Analysis and Prediction of Drug Transfer into Human Milk Taking into Consideration Secretion and Reuptake Clearances across the Mammary Epithelia

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ABSTRACT:
Medication use during lactation is a matter of concern due to unnecessary exposure of infants to drugs. Although some studies have predicted the extent of drug transfer into milk from physicochemical parameters, drug concentration-time profiles in milk have not been predicted or even analyzed yet. In the present study, a drug transfer model was constructed by defining secretion and reuptake clearances (CLsec and CLre, respectively) between milk and plasma based on unbound drug concentrations. Through the use of this model, drug concentration-time profiles were analyzed in human milk and plasma based on data collected from the literature. CLsec and CLre values were obtained successfully for 49 drugs. Because the CLsec and CLre values were in general similar for each drug, transport across the mammary epithelia was mediated by passive diffusion in most cases. This study demonstrated that the logarithmically transformed values of CLsec and CLre can be predicted from physicochemical parameters with adjusted $R^2$ values of 0.705 and 0.472, respectively. Moreover, 66.7% and 77.8% of predicted CLsec and CLre values were within 3-fold error ranges of the observed values for 45 and 27 drugs, respectively. Finally, time profiles of drug concentrations in milk were simulated from physicochemical parameters. The milk-to-plasma area under the concentration-time curve ratios also were predicted successfully within 3-fold error ranges of the observed values for 71.9% of the drugs analyzed. The method described herein therefore may be useful in predicting drug concentration-time profiles in human milk for newly developed drugs.

Introduction
Feeding babies with breast milk rather than artificial formula is recommended by many experts for a number of reasons. Breast milk is thought to provide the most complete array of nutritional benefits for infants given that it contains immunoglobulins, such as IgA, resulting in protection from infections (Downham et al., 1976). In addition, breast feeding may strengthen the emotional bond between mother and infant (Falceto et al., 2004). However, medical treatment by the mother may be transferred into breast milk to a significant degree, resulting in unnecessarily exposure of the infant to pharmacological agents. In the cases of certain drugs, adverse reactions in infants have been demonstrated after breast feeding (Patrick et al., 1972; Schimmel et al., 1989). Under such situations, mothers must choose between giving up breast feeding and abstaining from the medication. Hence, the selection of drugs that have an improved safety profile for infants is of the utmost importance to the health of the mother and child alike. However, the risk of adverse effects in breast-fed infants remains to be clarified for most drugs. In addition, information on drug transfer into breast milk is available for only a limited number of drugs, because such information is not usually requested when a new drug is approved. Thus, a method to predict drug transfer into milk has been demanded frequently so that rational criteria can be formulated for the selection of safer drugs for breast feeding infants from both drug discovery and therapeutic points of view. Moreover, it also would be useful to quantitatively evaluate the epidemiological risks posed by the exposure of breast feeding infants to drugs.

Several methods, or models, have been proposed previously to predict drug transfer into milk (Fleishaker, 2003). For example, drug distribution between plasma and milk was explained in part by the pH partition theory in early animal studies (Miller et al., 1967). By extending this theory, Atkinson and Begg (1990) proposed a “phase distribution model” in which drug distribution into milk was estimated...
by pH partition, protein binding, and distribution into milk lipid (Atkinson and Begg, 1990). In the phase distribution model, the authors hypothesized that rapid equilibrium of drug concentrations exists between plasma and milk. In a recent report, a population-based pharmacokinetic approach was applied to estimate the fluoxetine milk-to-plasma ratio and the exposure of infants to this drug through breast milk, assuming that plasma and milk drug concentrations are roughly parallel (Panchaud et al., 2011). However, the time course of drug concentrations in plasma and milk does not usually increase or decrease in parallel (Somogyi and Gugler, 1979; Shyu et al., 1992), and in these cases, the drug profiles cannot be explained by rapid equilibration. Further improvement of the predictive model to estimate the time profiles of drug concentrations in milk would be useful to minimize the exposure of breast feeding infants to drugs.

The three-compartment model was proposed to describe time profiles of certain drugs in milk (Stec et al., 1980). However, it is difficult to apply the three-compartment model to the pharmacokinetics of many drugs, because information is limited regarding the time profiles of drug concentrations in plasma and milk. Moreover, examples of transporter-mediated drug transfer into milk have been reported increasingly in animal studies (Jonker et al., 2005; Merino et al., 2005), and this mechanism of drug transfer likely also occurs in humans. Therefore, a new model of drug distribution is required for the accurate, quantitative analysis of the rate of drug transfer across the mammary epithelia in transporter-mediated processes.

In the present study, the hypothesis was explored that drug distribution between plasma and milk is not always in rapid equilibrium. A new model was constructed that permits the consideration of permeability-limited or transporter-mediated drug transfer across the mammary epithelia. On the basis of this model, secretion and reuptake clearances (CLsec and CLre, respectively) of drug transfer into milk were obtained by curve fitting. Comparison of the ratio of unbound drug concentrations in milk with those in plasma based on CLsec and CLre values suggested that drug partition between milk and plasma is mediated by passive diffusion in many cases, whereas involvement of transporter-mediated transfer must be taken into account in others. Finally, multiple linear regression analysis was performed to obtain equations relating CLsec and CLre values to physicochemical parameters for drugs that are transferred into milk predominantly via passive diffusion. Through the use of unbound concentration-based clearances predicted from physicochemical parameters and unbound fractions in plasma and in milk, time profiles of drug concentrations in milk and milk-to-plasma area under the curve ratios [M/P (AUC)] were predicted successfully.

Materials and Methods

Materials. Acetaminophen, carbencillin disodium salt, cefalothin sodium salt, clindamycin hydrochloride, digoxin, disopyramide phosphate salt, labetalol hydrochloride, nitrofurantoin, (-)-propranolol hydrochloride, terbutaline hemisulfate salt, and (-)-verapamil hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO). Acyclovir, alprazolam, atenolol, cefotaxime sodium salt, cephapirin sodium salt, diltiazem hydrochloride, hydrochlorothiazide, methotrexate, metronidazole, (-)-nicotine, nitrazepam, prednisolone, 6-propyl-2-thiouracil, trazodone hydrochloride, and thiopental sodium salt containing sodium carbonate were from Wako Pure Chemicals (Osaka, Japan). Penicillin G potassium salt, chloramphenicol, and cimetidine were from Nacalai Tesque (Kyoto, Japan). Budesonide, clarithromycin, flucloxacin, methimazole, methylkopa, metoprolol, minoxidil, mirtazapine, ofloxacin, praziquantel, and quetiapine fumarate were from LKT Laboratories (St. Paul, MO). Bupropion hydrochloride and triprolidine hydrochloride were from MP Biomedicals (Solon, OH). Metformin was from Enzo Life Sciences, Inc. (Farmington, NY). Moclobemide was from Toronto Research Chemicals Inc. (North York, ON, Canada). Other reagents were of analytical grade.

Model Construction of Drug Transfer into Milk. Clearances (CLsec and CLre) were defined as the processes of drug transfer from plasma to milk and milk to plasma, respectively (Fig. 1). Presuming that these clearances are constant during breast feeding, the assumption was made that drug transfer into milk follows a mass balance equation, given by eq. 1:

$$\frac{dC_{m}(t)}{dt} = CL_{sec} \cdot f_p \cdot C_{p}(t) - CL_{re} \cdot f_{m,total} \cdot C_{m}(t)$$

where \(C_{m}(t)\), \(C_{p}(t)\), \(f_p\), and \(f_{m,total}\) represent milk volume (500 ml), drug concentrations in plasma and milk, and unbound fractions of drug in plasma and milk, respectively. Although \(V_m\) may be altered because of breast feeding, the alternation was presumed to be insignificant for the sake of simplification. This simplification was based on the consideration that the volume of milk provided for a single feeding is small (approximately 150 ml) compared with the total volume of milk accumulated in the breasts (approximately 500 ml on average) (Dalry et al., 1993; Ramsay et al., 2005). Finally, the \(f_p\) values used in the present study were obtained from the DrugBank database (http://www.drugbank.ca) or the package insert, as summarized in Table 1.

Overview of the Data Analysis Procedure. A flow chart of the present study is shown in Fig. 2. Approximately 300 published works were collected initially from the literature that reported drug concentration data in human milk and plasma. The data were sorted in a stepwise procedure based on the criteria noted in Fig. 2 and according to the details provided in the following sections.

Determination of \(f_{m,total}\). Sixty-four drugs described in the collected literature works had calculable CLsec and CLre values (see Calculation of CLsec and CLre, Values by Curve Fitting and Fig. 2). Among these, the \(f_{m,total}\) Value was measured for 44 drugs (27 drugs in group A, 4 drugs in group B, and 13 drugs in group D; Fig. 2) using human milk. The milk samples were obtained from healthy nursing mothers whose children were admitted to the University of Tokyo Hospital. The mothers did not take any medications for at least 72 h before milk sampling. Pooled human milk samples were centrifuged (2000g, 15 min, room temperature) and separated into skim and lipid portions. The specimens then were mixed to reconstitute 5% milk lipid. The pH of the reconstituted milk was 7.2. An aliquot of the reconstituted milk (450 µl) was mixed with a drug solution (50 µl) to give a milk sample containing 4.5% lipid and 1 µM drug. Samples then were transferred to a siliconized 0.6-ml tube (Watson, Tokyo, Japan) and

![Fig. 1. Model of drug transfer between plasma and milk. The relationship between the plasma compartment and the milk compartment is shown. C_{p}(t) and C_{m}(t) represent drug concentrations in plasma and milk, respectively. V_{m} represents the milk compartment volume. CL_{sec} and CL_{re} are drug transport clearances from plasma into milk and milk into plasma defined by unbound drug concentrations in plasma and milk, respectively. F × dose represents bioavailable dose (F represents bioavailability). Only unbound drug (not associated with protein or lipid) can transfer between plasma and milk.](attachment://image.jpg)
### TABLE 1

Free fraction in plasma and milk and observed and predicted values of \( \log(\text{CL}_{\text{sec}}) \), \( \log(\text{CL}_{\text{re}}) \), and \( \text{M/P} \) (AUC)

<table>
<thead>
<tr>
<th>Drug</th>
<th>( f_P )</th>
<th>Measured</th>
<th>Predicted</th>
<th>Observed</th>
<th>Predicted</th>
<th>Predicted</th>
<th>Predicted</th>
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</tr>
</thead>
</table>
|       | \( \text{f}_{\text{true}} \) | \( \text{f}_{\text{true}} \) | \( \text{f}_{\text{true}} \) | \( \text{f}_{\text{true}} \) | \( \text{f}_{\text{true}} \) | \( \text{f}_{\text{true}} \) | \( \text{f}_{\text{true}} \) | \( \text{f}_{\text{true}} \) | \( \text{f}_{\text{true}} \) |}
and the ratio of each drug dissolved in ultrafiltrated milk. Any nonspecific adsorption to the device was corrected by using the recovery (Triton X-100; see Supplemental Table 1) to minimize nonspecific adsorption. The lipid layer on the top of each centrifuged sample was excised, and the lipid layer on the top of each centrifuged sample was excised, and the lipid was pretreated with a surfactant (5% Tween 20, 5% SDS, or 5% Triton X-100) to separate the lipid layer from the aqueous phase.

Ultrafiltrated milk samples (50 μl) were mixed with an equivalent amount of water or 2% formic acid containing 200 nM carbamazepine, and drug concentrations were determined by liquid chromatography tandem mass spectrometry using an ultraperformance liquid chromatography system and a Quattro Premier XE (Waters, Milford, MA) with an ACQUITY ultraperformance liquid chromatography bridged ethyl hybrid C18 analytical column (2.1 × 100 mm, internal diameter 1.7 μm; Waters). Carbamazepine was used as the internal standard. The oven temperature was 40°C, and the flow rate was 0.3 ml/min. Samples were kept at 4°C in the sample injector, and aliquots of 7.5 μl were injected (see Supplemental Table 1 for a summary of detailed analytical conditions). Finally, the drug concentrations in the ultrafiltrate were divided by 1 μM (the initial concentration in whole milk) to obtain \( f_{m,\text{total}} \) values.

For an additional 20 drugs (18 drugs in group C and 2 drugs in group D; Fig. 2), the \( f_{m,\text{total}} \) value was not measured due to 1) the difficulty in obtaining the compounds (narcotic compounds including codeine, hydrodromorphine, methadone, morphine, and pseudoephedrine as well as other compounds including cefprozil, citalopram, escitalopram, fluvoxamine, meperidine, metoclopramide, olanzapine, rofecoxib, sumatriptan, zaleplon, and zopiclone; 2) high background signals in control milk samples (caffeine and theophylline contamination as a result of drinking coffee); and 3) difficulty in establishing detection conditions for certain drugs (captopril and tacrolimus). In these cases, \( f_{m,\text{total}} \) values were approximated using eq. 2, where protein binding in milk (\( f_{m,\text{protein}} \)) and partition into milk lipid (\( P_{app} \)) were taken into account as reported previously (Atkinson and Begg, 1990) (drugs shown with * in Table 1):

\[
f_{m,\text{total}} = \frac{1}{0.955 f_{m,\text{protein}} + 0.045 \times P_{app}}
\]

In eq. 2, 0.955 represents the mean value of the composition ratio of skim milk, and 0.045 represents the mean value of the composition ratio of lipid in milk. These values were used on the basis of the data presented in a previous report (Jensen et al., 1980). Atkinson and Begg (1988a) approximated \( f_{m,\text{protein}} \) from \( f_p \) using eq. 3:

\[
f_{m,\text{protein}} = \frac{f_p^{0.448}}{6.94 \times 10^{-7} f_p^{0.448} + f_p^{0.005}}
\]

The authors also approximated \( P_{app} \) from log \( D_{7.2} \) using eq. 4 (Atkinson and Begg, 1988b):

\[
\log P_{app} = -0.88 + 1.29 \times \log D_{7.2}
\]

where log \( D_{7.2} \) represents the octanol-water distribution coefficient at pH 7.2, the mean pH of human milk. In the present study, log \( D_{7.2} \) values were calculated using the MarvinSketch program (ChemAxon, Budapest, Hungary), and log \( P \) values were collected from the DrugBank database.
Calculation of CL_{sec} and CL_{re} Values by Curve Fitting. The literature on drug transfer into human milk was researched exhaustively via reference to Medications and Mothers’ Milk (Hale, 2008) and performance of a thorough PubMed search. The search was performed until January 2009 using keywords such as “drug,” “human,” “milk,” “concentration,” “transfer,” “mother,” and so on. The net result was the collection of approximately 300 publications (Fig. 2), as noted above. Next, 179 data sets were selected that covered 73 drugs, providing a sufficient number of data points to perform curve fitting, i.e., 1) >5 or 7 data points of CL_{p} were available for analysis with the one- or two-compartment models, respectively; 2) >4 data points of CL_{m} were available; and 3) the elimination phase was observed for the drug in question (Fig. 2).

Calculation of CL_{sec} and CL_{re} values was performed as follows. First, CL_{p} was fitted to the conventional one- or two-compartment model with first-order absorption including lag time, and the corresponding kinetic parameters were obtained. In cases where both models were applicable to the data, the model with the higher model selection criterion, an expanded model selection criterion of Akaike’s information criterion as defined in the Scientist 3.0 program (MicroMath Inc., Salt Lake City, UT), was adopted. In the case of tacrolimus, drug concentrations in whole blood rather than in plasma were available. For this reason, the concentration of tacrolimus in blood was converted to the concentration in plasma using a ratio of drug concentration in whole blood to plasma (R_{b}) of 10, as described in an interview form for Prograf provided by Astellas Pharma Inc. (Tokyo, Japan). Among the 179 data sets collected from the literature, at least one of the compartment models was applicable for 172 data sets, covering 72 drugs. These 172 data sets were used for further analysis (Fig. 2).

Second, to obtain CL_{sec} and CL_{re} values, CL_{p} was fitted to eq. 1 in a numerical manner with CL_{m} calculated from the corresponding compartment model. These clearances were calculable for 160 data sets covering 64 drugs, which were used for subsequent analyses (Fig. 2). For another 12 data sets (aminopyrine, ampicillin, cefoperazone, erythromycin, fluoxetine, imipramine, kanamycin, and risperidone drug data sets and portions of the atenolol, caffeine, metformin, and terbutaline data sets), it was difficult to describe C_{p} based on the curve-fitting model described herein, because the CL_{m} values were not stable during lactation and largely deviated from fitted lines. Therefore, the CL_{sec} and CL_{re} values for these drugs were excluded from further analysis. The numerical calculation by the Runge-Kutta method, nonlinear least-squares fitting, and simulation were performed using the Scientist 3.0 program. If more than three data sets were available for the same drug, then the geometric mean and S.D. of the CL_{sec} and CL_{re} values were calculated for that drug.

Relationship between CL_{sec} and CL_{re} Values and Physicochemical Parameters. The relationship between CL_{sec} and CL_{re} values and physicochemical parameters was analyzed for drugs with CL_{sec} and CL_{re} values that were obtained in a reliable manner and with milk transfer that was not reported to be mediated by transporters (details provided under Results). The physicochemical parameters used for the analysis were MW or log(MW), log(D_{f},P), log(D_{f},P/CL_{m}), log(D_{f},P/MW^{1/2}), log(D_{f},P/MW^{1/2}), log(D_{f},P/MW^{1/2}), polar surface area (PSA) or log(PSA), hydrogen bond acceptor (HBA), and hydrogen bond donor (HBD). The MW and log P parameters were calculated using the MarvinSketch program. Regression analyses were performed for CL_{sec} and CL_{re} values using the SPSS 13.0J program (SPSS Inc., Chicago, IL) by the stepwise method. Combinations of parameters were selected so as to not exceed the absolute correlation coefficient, r, of 0.9. Details of parameter combinations and regression results are shown in the appendix (Supplemental Table 2). The best fit equations for CL_{sec} and CL_{re} values were selected according to the highest adjusted R² values.

Prediction of M/P (AUC). By integrating eq. 1 from time 0 to infinity and with rearrangements, M/P (AUC) can be predicted by eq. 5:

\[
M/P(AUC) = \frac{AUC_p}{AUC_m} = \frac{CL_{sec}}{CL_{re}} \times f_p \times \frac{\text{fm, total}}{f_m, total}
\]

As described by eq. 5, if CL_{sec} and CL_{re} values are predicted precisely from a regression using physicochemical parameters, then M/P (AUC) may be predicted from measured or approximated f_p and fm, total values. Predicted M/P (AUC) values calculated using eq. 5 were compared with observed M/P (AUC) values (obtained from AUC_p and AUC_m for clinical data, determined by the trapezoidal rule extrapolating C_p(t) and C_m(t) for infinite time) to verify the accuracy of the prediction. In the case of multiple data sets for observed M/P (AUC) values, the arithmetic mean and S.D. values were calculated.

Results

Analysis of CL_{sec} and CL_{re} Values. Curve fitting of C_{p} and C_{m} data collected from the literature with a model described by eq. 1 allowed the initial calculation of CL_{sec} and CL_{re} values for 64 drugs. However, the clearance values were not always reliable for certain drugs whose concentrations in milk and blood plasma were in rapid equilibrium. Indeed, sensitivity analysis indicated that the absolute values of CL_{sec} and CL_{re} were not reliably obtained for 15 drugs whose fm, total · CL_{re} values were >5000 ml/h [i.e., log(fm, total · CL_{re}) exceeded 3.7], drugs shown with d in Table 1 and belonging to group D in Fig. 2]. These 15 drugs were excluded from the initial set of 64 drugs, yielding 49 drugs with reliable CL_{sec} and CL_{re} values (observed values in Table 1).

Typical examples of curve fitting are shown in Fig. 3. Secretion and reuptake clearances were plotted in Fig. 4, revealing a strong positive correlation between CL_{sec} and CL_{re} values. Moreover, absolute values of these two parameters were found to be similar for most of the drugs analyzed, with some exceptions (Table 1). For example, CL_{sec} values of clindamycin and nitrofurantoin were 10-fold higher than their respective CL_{re} values. In contrast, CL_{sec} values of captopril, carbamazepine, methotrexate, and rifocexib were 10-fold lower than their respective CL_{re} values.

Measurement of fm, total Values. To describe the drug transfer process across the mammary epithelia, a consideration of unbound drug concentrations in plasma and milk is crucial. Although information regarding unbound fractions in plasma is widely available from the literature, those in milk are poorly reported. In the present study, fm, total values of 44 drugs of the original 64 drugs with calculable CL_{sec} and CL_{re} values were determined experimentally (shown as fm, total without c in Table 1). These 44 drugs corresponded to 27 drugs in group A, 4 drugs in group B, and 13 drugs in group D (Fig. 2).

Among the 44 drugs, fm, total values were reported previously for digoxin, prednisolone, propranolol, and verapamil (Atkinson and Begg, 1988a,b), but not for any of the other drugs. The previously reported values were comparable with the experimentally determined values measured in this study, validating the methodology employed. The fm, total values reported in the literature and those measured herein were 0.99 and 1.06 for digoxin, 0.92 and 0.86 for prednisolone, 0.41 and 0.41 for propranolol, and 0.17 and 0.21 for verapamil, respectively. For an additional 20 drugs (18 drugs in group C and 2 drugs in group D; Fig. 2), fm, total values were not determined experimentally but were instead predicted from f_p and log(D_{f},P) values using eqs. 2 to 4 (Table 1).

Multiple Linear Regression Analysis of CL_{sec} and CL_{re} Values. Forty-nine drugs with reliable CL_{sec} and CL_{re} values were obtained as described above (groups A–C in Fig. 2). Multiple linear regression analysis was performed for these drugs after eliminating particular drugs based on the following exclusion criteria: four drugs (acyclovir, cimetidine, methotrexate, and nitrofurantoin) were excluded for the analysis of both CL_{sec} and CL_{re} values because the contribution of a transporter in the milk transfer was suggested for these drugs (group B in Fig. 2); these drugs are reportedly transported by breast cancer resistance protein/ATP-binding cassette transporter G2 (BCRP/ ABCG2) (Volk and Schneider, 2003; van Herwaarden and Schinkel, 2006). Furthermore, 18 drugs were excluded from the analysis of CL_{re} values, because fm, total values were not measured experimentally (group C in Fig. 2). Consequently, 45 drugs (4 drugs excluded from 49 drugs) and 27 drugs (18 drugs further excluded from these 45
drugs) were subjected to multiple linear regression analysis of CL\textsubscript{sec} and CL\textsubscript{re} values, respectively, with physicochemical parameters as explanatory variables (Table 2). The logarithmically transformed CL\textsubscript{sec} values were found to be described by PSA, log\((MW)\), and explanatory variables (Table 2). The logarithmically transformed CL\textsubscript{re} values were found to be described by PSA, log\((MW)\), and log\((P/D_{1.4})\) (eq. 6) (Fig. 5A; Table 3).

\[
\log(\text{CL}_{\text{sec}}) = -3.912 - 0.015 \times \text{PSA} + 3.367 \times \log(\text{MW}) - 0.164 \times \log(P/D_{1.4})
\]  

(6)

In addition, the logarithmically transformed CL\textsubscript{re} values were found to be described by log\(P\) and HBD (eq. 7) (Fig. 5B; Table 3).

\[
\log(\text{CL}_{\text{re}}) = 2.793 + 0.179 \times \log(P) - 0.132 \times \text{HBD}
\]  

(7)

Adjusted \(R^2\) values for the prediction of CL\textsubscript{sec} and CL\textsubscript{re} values were 0.705 and 0.472, respectively. Thirty of 45 drugs (66.7%) and 21 of 27 drugs (77.8%) were within a 3-fold error range of predicted CL\textsubscript{sec} and CL\textsubscript{re} values, respectively (Fig. 5, A and B; Table 1). The relationship between predicted CL\textsubscript{sec} values and predicted CL\textsubscript{re} values (Fig. 5C) was quite similar in shape as that between observed CL\textsubscript{sec} values and observed CL\textsubscript{re} values (Fig. 4), showing that the results of the regression analysis were valid. We also predicted CL\textsubscript{sec} and CL\textsubscript{re} values for drugs in group D, which were excluded from the regression analysis. As a result, the predicted \(f_{\text{m,total}} \times \text{CL}_{\text{re}}\) values were <5000 ml/h for the 15 drugs examined (123–1095 ml/h). Moreover, the relationship between predicted CL\textsubscript{sec} values and predicted CL\textsubscript{re} values of group D drugs (Fig. 5C, gray diamonds) fell into the same plot area as group A to C drugs (Fig. 5C, open circles and open squares).

**Prediction of M/P (AUC) Values from Physicochemical Parameters.** The M/P (AUC) values were predicted for 64 drugs using eq. 5 and CL\textsubscript{sec} and CL\textsubscript{re} values that were predicted from physicochemical parameters using eqs. 6 and 7. Reported \(f_p\) values and measured (44 drugs) or predicted (20 drugs) \(f_{\text{m,total}}\) values were used in the calculation. The majority (71.9%) of predicted M/P (AUC) values were within a 3-fold error range of the values observed in clinical studies (Fig. 6).

**Simulation of Drug Concentration in Milk.** Drug concentration-time profiles in milk were simulated for 160 data sets (1056 data...
point) covering 64 drugs, using reported \( f_{o/s} \) values, measured or predicted \( f_{o/s,obs} \) values, and \( CL_{m,obs} \) and \( CL_{m,calc} \) values that were predicted from physicochemical parameters. Plots of observed versus simulated \( C_{m,obs} \) values for all of the drugs are shown in Fig. 7. Least-squares linear fitted lines did not deviate from theoretical best fit lines for drugs in groups A, C, and D; hence, there is no bias such as overestimation or underestimation of drug concentration in this simulation. However, apparent underestimations of drug concentration in milk are observed for drugs in group B.

### Discussion

Several reports are available that characterize drug transfer into the milk from plasma. However, time profiles of drug concentrations in plasma and milk (\( C_{p} \) and \( C_{m} \)) were not fully used in these analyses even though some reports have noted that drug concentrations in milk do not increase or decrease in parallel to drug concentrations in plasma (McNamara et al., 1991, 1992). In the present study, \( CL_{m,obs} \) and \( CL_{m,calc} \) values were defined to construct a pharmacokinetic model that dynamically describes drug transport between plasma and milk. The model revealed that drug concentration-time profiles in milk and plasma were not parallel and exhibited a difference in \( T_{\text{max}} \) (time of maximum concentration) between plasma and milk profiles for drugs whose net reuptake clearances \( (f_{o/s,calc} \cdot CL_{m}) \) were relatively low (<5000 ml/h). For these drugs, time profiles in milk may be predicted successfully by the pharmacokinetic model (Figs. 3 and 7). Such an analysis would be helpful in clinical situations, making it possible to advise mothers to avoid breast feeding when the \( C_{m} \) of the drug reaches its maximum, which is the time that most likely will result in maximum exposure of the infant to the drug with a consequent risk of adverse effects on infant health. However, \( CL_{sec} \) and \( CL_{re} \) values could not be well differentiated for drugs with \( f_{o/s,calc} >5000 \) ml/h due to rapid equilibrium between milk and plasma.

The \( CL_{sec} \) and \( CL_{re} \) values found to be nearly identical for most drugs (Fig. 4). The fact that \( CL_{sec} \) and \( CL_{re} \) values between plasma and milk for unbound drugs were in essence equivalent suggests that drug transport through the mammary epithelia occurs primarily via passive diffusion, as is accepted generally (Rivera-Calimlim, 1977). It is also noteworthy that the expression of transporters at the mammary epithelia and their contribution to the transfer of drugs into the milk have been studied extensively (Alcorn et al., 2002; Gilchrist and Alcorn, 2010). If a transporter mediates secretion from plasma to milk, then the observed \( CL_{re} \) value would be larger than predicted. However, if a transporter mediates reuptake from milk to plasma, then the observed \( CL_{sec} \) value would be larger than predicted. Actually, the \( CL_{sec} \) values of nitrofurantoin and cinetidine were higher than their \( CL_{re} \) values (Table 1). In addition, the observed \( CL_{sec} \) values also were higher than the \( CL_{re} \) values predicted from physicochemical parameters (Fig. 5A; Table 1). These are consistent with the fact that secretion of these two drugs into milk is mediated by BCRP (Jonker et al., 2005; Merino et al., 2005; Wang et al., 2008). It is also noteworthy that the expression level of the transporter changes depending on the lactating stage (Alcorn et al., 2002). This means that \( CL_{sec} \) and/or \( CL_{re} \) values are not necessarily stable throughout the lactation period and that inter- or intraindividual deviation of drug transfer into milk might emerge depending on the lactating stage, especially for drugs whose transfer across the mammary epithelia is mediated by transporters.

Multiple regression analysis revealed that \( CL_{sec} \) values were described by PSA, log(MW), and log(PD). The PSA is an index of the ability to participate in hydrogen bond formation and negatively correlates with permeability across cell monolayers (Stenberg et al., 2001), and as such, the PSA is related to drug transport across the
mammary epithelia. Furthermore, the MW of a drug is known to correlate with its diffusion constant, and therefore, log(MW) may be related to intramembrane and intracellular drug diffusion. In addition, log\( (P/D_{7.4}) \) represents the extent of ionization in the plasma at pH 7.4. Log \( P \) is an index of lipophilicity, as is log \( D \) (common logarithm of octanol-water distribution coefficient, taking into consideration the ionization of the compound at solvent pH). Because log\( (P/D_{7.4}) \) negatively affects CLsec values, drugs with a greater extent of ionization are suggested to be associated with a lesser degree of transfer into milk. In contrast, CLre values were described best by log \( P \) and HBD. In accordance with the positive correlation between log \( P \) and permeability through both the cell monolayer and an artificial membrane.

![Diagram](image-url)
(Barratt, 1995; Nakao et al., 2009), log $P$ positively affects $\text{CL}_{\text{re}}$ values. However, HBD negatively affects $\text{CL}_{\text{re}}$ values (eq. 7), consistent with previous observations that hydrogen bond donors decrease the permeability of the cell membrane due to increased interactions with the lipid bilayer (Winiwarter et al., 1998; Kokate et al., 2009). Given that $\text{CL}_{\text{re}}$ and $\text{CL}_{\text{re}}$ values were almost identical for many drugs (Fig. 4), it was unexpected that the physicochemical parameters best describing these two clearances were different from each other. One of the possible reasons is the poor correlation between $\text{CL}_{\text{sec}}$ and $\text{CL}_{\text{re}}$ values of drugs with a $\text{CL}_{\text{re}}$ value of $<100$ ml/h (Fig. 4), which could have contributed somehow to the apparently different equations for $\text{CL}_{\text{sec}}$ and $\text{CL}_{\text{re}}$ values. Alternatively, although known BCRP substrates were removed from the regression analysis, we cannot exclude the possibility that other asymmetrical transport substance drugs were included in the regression analysis. Further experimentation will be required to determine which of these possibilities is correct.

Taken together, the present analyses of human data successfully differentiate between drugs that are transferred into milk by passive diffusion and drugs that are transferred into milk by some other mechanism (e.g., transporter-mediated transfer). This drug classification is likely to assist in providing a comprehensive understanding of drug transfer mechanisms into milk and further improve the ability to predict drug transfer. Nonetheless, limitations of the present study must be addressed.

First, the milk compartment of the mammary gland was assumed to be a closed system that is only accessible from the central compartment of the body, and the milk compartment further was assumed to have a constant volume. However, drug concentrations in the milk compartment are decreased by lactation, and the volume of the milk compartment changes due to milk production and lactation. These facts may create a certain amount of bias in calculating $\text{CL}_{\text{sec}}$ and $\text{CL}_{\text{re}}$ values. For example, in the current pharmacokinetic model, drug elimination from the milk compartment is defined as transfer into the plasma compartment with a clearance given by the $\text{CL}_{\text{re}}$ value. This definition may lead to an overestimation of $\text{CL}_{\text{re}}$ values, particularly for drugs whose $\text{CL}_{\text{re}}$ values are $<100$ ml/h as shown in Fig. 4. The analysis described in this study suggested that $\text{CL}_{\text{re}}$ has a minimum value of approximately $20$ ml/h (i.e., $\log(\text{CL}_{\text{re}})$ of approximately $1.3$) (Fig. 4; Table 1), consistent with the fact that the milk production rate is approximately $15$ to $20$ ml/h on average and may reach as high as $60$ ml/h (Lai et al., 2010), which is also the mean rate for lactation. The correlation between $\text{CL}_{\text{sec}}$ and $\text{CL}_{\text{re}}$ values tended to deviate for drugs with a $\text{CL}_{\text{re}}$ value of $<100$ ml/h, including captopril, carbenicillin, and methotrexate (Fig. 4; Table 1).

Second, calculation of $\text{CL}_{\text{re}}$ values requires accurate determination of $f_{\text{m, total}}$ values. Although $f_{\text{m, total}}$ values are predictable from $f_p$ and log $D_{2, 4}$ values using eq. 2 (Atkinson and Begg, 1990), application of this equation does not always lead to reliable values (Supplemental Fig. 1; Table 1). For example, $f_{\text{m, total}}$ values experimentally measured in the present study and those predicted by eq. 2 are $1.06$ and $0.200$ for doxigoxin and $0.41$ and $0.92$ for propranolol, respectively. Therefore, the predictability of $\text{CL}_{\text{re}}$ values as well as $\text{M/P (AUC)}$ values may be affected inevitably by a prediction error for $f_{\text{m, total}}$ values for 20 drugs whose $f_{\text{m, total}}$ values were not determined experimentally (Table 1; Fig. 6).

Third, $f_{\text{m, total}}$ value may be affected by the lipid content in milk. The lipid content in milk changes to some degree depending on the

---

**TABLE 3**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Partial Regression</th>
<th>Standardized Partial Regression</th>
<th>$P$ Value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{CL}_{\text{sec}}$</td>
<td>$-3.912$</td>
<td>$-0.850$</td>
<td>$&lt;0.001$</td>
<td>$-7.018$ to $-0.806$</td>
</tr>
<tr>
<td>PSA</td>
<td>$-0.015$</td>
<td>$0.522$</td>
<td>$&lt;0.001$</td>
<td>$2.073$ to $4.661$</td>
</tr>
<tr>
<td>log($\text{MW}$)</td>
<td>$3.367$</td>
<td>$-0.264$</td>
<td>$0.005$</td>
<td>$-0.274$ to $-0.053$</td>
</tr>
<tr>
<td>log($\text{PSD}_{2, 4}$)</td>
<td>$-0.164$</td>
<td>$&lt;0.001$</td>
<td>$2.244$ to $2.627$</td>
<td></td>
</tr>
<tr>
<td>$\text{CL}_{\text{re}}$</td>
<td>$2.793$</td>
<td>$0.437$</td>
<td>$0.026$</td>
<td>$0.023$ to $0.335$</td>
</tr>
<tr>
<td>log $P$</td>
<td>$0.179$</td>
<td>$0.019$</td>
<td>$0.011$</td>
<td>$2.073$ to $4.661$</td>
</tr>
<tr>
<td>HBD</td>
<td>$-0.132$</td>
<td>$0.053$</td>
<td>$0.019$</td>
<td>$2.073$ to $4.661$</td>
</tr>
</tbody>
</table>

* $n = 45$, adjusted $R^2 = 0.705$, analysis of variance $P < 0.001$.

† $n = 27$, adjusted $R^2 = 0.472$, analysis of variance $P < 0.001$.
postpartum period; it is relatively low in the colostrum and increases after delivery (Hyttén, 1954). Moreover, the lipid content also changes during breast feeding, being low in foremilk and high in hindmilk (Wilson, 1983). Consequently, drug distribution into milk lipid may differ depending on the experimental conditions, particularly for lipophilic drugs. Although this study presumed a mean milk lipid content of 4.5%, lipid content actually ranges from 2.9 to 5.4% (Ferris and Jensen, 1984), which may result in deviations in the relationship between predicted and observed CLre values of group D drugs appears very similar to that of other drug types. Moreover, the M/P (AUC) values (Fig. 6, gray diamonds) and their time profiles (Fig. 7D) were well predicted as was the case for other drug types. These results are consistent with the fact that the predicted f_m,total · CLre values of group D drugs turned out to be <5000 ml/h. It seems reasonable to assume that values of >5000 ml/h were obtained erroneously due to subtle fluctuations in drug concentrations. Therefore, it is expected that f_m,total · CLre values were <5000 ml/h for most drugs; thus, the present prediction method can be considered to be widely applicable. However, the result should be taken with caution particularly when predicted f_m,total · CLre values exceed 5000 ml/h for the drug of interest.

In conclusion, secretion and reuptake clearances of 49 drugs between plasma and milk compartments were analyzed successfully in humans based on drug concentration profiles in milk and plasma. Although some limitations and exceptions exist, these clearances can be predicted on the basis of physicochemical parameters. This gives a tentative but valuable estimation of drug transfer into milk for newly developed drugs. Although further validation studies are required, this information will be useful in advancing medications that are efficacious for mothers and considered to be safe for infants during lactation.

Acknowledgments
We thank Dr. Naoki Ito (Department of Pediatrics, The University of Tokyo Hospital) for assistance with the collection of milk samples.

Authorship Contributions
Participated in research design: Koshimichi, Ito, Hisaka, Honma, and Suzuki.
Conducted experiments: Koshimichi.
Performed data analysis: Koshimichi.
Wrote or contributed to the writing of the manuscript: Koshimichi, Ito, Hisaka, Honma, and Suzuki.

References


Wang L, Leggas M, Goswami M, Empey PE, and McNamara PJ (2008) N-4-(2-[1,2,3,4-tetrahydro-6,7-dimethoxy-2-isooquinolinyl(ethyl)-phenyl)-9,10-dihydro-5-methoxy-4-acridine carbazamide (GF120918) as a chemical ATP-binding cassette transporter family G member 2 (Abc2) knockout model to study nitrofurantoin transfer into milk. Drug Metab Dispos 36:2591–2596.


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Drug Metabolism and Disposition

Appendix references

References of drug concentration time profiles in plasma and milk. Reference number corresponds to those shown in Table 1.


23. Oo, C.Y., Kuhn, R.J., Desai, N. & McNamara, P.J. Active transport of cimetidine


37. Peiker, G., Muller, B., Ihn, W. & Noschel, H. [Excretion of pethidine in mother's


53. Matheson, I., Lunde, P.K. & Bredesen, J.E. Midazolam and nitrazepam in the


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Hiroki Koshimichi, Kousei Ito, Akihiro Hisaka, Masashi Honma and Hiroshi Suzuki

Drug Metabolism and Disposition

Supplemental Figure 1
Measured and predicted $f_{m,total}$ values are plotted for 44 drugs whose $f_m$ total are experimentally measured in the present study (original data are shown in Table 1). Predicted $f_{m,total}$ values are calculated based on eq. 2 as described in the text. Solid line represents theoretical best fit line (1:1 correlation).
### LC-MS/MS Instrument Conditions

1. **MS-MS Conditions**
   - ESI ion mode:
     - Positive ion mode (+)
     - Negative ion mode (-)
   - Conditions of tune settings are described in the third section 3) Tune settings.

2. **UPLC Gradient Programs**
   - IS: Internal Standard
   - ESI: Electrospray Ionization
   - Dwell time and Delay time are 0.100 and 0.005 seconds, respectively.
   - Details of gradient program are described in the second section 2) UPLC gradient program.

### Supplemental Table 1

<table>
<thead>
<tr>
<th>Drug</th>
<th>Filter pretreatment</th>
<th>Diluent solvent</th>
<th>Gradient program</th>
<th>ESI ion mode</th>
<th>Cone Voltage (V)</th>
<th>Collision energy (eV)</th>
<th>Parent mass (m/z)</th>
<th>Daughter mass (m/z)</th>
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<tr>
<td>acetaminophen</td>
<td>5% SDS</td>
<td>Water</td>
<td>A +</td>
<td>21</td>
<td>188.10</td>
<td>70.0</td>
<td>10.0</td>
<td>16.0</td>
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<td>aciphex</td>
<td>5% AC</td>
<td>Water</td>
<td>A +</td>
<td>49</td>
<td>208.02</td>
<td>70.0</td>
<td>10.0</td>
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<td>Water</td>
<td>A +</td>
<td>25</td>
<td>267.32</td>
<td>140.0</td>
<td>12.6</td>
<td>24.1</td>
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<td>aspirin</td>
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<td>Water</td>
<td>C +</td>
<td>31</td>
<td>268.48</td>
<td>140.0</td>
<td>12.6</td>
<td>24.1</td>
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<td>brompheniramine maleate</td>
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<td>2% Formic acid</td>
<td>C +</td>
<td>17</td>
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<td>24.1</td>
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<td>Water</td>
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<td>23</td>
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<td>41.1</td>
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<td>Water</td>
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<td>2% Formic acid</td>
<td>A +</td>
<td>34</td>
<td>241.84</td>
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</table>

### Analysis and prediction of drug transfer into human milk taking into consideration secretion and reuptake clearances across the mammary epithelia

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Drug Metabolism and Disposition
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Hiroki Koshimichi, Kousei Ito, Akihiro Hisaka, Masashi Honma and Hiroshi Suzuki
Drug Metabolism and Disposition

Supplemental Table 2

Results of multiple linear regression analysis for all combinations; combination of tried parameters, significant variables, R² values and adjusted R² values.

### CLsec

<table>
<thead>
<tr>
<th>Combination of tried parameters</th>
<th>Significant variables</th>
<th>R² value</th>
<th>Adjusted R²</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>sec-a</td>
<td>log(P/MW^{0.52}), PSA, HBD, HBA, log(P/D_{7.4})</td>
<td>log(P/MW^{0.52}), PSA, log(P/D_{7.4})</td>
<td>0.613</td>
<td>0.584</td>
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<tr>
<td>sec-b</td>
<td>log(P/MW^{0.52}), log(PSA), HBD, HBA, log(P/D_{7.4})</td>
<td>log(P/MW^{0.52}), log(PSA), log(P/D_{7.4})</td>
<td>0.593</td>
<td>0.563</td>
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<tr>
<td>sec-c</td>
<td>log(D_{7.4}/MW^{0.52}), PSA, HBD, HBA, log(P/D_{7.4})</td>
<td>log(D_{7.4}/MW^{0.52}), PSA</td>
<td>0.611</td>
<td>0.593</td>
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<td>sec-d</td>
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<td>0.589</td>
<td>0.569</td>
<td>p &lt; 0.001</td>
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<tr>
<td>sec-e</td>
<td>log(P/ MW, PSA, HBD, HBA, log(P/D_{7.4})</td>
<td>MW, PSA, log(P/D_{7.4})</td>
<td>0.696</td>
<td>0.674</td>
</tr>
<tr>
<td>sec-f</td>
<td>log(P, MW, log(PSA), HBD, HBA, log(P/D_{7.4})</td>
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<td>0.626</td>
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<tr>
<td>sec-g</td>
<td>log(D_{7.4}/ MW, PSA, HBD, HBA, log(P/D_{7.4})</td>
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<td>0.674</td>
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<td>log(D_{7.4}/ MW, log(PSA), HBD, HBA, log(P/D_{7.4})</td>
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<td>0.573</td>
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<tr>
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<td>log(P, log(MW), PSA, HBD, HBA, log(P/D_{7.4})</td>
<td>log(MW), PSA, log(P/D_{7.4})</td>
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<td>0.705</td>
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<td>log(D_{7.4}, log(PSA)</td>
<td>0.593</td>
<td>0.573</td>
</tr>
</tbody>
</table>

Step-wise method, include variables if p < 0.05 and exclude them if p > 0.10.

### CLre

<table>
<thead>
<tr>
<th>Combination of tried parameters</th>
<th>Significant variables</th>
<th>R² value</th>
<th>Adjusted R²</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
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<td>log(P/MW^{0.52}), PSA, HBD, HBA, log(P/D_{7.4})</td>
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<td>0.510</td>
<td>0.470</td>
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<td>log(P/MW^{0.52}), HBD</td>
<td>0.510</td>
<td>0.470</td>
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<td>re-c</td>
<td>log(D_{7.4}/MW^{0.52}), PSA, HBD, HBA, log(P/D_{7.4})</td>
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<td>0.509</td>
<td>0.468</td>
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<td>re-d</td>
<td>log(D_{7.4}/MW^{0.52}), log(PSA), HBD, HBA, log(P/D_{7.4})</td>
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<td>0.468</td>
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<td>re-e</td>
<td>log(P/ MW, PSA, HBD, HBA, log(P/D_{7.4})</td>
<td>log(P, HBD</td>
<td>0.513</td>
<td>0.472</td>
</tr>
<tr>
<td>re-f</td>
<td>log(P, MW, log(PSA), HBD, HBA, log(P/D_{7.4})</td>
<td>log(P, HBD</td>
<td>0.513</td>
<td>0.472</td>
</tr>
<tr>
<td>re-g</td>
<td>log(D_{7.4}/ MW, PSA, HBD, HBA, log(P/D_{7.4})</td>
<td>log(D_{7.4}, HBD</td>
<td>0.511</td>
<td>0.471</td>
</tr>
<tr>
<td>re-h</td>
<td>log(D_{7.4}, MW, log(PSA), HBD, HBA, log(P/D_{7.4})</td>
<td>log(D_{7.4}, HBD</td>
<td>0.511</td>
<td>0.471</td>
</tr>
<tr>
<td>re-i</td>
<td>log(P, log(MW), PSA, HBD, HBA, log(P/D_{7.4})</td>
<td>log(P, HBD</td>
<td>0.513</td>
<td>0.472</td>
</tr>
<tr>
<td>re-j</td>
<td>log(P, log(MW), log(PSA), HBD, HBA, log(P/D_{7.4})</td>
<td>log(P, HBD</td>
<td>0.513</td>
<td>0.472</td>
</tr>
<tr>
<td>re-k</td>
<td>log(D_{7.4}/ MW, log(PSA), PSA, HBD, HBA, log(P/D_{7.4})</td>
<td>log(D_{7.4}, HBD</td>
<td>0.511</td>
<td>0.471</td>
</tr>
<tr>
<td>re-l</td>
<td>log(D_{7.4}/ MW, log(PSA), PSA, HBD, HBA, log(P/D_{7.4})</td>
<td>log(D_{7.4}, HBD</td>
<td>0.511</td>
<td>0.471</td>
</tr>
</tbody>
</table>

Step-wise method, include variables if p < 0.10 and exclude them if p > 0.15.

Multiple linear regression analysis was performed for CL_{sec} (45 drugs) and CL_{re} (27 drugs) using physicochemical parameters (shown in Table 2) as explanatory variable.

Combination of tried parameters were selected not to exceed absolute correlation coefficient, r, of 0.9.

Best equations were obtained describing CL_{sec} and CL_{re} with the highest adjusted R² values as shown in Table 3.
### Table 1: Regression Analysis for Drug Metabolism Disposition

<table>
<thead>
<tr>
<th>Variable</th>
<th>Partial Regression Coefficient</th>
<th>Standardized Partial Regression Coefficient</th>
<th>P-value</th>
<th>95% CI</th>
<th>Partial Regression Coefficient</th>
<th>Standardized Partial Regression Coefficient</th>
<th>P-value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>3.992</td>
<td>0.004</td>
<td>0.470</td>
<td>0.470</td>
<td>2.789 to 5.307</td>
<td>0.470</td>
<td>0.470</td>
<td>2.789 to 5.307</td>
</tr>
<tr>
<td>PSA</td>
<td>0.747</td>
<td>0.434</td>
<td>0.001</td>
<td>0.001</td>
<td>0.022 to 0.035</td>
<td>0.434</td>
<td>0.001</td>
<td>0.022 to 0.035</td>
</tr>
<tr>
<td>MW</td>
<td>0.436</td>
<td>0.434</td>
<td>0.001</td>
<td>0.001</td>
<td>0.022 to 0.035</td>
<td>0.434</td>
<td>0.001</td>
<td>0.022 to 0.035</td>
</tr>
<tr>
<td>HBD</td>
<td>0.321</td>
<td>0.321</td>
<td>0.001</td>
<td>0.001</td>
<td>0.022 to 0.035</td>
<td>0.321</td>
<td>0.001</td>
<td>0.022 to 0.035</td>
</tr>
<tr>
<td>HBA</td>
<td>0.232</td>
<td>0.232</td>
<td>0.001</td>
<td>0.001</td>
<td>0.022 to 0.035</td>
<td>0.232</td>
<td>0.001</td>
<td>0.022 to 0.035</td>
</tr>
</tbody>
</table>

*(n=27, Adjusted R² = 0.471, ANOVA p<0.001) (n=45, Adjusted R² = 0.472, ANOVA p<0.001)