Mass Fragmentographic Determination of Methadyl Acetate in Urine Using Stable Isotope Labeled Analog as Internal Standard

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Abstract □ A quantitative GLC–mass spectrometric assay was developed for the determination of methadyl acetate in urine. The assay utilizes selective ion focusing to monitor, in a GLC effluent, the M − 15 ion generated by electron-impact ionization of methadyl acetate. Methadyl acetate-d_4 was used as an internal standard. The assay can measure 10 ng of drug/ml with about 6% precision. The curve relating the amounts of drug added to control urine *versus* the amounts experimentally found over a large concentration range is a straight line with a slope of 0.98 ± 0.02 and a nearly zero intercept. Assay specificity was confirmed by complete identity of the mass spectrum of methadyl acetate in the biological extract with that of the authentic material. The method was used for the urinary analysis of methadyl acetate in a rabbit given a single intravenous dose. The animal excreted less than 1% of the intact drug with a half-life of approximately 15 hr. Consequently, the long-acting characteristic of methadyl acetate must be attributed to its metabolism into active metabolites.

Keyphrases □ Methadyl acetate—GLC–mass spectrometric analysis in urine □ GLC–mass spectrometry—analysis, methadyl acetate in urine □ Narcotic analgesics—methadyl acetate, GLC–mass spectrometric analysis in urine

Methadyl acetate[1], 6-(dimethylamino)-4,4-diphenyl-3-heptanol acetate (I), a potent, long acting, synthetic narcotic analgesic (1, 2), is currently under clinical evaluation for the treatment of opiate addiction (3–5). Its usefulness as a substitute for methadone in opiate addiction therapy prompted the development of a sensitive and specific assay in biological fluids. Methadyl acetate is biotransformed to several metabolites (5, 6), some of which are structurally similar to the parent drug. Consequently, an assay must be specific and free from interference from these metabolites.

This report presents a GLC–mass spectrometric assay for I in urine. Selective ion monitoring, the technique built on combined GLC–mass spectrometry, with selective focusing on a suitable fragment of the molecular ion (mass fragmentography) is a well-established technique in biomedical research (7, 8). This technique was used to develop a very sensitive and specific assay of I in urine utilizing deuterium-labeled I as an internal standard.

EXPERIMENTAL

Materials—Analytical grade I hydrochloride[2], sodium borodeuteride[3], and acetic anhydride[4] were used without further purification. All solvents were analytical grade[4]. Silanized tubes[5] (10 ml) with screw caps[6] were used for urine extraction; final solvent evaporation was performed in 5-ml glass-stoppered centrifuge tubes[7]. Pasteur pipets with hand-drawn constricted tips were utilized for all solution transfers. Urine was collected by catheterization, an aliquot was taken, an appropriate amount of internal standard was added, and the mixture was stored frozen until analysis.

Methadone[8]-d_3 [6-(dimethylamino)-4,4-diphenyl-1,1,1-trideutero-3-heptanone], on treatment with sodium borodeuteride using an established procedure for reduction of carbonyl compounds (9), gave dimepheptanol-d_4. On subsequent reaction with acetic anhydride (10), dimepheptanol-d_4 afforded I-d_4 [6-(dimethylamino)-4,4-diphenyl-1,1,1,3-tetradeutero-3-heptanol acetate] in an overall yield of 80%. The labeled compound showed satisfactory mass spectral (electron-impact ionization) characteristics; a multiple-ion detection analysis showed the presence of an ion equivalent to 99 ± 0.4% (n = 4) I-d_4 and 0.61 ± 0.1% I-d_0.

Extraction of I from Urine—To 5 ml of urine was added an appropriate amount of I-d_4 (typically 97 ng/ml) as an internal standard. The solution was adjusted to pH 8.5 with 1 N NH_4OH and extracted twice with 10 ml of ether. The organic fractions were combined. To this solution was added 1 ml of 0.1 N HCl; the solution was shaken for 15 min and centrifuged. The organic phase was discarded, and the aqueous phase was adjusted to pH 8.5 with 0.4 N NH_4OH and extracted twice with 4 ml of cyclohexane.

The organic fractions were combined, dried with sodium sulfate, and filtered. Then the filtrate was evaporated to dryness at 40° under a gentle stream of nitrogen. The residue was taken up in 20 μl of benzene, and an aliquot was injected into the GLC–mass spectrometer for selective ion monitoring. Recovery of I, added to control urine, was studied at the 20-ng/ml level.

Instrumentation—A magnetic sector, single-focusing mass spectrometer[9] interfaced with a gas chromatograph and equipped with a multiple-ion detector/peak matcher accessory was used (7, 8). GLC was performed on a 1.8-m glass column (2 mm i.d.). The column was silanized with 5% dimethyldichlorosilane in toluene, packed with 1.5% OV-1 on 100–200-mesh Gas Chrom Q, and conditioned for 24 hr at 280° with a flow rate of 20 ml of helium/min.

During the mass fragmentography assay, the column temperature was maintained at 185°, the flash heater was at 230°, the separator was at 235°, and the ion source was at 250°. The accelerating voltage was 3.5 kv. The ionization potential was 70 ev in the scan mode and 30 ev in the multiple-ion detector mode, and the trap current was set at 60 μamp. The magnetic field was kept constant by focusing on the background ion (column bleed) at m/e 355; the additional voltages were 173 and 130 v for measuring ion intensities at m/e 338 and 342, respectively. The retention time for I was 6 min.

RESULTS AND DISCUSSION

The mass spectrum of I (Fig. 1A) shows a molecular ion, an ion at m/e 338 (M − 15), and a base peak at m/e 72 [$CH_3CH{=}N^+(CH_3)_2$]. The fragmentation pattern is consistent with the structure and is in agreement with the spectra of similar compounds (11, 12). The mass spectrum of I-d_4 is similar to the spectrum of unlabeled isomer. Most ions are shifted to higher mass by 4 amu except the ion at m/e 72, which is a common ion from both labeled and unlabeled I.

The ion at m/e 338 is specific for I (m/e 342 for I-d_4) and is not observed in the electron-impact ionization spectra of methadone, methadone metabolites, and known metabolites of α-l-acetylmethadone (6, 13–15). Consequently, I was quantitated by measuring the ion intensities at m/e 338 and 342.

Control urine samples subjected to the described procedure for I showed no significant background ions at m/e 338 and 342. Known amounts of I along with the internal standard I-d_4 in "fixed" amount were added to control urine and processed as already described. Analysis of the data (Fig. 2) gave a slope of 0.98 ± 0.02 and an intercept of 0.15 ± 0.2

[1] The International Nonproprietary Name is α-l-acetylmethadol.
[2] Gift from Dr. K. Verebey, N.Y. State Department Drug Addiction Control Commission, Brooklyn, N.Y.
[3] ICN Pharmaceuticals, Plainview, N.Y.
[4] Fisher Scientific Co., Pittsburgh, Pa.
[5] Kimble, Owens, Illinois.
[6] Pyrex 8084.
[7] Lined with Teflon (Dupont).
[8] Gift from Dr. J. C. Hsia, University of Toronto, Toronto, Canada.
[9] LKB 9000, LKB, Stockholm, Sweden.

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Table I—Urinary I Levels after Injection of 15 mg iv in a Rabbit [a]

Sample	Hours	I, ng/ml	Total I, μg
1	5.5	384.6 ± 4.5[b]	13.944
2	27.0	269.8 ± 5.3	20.774
3	51.5	332.7 ± 4.92	22.956
4	75.0	74.0 ± 3.10	3.700
5	101.5	6.0 ± 0.75	0.420
6	168.5	13.0 ± 0.55	0.975
7	216.5	12.2 ± 0.54	0.817

[a] At varying intervals during 9 days, seven urine specimens (1–7) were obtained following administration of 15 mg iv of I hydrochloride to a 6-kg rabbit. [b] Mean of duplicate determinations.

ng. These data affirm a linear relationship between the ion intensity ratios (*m/e* 338/342) and the concentration of I in the biological fluid and exclude any significant isotopic fractionation during extraction, GLC column adsorption, and the fragmentation process.

Five samples of control urine containing 20 ng of I/ml were analyzed by this method with 22 ng of I-d_4/ml as the internal standard. The result for these samples was 19.3 ± 0.7 ng. These samples were assayed in duplicate; exactly the same amounts were taken in this set as in the previous experiment, but the internal standards were added after the extraction. The recoveries for these samples, based on comparison of the ion intensity

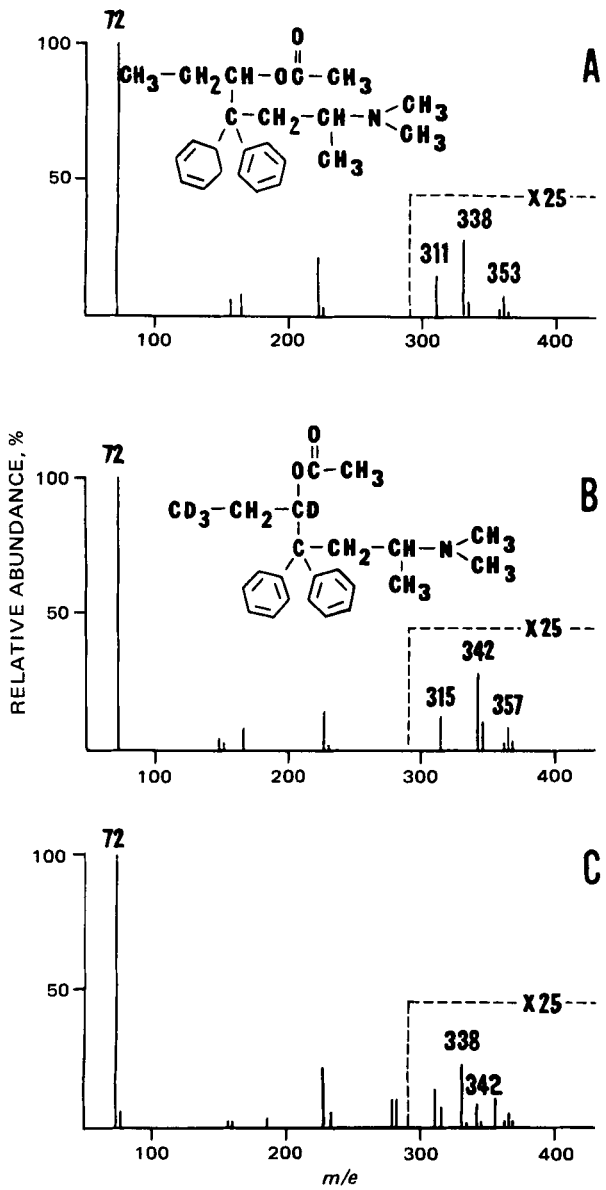

Figure 1—*Electron ionization (70 ev) mass spectrum of I (A), I-*d_4 *(B), and I in urine extract along with added I-*d_4 *(C).*

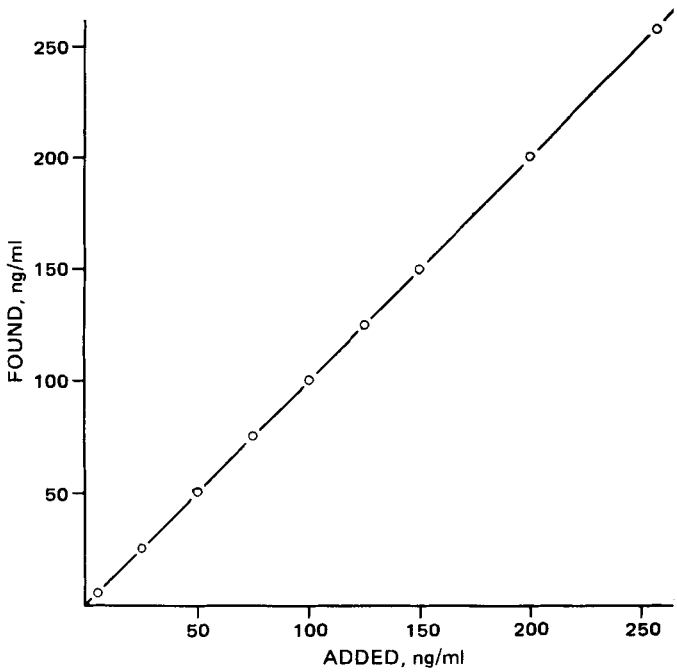

Figure 2—*Curve relating amount of I (0–250 ng) added to control urine versus the amounts found using the method described. For these samples, 97 ng of I-*d_4*/ml was used as the internal standard.*

ratios of the two sets, were 82 ± 8% (*m/e* 338/342). The variable recoveries observed are expected in the field of trace analysis and are attributed to variable glassware and GLC column adsorption. The use of I-d_4 as an internal standard in the assay presented here circumvents these problems.

A number of experiments were performed on rabbits[10] to determine urinary excretion after a single intravenous dose. In a typical experiment, a 6-kg rabbit was given 15 mg iv of I hydrochloride in water. Urine was collected by catherization periodically, starting at 5.5 hr. The last sample was collected 216 hr after the injection. Aliquots of urine samples were taken, appropriate amounts of the internal standard were added, and the samples were processed as described. The mass chromatograms (Fig. 3) at *m/e* 338/342 obtained from these samples show clean, symmetrical peaks and were used for analysis. The practical sensitivity of the assay in its current form is about 10 ng/ml of urine; furthermore, the assay is specific. Assay specificity was confirmed by a complete mass spectrum

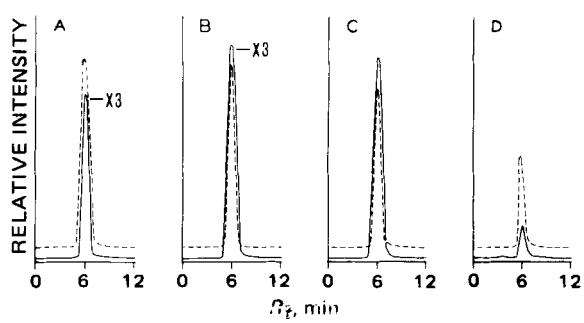

Figure 3—*Selective ion chromatograms for I (—,* m/e *338) along with I-*d_4 *(– –,* m/e *342). A: Urine was obtained 27 hr after the dose. The internal standard I-*d_4 *(*m/e *342) concentration was 97 ng/ml, and the I concentration was 269.8 ng/ml. B: Urine was obtained 51.5 hr after the dose. The internal standard I-*d_4 *(*m/e *342) concentration was 97 ng/ml, and I (*m/e *338) concentration was 332.7 ng/ml. C: Urine was obtained 75 hr after the dose. The internal standard I-*d_4 *(*m/e *342) concentration was 68 ng/ml, and the I (*m/e *338) concentration was 74 ng/ml. D: Urine was obtained 168.5 hr after the dose. The internal standard I-*d_4 *(*m/e *342) concentration was 39 ng/ml, and the I (*m/e *338) concentration was 13 ng/ml.*

[10] White New Zealand.

of the material (Fig. 1C) in the biological extract with added internal standard.

Table I shows the urinary analysis of I in the rabbit as a function of time. The cumulative urinary excretion indicates the half-life of excretion of the intact drug to be approximately 15 hr. Significantly, the animal excreted less than 1% of the drug in its native form. Evidently, its long acting characteristic must be attributed to its biotransformation into active metabolites.

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Color Analysis of Dextrose Solutions Using a Color Difference Meter

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Abstract□ A method for quantitating color measurements in dextrose solutions by using a color difference meter is described. This method was shown to correlate well with standard American Public Health Association (APHA) color measurements. A mathematical relationship was developed relating yellowness index values to APHA numbers as described in the USP for dextrose solutions. This relationship was tested by comparing the results from standard APHA color tests on laboratory samples of autoclaved dextrose solutions to APHA numbers calculated from yellowness index values for the same samples.

Keyphrases □ Dextrose solutions—color analysis, color difference meter compared to standard measurements □ Color—analysis, dextrose solutions, color difference meter compared to standard measurements

After sterilization, dextrose solutions may exhibit a brownish-yellow color as a result of dextrose degradation (1, 2). Currently, color measurement in many pharmaceutical solutions is performed by the American Public Health Association (APHA) method, which utilizes a concentration gradient of platinum–cobalt solutions in 100-ml Nessler tubes (3). The sample solution color can be measured by matching the color of the sample tube with one corresponding standard. Color is reported as parts per million of platinum–cobalt.

This study examined the correlation of yellowness index values, determined by a color difference meter[1], to the standard APHA colors. The practical use of the yellowness index for color evaluation of pharmaceutical dextrose solutions also was demonstrated. The advantage of this technique is that it allows an objective quantitation of color.

EXPERIMENTAL

A color difference meter, standardized using a calibrated white plate[2], was used for color measurement. Calibration values were derived by direct comparison to master standards traceable to measurements at the National Bureau of Standards.

A 6.4-cm optical cell[3] was the sample holder for each solution. The cell was filled with 80 ml of solution and placed over the 5.1-cm aperture of the detection head. A white reflective plate was placed over the cell; then a lightproof cover was placed over the detection head to exclude any ambient light. Light was transmitted through the bottom of the cell and reflected off the reflector plate into the detector head where an optical sensor transmitted a signal to the signal processor. The color meter then presented the color of solutions as four variables: L (brightness), a (green and red), b (blue and yellow), and YI (yellowness index) (4, 5). Color values were displayed by a digital readout to one decimal place.

Color values for a concentration gradient of platinum–cobalt solutions (0–80 ppm) were obtained using a color difference meter. Three readings were taken at each concentration level.

The practical application of the color difference meter was demonstrated through a comparison of observed APHA values for various autoclaved dextrose solutions to mathematically derived APHA values calculated from YI values. Solutions were made from various lots of dextrose[4] and were tested by USP methods (6). The particular solutions were chosen at random to represent various products with potential color variation.

The colors of five solutions of different dextrose concentrations were determined by six observers using the APHA method. The colors of these

[1] D25L-2, Hunter Associates Laboratory, Fairfax, Va.

[2] C2-6664, Hunter Associates Laboratory, Fairfax, Va.

[3] Agtron 13851210, Hunter Associates Laboratory, Fairfax, Va.

[4] Corn Products, Englewood Cliffs, NJ 07632.

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