Table III—Comparison of Ibuprofen Pharmacokinetics in Venous and Capillary Plasma Following the Administration of One 400-mg Ibuprofen Tablet to Four Normal Healthy Subjects

Subject	C_{max}, μg/mL		t_{max}, h		AUC_{10}, μg/mL · h		$t_{1/2}$, h	
	Capillary	Venous	Capillary	Venous	Capillary	Venous	Capillary	Venous
1	32.8	32.1	4.0	4.0	180	162	1.65	2.54
2	35.2	31.4	2.0	2.0	151	148	1.95	2.01
3	33.9	32.6	3.0	3.0	107	106	1.50	1.45
4	33.1	32.1	3.0	3.0	138	132	2.49	2.76
Mean	33.7	32.1	3.0	3.0	144	137	1.90	2.19
SD	1.07	0.493	0.816	0.816	30.3	24.0	0.439	0.585

than drug-free plasma[17]. All saliva concentrations were below assay sensitivity, indicating the unsuitability of saliva sampling as a noninvasive alternative to the collection of blood samples.

[17] The precision, sensitivity, and accuracy of the method were comparable whether the biological matrix was plasma or saliva.

REFERENCES

(1) S. S. Adams, K. F. McCullough, and J. S. Nicholson, *Arch. Int. Pharmacodyn. Ther.*, **178**, 115 (1969).

(2) C. D. Brooks, C. A. Schlagel, N. C. Sekhar, and J. T. Sobota, *Curr. Ther. Res. Clin. Exp.*, **15**, 180 (1973).

(3) E. F. Davies and G. S. Avery, *Drugs*, **2**, 416 (1971).

(4) T. G. Kantor, *Ann. Intern. Med.*, **91**, 877 (1979).

(5) M. Thompson, A. W. Craft, M. S. Akyol, and R. Porter, *Curr. Med. Res. Opin.*, **3**, 594 (1975).

(6) F. E. Scheldrake and B. M. Ansell, *Curr. Med. Res. Opin.*, **3**, 604 (1975).

(7) S. Simila, K. Kouvalainen, and S. Keinsnew, *Scand. J. Rheumatol.*, **5**, 81 (1976).

(8) D. G. Kaiser and R. S. Martin, *J. Pharm. Sci.*, **67**, 627 (1978).

(9) L. P. Hackett and L. J. Dusci, *Clin. Chim. Acta*, **87**, 301 (1978).

(10) K. K. Midha, J. K. Cooper, J. W. Hubbard, and I. J. McGilveray, *Can. J. Pharm. Sci.*, **12**, 29 (1977).

(11) D. J. Hoffman, *J. Pharm. Sci.*, **66**, 749 (1977).

(12) G. J. Vangiessen and D. G. Kaiser, *J. Pharm. Sci.*, **64**, 798 (1975).

(13) D. G. Kaiser and G. J. Vangiessen, *J. Pharm. Sci.*, **63**, 219 (1974).

(14) G. L. Kearns and J. T. Wilson, *J. Chromatogr.*, **226**, 183 (1981).

(15) J. L. Shimek, N. G. S. Rao, and S. K. WahbaKhalil, *J. Pharm. Sci.*, **70**, 514 (1981).

(16) D. Pitre and M. Grandi, *J. Chromatogr.*, **170**, 278 (1979).

A New Method for High-Performance Liquid Chromatographic Determination of Drotaverine in Plasma

J. MEZEI ˣ, S. KÜTTEL, P. SZENTMIKLÓSI, S. MARTON, and I. RÁCZ

Received December 7, 1982, from the *Institute of Pharmacy, Semmelweis University of Medicine, Högyes E. 7., 1092. Budapest, Hungary.* Accepted for publication August 30, 1983.

Abstract □ A sensitive, specific high-performance liquid chromatographic procedure was developed for the determination of plasma drotaverine levels. Basic plasma samples were adjusted to pH 1.5 and extracted with chloroform. HPLC [*n*-heptane–dichloromethane–diethylamine (50:25:2)] on a microporous silica column, with a variable-wavelength UV detector set at 302 nm allowed the measurement of drotaverine at the 50-ng/mL level. The utility of this method for determination of drotaverine in dog and rat plasma was demonstrated.

Keyphrases □ Drotaverine—liquid chromatography, plasma □ Liquid chromatography—determination of plasma drotaverine levels

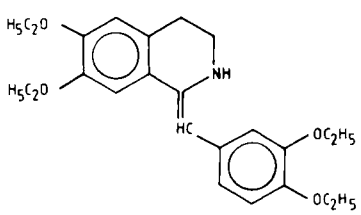
Drotaverine, [1-(3,4-diethoxybenzylidene)-6,7-diethoxy-1,2,3,4-tetrahydroisoquinoline] (I), an effective spasmolytic drug (1), is also marketed as a salt developed with theophylline-7-acetic acid (2).

There are only a few reports in the literature describing the measurement of drotaverine in plasma. ^{14}C-Labeled drotaverine was used to study the pharmacokinetics of the drug in mice (3) and humans (4). The spectrophotometric method (5) and the procedure suggested for the assay of papaverine (6) was found to be neither specific nor sensitive. This paper describes a modification of the high-performance liquid chromatographic method (HPLC) which has been reported for the measurement of papaverine in plasma (7).

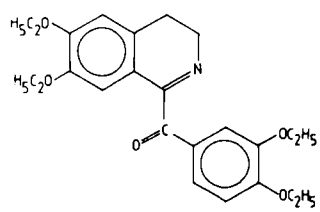
EXPERIMENTAL SECTION

Reagents—Drotaverine[1], papaverine hydrochloride[1], drotaveraldine[1] (II), chloroform[2], hydrochloric acid[3], *n*-heptane[4], diethylamine[3], dichloromethane[3], glacial acetic acid[5], and anhydrous sodium sulfate[3] were used without any further purification.

Apparatus—The liquid chromatograph[6] was fitted with a stopped-flow injector[6], a variable-wavelength UV detector[7] (set at 302 nm), and a microporous silica column[8]. The flow rate of the mobile phase was 1.6 mL/min. Chromatograms were recorded[9] at 1 cm/min chart speed.



I



II

[1] Chinoin Chemical Works Ltd., Budapest, Hungary.
[2] Merck, Darmstadt, Federal Republic of Germany.
[3] Reanal Chemical Works, Budapest, Hungary.
[4] Reachim, Soviet Union.
[5] Erdökémia Chemical and Industrial Co., Budapest, Hungary.
[6] Model Liquochrom 307; Labor MIM, Budapest, Hungary.
[7] Model Liquodet 308; Labor MIM, Budapest, Hungary.
[8] Chromspher-Sil 10 μm; 3.9 × 250 mm; Labor MIM, Budapest, Hungary.
[9] NE-230 recorder; EMG, Budapest, Hungary.

0022-3549/84/1000-1489$01.00/0

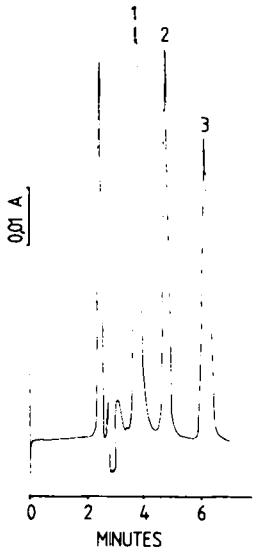


Figure 1—*Chromatograms of a mixed sample of drotaveraldine (0.5 μg), drotaverine (1.0 μg), and papaverine (2.5 μg). Key: (1) drotaveraldine; (2) drotaverine; (3) papaverine.*

A solution containing *n*-heptane–dichloromethane–diethylamine (50:25:2) was filtered[10] and deaerated by refluxing in a water bath. Elution was performed at room temperature. The mobile phase was prepared fresh daily.

Standard Solutions—Solutions of drotaverine hydrochloride in water (25, 50, 100, 150, and 200 μg/mL) were used to spike plasma samples. Papaverine hydrochloride dissolved in chloroform (1000 μg/mL) was used as the internal standard.

Quantitation—Control plasma from rat and dog whole blood was drawn into heparinized tubes, and 10 μL of each standard solution was added to 1.00 mL of the plasma. The spiked plasma samples were adjusted to pH 1.5 using a few drops of 1 M hydrochloric acid. Following the addition of 10.0 mL of chloroform to each tube, the tubes were capped and then shaken on a shaker[11] for 30 min. The mixtures were centrifuged[12] for 10 min, at 4000×*g*. The separated chloroform layers were acidified with 40 μL of glacial acetic acid and then dried with anhydrous sodium sulfate. Eight milliliters of the solution was evaporated to dryness under reduced pressure. Each residue was redissolved in a mixture of 70 μL of chloroform and 10 μL of the internal standard solution. Twenty microliters of the solution was injected onto the column and chromatographed.

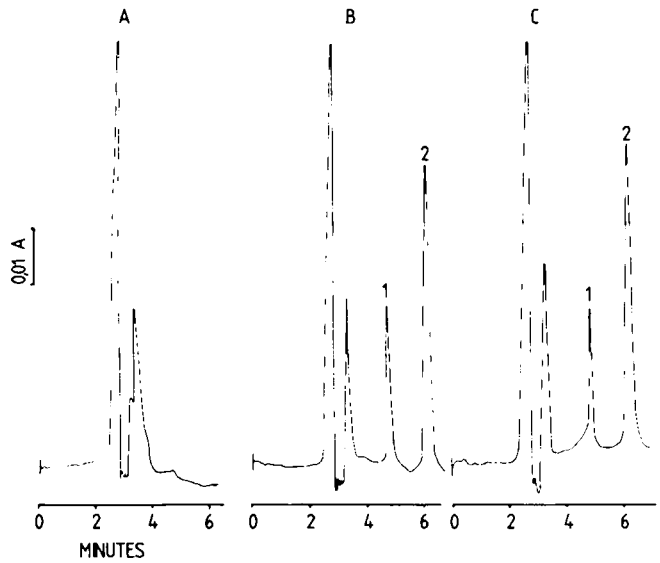


Figure 2—*Chromatograms of extracts from a plasma blank (A), spiked plasma (2.0 μg/mL) (B), and plasma from a rat administered drotaverine (C). Key: (1) drotaverine; (2) papaverine, internal standard.*

[10] G-4 type, 5–15 μm pore size; Schott Jena, Democratic Republic of Germany.
[11] Vibrotherm; Labor MIM, Budapest, Hungary.
[12] Janetzky, Model T-30; Jena, Democratic Republic of Germany.

Table I—Linearity of the Assay Method[a]

Drotaverine Concentration, μg/mL	Peak Height of Drotaverine, mm	Peak Height of Papaverine, mm
0.25	8.3 ± 0.8	103 ± 2.1
0.50	15.0 ± 1.1	108 ± 4.3
1.00	31.3 ± 3.0	103 ± 4.3
1.50	47.5 ± 3.7	108 ± 5.7
2.00	63.1 ± 5.9	108 ± 2.7

[a] Mean ± *SD*; $n = 8$.

A standard curve was prepared by plotting the ratios of peak heights (drotaverine from the extracted plasma samples–internal standard) *versus* the drotaverine concentrations expressed as micrograms per milliliter of plasma. Plasma drotaverine recovery levels were determined by comparing the peak heights of the spiked plasma standards with those of unextracted drotaverine standards (taking into consideration that only 8.0 mL of the original 10.0 mL of chloroform was analyzed).

Sample Preparation and Assay—Drotaverine was orally administered as an aqueous solution of 1.2% to Long Evans rats (200 g) through a gastric tube in doses of 60 mg/kg, and to beagle dogs (10–11 kg) in capsules containing 300 mg of the active substance. Before drug administration, the animals had been fasted overnight with water *ad libitum*. Blood samples were taken prior to dosing and at specified times (0.5, 1, 2, 4, 6, 8, and 24 h) postdose. In the case of rats, three animals at each sampling time were exsanguinated by aortal puncture, and the blood samples were collected separately. For the three beagle dogs, blood samples (2.0 mL) were withdrawn at different time intervals through the saphenous vein. The samples were collected in heparinized, evacuated tubes and centrifuged. One milliliter of each plasma sample was assayed immediately, using the procedure described above for drotaverine-spiked plasma.

Having measured the peak-heights, the ratios (extracted drug–internal standard) were calculated, and the plasma drotaverine levels were read from the calibration curve.

RESULTS AND DISCUSSION

Under the described chromatographic conditions, drotaverine was eluted from the column in 4.7 min and it could be separated from its metabolite, drotaveraldine, which appeared at 3.6 min. The retention time of the internal standard was 6 min (Fig. 1).

After 20–30 samples, the retention time of drotaverine changed gradually to ~4.3 min, due to the decreased retention capacity of the column. To remove impurities from the plasma, the column was washed with methanol[3]; thereafter, the retention capacity was controlled by injecting standard solutions of drotaverine and papaverine onto the column.

Representative chromatograms of blank and spiked plasma extracts and

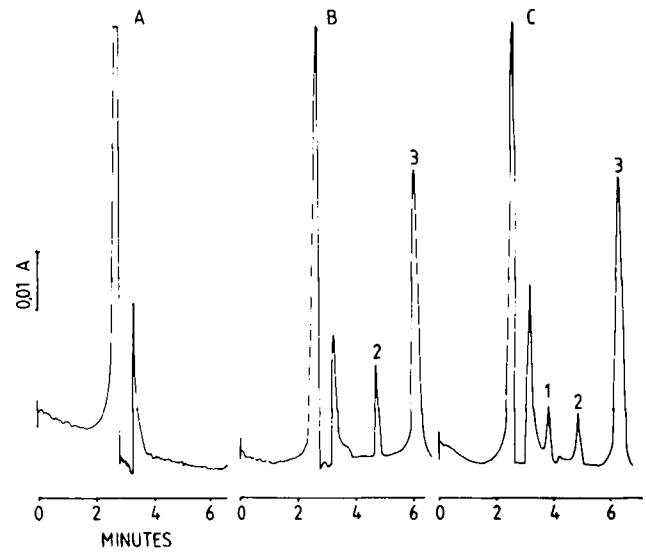


Figure 3—*Chromatograms of extracts from a plasma blank (A), spiked plasma (1.0 μg/mL) (B), and plasma from a dog administered drotaverine (C). Key: (1) drotaveraldine; (2) drotaverine; (3) papaverine, internal standard.*

Table II—Recoveries of Drotaverine from Spiked Rat and Dog Plasma [a]

Drotaverine Concentration, μg/mL	Recovery from Rat Plasma, %	Recovery from Dog Plasma, %
0.25	83.2 ± 4.7	82.8 ± 6.8
0.50	77.0 ± 9.1	84.4 ± 5.2
1.00	82.3 ± 6.5	85.8 ± 8.8
1.50	89.8 ± 6.0	87.9 ± 4.9
2.00	94.3 ± 7.3	88.7 ± 7.1

[a] Mean ± *SD*; $n = 8$.

plasma extracts from a rat given 60 mg/kg of drotaverine, and from a dog given 30 mg/kg of drotaverine, are shown in Figs. 2 and 3.

The drotaverine ultraviolet spectrum has maxima at 242, 302, and 350 nm wavelengths. A variable-wavelength UV detector set at 302 nm was used to eliminate background contributions to the drotaverine peak at 242 nm in blank plasma extracts. The intensity of the maximum at 350 nm is lower than that of the maximum at 302 nm. The detection limit was found to be 50 ng of drotaverine/mL of plasma.

Data in Table I show the linearity of the assay method. A linear regression analysis was performed for the calibration curve prepared from drotaverine-spiked plasma samples. It resulted in the equation $y = 0.2912x + 0.0038$, where y is the ratio of the peak height of the drug divided by the peak height of the internal standard, and x is the drug concentration in the plasma. As evidenced by the experimental data, peak heights were proportional to the drotaverine concentrations for the range examined (from 0.25 to 2 μg of drotaverine/mL of plasma).

Recoveries of drotaverine were nearly quantitative at plasma concentrations of 0.25–2.0 μg/mL, with average recoveries ranging from 77.0 to 94.3% and coefficients of variation ranging from 4.7 to 9.1% (Table II).

REFERENCES

(1) Z. Mészáros, P. Szentmiklósi, and G. Czibula, U.S. Pat. No. 3,337,539, August 22, 1967.

(2) P. Szentmiklósi, Z. Mészáros, *et al.*, U.S. Pat. No. 4,035,366, July 12, 1977.

(3) P. Szentmiklósi and S. Marton, *Acta Pharm. Hung.*, **49**, 50 (1979).

(4) M. H. Rutz-Coudray, L. Balant, C. Revillard, P. Buri, and J. Giust, *Pharm. Acta Helv.*, **51**, 258 (1976).

(5) K. U. Ushbaev and V. A. Katasov, *Gyógyszerészet*, **22**, 424 (1978).

(6) J. Axelrod, R. Schafer, J. K. Inscoe, W. M. King, and A. Sjoerdsma, *J. Pharmacol. Exp. Ther.*, **124**, 9 (1958).

(7) E. Renier, K. Kjeldsen, and M. Pays, *Feuill. Biol.*, **106**, 111 (1979).

Influence of α- and β-Adrenergic Antagonists on Dopamine-Induced Responses in the Isolated Heart of *Mercenaria mercenaria*

DAVID E. MANN, JR. [x] **and RONALD F. GAUTIERI**

Received December 6, 1982, from the *Department of Pharmacology, School of Pharmacy, Temple University, Philadelphia, PA 19140* Accepted for publication September 8, 1983.

Abstract □ Agents with predominantly adrenergic antagonistic properties (the α-blockers, phentolamine and tolazoline, and the β-blocker propranolol), and those with suspected α-blocking capabilities [hydralazine and 3,3′-(4,4′-biphenylene)bis(2,5-diphenyl-2*H*-tetrazolium chloride) (neotetrazolium chloride); I] were added individually to a bath containing an isolated *Mercenaria mercenaria* heart. Two and one-half minutes later, dopamine was added to the bath as the second drug and cardiac responses were noted. Pretreatment with saline controls, followed by dopamine 2.5 min later, produced results that were identical with those which occurred after the administration of dopamine alone, *i.e.*, marked stimulation and cardiac arrest. Pretreatment with phentolamine and I were the only procedures that prevented dopamine-induced cardiac arrest. Phentolamine, tolazoline, and hydralazine generally produced positive inotropic responses when initially added to the bath, whereas propranolol mimicked the effects caused by the addition of dopamine alone or after saline pretreatment. The *M. mercenaria* heart appears to possess an adrenergic receptor of an α-configuration.

Keyphrases □ α- and β-Adrenergic antagonists—dopamine-induced response, heart of *Mercenaria mercenaria* □ Dopamine—α- and β-adrenergic antagonists, effect on heart of *M. mercenaria*, pretreatment controls.

A number of studies have suggested the presence of an adrenergic receptor in the smooth muscle of the heart of *Mercenaria mercenaria*, the hard-shelled clam or quahog. Welsh and Taub (1) administered various drugs to the isolated heart of *M. mercenaria* and noted that epinephrine evoked negative inotropic responses and tachyphylaxis. Fujita and Mann (2) reported similar responses with norepinephrine under comparable experimental conditions. Ciuchta and Mann (3) examined the relative effectiveness of ephedrine isomers in preventing the onset of norepinephrine-induced tachyphylaxis in the *M. mercenaria* heart and found that the *l*-isomer was the most potent, the racemate less potent, and the *d*-isomer least potent. Orzechowski and Mann (4) performed a similar experiment with amphetamine isomers and observed that norepinephrine-induced tachyphylaxis was blocked most effectively by the *d*-isomer, while the *d,l*- and *l*-isomers were equal but less potent. When higher doses of amphetamine isomers were given, norepinephrine elicited positive inotropic responses, suggesting that these agents act upon common receptor sites. It was further speculated that amphetamine, like ephedrine, may block cardiac inhibitory sites on which norepinephrine acts, thus allowing ephedrine to cause stimulation.

Dopamine, which is present in *M. mercenaria* nervous tissue (5), appears to activate excitatory receptors in the clam heart in contrast to epinephrine and norepinephrine, which affect inhibitory receptors. Tachyphylaxis also occurs in the *M. mercenaria* heart after successive, equal doses of dopamine[1].

The purpose of this study was twofold:

1. To determine whether the α-blockers (tolazoline, phentolamine, and hydralazine) and the β-blocker (propranolol) could prevent the excitatory effects of dopamine on the isolated *M. mercenaria* heart, thereby elucidating the nature of its adrenergic receptor;

2. To ascertain whether the hypotensive agent, 3,3′(4,4′-biphenylene)bis(2,5-diphenyl-2*H*-tetrazolium chloride) (neotetrazolium chloride; I), which has both adrenergic α-blocking and gangliolytic actions (6–8), could antagonize

[1] Unpublished results.

0022-3549/84/1000-1491$01.00/0