SUMMARY

A GLC method, capable of measuring plasma levels as low as 50 ng./ml. of iodochlorhydroxyquin, was developed. Volunteers given single oral doses of 250, 750, and 1500 mg. of iodochlorhydroxyquin powder exhibited a clear dose–plasma concentration relationship. Calculation of the area under the plasma concentration curves showed that the relative absorption of the three doses was similar. The half-life was between 11 and 14 hr. Volunteers given orally 3 × 500 mg. of the powder daily for 7 days followed by 3 × 250 mg. daily for an additional 7 days had blood samples withdrawn 24 hr. after the daily dose. Equilibrium between the amount absorbed and the amount eliminated per 24-hr. period appeared to be reached by the 5th day of administration. After discontinuation of treatment, the plasma levels fell to undetectable levels after 3 days, a finding that agreed well with the results of the single-dose study. No evidence of the accumulation of iodochlorhydroxyquin was found.

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Blood Level Profile in Man following Chronic Oral Administration of Flurazepam Hydrochloride

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Abstract □ The blood level profile of flurazepam and its major metabolites was determined in man following the oral administration of 30 mg. daily for 2 weeks. The levels of the intact drug were below the sensitivity limit of the assay (<3–4 ng./ml.). The hydroxyethyl metabolite was measurable only during the early hours after a 30-mg. oral dose and was not detectable after 24 hr. The major metabolite in blood was *N*-desalkyl-flurazepam, which reached steady-state (plateau) levels after 7 days. The half-life of elimination of the *N*-desalkyl metabolite ranged from 47 to 100 hr. No measurable amounts of the *N*-desalkyl-3-hydroxy metabolite were seen in blood.

Keyphrases □ Flurazepam hydrochloride and metabolites—blood level profile in man following 14 days administration, half-lives □ Blood level profile—flurazepam hydrochloride and metabolites after 14 days administration, man □ Biotransformation—flurazepam hydrochloride after chronic administration, blood level profile including metabolites, half-lives □ Benzodiazepines, flurazepam hydrochloride—biotransformation, blood level profile, metabolites

Flurazepam[1], 7-chloro-1-[2-(diethylamino)ethyl]-5-(*o*-fluorophenyl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one dihydrochloride (I), was synthesized by Sternbach *et al.* (1) and is marketed as a hypnotic for the treatment of insomnia (2). Studies on the metabolism of flurazepam (3) showed that it was extensively metabolized in both man and dog to yield measurable amounts of the hydroxyethyl (II) and *N*-desalkyl (III) metabolites in the blood. The latter was further metabolized to the 3-hydroxy analog (IV), which appeared in trace amounts in urine as a glucuronide conjugate. In addition, they showed that both flurazepam and the hydroxyethyl metabolite were metabolized extensively in the dog to an acidic compound, flurazepam-*N*-yl-acetic acid (V), by the oxidation of the alcohol side chain to a carboxylic acid. Flurazepam-*N*-yl-acetic acid is a minor urinary metabolite in man.

The characterization of these metabolites and the synthesis of authentic reference compounds were reported previously (4, 5). The objective of the present study was to define the blood level profile of flurazepam and its major biotransformation products when the drug is administered once daily for 14 days at the usually recommended therapeutic dose of 30 mg./day.

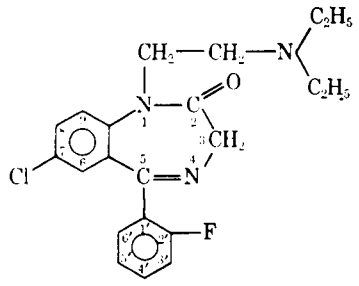
PROCEDURE

Blood levels of flurazepam and its major biotransformation products were analyzed by a spectrofluorometric method (6), which involves selective extraction of flurazepam and its metabolites (II, III, and IV) from blood buffered to pH 9.0 into ether, back-extraction into 6 *N* HCl, and hydrolysis to their benzophenones followed by cyclization to the highly fluorescent 9-acridanone derivatives. These derivatives are extracted into ether and separated by TLC, and their fluorescence is determined in methanol–0.1 *N* HCl (80:20) (after elution from the silica gel) at their respective activation and emission maxima.

The sensitivity of the assay is of the order of 3–10 ng. of each compound/ml. of blood using a 4-ml. specimen per analysis. The spectrofluorometric assay does not specifically differentiate *N*-desalkyl-flurazepam from the *N*-desalkyl-3-hydroxy metabolite if present, since they both give rise to the same 9-acridanone derivative (6). However, the presence of *N*-desalkyl-flurazepam as the major metabolite in the blood of these subjects was confirmed by electron-capture GLC analysis[2], which completely resolves the *N*-

[1] Flurazepam hydrochloride is the active drug substance in Dalmane, Hoffmann-La Roche Inc., Nutley, N. J.

[2] J. A. F. de Silva and C. V. Puglisi, unpublished data on file, Hoffmann-La Roche Inc., Nutley, NJ 07110, 1973.



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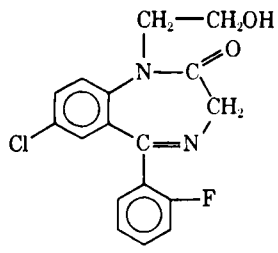

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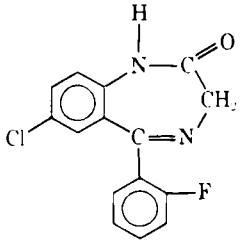

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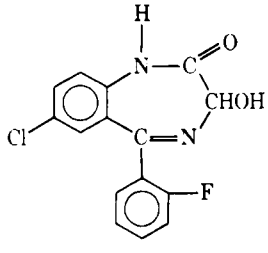

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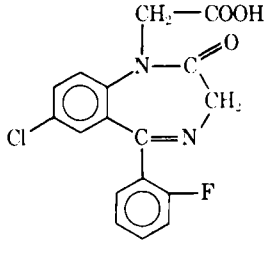

flurazepam-*N*-1-yl-acetic acid (V)

desalkyl metabolite from the *N*-desalkyl-3-hydroxy metabolite. The specimens from Days 14, 15, and 16 in Subjects 2, 3, and 4 were analyzed by the GLC procedure and clearly showed that the *N*-desalkyl-3-hydroxy metabolite was not present in the blood and confirmed that the *N*-desalkyl metabolite was the major blood component present. Therefore, the metabolite levels represented as the *N*-desalkyl metabolite by the fluorometric assay are due only to this component in these subjects.

Four adult healthy male volunteers (age and weight listed here) were selected for this study:

Subject	Age	Weight, kg.
1	44	90.0
2	25	76.8
3	28	69.5
4	42	85.5

Each subject was given a 30-mg. capsule of flurazepam hydro-

Table I—Blood Level Profile of Flurazepam and Its Major Metabolites in Subject 1 following the Administration of 30 mg. Flurazepam Hydrochloride Every 24 hr. for 14 Days[a]

Day	Hour	Hydroxyethyl-flurazepam (II), ng./ml.	*N*-Desalkyl-flurazepam (III), ng./ml.
1	0	n.m.	n.m.
	1	7.0	13.6
	3	3.0	10.5
	6	n.m.	19.8
	12	n.m.	6.8
	24	n.m.	14.5
2	24	n.m.	23.4
3	24	n.m.	30.0
4	24	n.m.	30.5
5	24	n.m.	40.0
6	24	n.m.	53.0
7	1	5.4	36.0
	3	5.4	62.0
	6	n.m.	79.0
	12	n.m.	58.0
	24	n.m.	61.0
8	24	n.m.	139.0
9	24	n.m.	40.0
10	24	n.m.	80.0
11	24	n.m.	70.0
12	24	n.m.	109.0
13	24	n.m.	134.0
14	1	15.5	113.0
	3	16.9	142.0
	6	4.8	60.0
	12	2.5	90.0
	24	n.m.	70.0
15	24	n.m.	66.0
16	24	n.m.	58.0
18	24	n.m.	33.0
20	24	n.m.	27.0
27	24	n.m.	2.4
34	24	n.m.	n.m.

[a] n.m. = not measurable <2-3 ng./ml. Flurazepam levels were measurable only on Day 14 at 1 hr. (3 ng./ml.) and at 6 hr. (4 ng./ml.).

Table II—Blood Level Profile of Flurazepam and Its Major Metabolites in Subject 2 following the Administration of 30 mg. Flurazepam Hydrochloride Every 24 hr. for 14 Days[a]

Day	Hour	Hydroxyethyl-flurazepam (II), ng./ml.	*N*-Desalkyl-flurazepam (III), ng./ml.
1	0	n.m.	n.m.
	1	4.3	5.6
	3	3.4	6.8
	6	n.m.	9.8
	12	n.m.	10.2
	24	n.m.	8.4
2	24	n.m.	10.1
3	24	n.m.	15.4
4	24	n.m.	20.0
5	24	n.m.	15.9
6	24	n.m.	22.7
7	1	6.8	36.8
	3	3.6	41.2
	6	n.m.	42.7
	12	n.m.	37.3
	24	n.m.	33.2
8	24	n.m.	35.5
9	24	n.m.	34.1
10	24	n.m.	33.0
11	24	n.m.	31.7
12	24	n.m.	42.2
13	24	2.5	38.7
14	1	11.5	44.4
	3	5.3	49.2
	6	2.8	38.9
	12	n.m.	42.8
	24	n.m.	39.4
15	24	n.m.	29.9
16	24	n.m.	15.1
18	24	n.m.	9.5
20	24	n.m.	n.m.
27	24	n.m.	n.m.
34	24	n.m.	n.m.

[a] n.m. = not measurable <2-3 ng./ml. Flurazepam levels were unmeasurable throughout the study.

Table III—Blood Level Profile of Flurazepam and Its Major Metabolites in Subject 3 following the Administration of 30 mg. Flurazepam Hydrochloride Every 24 hr. for 14 Days[a]

Day	Hour	Hydroxyethyl-flurazepam (II), ng./ml.	N-Desalkyl-flurazepam (III), ng./ml.
1	0	n.m.	n.m.
	1	14.8	6.9
	3	8.6	15.2
	6	3.3	13.3
	12	n.m.	20.6
	24	n.m.	21.8
2	24	n.m.	41.4
3	24	n.m.	34.8
4	24	n.m.	23.8
5	24	n.m.	36.0
6	24	n.m.	24.3
7	1	2.7	26.6
	3	10.5	35.6
	6	3.8	35.9
	12	n.m.	37.2
	24	n.m.	53.8
8	24	n.m.	34.3
9	24	n.m.	44.1
10	24	n.m.	32.4
11	24	n.m.	27.1
12	24	n.m.	41.1
13	24	n.m.	58.0
14	1	n.m.	56.1
	3	n.m.	48.5
	6	n.m.	42.2
	12	n.m.	50.4
	24	n.m.	36.5
15	24	n.m.	29.0
16	24	n.m.	24.7
18	24	n.m.	11.9
20	24	n.m.	5.4
27	24	n.m.	n.m.
34	24	n.m.	n.m.

[a] n.m. = not measurable <2–3 ng./ml. Flurazepam levels were unmeasurable throughout this study.

Table IV—Blood Level Profile of Flurazepam and Its Major Metabolites in Subject 4 following the Administration of 30 mg. Flurazepam Hydrochloride Every 24 hr. for 14 Days[a]

Day	Hour	Hydroxyethyl-flurazepam (II), ng./ml.	N-Desalkyl-flurazepam (III), ng./ml.
1	0	n.m.	n.m.
	1	6.1	8.3
	3	3.9	11.2
	6	n.m.	10.7
	12	n.m.	11.5
	24	n.m.	13.5
2	24	n.m.	27.6
3	24	n.m.	47.0
4	24	n.m.	42.0
5	24	n.m.	46.1
6	24	n.m.	69.8
7	1	2.5	55.7
	3	6.6	71.2
	6	2.8	72.1
	12	n.m.	75.7
	24	n.m.	71.6
8	24	n.m.	62.4
9	24	n.m.	83.3
10	24	4.0	87.1
11	24	n.m.	89.1
12	24	n.m.	98.4
13	24	n.m.	106.0
14	1	4.2	117.8
	3	4.4	115.5
	6	n.m.	99.0
	12	n.m.	115.9
	24	n.m.	113.8
15	24	n.m.	84.4
16	24	n.m.	78.4
18	24	n.m.	47.6
20	24	n.m.	34.8
27	24	n.m.	12.7
34	24	n.m.	4.8

[a] n.m. = not measurable <2–3 ng./ml. Flurazepam levels were unmeasurable throughout the study.

chloride at approximately 10 p.m. for 14 days. Ten milliliters oxalated blood specimens were obtained as follows: Days 1, 7, and 14—just prior to drug administration and at 1, 3, 6, 12, and 24 hr. postadministration; Days 2 through 6 and 8 through 13—just prior to drug administration (24 hr. postadministration); and Days 15, 16, 17, 19, 21, 28, and 35—at 10 p.m. (1, 2, 3, 5, 7, 14, and 21 days following the last administration on Day 14).

All blood specimens were frozen immediately and maintained frozen until analyzed.

RESULTS AND DISCUSSION

The blood level data are tabulated in Tables I–IV for Subjects 1–4. The blood levels of the N-desalkyl metabolite in each subject are summarized in Fig. 1. The overall blood level profile parameters are summarized in Table V.

Blood levels of flurazepam were generally below 2 ng./ml. Only Subject 1 exhibited trace amounts of flurazepam of 3–4 ng./ml. on Day 14 of the study.

Blood levels of the hydroxyethyl metabolite were seen in the early hour samples measured on Days 1, 7, and 14 (Tables I–IV). The elimination rate of the hydroxyethyl metabolite was very rapid (data insufficient for half-life calculation), and this metabolite was not evident in the blood 24 hr. postadministration.

The N-desalkyl metabolite was the major detectable blood component following flurazepam hydrochloride administration (Fig. 1). The half-lives of elimination of this component from the blood were calculated to be 64, 47, 51, and 100 hr. for Subjects 1, 2, 3, and 4, respectively.

Subjects 2 and 4 exhibited a typical steady-state blood level profile of the N-desalkyl metabolite. In both subjects, the blood levels appeared to reach a plateau on Days 7–10 of the study. Subject 2 exhibited a minimum steady-state level (24 hr. postdosing), ranging between 33 and 42 ng./ml. The corresponding range for Subject 4 was 84–114 ng./ml. These values correlate well with the "calculated" minimum steady-state levels of 28 and 88 ng./ml. for Subjects 2 and 4, respectively (Table V). The calculated value was obtained using the observed half-life and the 24-hr. blood level on Day 1 for each subject. This close correlation of actual and calculated minimum steady-state levels indicates that the levels of the N-desalkyl metab-

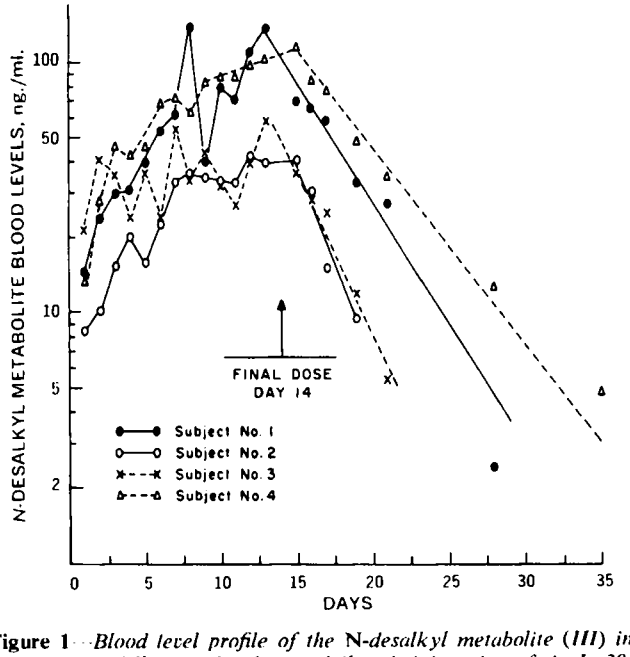

Figure 1—*Blood level profile of the* N-*desalkyl metabolite (III) in Subjects 1–4 following the chronic daily administration of single 30-mg. oral doses of flurazepam hydrochloride.*

Table V—Blood Level Profile Parameters

	Subject			
	1 (90.0 kg.)[a]	2 (76.8 kg.)	3 (69.5 kg.)	4 (85.5 kg.)
N-Desalkyl metabolite (III):				
Maximum blood level on Day 1	20 ng./ml. at 6 hr.	10 ng./ml. at 12 hr.	22 ng./ml. at 24 hr.	14 ng./ml. at 24 hr.
Maximum blood level on Day 14	142 ng./ml.	49 ng./ml.	56 ng./ml.	118 ng./ml.
Asymptotic levels commence	≃Day 10-erratic	Day 7	≃Day 2-erratic	Day 9
Asymptotic 24-hr. level (minimum)	~70–134 ng./ml.	≃33–42 ng./ml.	≃24–58 ng./ml.	≃84–114 ng./ml.
24-hr. blood level on Day 1	15 ng./ml.	8 ng./ml.	22 ng./ml.	14 ng./ml.
Half-life calculated after last dose on Day 14	64 hr.	47 hr.	51 hr.	100 hr.
Calculated minimum steady-state level based on Day 1–24-hr. level	63 ng./ml.	28 ng./ml.	78 ng./ml.	88 ng./ml.
Hydroxyethyl metabolite (II):				
Maximum blood level on Day 1	7 ng./ml.	4.3 ng./ml.	14.8 ng./ml.	6.1 ng./ml.
Maximum blood level on Day 7	5.4 ng./ml.	6.8 ng./ml.	10.5 ng./ml.	6.6 ng./ml.
Maximum blood level on Day 14	16.9 ng./ml.	11.5 ng./ml.	N.D.	4.4 ng./ml.
Half-life	Rapid	Rapid	Rapid	Rapid
Flurazepam (I):				
Blood levels	Barely detectable on Day 14, only at 3–4 ng./ml.	N.D.[b]	N.D.	N.D.

[a] Body weight. [b] N.D. = not detectable <2–3 ng./ml.

olite reach a predictable steady-state plateau within 7–10 days upon chronic drug administration. The actual minimum steady-state blood levels were approximately four- to sixfold greater than the 24-hr. blood level observed on Day 1.

The daily 24-hr. blood levels of the *N*-desalkyl metabolite in Subjects 1 and 3 were more variable than those found in Subjects 2 and 4. The correlation of the actual 24-hr. blood levels and the calculated steady-state level was fair in Subject 1, with the calculated value falling at the lower end of the actual 24-hr. blood level range (Table V). Subject 1 exhibited 24-hr. blood levels from Days 10 to 14, which were approximately five- to sixfold greater than the observed Day 1 level, which is consistent with the findings in Subjects 2 and 4. The actual 24-hr. levels in Subject 3 were lower than the calculated minimum steady-state levels.

The blood level profile following the oral administration of 30 mg. of flurazepam hydrochloride every 24 hr. indicates that: (*a*) the blood level of flurazepam was <2 ng./ml. in blood, (*b*) the hydroxyethyl metabolite was present in the blood in the early hours following each administration and was rapidly eliminated, and (*c*) the *N*-desalkyl metabolite was the major blood component.

The blood level profiles indicate that the *N*-desalkyl metabolite was eliminated with half-lives of 64, 47, 51, and 100 hr. in the four subjects studied. In three subjects, blood levels of this metabolite exhibited a "typical," predictable minimum steady-state profile: *i.e.*, the actual and calculated steady-state levels correlated well. This would suggest that the physiological disposition of the *N*-desalkyl metabolite follows apparent first-order kinetic patterns. The minimum steady-state levels observed, commencing 7–10 days following the onset of a once every 24-hr. dosing regimen, were approximately five- to sixfold greater than the 24-hr. levels observed on Day 1.

SUMMARY

Following the oral administration of 30 mg. of flurazepam hydrochloride in man every 24 hr. for 14 days, blood samples were analyzed for flurazepam and its major metabolites. The method used for the analysis of flurazepam was not sufficiently sensitive to measure the concentration of unchanged drug in blood (<2 ng. flurazepam/ml. of blood was present). The hydroxyethyl metabolite was present in the blood shortly after each administration and was rapidly eliminated. The *N*-desalkyl metabolite was the major metabolite in blood. It was eliminated with half-lives of 47–100 hr. in the four subjects. It exhibited a typical minimum steady-state profile, with plateau levels reached after 7–10 days of dosing, approximately five- to sixfold greater than the 24-hr. levels observed on Day 1.

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