Prediction of human intestinal absorption using micellar liquid chromatography with an aminopropyl stationary phase

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Abstract:

The extent of human intestinal absorption (HIA) for a drug is considered to be an important pharmacokinetic parameter which must be determined for orally administered drugs. Traditional experimental methods relied upon animal testing and are renowned for being time consuming, expensive as well as being ethically unfavourable. As a result, developing alternative methods to evaluate a drug's pharmacokinetics is crucial. Micellar liquid chromatography (MLC) is considered to be one of these methods that can replace the use of animals in prediction of HIA. In this study, the combination of an aminopropyl column with the biosurfactant sodium deoxycholate (NaDC) bile salt were used in the experimental determination of micelle-water partition coefficients (log P_{mw}) for a group of compounds. Multiple linear regression (MLR) was then used for the prediction of HIA using the experimentally determined log P_{mw} along with other molecular descriptors leading to the construction of a model equation of R^2= 85 % and a prediction power represented by $R^2_{Pred.}$ =72 %. The use of MLC with an aminopropyl column in combination with NaDC was found to be a good method for the prediction of human intestinal absorption, providing data for a far wider range of compounds compared with previous studies.

Keywords:

Aminopropyl column; biosurfactant; human intestinal absorption; micellar liquid chromatography; multiple linear regression; pharmacokinetics; sodium deoxycholate.

1.Introduction:

The use of micelles in HPLC was first introduced by Armstrong and Henry in 1980 (Berthod and Garcia-Alvarez-Coque 2000), now more commonly known as micellar liquid chromatography (MLC), and was used to enhance retention and selectivity of various solutes that would otherwise be inseparable or poorly resolved. MLC is an interesting technique for green chemistry as it uses a mobile phase containing 90 % or more water, these micellar mobile phases have low toxicity, are non-flammable and do not produce hazardous waste (Kanakaiah 2013). Micellar liquid chromatography uses mobile phases containing a surfactant (ionic or non-ionic) above its critical micellar concentration (CMC) along with columns such as C18 (and to a lesser extent cyanopropyl (CN)) (Kalyankar, Kulkarni et al. 2014; Rambla-Alegre 2012). In MLC, surfactant monomers incorporated in the mobile phase adsorb on the porous RPLC packing altering the various surface properties of the stationary phase, such as surface area, polarity, structure, and pore volume which majorly influences chromatographic retention. The stationary phase pores are also coated by the surfactant molecules, decreasing their volume (Kalyankar, Kulkarni et al. 2014). Different stationary phases have different chemical characteristics which influence the elution of different groups of compounds where the formation of an aqueous layer over the stationary phase depends on the nature and the number of the chemically bonded groups therefore, determining the solutes' partitioning. As a result, the use of a aminopropyl column was attempted in this work to help enhance the applicability of chromatography for absorption prediction where higher reactivity and polar interactions are important characteristics of amino columns as discussed in literature (Rambla-Alegre, Carda-Broch et al. 2009; Gama, da Costa Silva et al. 2012).

MLC has been applied to a variety of applications including prediction of transdermal permeation (Waters, Shahzad et al. 2013) through to oral drug absorption (Escuder-Gilabert, Martınez-Pla et al. 2003), blood brain barrier penetration (Escuder-Gilabert, Molero-Monfort et al. 2004) and ocular tissue permeability (Martın-Biosca, Molero-Monfort et al. 2003). In recent years, studies have also attempted to link chromatographically derived retention constants with pharmacokinetic predictors determined *in silico* and although linear relationships have been found (Milošević, Stojanović, Penov-Gaši, Perišić-Janjić, Kaliszan 2014), these previously published studies depend upon the reliability of calculated data whereas this work attempts to correlate chromatographic data with published experimental data. Solutes injected within an MLC system are classified according to their elution behaviour into three categories; binding, antibinding and non-binding. Binding solutes are those which bind or

associate to micelles, they show decreased retention when the micelle concentration is increased. Antibinding solutes display increased retention with an increased concentration of micelles. Finally, non-binding solutes do not bind or associate to micelles displaying unaltered retention with changing micellar concentration.

Previous work by the authors of this study presented the application of a cyanopropyl column as a stationary phase in an MLC system using a mixture of bile salts to determine human intestinal absorption (HIA) (Waters, Shokry et al. 2016; Shokry, Waters et al. 2018). However, for this study, an aminopropyl column was investigated as the stationary phase to determine the suitability of this alternative column for facilitating prediction of HIA.

In summary, the work presented in this paper utilises micellar liquid chromatography to predict human intestinal absorption with sodium deoxycholate as the mobile phase and an aminopropyl column as the stationary phase. Previous studies have successfully utilised a system similar to this yet have been restricted by the variety of compounds that can be analysed based upon limitations as a consequence of the stationary phase employed. Through incorporating an aminopropyl column in to the system it is envisaged that a far wider range of compounds will be suitable for analysis thus expanding the potential applicability of the technique.

2. Materials and Methods

2.1 Materials

Sodium deoxycholate (NaDC) was used as purchased from Sigma Aldrich, Dorset, UK (97 %). Analysed compounds using MLC were acetaminophen (99 %, Sigma Aldrich, Dorset, UK), acetyl salicylic acid (99 %, Acros organics, Geel, Belgium), caffeine (97 %, Sigma Aldrich, Dorset, UK), carbamazepine (99 %, Sigma Aldrich, Dorset, UK), cimetidine (Sigma Aldrich, Dorset, UK), diclofenac (98 %, TCI, Zwijndrecht, Europe), fenoprofen (97 %, Fluka, Dorset, UK), fluconazole (98 %, Sigma Aldrich, Dorset, UK), flurbiprofen (98 %, TCI, Zwijndrecht, Europe), gemfibrozil (98 %, TCI, Zwijndrecht, Europe), ibuprofen (98 %, BASF, Cheshire, UK), indomethacin (99 %, Sigma Aldrich, Dorset, UK), ketoprofen (98 %, Sigma Aldrich, Dorset, UK), lidocaine (98 %, Sigma Aldrich, Dorset, UK), lornoxicam (>98 %, TCI, Zwijndrecht, Europe), meloxicam (98 %, TCI, Zwijndrecht, Europe), naproxen (98 %, Sigma Aldrich, Dorset, UK), nicotinic acid (99.5 %, Sigma Aldrich, Dorset, UK), phenylbutazone (98.5 %, Sigma Aldrich, Dorset, UK), piroxicam (98 %, Sigma Aldrich, Dorset, UK), salicylic acid (99 %, Fisher Scientific, Loughborough, UK), theophylline (98 %, TCI, Oxford, UK) and terbutaline (96 %, Sigma Aldrich, Dorset, UK) used as purchased. Deionised water was prepared using a Barnstead™ Ultrapure system.

2.1 Preparation of NaDC solutions used as mobile phase:

A 20 mM stock solution of NaDC bile salt in water was prepared by transferring an accurately weighed amount of NaDC to a 250 mL volumetric flask and completing to the mark with deionised water. Accurately measured aliquots were then transferred from the stock solution to 50 mL volumetric flasks; solutions were then completed to the final volume with deionised water to give NaDC solutions of concentrations within the range of (5-20 mM).

2.2 Experimental Procedure

Micellar liquid chromatography required injection of 20 µL samples of twenty-three compounds (0.2 mM) into a Rheodyne injector and pumped with the mobile phase through a reversed phase aminopropyl column (APS) (Hypersil 5µm, 15 cm x 4.6mm, Thermo Fisher Scientific, MA, USA) using an Agilent1100 Series Binary pump at a flow rate of 1.34 mL/min. The retention of the solutes within the column was detected using a UV detector (Perseptive Biosystems UVIS-205, MA, USA), set at a wavelength appropriate for each drug and recorded via Picolog software indicating retention times. The mobile phase was filtered through a 0.45 µm Nylon filter and degassed in an ultrasonic bath. The recorded data were analysed to obtain retention factors and each sample was repeated in triplicate to ensure that reasonable accuracy and precision were achieved using a series of mobile phases of NaDC concentrations. A minimum of five concentrations were analysed per drug ranging from 5-20 mM. All experiments were performed at room temperature.

2.3 Determination of Dead time (t_0)

The dead time (t_0) (time taken by the solvent front to reach the detector) was measured by the injection of water in to the system (Pramauro, Minero et al. 1988) for the appearance of the first major perturbation to the baseline. The dead time was calculated from an average of ten results.

2.4 Calculation of Log P_{mw}

From the recorded retention times for each compound and the dead time, retention factors (k) were calculated as follows:

$$k = \frac{(\text{Retention time} - \text{dead time})}{\text{dead time}} \qquad \text{Eq. (1)}$$

The CMC value of NaDC was taken to be 5 mM (Olesen, Westh et al. 2015) and the micellar concentration (C_M) was then calculated for each NaDC concentration used as follows:

$$(C_M) = \text{Total surfactant concentration} - \text{Critical micellar concentration (CMC)} \quad \text{Eq. (2)}$$

On the basis of a linear relationship between micellar concentration (C_M) and (1/k) according to the equations described by Arunyanart and Love (1985), the log of the partition coefficient (log P_{mw}) can be determined for each compound by:

$$\text{Log } P_{mw} = \log[\text{intercept}/\text{slope}] \qquad \text{Eq. (3)}$$

3. Results:

Prior to this study, the use of NaDC as a mobile phase (with an alternative column) confirmed the potential of MLC in the prediction of HIA (Waters, Shokry et al. 2016). A further study considered the effect of changing the mobile phase composition confirming the use of a mixture of NaDC along with phospholipid was a better predictive system of HIA than NaDC alone (Shokry, Waters et al. 2018). This current study explores the effect of an alternative stationary phase regarding HIA predictive capability.

3.1 Selection of Aminopropyl column

According to literature, aminopropyl columns are widely used especially in hydrophilic interaction liquid chromatography (HILIC). They also offer a more reactive, selective and efficient stationary phase and polar interactions are more abundant than in cyanopropyl columns (Rambla-Alegre, Carda-Broch et al. 2009; Gama, da Costa Silva et al. 2012). Also aminopropyl columns offer the possibility of anion exchange mechanisms around neutral pH (Olsen 2001). Compounds such as amines, ethers, esters and ketones are preferentially retained on amino columns compared with cyano columns. This could be the reason why more compounds were analysed using this column (rather than a cyano column) in the previous study. Furthermore, because of general concerns about stability and reproducibility, cyanopropyl columns are less commonly used overall.

3.2 Effect on retention behaviour

Experimental data for the calculated log P_{mw} values, along with published physicochemical data utilised for analysis are presented within 'Supplementary Information' (Table S1). The use of an aminopropyl column as a stationary phase had a great impact on the retention profiles for a number of the analysed compounds (including neutral, anionic and cationic compounds) using MLC. Therefore, there was an appreciable effect on the obtained log P_{mw} values which is a reflection of the partitioning process of the compounds under study. The dead time average value was determined to be 79.40 seconds.

Ideally, neutral and cationic drugs are expected to show binding behaviour whilst anionic drugs are expected to show antibinding behaviour with the increase in the bile salt concentration. Interestingly, the opposite to what was expected was observed in that neutral compounds

displayed antibinding behaviour whilst cationic and anionic drugs displayed both binding and antibinding behaviour according to their molecular weight.

3.2.1 Theory behind retention behaviour

Salt bridge formation is assumed to be the justification behind the change in the way drugs interacted with the stationary phase (aminopropyl column) that led to unconventional patterns of elution. This assumption is supported by the work of Takeuchi *et al.* who presented the possibility of using bile acids as stationary phases in liquid chromatography through their immobilisation on aminopropyl silica through electrostatic interactions (Takeuchi, Chu et al. 1998). A salt bridge is a combination of two noncovalent interactions which are hydrogen bonding and electrostatic interactions. Although such bridges are abundant in protein folded conformations (to provide stability) they are also found in supramolecular chemistry. Since the pH of the medium was found to be in the range of (6.4-8.0) the amino group ($-NH_2$) is thought to undergo protonation converting to the ammonium ion ($-NH_3^+$) and, in this case, rendering the column positively charged. As a result, a salt bridge is assumed to have formed through electrostatic attraction between the negatively charged carboxylic group ($–COO^-$) of NaDC and the positively charged ammonium group ($-NH_3^+$) of the column also, through hydrogen bonding between the hydrogen atom of the ammonium group ($-NH_3^+$) and the oxygen atom of the carboxylic group ($–COO^-$) which adds up to the overall stability of the formed network as it acts as a small stabilising interaction (Anslyn and Dougherty 2006). The charge on both the column and the bile salt adsorbed on its surface are masked by their electrostatic attraction. Salt bridges form between the bile salt monomers and the column creating a stable network. Also, hydrogen bonds form between the bile salts hydroxyl groups as well as the nonpolar binding of the hydrophobic moiety of NaDC molecules, creating a network with free monomers from the mobile phase leading to the formation of the appearance of bilayers of bile salt.

Although some anionic drugs displayed antibinding behaviour (which is typical for conventional retention), a number of anionic drugs exhibited the opposite behaviour i.e. a binding interaction with NaDC. Both cases can be explained according to the previously mentioned theory for bile salt (micellar mobile phase) interactions with the aminopropyl column used in this method.

The anionic drugs (fenoprofen, ibuprofen, gemfibrozil and phenylbutazone) displayed a retention behaviour typical for that expected for anionic compounds with anionic surfactant with an antibinding interaction with the NaDC micelles. On the other hand, three anionic drugs, namely lornoxicam, meloxicam and piroxicam displayed an opposite pattern of interaction as

they acted as binding solutes. This is unusual for anionic drugs when analysed with anionic surfactants in MLC.

The typical antibinding behaviour of anionic compounds can be attributed to the electrostatic repulsion taking place between the negatively charged compounds and the negatively charged surfactant. As a result of this repulsion the compound bound to the column displayed an increase in retention on the column with the increase in the surfactant concentration. In this case the drug must have a low molecular weight in order to be entrapped inside the layers of the bile salt network structure formed with the aminopropyl column by means of electrostatic attraction and hydrogen bonding. As a result, fenoprofen, ibuprofen, gemfibrozil and phenylbutazone, having relatively low molecular weights of 242.3, 206.3, 250.3 and 308.4 g/mol respectively (http://www.chemspider.com/), were entrapped inside the bile salt network structure displaying an antibinding interaction. However, lornoxicam, meloxicam and piroxicam, having relatively higher molecular weight values of 371.8, 351.4 and 331.4 g/mol respectively (http://www.chemspider.com/), could not be entrapped inside the bile salt network structure. Instead, they were entrapped inside the micellar core, overcoming the repulsion forces with the micelles as a consequence of their high molecular weights (Figure 1).

For neutral drugs (acetaminophen, caffeine, fluconazole and theophylline), an antibinding retention behaviour was observed which is again, against convention, where neutral drugs traditionally undergo a binding interaction. This can be attributed to the preference of these drugs to bind to the more stable hydrophobic core of the bile salt network structure rather than that of the bile salt micelles in the mobile phase. Also these drugs have comparatively low molecular weights of 151.2, 194.2, 306.27, 180.2 g/mol respectively (http://www.chemspider.com/), facilitating entrapment within the hydrophobic core of the bile salt network structure within the column therefore showing antibinding retention behaviour.

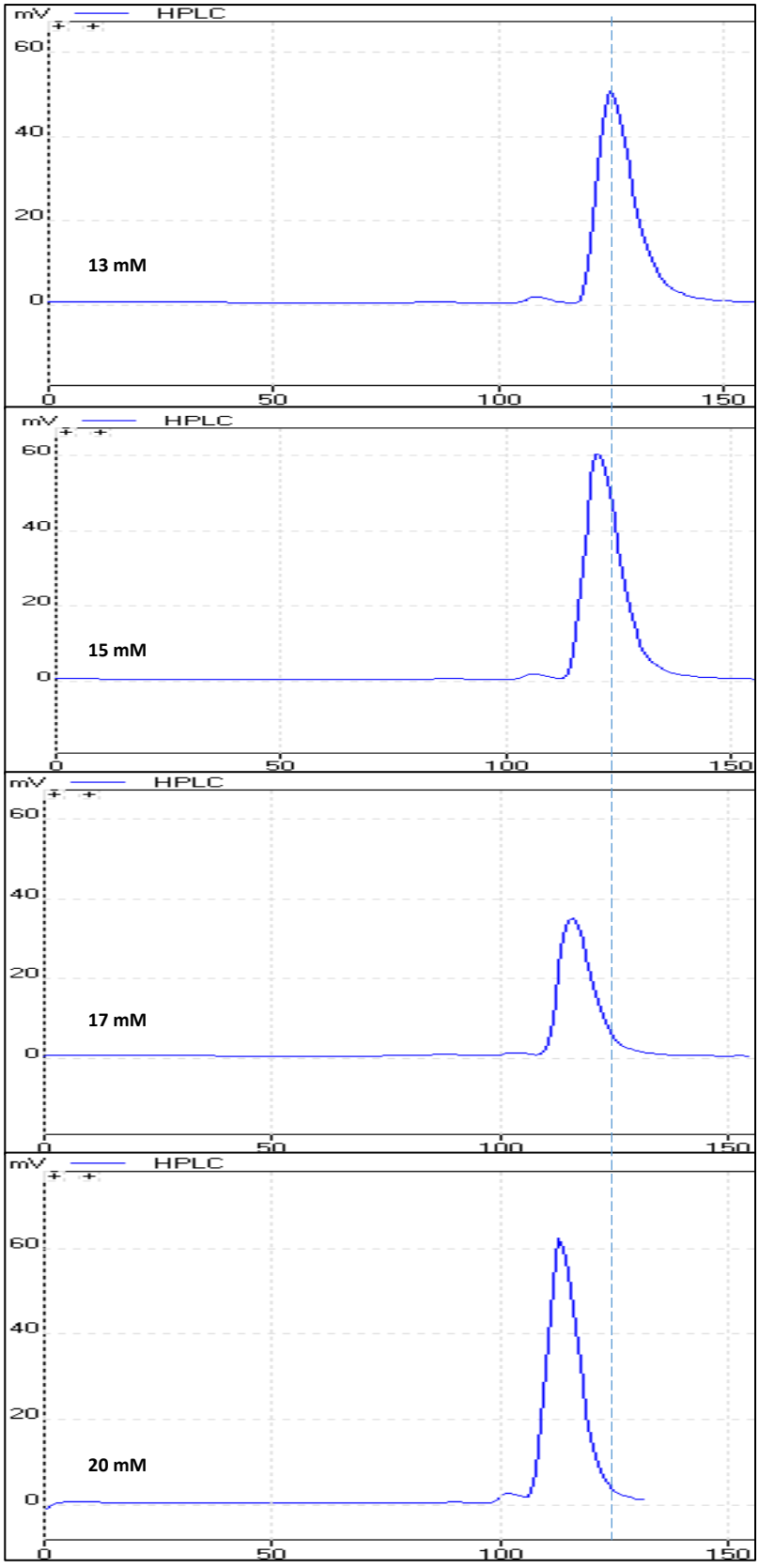

Figure 1: Chromatograms displaying the binding behaviour of meloxicam over a series of mobile phase concentrations with an aminopropyl column as a stationary phase. (The dotted line is only used for visual guidance).

3.3 Prediction of human intestinal absorption (HIA)

Statistical data analysis was conducted using Minitab 17® software. Multiple linear regression was carried out where different molecular descriptors collected from literature were regressed against the dependant variable %HIA and a backward elimination modelling strategy was carried out. To take variance inflation factors (VIF) to acceptable limits, variables with high (VIF) were removed. Finally, an optimum model was obtained that provides a good summary of data, this was undertaken in a similar manner to that previously published (Waters, Shokry et al. 2016).

The variables remaining in the optimal model were assessed for significance and relative importance by standardised coefficients and the associated p-values.

The predictive ability of the final model was assessed using adjusted-R^2 and R^2 for prediction (R^2_{PRED}) derived from predicted residual error sum of squares (PRESS statistic). The predictive ability of the model was indicated by R^2_{PRED} which consequently reflects the model's applicability.

In this study, a group of twenty-three drugs were analysed using the MLC system using an aminopropyl (APS) column and log P_{mw} was calculated for each compound, a number of molecular descriptors such as molecular weight (Mwt), polar surface area (PSA), freely rotating bonds (FRB), molar volume (V_M), dissociation constant (pK_a), aqueous solubility (S_w), number of hydrogen bond donors (nHD) and number of hydrogen bond acceptors (nHA) were used along with the obtained log P_{mw} to develop a mathematical model for prediction of %HIA. Logit (%HIA) was used to improve the linear relationship between published (%HIA) and experimental log P_{mw} values as seen in studies of a similar type (Norinder, Österberg et al. 1999; Raevsky, Fetisov et al. 2000; Zhao, Abraham et al. 2002). The human intestinal absorption values were transformed to logit by substitution in Equation 4.

$$\text{Logit (\%HIA)} = \log (\%HIA / (100-\%HIA)) \qquad \text{Eq. (4)}$$

For simplification, all drugs of 100 or 0 % HIA were excluded from the training set.

Log P_{mw} was successfully included with 2 other molecular descriptors in the final model equation with %HIA experimental values for orally administered drugs which successfully predicted the %HIA with 72 % predictability. The final model was validated using a set of seven compounds.

The model obtained for the prediction of %HIA is given by Equation 5:

$$\text{logit \%HIA} = -0.758 - 0.369 \log P_{mw} + 0.01157 V_M + 0.0714 S_w \qquad \text{Eq. (5)}$$

Sixteen drugs were used in the development of the final model. The model's R^2 = 84.62 %,

$R^2_{adjust.}$= 80.77 % , R^2_{PRED} = 71.51 %, S= 0.203

A 95 % confidence interval for log P_{mw} is given by (-0.726, -0.011), t-statistic and standardised coefficient of log P_{mw} are –2.25 ($p<0.05$) and -0.294 respectively suggesting statistical significance of log P_{mw} as a predictor. Also the F-ratio of the overall model is statistically significant, F= 22 and P value 0.000 ($p<0.05$). Figure 2 shows no marked relationship between residuals and predicted values while Figure 3 summarises the model.

Seven drugs (cimetidine, fenoprofen, lornoxicam, nicotinic acid, piroxicam, salicylic acid and terbutaline) were used to test the model predictability. The model was able to predict the %HIA for these drugs within a minimum of 0.1 % and a maximum of 8 % difference between the predicted %HIA and the published %HIA. The model appears to have underestimated %HIA for both lornoxicam and salicylic acid with a 12 % and 24 % difference between the two predicted and published values. The relationship between the predicted and the experimental %HIA values is shown in Table 1 and Figure 4.

Comparing this current model with that obtained with the MLC system using a cyanopropyl column; logit HIA = -0.410 - 0.482 log P_{mw} + 0.00852 Mwt + 0.04799 S_w (Waters, Shokry et al. 2016), it is observed that three predictors were included in each model: (log P_{mw} and S_w) in both models, Mwt (in CN column derived model) and V_M (in APS column derived model). According to literature, molecular weight (Mwt) and molar volume (V_M) are both estimates of size however, V_M takes into consideration both the size and shape as it is very much related to molecular surface area which offers a better guide to estimate the potential for permeability (Smith, Walker et al. 2006). Although both models have nearly the same HIA prediction ability, this current model was obtained based on a larger data set where the change of the used column from CN to APS allowed the analysis of a larger number of compounds. This is because the latter column resulted in compound-stationary phase interactions that facilitated calculation of values for compounds that had been unsuccessfully analysed using the former column type.

Finally, combining the results of changing the column type from CN to APS and changing the type of micellar mobile phase from NaDC to a physiological mixture of bile salts with lecithin, it is hypothesised that the use of an MLC system that combines the use of an APS column with bile salts with lecithin as a mobile phase would provide a more reliable HIA predictive model covering a wider range of %HIA prediction.

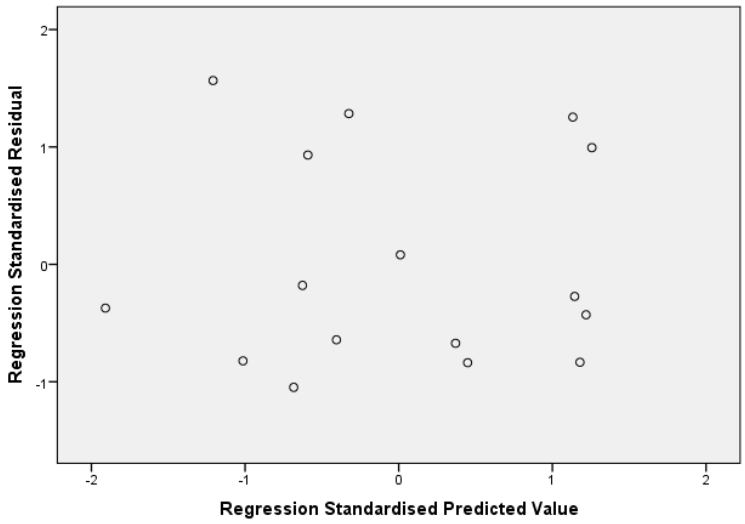

Figure 2: Residual plot for optimal logit HIA regression model.

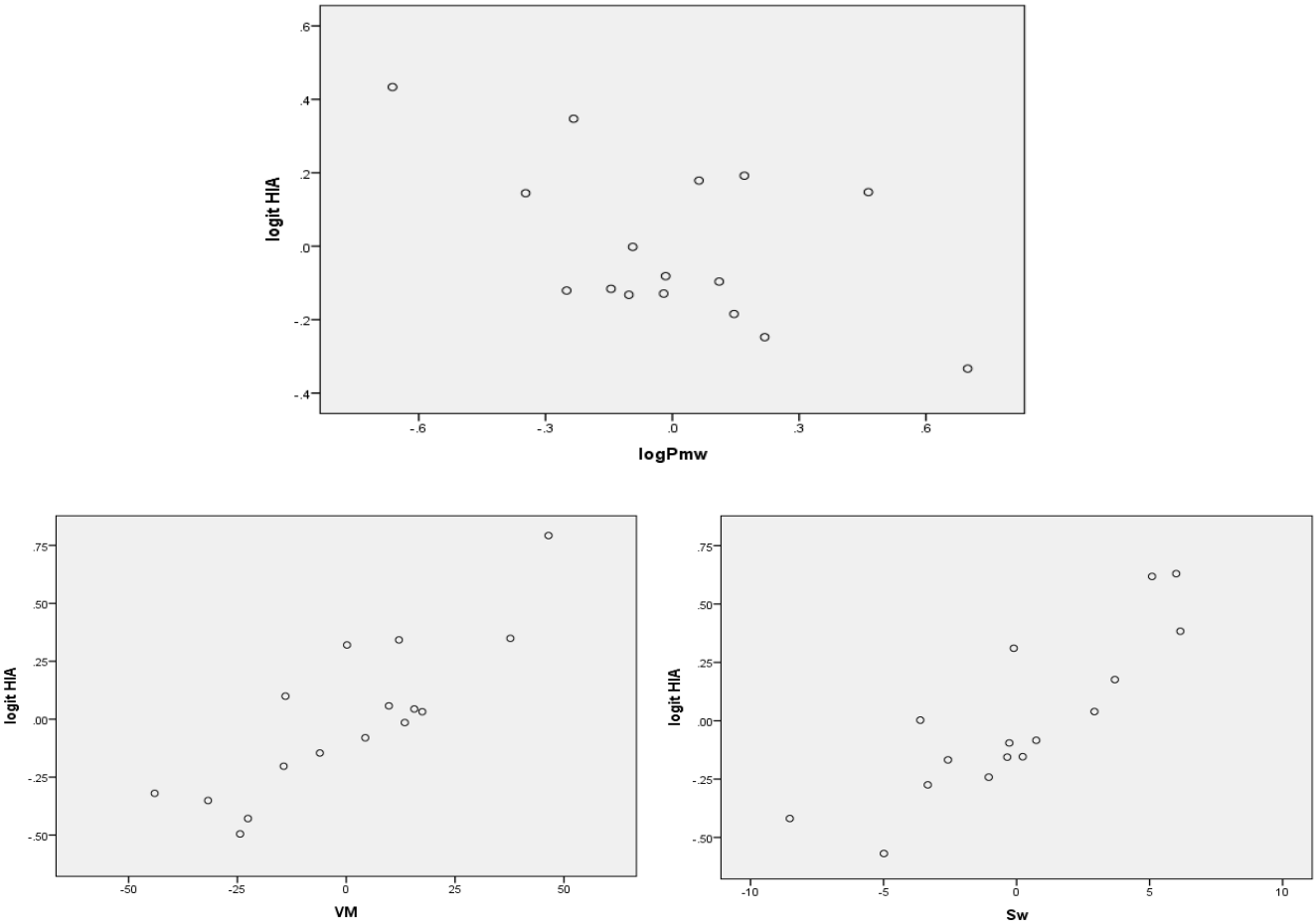

Figure 3: Partial regression plots of experimental logit HIA. values against log P_{mw}, V_M and S_w .

320 **Table 1**: Experimental and predicted values for %HIA.

Drug	Expt. %HIA	Pred. %HIA	Reference
Acetaminophen	95.00	94.82 (±0.04)	(Castillo-Garit, Cañizares-Carmenate et al. 2014)
Acetylsalicylic acid	82.00	87.00 (±0.04)	(Castillo-Garit, Cañizares-Carmenate et al. 2014)
Caffeine	99.00	98.42 (±0.01)	(Yan, Wang et al. 2008)
Carbamazepine	70.00	73.53 (±0.09)	(Varma, Sateesh et al. 2005)
Cimetidine*	73.50	73.60 (±0.05)	(Veber, Johnson et al. 2002; Yan, Wang et al. 2008)
Diclofenac	90.00	90.73 (±0.01)	(Molero-Monfort, Escuder-Gilabert et al. 2001)
Fenoprofen*	85.00	93.07 (±0.06)	(Hou, Wang et al. 2007)
Fluconazole	97.50	98.29 (±0.05)	(Castillo-Garit, Cañizares-Carmenate et al. 2014)
Flurbiprofen	92.00	84.68 (±0.01)	(Raevsky 2004)
Gemfibrozil	95.00	96.57 (±0.05)	(Paixão, Gouveia et al. 2012)
Ibuprofen	85.00	90.25 (±0.01)	(Paixão, Gouveia et al. 2012)
Indomethacin	99.00	98.22 (±0.04)	(Chu 2009)
Ketoprofen	96.00	92.94 (±0.05)	(Castillo-Garit, Cañizares-Carmenate et al. 2014)
Lidocaine	95.00	96.30 (±0.02)	(Molero-Monfort, Escuder-Gilabert et al. 2001; Chu 2009)
Lornoxicam*	100.00	88.67 (±0.06)	(Newby, Freitas et al. 2015)
Meloxicam	90.00	92.40 (±0.06)	(Castillo-Garit, Cañizares-Carmenate et al. 2014)
Naproxen	94.00	91.02 (±0.05)	(Castillo-Garit, Cañizares-Carmenate et al. 2014)
Nicotinic acid*	94.00	100.00 (±0.01)	(Yan, Wang et al. 2008)
Phenylbutazone	98.00	98.36 (±0.01)	(Hou, Wang et al. 2007)
Piroxicam*	99.00	92.28 (±0.05)	(Chu 2009)
Salicylic acid*	99.00	75.44 (±0.08)	(Raevsky 2004)
Theophylline	98.00	98.24 (±0.01)	(Kansy, Senner et al. 1998)
Terbutaline*	80.00	84.25 (±0.01)	(Grès, Julian et al. 1998)

321 The asterisk (*) indicates the validation compounds.

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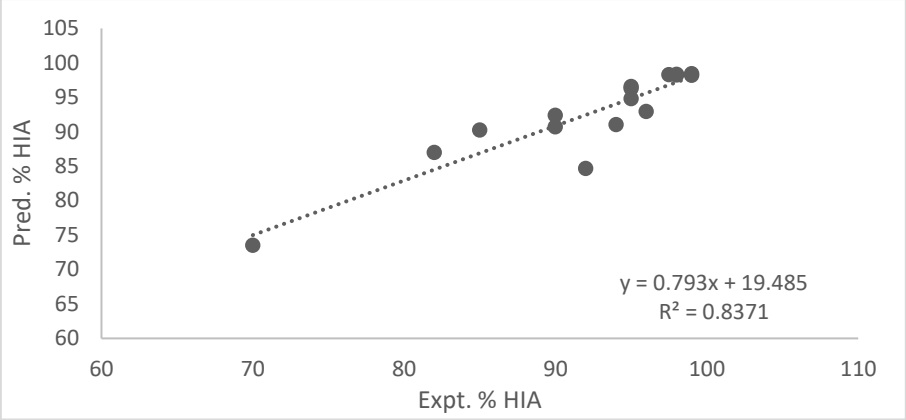

Figure 4: Plot of experimental vs. predicted %HIA.

4. Conclusion:

The change in the type of the stationary phase used in the MLC method from cyanopropyl to aminopropyl (APS) had a significant impact on the interaction of the analysed drugs with both the micellar mobile phase and the stationary phase used and consequently on their elution. This method was able to predict %HIA using a reliable model. This MLC method has one major advantage over that previously published with a different column in that it permits analysis of a greater number of compounds than that analysed prior to this study. This is because some compounds displayed non-binding behaviour in the previously published method (i.e. it was not possible to calculate log P_{mw} for these compounds) yet they displayed binding or antibinding behaviour using this method. This expanded study helps in the establishment of a reliable model for prediction of HIA from a wider dataset. It is hypothesised that by combining the physiologically relevant mixture of bile salts that displayed good HIA predictability with the column used in this study ($R^2_{pred.}$=81%) could create a mathematical model with an even higher predictive ability that could be developed based on a greater number of compounds in both the training and validation sets.

References:

Anslyn, E. V. and D. A. Dougherty (2006). Modern physical organic chemistry, University Science Books.

Arunyanart, M. and L.J.Cline-Love (1984). "Model of micellar effects on liquid chromatography capacity factors and for determination for micelle solute equilibrium constants". Anal. Chem. 56: 1557–1561.

Berthod, A. and C. Garcia-Alvarez-Coque (2000). Micellar liquid chromatography, CRC Press.

Castillo-Garit, J., Y. Cañizares-Carmenate, et al. (2014). "Prediction of ADME properties, part 1: classification models to predict Caco-2 cell permeability using atom-based bilinear indices." Afinidad 71(566): 129-138.

Chu, K. A. (2009). "Predicting Passive Intestinal Drug Absorption: An Interesting Relationship between Fraction Absorbed and Melting Point." The University of Arizona, Tucson, AZ;

Escuder-Gilabert, L., J. Martınez-Pla, et al. (2003). "Biopartitioning micellar separation methods: modelling drug absorption." Journal of Chromatography B 797(1): 21-35.

Escuder-Gilabert, L., M. Molero-Monfort, et al. (2004). "Potential of biopartitioning micellar chromatography as an in vitro technique for predicting drug penetration across the blood–brain barrier." Journal of Chromatography B 807(2): 193-201.

Gama, M. R., R. G. da Costa Silva, et al. (2012). "Hydrophilic interaction chromatography." TrAC Trends in Analytical Chemistry 37: 48-60.

Grès, M.-C., B. Julian, et al. (1998). "Correlation between oral drug absorption in humans, and apparent drug permeability in TC-7 cells, a human epithelial intestinal cell line: comparison with the parental Caco-2 cell line." Pharmaceutical research 15(5): 726-733.

Hou, T., J. Wang, et al. (2007). "ADME evaluation in drug discovery. 7. Prediction of oral absorption by correlation and classification." Journal of chemical information and modeling 47(1): 208-218.

http://www.chemspider.com/ (December, 2018).

Kalyankar, T., P. Kulkarni, et al. (2014). "Applications of Micellar Liquid Chromatography in Bioanalysis: A Review." Journal of Applied Pharmaceutical Science 4(1): 128-134.

Kanakaiah, B. (2013). "Micellar Liquid Chromatography." International Journal of Bio-Pharma Research 2(04): 117-122.

Kansy, M., F. Senner, et al. (1998). "Physicochemical high throughput screening: parallel artificial membrane permeation assay in the description of passive absorption processes." Journal of medicinal chemistry 41(7): 1007-1010.

Martın-Biosca, Y., M. Molero-Monfort, et al. (2003). "Rapid in vitro test to predict ocular tissue permeability based on biopartitioning micellar chromatography." European journal of pharmaceutical sciences 20(2): 209-216.

Milošević, N.P., S.Z. Stojanović, , K. Penov-Gaši, , N. Perišić-Janjić, R.Kaliszan (2014) "Reversed- and normal-phase liquid chromatography in quantitative structure retention-property relationships of newly synthesized seco-androstene derivatives." Journal of Pharmaceutical and Biomedical Analysis 88: 636-642.

Molero-Monfort, M., L. Escuder-Gilabert, et al. (2001). "Biopartitioning micellar chromatography: an in vitro technique for predicting human drug absorption." Journal of Chromatography B: Biomedical Sciences and Applications 753(2): 225-236.

Newby, D., A. A. Freitas, et al. (2015). "Decision trees to characterise the roles of permeability and solubility on the prediction of oral absorption." European Journal of Medicinal Chemistry 90: 751-765.

Norinder, U., T. Österberg, et al. (1999). "Theoretical calculation and prediction of intestinal absorption of drugs in humans using MolSurf parametrization and PLS statistics." European journal of pharmaceutical sciences 8(1): 49-56.

Olesen, N. E., P. Westh, et al. (2015). "Determination of thermodynamic potentials and the aggregation number for micelles with the mass-action model by isothermal titration calorimetry: A case study on bile salts." Journal of colloid and interface science 453: 79-89.

Olsen, B. A. (2001). "Hydrophilic interaction chromatography using amino and silica columns for the determination of polar pharmaceuticals and impurities." Journal of chromatography A 913(1-2): 113-122.

Paixão, P., L. F. Gouveia, et al. (2012). "Prediction of the human oral bioavailability by using in vitro and in silico drug related parameters in a physiologically based absorption model." International journal of pharmaceutics 429(1): 84-98.

Pramauro, E., C. Minero, et al. (1988). "Partition equilibria of phenols between water and anionic micelles: Correlation with the octanol/water system." Analytica Chimica Acta 212: 171-180.

Raevsky, O. A. (2004). "Physicochemical descriptors in property-based drug design." Mini reviews in medicinal chemistry 4(10): 1041-1052.

Raevsky, O. A., V. I. Fetisov, et al. (2000). "Quantitative Estimation of Drug Absorption in Humans for Passively Transported Compounds on the Basis of Their Physico-chemical Parameters." Quantitative Structure-Activity Relationships 19(4): 366-374.

Rambla-Alegre, M. (2012). "Basic principles of MLC." Chromatography Research International 2012: Article ID 898520.

Rambla-Alegre, M., S. Carda-Broch, et al. (2009). "Column classification and selection for the determination of antibiotics by micellar liquid chromatography." Journal of Liquid Chromatography & Related Technologies® 32(8): 1127-1140.

Shokry, D. S., L. J. Waters, et al. (2018). "Incorporating physiologically relevant mobile phases in micellar liquid chromatography for the prediction of human intestinal absorption." Biomedical Chromatography 32(12) 1-9.

Smith, D. A., H. Waterbeemd, D. K. Walker, et al. (2006). "Pharmacokinetics and metabolism in drug design." Methods and principles in medicinal chemistry 31: 8.

Takeuchi, T., J. Chu, et al. (1998). "Bile acids as stationary phase in liquid chromatography." Chromatographia 47(3-4): 183-188.

Varma, M. V., K. Sateesh, et al. (2005). "Functional role of P-glycoprotein in limiting intestinal absorption of drugs: contribution of passive permeability to P-glycoprotein mediated efflux transport." Molecular pharmaceutics 2(1): 12-21.

Veber, D. F., S. R. Johnson, et al. (2002). "Molecular properties that influence the oral bioavailability of drug candidates." Journal of medicinal chemistry 45(12): 2615-2623.

Waters, L. J., Y. Shahzad, et al. (2013). "Modelling skin permeability with micellar liquid chromatography." European Journal of Pharmaceutical Sciences 50(3): 335-340.

Waters, L. J., D. S. Shokry, et al. (2016). "Predicting human intestinal absorption in the presence of bile salt with micellar liquid chromatography." Biomedical Chromatography 30(10): 1618-1624.

Yan, A., Z. Wang, et al. (2008). "Prediction of human intestinal absorption by GA feature selection and support vector machine regression." International journal of molecular sciences 9(10): 1961-1976.

Zhao, Y. H., M. H. Abraham, et al. (2002). "Rate-limited steps of human oral absorption and QSAR studies." Pharmaceutical research 19(10): 1446-1457.