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Pharmacokinetic Analysis of Concentration–Time Data Obtained Following Administration of Drugs that are Recycled in the Bile

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Abstract □ A pharmacokinetic model for calculating the pharmacokinetic parameters for a compound that is recycled in the bile is presented and tested using theoretical as well as experimental data. The results indicate that this method is stable and only slightly susceptible to sampling and recycling times. It is apparent from the present study that pharmacokinetic terms that have been used in classical situations are not directly applicable to drugs that enter the enterohepatic circulation. Effective half-life and effective clearance are used to describe the intrinsic ability of the eliminating organs to remove drug from the blood, whereas net half-life and net clearance are used to describe the irreversible elimination of the drug from the body.

Keyphrases □ Pharmacokinetics—biliary recycling of drugs, theoretical model, applications to cimetidine and isotretinoin □ Biliary excretion—incorporation in pharmacokinetics, model, applications to cimetidine and isotretinoin □ Mathematical models—incorporation of biliary recycling in pharmacokinetics, application to cimetidine and isotretinoin

The ability to predict plasma concentration–time profiles observed following repetitive doses from data obtained following a single dose is an important aspect of pharmacokinetic analysis. In most cases, drugs that follow linear pharmacokinetic models allow these predictions to be made with reasonable certainty. However, when plasma concentration–time data cannot be adequately described by classical pharmacokinetic equations, *i.e.*, saturable processes or enzyme induction, this predictive capability becomes impaired. When a compound is excreted in the bile and subsequently reabsorbed from the GI tract, a similar situation exists in that classical equations cannot be used to sufficiently characterize the erratic and fluctuating plasma concentration–time curves. The purpose of the present investigation was to develop a biliary recycling model, to test its susceptibility to sampling times and experimental error, and to apply it to experimental data from drugs that are known to recycle in the bile.

THEORETICAL

Two distinct types of compounds must be considered when discussing biliary excretion and enterohepatic circulation. In the first category, there are compounds that are recycled but still are eliminated in ≤24 h; indomethacin (1), cimetidine (2), and imipramine (3) are examples from this category. In the second category are compounds that persist for much longer than 24 h; isotretinoin (4), digitoxin (5), and phenprocoumon (6) are examples from this category of substances. For compounds that are eliminated from the body in ~24 h or less, extensive sampling of body fluids is required over the entire interval, whereas for compounds that take substantially longer than 24 h to be eliminated from the body, extensive sampling during the first 24 h and subsequent samples at 24-h intervals are required. The modeling procedure developed herein considers both types of compounds.

Model Development—The following compartmental models (Fig. 1) are among those applicable to the study of blood concentration–time data profiles of compounds that undergo enterohepatic circulation. The models differ only in the sites of elimination. It can be shown that models

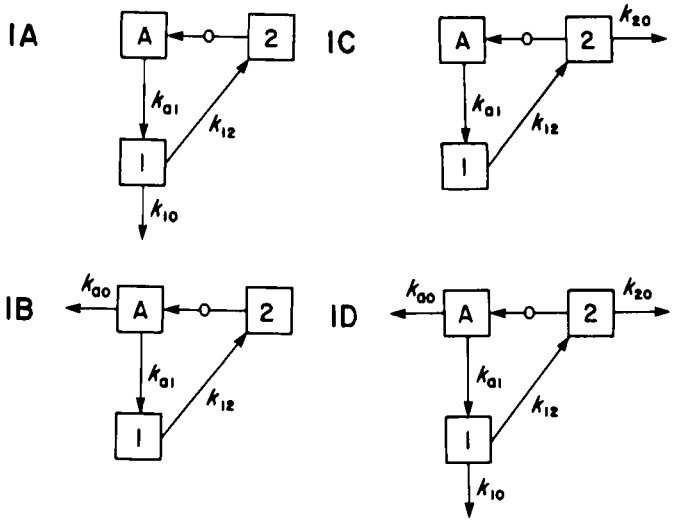

Figure 1—*Four possible enterohepatic recycling models. Compartment A is the absorption site, compartment 1 is the sampled blood compartment, and compartment 2 is the storage compartment that includes the gallbladder and the transit-time factor. The first-order rate constants* k_{a1} *and* k_{12} *represent the transfer of drug from the absorption site to the sampled blood compartment and from the sampled compartment to the gallbladder storage compartment whereas* k_{a0}, k_{10}, *and* k_{20} *are rate constants that represent first-order elimination from the absorption site, sampled compartment, and gallbladder storage compartment. The arrow between compartments 2 and A represent the discontinuous emptying process of the gallbladder such that the amount of drug in the gallbladder is transferred instantaneously to the absorption site at the time* (t_{bile}) *that reabsorption begins and V is the volume of the sampled compartment.*

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Table I—Schematic Representation of the Blood Sampling Schedules

Time, h	Schedule Designation A	B	C	D
0	+	+	+	+
1	+	+	+	+
2	+	+	+	+
3	+	+	+	+
4	+	+	+	+
5	+			
6	+	+	+	+
7	+			
8	+	+		
9	+		+	+
10	+	+		
11	+			
12	+	+	+	+
13	+			
14	+	+		
15	+		+	
16	+	+		
17	+			
18	+	+	+	+
19	+			
20	+	+		
21	+	+		
22	+	+		
23	+			
24	+	+	+	+

1A and 1B are pharmacokinetically differentiated from model 1C when recycling occurs at nonuniform intervals. However, within the limits of analytical precision and considering the biological variation that is associated with experimental data, the four models are not structurally identifiable when blood concentration–time data are evaluated. The four models are pharmacokinetically unique only if blood, urine, feces, and bile are collected following both intravenous and oral doses and analyzed for both parent drug and all metabolites.

One of these models (1A) has been previously described by Veng-Pedersen and Miller (7) and Steimer *et al.* (8), but the integrated equation derived in these manuscripts included only one recycling of bile during the experiment (7). For the purpose of testing the impact of sampling time and experimental error on the model predictions, data will be simulated assuming that the gallbladder empties at each meal ingestion (9) (e.g., at 0700, 1300, 1900 on each day of the study) and that drug is administered at 0700 on day 1 with no meal being ingested at that time. Therefore, the first gallbladder emptying occurs at 1300 on day 1. In the first example (case I) it is assumed that reabsorption begins each time (t_{bile}) that the gallbladder empties. However, in many cases, a conjugate of parent compound is excreted in the bile, deconjugated in the lower intestinal contents, then reabsorbed (10–12). This situation could result in substantial time delay between meal ingestion and subsequent reabsorption (13). For this purpose, blood concentration–time data were simulated after incorporating a 3-h (case II) or 6-h (case III) transit-time lag between gallbladder emptying and the onset of reabsorption (t_{bile}) such that the first reabsorption begins 9 or 12 h after the dose, respectively.

Model Testing—The ability of the recycling model to adequately describe simulated blood concentration–time data following a single dose of drug was tested under the following experimental conditions:

1. Ideal data sets (cases I–III) with four different sampling schedules (see Table I).
2. Ten errant data sets (case II only) with uniformly distributed random error using each of the four different sampling schedules.
3. Ten errant data sets assuming that gallbladder emptying occurred at 5 and 11 h and 10 errant data sets assuming gallbladder emptying occurred at 7 and 13 h during the first 24-h period after dosing to determine the effect of nonsampled recycling times.

In addition, two sets of experimental data were fitted with the model to assess the impact of repeated recycling during the experimental interval. In the first example, cimetidine concentration–time data (14) were *fitted with* model 1A. Patient data were obtained following a single 300-mg oral dose 3 h prior to breakfast (14); no mention was made of subsequent meal times. The results from the present curve-fitting technique are compared with previous attempts to fit the data with the single recycling technique (2). For the second example, blood concentration–time data from a recently completed single 100-mg oral dose pharmacokinetic study with isotretinoin (4) were fitted with model 1A.

Table II—Ideal Theoretical Data Sets Curve-Fitted Using the Different Sampling Schedules

	t_{bile}, h	k_a, h^{-1}	k_{12}, h^{-1}	k_{10}, h^{-1}	V, L
		Case I			
Ideal Schedule	6.00	0.693	0.420	0.0800	400
A	6.00	0.695	0.419	0.0801	401
B	6.00	0.694	0.420	0.0802	400
C	6.00	0.696	0.418	0.0800	401
D	6.00	0.696	0.419	0.0801	401
		Case II			
Ideal Schedule	9.00	0.693	0.420	0.0800	400
A	9.00	0.695	0.419	0.0800	401
B	9.00	0.694	0.420	0.0800	400
C	9.00	0.695	0.419	0.0801	401
D	9.00	0.706	0.411	0.0800	406
		Case III			
Ideal Schedule	12.0	0.693	0.420	0.0800	400
A	12.0	0.695	0.419	0.0801	400
B	12.0	0.695	0.419	0.0800	400
C	12.0	0.696	0.418	0.0800	401
D	12.0	0.695	0.419	0.0802	401

In this second example, it was assumed that the gallbladder emptied at meal ingestion and that the time to transit in the intestine to sites where deglucuronidation could occur was constant after each meal. In this way, a single recycling parameter could be estimated and used to calculate subsequent recycling times on the basis of meals ingested. All concentration (C_B) data were fitted with the differential equations required to describe model 1A in conjunction with the nonlinear least-squares regression program, NONLIN (15), using $1/C_B$ weighting[1].

In addition, a net elimination rate constant during recycling (β_R) can be determined by performing log-linear regression on concentration–time data at 24-h intervals during the elimination phase, *e.g.*, 24, 48, 72, and 96 h or 12, 36, 60, and 84 h. The determination of β_R is based on the assumption that gallbladder emptying and subsequent reabsorption occur at the same times each day. Therefore, if recycling occurs at the same time during each 24-h interval, the β_R obtained by fitting data points collected at 24-h intervals should reflect the true elimination rate since the concentration–time points fall on the same portion of the sinusoidal recycling curve. Experimentally, this can be achieved by controlling the time and type of food intake. This curve-fitting procedure was tested by introducing uniform random error into theoretical concentrations.

RESULTS

The parameters estimated for ideal theoretical data sets from cases I–III with the four different sampling schedules (Table I) are presented in Table II. The parameters estimated for 10 errant data sets from case II using the four different sampling schedules are presented in Table III. The parameters estimated for 10 errant data sets from 5- and 11-h recycling data and from 7- and 13-h recycling data are presented in Table IV.

It is apparent from the parameter estimates in Table II, that when no error is present in the data, the curve-fitting procedure is not dependent on either the times of recycling or sampling. Similarly, the mean parameter estimates presented in Tables III and IV would indicate that, on the average, the curve-fitting procedure is not strongly influenced by either recycling or sampling times. However, the variation around the mean values are greater for sampling schedules C and D than for schedules A and B, *i.e.*, when nonsampled intervals (Table I) become >2 h.

By introducing ±10% error into the 24-, 48-, 72-, 96-, and 120-h samples, it was possible to assess the impact of random error on the estimation of β_R. For the purpose of testing an extreme case, 10% error was added to the 24-h concentration and subtracted from either a 48-, 72-, 96-, or 120-h concentration. When 10% error was subtracted from the 48-, 72-, 96-, and 120-h concentration, the error introduced into β_R was 29.0, 14.5, 9.7, and 7.3%, respectively. It is apparent that the more time points and the longer the time used to calculate β_R, the more precise will be the estimate.

The results from curve-fitting the cimetidine blood concentration–time

[1] A listing of the FORTRAN program used in this study is available from the author on request.

Table III—Parameters Estimated from 10 Sets of Theoretical Concentration–Time Data Containing ±10% Uniformly Distributed Random Error and Simulated According to Case II

	Parameters				
	t_{bile}, h	k_a, h^{-1}	k_{10}, h^{-1}	k_{12}, h^{-1}	V, L
Ideal	9.00	0.693	0.0800	0.420	400
Schedule A					
Mean ± *SD*	9.04 ± 0.06	0.742 ± 0.095	0.0786 ± 0.0053	0.405 ± 0.036	418 ± 35.6
Range	(8.99–9.16)	(0.579–0.882)	(0.0714–0.0873)	(0.362–0.487)	(350–460)
Schedule B					
Mean ± *SD*	8.99 ± 0.13	0.749 ± 0.135	0.0774 ± 0.0057	0.407 ± 0.048	420 ± 43.3
Range	(8.78–9.12)	(0.565–0.972)	(0.0711–0.0879)	(0.337–0.501)	(343–486)
Schedule C					
Mean ± *SD*	9.11 ± 0.20	0.763 ± 0.149	0.0846 ± 0.0110	0.392 ± 0.058	430 ± 61.1
Range	(9.00–9.64)	(0.573–1.014)	(0.0640–0.0999)	(0.269–0.500)	(351–536)
Schedule D					
Mean ± *SD*	9.26 ± 0.24	0.747 ± 0.143	0.0865 ± 0.0122	0.402 ± 0.050	422 ± 53.9
Range	(9.00–9.67)	(0.553–0.988)	(0.0637–0.1090)	(0.319–0.491)	(349–494)

Table IV—Parameters Estimated from 10 Sets of Theoretical Concentration–Time Data Containing ±10% Uniformly Distributed Random Error and Simulated With Recycling Occurring at Nonsampled Times

	Parameters				
	t_{bile}, h	k_a, h^{-1}	k_{10}, h^{-1}	k_{12}, h^{-1}	V, L
Ideal	5.00	0.693	0.0800	0.420	400
Schedule D					
Mean ± *SD*	5.01 ± 0.14	0.735 ± 0.168	0.0790 ± 0.0177	0.421 ± 0.056	412 ± 59.6
Range	(4.71–5.18)	(0.570–0.954)	(0.0590–0.0950)	(0.338–0.496)	(346–487)
Ideal	7.00	0.693	0.0800	0.420	400
Schedule D					
Mean ± *SD*	7.09 ± 0.37	0.728 ± 0.166	0.0807 ± 0.0208	0.431 ± 0.057	408 ± 60.1
Range	(6.47–7.60)	(0.559–1.000)	(0.0510–0.1093)	(0.322–0.503)	(348–498)

data are presented in Table V, and a representative plasma concentration–time profile is presented in Fig. 2. Similarly, the results obtained by curve-fitting isotretinoin blood concentration–time data are presented in Table VI, and a representative curve is presented in Fig. 3. The parameter estimates determined by curve-fitting experimental data from the literature for cimetidine (14) and blood concentration–time data presently being generated in this laboratory for isotretinoin show the applicability of the procedure.

Comparing the results of the present analysis of cimetidine blood concentration–time data with those reported in the literature using the single-recycling model of Veng-Pedersen and Miller (2), it appears that the complexities associated with the previously reported model may not be required to fit the data. If one assumes that a second recycling occurs during the time-course of the study, one can adequately fit the data without having to involve the complexities inherent in the two-compartment distribution model of Veng-Pedersen and Miller. It is difficult to justify the more complex model when recycling occurs at 2–6 h intervals and sampling is not sufficiently frequent to differentiate biphasic distribution characteristics from the recycling characteristics. In fact, it can be shown that when sampling is infrequent a recycled drug that conforms to model 1A in Fig. 1 exhibits what appears to be a two-compartment model profile. In addition, when it is not possible to simultaneously fit data from several routes and modes of administration, parsimony dictates that the simplest model should be chosen.

DISCUSSION

A model-dependent method for calculating the pharmacokinetic parameters for a compound that is recycled in the bile has been presented and tested using simulated theoretical data as well as experimental data. The results indicate the the method is stable, only slightly susceptible to changes in sampling and recycling times, and adequate to describe the blood concentration–time profiles of drugs that recycle in the bile.

The results of the present analysis suggest that more explicit terms are required to describe the pharmacokinetic parameters of compounds that are recycled in the bile. The blood clearance (CL_B) determined by the classical method:

$$CL_B = \frac{\text{Dose}}{AUC_{0-\infty}} \qquad \text{(Eq. 1)}$$

does not reflect the *effective* clearance (CL_E) of the drug by the liver. From model 1A, it can be shown that:

$$CL_E = V \cdot K \qquad \text{(Eq. 2)}$$

where the *effective* elimination rate constant (K) is the sum of k_{10} and k_{12} in model 1A. However, the *net* clearance (CL_N) derived from model 1A according to the following equation:

$$CL_N = V \cdot k_{10} \qquad \text{(Eq. 3)}$$

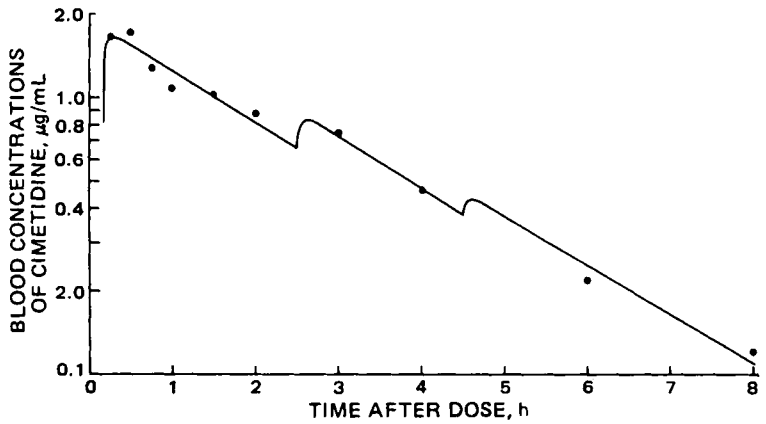

Figure 2—*Representative blood concentration–time profile of cimetidine along with the model-predicted line for subject 5.*

Table V—Parameters Estimated by Curve Fitting Cimetidine Blood Concentration–Time Data Following a Single 300-mg Oral Dose [a]

Subject	Parameters							
	t_{lag}, h	$t_{bile(1)}$ [b], h	$t_{bile(2)}$ [b], h	k_a, h^{-1}	k_{10}, h^{-1}	k_{12}, h^{-1}	V, L	r [c]
1	0.20	2.65	5.85	1.46	0.790	0.700	87.4	0.998
2	0.15	1.50	3.90	2.24	0.340	0.490	109	0.995
3	0.15	1.98	5.98	8.97	0.312	0.393	123	0.988
4	0.10	1.92	3.92	4.83	0.566	0.089	103	0.997
5	0.20	1.98	—	35.4	0.022	0.538	220	0.996
6	0.15	2.50	4.50	39.3	0.340	0.077	172	0.995
7	0.10	1.17	3.67	1.38	0.452	0.124	88.4	0.982
8	0.20	1.94	3.74	4.13	0.212	0.434	144	0.996
9	0.00	2.98	—	2.42	0.241	0.148	192	0.996
10	0.00	1.80	4.60	1.81	0.320	0.006	172	0.973
11	0.20	1.83	5.83	5.00	0.459	0.020	145	1.000
12	0.05	1.96	—	3.04	0.171	0.113	316	0.937
Mean	0.125	2.02	4.67	9.17	0.352	0.261	156	
SD	0.075	0.49	0.97	13.4	0.199	0.235	65.5	
Range	0–0.2	1.17–2.98	3.67–5.98	1.38–39.3	0.022–0.790	0.006–0.700	87.4–316	

[a] Concentration–time data obtained from Ref. 14. [b] $t_{bile(1)}$ and $t_{bile(2)}$ represent the times of the first and second recycling, respectively. [c] Correlation coefficient.

Table VI—Parameters Estimated by Curve Fitting Blood Concentrations of Isotretinoin Following a Single 100-mg Oral Dose to Five Normal Male Subjects

Parameter	Subject					Mean ± 1 *SD*
	1	2	3	4	5	
t_{lag}, h	1.95	0.71	0.10	0.87	0.77	0.88 ± 0.67
t_{bile}, h	7.83	11.8	9.53	9.83	17.9	11.4 ± 3.9
k_a, h^{-1}	1.33	0.609	0.635	0.456	0.825	0.771 ± 0.339
k_{10}, h^{-1}	0.182	0.099	0.052	0.072	0.087	0.098 ± 0.050
k_{12}, h^{-1}	0.333	0.478	0.125	0.152	0.299	277 ± 0.144
β_R, h^{-1}	0.0521	0.0206	0.0265	0.0312	0.0247	0.0310 = 0.0214
$t_{1/2\beta}$, h	13.3	33.6	26.2	22.2	28.1	22.4[a]
V/F, L	379	116	421	739	180	367 ± 245
AUC_D [b] ng·h/mL	1450	8708	4568	1879	6386	4598 ± 3057
r	1.000	.999	.999	.998	1.000	

[a] Harmonic mean. [b] Model-dependent area (AUC_D) calculated as $(F\cdot\text{Dose})/(V\cdot k_{10})$ and model.

is equivalent to CL_B. This distinction in clearance terms is similar to the approach previously employed by Kwan *et al.* (1) with indomethacin. However, it is a more unified approach which incorporates the differences in clearance into a single pharmacokinetic modeling technique.

Levy (16) has stated that fitting concentration–time data obtained following the administration of drugs that are involved in the enterohepatic circulation to classical pharmacokinetic models results in an overestimate of the true volume of distribution and an overestimation of the true half-life of the compound. In fact, curve-fitting data from compounds that are recycled to classical bi- or triexponential equations does overestimate the true volume of distribution and the *effective* elimination half-life ($t_{1/2E}$), derived from $0.693/K$. However, curve-fitting with classical equations may not, depending on the erratic nature of the blood concentration–time profile, overestimate the *net* elimination half-life ($t_{1/2N}$), derived from $0.693/\beta_R$ where β_R is the net elimination rate constant. β_R is related to the elimination rate constant (k_{10}) by:

$$\beta_R = F_1 \cdot k_{10} \qquad \text{(Eq. 4)}$$

where F_1 is the average fraction of the total amount of drug in the body that is in the blood compartment during any 24-h interval. This net elimination half-life ($t_{1/2N}$) is in fact the true elimination half-life, since it reflects the fraction of a dose that still remains to be irreversibly eliminated (17).

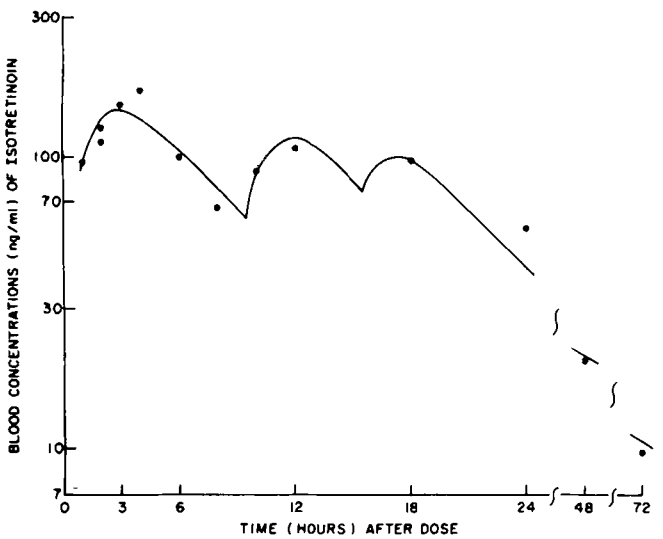

Figure 3—*Representative blood concentration–time profile of isotretinoin from subject 8 along with the model-predicted curve.*

If one applies these concepts to the data obtained for cimetidine and isotretinoin, interesting results are obtained. For cimetidine CL_E = 1393 mL/min (range 849–2170) and CL_N = 777 mL/min (range 81–1151), suggesting a first-pass effect ($E = CL_E/Q + CL_E$) of 0.36–0.59. However, using the net clearance to calculate the apparent first-pass effect ($E = CL_N/Q + CL_N$) yields values of 0.05–0.43, which are consistent with the conclusions from the original report (14). For isotretinoin, CL_E = 1906 mL/min (range 1116–3253) and CL_N = 571 mL/min (range 191–1150), suggesting a true first-pass effect of ~0.40. However, since isotretinoin is eliminated solely by liver clearance, 65–77% of the drug that is cleared by first-pass will be returned through enterohepatic circulation, resulting in an apparent first-pass effect of ~0.28. The average fraction (F_1) of the total amount of drug in the body that was in the blood compartment was 0.32.

It became apparent during these curve-fitting procedures that the pharmacokinetics of drugs that are incorporated into the enterohepatic circulation are complex, and development of their profiles will require specialized clinical studies. Two important aspects of performing clinical pharmacokinetic studies with compounds that recycle in the bile is to establish an appropriate experimental design and to maintain well-controlled conditions. Sufficient blood samples must be obtained to adequately characterize the recycling time course during a 24-h interval, and the time of meal ingestion must be controlled to ensure that the variation in recycling times is minimized.

In conclusion, a model-dependent method for analyzing pharmacokinetic data from drugs that are recycled in the bile has been developed and tested. It is apparent that the pharmacokinetic terms used in other situations are not directly applicable to drugs that enter the enterohepatic circulation. Effective half-life and effective clearance have been introduced to describe the intrinsic ability of the liver to remove drug from

the blood, whereas net half-life and net clearance have been used to describe the permanent elimination of the drug from the body.

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Quantitative Determination of *N*-(*trans*-2-Dimethylaminocyclopentyl)-*N*-(3′,4′-dichlorophenyl)propanamide and Its *N*-Demethyl Metabolite in Dog Serum by Gas Chromatography

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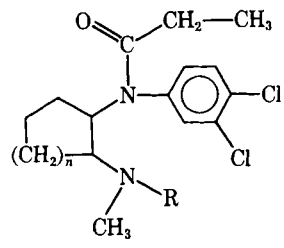
Abstract □ A gas chromatographic–electron capture (GC–EC) method has been developed for the determination of *N*-(*trans*-2-dimethylaminocyclopentyl)-*N*-(3′,4′-dichlorophenyl)propanamide, a potential antidepressant drug, and its *N*-demethyl metabolite in serum. The GC–EC system employed a 3% OV-17 on 100/120 mesh Supelcoport, 2-m × 2-mm i.d. glass column and an isothermal temperature of 195°C. The parent drug and metabolite were extracted from alkalinized serum (pH ~ 13) with toluene, back-extracted into an acidic solution (pH ~ 1), and finally, after adjusting to pH 13, extracted again with toluene. The extensive sample cleanup was necessary to remove serum components which interfered with the analysis. The analytical method was shown to give quantitative recovery of the drug and metabolite, to be linear over a 100-fold concentration range, and to have the necessary precision and sensitivity to detect and quantify as little as 1 ng/mL of the drug or its metabolite. The method has been employed to determine the serum level of drug and metabolite in dogs receiving a single oral dose and to determine the possible correlation between the administered dose and serum levels.

Keyphrases □ *N*-(*trans*-2-Dimethylaminocyclopentyl)-*N*-(3′,4′-dichlorophenyl)propanamide—quantitative determination, *N*-demethyl metabolite in dog serum, gas chromatography–electron capture □ Gas chromatography—electron capture, quantitative determination of *N*-(*trans*-2-dimethylaminocyclopentyl) -*N*- (3′,4′-dichlorophenyl) propanamide and its *N*-demethyl metabolite, dog serum

Some tricyclic antidepressants (*e.g.*, imipramine) used in the treatment of endogenous depression require an induction period of several days before improvement is noted and have been reported to have frequent side effects (1, 2). A potential antidepressant drug, *N*-(*trans*-2-dimethylaminocyclopentyl)-*N*-(3′,4′ - dichlorophenyl)propanamide (I) which may have a more rapid onset of therapeutic activity and fewer side effects was recently reported (3).

For definition of the pharmacokinetic and metabolic profiles of this potential antidepressant drug, a sensitive, precise, and specific analytical method is required. The presence of two chlorine moieties in the aromatic ring provides sufficient electronegativity for electron-capture (EC) detection after gas chromatographic (GC) separation. Initial studies[1] had shown that the drug was quickly demethylated at the dimethylamine functional group to give II. Also, a homologue of I with a cyclohexyl ring in place of the cyclopentyl ring was available (III) and was an ideal internal standard.

This report describes the GC–EC method for the quantitative determination of I, its *N*-demethyl metabolite, and the internal standard and the sample preparation procedure necessary to isolate the compounds from serum and to provide a sample amenable to GC–EC determination. Application of the methodology was demonstrated by the determination of serum levels of I and II in dogs



I: R = CH_3, n = 1
II: R = H, n = 1
III: R = CH_3, n = 2

[1] Unpublished data.

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