Table II—Activity of Podocarpic Acid Derivatives Against L1210 Lymphoid Leukemia

Compound	Host Mice[a]	Weight Difference,[b] g	Dose, mg/kg	T/C[c]
IIb	BDF_1	−0.8	400	101
		−0.2	200	101
		0.5	100	97
	CDF_1[d]	−0.8	200	112
		−0.2	100	100
		0.9	50	103
VII	BDF_1	0.7	400	87
		0.7	200	90
		−0.3	100	90
	CDF_1	−0.5	400	103
		−1.0	200	106
		−1.1	100	104
VIII	BDF_1	−0.2	400	101
		0.0	200	100
		−0.1	100	103
IX	BDF_1	1.1	400	87
		−0.3	200	89
		−0.3	100	92
X	BDF_1[e]	−0.4	400	90
		−0.4	200	88
		−0.4	100	97
		−0.2	50	100
		−0.1	25	100

[a] Intraperitoneal injections with saline with Tween 80 as a vehicle unless otherwise indicated. [b] Average weight change of test group minus average weight change of control animals. [c] Ratio of the mean survival time of the test animals to the control animals. [d] P388 lymphocytic leukemia screen. [e] Tested using another screen.

49 g (81%) of bromoketone IIb as a white crystalline solid, mp 142–144°C [lit. mp 142–144.5°C (1), 141–142°C (12), 135–137°C (13), and 123–126°C (14)]. IR (KBr): 2950, 1725, 1684, and 1604 cm^{-1}; ^1H-NMR ($CDCl_3$): δ 0.85 (s, 3, C_{10}-CH_3) [lit. (7, 13) 0.85], 1.54 (s, 3, C_4-CH_3) [lit. (7, 13) 1.53], 3.70 (s, 3, OCH_3, ester), 3.84 (s, 3, OCH_3, ether), 5.80 (d, 1, J = 7 cps, CBrH) [lit. (7, 13) J = 7 cps], 6.85 (m, 2, ArH), and 7.80 ppm (d, 1, J = 9 cps, ArH).

Biological Procedures All biological tests were performed at the Cancer Chemotherapy National Service Center, Bethesda, Md. The general procedure, protocols, and data interpretation used at the National Cancer Institute have been published previously (15, 16).

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Isocratic Liquid Chromatographic Method for the Determination of Amoxapine and its Metabolites

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Received October 1, 1982; from *The Pharmacokinetic Drug Analysis Laboratory, Pharmaceutical Science Dept., College of Pharmacy, North Dakota State University*, and *Veterans Administration Medical Center, Fargo, ND 58105.* Accepted for publication March 23, 1983.

Abstract □ An isocratic reverse-phase liquid chromatographic method for the determination of amoxapine and its major metabolites in human plasma utilizing UV detection is described. Plasma samples were extracted with ethyl acetate after pH adjustement. The reconstituted extracts were injected onto a cyanopropylsilane column and eluted with a mobile phase consisting of 65% acetonitrile and 35% sodium acetate buffer 0.03 M and pH 6. The minimum detectable limit was <10 ng/mL of plasma. Possible interferences from other drugs which might be administered concurrently were studied. The reproducibility and precision of the method are demonstrated by the analysis of samples containing 25–600 ng/mL of plasma. The method is being applied successfully in our laboratory for the analysis of plasma from patients receiving amoxapine.

Keyphrases □ Amoxapine—isocratic HPLC method, determination of metabolites □ Metabolites—isocratic HPLC method, determination of amoxapine

Amoxapine[1], 2-chloro-11-(1-piperazinyl)dibenz[*b,f*]-[1,4]oxazepine, a relatively new antidepressant of the dibenzoxazepine class, has been shown to be a potent antidepressant (1–3). The relationship between tricyclic antidepressant plasma concentrations and therapeutic response has been studied extensively, with inconclusive results (4, 5). The side effects of tricyclic antidepressants have also been correlated to plasma concentrations with mixed results (6). Since the major metabolic pathway of amoxapine is hydroxylation (7), monitoring therapeutic levels of the parent drug and its active

[1] Lederle Laboratories, Pearl River, N.Y.

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Table I—Precision and Accuracy of the Determination of Amoxapine and Its Metabolites in Human Plasma [a]

Amount Added, ng/mL	Amount Found, ng/mL					
	Day 1			Day 2		
	I	II	III	I	II	III
25	29.3 ± 4.7	30.9 ± 2.4	26.6 ± 0.9	30.5 ± 5.7	33.4 ± 7.3	27.5 ± 6.9
50	49.2 ± 4.7	52.7 ± 5.1	48.1 ± 4.2	—	—	—
100	99.5 ± 4.0	99.9 ± 6.4	96.4 ± 4.9	99.1 ± 6.4	92.6 ± 2.4	97.2 ± 7.1
200	202.5 ± 7.5	202.2 ± 2.6	197.4 ± 55.7	—	—	—
300	305.9 ± 14.9	305.9 ± 13.0	309.6 ± 13.6	308.8 ± 18.6	308.4 ± 20.0	303.3 ± 13.4
400	413.9 ± 17.6	396.6 ± 7.3	404.2 ± 15.8	—	—	—
600	600.9 ± 16.2	593.1 ± 20.2	590.5 ± 14.9	607.4 ± 27.3	594.1 ± 24.8	593.5 ± 21.6
Correlation coefficient (r)	0.996	0.995	0.995	0.997	0.997	0.998
n	35	35	35	20	20	20
r^2	0.993	0.991	0.990	0.994	0.993	0.996

[a] Mean ± *SD*.

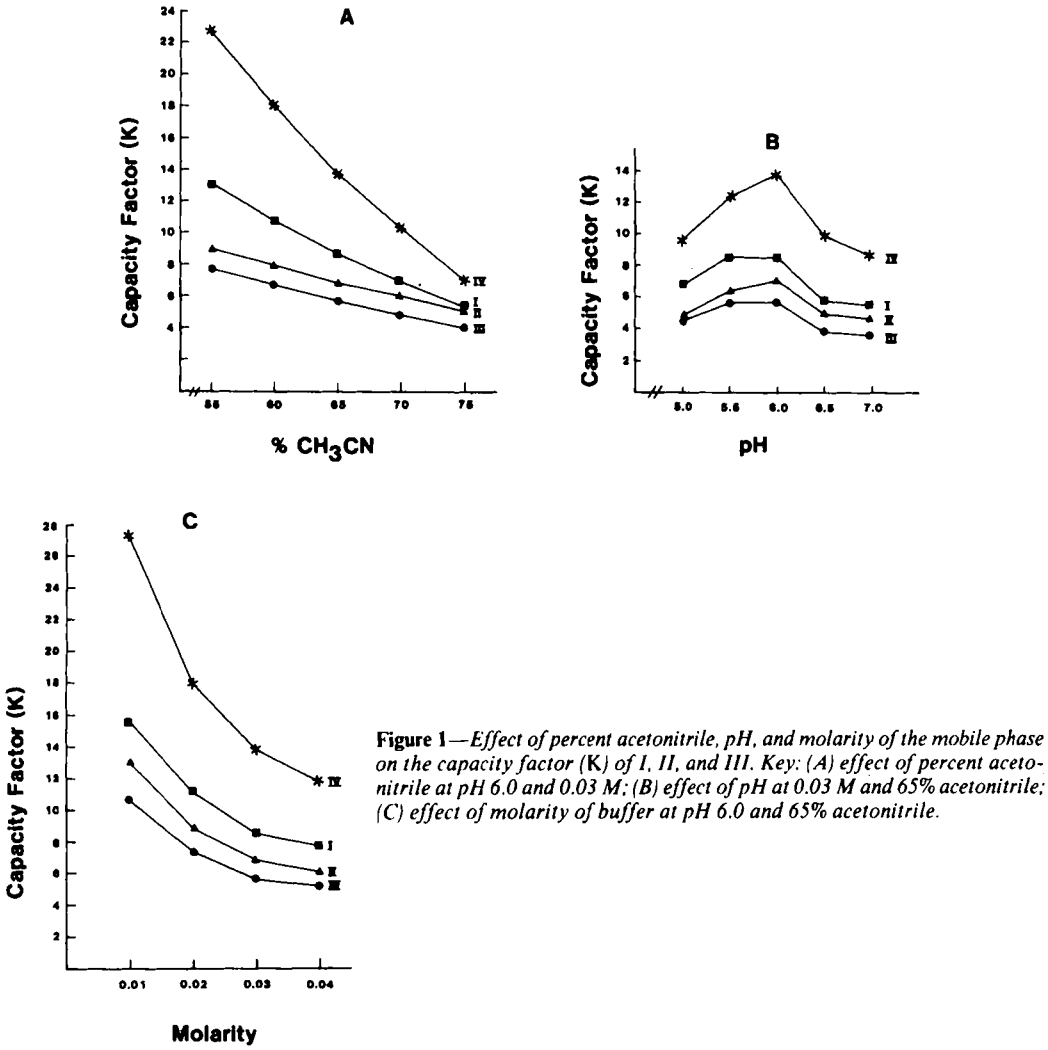

Figure 1—*Effect of percent acetonitrile, pH, and molarity of the mobile phase on the capacity factor (K) of I, II, and III. Key: (A) effect of percent acetonitrile at pH 6.0 and 0.03 M; (B) effect of pH at 0.03 M and 65% acetonitrile; (C) effect of molarity of buffer at pH 6.0 and 65% acetonitrile.*

hydroxylated metabolites is important for studying the kinetics and correlating the plasma concentrations with therapeutic response and side effects.

Methodology to determine amoxapine and its metabolites in serum has been limited to GC using derivatization and electron-capture detection (8, 9). However, recently a high-performance liquid chromatographic (HPLC) method (10) was developed using an ODS reverse-phase column and an elaborate three-step extraction procedure. The structurally related compound loxapine (*N*-methylamoxapine) has been analyzed by GC using derivatization and electron-capture detection (8, 9), nitrogen–phosphorus detection (11), or MS (9, 11).

This paper describes the separation and quantitation of amoxapine and its 7- and 8-hydroxylated metabolites from human plasma using HPLC with a reverse-phase cyanopropylsilane column and UV detection. The method has the advantages of involving only one extraction step and no derivatization. The method is sensitive and applicable to the direct determination of plasma levels in the presence of one or more

Table II—Drugs Tested for Possible Interference

Compound	Retention time, min
Diazepam	1.25
Trazodone	1.43
Lidocaine	1.43
8-Hydroxyloxapine	2.42
7-Hydroxyloxapine	2.83
8-Methoxyloxapine	3.09
Loxapine	3.23
Fluphenazine	3.20
Procaine	4.02
Perphenazine	4.65
8-Hydroxyamoxapine (III)	4.65
7-Hydroxyamoxapine (II)	5.57
Haloperidol	5.59
N-Acetylprocainamide	6.22
Amoxapine (I)	6.61
Procainamide	7.27
10-Hydroxyamitriptyline	8.27
Quinidine	8.38
Trimipramine (IV)	8.42
Thiothixene	8.72
10-Hydroxynortriptyline	9.17
p-Chlorodisopyramide	9.82
Disopyramide	10.08
Doxepin	10.44
Chlorprothixene	10.74
Trifluoperazine	11.35
Amitriptyline	11.39
Demethyldoxepin	11.65
Clomipramine	11.97
Protriptyline	12.15
Imipramine	12.33
Nortriptyline	12.35
Maprotiline	12.36
Desipramine	12.66
Chlorpromazine	13.09
Thioridazine	14.96

of several drugs which might be prescribed concurrently. The applicability of the method has been demonstrated by the analysis of plasma from patients receiving amoxapine.

EXPERIMENTAL

Instrumentation—A high-performance liquid chromatograph[2] was equipped with a cyanopropylsilane column[3], 250 mm × 4.6 mm i.d. The deaerated mobile phase was pumped through the column at 3.0 mL/min (13–15 mPa). The column compartment was maintained at 45°C, with UV monitoring at 250 nm.

Chemicals and Reagents—Sodium hydroxide, acetic acid, and sodium acetate were reagent grade. HPLC-grade ethyl acetate, acetonitrile, and methanol were used. Amoxapine (I)[1], 7-hydroxyamoxapine (II)[1], 8-hydroxyamoxapine (III)[1], and trimipramine hydrochloride (IV)[4] were supplied by the manufacturers.

Solutions—Sodium acetate solution, 0.03 M, was prepared in deionized distilled water, and the pH was adjusted to 6 by the gradual addition of acetic acid. The mobile phase consisted of 65% acetonitrile and 35% buffer.

Separate solutions of the drug, each metabolite, and the internal standard were made containing 5 mg/10 mL of methanol. A dilution to a working concentration (5 ng/μL for each) was made in methanol for the drug-metabolite group (I, II, and III). The internal standard stock solution (IV) was diluted with ethyl acetate to a concentration of 20 ng/mL.

Sample Preparation—To 1.0 mL of heparinized plasma in a 15-mL screw-capped centrifuge tube was added an aliquot (5–120 μL) of the drug and metabolite working solution containing 25–600 ng, 0.5 mL of 0.1 M NaOH, and 10 mL of extraction solution. The tubes were vortexed for 10 s and centrifuged for 5 min at 900×*g*. A 9-mL volume of the organic phase was transferred to special concentration tubes[5] and evaporated to dryness at 50°C under a gentle stream of nitrogen.

Separation and Quantitation—The residue was dissolved in 100 μL of methanol and transferred to a polypropylene microvial[6] before injection of 30 μL into the chromatograph. A standard curve was constructed utilizing a minimum of four replicate plasma extractions simulating concentrations of drug and metabolites from 25 to 600 ng/mL. The chromatograms were recorded at a chart speed of 5 mm/min. The peak heights were measured, and the ratios (drug/internal standard and metabolite/internal standard) were calculated and plotted *versus* concentration expressed as nanograms per milliliter of plasma.

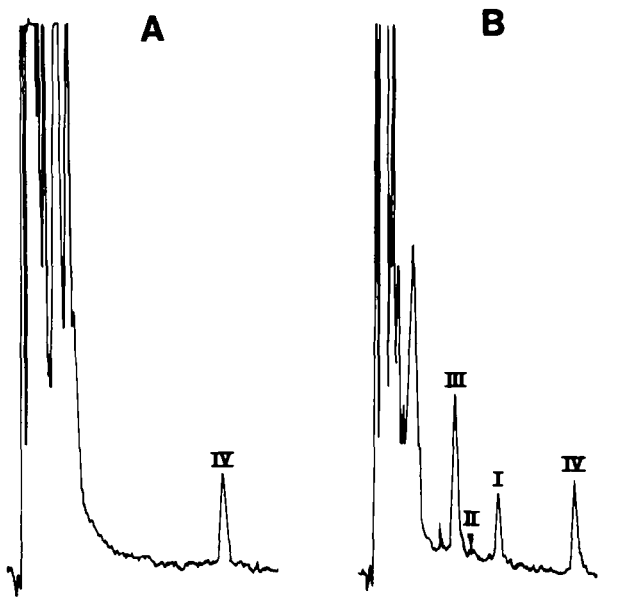

Figure 2—*Typical chromatograms of ethyl acetate extracts of 1.0 mL of plasma. The extracts were reconstituted with 100 μL of methanol, injection volume 30 μL, absorbance at 250 nm. Key: (A) drug free; (B) patient receiving I orally, concentrations of compounds expressed as ng/mL of plasma are I = 72, II = <10, and III = 227.*

Patient Samples—Heparinized plasma samples from patients receiving oral amoxapine were extracted in duplicate using the same procedure. The amounts of drug and metabolites in patient samples were calculated by comparison with a standard curve prepared daily. Possible interferences from normal plasma constituents, as well as other drugs and metabolites, were also studied.

RESULTS AND DISCUSSION

Amoxapine can be extracted readily from basified plasma using a solvent mixture (hexane containing 0.5% diethylamine) reported for the extraction of several tricyclic antidepressants and their metabolites (12). The active hydroxylated metabolites of amoxapine cannot, however, be extracted with that system and, therefore, the polar solvent ethyl acetate was selected.

The use of a cyanopropylsilane column in a reverse-phase mode with a mobile phase of acetonitrile–0.03 M acetate buffer at pH 6 (65:35, v/v) produced excellent separation of the drug from its metabolites and the internal standard. The separation depends on the pH and molarity of the buffer as well as the percentage of acetonitrile in the mobile phase. The mobile phase composition selected for this assay was based on the capacity factor (K) data plotted in Fig. 1. Detection at 250 nm was optimum for the drug, its metabolites, and the internal standard. Monitoring at 254 nm resulted in only a slight loss of sensitivity.

The ratios of peak heights of the drug and metabolites to the peak height of the internal standard were calculated. Linear regression analysis of the data on two separate days (Table I) indicated linearity and reproducibility in the range of 25–600 ng/mL of plasma. Extension of the standard curve beyond 600 ng/mL exhibited nonlinearity. A normal therapeutic range has not been established for amoxapine or its metabolites, but the linear portion of the standard curve encompasses the plasma concentrations which are expected based on normal dosages of amoxapine. Absolute recovery of the drug and its 7- and 8-hydroxy metabolites from plasma were 63, 69, and 68%, respectively, of the theoretical amounts. These values correlate well with data published recently (10). This low recovery may be responsible for the variation in the amount found at low concentrations (Table I). The minimum detectable limits of the assay are 6.5, 9.4, and 8.5 ng/mL of plasma for I, II, and III.

[2] Model 1084B chromatograph with variable-wavelength UV detector and autoinjector; Hewlett-Packard Co., Avondale, Pa.
[3] Zorbax-CN; Dupont Co., Wilmington, Del.
[4] Ives Laboratories, Inc., New York, N.Y.
[5] Concentratubes; Laboratory Research Co., Los Angeles, Calif.
[6] Micro-volume sample flask; P. Weidmann & Co., Romanshorn, Switzerland.

No interference with the drug or its metabolites from normal plasma constitutes was observed (Fig. 2A). The retention times of several drugs that might be prescribed simultaneously with amoxapine were determined (Table II). Little interference would be expected under the experimental conditions.

The method has been applied to the analysis of many samples obtained from patients receiving amoxapine orally. The drug and its 8-hydroxy metabolite are present in relatively high concentrations (Fig. 2B). However, the 7-hydroxy metabolite is found in low concentration. This may be due to its short half-life (8).

Major advantages of the method are the small sample volume required, the simplicity and high recovery using a single extraction step with no derivatization, and the use of an isocratic mobile phase. The sensitivity is sufficient for routine analysis of patient samples and for pharmacokinetic studies.

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Isosorbide Dinitrate Plasma Concentrations and Bioavailability in Human Subjects after Administration of Standard Oral and Sublingual Formulations

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Received September 13, 1982, from the *Department of Metabolism and Pharmacokinetics, Huntingdon Research Centre, Huntingdon, PE18 6ES England and the Institute of Clinical Pharmacology, Dublin, Ireland.* Accepted for publication December 23, 1982.

Abstract □ The bioavailability of isosorbide dinitrate from formulations containing 5, 10, and 20 mg in tablets and 10 mg in solution for oral use and 5 mg in tablets for sublingual use, has been compared. When adjusted for dose, the peak mean plasma drug concentrations after oral administration were similar (*e.g.*, 9.2 ng/mL after a 10-mg tablet) and about one-half that obtained after sublingual administration. Drug concentrations declined monoexponentially with mean half-lives ranging from 25–36 min. The relative bioavailability of isosorbide dinitrate from the oral formulations was not significantly different ($p > 0.05$) over the dose range studied, whereas the relative bioavailability after sublingual administration was about twice as great ($p < 0.01$) as that after oral administration. The plasma drug concentration–time profile after administering the 5-mg sublingual tablet was similar to that obtained after administering orally a solution containing 10 mg, indicating that the latter should be as clinically effective as the former.

Keyphrases □ Isosorbide dinitrate—rate and extent of bioavailability from various oral formulations compared, oral and sublingual formulations compared, humans □ Bioavailability—isosorbide dinitrate, oral and sublingual formulations compared, human □ Vasodilators—isosorbide dinitrate, oral and sublingual formulations compared, humans

Isosorbide dinitrate is an anti-anginal organic nitrate vasodilator that is in extensive clinical use. Following the development of suitably sensitive GC assays (1–3) for the measurement of isosorbide dinitrate in plasma, there have been several reports of the plasma levels of isosorbide dinitrate after the administration of different formulations of the drug (4–9). Sustained-release formulations have proven to be of particular interest. However, none of these reports have compared the relative bioavailability of isosorbide dinitrate from increasing doses of standard oral formulations. Such studies are described in this paper.

EXPERIMENTAL

Drug Administration—Two studies were conducted: the first compared a 5-mg sublingual tablet formulation with a 10-mg oral solution and 10- and 20-mg standard oral tablet formulations of isosorbide dinitrate[1]. The second study compared a 5-mg standard oral tablet formulation[1] with its 10-mg counterpart investigated in the first study. In each study, separate groups of 12 male volunteers each were involved, and the experimental conditions were the same. All subjects (18–40 years old and 58–85 kg) gave their written consent. Within 7 d before and after either study, each subject was given a complete physical examination including routine laboratory screening tests. During the study, the subjects remained under medical supervision. No adverse reactions, apart from headache (4 subjects), were reported by any subject. The studies were approved by the Institutional Review Board. Each of the oral dosage formulations was administered with 100 mL of water according to a complete crossover repeated Latin-square design with an interval of 1 week between doses. The sublingual formulation was retained under the tongue until it completely disintegrated; the subjects were instructed to avoid swallowing during this period. For at least 12 h predosing and for 4 h postdose the subjects fasted, and activity and subsequent diet were standardized.

At predose and after dosing, blood samples were collected into heparinized tubes by venipuncture, immediately cooled, and centrifuged. The resultant plasma was removed and stored at −20°C under conditions in which the drug was stable throughout the assay period.

Drug Assay—Isosorbide dinitrate in plasma was measured by an electron

[1] Formulations (Risordan) were provided by Theraplix, Paris, France. The respective batch numbers were 416, 430, 6659, 6665, 6747 for Risordan 5 mg (sublingual), 5 mg (oral), 10 mg (tablet), 20 mg and 10 mg (solution), respectively.

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