Table I—Pharmacological Properties of *O*-Acylmorphines

Morphine Derivatives[a]	ED_{50}, mg/kg[b]	PDC[c]	Duration of Action, hr (Monkeys)
I	0.5 (0.4–0.6)	High (1.0)[d]	3–4
II	0.6 (0.5–0.8)	—[e]	—
III	0.3 (0.2–0.4)	High (0.8)[d]	3–4
IV	0.5 (0.4–0.6)	High (1.0)[d]	—[f]
V	0.3 (0.2–0.4)	High (1.6)[d]	12
VI	0.3 (0.2–0.4)	Medium high (2.0)[d]	>6
VII[g]	1.3 (1.2–1.5)	High (2.0)[d]	3
Morphine[a]	1.2 (0.9–1.3)	High (3.0)[d]	6

[a] Subcutaneously administered as hydrochloride salts, unless otherwise noted, to mice. [b] Determined by the hot-plate method (6, 7). Numbers in parentheses are the 95% *SE* limits, as obtained by probit analysis. [c] Physical dependence capacity as determined in monkeys (8, 9). [d] Equivalence to 3 mg/kg of morphine sulfate. [e] Too unstable to test in monkeys. [f] Of shorter duration than morphine. [g] Sulfamate salt.

(to the appearance of crystals) and cooled finally to 0° for 1 hr to give 1.8 g, mp 155–165°.

Anal.—Calc. for $C_{20}H_{24}ClNO_4 \cdot 0.5H_2O$: C, 62.07; H, 6.47; Cl, 9.16; N, 3.62. Found: C, 61.70; H, 6.43; Cl, 9.18; N, 3.29.

RESULTS AND DISCUSSION

As seen in Table I, the 3,6-diformyl (II), 3,6-dipropanoyl (III), and 6-propanoyl (VI) derivatives were comparable to heroin (I) and 6-acetylmorphine (IV) in analgesic potency and were three to four times as potent as morphine (6, 7). 3-Acetylmorphine (VII) sulfamate (4) was nearly identical in potency to morphine. As expected, all had good capacity to sustain morphine dependence in monkeys (8, 9) with a wide variation in duration of action. Superiority in this respect was shown by V (12 hr) and VI (>6 hr), both longer acting than morphine, heroin, or 6-acetylmorphine[2].

The mode of action of the 6-acylated compounds, V and VI, is not known. Like 6-acetylmorphine, they are probably metabolized to morphine (10), albeit less rapidly. Their duration of action for analgesia (mice) was much longer than that for heroin but similar to morphine. Their considerably longer action than heroin and even morphine in higher animals (monkeys) may be more indicative of their behavior in humans.

The oral activity of V and VI (in mice) was relatively good (ED_{50} 11.6 and 7.4 mg/kg, respectively). Thus, V and VI would appear to be the most interesting compounds of this group for further study as possible maintenance drugs or in detoxification therapy. The diformyl compound (II) was too unstable to be tested in monkeys.

Qualitatively, the order of stability of these compounds in the salt form noted in Table I appeared to be: 3,6-diacetyl ≅ 3,6-dipropanoyl > 6-propanoyl ≅ 6-acetyl ≅ 3-acetyl > 6-formyl > 3,6-diformyl derivatives at 25°. This order seems to be at variance with the durations of action in supporting morphine dependence in monkeys. The impression is given by these qualitative studies that the acylmorphines (as salts), except II, are more stable in aqueous solution than is generally believed.

[2] Extracted from Addenda to the Proceedings of several meetings of the Committee on Problems of Drug Dependence in early to mid-1950's.

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Liquid Chromatography in Pharmaceutical Analysis VI: Determination of Dantrolene Sodium in a Dosage Form

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Abstract □ Operating conditions are described for the qualitative and quantitative determination of dantrolene sodium by high-pressure liquid chromatography. A 10-μm porous silica column was employed, using carbon tetrachloride–dimethylformamide (90:10) as the mobile phase. The flow rate was 2.0 ml/min (1800 psig), and the peaks were detected at 375 nm. The analysis of a dosage form can be carried out within 30 min with an accuracy of 3.1%. The results agree favorably with those obtained with a modified spectrophotofluorometric method.

Keyphrases □ Dantrolene sodium—high-pressure liquid chromatographic analysis, commercial dosage forms □ High-pressure liquid chromatography—analysis, dantrolene sodium, commercial dosage forms □ Relaxants, skeletal muscle—dantrolene sodium, high-pressure liquid chromatographic analysis, commercial dosage forms

Dantrolene sodium, 1-[[5-(*p*-nitrophenyl)furfurylidene]amino]hydantoin sodium salt hydrate, was first reported by Snyder *et al.* (1) as a representative of a new class of muscle relaxants which apparently acts directly on the skeletal muscle.

Previous reported analyses of dantrolene sodium include spectrophotofluorometry (2, 3), differential pulse polarography (4), and a colorimetric procedure for qualitative

Table I—Calibration Data for Standard Drug Solutions

Analytical Method	Initial Concentration of Dantrolene Sodium, mg/ml	Observed Results	Slope	Intercept	$r \pm s_{yx}$[a]
HPLC	0.2	1.1192 ± 0.0010[b]			
	0.5	2.8129 ± 0.0100	0.5362	0.0795	0.9997 ± 0.0369
	1.0	5.4219 ± 0.0099			
Spectrophotofluorometric	0.2	28.5[c]			
	0.5	55.0	8.9439	10.4847	0.9999 ± 0.1458
	1.0	100.0			

[a] r is the correlation coefficient determined from linear regression analysis, and s_{yx} is the sample standard deviation, also from regression analysis. [b] The D/IS ratio is based on four replicate injections of standard solution. The table value is the mean ± *SD*. The D/IS is the ratio of the integrated area of the drug at some concentration to the integrated area of furil at a concentration of 4 mg/ml. [c] Arbitrary fluorescence units.

determination (5). Although the use of liquid chromatography *via* a reversed-phase process has been successful with various drugs (6–8), the limited solubility of dantrolene sodium in polar mobile phases has precluded use of this technique. This paper reports the conversion of the drug to its free acid followed by extraction, chromatographic separation, and quantification using adsorption chromatography. The analysis of dosage forms can be performed within 30 min. The results of the high-pressure liquid chromatographic (HPLC) procedure are compared to those of a spectrophotofluorometric method, modified from that reported by Hollifield and Conklin (3).

EXPERIMENTAL

Reagents and Chemicals—A powdered sample of dantrolene sodium[1] was used; chemicals and reagent grade solvents were obtained commercially and used as received. The chromatographic solvents, carbon tetrachloride and dimethylformamide, were filtered through a 0.5-μm filter[2] prior to use. The pH of a 0.5 *M* aqueous solution of dimethylformamide was consistently 6.8 ± 0.1.

Mobile Phase—The mobile phase consisted of carbon tetrachloride–dimethylformamide (90:10) and was used after degassing.

Internal Standard Solution—The stock internal standard solution (20 mg/ml) was prepared by dissolving powdered furil[3] in water-saturated 1-butanol–chloroform (30:70). It was filtered through a 5.0-μm filter[4] before use.

Standard Solution for Calibration Curve—A stock solution of dantrolene sodium was prepared by dissolving 25 mg of dantrolene sodium powder in 5 ml of dimethylformamide in a 25-ml volumetric flask. The solution was then diluted to 25 ml with distilled water to yield a solution of 1 mg/ml.

Determination of Calibration Curve—Accurately pipetted volumes of 2.0, 5.0, and 10.0 ml of the dantrolene sodium stock solution were each placed in a 60-ml separator. Where necessary, the volume of each solution was adjusted to 10 ml with distilled water. Then 5.0 ml of 2 *N* HCl solution was added to each separator to precipitate dantrolene. The aqueous solutions were extracted with 2 × 10-ml portions of 1-butanol–chloroform (30:70), and the organic layer from each separator was separated and transferred to 25-ml volumetric flasks.

The volume of each flask was then adjusted to 25 ml with additional organic solvent, and the organic extract was filtered through a 5.0-μm filter[4]. Four milliliters of the filtered extract was pipetted into a 5-ml volumetric flask followed by the addition of 1 ml of the internal standard stock solution. Twenty microliters of each solution was injected into the chromatograph.

Determination of Percent Extraction of Dantrolene Sodium—A solution of dantrolene[5] (0.0522 mg/ml) was prepared in 1-butanol–chloroform (30:70). Four milliliters was pipetted into a 5-ml volumetric flask, 1 ml of internal standard solution was added, and the solution was chromatographed. The ratio of the dantrolene peak area to the area of the internal standard (D/IS) was compared with the D/IS ratio obtained by a theoretically equivalent amount of dantrolene free acid, extracted from dantrolene sodium, which was prepared as follows. Two milligrams of dantrolene sodium was dissolved in 10 ml of water, converted to dantrolene, and extracted into 3 × 10-ml portions of 1-butanol–chloroform (30:70) to give a solution with a theoretical dantrolene concentration of 0.0522 mg/ml. Four milliliters of this solution was pipetted into a 5-ml volumetric flask, 1 ml of internal standard solution was added, and the solution was chromatographed.

Determination of Dantrolene Sodium in Dosage Form—The contents of two 25-mg capsules[6] were quantitatively transferred to separate 50-ml volumetric flasks with the aid of a small amount of distilled water. The contents were dissolved in 10 ml of dimethylformamide and

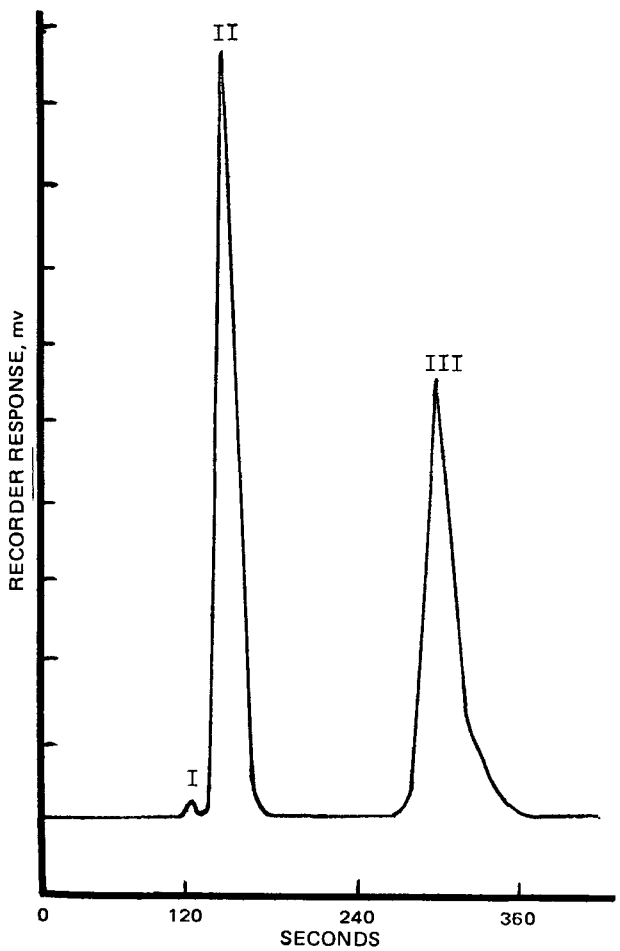

Figure 1—*Typical chromatogram of dantrolene sodium using a porous silica column (room temperature) at a flow rate of 2.0 ml/min. Mobile phase was carbon tetrachloride–dimethylformamide (90:10). Key: I, solvent front; II, furil (internal standard); and III, dantrolene.*

[1] Courtesy of Eaton Laboratories, Norwich, N.Y.
[2] Catalog No. FHL PO 4700, Millipore Corp., Bedford, Mass.
[3] Catalog No. 2040, Eastman Organic Chemicals, Rochester, N.Y.
[4] Catalog No. LSWF01300, Millipore Corp., Bedford, Mass.
[5] Free acid, courtesy of Eaton Laboratories.
[6] Dantrium, Eaton Laboratories, Norwich, N.Y.

Table II—Determination of Dantrolene Sodium in Dosage Form[a]

Capsule	Labeled Amount, mg	Percent Found	
		HPLC[b]	Spectrophotofluorometric Method[c]
1	25	98.50 ± 1.66[d]	100.28 ± 0.73[d]
2	25	104.15 ± 1.17	106.03 ± 0.90

[a] There was no significant difference between the HPLC results and the fluorometric results at the $p = 0.01$ level. [b] Based on four replicate injections. [c] Based on three replicate determinations. [d] Confidence limits at $p = 0.05$.

diluted to volume with distilled water. The solutions were then filtered[7]. Ten milliliters of the filtrate was transferred to a 60-ml separator, acidified, extracted, and analyzed as described under *Determination of Calibration Curve*.

Conditions for Chromatographic Quantification—The liquid chromatograph was equipped with a pump[8], a variable wavelength detector[9], an integrator with digital printout[10], and a column containing a porous silica packing[11]. The degassed mobile phase was pumped through the column at a flow rate of 2.0 ml/min (1800 psig) at room temperature (23 ± 2°) until a stable baseline was obtained. Replicate 20-μl injections of sample and standard solutions were made using a 25-μl syringe[12]. The chart recorder provided a record of drug elution from the column as peaks on a chromatogram. The peaks were detected at 375 nm. In all cases, the solute was measured by digital integration of the peak area.

Determination of Dantrolene Sodium by Fluorescence—One milliliter of the 1-butanol–chloroform extract from both the calibration curve and the dosage form was added to a 100-ml volumetric flask and diluted to volume with 1-butanol–chloroform (30:70). The fluorescence was measured in a spectrophotofluorometer[13] with the following operating parameters: slit width for excitation and emission, 5 nm; sensitivity, 3.0; excitation wavelength, 395 nm; and emission wavelength, 575 nm. By using the fine sensitivity control, the recorder was set to read full scale (100 units) for the 1-butanol–chloroform solution containing the highest concentration of dantrolene. Measurements for the standard and dosage form were made respective to this arbitrary setting.

RESULTS AND DISCUSSION

Studies on the dosage form of dantrolene sodium showed that dantrolene free acid could be precipitated in acidic media between pH 2.5 and 4.0 using 2 *N* HCl. The free acid was then extracted into 1-butanol–chloroform with a percent extraction of 90.97 ± 0.14 as compared to the theoretical amount of known dantrolene free acid. When injected into the chromatograph, dantrolene was eluted within 6 min (Fig. 1). Furil was found to be a suitable internal standard. Although furil and dantrolene have absorption maxima at 305 and 395 nm, respectively, an intermediate wavelength of 375 nm was selected to obtain the sensitivity necessary to detect both components.

Standard solutions of dantrolene sodium were chromatographed using the microsilica column. The area under the curve for each peak on the chromatogram was digitally integrated. The ratio of the dantrolene peak area to the area of the internal standard was calculated for each chromatogram. A linear regression analysis of the data at three concentrations of dantrolene sodium gave the results shown in Table I. The calibration data for the fluorometric method are also listed.

Dantrolene sodium in a commercial dosage form was dissolved, extracted, and chromatographed in a manner analogous to the standard solutions, and the D/IS ratio was calculated. The constants (slope and intercept) from the linear regression equation shown in Table II were used to solve for drug concentration [D/IS = (slope × concentration) + intercept]; a programmable calculator[14] was used. The same extraction solutions were analyzed fluorometrically after appropriate dilutions.

The data in Table II demonstrate the quantitative results obtained for the dosage form. The utility of HPLC in the analysis of dantrolene sodium is clearly indicated, and the results agree favorably with those obtained with the spectrophotofluorometric method.

With various concentrations of dantrolene sodium as unknowns, the accuracy of the overall chromatographic procedure was shown to be 3.1%.

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[8] Waters Associates liquid chromatograph, model ALC/GPC 201, equipped with an M-6000 pump.

[9] Perkin-Elmer model LC-55 UV-visible.

[10] Infotronics integrator, model CRS-204.

[11] Waters packed 10-μm μPorasil column, 4 mm i.d. × 30 cm.

[12] Model B-110, Precision Sampling Corp., Baton Rouge, La.

[13] Perkin-Elmer model MPF-4.

[14] Olivetti-Underwood programma No. 101.