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Determination of Orally Coadministered Nadolol and Its Deuterated Analogue in Human Serum and Urine by Gas Chromatography with Selected-Ion Monitoring Mass Spectrometry

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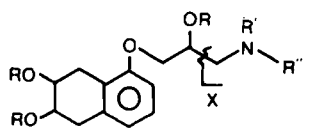
Abstract □ A cartridge serum and urine extraction procedure of the β-adrenergic antagonist, nadolol, employing a cross-linked styrene-divinyl benzene macroreticular resin is described. Samples were analyzed as the silylated derivative by gas chromatography–mass spectrometry (GC–MS) using selected-ion monitoring. When nadolol was orally coadministered with its deuterated analogue, relative bioavailability could be demonstrated with six or fewer subjects. Employing a base-deactivated GC phase, the limit of detection is 1 ng and 0.5 ng/mL of serum for nadolol and the deuterated analogue, respectively. For levels of <10 ng/mL, the respective coefficients of variation are 4 and 2%. For concentrations of >10 ng/mL, the *CV* is 1% for nadolol and nadolol-d_9.

Keyphrases □ Nadolol—human serum and urine, GC–MS, selected-ion monitoring, nadolol-d_9 □ GC–MS—selected-ion monitoring, nadolol, human serum and urine, nadolol-d_9

Nadolol (I) is a nonselective β-adrenergic antagonist recommended for the treatment of hypertension and angina pectoris (1, 2). Dreyfuss *et al.* have determined bioavailability parameters of orally and intravenously administered [^{14}C]-nadolol (3, 4) in mildly hypertensive humans. Nadolol is partially and variably absorbed (1), like other β blockers, but it is eliminated without metabolism. With oral administration, the pharmacokinetic profile of nadolol is described by an open two-compartment model with respective absorption, distribution, and elimination half-lives of ~1, 1.5, and 17 h (3, 4).

A gas chromatography–selected-ion monitoring mass spectrometry (GC–SIM MS) determination of nadolol serum levels was previously reported (5). Carlin *et al.* (6) have recently presented an elegant application of GC–SIM MS to the measurement of low levels of the β-blocker timolol and its deuterated analogue in a coadministration bioavailability evaluation. Coadministration bioavailability studies for other drug types have been reported by Strong *et al.* (7), Wolen *et al.* (8), d'A. Heck *et al.* (9), Walle and Walle (10), Alkalay *et al.* (11), Murphy and Sullivan (12), and most recently by Cohen *et al.* (13). The extraction and isolation procedure (5) has been simplified for more effective sample processing by GC–SIM MS (13). The bioavailability of two 80-mg formulations of nadolol (I) given to six subjects, administered either as a tablet or solution along with a concomitant 80-mg solution of nonadeuterated nadolol (II) is presented.



		R	R'	R''	X EI	MH$^+$ PCI
Nadolol	I	H	H	$C(CH_3)_3$		
Deuterated nadolol	II	H	H	$C(CD_3)_3$		
Internal reference	III	H	CH_3	$C(CH_3)_3$		
	IV	$Si(CH_3)_3$	H	$C(CH_3)_3$	86	526
	V	$Si(CH_3)_3$	H	$C(CD_3)_3$	95	535
	VI	$Si(CH_3)_3$	CH_3	$C(CH_3)_3$	100	540

EXPERIMENTAL SECTION

Reagents—Nadolol (I)[1] and its *N*-methyl derivative (III)[1] were characterized, pharmaceutical-grade materials (14). The structures and purity were confirmed by elemental analyses, ^{1}H-NMR, and MS (15) and by GC–MS after silylation. The nonadeuterated nadolol (II) was prepared from the

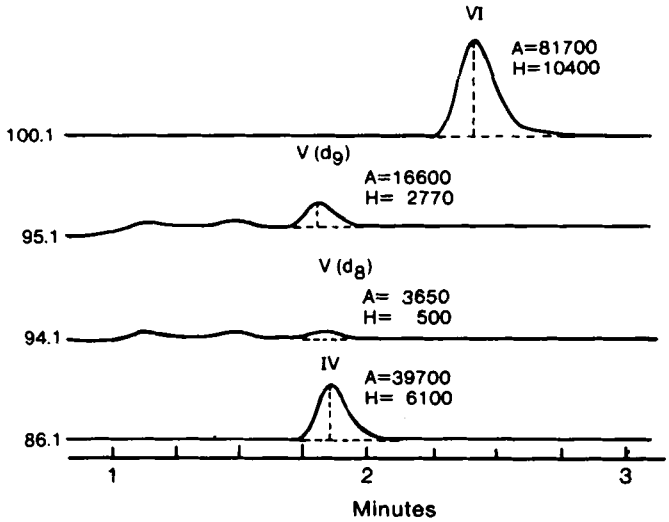


Figure 1—*EI–SIM of a typical processed serum extract containing 133, 83.4, and 250 ng/mL of I, II, and III, respectively, measured as their trimethylsilylated derivatives, IV, V, and VI.*

[1] Nadolol (CAS 42200-33-9), *N*-methyl derivative (CAS 67247-47-6); E. R. Squibb & Sons, Princeton, N.J. Deuterium-labeled *tert*-butylamine; Merck, Sharp and Dohme of Canada.

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Table I—Serum Concentrations of Nadolol for Subjects 1-6 (Solution *versus* Deuterated Solution)

Hours Postdose	Serum Concentration, ng/mL: Deuterated Solution (II) 1	2	3	4	5	6	Solution (I) 1	2	3	4	5	6
1	89.3	39.0	37.5	50.1	29.7	33.0	113.0	46.1	44.7	63.7	31.7	35.6
2	178.0	74.2	33.6	49.6	21.5	26.9	197.0	92.2	39.3	59.5	23.8	30.7
3	118.0	314.0	76.4	70.7	45.3	209.0	136.0	369.0	100.0	86.5	53.6	247.0
4	98.4	282.0	88.8	61.8	127.0	184.0	112.0	314.0	110.0	80.5	122.0	209.0
5	90.5	179.0	65.9	64.9	90.7	83.3	113.0	208.0	82.4	86.0	129.0	97.9
6	55.8	115.0	69.9	67.4	69.8	75.2	68.3	134.0	86.9	68.7	85.4	92.2
8	51.2	113.0	58.5	51.0	68.0	78.0	64.1	131.0	72.0	62.8	71.0	80.2
12	41.9	62.1	32.8	29.0	41.2	47.1	48.8	74.6	40.5	39.8	50.4	59.1
24	22.0	31.5	19.3	20.6	26.8	18.9	27.1	36.1	26.6	24.6	31.3	22.2
48	11.5	6.5	7.9	9.4	2.5	2.2	10.6	8.3	10.7	13.5	4.0	3.0

epoxyketal intermediate (VII) (14) by the reaction with 98 atom-% deuterium-labeled *tert*-butylamine (VIII)[1].

Synthesis of II—A solution of 17 mL of methanol containing 4.9 g (17.7 mmol) of VII and 4.3 g (52.4 mmol) of V was refluxed for 2.5 h using a dry-ice condenser. The volatiles were then distilled into a dry-ice trap at 60–70°C at 20 torr and used again until the remaining VIII was <10% of the original amount. The combined residues were purified by extraction with 100 and 50 mL of 6% w/v aqueous citric acid from 100 mL of ether. The aqueous layer was adjusted to pH 10.8 with 5 M NaOH and then was extracted with 200 mL of ether (4×). The dried residue was dissolved in 60 mL of warm water containing 8 mL concentrated hydrochloric acid, and was allowed to stand for 3 h. The pH of the cooled aqueous solution was adjusted to pH 7.8 with 5 M NaOH, washed with 100 mL of dichloromethane, then made to pH 10.2 with 10 M NaOH, saturated with sodium chloride, and exhaustively extracted with dichloromethane. The pooled extract was then treated with activated charcoal and concentrated under reduced pressure. The crude product was crystallized from 350 mL of warm acetone and then cooled to room temperature. The slurry was cooled in an ice bath, and 350 mL of hexane was gradually added. The crystalline material was collected by filtration, and then the material was washed with 100 mL of cold acetone–hexane (1:1) and dried, to give (70% yield) a crystalline powder, mp 128–130°C; IR (KBr): 2208 cm^{-1} (C—D stretch); 100-MHz ^{1}H-NMR (methanol-d_4): δ 2.5–3 (m, 6, CH_2N, 2 CH_2Ar), 6.5–7.1 (m, 3, ArH), and 3.8–4.1 ppm (m, 5, CH—O) [identical patterns as I except δ 1.12 ppm (s, 9, CH_3) is replaced with a broad weak peak (CD_2H—)]; EI-MS M^+: *m/z* 318, 300 ($M^+ - CD_3$), 274 (rearrangement, $M^+ - C_2H_4O$), and 95 [$CH_2NHC(CD_3)_3$ base ion] (estimated octadeutero analogue 10–15%). EI GC-MS of V M^+: *m/z* 534 (absent), 519 ($M^+ - CH_3$), 516 ($M^+ - CD_3$), 95 (base ion) (*c.f.*, Fig. 1); PCI GC-MS of V MH^+: *m/z* 535 (*c.f.*, Fig. 2) (estimated octadeutero analogue 10–15%).

Anal.—Calc. for $C_{17}H_{18}D_9NO_4$: C, 64.12; D and H, 11.39 (8.55 as H); N, 4.40. Calc. for $C_{17}H_{27}NO_4$: C, 65.99; H, 8.80; N, 4.53. Found: C, 64.10; H, 8.69 (H equivalent to 27 atoms/mol), N, 4.38.

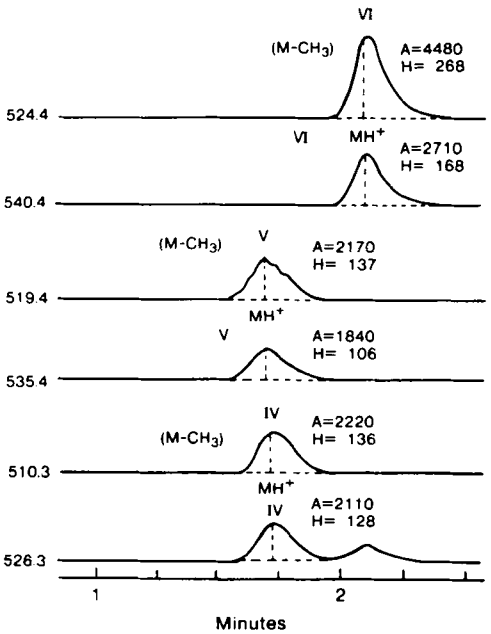

Figure 2—*PCI-SIM of a typical processed serum extract containing 122, 127, and 250 ng/mL of I, II, and III, respectively, measured as their trimethylsilylated derivatives, IV, V, and VI.*

The extraction cartridges[2] were used as received. *N*-(Trimethylsilyl)imidazole in pyridine[3] (1.5 mEq/mL) was obtained in 1-mL sealed ampules.

Standard Solutions—Stock solutions of I, II, and III prepared in 0.1 M HCl were refrigerated and used for at least 6 months. Diluted working solutions were prepared daily in 0.1 M HCl.

Equipment—Various equipment used in the sample preparation include: evaporators[4,5], vortex mixer[6], reaction vials[7], vial seals[8], crimper[9], centrifuge[10], screw-cap culture tubes[11], disposable Pasteur pipets[12], syringes[13], repipet dispenser[14], and dessicators.

Extraction Procedure—*Serum*—Thawed and centrifuged 1-mL serum samples, processed in groups of 35, were transferred to the extraction cartridges[2], placed on 150 mL screw-top culture tubes, and 250 ng of internal reference solution III and 1 mL of distilled water were added to each cartridge. A 1.0-mL blank serum sample containing 500 ng of I, 500 ng of II, and 250 ng of III was processed with each set of samples. A calibration sample curve was prepared using blank serum to which 0–500 ng each of I and II and 250 ng of III were added.

The cartridges were centrifuged at 800 rpm for 30 s. The cartridge caps were removed, and the cartridges were then centrifuged for 5 min at 1000 rpm, and, if required at 1500 rpm until centrifugation was complete. After the addition of 4 mL of water, the cartridge was centrifuged at 1000 rpm for 4 min, followed by 1500 rpm for 1 min. To each cartridge, placed on a scintillation vial, 2 mL of methanol was added, and the cartridges were centrifuged at 1000 rpm for 5 min. The contents of the cartridge was evaporated in a sample concentrator at 65°C, reconstituted in 0.5 mL of methanol, and transferred to a 1-mL reaction vial. A solution of I, II, and III, which constitutes the unextracted control, was evaporated[4]. Each solution was evaporated[5] to dryness under nitrogen at 60°C. After drying under reduced pressure in a desiccator for 30 min, the samples were capped.

Urine—To each cartridge 0.5 or 0.1 mL of thawed urine was added, followed by 0.5 μg of III internal reference solution and 3 mL of H_2O. These samples were then processed in the same manner as serum samples.

Calibration and Preparation of Control Blood and Urine Samples—Varying amounts of I (and an equal amount of II for simultaneous measurements) were added to cartridges containing 1 mL of serum to yield concentrations from 0 to 500 ng/mL. To each cartridge, 250 ng of internal reference III was added. The control sample represents the calibration point equal to 500 ng of I and II and 250 ng of III.

The urine standard curve was prepared from urine samples containing from 0 to 1 μg of I and II and 0.5 μg of III. Only 0.1 mL of urine was needed from samples collected within the first 4 h, and 0.5 mL was required after 4 h. Like the serum control, the control urine represents the 2:1 calibration point. The ratio of the maximum peak heights *versus* the respective nanogram (or microgram) ratio was fitted by linear regression[15] (13). For serum:

$$R_1 = 0.01916 + 1.0654R'_1 \text{ (nadolol)} \qquad \text{(Eq. 1)}$$

$$R_2 = 0.00467 + 0.7969R'_2 \text{ (deuterated nadolol)} \qquad \text{(Eq. 2)}$$

[2] Type W, cross-linked styrene-divinyl benzene macroreticular resin with activators; E. I. DuPont Co., Wilmington, Del.
[3] Tri Sil Z; Pierce Chemical Co., Rockford, Ill.
[4] Model SC248 with scintillation adapter No. 3500750-4; Brinkmann Instruments.
[5] SC-3 concentrator with DB-3 dri-block heating bath; Techne.
[6] Cat. No. 12-812; Fisher Scientific Co.
[7] Cat. No. 3-3123, 1-mL; Supelco, Bellefonte, Pa.
[8] Cat. No. 5080-8713; Hewlett-Packard Co.
[9] Cat. No. 6-406-20; Fisher Scientific Co.
[10] International Centrifuge Co.
[11] Cat. No. 14-930-10J, 25 × 150 mm; Fisher Scientific Co.
[12] Cat. No. 13-678-6B, Pasteur Type, 23-cm long; Fisher Scientific Co.
[13] Size 100A-FN, Cat. No. 2-1536, fine needle 100 μL; Supelco.
[14] 1 mL; Lab Industries.
[15] The BASIC program for the HP 5985B GC–MS is available on request.

Table II—Serum Concentrations of Nadolol for Subjects 1–6 (Tablet *versus* Deuterated Solution)

Hours Postdose	Serum Concentration, ng/mL: Deuterated Solution (II) 1	2	3	4	5	6	Tablet (I) 1	2	3	4	5	6
1	47.8	56.3	18.5	52.9	19.7	13.1	67.4	77.9	8.7	67.1	20.4	14.9
2	54.0	94.7	81.6	73.6	16.9	41.0	68.0	124.0	84.8	92.3	17.8	47.7
3	136.5	234.0	114.0	126.0	29.8	83.4	165.0	269.0	128.0	153.0	38.8	133.0
4	119.0	177.0	132.0	92.9	80.8	85.5	140.0	210.0	143.0	116.0	109.0	144.0
5	121.0	118.0	105.0	68.6	61.6	56.2	111.0	141.0	117.0	85.7	80.8	91.9
6	79.3	107.0	92.4	62.9	43.7	47.1	90.9	128.0	106.0	79.4	61.2	77.4
8	64.9	90.0	86.1	50.9	37.4	42.9	78.0	116.0	100.0	65.1	48.9	69.5
12	51.0	59.3	52.2	30.9	26.7	37.5	48.5	74.9	59.3	39.0	34.8	55.7
24	19.9	23.8	27.0	18.2	9.8	15.1	23.3	28.4	31.8	26.6	13.2	21.2
48	4.4	6.6	7.0	7.5	3.9	2.0	6.1	9.4	7.8	10.7	4.7	4.8

For urine:

$$R_1 = 0.05734 + 0.9781R'_1 \text{ (nadolol)} \quad \text{(Eq. 3)}$$

$$R_2 = 0.04410 + 0.8248R'_2 \text{ (deuterated nadolol)} \quad \text{(Eq. 4)}$$

where R'_1 and R'_2 are concentration ratios and R_1 and R_2 are the measured ratios (13).

Selected-Ion Monitoring—The extracted samples were reconstituted in 40 μL of *N*-(trimethylsilyl)imidazole in pyridine[3] at least 1 h prior to analysis. *N*-(Trimethylsilyl)imidazole (0.25 μL in pyridine) was drawn into a 10-μL syringe, followed by 1 μL of solution. The appropriate ion profiles (*m/z* 86.1, *m/z* 95.2, and *m/z* 100.2) were obtained by selected-ion monitoring (SIM). The maximum peak heights were measured using a program written especially for the batch processing of data (13).

Instrumental Procedure—The combined GC–MS, with the data system[16], was tuned[17] for electron-impact (EI) MS from 69 to 502 D using perfluorobutylamine at an ionization voltage of 70 eV and at an emission of 300 μA. The ion source was controlled at a constant temperature of 200°C. For positive chemical ionization (PCI) MS the source parameters were optimized at an ionization voltage of 300 eV.

A septum guide[18] was attached to the GC injector. The analysis was carried out on an 80-cm × 2-mm i.d. silanized glass column packed with 3% SP-2100DB[19], which was connected through a single-stage jet separator. The transfer line and separator were maintained at 275°C, while the column was kept at an isothermal temperature of 230°C and the injector was heated to 265°C. Typical retention times of 1.9 min for the silylated compounds (IV and V) and 2.5 min for the silylated internal reference (VI) were obtained at a helium flow rate of 30 mL/min (Fig. 1). Helium, otherwise diverted, was allowed to flow into the ion source at 1.3 min after sample injection until the end of the measurement. The multiplier and electron voltages were only applied during the data collection period, from 1.5 min after injection until the termination of the run.

Positive CI GC–MS was obtained with methane as the GC carrier gas, at a flow rate of 20 mL/min, flowing directly into the source to create a 1-torr source pressure (Fig. 2). Otherwise, the chromatographic conditions were identical to that of the EI GC–SIM MS.

Drug Administration—All subjects fasted for 8 h prior to and for 4 h after dosing. Twenty-milliliter blood samples were drawn[20] from an antecubital vein, under sterile conditions. The whole blood was allowed to clot and was then centrifuged. Urine was collected for 0–4, 4–8, 8–12, 12–24, and 24–48 h after dosing. After measurement of the total volume for each collection, a 15-mL aliquot was transferred to a small, labeled plastic container and frozen.

RESULTS AND DISCUSSION

The cartridge extraction procedure (16) is based on a column packed with cross-linked styrene–divinyl benzene macroreticular resin, processed manually with a conventional laboratory centrifuge. Timolol samples were separated on a reverse-phase C_{18} cartridge, supplemented with acid–base extractions (6). Using [^{14}C]nadolol as a tracer, nadolol is quantitatively absorbed on the column from pH 2–13.5 solutions. Washing with distilled water removed potentially interfering serum components without disturbing the absorbed I. Nadolol is quantitatively displaced with methanol, but <80% is displaced by equal amounts of acetone. Lower polarity solvents such as dichloromethane did not displace I at all. On the other hand, solvent mixtures containing ≥25% methanol also produced quantitative elution. The radioactivity was quantitatively recovered with 0.5–2 mL of methanol. The dried eluate was sufficiently pure to be directly silylated for GC–MS analysis. Similarly, processed urine samples yielded excellent recoveries for microgram quantities of I. This isolation procedure directly replaces the fluorometric (17) and MS (5) extraction procedures. It is also likely that other β-adrenergic agents would behave similarly in this extraction system.

The GC–MS SIM processing efficiency was improved by reducing "ghosting" and tailing of the underivatized secondary amine. A glass column containing a specially base-deactivated methyl silicone polymer packed phase[19] was operated isothermally. Background components with interfering ions were minor, although the background at *m/z* 95 was somewhat greater than at the even-mass *m/z* 86 and 100 ions (Fig. 1). Confirmation of the structures of the silylated derivatives was made by PCI GC–MS SIM (Fig. 2). For I the limit of detection is at least 1 ng/mL, and for the deuterated analogue (II) it is ~50% of that value. Nine of twelve time-zero samples were found to have no detectable I, while two contained 0.1 ng and another 0.3 ng per milliliter. All 12 deuterated analogue (II) values were measured as zero. All 48-h serum samples had nonzero values (Tables I–III). The estimated *CV* for measurements at 10 ng/mL are 4% for I and 2% for II, which decreases to 1% for both I and II at greater concentrations.

Urine contained from 0.2 to 24 μg/mL of I and II. As a percentage of the dose, the amounts excreted with time were not necessarily influenced by urine

Table III—Mean Serum Concentrations of Nadolol After Concomitant Administration of Perdeuterated Solution with Either a Tablet or an Oral Solution[a]

Hours Postdose	Mean Serum Concentration, ng/mL: Tablet (I)	Deuterated Solution (II)	Solution (I)	Deuterated Solution (II)
1.0				
Mean	42.7	34.7	55.8	46.4
SEM	12.7	8.0	12.3	9.0
2.0				
Mean	72.4	60.3	73.8	64.0
SEM	15.1	11.7	26.6	24.1
3.0				
Mean	147.8	120.6	165.4	138.9
SEM	30.3	27.6	49.0	42.2
4.0				
Mean	143.7	114.5	157.9	140.3
SEM	14.6	14.9	35.9	33.0
5.0				
Mean	104.6	88.4	119.4	95.7
SEM	9.3	12.1	19.1	17.3
6.0				
Mean	90.5	71.7	89.2	75.5
SEM	9.7	10.3	9.8	8.3
8.0				
Mean	79.6	62.0	80.2	70.0
SEM	10.0	9.1	10.5	9.6
12.0				
Mean	51.7	42.9	52.2	42.4
SEM	6.1	5.3	5.3	4.8
24.0				
Mean	24.1	19.0	28.0	23.2
SEM	2.7	2.5	2.0	2.0
48.0				
Mean	7.2	5.2	8.4	6.7
SEM	1.0	0.9	1.7	1.5

[a] All doses were 80 mg.

[16] Model HP5985B; Hewlett-Packard, Palo Alto, Calif.
[17] AUTOTUNE, Hewlett-Packard.
[18] Cat. No. 2-0839; Supelco.
[19] One 80/100 Supelcoport; Supelco.
[20] Vacutainers; Becton, Dickinson and Co.

Table IV—Urinary Excretion of Nadolol for Subjects 1–6 (Solution *versus* Deuterated Solution)

Hours Postdose	Urinary Excretion, % of Dose[a]											
	Deuterated Solution (II)						Solution (I)					
	1	2	3	4	5	6	1	2	3	4	5	6
0–4	1.58	3.96	1.47	1.36	1.34	1.38	1.29	4.70	1.82	1.68	1.61	1.71
0–8	4.24	8.39	3.88	2.92	3.11	5.27	5.04	9.79	4.78	3.64	3.73	6.57
0–12	5.50	10.30	4.73	3.99	5.11	6.63	6.59	12.04	5.82	5.02	6.20	8.29
0–24	7.87	13.36	5.84	6.28	7.40	7.68	9.46	15.71	7.22	7.82	9.03	9.59
0–48	10.63	17.01	7.72	8.68	8.72	8.75	12.74	20.09	9.50	10.82	10.67	10.91

[a] All doses were 80 mg.

Table V—Urinary Excretion of Nadolol for Subjects 1–6 (Tablet *versus* Deuterated Solution)

Hours Postdose	Urinary Excretion, % of Dose[a]											
	Deuterated Solution (II)						Tablet (I)					
	1	2	3	4	5	6	1	2	3	4	5	6
0–4	1.65	1.53	1.38	2.33	0.81	2.41	2.03	1.82	1.35	2.94	0.88	2.93
0–8	5.12	3.94	2.04	4.31	2.70	5.20	6.02	4.70	2.11	5.42	3.27	7.51
0–12	6.21	5.30	3.06	5.49	5.05	6.26	7.37	6.38	3.30	7.00	6.26	9.12
0–24	8.81	10.27	5.54	7.97	5.57	8.51	10.54	12.42	6.15	10.14	6.94	12.34
0–48	11.44	12.80	7.06	10.52	7.09	9.27	13.75	15.56	7.96	13.37	8.87	13.35

[a] All doses were 80 mg.

volume (Tables IV and V). The amount of drug excreted is directly related to the circulating serum levels just prior to or during the collection period.

Since the deuterated analogue (II) was administered to all subjects, an evaluation of the variation bioavailability can be readily assessed. As a rule, no subject exhibited identical bioavailability for II with respect to maximum concentration levels, time to maximum, and area under the curve. Consequently comparison of intrasubject data is unreliable, which then requires treating the composite data (Table III). With this limited data set of six subjects, sufficient smoothing has occurred to begin to show equivalence between the legs of the administration of the deuterated analogue (Table III).

Table VI—Area Under the Serum Concentration–Time Curve from 0 to 48 h

Subject	AUC_{0-48}, ng·h/mL					
	Tablet (I)	Deuterated Solution (II)	Relative Area (Ratio)	Solution (I)	Deuterated Solution (II)	Relative Area (Ratio)
1	1802	1608	1.12	1971	1681	1.17
2	2585	2092	1.24	2970	2542	1.17
3	2081	1836	1.13	1654	1287	1.28
4	1732	1326	1.31	1591	1267	1.26
5	1078	823	1.31	1716	1465	1.17
6	1641	1074	1.53	1908	1626	1.17
Mean	1820	1460	1.27	1968	1645	1.20
SEM	204	195	0.06	209	192	0.02
	Tablet *versus* Solution[a]					
			Absolute Area			Relative Area
	RSD, %		14.4			11.0
	Number of Subjects Required		7.2 (=8)			4.2 (=6)

[a] No statistically significant differences were observed between tablet and solution or between deuterated solutions.

Table VII—Mean Cumulative Urinary Excretion of Nadolol

Interval	Mean Cumulative Excretion, % of Dose							
	Tablet (I)	Deuterated Solution (II)	Relative Excretion (Ratio)	Solution (I)	Deuterated Solution (II)	Relative Excretion (Ratio)	No. of Subjects Required[a]	
							Absolute	Relative
0–4 h								
Mean	1.99	1.68	1.16	2.24	1.85	1.22	98	2
SEM	0.34	0.25	0.04	0.49	0.42	0.01		
0–8 h								
Mean	4.84	3.88	1.22	5.59	4.63	1.21	54	4
SEM	0.79	0.52	0.05	0.95	0.83	0.01		
0–12 h								
Mean	6.57	5.23	1.24	7.32	6.04	1.22	36	2
SEM	0.78	0.48	0.05	1.04	0.92	0.01		
0–24 h								
Mean	9.76	7.78	1.25	9.80	8.07	1.22	12	2
SEM	1.09	0.77	0.05	1.24	1.11	0.01		
0–48 h								
Mean	12.14	9.70	1.25	12.44	10.25	1.22	10	2
SEM	1.23	0.95	0.04	1.57	1.41	0.01		

[a] No statistically significant differences were observed between tablet and solution or between deuterated solutions.

The urinary excretion patterns of nadolol (I) and nadolol-d_9 (II) are summarized in Tables IV and V. Depending on the drug form, subject, and leg, 8–20% (mean 12%) and 7–17% (mean 10%) of the respective doses of I and II are excreted in 48 h. Although not entirely unexpected, the deuterated solution formulation (II) has lower bioavailability than either the solution or tablet formulations of I. From the statistical criteria applied to absolute bioavailability data, the deuterated solution formulation is marginally bioequivalent, differing by <20% from the nondeuterated tablet and solution formulations. The tablet and solution formulations of I are bioequivalent, on the other hand, and the urinary excretion values also parallel these observations.

The use of the nonadeuterated analogue was based on the availability of intermediates. Apparently extensive deuterium labeling in the side chain resulted in reduced absorption and lower bioavailability. While it might have been more appropriate to have used ^{13}C- and ^{15}N-labeled nadolol to reduce the isotope effects, the deuterated form yielded reliable results which paralleled those of the nonlabeled forms of I.

The mean serum values of the area under the bioavailability curve can be calculated from the absolute areas in the conventional way (18)[21] or from the relative area, defined as absolute area the formulation of I divided by the value for the coadministered II (Table VI). From the absolute area, seven to eight subjects would be required as the minimum number to demonstrate bioequivalence, while only four to five subjects would be required to show the bioequivalence using coadministration[21]. Increasing this number to six ensures staying within the confidence of establishing bioequivalence. The use of the relative parameter is even more striking in the evaluation of the urinary excretion data, which required a minimum of 10 subjects in the normal approach and either 2 or 4 subjects using relative bioavailability (Table VII).

For studies employing analytical methods with reasonable precision, the limitation in bioavailability studies continues to be in the variability of the disposition by the subjects. In these instances, the use of coadministration represents the optimum method of generating a reliable data set with a minimum of subjects. Rapidly processed, an early evaluation of the bioavailability of a new dosage form can be made. It also takes the greatest advantage of the MS selective capability. Finally, the introduction of new low-priced high-performance EI mass selective detectors should result in a wider acceptance of coadministration relative bioavailability and absolute bioavailability MS evaluations.

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A New Series of Reductive Amination Derivatives of Daunorubicin: Syntheses, Partition Coefficients, and DNA Binding

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Abstract □ A series of daunorubicin derivatives were prepared by sodium cyanoborohydride reductive amination of daunorubicin with appropriate amines. All derivatives were found to bind quite strongly to DNA and viscosity increases with linear DNA indicated that each formed an intercalation complex. A range of octanol-aqueous buffer partition coefficients was obtained, around the values of daunorubicin and dauxorubicin hydrochloride, by varying the character of the starting amine. All monoamine derivatives had activity against P388 leukemia in mice which was similar to that of daunorubicin. A diamine derivative had reduced activity against P388. Several anthracyclines administered as DNA complexes had similar activity against P388 but significantly reduced toxicity compared to the uncomplexed compounds. For anthracyclines which bind strongly to DNA, optimum activity against P388 leukemia in mice seems to be centered on compounds with octanol-buffer partition coefficients in the range of 0.5–0.8.

Keyphrases □ Anthracyclines—new derivatives, antineoplastic agents □ Toxicity—reduced cardiotoxicity, anthracyclines

The glycosidic anthracycline antibiotics daunorubicin (I) and dauxorubicin hydrochloride (II), are being clinically used as antineoplastic agents (1–8). Unfortunately, these drugs are severely limited by their dose related cardiotoxicity (1–5). Although the molecular basis of the cardiotoxicity is not clear, two proposed mechanisms are widely accepted. First, the anthracyclines are reduced to semiquinone free radicals and, subsequently, generate oxygen radicals which cause membrane lipid peroxidation and DNA strand scission (9–11). Second, the chemically labile glycosidic bonds of I and II are cleaved by glycosidases to form the respective aglycons during *in vivo* metabolism (1–4, 9, 12). The water insoluble aglycons accu-

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