# Qualitative Method for Dantrolene and a Related Metabolite in Urine

JOHN D. CONKLIN▲ and R. J. SOBERS

**Abstract** □ A qualitative method is described for the determination of dantrolene, a new skeletal muscle relaxant, and a related nonreduced metabolite in human urine. The method consists of the direct extraction of the drug and the metabolite from urine into nitromethane. Addition of a hyamine hydroxide solution to the solvent extract from urines containing the drug and the metabolite results in a yellow color which is linearly related to the amount of drug and metabolite present. A yellow color is not apparent when the alkaline solution is added to solvent extracts from control urine. The method is specific for dantrolene plus the nonreduced metabolite in the presence of a reduced metabolite. Data obtained from urine specimens, collected from humans receiving sodium dantrolene and analyzed by the qualitative method, are also presented.

**Keyphrases** □ Dantrolene and metabolite—qualitative colorimetric determination in urine □ Colorimetry—qualitative determination of dantrolene and metabolite in urine

Sodium dantrolene[1], 1-{[5-(*p*-nitrophenyl)furfurylidene]amino}hydantoin sodium hydrate, is a new skeletal muscle relaxant (1–3). By utilizing a combination of solvent extractions and chromatography followed by fluorometric measurement, dantrolene is quantitatively determined in biological specimens (4). There still is need, however, for a qualitative method which the clinical laboratory can readily perform. This report describes a simple and rapid colorimetric method for dantrolene and a related nonreduced metabolite, designated as Metabolite A, in human urine.

## EXPERIMENTAL

**Reagents and Instrument**—The reagents used included: crystalline dantrolene[2]; ammonium sulfate[3], special enzyme grade; *N,N*-dimethylformamide[4], reagent grade; nitromethane[5], practical grade; methanol[6], analytical reagent grade; and hyamine hydroxide[7], 1 *M* in methanol. The 1 *M* hyamine solution (2 ml.) is diluted to 25 ml. with absolute methanol to obtain a 0.08 *M* solution.

A spectrophotometer[8] was used to measure absorbances.

**Procedure**—To 2 ml. of urine in a tube, add 1 ml. of water and then 3 ml. of saturated ammonium sulfate solution and mix thoroughly. Add 5 ml. of nitromethane, mix the tube contents vigorously for 1 min., and centrifuge at 2000 r.p.m. for 10 min. Transfer 3 ml. of the solvent extract (top layer) to a test tube. Then add 5 drops of 0.08 *M* hyamine hydroxide solution and mix the contents.

In the qualitative test, the color of the extract from the unknown sample is immediately compared with the color of the corresponding extract from a control sample against a white background. Under these conditions, extracts from urine specimens containing dantrolene and Metabolite A exhibit a yellow color which is linearly related to the amount of drug and metabolite present. Control urine extracts do not exhibit any apparent yellow color when the hyamine reagent is added.

[1] Dantrium, Eaton Laboratories.
[2] Eaton Laboratories.
[3] Schwarz/Mann, Catalog No. 1946.
[4] Matheson, Coleman and Bell, Catalog No. DX1730.
[5] Commercial solvents.
[6] Mallinckrodt Chemical Works, Catalog No. 3016.
[7] Packard Instrument Co., Catalog No. 6003005.
[8] Hitachi model 139.

**Table I—Determinations of Dantrolene and Metabolite A Standards by the Nitromethane–Hyamine Method**

| Sample, mcg./ml. | Absorbance[a], 400 nm. Dantrolene Water | Dantrolene Urine | Metabolite A Water | Metabolite A Urine |
|---|---|---|---|---|
| Control | 0.009 | 0.033 | 0.006 | 0.037 |
| 2.5 | 0.079 | 0.083 | 0.078 | 0.077 |
| 5.0 | 0.157 | 0.162 | 0.154 | 0.158 |
| 15.0 | 0.478 | 0.483 | 0.464 | 0.469 |
| 25.0 | 0.780 | 0.799 | 0.759 | 0.765 |

[a] Mean based on three samples. Absorbances for standards are control corrected.

If an estimate of the drug concentration is desired, the solvent extract is then subjected to spectrophotometric measurement at 400 nm. within 1 min. after addition of the hyamine reagent. Pure nitromethane is used to set the instrument to zero absorbance. The absorbance of the control sample is subtracted from the absorbance of the unknown sample, and the estimated drug and metabolite present are calculated from dantrolene standards subjected to the method. The results are expressed as dantrolene equivalents. A dantrolene standard with a concentration of 25 mcg./ml. exhibits an absorbance of about 0.800. Dilutions are necessary when a nitromethane extract from a sample subjected to the procedure exhibits an absorbance greater than 0.800 after addition of the hyamine reagent. If this occurs, some of the remaining nitromethane extract, not reacted with the hyamine reagent, is diluted with pure nitromethane and then the hyamine reagent is added.

In preparing standard solutions, 50 mg. of crystalline dantrolene is dissolved in 50 ml. of dimethylformamide. Ten milliliters of this solution is placed in a 100-ml. volumetric flask, 40 ml. of dimethylformamide is added, and the solution is diluted to volume with water to obtain a drug concentration of 100 mcg./ml. This solution is then diluted with water to obtain the required drug concentrations. Standards[2] of Metabolite A and acetylated dantrolene[9] are prepared in an identical manner.

Urine reference standards are prepared with 2 ml. of urine, 1 ml. of standard solution, and 3 ml. of saturated ammonium sulfate solution. Aqueous standards are prepared in an identical manner except that 2 ml. of water is substituted for the 2 ml. of urine. Water or the human urine used to prepare the reference standards serves as a control.

## RESULTS

The results presented in Table I show that both dantrolene and Metabolite A are susceptible to the nitromethane–hyamine procedure. Standard curves for each compound are linear from 2.5 to 25.0 mcg./ml. Drug concentrations as low as 2.5 mcg./ml. (5 mcg./tube) are visually identified by a distinct yellow color. Agreement is apparent between aqueous and human urine reference standards for both dantrolene and Metabolite A (Table I). Average recoveries of added dantrolene and Metabolite A from human urine were 102.9 ± 1.6 and 100.8 ± 1.6%, respectively, based on aqueous standards. Acetylated dantrolene, another metabolite, did not exhibit appreciable absorbance when subjected to the nitromethane–hyamine procedure.

Urine specimens collected from healthy humans who had received sodium dantrolene as an encapsulated formulation orally were subjected to the nitromethane–hyamine method. Nitromethane

[9] 1-{[5-(*p*-Acetamidophenyl)furfurylidene]amino} hydantoin.

Table II—Determinations of Urine Specimens from Humans Administered Sodium Dantrolene by the Nitromethane–Hyamine Method[a]

| Subject | Absorbance[b], 400 nm. | Concentration[c], mcg./ml. |
|---|---|---|
| 3 | 0.098 (0.062) | 3.06 |
| 6 | 0.149 (0.034) | 4.65 |
| 2 | 0.719 (0.013) | 22.47 |
| 5 | 0.745 (0.015) | 23.28 |
| 11 | 0.541 (0.032) | 16.90 |

[a] An encapsulated sodium dantrolene formulation was administered orally as a single dose at 100 mg. to Subjects 3 and 6 and at 100 mg. q.i.d. to Subjects 2, 5, and 11. Urine specimens were collected from 0–24 hr., pooled, and frozen. A control urine specimen was collected just before drug administration. [b] Control corrected; figure in parentheses represents the control urine absorbance. [c] Estimated drug concentration for 0–24 hr., expressed as dantrolene equivalents, represents dantrolene plus Metabolite A.

extracts from each of the specimens, collected after drug administration, exhibited a visual yellow color following the addition of the hyamine reagent (Table II). Under these conditions, a yellow color was not apparent with extracts from corresponding control urines, collected before drug administration.

The dantrolene–hyamine complex in nitromethane exhibits an absorbance maximum near 400 nm., while the corresponding Metabolite A–hyamine complex displays a maximum near 395 nm. When subjected to the nitromethane–hyamine procedure, the human urines collected after the administration of sodium dantrolene yielded an absorbance maximum from 380 to 385 nm.

An estimate of the drug and metabolite concentration present in the urine specimens was obtained by spectrophotometric measurement (Table II). These results are expressed as dantrolene equivalents, based on dantrolene standards, and represent dantrolene plus Metabolite A.

## DISCUSSION

At present, two of the major dantrolene-related metabolites recovered from human urine after the administration of sodium dantrolene are reduced acetylated dantrolene and a nonreduced metabolite designated as A (5). The amount of each of these metabolites excreted in urine is usually much greater than the corresponding amount of dantrolene (5). It is assumed that dantrolene plus Metabolite A is primarily responsible for the yellow color observed when urine specimens collected from humans receiving sodium dantrolene are analyzed by the nitromethane–hyamine procedure, since standards of acetylated dantrolene do not exhibit an appreciable absorbance when subjected to the qualitative method. A partial explanation for this specificity is that acetylated dantrolene does not contain the nitro group on the benzene ring which is present in both dantrolene and Metabolite A.

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▲ To whom inquiries should be directed.

# *COMMUNICATIONS*

## Dansyl Derivatives of $\Delta^9$- and $\Delta^8$-Tetrahydrocannabinols

**Keyphrases** □ Dansyl derivatives of $\Delta^9$- and $\Delta^8$-tetrahydrocannabinols—synthesis, physical and chemical properties □ Tetrahydrocannabinols—*synthesis of dansyl derivatives*, physical and chemical properties

*Sir:*

The use of dansyl chloride (5-dimethylamino-1-naphthalenesulfonyl chloride, I) in the fluorometric determination of amines and phenols is well documented (1). Dansylated cannabinol-related compounds were recently derived from blood, urine, and saliva samples following hashish administration (2). As a prerequisite for the development of suitable methodology for the assay of compounds and metabolites derived from *Cannabis sativa*, a series of dansyl derivatives of several cannabinoid compounds was prepared in micromolar quantities and their TLC properties were reported (3). The preparation of larger quantities of dansyl-$\Delta^9$-tetrahydrocannabinol (II) and dansyl-$\Delta^8$-tetrahydrocannabinol (III) was accomplished to determine the physical and chemical properties of these derivatives.

The reaction of $\Delta^9$- or $\Delta^8$-tetrahydrocannabinol and excess dansyl chloride was performed in acetone–water solution saturated with sodium carbonate at 40° for 2 hr. After excess dansyl chloride was hydrolyzed by treatment with base, the reaction mixture was extracted with ethyl acetate. Compounds II and III were isolated as thick oils. Crystallization could only be induced by prolonged storage at −5° in hexane. Recrystallization from hexane or heptane yielded crystalline solids which provided microanalytical values within acceptable limits and the correct molecular weight by mass spectrometry. The physical and spectral characteristics of Compounds II and III are summarized in Tables I and II.

The reaction of I would be expected to occur on the phenolic group of tetrahydrocannabinol. Examples are known with reactions similar to dansylation where the substituent does not add onto the phenolic oxygen but