Evaluation of common *in vitro* assays for the prediction of oral bioavailability and hepatic metabolic clearance in humans

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ABSTRACT

Introduction Intrinsic hepatic metabolic clearance (CL_{int}) measured with human hepatocytes, apparent intestinal permeability (P_{app}) obtained using the Caco-2 model, unbound fraction in plasma (f_u) and blood-to-plasma concentration ratio (C_{bl}/C_{pl}) are commonly used for predicting the hepatic clearance (CL_H) and oral bioavailability (F) of drug candidates in humans. The primary objective was to select drugs whose *in vitro* hepatocyte CL_{int}, Caco-2 P_{app}, f_u and C_{bl}/C_{pl} have been measured in various laboratories and studies, and estimate correlation coefficients (R^2) for predicted and observed F and log plasma CL_H. Secondary aims were to estimate the laboratory/study variability and its impact on predictions and to compare results to *in silico* and animal model-based predictions.

Materials and Methods A literature search was done in order to find unbound hepatocyte CL_{int} (and corresponding predicted *in vivo* CL_{int}), Caco-2 P_{app}, f_u and C_{bl}/C_{pl} data. Compounds with multiple measurements for the four assays, without significant *in vivo* solubility/dissolution limitations and with known *in vivo* CL_H and F, were selected. Min, max and mean estimates were used in the analysis.

Results and Discussion Thirty-two compounds with data (in total 561 estimates) produced by 21 major pharmaceutical companies and universities met the inclusion criteria. The predicted *vs* observed R^2 for log mean CL_{int}, log mean CL_H and mean F were 0.32, 0.08 and 0.20, respectively. Exclusion of atenolol increased the R^2 for CL_H to 0.20. R^2-values were considerably lower than those presented in many studies, which seems to be explained by selection bias (choosing favorable reference values). There was considerable interstudy variability for measured and predicted CL_{int} (80- and 1,476-fold mean and max differences, respectively) and measured f_u (6.6- and 50-fold mean and max differences, respectively). For F, higher predictive performance was found for *in silico* (Q^2=0.58; head-to-head) and animal *in vivo* models (R^2=0.30).

Conclusion The combination of data from many laboratories and the use of mean values resulted in reduced selection bias and predictive accuracy. Overall, the predictive accuracy (here R^2) for log CL_{int}, log CL_H and F was low to moderately low (0.08-0.32). The halved R^2 compared to individual studies where high performance was demonstrated seems to be explained be selection bias (enabled by large data variability). Animal *in vivo* models, and in particular, *in silico* methodology, outperformed *in vitro* methodology for the prediction of F in man.

INTRODUCTION

Intrinsic hepatic metabolic clearance (CL_{int}) measured with human hepatocytes, apparent intestinal permeability (P_{app}) measured using the Caco-2 cell model, unbound fraction in plasma (f_u) and blood-to-plasma concentration ratio (C_{bl}/C_{pl}) can be used for predicting the oral bioavailability (F) of drug candidates in humans. Such studies and reports are, however, rare. It is more common that human hepatocyte CL_{int}, f_u and C_{bl}/C_{pl} are used for prediction of the hepatic metabolic clearance (CL_H).

Some reports in the literature show high correlation coefficients (R^2) (up to 0.7-0.9; with slopes close to 1) between predicted and observed log *in vivo* CL_{int} and CL_H (Riley et al. 2005; Hallifax et al. 2010; Sohlenius-Sternbeck et al. 2010; Yamagata et al. 2017), whereas other show low to moderate correlations (down to ca 0.2; with slopes clearly deviating from 1) (Stringer et al. 2008; Wood et al. 2017).

Variability within and between laboratories and differences between *in vitro* and *in vivo* conditions are known sources behind uncertainties and errors of *in vivo* predictions. For example, 3- to 3.5-fold mean interlaboratory variability (calculated as ratios between maximum and minimum values for each compound) was found for human hepatocyte CL_{int}, Caco-2 P_{app} and f_u (Fagerholm 2022). An extensive investigation of interlaboratory variability of human hepatocyte CL_{int} and unbound CL_{int} by Louisse et al. 2020 showed coefficients of variance between 26 and 231 % and that values for the majority of compounds differed by more than one order of magnitude.

In vitro to *in vivo* predictions of *in vivo* $CL_{int,}$ CL_H and F are sensitive to subjective bias (for example, selection of favorable or unfavorable f_u- and C_{bl}/C_{pl}-data). Our previous investigation showed the impact of selecting favorable data for maximizing the shown predictive accuracy of *in vivo* CL_{int} (Fagerholm et al. 2022). It was possible to select f_u- and C_{bl}/C_{pl}-data to reach a R^2 for CL_{int} of ca 0.7-0.9, but also to reach R^2s below 0.1. With the use of mean f_u- and C_{bl}/C_{pl}-data a R^2 of 0.38 was reached for CL_{int} (n=17).

The primary objective of the present study was to find and select drugs whose *in vitro* hepatocyte CL_{int} (and corresponding predicted *in vivo* CL_{int}), Caco-2 P_{app}, f_u and C_{bl}/C_{pl} have been measured in various laboratories and studies, and estimate the R^2 for predicted *vs* observed F and plasma CL_H. Secondary aims were to estimate the laboratory/study variability and its impact on predictions and to compare results to *in silico* and animal model-based predictions.

MATERIALS & METHODS

The literature was searched for studies with data for human hepatocyte CL_{int}-predicted *in vivo* unbound CL_{int}, Caco-2 P_{app} and *in vivo* fraction absorbed (f_a), f_u, C_{bl}/C_{pl}, non-renal CL (surrogate for CL_H), F and gut-wall bioavailability (F_{gut}).

Suitable data were found in the following references – Predicted unbound *in vivo* CL_{int} (Albaugh et al, 2012; Bowman and Benet 2019; Keefer et al. 2023), Caco-2 P_{app} (Skolnik et al. 2010; Lin et al. 2011, McGinnity et al. 2007, Thomas et al. 2005, Matsson et al. 2005, Lee et al. 2017), Li et al. 2018, Gertz et al. 2010, Sköld et al. 2006, Irvine et al. 1999, Yee 1997, Lau et al. 2004, Fujikawa et al. 2005, Li et al. 2007), f_u (Fagerholm et al. 2021a; Wang et al. 2014; Hallifax et al. 2010; Soars et al. 2002; Sohlenius-Sternbeck et al. 2010; Obach 1999; Obach et al. 2008; Yau et al. 2017; Yamagata et al. 2017; Keefer et al. 2023, Prosilico AB databank), C_{bl}/C_{pl} (Hallifax et al. 2010; Sohlenius-Sternbeck et al. 2010; Obach 1999; Yamagata et al. 2017; Keefer et al. 2023, Yau et al. 2017; Uchimura et al. 2010; Prosilico AB databank), CL_H (Hallifax et al. 2010; Sohlenius-Sternbeck et al. 2010; Obach 1999; Yamagata et al. 2017; Keefer et al. 2023; Obach et al. 2008; Prosilico AB databank), F (Varma et al. 2010; Yau et al. 2017; Musther et al. 2014; Fagerholm et al. 2021b; Prosilico AB databank), and F_{gut} (Yau et al. 2017).

Caco-2 P_{app} *vs in vivo* f_a-relationships were established for each report with Caco-2 P_{app}-data (including data for many other reference substances), and f_a for the selected compounds was predicted based in P_{app}-f_a-relationships obtained for each study.

Only compounds with complete *in vivo* dissolution and *in vitro* CL_{int}>limit of quantification (LOQ) were selected.

Selected data were produced by companies including Astellas Research Institute, AstraZeneca, Boehringer-Ingelheim, Chugai, Cyprotex, GSK, Millennium Pharmaceuticals, Novartis, Pfizer, Roche and Schering Plough, RIKEN Innovation Center, and Universities of Chungnam, Kyoto, Manchester, New Jersey, Nottingham, Shanghai, South Dakota, Sungkyunkwan and Uppsala.

Plasma CL_H and the corresponding F_H were estimated using the well-stirred liver model with a liver blood flow rate of 1500 mL/min and by considering C_{bl}/C_{pl} for F-predictions. The F was estimated as $f_a \bullet F_{gut} \bullet F_H$, where f_a was predicted from P_{app} *vs* f_a-relationships in each Caco-2 study.

For each selected compound, minimum, maximum and mean F- and plasma CL_H-values were estimated, and predicted mean values were compared to corresponding measured *in vivo* mean values. To further evaluate the results for compounds with known gut-wall metabolism by CYP3A4 and available *in vivo* F_{gut}-estimates these were either removed or their F_{gut} was considered.

In silico (forward-looking results by Prosilico AB; head-to-head) and animal model (mice, rats and dogs) predicted data were taken from Fagerholm et al. 2021 and compared to *in vitro* data based predictions.

RESULTS & DISCUSSION

Selected compounds and variability between studies and data sets

Thirty-two compounds that met the inclusion criteria were found and selected. Their measured and predicted data are shown in Tables 1 and 2. In total, 175 predicted *in vivo* CL_{int}, 126 P_{app} (over 500 estimates including references), 172 and 88 f_u and C_{bl}/C_{pl}-values were found and used (in total 561 estimates), which corresponds to an average of 5.5, 3.9, 5.4 and 2.8 CL_{int}-, P_{app}-, f_u- and C_{bl}/C_{pl}-estimates per compound, respectively.

Estimate ranges for CL_{int}, f_a, f_u, C_{bl}/C_{pl}, CL_H and F were 6-57,500 mL/min, 0.50-1.00, 0.0009-0.97, 0.56-1.67, 2-1222 mL/min and 0.08-0.98, respectively. Thus, there were wide estimate ranges for all parameters except for f_a.

The average, median and maximum CL_{int}-ratios (based on differences between studies) were 80-, 6.7- and 1,476-fold, respectively. Corresponding values for f_a were 1.34-, 1.13- and 2.63-fold, respectively. The average, median and maximum f_u-ratios were 6.64-, 2.62- and 50-fold, respectively. Corresponding values for C_{bl}/C_{pl} were 1.24-, 1.16- and 1.78-fold, respectively. Thus, there was considerable interstudy variability for measured and predicted CL_{int} and measured f_u, which is in line with findings in previous reports (Bowman and Benet 2019; Fagerholm et al. 2021a; Fagerholm 2022).

More than 3-fold differences between maximum and minimum predicted F were found for 8 compounds – alprenolol (3.6-fold; predicted F=0.27-0.98; observed F=0.08), atenolol (5.0-fold; predicted F=0.14-0.71; observed F=0.50), cimetidine (6.5-fold; predicted F=0.10-0.62; observed F=0.68), diclofenac (>99-fold; predicted F=0-0.99; observed F=0.54), metoprolol (3.1-fold; predicted F=0.30-0.92; observed F=0.40), nifedipine (6.2-fold; predicted F=0.16-0.98; observed F=0.50), propranolol (4.7-fold; predicted F=0.20-0.94; observed F=0.24), verapamil (22-fold; predicted F=0.04-0.858; observed F=0.24).

Very large differences between maximum and minimum predicted CL_H were found for some compounds. For example, diclofenac (CL_H predicted to be low to high; 162-fold difference between lowest and highest predicted CL_H), montelukast (CL_H predicted to be low to very high; 245-fold difference between lowest and highest predicted CL_H), gemfibrozil (CL_H predicted to be very low to moderate; 1,083-fold difference between lowest and highest predicted CL_H), fenoprofen (CL_H predicted to be very low to low; 205-fold difference between lowest and highest predicted CL_H) and naproxen (CL_H predicted to be very low to low; 135-fold difference between lowest and highest predicted CL_H).

The R^2 for mean predicted *vs* observed f_a and log CL_{int} were 0.75 (Figure 1) (average R^2=0.5 for all studies) and 0.32 (Figure 2), respectively. The moderately low predictive accuracy for hepatocyte-

based predictions of *in vivo* log CL_{int} (R^2=0.32) was similar to that found for 16 Pfizer drug discovery compounds by Keefer et al. 2023 (0.43) and by Stringer et al. 2008, but about half of that found by, for example, Sohlenius-Sternbeck et al. 2010 (0.72; 0.38 after correction for reference data) and Hallifax et al. 2010 (0.62).

Based on the results it appears that a R^2 of ca 0.3±0.2 is appropriate for human hepatocyte-based predictions of *in vivo* log CL_{int}.

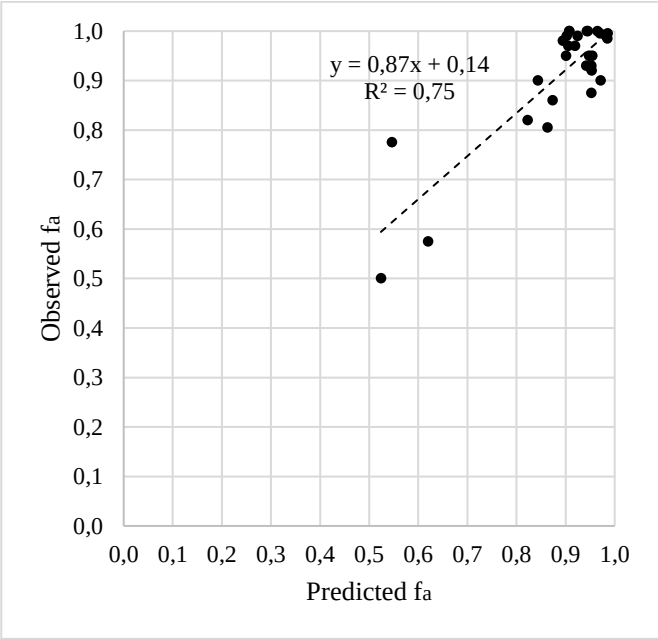

Figure 1. The correlation between predicted and observed mean f_a (n=29).

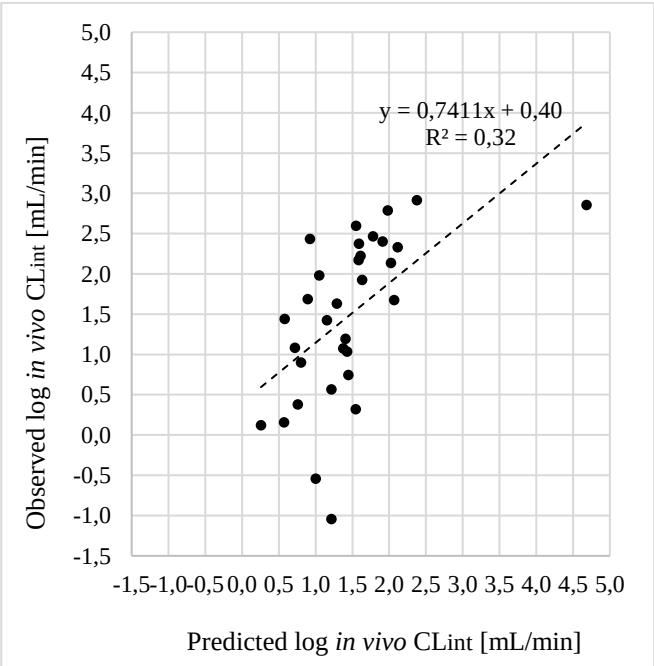

Figure 2. The correlation between predicted and observed mean log CL_{int} (n=32).

Prediction of oral bioavailability

Twenty-nine of the 32 compounds qualified for evaluation of prediction of F. The R^2 between predicted and observed mean F was 0.20 (Figure 3). Consideration of F_{gut} for and removal of 5 CYP3A4-substrates with known measure F_{gut} had only slight impact on the F-predictions (R^2=0.26). Median and max ratios for highest and lowest predicted F were 2.1- and >99-fold, respectively. 72 % of observed F-estimates were within prediction ranges (Figure 4).

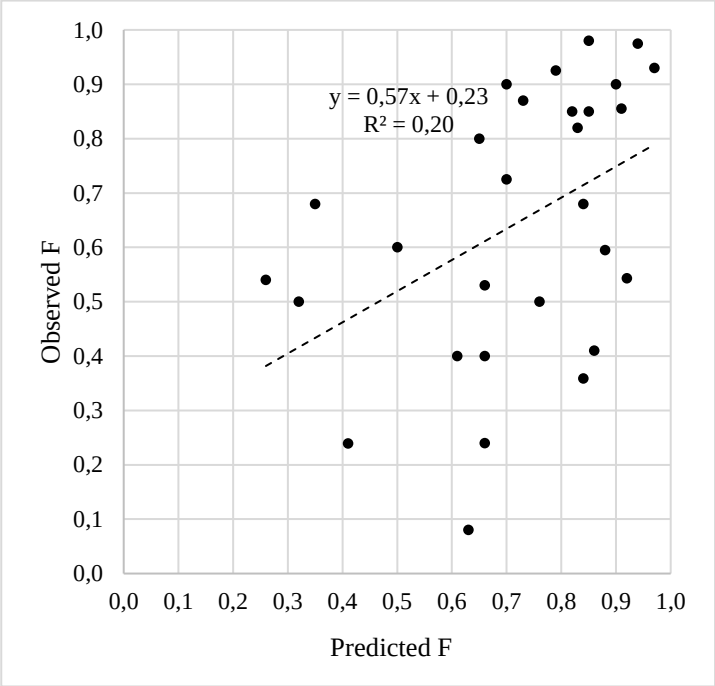

Figure 3. The correlation between predicted and observed mean F (n=29).

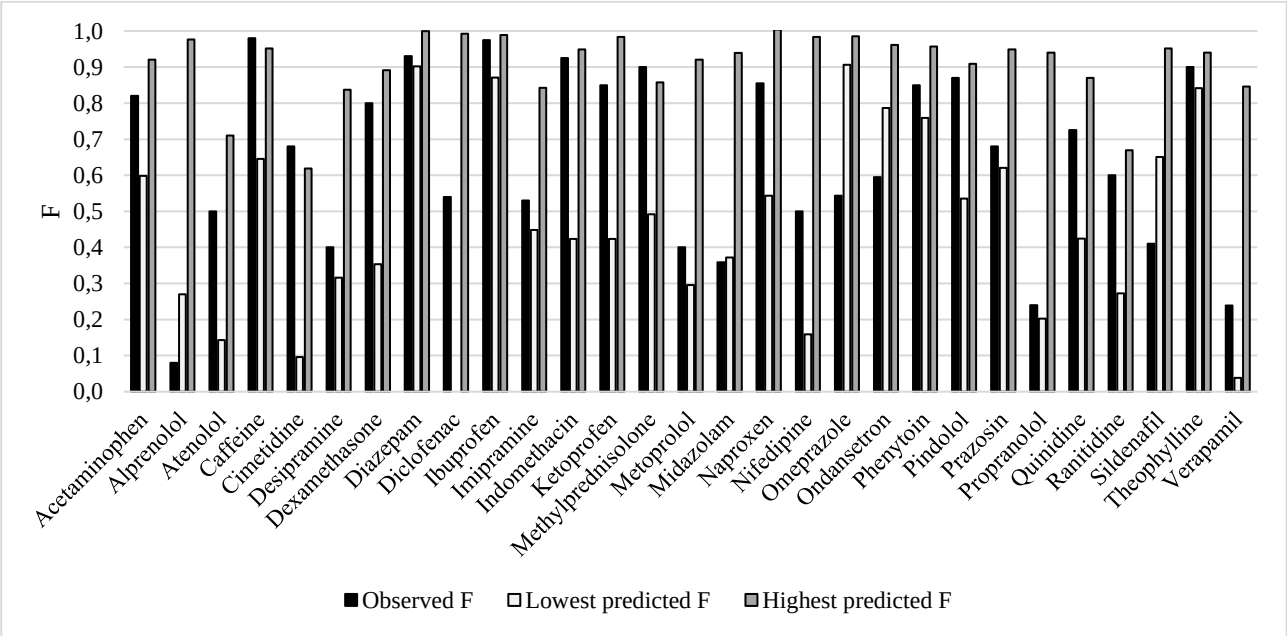

Figure 4. Observed mean F and predicted min and max F (n=29).

The R^2 for F based on mean data from many sources was considerably lower than that reported from one laboratory by Li et al. 2007. With human hepatocyte CL_{int} and Caco-2 P_{app} they reached a R^2 of 0.66 for 30 compounds (including 10 of the compounds in the present study).

Prediction of plasma hepatic clearance

The R^2 between predicted and observed mean log plasma CL_H was 0.08 (Figure 5). Exclusion of atenolol (with only one literature *in vitro* CL_{int}-estimate) the increased the R^2 for plasma CL_H to 0.20. Mean, median and max ratios for highest and lowest predicted CL_H were 81-, 10- and 1,083-fold, respectively. 69 % of observed CL_H-estimates were within prediction ranges (Figure 6).

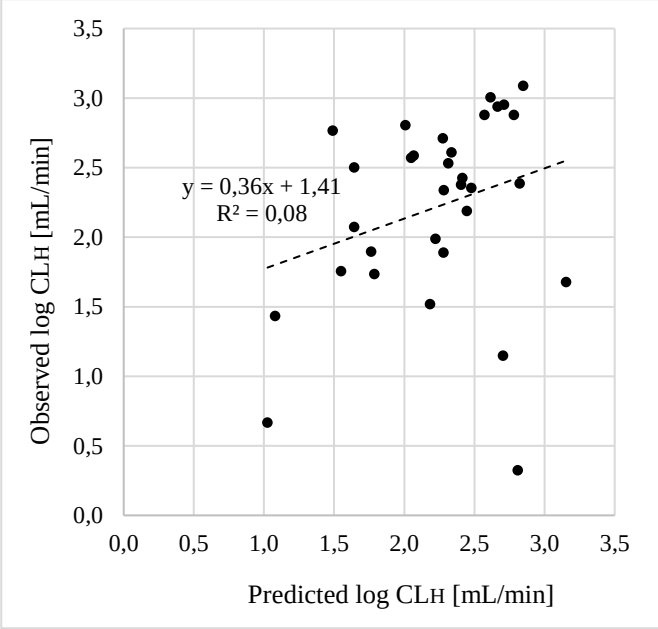

Figure 5. The correlation between predicted and observed mean log plasma CL_H (n=32).

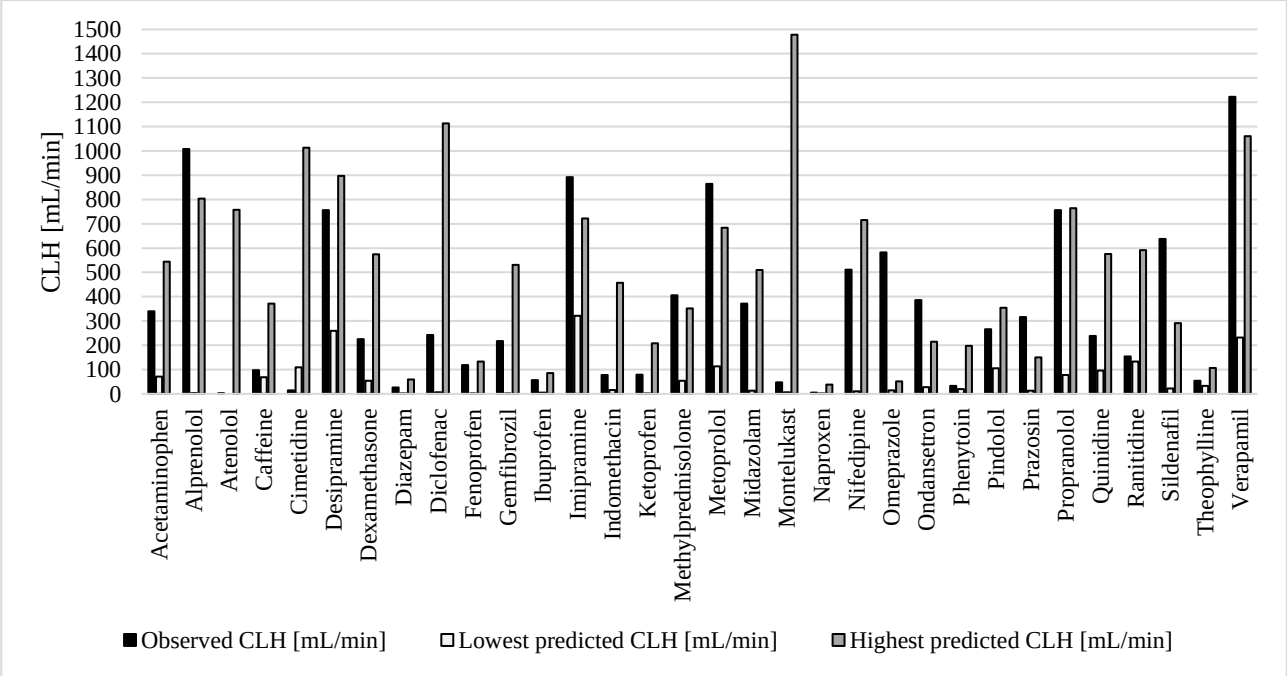

Figure 6. Observed plasma CL_H and predicted min and max plasma CL_H (n=32).

In line with what was found for F, the R^2 for log plasma CL_H based on mean data from many sources was considerably lower than that reported by, for example, Riley et al. 2005; Hallifax et al. 2010, Sohlenius-Sternbeck et al. 2010 and Yamagata et al. 2017) (ca 0.7-0.9). It was closer to estimates by Stringer et al. 2008; Wood et al. 2017 and Fagerholm et al. 2021a and for a test set by Keefer et al. 2023 (ca 0.2-0.4). The low reproducibility and high predictive accuracy in some studies is interesting.

The impact on data selection for predictions

The reason to higher R^2-values in separate reports compared to those reached with mean data from multiple sources could be retrospective selection of favorable f_u, C_{bl}/C_{pl} and *in vivo* CL_H-data. Support for that is given by interstudy differences in selected data by companies and researchers.

F_u- and C_{bl}/C_{pl}-data for 23 compounds from 3 studies were used in prediction studies by one player in the pharmaceutical industry, and in 82 % of cases different data were used for each study (Figure 7). The mean and maximum interstudy differences for f_u were 1.89- and 4.50-fold, respectively. Corresponding estimates for C_{bl}/C_{pl} were 1.14- and 1.59-fold, respectively. For every other compound it was possible to select f_u- and C_{bl}/C_{pl}-data to adjust for 2- to 5-fold differences. Replacement of selected f_u and C_{bl}/C_{pl} with average values resulted in large decrease of R^2 and increase of prediction errors.

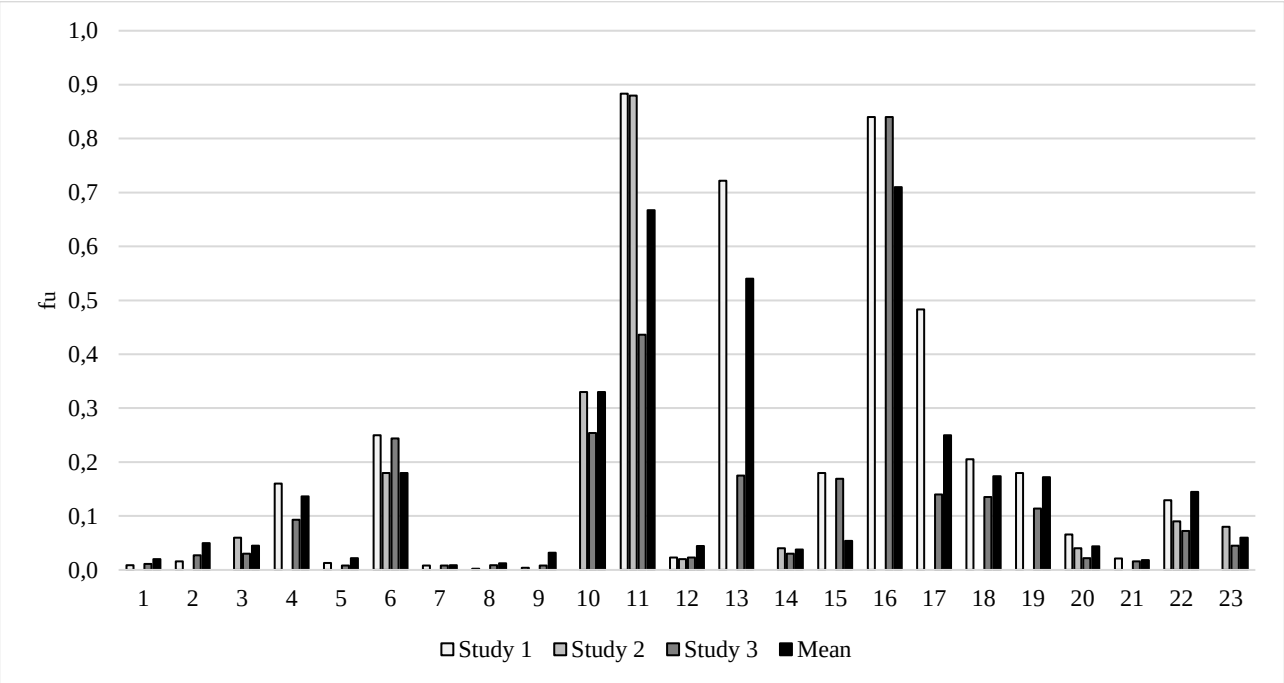

Figure 7. F_u-values for 23 compounds selected in different studies by one player in the pharmaceutical industry, including mean f_u-values estimated from more studies.

F_u-data for 28 compounds from 4 studies were used in prediction studies by another player, and in all (100 %) cases different data were used for each study. The mean and maximum interstudy differences for f_u were 3.04- and 15-fold, respectively. Also in this case, replacement of selected f_u and C_{bl}/C_{pl} with average values resulted in large decrease of R^2 and increase of prediction errors.

Another major pharmaceutical company reported 5.9- and 19-fold mean and maximum differences in hepatocyte CL_{int}-estimates between studies (n=19). In 91 % of cases, different f_u- and C_{bl}/C_{pl}-data were used for each study. The mean and maximum interstudy differences for f_u were 1.95- and 14.5-fold, respectively. Corresponding estimates for C_{bl}/C_{pl} were 1.16- and 1.63-fold, respectively.

Thus, it is common that researchers and companies select different reference estimates for different studies. An apparent consequence is that exaggerated predictive performances are demonstrated. With the use of mean values from different sources/studies a more accurate and less biased picture is reached and shown.

Another way to improve the R^2-values is to exclude/remove non-quantifiable compounds (e.g. CL_{int} and f_u<LOQ) and compounds with high lipophilicity (low solubility and poor dissolution) and low recovery. Such compounds are seldom included and reported. Atenolol is a compound with negligible *in vivo* metabolism, but with reported moderate/high *in vitro* CL_{int}. As shown above, inclusion of results for this compound has a major negative effect on the R^2 for plasma CL_H (3-fold higher R^2 when excluding atenolol). There are also examples of studies where compounds with low f_u are avoided, which reduces uncertainty and errors for CL-predictions.

The unbound fraction in hepatocyte incubations ($f_{u,inc}$) is another source of variability and uncertainty. The $f_{u,inc}$ for the 32 selected compounds averages 0.80. Only, two of them has a $f_{u,inc} < 0.5$ (diazepam; $f_{u,inc} = 0.15$, montelukast; $f_{u,inc} = 0.0002$). The mean and maximum of ratios between highest and lowest reported estimates were 1.47- and 2.73-fold, respectively. This was more and less extensive than for C_{bl}/C_{pl} and f_u, respectively.

Based on the average choice of f_u, C_{bl}/C_{pl} and $f_{u,inc}$ data a 4-fold difference (2.29 • 1.15 • 1.47) of predicted CL_H can be produced. The corresponding maximum difference is 65-fold (15 • 1.59 • 2.73).

Comparing predictions based on *in vitro*, *in silico* and animal *in vivo* data

For the 29 compounds for which *in vitro* data-based F-predictions resulted in a R^2 of 0.20 (slope=0.57; intercept=0.23; see above) *in silico* predictions (using methodology by Prosilico AB) reached a Q^2 (R^2 for forward-looking predictions; compounds not included in training sets of models) of 0.58 (slope=0.82; intercept=0.13; Figure 8). Thus, the *in silico* methodology clearly outperformed the *in vitro*-to-*in vivo*-methodology with average data from many sources.

In a previous study, this *in silico* method outperformed animal models (mice, rats and dogs) in a head-to-head comparison (Q^2=0.50 for *in silico* method *vs* R^2=0.30 for animal models combined; n=156) (Fagerholm et al. 2021b) (Figure 8). The *in silico* method also produces a higher Q^2 compared to a new *in silico* method by AstraZeneca (Q^2 ca 0.1-0.3) (Miljković et al. 2021) (Figure 8).

The poorer predictive performance of *in vitro* methodology *vs* animal models is in line with previous findings (Poulin et al. 2011).

Other advantages with the *in silico* method is that it is devoid of prediction data selection bias and can predict CL_H and F for compounds out of reach for *in vitro* methods, such as those with CL_{int} and f_u<LOQ and high lipophilicity, low solubility, poor dissolution and low recovery, and for low permeability compounds.

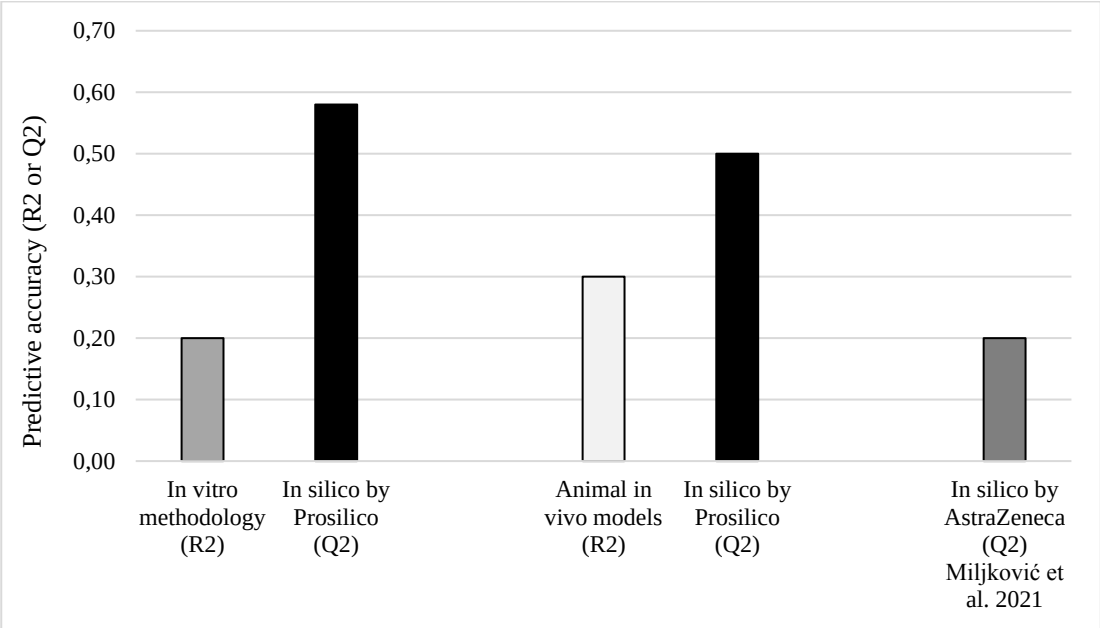

Figure 8. Predictive performance of *in vitro* (R^2), *in silico* (Q^2) and animal *in vivo* models (R^2) for F. Head-to-head comparisons were done for *in vitro vs in silico* (n=29) and animal *in vivo vs in silico* (n=156).

CONCLUSION

The combination of data from many laboratories and the use of mean values resulted in reduced selection bias and predictive accuracy. Overall, the predictive accuracy (here R^2) for log CL_{int}, log CL_H and F was low to moderately low (0.08-0.32). The halved R^2 compared to individual studies where high performance was demonstrated seems to be explained be selection bias (enabled by large data variability). Animal *in vivo* models, and in particular, *in silico* methodology, outperformed *in vitro* methodology for the prediction of F in man. The encouraging results for *in silico* methodology is a major step forward in the process of replacing an reducing animal experiments in pharmacokinetics and drug discovery and development.

REFERENCES

Albaugh DR, Fullenwider CL, Hoelscher S, Freeman KM, Smith CM, Whisnant R, Ferguson SS. 2012. Optimization of a plated cryopreserved human hepatocyte model for assessment of the metabolic stability of low clearance compounds using dextrometorphan, midazolam, tolbutamide, and theophylline. Conference paper in Drug Met Rev. 44.

Bowman CM, Benet LZ. 2019. Interlaboratory variability in human hepatocyte intrinsic clearance values and trends with physicochemical properties. Pharm Res. 36:113.

Fagerholm U, Spjuth O, Hellberg S. 2021a. Comparison between lab variability and *in silico* prediction errors for the unbound fraction of drugs in human plasma. Xenobiot. 51:1095-1100.

Fagerholm U, Hellberg S, Spjuth O. 2021b. Advances in predictions of oral bioavailability of candidate drugs in man with new machine learning methodology. Molecules 26:2572.

Fagerholm U. An analysis of laboratory variability and thresholds for human *in vitro* ADME/PK methods. bioRxiv, Sept. 2022.

Fagerholm U, Spjuth O, Hellberg S. 2022. The impact of reference data selection for the prediction accuracy of intrinsic hepatic metabolic clearance. J Pharm Sci. 111:2645-2649.

Fujikawa M, Ano R, Nakao K, Shimizu R, Akamatsu M. 2005. Relationships between structure and high-throughput screening permeability of diverse drugs with artificial membranes: application to prediction of Caco-2 cell permeability. Bioorg Med Chem. 13:4721-4732.

Gertz M, Harrison A, Houston JB, Galetin A. 2010. Prediction of human intestinal first-pass metabolism of 25 CYP3A substrates from *in vitro* clearance and permeability data. Drug Metab Dispos. 38(7):1147-58.

Hallifax D, Foster JA, Houston JB. 2010. Prediction of human metabolic clearance from *in vitro* systems: Retrospective analysis and prospective view. Pharm Res. 27:2150-2161.

Irvine JD, Takahashi L, Lockhart K, Cheong J, Tolan JW, Selick HE, Grove JR. 1999. MDCK (Madin-Darby Canine Kidney) Cells: A tool for membrane permeability screening. *J Pharm Sci.* 88:28-33.

Keefer CE, Chang G, Di L, Tess DA, Osgood SM, Kapinos B, Racich J, Carlo AA, Balesano A. 2023. The comparison of machine learning and mechanistic *in vitro–in vivo* extrapolation models for the prediction of human intrinsic clearance. Mol Pharmaceut. 20:5616-5630.

Lau YY, Chen Y-H, Liu T-T, Li C, Cui X, White RE, Cheng K-C. 2004. Evaluation of a novel in vitro Caco-2 hepatocyte hybrid system for predicting in vivo oral bioavailability. Drug Metab Disp. 32:937-942.

Lee JB, Zgair A, Taha DA, Zang X, Kagan L, Kim TH, Kim MG, Yun H-Y, Fischer PM, Gershkovich P. 2017. Quantitative analysis of lab-to-lab variability in Caco-2 permeability. Eur J Pharm Biopharm. 114:38-42.

Li A, Sui Z, Liu Y, Wang D, Ge G, Yang L. 2018. A fast screening model for drug permeability assessment based on native small intestinal extracellular matrix. Royal Soc of Chem Adv. 8:34514.

Li C, Liu T, Cui X, Uss AS, Cheng K-C. 2007. Development of *in vitro* pharmacokinetic screens using Caco-2, human hepatocyte, and Caco-2/human hepatocyte hybrid systems for the prediction of oral bioavailability in humans. J Biomol Screening 12.

Lin X, Skolnik S, Chen X, Wang J. 2011. Attenuation of intestinal absorption by major efflux transporters: quantitative tools and strategies using a Caco-2 model. Drug Met Disp. 39: 265-274.

Louisse J, Alewijn M, Peijnenburg AACM, Cnubben NHP, Hering MB, Coecke S, Punt A. 2020. Towards harmonization of test methods for in vitro hepatic clearance studies. Toxicology in vitro 63.

Matsson P, Bergström CAS, Nagahara N, Tavelin S, Norinder U, Artursson, P. 2005. Exploring the role of different drug transport routes in permeability screening. J Med Chem. 48:604-613.

McGinnity DF, Collington J, Austin RP, Riley RJ. 2007. Evaluation of human pharmacokinetics, therapeutic dose and exposure predictions using marketed oral drugs. Current Drug Met. 8:463-479.

Miljković F, Martinsson A, Obrezanova O, Williamson B, Johnson M, Sykes A. 2021. Machine learning models for human *in vivo* pharmacokinetic parameters with in-house validation. Mol Pharmaceut. 18:4520-4530.

Musther, H, Olivares-Morales, A, Hatley, OJD, Liu, B, Rostami Hodjegan, A. 2014. Animal versus human oral drug bioavailability: Do they correlate? Eur J Pharm Sci. 57:280-291.

Obach RS. 1999. Prediction of human clearance of twenty-nine drugs from hepatic microsomal intrinsic clearance data: an examination of *in vitro* half-life approach and nonspecific binding to microsomes. Drug Metab Disp. 27:1350-1359.

Obach RS, Lombardo F, Waters NJ. 2008. Trend analysis of a database of intravenous pharmacokinetic parameters in humans for 670 drug compounds. Drug Metab Disp. 36:1385-1405.

Poulin P, Jones RDO, Jones HM, Gibson CT, Rowland M, Chien JY, Ring BJ, Adkinson KK, Ku S, Vuppugalla R, Marathe P, Fischer O, Dutta S, Sinha VK, Björnsson T, Lavé T. Yates JW. 2011. PHRMA CPCDC initiative on predictive models of human pharmacokinetics, Part 5:

Prediction of plasma concentration–time profiles in human by using the physiologically-based pharmacokinetic modeling approach. J Pharm Sci. 100:4127-4157.

Riley RJ, McGinnity DF, Austin RP. 2005. A unified model for predicting human hepatic, metabolic clearance from *in vitro* intrinsic clearance data in hepatocytes and microsomes. Drug Metab Disp. 33:1304-1311.

Skolnik S, Lin X, Wang J, Chen X-H, He T, Zhang B. 2010. Towards prediction of *in vivo* intestinal absorption using a 96-well Caco-2 assay. J Pharm Sci. 99:3246-3265.

Sköld C, Winiwarter S, Wernevik J, Bergström F, Engström L, Allen R, Box K, Comer J, Mole J, Hallberg A, Lennernäs H, Lundstedt T, Ungell A-L, Karlén A. 2006. Presentation of a structurally diverse and commercially available drug data set for correlation and benchmarking studies. J Med Chem. 49:6660-6671.

Soars MG, Burchell B, Riley RJ. 2002. *In vitro* analysis of human drug glucuronidation and prediction of *in vivo* metabolic clearance. J Pharmacol Expl Ther. 301:382-390.

Sohlenius-Sternbeck A-K, Afzelius L, Prusis P, Neelissen J, Hoogstraate J, Johansson J, Floby E, Bengtsson A, Gissberg O, Sternbeck J, Petersson C. 2010. Evaluation of the human prediction of clearance from hepatocyte and microsome intrinsic clearance for 52 drug compounds. Xenobiot. 40:637-649.

Stringer R, Nicklin PL, Houston JB. 2008. Reliability of human cryopreserved hepatocytes and liver microsomes as *in vitro* systems to predict metabolic clearance. Xenobiot. 38:1313-1329.

Thomas S, Brightman F, Gill H, Lee S, Pufong B. 2005. Simulation modelling of human intestinal absorption using Caco-2 permeability and kinetic solubility data for early drug discovery. J Pharm Sci. 48:604-613.

Uchimura T, Kato M, Saito T, Kinoshita H. Prediction of human blood-to-plasma drug concentration ratio. Biopharm Drug Disp 2010;31:286-297.

Varma MVS, Obach RS, Rotter C, Miller HR, Chang G, Steyn SJ, El-Kattan A, Troutman MD. 2010. Physicochemical space for optimum oral bioavailability: Contribution of human intestinal absorption and first-pass elimination. J Med Chem. 53:1098-1108.

Wang H, Zrada M, Anderson K, Katwaru R, Harradine P, Choi B, Tong V, Pajkovic N, Mazsenko R, Cox K, Chien LH. 2014. Understanding and reducing the experimental variability of *in vitro* plasma protein binding measurements. J Pharm Sci. 103;3302-3309.

Wood FL, Houston JB, Hallifax D. 2017. Clearance prediction methodology needs fundamental improvement: Trends common to rat and human hepatocytes/microsomes and implications for experimental methodology. Drug Met Disp. 45:1178-1188.

Yamagata T, Zanelli U, Gallemann D, Perrin D, Dolgos H, Petersson C. 2017. Comparison of methods for the prediction of human clearance from hepatocyte intrinsic clearance for a set of reference compounds and an external evaluation set. Xenobiot. 47:741-751.

Yau E, Petersson C, Dolgos H, Peters SH. 2017. A comparative evaluation of models to predict human intestinal metabolism from nonclinical data. Biopharm Drug Dispos. 38:163-186.

Yee S. 1997. *In vitro* permeability across Caco-2 cells (colonic) can predict *in vivo* (small intestinal) absorption in man-fact or myth. Pharm Res. 14:763-766.

Table 1. The selected compounds and their CL_{int} (predicted from human hepatocyte unbound CL_{int}) and f_a (predicted from Caco-2 P_{app}).

	CL_{int} [mL/min]		**f_a**	
	Min	Max	Min	Max
Acetaminophen	150	1069	0,94	0,96
Alprenolol	18	9613	0,90	0,98
Atenolol	-	ca 1550	0,27	0,71
Caffeine	112	618	0,94	1,00
Cimetidine	227	3857	0,34	0,67
Desipramine	3374	12390	0,83	0,94
Dexamethasone	434	2905	0,68	0,93
Diazepam	100	1701	0,97	1,00
Diclofenac	3444	43260	0,97	1,00
Fenoprofen	98	7272	-	-
Gemfibrozil	245	22807	-	-
Ibuprofen	805	4994	0,97	0,99
Imipramine	6594	8190	0,91	0,97
Indomethacin	4130	6580	0,93	0,96
Ketoprofen	431	4017	0,56	0,99
Methylprednisolone	393	2002	0,65	0,89
Metoprolol	279	1400	0,52	0,98
Midazolam	777	10710	0,95	0,95
Montelukast	6739	9949084	-	-
Naproxen	395	2180	0,57	1,00
Nifedipine	1274	20119	0,93	0,99
Omeprazole	351	784	0,96	1,00
Ondansetron	105	368	0,95	0,98
Phenytoin	403	1834	0,97	0,98
Pindolol	196	553	0,81	0,97
Prazosin	440	1965	0,74	0,96
Propranolol	1113	5677	0,52	0,99
Quinidine	896	4055	0,82	0,94
Ranitidine	189	1029	0,46	0,73
Sildenafil	1015	5465	0,94	0,97
Theophylline	84	188	0,92	0,97
Verapamil	3801	16590	0,52	1,00

Table 2. The selected compounds and their predicted and observed plasma CL_H and F and measured F_{gut}.

	Plasma CL_H			[mL/min]	**F**				**Fgut**
	Predicted			Observed	Predicted			Observed	Observed
	Min	Max	Mean	Mean	Min	Max	Mean	Mean	
Acetaminophen	71	545	206	340	0,60	0,92	0,83	0,82	
Alprenolol	3	803	411	1008	0,27	0,98	0,63	0,08	CYP3A4
Atenolol	-	757	645	2,1	0,14	0,71	0,32	0,50	
Caffeine	68	372	167	97	0,65	0,95	0,85	0,98	CYP3A4
Cimetidine	109	1013	506	14	0,10	0,62	0,35	0,68	CYP3A4
Desipramine	259	897	605	756	0,32	0,84	0,61	0,40	
Dexamethasone	54	574	300	225	0,35	0,89	0,65	0,80	CYP3A4
Diazepam	1	59	12	27	0,90	1,00	0,97	0,93	CYP3A4
Diclofenac	6,86	1114	664	243	0	0,99	0,26	0,54	
Fenoprofen	0,65	133	44	118	-	-	-	-	
Gemfibrozil	0,49	531	191	217	-	-	-	-	
Ibuprofen	5	86	35	57	0,87	0,99	0,94	0,98	
Imipramine	321	722	512	892	0,45	0,84	0,66	0,53	
Indomethacin	16	457	191	77	0,42	0,95	0,79	0,93	
Ketoprofen	2	208	58	78	0,42	0,98	0,85	0,85	
Methylprednisolone	54	352	217	406	0,49	0,86	0,70	0,90	CYP3A4
Metoprolol	113	684	462	865	0,30	0,92	0,66	0,40	
Midazolam	13	509	112	371	0,37	0,94	0,84	0,36	0,57
Montelukast	6,04	1478	1421	48	-	-	-	-	
Naproxen	0	38	11	5	0,54	1,00	0,91	0,86	
Nifedipine	10	716	189	511	0,16	0,98	0,76	0,50	0,68
Omeprazole	14	51	31	582	0,91	0,99	0,92	0,54	CYP3A4
Ondansetron	28	214	116	386	0,79	0,96	0,88	0,60	CYP3A4
Phenytoin	20	197	153	33	0,76	0,96	0,82	0,85	
Pindolol	106	355	259	266	0,54	0,91	0,73	0,87	
Prazosin	13	150	44	316	0,62	0,95	0,84	0,68	
Propranolol	78	764	373	756	0,20	0,94	0,66	0,24	CYP3A4
Quinidine	96	575	254	238	0,42	0,87	0,70	0,73	0,90
Ranitidine	132	592	279	154	0,27	0,67	0,50	0,60	
Sildenafil	22	291	102	637	0,65	0,95	0,86	0,41	0,72
Theophylline	33	106	61	54	0,84	0,94	0,90	0,90	CYP3A4
Verapamil	231	1060	703	1222	0,04	0,85	0,41	0,24	0,57