenyl stock solution. A 4-μl. portion of each standard was analyzed by GLC, and the peak areas were determined by triangulation. The area ratio of methadone hydrochloride to 4-benzylbiphenyl and the weight of methadone hydrochloride added to each standard were utilized in a least-squares program on an IBM 360-65 computer to obtain a best straight-line equation for the six standards. Since the weight of methadone hydrochloride was the most accurately determined input value, it was used as the independent variable in the least-squares calculations. A plot of area ratio of methadone hydrochloride to 4-benzylbiphenyl *versus* the weight of methadone hydrochloride added to each standard also demonstrated linearity.

Evaluation of Assay Method—Thirty-eight urine samples, which had known amounts of methadone hydrochloride added at 12 different concentration levels, were used in the method evaluation. In some instances, urine from a single individual was used; in other cases, pooled urine from several persons was used. A control sample from each lot of urine used was also evaluated for possible interferences with the determination of methadone. Each urine sample was extracted by the procedure already described, and the resultant residue was dissolved in 2 ml. of the 4-benzylbiphenyl stock solution. A 4-μl. portion of this solution was analyzed by GLC, and the peak areas for 4-benzylbiphenyl and methadone were determined by triangulation. The area ratio of methadone to 4-benzylbiphenyl was determined and substituted into the best straight-line equation for the six standards as determined by the least-squares program, and the milligrams of methadone[5] found was obtained.

Table I gives the average milligrams of methadone found, as well as precision and accuracy values.

Clinical Utility of Assay Method—To evaluate the clinical utility of the present assay method for methadone, the 24-hr. pooled urines of three female patients participating in a methadone maintenance program[6] were assayed. Urines were collected on 2 different days from each patient and frozen immediately. Samples were thawed at room temperature prior to analysis, and 100 ml. of each sample was extracted and analyzed using the described procedures. A set of standards prepared as already described were randomly interspersed among the samples analyzed by GLC to assure greater accuracy. Results for this study are summarized in Table II.

RESULTS AND DISCUSSION

A GLC assay method for methadone was developed and evaluated in a concentration range of 3–30 mcg./ml. in human urine. The precision of all determinations was improved by using a least-squares best straight-line equation rather than a standard curve to obtain appropriate recovery values. The precision and accuracy of the method were shown to be approximately $\pm 7\%$ for the relative standard deviation (*RSD*) and relative error (*RE*), with a 93.2% average overall recovery. Control samples of normal human urine were evaluated by this method and demonstrated the lack of urine components which might interfere with the identification of methadone.

Evaluation of urines from three tolerant patients receiving three different dosages of methadone clearly demonstrated the clinical utility of the assay method. The two known metabolites of methadone in humans were detected for each of these patients.

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[4] Analyzed as the perchlorate and hydrochloride salts, respectively, dissolved in the 4-benzylbiphenyl stock solution.

[5] The value determined was actually the weight of methadone hydrochloride and must be multiplied by 0.8946 to obtain the weight of methadone.

[6] Urine samples were supplied by Dr. R. Knowles, St. Louis State Hospital, St. Louis, Mo. Additional excretion data obtained with the present method will be reported by Dr. Knowles and his associates.