Prediction of Human Volume of Distribution Values for Neutral and Basic Drugs. 2. Extended Data Set and Leave-Class-Out Statistics

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We present an extension and confirmation of our previously published method (*J. Med. Chem.* **2002**, *45*, 2867−2876) for the prediction of volume of distribution (VD) in humans for neutral and basic compounds. It is based on two experimentally determined physicochemical parameters, ElogD(7.4) and $f_{i(7.4)}$, the latter being the fraction of compound ionized at pH 7.4, and on the fraction of free drug in plasma (f_u). By regressing the fraction unbound in tissues, f_{ut}, vs the above parameters, we demonstrate the ruggedness of the method in predicting VD through the Oie−Tozer equation, via the use of several testing approaches. A comparison is also presented between several methods based on animal pharmacokinetic data, using the same set of proprietary compounds, and it lends further support for the use of this method, as opposed to methods that require the gathering of pharmacokinetic data in laboratory animals. The reduction in the use of animals and the overall faster and cheaper accessibility of the parameters used make this method highly attractive for prospectively predicting the VD of new chemical entities in humans.

Introduction

The complex, costly, and often uncertain outcome of the drug discovery and development process requires the simultaneous optimization of several properties. It has now long been recognized that favorable potency and selectivity characteristics are not the sole hallmarks of a successful drug discovery program, nor is the safety profile considered to be the only hurdle to be overcome, although it is of paramount importance.

The ability to prospectively predict the pharmacokinetics of new chemical entities in humans is a powerful means by which scientists involved in the discovery of new drugs can select for further development only those compounds with the potential to be successful therapeutic agents.

The half-life of a drug is a major contributor to the dosing regimen,[1] and it is a function of the clearance and apparent volume of distribution (VD), each of which can be predicted and combined to predict the half-life. Drugs with short half-lives are more likely to be required to be administered more frequently than those with long half-lives. Dosing regimen is also intrinsically linked to other factors such as the pharmacodynamics of the drug and the difference between systemic concentrations associated with side effects vs those minimally required for efficacy. However, these latter attributes are much more difficult to predict from in vitro or animal data and will be different for each therapeutic target. Thus, a great deal of focus has been placed on the prediction of human half-life. While methods using allometric scaling or correlative methods exist for prediction of half-life,[2−4] greater success is attained if the two major components of half-life, clearance and volume of distribution, are predicted separately and combined to generate a half-life prediction.[5]

Volume of distribution represents a complex combination of multiple chemical and biochemical phenomena. It is a measure of the relative partitioning of drug between plasma (the central compartment) and the tissues. Thus, the volume of distribution term considers all of the tissues as a single homogeneous compartment.

As a result, compounds that are equally bound to plasma proteins may yield different volumes of distribution, since the compound with the greater tissue binding will yield the larger VD. Conversely, compounds with equal tissue binding may differ in VD, with the compounds having the greater plasma protein binding yielding the smaller volume of distribution. Drug partitioning into tissues is a function of the sum of binding interactions with tissue components vs binding to plasma proteins, provided that the drug can readily penetrate into tissues. It should be noted that, realistically, binding to the various tissues is a function of the composition of each tissue, which dictates the binding affinities and capacities for various drugs. However, while it is simple to measure plasma protein binding using human plasma, measurement of tissue binding in humans is not practical.

In a previous report[6] we described a method for prediction of human volume of distribution for cationic and neutral drugs via the prediction of the theoretical unbound fraction in tissues (f_{ut}) for each of these drugs by the Oie−Tozer equation.[7] Once the predicted f_{ut} value is available, VD can be calculated from this value and the fraction unbound in plasma, f_u, to generate the predicted volume of distribution values. This method was generally successful, yielding approximately a 2-fold mean accuracy for predictions.

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In this report, we have further expanded this method and have demonstrated the robustness of the overall approach. The scientific literature was exhaustively mined for human volume of distribution data, resulting in an expansion of the data set used, with the original references provided in the Supporting Information section. Several statistical approaches, as well as an external test set, have been used to validate the model. The obvious advantage is in the application of this method to the prediction of volume of distribution and, of course, in the drastic reduction in the amount of resources needed and in the reduction in the use of animals. Details of this method are described herein, and a discussion of the general applicability of the method is offered.

Results and Discussion

In the Introduction we have outlined the importance of the volume of distribution (VD) for the prediction of the half-life of a drug and the usefulness of our previously reported method[6] in predicting VD from physicochemical properties and the fraction unbound in plasma. The following discussion will further illustrate these points and will show the suitability and ruggedness of this approach, with an extended data set and test statistics. We have mined the literature extensively for volume of distribution at steady state, VD_{ss}, from clinical data, and we have assembled a data set of 120 compounds comprising neutral and basic drugs. In the vast majority of cases these data are indeed VD_{ss} values, and in many cases they represent the weighted average of multiple reports. In some cases we have used VD_{β}, or the volume of distribution of the terminal phase, if that was the only value available. We are aware of the potential variability, from drug to drug, of the two parameters, but we considered the extended data set a higher priority. Furthermore, in considering these data, it should be borne in mind that variability is encountered from protocol to protocol, laboratory to laboratory, and among individuals, as well as between healthy subjects and patients, and it is not possible to avoid such variability because it is not possible to have access to self-consistent clinical studies for a wide variety of compounds. It should also be emphasized that only studies using intravenous administration offer a legitimate basis for calculating VD from concentration vs time data, and those were the only data considered, although this "filter" resulted in the elimination of many compounds from inclusion in the data set. In the course of the discussion we will refer to VD_{ss} unless otherwise specified.

VD_{ss} is, of course, a composite parameter because it depends on a plethora of factors, and the basic premise of our previous work was to reduce its complexity by choosing the fraction unbound in tissues, f_{ut}, as the target of the quantitative structure−pharmacokinetic relationship, or QSPkR,[8] we wished to pursue. Other authors have pursued direct correlations between VD_{ss} and physicochemical parameters, especially logD(7.4), but these attempts have generally been confined to very small sets of compounds and in some cases to sets of analogues.[9−14]

To derive the f_{ut} values to be used in our QSPkR efforts, we used the Oie−Tozer equation, which relates VD_{ss} to f_{ut} and f_u with some species-dependent parameters:

$$VD_{ss} = V_P(1 + R_{E/I}) + f_u V_P(V_E/V_P - R_{E/I}) + \frac{V_R f_u}{f_{ut}} \quad (1)$$

The parameters V_P, V_E, and $R_{E/I}$ are taken to be the plasma volume, the extracellular fluid volume, and the ratio of extravascular to intravascular proteins, respectively, with corresponding values in human of 0.0436 and 0.151 L/kg body weight for V_P and V_E, respectively, and approximately 1.4 for the ratio. $R_{E/I}$, in particular, only takes into account the distribution of albumin. V_R is defined as the physical volume into which the drug is distributed minus the extracellular space, and its value is taken to be 0.380 L/kg body weight. f_u and f_{ut} are defined respectively as the fraction of drug unbound in plasma and the overall fraction unbound in tissues. The value of f_{ut} is, of course, an oversimplified "average" value, which actually arises from numerous, undeterminable binding interactions with various tissue components. It is a fundamental assumption of this approach that these binding interactions are of a "nonspecific" type and rely heavily on the physicochemical properties of the drug. Drugs for which the volume of distribution is heavily driven by a specific type of binding interaction will likely fail to have their VD values accurately predicted.

A useful rearrangement of the Oie−Tozer equation, described in the Experimental Section, allows the calculation of f_{ut}, and the f_{ut} data were used to derive our correlation. We further transformed, as in previous work, the values f_u and f_{ut} into their respective logarithms, and we sought to establish a correlation with lipophilicity and the fraction of drug ionized at pH 7.4, or $f_{i(7.4)}$.

Table 1 shows the compounds used in the present study together with the pharmacokinetic (PK) data used and the respective references. In the calculations, when only a value in liters was reported, a 70 kg average human weight was assumed, and the f_{ut} data were calculated from the rearranged form of the Oie−Tozer equation described in the Experimental Section.

Using only physicochemical parameters, such as ElogD(7.4) determined via our published method,[15] and an experimentally determined pK_a for the calculation of $f_{i(7.4)}$ together with the logarithm of f_u values, we cast an equation for these 120 compounds that is similar to the previously reported one for the set of 64 compounds. We note here that this approach has the advantage of relying only on in vitro parameters that can be generated via high-throughput methods.[16−20] Table 2 reports all the physicochemical data used, with their respective references, and the QSPkR equation used is

$$\log f_{ut} = 0.0080(\pm 0.0747) - 0.2294(\pm 0.0410)\ \text{ElogD} - 0.9311(\pm 0.0777)\ f_{i(7.4)} + 0.8885(\pm 0.0956) \log f_u \quad (2)$$

$$N = 120;\quad R^2 = 0.8665;\quad \text{rmse} = 0.3661;\quad Q^2 = 0.8542;\ F_{3,116} = 250.9;\ p < 0.0001;\quad \text{mean-fold error for the prediction of } VD_{ss} = 2.08$$

while a plot of predicted vs calculated (Oie−Tozer, from clinical data) log f_{ut} values is shown in Figure 1.

Table 1. Pharmacokinetic Data for the 120 Compounds in the Training Set

compd	CAS no.	VD_{ss} [a] (obsd) (L/kg)	f_u [b]	f_{ut} [c]	VD_{ss} [d] (predicted) (L/kg)	ref[e]
acebutolol	37517-30-9	1.2	0.74	0.273	2.65	29−31
acetamidophenol	103-90-2	0.95	1	0.503	0.65	32
alfentanil	70879-28-6	0.75	0.08	0.048	1.38	33
allopurinol	315-30-0	0.6	0.95	0.881	0.54	34
alprazolam	28981-97-7	0.72	0.29	0.187	1.15	35−36
alprenolol	13655-52-2	3.2	0.24	0.030	3.85	37−39
amantadine	768-94-5	6.6	0.33	0.019	1.96	40
amiodarone	1951-25-3	66	0.0002	0.000	26.00	41
amitriptyline	50-48-6	8.3	0.06	0.003	10.87	42−43
amitriptyline *N*-oxide	4317-14-0	1.2	0.2	0.071	1.76	42, 44
antipyrine	60-80-0	0.6	0.9	0.825	0.63	45
atenolol	29122-68-7	0.93	0.91	0.465	1.59	46−52
atomoxetine	83015-26-3	0.85	0.02	0.010	4.18	53
atropine	51-55-8	2	0.82	0.171	3.02	54
azelastine	58581-89-8	15	0.17	0.004	7.25	55
azithromycin	83905-01-5	33	0.88	0.010	38.88	56
betamethasone	378-44-9	1.32	0.36	0.116	1.28	57−58
betaxolol	63659-18-7	6.08	0.42	0.027	3.71	59−63
bisoprolol	66722-44-9	3.31	0.7	0.085	2.63	64−66
bromazepam	1812-30-2	0.91	0.39	0.192	0.84	67
butorphanol	42408-82-2	8.8	0.18	0.008	4.38	68−70
caffeine	58-08-2	0.61	0.64	0.543	0.52	71
chloramphenicol	56-75-7	0.94	0.47	0.225	0.92	72
chlordiazepoxide	58-25-3	0.34	0.04	0.066	1.43	73−80
chlorpheniramine	132-22-9	3.2	0.3	0.037	6.29	81
chlorpromazine	50-53-3	10.1	0.03	0.001	11.43	82−84
cimetidine	51481-61-9	1	0.81	0.374	0.98	85
citalopram	59729-33-8	13	0.2	0.006	5.32	86−88
clomipramine	303-49-1	20	0.04	0.001	14.83	89
clonidine	4205-90-7	2.1	0.8	0.158	2.62	90
clozapine	5786-21-0	5.4	0.05	0.004	6.24	91
cocaine	50-36-2	2	0.09	0.018	2.94	92
codeine	76-57-3	3.5	0.93	0.107	3.08	93
colchicine	64-86-8	5.2	0.61	0.046	0.73	94−95
delorazepam	2894-67-9	1.7	0.05	0.012	1.53	94
Δ9-THC	1972-08-3	9.8	0.03	0.001	10.23	97−98
desipramine	50-47-5	20	0.15	0.003	5.23	99−100
desmethyldiazepam	1088-11-5	0.64	0.03	0.021	1.52	101
dexamethasone	50-02-2	1.14	0.32	0.121	1.09	102−104
diazepam	439-14-5	1.3	0.02	0.006	1.27	101, 105−124
diltiazem	33286-22-5	3.1	0.22	0.028	5.38	125
diphenhydramine	58-73-1	4.5	0.22	0.019	5.46	126
domperidone	57808-66-9	5.7	0.08	0.005	7.77	127
ergotamine	113-15-5	2.7	0.02	0.003	2.96	128
estradiol	50-28-2	1.2	0.015	0.005	1.94	129
felodipine	72509-76-3	10	0.004	0.000	2.30	130
fentanyl	990-73-8	5.1	0.16	0.012	7.76	33, 131−135
flecainide	54143-55-4	4.9	0.39	0.031	3.76	136
fluconazole	86386-73-4	0.6	0.89	0.814	0.71	137
flumazenil	78755-81-4	1.1	0.5	0.200	0.67	138−139
galanthamine	357-70-0	2.46	0.82	0.137	2.94	140−141
haloperidol	52-86-8	18	0.08	0.002	7.97	142
hydrocortisone	50-23-7	0.44	0.09	0.105	0.74	143−145
imipramine	50-49-7	21	0.1	0.002	6.95	100, 146−150
itraconazole	84625-61-6	3.9	0.028	0.003	5.75	151
labetalol	36894-69-6	4.8	0.5	0.041	2.46	14
levomepromazine	60-99-1	14	0.034	0.001	10.62	152
lidocaine	137-58-6	0.72	0.3	0.194	3.54	153−154
lorazepam	846-49-1	1.6	0.09	0.023	1.36	124, 155−166
lormetazepam	848-75-9	1.5	0.12	0.033	1.39	167[f]
maprotiline	10262-69-8	43	0.12	0.001	7.48	168−169
meperidine	57-42-1	2.7	0.42	0.062	4.83	170
methadone	76-99-3	6.2	0.11	0.007	3.91	171−173
methylprednisolone	83-43-2	1.38	0.22	0.067	1.26	174
metoclopramide	364-62-5	3.4	0.6	0.070	4.51	175
metoprolol	56392-17-7	3.73	0.89	0.095	2.43	176−180
metronidazole	443-48-1	0.74	0.89	0.609	0.58	181
mexiletine	31828-71-4	4.9	0.37	0.030	3.24	182
midazolam	59467-70-8	1.4	0.02	0.006	1.49	107, 183
mirtazapine	61337-67-5	4.8	0.15	0.012	3.20	184
morphine	64-31-3	2.6	0.61	0.095	2.79	154, 185−196
nadolol	42200-33-9	1.94	0.7	0.150	2.18	197
naloxone	465-65-6	4.3	0.54	0.049	4.09	198
nebivolol	99200-09-6	11.2	0.02	0.001	6.78	14

Table 1 (Continued)

compd	CAS no.	VD_{ss} [a] (obsd) (L/kg)	f_u [b]	f_{ut} [c]	VD_{ss} [d] (predicted) (L/kg)	ref[e]
nefazodone	83366-66-9	0.51	0.009	0.008	3.94	199
nicotine	54-11-5	2.6	0.95	0.150	2.69	200
nifedipine	21829-25-4	0.78	0.04	0.023	1.28	201
nizatidine	76963-41-2	1.2	0.78	0.289	0.67	175
nortriptyline	72-69-5	19.1	0.12	0.002	6.94	202
omeprazole	73590-58-6	0.34	0.05	0.082	0.88	203
oxazepam	604-75-1	0.59	0.04	0.032	1.34	204
oxycodone	76-42-6	2.3	0.55	0.097	3.28	205−207
paclitaxel	33069-62-4	2.4	0.07	0.012	3.10	208
paroxetine	61869-08-7	17.2	0.05	0.001	6.97	209
pentoxifylline	6493-05-6	4.2	1	0.095	0.62	210
perphenazine	58-39-9	20	0.07	0.001	13.48	211
pindolol	13523-86-9	2.01	0.58	0.119	2.81	212−214
prednisolone	50-24-8	0.52	0.075	0.070	0.76	215−222
prednisone	53-03-2	0.97	0.25	0.113	0.74	220, 222−223
procainamide	614-39-1	1.9	0.84	0.186	2.42	224
promazine	58-40-2	7.5	0.11	0.006	10.19	83
promethazine	60-87-7	14	0.07	0.002	13.27	83
propafenone	54063-53-5	3.6	0.05	0.005	4.98	225
propofol	2078-54-8	3.9	0.02	0.002	2.31	226−235
propranolol	525-66-6	4.02	0.1	0.010	4.06	236−241
quinacrine	69-05-6	124	0.103	0.000	18.45	242[f]
quinidine	56-54-2	3.5	0.13	0.015	3.92	243−244
ranitidine	66357-35-5	1.3	0.85	0.289	2.21	245
remoxipride	80125-14-0	0.64	0.28	0.209	3.89	246
risperidone	106266-06-2	1.1	0.11	0.042	4.65	247
rivastigmine	123441-03-2	2.2	0.6	0.112	3.62	248
sotalol	3930-20-9	0.9	1	0.539	1.66	249−250
sufentanil	56030-54-7	2.9	0.07	0.010	7.99	33
sumatriptan	103628-46-2	0.65	0.82	0.661	2.66	251
tacrine	1684-40-8	8.22	0.45	0.021	3.30	252−254
tebufelone	112018-00-5	31	0.0007	0.000	3.33	255
terbutaline	23031-32-5	1.8	0.8	0.187	1.45	256
testosterone	58-22-0	1.03	0.02	0.008	1.39	257
theophylline	58-55-9	0.57	0.46	0.412	0.59	258
timolol	26839-75-8	3.5	0.9	0.103	2.55	259
tolamolol	38103-61-6	3.2	0.09	0.011	3.90	260
tolterodine	124937-51-5	1.3	0.037	0.012	3.89	261
trazodone	19794-93-5	1	0.07	0.030	2.14	262
triazolam	28911-01-5	0.67	0.1	0.068	1.51	263
trimethoprim	738-70-5	1.6	0.63	0.166	1.36	264
trimipramine	739-71-9	31	0.05	0.001	11.48	265
venlafaxine	93413-69-5	4.4	0.73	0.066	4.95	266
verapamil	52-53-9	4.68	0.1	0.008	7.67	267−269
voriconazole	137234-62-9	4.6	0.42	0.036	1.20	270
zidovudine	30516-87-1	1.7	0.77	0.192	0.56	271−274

[a] VD_{ss} data from iv clinical studies. See Experimental Section for further details. [b] Experimentally determined fraction unbound in human plasma, from literature or in-house data. [c] Calculated via a rearranged form of the Oie−Tozer equation and experimental VD_{ss} and f_u values. See Experimental Section. [d] VD_{ss} predicted using predicted f_{ut} values and experimentally determined f_u. [e] References for the volume of distribution data reported from clinical iv studies. Available as Supporting Information. [f] The experimental VD_{ss} value was calculated using the data obtained from the reported plot after digitization.

Equation 2 was derived directly from a multiple linear regression, but it was checked, together with its statistics, via a principal component regression analysis. As previously reported,[6] we took this approach to check the potential impact of collinearity between ElogD and log f_u data. The principal component regression analysis showed that all the three principal components, derived from all three variables, are statistically significant. Furthermore, we have performed a randomization experiment (1000 cycles, data not shown) that yielded R^2 values well below 0.2 in all cases, with a high distribution of zero and near-zero values. In a similar randomization experiment with 1000 cycles, we found that the mean-fold error was centered about a value of 6, with a minimum value above 4, while the actual value of mean-fold error for the prediction of VD_{ss} is 2.08 for the training set. These findings further confirm the validity and stability of eq 2 and of our approach. We note that ElogD and the fraction ionized increased their respective coefficients when compared to the previously reported equation, while the log f_u term yielded a lower value for its coefficient. However, the fraction ionized and the log f_u parameters are still the largest contributors to the overall equation, and all the coefficients are reasonably close to the ones reported for the equation based on 64 compounds. Thus, the near-doubling of the compounds in the training set did cause some change in the coefficients observed, but the overall statistical quality and predictive power was unchanged. This is, in itself, an indication of the ruggedness of the approach, especially when considering that, together with a wider parameter space, we have introduced more error, in particular from the variability of clinical and biological data.

The signs of the coefficients are physically reasonable and they reflect, for example, an increase in tissue binding (lower log f_{ut}) with an increase in the fraction ionized. This may be rationalized by considering the

Table 2. Physicochemical Data for the 120 Compounds in the Training Set

compd	CAS no.	ElogD [a]	$f_{i(7.4)}$ [b]	pK_a [c]	ref[d]	compd	CAS no.	ElogD [a]	$f_{i(7.4)}$ [b]	pK_a [c]	ref[d]
acebutolol	37517-30-9	−0.39	0.995	9.67	275	maprotiline	10262-69-8	2.04	0.999	10.5	290
acetamidophenol	103-90-2	0.38	0.000	n/a		meperidine	57-42-1	1.11	0.952	8.7	285
alfentanil	70879-28-6	2.39	0.112	6.5	276	methadone	76-99-3	1.3	0.876	8.25	291
allopurinol	315-30-0	−0.1	0.000	n/a		methylprednisolone	83-43-2	2.42	0.000	n/a	
alprazolam	28981-97-7	2.16	0.000	n/a		metoclopramide	364-62-5	0.73	0.992	9.51	e, 277
alprenolol	13655-52-2	0.62	0.994	9.6	*e*, 275, 277	metoprolol	56392-17-7	−0.62	0.995	9.7	275
amantadine	768-94-5	−0.81	0.999	10.68	*e*	metronidazole	443-48-1	0.12	0.000	n/a	
amiodarone	1951-25-3	5.95	0.955	8.73	278	mexiletine	31828-71-4	0.23	0.983	9.15	*e*, 277
amitriptyline	50-48-6	2.94	0.990	9.4	279	midazolam	59467-70-8	3.31	0.000	n/a	
amitriptyline *n*-oxide	4317-14-0	3.14	0.000	n/a		mirtazapine	61337-67-5	2.6	0.443	7.3	292−293
antipyrine	60-80-0	0.34	0.000	n/a		morphine	64-31-3	0.32	0.858	8.18	282
atenolol	29122-68-7	−1.51	0.994	9.6	275, 277	nadolol	42200-33-9	−0.77	0.995	9.67	275
atomoxetine	83015-26-3	1.3	0.998	10.1	*f*	naloxone	465-65-6	1.44	0.776	7.94	285
atropine	51-55-8	−0.16	0.996	9.84	*e*	nebivolol	99200-09-6	2.76	0.869	8.22	14
azelastine	58581-89-8	1.93	0.993	9.54	*e*	nefazodone	83366-66-9	4.95	0.112	6.5	*e*
azithromycin	83905-01-5	0.9	1.950	8.74	280	nicotine	54-11-5	0.23	0.834	8.1	*e*
				9.45		nifedipine	21829-25-4	2.84	0.000	n/a	
betamethasone	378-44-9	2.33	0.000	n/a		nizatidine	76963-41-2	0.06	0.134	6.59	*e*
betaxolol	63659-18-7	0.46	0.985	9.21	281	nortriptyline	72-69-5	1.9	0.998	10.1	*e*
bisoprolol	66722-44-9	−0.38	0.993	9.57	14	omeprazole	73590-58-6	2	0.000	n/a	
bromazepam	1812-30-2	1.38	0.000	n/a		oxazepam	604-75-1	2.94	0.000	n/a	
butorphanol	42408-82-2	1.48	0.860	8.19	*g*	oxycodone	76-42-6	0.37	0.931	8.53	294
caffeine	58-08-2	−0.01	0.000	n/a		paclitaxel	33069-62-4	4.5	0.000	n/a	
chloramphenicol	56-75-7	1.55	0.000	n/a		paroxetine	61869-08-7	2.12	0.992	9.51	f
chlordiazepoxide	58-25-3	3.08	0.000	n/a		pentoxifylline	6493-05-6	0.24	0.000	n/a	
chlorpheniramine	132-22-9	1.56	0.986	9.26	*e*	perphenazine	58-39-9	3.94	0.837	8.11	277
chlorpromazine	50-53-3	3.2	0.986	9.25	275, 279, 282	pindolol	13523-86-9	−0.2	0.993	9.54	275
cimetidine	51481-61-9	0.4	0.271	6.97	*e*	prednisolone	50-24-8	1.6	0.000	n/a	
citalopram	59729-33-8	1.31	0.990	9.38	*f*	prednisone	53-03-2	1.22	0.000	n/a	
clomipramine	303-49-1	3.62	0.990	9.38	279	procainamide	614-39-1	−0.57	0.986	9.24	295
clonidine	4205-90-7	0.29	0.817	8.05	283	promazine	58-40-2	2.7	0.987	9.28	277
clozapine	5786-21-0	3.38	0.629	7.63	*e*	promethazine	60-87-7	3.33	0.980	9.1	296
cocaine	50-36-2	0.48	0.952	8.7	284	propafenone	54063-53-5	1.49	0.987	9.27	*e*
codeine	76-57-3	0.39	0.863	8.2	282, 285	propofol	2078-54-8	4.19	0.000	n/a	
colchicine	64-86-8	0.9	0.000	n/a		propranolol	525-66-6	0.93	0.991	9.45	275
delorazepam	2894-67-9	3.16	0.000	n/a		quinacrine	69-05-6	1.1	1.664	10.2	297
Δ9-THC	1972-08-3	6.99	0.000	n/a						7.73	
desipramine	50-47-5	1.3	0.999	10.23	*e*	quinidine	56-54-2	1.51	0.817	8.05	298
desmethyldiazepam	1088-11-5	3.26	0.000	n/a		ranitidine	66357-35-5	−0.5	0.922	8.47	*e*
dexamethasone	50-02-2	2.03	0.000	n/a		remoxipride	80125-14-0	0.71	0.969	8.9	299
diazepam	439-14-5	2.98	0.000	n/a		risperidone	106266-06-2	1.59	0.888	8.3	*f*
diltiazem	33286-22-5	2	0.820	8.06	*e*	rivastigmine	123441-03-2	0.37	0.975	8.99	*f*
diphenhydramine	58-73-1	1.38	0.980	9.1	*e*	sotalol	3930-20-9	−1.45	0.996	9.76	14, 275
domperidone	57808-66-9	3.17	0.760	7.9	286	sufentanil	56030-54-7	3.34	0.738	7.85	*e*, 276
ergotamine	113-15-5	4.38	0.074	6.3	287	sumatriptan	103628-46-2	−0.4	0.992	9.5	300
estradiol	50-28-2	3.9	0.000	n/a		tacrine	1684-40-8	0.17	0.996	9.8	301
felodipine	72509-76-3	4.52	0.000	n/a		tebufelone	112018-00-5	5.63	0.000	n/a	
fentanyl	990-73-8	2.39	0.915	8.43	276	terbutaline	23031-32-5	−1.49	0.952	8.7	278
flecainide	54143-55-4	0.49	0.988	9.3	288	testosterone	58-22-0	3.17	0.000	n/a	
fluconazole	86386-73-4	0.66	0.000	n/a		theophylline	58-55-9	0.49	0.000	n/a	
flumazenil	78755-81-4	0.78	0.000	n/a		timolol	26839-75-8	−0.39	0.962	8.8	275
galanthamine	357-70-0	0.21	0.893	8.32	289	tolamolol	38103-61-6	1.81	0.760	7.9	275
haloperidol	52-86-8	2.46	0.947	8.65	*e*	tolterodine	124937-51-5	1.04	0.996	9.8	*e*, 302
hydrocortisone	50-23-7	1.49	0.000	n/a		trazodone	19794-93-5	2.97	0.197	6.79	*e*
imipramine	50-49-7	1.97	0.991	9.45	*e*, 279	triazolam	28911-01-5	2.53	0.112	6.5	303
itraconazole	84625-61-6	5.9	0.000	n/a		trimethoprim	738-70-5	0.61	0.420	7.26	*e*, *f*
labetalol	36894-69-6	1.57	0.500	7.4	275	trimipramine	739-71-9	3.1	0.986	9.24	*e*
levomepromazine	60-99-1	3.04	0.984	9.19	277	venlafaxine	93413-69-5	0.87	0.992	9.5	292, *f*
lidocaine	137-58-6	1.29	0.776	7.94	282	verapamil	52-53-9	2.24	0.971	8.92	304−305
lorazepam	846-49-1	2.8	0.000	n/a		voriconazole	137234-62-9	2.15	0.000	n/a	
lormetazepam	848-75-9	2.77	0.000	n/a		zidovudine	30516-87-1	0.12	0.000	n/a	

[a] As described in ref 15. [b] Fraction ionized at pH 7.4 calculated from experimental pK_a values. [c] Experimental pK_a values. For compounds having only a single pK_a value and a value less than 5, the notation "not applicable" (n/a) is used. [d] References for experimental pK_a data reported. Available as Supporting Information. [e] Potentiometri titration. [f] Capillary electrophoresis. [g] Estimated to be similar to codeine and morphine.

binding of cations (ionized basic compounds) to negatively charged membranes in tissue and organelles. An increase in lipophilicity would also decrease the amount of the free drug in tissues and thus increase its VD_{ss}. Thus, lipophilicity and ionic interactions capture the nonspecific drug−tissue binding to a very large extent.[21] The introduction of the predicted log f_{ut} values (as f_{ut}) into the Oie−Tozer equation yielded a mean-fold error of 2.08 for the prediction of VD_{ss} for the training set, and the corresponding plot of the predicted vs clinical VD_{ss} data is shown in Figure 2.

To further explore the usefulness and ruggedness of eq 2, we undertook additional statistical testing on the basis of the adoption of a leave-class-out (LCO) approach and the use of an external test set, and we used VD_{ss} as the end-point of the prediction. Tables 3 and 4 show the results of these two tests. It can be seen from Table 3 that the statistical quality of the equation (eq 2) does

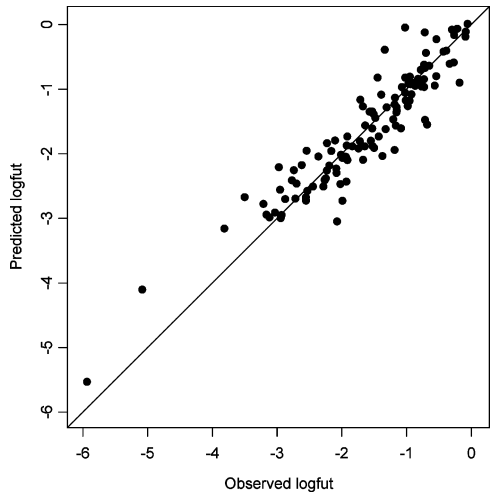

Figure 1. Plot of predicted log f_{ut} vs observed log f_{ut} for the 120 compounds in the training set.

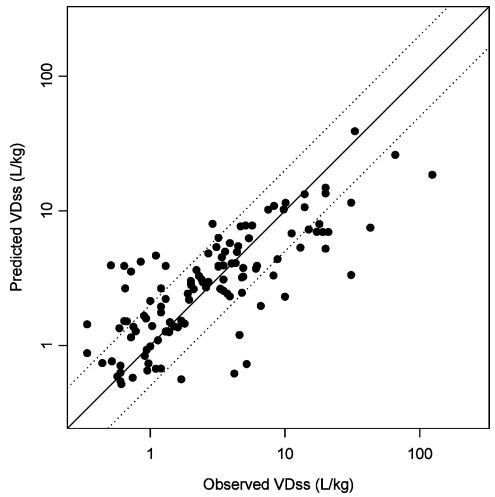

Figure 2. Plot of predicted VD_{ss} vs observed VD_{ss} for the 120 compounds in the training set. The dotted lines represent the 2-fold error limits.

Table 3. Leave-Class-Out Statistics

class	no. of compds in class	R^2 of predictive equation[a]	mean-fold error of predicted VD
antipsychotics	4	0.8608	1.26
β-blockers	14	0.8604	1.54
steroids	8	0.8651	1.36
tricyclic antidepressants	7	0.8651	2.51[b]
morphine analogues	4	0.8659	1.41
benzodiazepines	12	0.8737	1.86[c]
fentanyl analogues	3	0.8690	2.10

[a] Based on 120-N observations and calculated for the log f_{ut} regression as in eq 2. [b] Desipramine, imipramine, and trimipramine were underestimated by greater than a 3-fold factor. See text. [c] Chlordiazepoxide was overestimated by a factor of 4.2.

not depend on any particular class of analogues, since the removal of each class yields predictive equations of similar statistical power, together with allowing the prediction of the VD_{ss} for each class very close to or within a factor of 2. The fact that this method remains robust after LCO testing is important in the investigation of new compounds. In most drug discovery efforts, researchers are working with novel classes of structures and they must be confident that a predictive approach based on well-established classes of drugs will be

Table 4. Physicochemical and Pharmacokinetic Parameters for the Test Set Compounds

compd	ElogD	pK_a	$f_{i(7.4)}$ [a]	f_u [b]	f_{ut} [c]	obsd VD_{ss} [d] (L/kg)	predicted VD_{ss} [e] (L/kg)	fold error[f]
1	0.79	6.99	0.280	0.120	0.055	0.7	0.93	1.33
2	4.44	7.2	0.387	0.001	0.000[g]	1.5	4.24	2.82
3	0.68	7.26	0.420	0.603	0.180	1.5	1.40	1.07
4	1.01	9.09	0.980	0.191	0.017	6.6	4.44	1.49
5	−0.09	8.98	0.974	0.603	0.085	5.5	2.87	1.91
6	2.99	7.24	0.409	0.010	0.001	1	2.71	2.71
7	0.53	1.76	0.000	0.891	0.667	0.7	0.67	1.04
8	1.54	8.66	0.948	0.020	0.002	15.1	4.26	3.55
9	1.00	7.13	0.349	0.427	0.130	1.5	1.36	1.10
10	−0.50	8.2	0.863	0.020	0.007	9	1.28	7.01
11	0.85	8.03	0.810	0.363	0.046	2.8	3.10	1.11
12	1.41	9.82	0.996	0.120	0.009	2.1	5.36	2.55
13	2.33	9.09	0.980	0.030	0.002	21	7.17	2.93
14	3.09	6.8	0.200	0.040	0.007	1.5	2.15	1.44
15	1.57	7.11	0.339	0.250	0.061	4.7	1.64	2.86
16	1.00	7.26	0.420	0.27	0.080	2.2	1.38	1.59
17	3.29	9.25	0.986	0.02	0.001	26	11.45	2.27
18	1.98	9.2	0.984	0.02	0.001	3	5.76	1.92

[a] Fraction ionized at pH 7.4. [b] Fraction unbound in human plasma. [c] Fraction unbound in tissues (f_{ut}) predicted from eq 2. [d] Experimental VD_{ss} value from iv clinical studies. [e] Calculated VD_{ss} value from the predicted f_{ut} data in this table, using the Oie−Tozer equation. [f] Mean-fold error is 2.26. No f_u filter was used (see text). [g] Actual value is 0.00009.

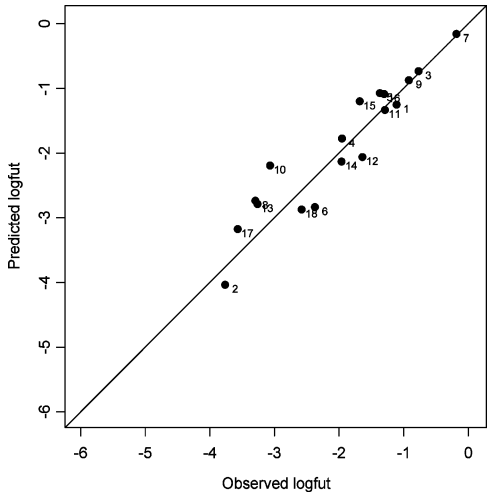

Figure 3. Plot of predicted log f_{ut} vs observed log f_{ut} for the 18 compounds in the test set.

applicable to novel structural classes. We observed some significant deviations for some tricyclic antidepressants, and we shall return to this point later, since these deviations might be due to tight and specific binding to cellular organelles, membranes, or DNA or to active influx or efflux mechanisms, while this method, as described earlier, assumes passive diffusion as the only mechanism of tissue penetration and "average" binding in all tissues.

Table 4 shows the prediction of VD_{ss}, via eq 2 and the Oie−Tozer equation, for a set of 18 structurally unrelated proprietary compounds, while Figures 3 and 4 show, respectively, the predicted vs calculated (Oie−Tozer, from clinical data) log f_{ut} and the predicted vs clinical VD_{ss} plots. Despite a very significant deviation (a factor of 7) for compound **10**, the mean-fold error is 2.26, or slightly above a factor of 2. No allowance was made in this case for the removal of the compounds having a fraction unbound in plasma (f_u) lower than

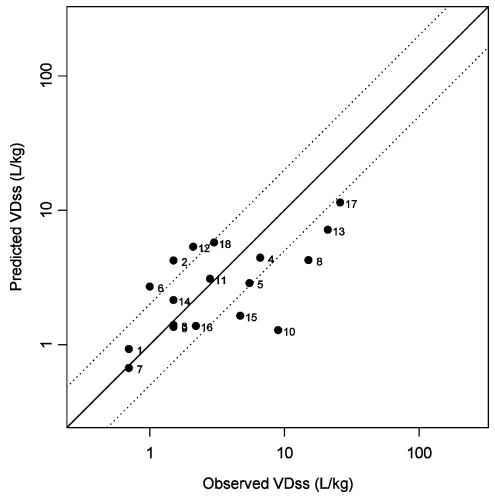

Figure 4. Plot of predicted VD_{ss} vs observed VD_{ss} for the 18 compounds in the test set. The dotted lines represent the 2-fold error limits.

0.02, by adopting the "f_u filter" as discussed in our previous work, but the removal of compound **10** as a possible outlier would yield a mean fold-error of 1.98. We do not have an explanation for the significant underprediction of VD_{ss} for compound **10**, but it is possible that specific binding in selected tissues may have occurred. The next largest deviation was observed for compound **8**, which is also underpredicted. Both compounds, however, are highly bound to plasma proteins, and some error may be introduced, as we postulated in our previous work, from the determination of f_u and the technical challenges associated with accurate measurement of the free fraction for very highly protein-bound drugs. It is important to emphasize that while plasma represents a small fraction of the total body mass (~4%),[22] the accuracy of f_u determinations has important consequences for VD_{ss}.

Specific binding, resulting in underprediction, could be invoked for several drugs used in this study and range from quinacrine, well-known for binding to DNA[23] and some peptide hormone producing cells,[24] to imipramine, reported to have high affinity for lysosomes and potentially susceptible to binding to lipophilic substances and to aggregation within lysosomes.[25−27] Furthermore, the structurally very similar trimipramine and desipramine, also underpredicted, may be specifically sequestered in lysosomes, and this aspect may contribute to explain the significant deviation observed. However, we do not have strong evidence allowing us the removal of those compounds or of the overpredicted chlordiazepoxide.

As a final comparison, we present the results obtained by comparing several methods based on animal pharmacokinetic data[5] with our physicochemical parameters and f_u approach based on eq 2. Table 5 shows the predicted VD values in humans using those methods and compares those values to the predictions reported in Table 4 and based on the present method. For 14 out of the 18 compounds in the present test set, the results are comparable, and in some cases the prediction yields essentially the same result than the more resource-demanding PK methods as in the case, for example, of compounds **1**, **3**, **4**, and **7**. Compound **2** is overpredicted by the present method, but it is underpredicted by a larger extent by all PK-based methods. For compound **15**, the first two methods yield the same level of accuracy as the present method, while the method based on allometric scaling yields a mean-fold error of about 6. Thus, the present method, based only on in vitro parameters and with the caveats discussed above in terms of specific binding and efflux/influx phenomena, performs comparably to the more resource-demanding PK-based methods.

Table 5. Comparison of VD Predictions Using Animal PK or Physicochemical Data

compd	V1 [a] (L/kg)	V2 [b] (L/kg)	V3 [c] (L/kg)	VD_{ss} [d] (L/kg)	VD_{ss} (obsd) (L/kg)
1	0.5	0.5		0.9	0.7
2	0.3	0.4	0.4	4.2	1.5
3	1.1	1.5	0.9	1.4	1.5
4	4.1	5.7	7.2	4.4	6.6
5	2.9	4.9	2.7	2.9	5.5
6	1.6	1.8	1.5	2.7	1
7	0.6	0.7	0.6	0.7	0.7
8	9.3	11.0		4.3	15.1
9	1.5	1.7	1.2	1.4	1.5
10	11.0	13.0	18.4	1.3	9
11	2.7	3.1	2.6	3.1	2.8
12	2.6	3.0	2.5	5.4	2.1
13	25.3	30.0	42.3	7.2	21
14	1.3	1.5	2.2	2.2	1.5
15	1.8	1.3	0.8	1.6	4.7
16	1.9	1.4	1.1	1.4	2.2
17	23.6	41.7	61.8	11.5	26
18	4.0	4.8	3.3	5.8	3

[a] Calculated via the Oie−Tozer equation, using a mean f_{ut} value derived from animal data and f_u in humans. [b] Dog−human proportionality method, corrected for f_u. [c] Allometric scaling, with correction for interspecies differences in f_u. [d] Calculated from eq 2 and the Oie−Tozer equation using the parameters from Table 4 (this work).

Conclusion

We have confirmed our previous findings and discussed a facile method for the prediction of VD_{ss} in human that does not require animal PK data. It is therefore more amenable to faster screening approaches, is less resource-demanding, and requires much smaller quantities of compound than previously described VD prediction methods.[5,28] We have confirmed with extended training and test sets and with a leave-class-out approach the good predictive power of this method, with particular regard to the actual work of drug metabolism scientists aimed at differentiating compounds belonging to similar classes, with a general equation. The method yields a mean-fold error close to 2.

This method should find application in the prediction of VD_{ss} in man and therefore should contribute to the prediction of half-life ($t_{1/2}$) and dosing regimen. The extension of this method to the prediction of VD_{ss} of acidic compounds, the application of computational methods to these predictions, and further extension of the scope of these methods are among the future objectives of our work.

Experimental Section

Materials and Methods. Most of the drugs were purchased directly from commercial sources (Aldrich, Fluka, ICN, RBI, Sigma, Tocris) and used as received in all cases. In several cases they were available through our Materials Management Group as either proprietary compounds or samples extracted

from commercial formulations. The ElogD data were determined using our recently published method,[15] which is based on a linear regression of capacity factors (as log k') obtained from polycratic RP-HPLC determinations and extrapolated to 0% of organic solvent. Its ruggedness, and similarity to the balance of forces present in classical "two-phase" systems, has been discussed in detail in the original work. In some cases, newer determinations were made even for compounds previously reported and then averaged, or some of the ElogD data were recast using the equation from our published ElogD work.[15] The data range spans over 7 units. The pK_a data were either taken from the literature or determined in house from potentiometric or CE determinations, either via a single capillary instrument or using a CombiSep 96-channnel CE instrument (CombiSep, Inc., Ames, IA). In several instances they were obtained from potentiometric determinations performed by pIon Inc., Woburn, MA, either on commercial or proprietary samples. When more than one source was available, the pK_a data were averaged. The $f_{i(7.4)}$ values were then determined using the pK_a, and the data range spans from 0 (neutral) to approximately 2 (dication).

Volume of Distribution and Plasma Protein Binding Data. Volume of distribution and plasma protein binding data for the 120 compounds constituting the training set were obtained in all cases from the original references, and they are reported in the Supporting Information. The f_u data for tebufelone and quinacrine were determined in-house using equilibrium dialysis. The f_u data range spans from 0.0002 (amiodarone) to 1 (acetaminophen). The VD_{ss} data, in either the training or the test set, comprise only data from studies in which a systemic dose was administered, since the accurate measurement of volume of distribution requires the entire dose to be completely available to the systemic circulation. If more than one reference was available, a weighted average based on the number of subjects in each reported study was used. In a few cases, VD data for the compounds used for the calculation of f_{ut} had been reported as VD_β values rather than VD_{ss}, and they were used as such. In a few other cases the VD_{ss} values were calculated from data extracted from the plot or available in tabular form. The data range spans from volumes well below 1 L/kg (e.g., hydrocortisone) to 124 L/kg in the case of quinacrine. In cases when only a volume of distribution in liters was reported, an average body weight of 70 kg for each study subject was assumed. The literature data used for the correlation are listed in Table 1.

Calculation of Fraction Unbound in Tissues. Literature data for VD_{ss} and f_u were used in the following rearrangement of the Oie−Tozer equation:[7]

$$f_{ut} = \frac{V_R f_u}{[VD_{ss} - V_P - (f_u V_E)] - [(1 - f_u) R_{E/I} V_P]}$$

In this equation, f_{ut} is the fraction unbound in tissues, f_u is the fraction unbound in plasma, VD_{ss} is the steady-state volume of distribution, and $R_{E/I}$ refers to the ratio of binding proteins in extracellular fluid vs plasma (1.4). V_P, V_E, and V_R refer to the volumes of plasma, extracellular fluid, and "remainder fluid" with values of 0.0436, 0.151, and 0.380 L/kg body weight, respectively, in human. In general, the use of logarithmic values is the most common means of data transformation. Veng-Pedersen[10] has discussed means of data transformation to linearize the response and stabilize the variance points in some detail. Therefore, we applied this transformation to the f_{ut} and f_u values. It is also worth mentioning that several other possible forms of the regression equation, using VD_{ss} or $VD_{ss,unbound}$ or their respective logarithmic values as the dependent variables as well as f_u instead of its logarithmic value as one of the independent variables, were tested but yielded significantly inferior statistics. The original form of the Oie−Tozer equation (eq 1) was used to calculate the VD_{ss} for the compounds in the test set, knowing their calculated f_{ut} (from eq 2) and experimental f_u.

Statistical Analysis. The statistical analysis was performed using S-PLUS 2000 (MathSoft, Inc.) and JMP, version 3.2.6 (SAS Institute Inc.). Ordinary least-squares method was used to fit the regression model for predicting f_{ut}, yielding eq 2.

All the predictor variables in the equation are statistically significant. We also examined the correlation between the predictor variables and noticed that the sample correlation coefficient between ElogD and log f_u was − 0.8393. We subsequently performed a principal component regression analysis, and observed that all three principal components derived from the three variables were statistically significant. This indicates that all three predictor variables contribute significantly in predicting log f_{ut}. We would have obtained the same regression equation by principal component regression analysis.

Randomization experiments on the training set and the use of an independent test set of 18 proprietary compounds were also part of the statistical assessment of the model, as described in Results and Discussion.

Leave-Class-Out Approach. We performed the leave-class-out cross-validation of our approach. We have identified seven classes of analogues in our data set. In the leave-class-out cross-validation exercise, we left out one class from our data set at a time and fit the regression model for log f_{ut} prediction based on the remaining data. We then used the model to predict log f_{ut} for compounds in the class being left out. Following that, we used the obtained predicted log f_{ut} in the Oie−Tozer equation to predict VD_{ss}. The results are discussed in the text and presented in Table 3.

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Supporting Information Available: The complete list of original references with the same numbering used in Tables 1 and 2. This material is available free of charge via the Internet at http://pubs.acs.org.

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