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PREDICTING CHROMATOGRAPHIC BEHAVIOR OF SEVERAL CHIRAL β-BLOCKERS FROM MOLECULAR STRUCTURE BY QSPR ANALYSIS

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ABSTRACT. The chiral HPLC separation parameters of a series of fourteen β-blockers previously performed on four polysaccharide-based chiral stationary phases (CSPs) was evaluated through computational techniques, using a set of 340 molecular descriptors (MD), calculated with the Molecular Operating Environment (MOE) software. Several semi-empirical mathematical models were built and refined by PLS, O2PLS multivariate data analysis and by PLS-Tree® clustering, correlating chromatographic data with the descriptors. The resulting models revealed the importance of certain analyze descriptors shaping chromatographic behaviour of the studied enantiomers. The chiral selector backbone as well as the presence of halogen atom(s) in the structure of the used stationary phase appears to exert an influence on the type of descriptors that significantly contribute either positively or negatively on the prediction power of the developed models. The influence of additive on the predictive power of models was also briefly analysed. This QSPR study generated models with a good predictive power. However, these results could be substantially improved in the future by including the descriptors for the chiral selectors and additives in the model and by performing docking studies.

Keywords: *Molecular descriptors, MOE, β-blockers, clustering, chiral HPLC*

INTRODUCTION

The development of methods for the separation of enantiomers is of significant importance in analytical chemistry, because of the identical nature of their physico-chemical properties and, at the same time, the highly stereospecific interaction behaviour with other chiral molecules, in specific conditions. In clinical

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practice it translates in differences in terms of biological activity, potency, toxicity. In analytical chemistry research, more specifically in direct chromatographic separations, it leads to the formation of distinct diastereomeric complexes between the chiral selector and each of the optical isomers. Researchers working on enantiomeric separations using chiral stationary phases (CSPs) are faced with the challenge of having to screen and select the most appropriate chiral selectors and understand whether the chiral discrimination can be indeed achievable. This is generally done by trial and error, resulting in a time consuming and expensive strategy [1]. In order to reduce costs and time, experiments have started to be coupled with attempts at rationalizing enantiospecific recognition by a chiral selector at a molecular level. In its review on separation mechanisms in stereoselective chromatographic and electromigration techniques, Scriba notes several factors that might be involved in the stereoselective interactions between enantiomers and chiral selectors: H-bonds and π–π interactions, fit or non-fit of the solute in a cavity or cleft of the selector, conformational changes of the selector during complex formation with the solute (induced-fit) [2].

A deeper understanding of the manner in which the solute and selector features correlate with the experimental outcomes can be achieved by building interaction and prediction models. A detailed discussion of the models of chiral separations can be found in the review by Lämmerhofer [3].

The approaches having been used so far to build such models are quantitative structure-property relationships (QSPR), quantitative structure-retention relationships (QSRR) and quantitative structure-activity relationships (QSAR). The idea of these computerized statistical chemometric techniques is to find a correlation between dependent variables, like chromatographic parameters and independent variables, which are various analyte or/and selector descriptors. These have been applied to predict retention for a new analyte and to identify unknown analytes, to investigate the molecular mechanism of separation in a chromatographic system and predicting retention factors [4-6], separation factors [7, 8] and resolution [9] or by performing docking studies [10]. Also, such models were used to quantitatively compare separation properties of different types of chromatographic columns, to evaluate properties like lipophilicity and dissociation constants, to estimate relative bioactivities within sets of drugs.

Aside from the mentioned applications, these chemometric techniques can be used for optimizing HPLC chiral separations by offering the possibility of rationalizing the selection of a chiral column with characteristics that can be provided by QSRR. Many papers are focused on the use of chirality descriptors in QSAR [11-13], some results being selected to be discussed in the following rows. Aires-de-Sousa and Gasteiger developed two different kinds of chirality codes named “conformation-independent chirality code” (CICC) and “conformation-dependent chirality code” (CDCC) to distinguish between enantiomers [14, 15], but when investigating further the efficiency along a more straightforward statistical technique [16], they did not satisfy. In order to generate more interpretable

results, Zhang et al. [17] introduced the Physicochemical Atomic Stereodescriptors (PAS) based on twenty-one physicochemical properties of the ligands attached to a chiral centre. Later, total and atom-level molecular descriptors relevant to QSAR/QSPR studies and 'rational' drug design were developed to define quadratic chiral indices for a molecule from its pseudograph considering either atom (vertex) adjacency [18-20] or bond (edge) adjacency [21]. Their drawback was caused by the use of 2D molecular information for their calculation. Their application was subsequently extended to consider 3D features of small to medium-sized molecules based on the trigonometric-3D-chirality-correction factor approach, using a new vector called the chirality molecular vector [22]. However, these descriptors and codes were not broadly used because of the complicated computational methods. Because of the vast number of molecular descriptors available, but which were not able to discriminate between enantiomers several research groups [23-27] developed chirality indices by applying correction factors to the topological indexes already existent.

A new class of chirality descriptors called Relative Chirality Index (RCI), calculated based on valence connectivity, the formula weights of the groups and the electrotopological state of the various atoms and groups in the four substituents at the asymmetric carbon were studied by Natarajan et al. [28]. They were found that RCI are not being applicable to large molecules. In 2012, five quantum chemical descriptors were applied in a study of Rasulev: HLG (gap between E_{HOMO} and E_{LUMO}), hardness (η, $\eta = E_{HOMO} - E_{LUMO}$=HLG), softness (σ, $\sigma = 1/\eta$), electronegativity (χ, $\chi = (E_{HOMO} + E_{LUMO}) / 2$), total energy ($E_{total}$) [29].

Nowadays, thousands of descriptors encoding the molecular structure features of analytes or selectors can be calculated by various software like Gaussian, Marvin Suite [30], DRAGON, CORINA, ADRIANA. Code, MOPAC, VolSurf, Molecular Operating Environment (MOE) [4, 8], PaDEL and many others.

Statistical analysis has been the most commonly used tool for interpreting correlations between experimental parameters and molecular descriptors. The most widely used model-building technique is multivariate data analysis. Aside from being most reported in the literature, multivariate statistical analysis and building semi-empirical models for prediction could contribute to the elucidation of the most significant chiral descriptors contributing to the separation of enantiomers. Various methods for data analysis exist today. Partial Least Square regression, also known as Projections to Latent Structures (PLS) has been used for over three decades. Several improvements have been introduced during this period of time; first, orthogonal PLS- OPLS [31] and then O2PLS [32, 33] were formulated, which are able to filter out variation that is not directly related to the response in various data analytical objectives related to classification, discrimination, regression or prediction. In contrast to PLS and OPLS, O2PLS is bidirectional and it is able to map how different types of variation in two datasets are connected, as well as to identify the unique information in each

dataset. The blocks X (chemical descriptors) and Y (chromatographic responses) represent two data matrices to be compared. X can be used to predict Y (as with PLS and OPLS), but at the same time Y can be used to predict X (unlike PLS and OPLS) [34]. With O2PLS one can model: the joint X-Y covariation, the Y-orthogonal variation in X and the X-orthogonal variation in Y.

To our knowledge there is no study already conducted on the entire set of β-blockers employed in the current analysis, dedicated to find correlations between their 3D structures derived molecular descriptors and the experimentally obtained chromatographic parameters. The present study aims to highlight some structural features, derived from significant molecular descriptors that are likely to be responsible for the observed enantio-selectivity and could possibly contribute to understanding the molecular characteristics that are involved in the chiral separation mechanism.

RESULTS AND DISCUSSIONS

Statistical analysis

MOE calculates 340 descriptors from three classes - 2D descriptors, i3D and x3D, internal and external 3D descriptors [35]. After the screening process and the calculation of molecular descriptors, the available data was compiled and analysed statistically.

Data analysis of MD and chromatographic parameters by PCA and OPLS/O2PLS

The available X- and Y-block variables, described above, were subjected to O2PLS analysis.

Multivariate data analysis began on the entire data set (all observations recorded on 4 columns with 3 additives, N= 336) with a principal component analysis (PCA). Standard scaling and mean-centering (centred and scaled to unit variance) was performed in the data pre-treatment step. Pre-treatment using PCA of the X-data gave an eighteen-component model, which explained 96.4% of the variation (R2X=0.964).The score scatter plot of the PCA-X model (data not shown), as in the case of the subsequent PLS models, indicated significant structural and chromatographic differences between R- and S- carvedilol and the rest of the enantiomers. As an additional exploratory data analysis hierarchical cluster analysis (HCA) and PLS-Tree®[33] was also performed for identifying more subtle clustering within the set of variables (X-data block) or observations (both X- and Y-block) that eventually might offer better models for parts of the data. As a next step multivariate regression analysis by PLS modelling on the entire mean centred and scaled data set has been carried out. Both, PLS

and O2PLS regression on the entire data set provided models with relatively modest predictive ability (PLS - Q2cum = 0.435; O2PLS - Q2cum = 0.454), where one of the possible reasons might be the absence of molecular descriptors related to the four different CSPs in the chemometric model. Therefore, the observations were grouped in four classes, according to the nature of the CSP. The corresponding O2PLS models presented significantly improved prediction abilities, where the cross-validated variances were Q2(cum) = 0.606 for column IA, Q2(cum) = 0.61 for column IB, Q2(cum) = 0.837 for column IC, Q2(cum)= 0.851 for column ID.

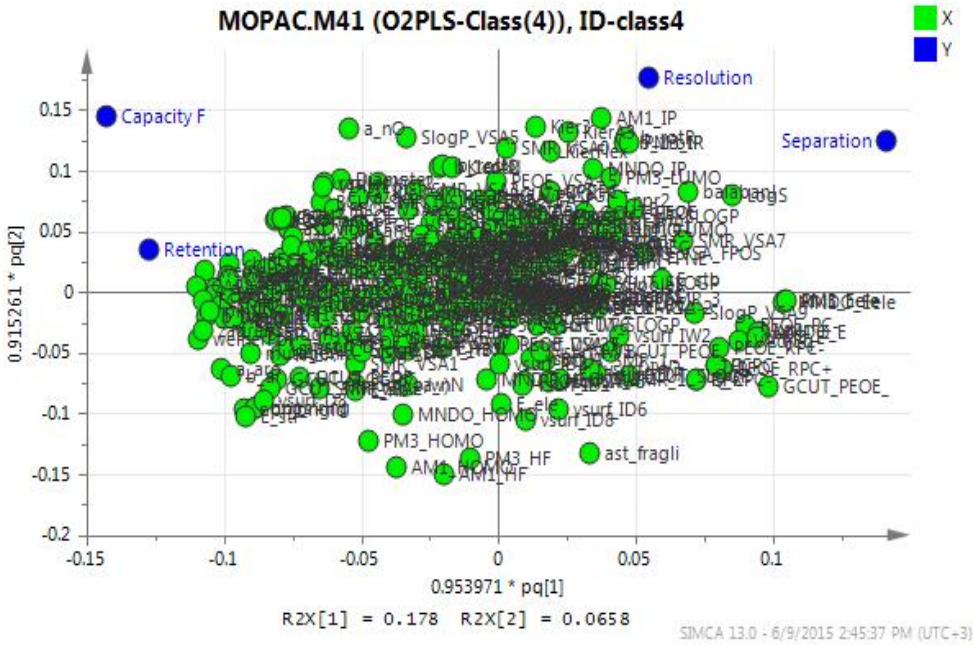

Figure 1. 2D loading scatter plot in function of the first two principal components of the O2PLS model (separated on ID column)

The loading scatter plot (Figure 1) indicates the relationship between all factors (X) and responses (Y), and how molecular descriptors correlate to each of the responses of interest (chromatographic parameters). Interpreting a model with so many components based on the scatter plots may be cumbersome; therefore a more appropriate approach was the examination of the Variable Importance in the Projection (VIP) plot, that summarizes the importance of the variables both to explain X and to correlate to Y. Usually VIP values larger than 1 indicate significant X-variables and values below 0.5 are considered to lack significance. Additionally, to correct for data skewness and to improve the efficiency of data analysis, further pre-processing (i.e. log-transformation)

of the Y-data (α, k', t_R) was performed. In order to conveniently compare the performance in terms of predictive power amongst the generated models, the Root Mean Square Error from cross-validation (RMSEcv) was calculated (data not shown). It should be noted that, with and without data pre-processing, the best predictive power is obtained in case of the separation factor, whereas the retention time seems to be modelled the poorest. Nevertheless, log-transformation of α, k' and t_R does offer improvements in the case of columns IA and IB, with a slight increase of the cross-validation residuals in the case of columns IC and ID. Because of the improvements offered by logarithmic transformation of chromatographic parameters, only data obtained with log-transform will be further considered.

Model improvement through external validation approach

External validation approach was also tested, dividing the observations into two sub-sets, where the training set represented the initial set of observations out of which every 5th observation was removed. The arbitrary sampling of the validation set was meant to additionally prove the reliability of the O2PLS model, where in principle from the systematic variability the part uncorrelated (orthogonal) to Y has already been removed. The resulting set of complementary observations (N = 5-6), grouped by the type of additive and used column, represented the validation set on which predictions of the chromatographic data were performed.

The measure of predictive ability, expressed as the Root Mean Square Error of Prediction (RMSEP), representing the fitted residuals for the observations from the validation set (Table 1), may serve for the comparison between the predictive powers of the models generated from the training sets, as well as for the validation of the entire dataset. Satisfactory predictive capability, which has been obtained in almost every case of the orthogonal PLS modelling, was further improved by the log-transformation of three of the chromatographic parameters (α, k' and t_R).By comparing the individual measure of predictive power (either RMSEcv or RMSEP) for a given model, one can observe that the lowest values, thus the best predictive ability, is obtained in case of the separation (α) and capacity (k') factors.

New OPLS models have been developed for each type of CSP and additive, subjecting them to an external validation procedure using the same validation set mentioned above (every 5th analysis of the entire dataset). The quality and improved predictive ability of the resulting models, as well as the ruggedness and validity of the dataset, was proven by the calculated statistical parameters (i.e. R^2Y, Q^2Y, and RMSEP) as well as by the predicted values of retention time for the enantiomers of the validation set.

In spite of the relatively limited number of observations in the calibration set the predicted retention time values in many cases are quite close to the observed ones. Nevertheless, in some cases relatively high bias between the two values is recorded, indicating the need for further improvement.

Table 1. Measure of predictive power related to the validation set of the generated O2PLS models from the training sets with pre-processing of Y-variables

Column	Additive	RMSEP			
		A	k'	t_R	R_S
IA	EA	0.0562	0.6310	1.4545	0.7216
	EDA	0.1055	0.6664	2.1430	0.8403
	DEA	1213.31	0.8300	3.5738	10.719
IB	EA	0.1189	0.7266	1.9881	0.6274
	EDA	0.2126	1.8689	6.4567	1.9854
	DEA	0.0823	1.6827	2.1845	0.5373
IC	EA	0.1848	0.6197	2.0120	2.4205
	EDA	0.7623	1.3828	3.1781	510.571
	DEA	0.1158	1.0064	2.5615	1.7668
ID	EA	0.2559	0.4868	4.8475	1.1110
	EDA	0.1156	0.6293	1.7575	0.8572
	DEA	0.4250	0.5786	6.1821	2.8185

Model improvement by cluster analysis

Because the above chemometric models do not include molecular descriptors weighing the structural and physicochemical particularities of the CSPs, and neither of the tested basic additives, additional exploratory data analysis has been performed on the available data set for the identification of a more subtle data clustering that might have been overlooked by PCA and which would enable the generation of daughter training sets that eventually could lead to an improved modelling of the chromatographic behaviour.

Among the two available clustering tools HCA (bottom-up approach) and PLS-Tree® (top-down approach), the later was preferred, since it accounts also for the Y-variables (chromatographic parameters) and offers cleaner dendrograms (classification trees). As algorithm parameters in the assessment of score value split points A = 0.1 and B = 0.3 were selected, with a maximum depth of 4 of the PLS-Tree. Running the PLS-Tree clustering on either the entire data set (all 4 columns and all 4 additives) or only on data sub-sets defined by the afore mentioned qualitative variables (type of column, type of basic additive) two main clusters of β-blockers (Figure 2) could be distinguished: group 1) containing oxprenolol, metoprolol, alprenolol, propranolol, pindolol and group 2) the remaining β-blocker representatives. When working on the IB and IC data subsets, esmolol, betaxolol and carazolol were also assigned into the first group. Due to its particular structure carvedilol represents a moderate outlier in several models; however, to build a predictive model that applies to the entire class of β-blocker drugs, it has been kept within the observations.

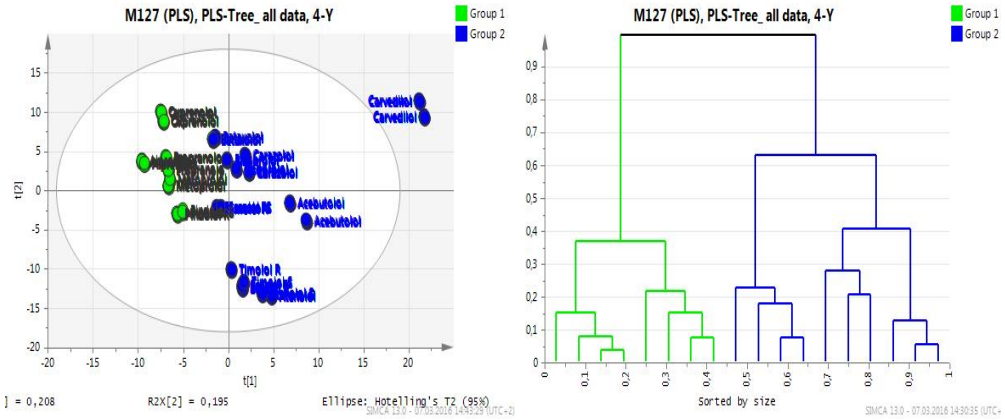

Figure 2. The two main clusters of b-blockers (left) and the corresponding dendrogram (right) by PLS-Tree® clustering on the entire data set

The two main observed clusters were used to create O2PLS class models for each individual column. As it turned out, the predictive ability (Q2cum) and RMSEP values (Table I and II) of the resulting models were far superior to those obtained before clustering of observations (β-blockers), therefore once again for each cluster a validation set has been assigned (every 5th observation), based on which retention time values predictions were performed. If in the case of columns IA and IB no consistent improvements in the prediction of retention time is observed (data not shown), in the case of IC and ID columns the prediction errors are significantly lower. In the case of IC and ID columns the influence of the mobile phase additives (EA, DEA, EDA) on the enantioseparation is almost negligible (around 62% on IC and 69% on ID of the 14 studied herein β-blockers were baseline or partially separated in the case of all the three basic additives employed), in comparison with their influence of the enantioselectivity using columns IA and IB (out of the 14 β-blockers 56% using EA, 25% using DEA, 50% using EDA on the IA column, and 56% using EA, 56% using DEA, 44% using EDA on the IB column, respectively were baseline or partially separated) The experimental results are taken from the study of Moldovan et al. [36].

For each column, the clustered data set was once again split according to the employed basic additive and O2PLS class models were once again generated. The overall predictive power (mean value of Q2Y and RMSEcv) for each pair of column - basic additive is significantly improved in comparison with the models obtained on the data sets that were not subjected to PLS-Tree® clustering (data not shown).

Table 2. Measure of predictive power related to the validation set of the generated PLS models upon PLS-Tree clustering

Column	Cluster	RMSEP			
		A	k'	t_R	R_S
IA	1(OXP, PRN et.)	0.1152	0.2816	0.8125	0.9989
	2	0.0883	1.0001	2.7539	1.3652
IB	1	0.2021	0.5987	1.9030	2.0035
	2	0.0361	2.5970	3.7774	0.3508
IC	1	0.0356	0.0932	0.2843	0.6766
	2	0.0127	0.3511	0.9316	0.2059
ID	1	0.1399	0.0375	0.1344	0.9984
	2	0.1108	0.3817	3.6494	0.8716

Unfortunately, a further splitting of the data sets into validation sets significantly reduces the number of observations making up the training sets in many cases less than 5 observations, which is not able to further provide any real improvements in their prediction ability. However, as mentioned earlier, extending the range of accounted variables with molecular descriptors of the employed CSPs and mobile phase modifiers, a stronger correlation structure between the nature of selector and selectand (ligand) may be identified, able to account for more intimate structural complementarities and particularities.

Relationship between retention times and significant MDs

The loading scatter plots convey information about the descriptors that are influential in the modelling of chromatographic parameters on columns IA, IB, IC and ID and how they are correlated. Among the descriptors showing a significant contribution to every predictive model are to be mentioned the surface area, volume and shape descriptors, number of heavy atoms, the sum of hydrogen bond donors and acceptors, the number of O and N atoms in the molecule, the entropy of element distribution, the van der Waals surface area and volume, molecular weight, molecular refractivity and polarizability, total polar surface area.

Columns IA and ID are based on amylose, while IB and IC are based on cellulose derivatives. Moreover, column IA and IB only differ on the polysaccharide type backbone, but the substituent is the same, 3,5-dimethylphenylcarbamate. The particularities induced by the increasing number of halogen atoms on the substituents of Columns IC and ID (3,5-diclorophenylcarbamate on IC and

3-cloropehnylcarbamate on ID) may also be revealed. Without considering whether the correlation is positive or negative, two classes of descriptors exhibit by far the strongest contributions: the class of 3D descriptors that depend on surface area, volume and shape and the class of 2D partial charge descriptors. The first one registered contribution scores of 21.08% on Chiralpak IA, 32.62% on Chiralpak IB, 30.68% on Chiralpak IC and 23.51% on Chiralpak ID. Correlating this information with the structures of chiral selectors, strong similarities in the percentage of contribution scores and the nature of polymeric backbone is to be identified (~20% on amylose and ~30% on cellulose based CSP, respectively). The second class of descriptors showed a greater contribution for columns IA and IC and slightly lower, but still significant for IB and ID. Medium contributions were observed for the classes of adjacency and distance matrix and atom count descriptors.

Partial charge descriptors and surface area, volume and shape descriptors were the two classes of MD that exhibited the highest positive correlation to the chromatographic parameters. However, in case of the partial charge type, contribution can be seen only in case of columns IA and IB, while for IC and ID there is no visible contribution to the model. Columns IA and IB have distinct polymer backbone, but the same substituent, which could be accountable for the behaviour.

A preferential behaviour of descriptors is also observed in case of MOPAC MDs, which for columns with cellulose-based polymer backbone correlate positively with the chromatographic parameters, while for amylose-based columns the correlation is insignificant. From the distribution of contribution scores on each of the two cellulose-based columns, it results that on IC the heat of formation and total potential energy, which have values of over 1,5, are highly significant for the prediction model. For IB column, the highest contribution is exhibited by MNDO_dipole (~1). The difference between the two columns is the presence on column IC of two halogen atoms, suggesting that these could be responsible for the particular behaviour. While in case of column IC the total potential energy is a descriptor that significantly correlates in a positive way, the same descriptor, for the other columns, correlates negatively with the chromatographic parameters, registering values of contribution scores of over 2, rendering it extremely significant for the predictive model. In case of column IC the MOPAC descriptors that contribute negatively are the dipole moment and ionization potential. In case of columns IA, IB and ID the class of molecular descriptors with the strongest negative correlation with the chromatographic parameters was the partial charge descriptors, which contribute to the model 9.9%, for IA, 15.26% for IB and 17.53% for ID. For column IC the contribution is significant, 22.99%, but its highest contribution is exhibited by the class of surface area, shape and volume 3D descriptors, with 30.68% of the entire model, compared to the other three columns which register values between 3% and 7%. The surface area, shape and volume descriptors depend on structure connectivity and conformation. Our study revealed a particular behaviour in terms of the negative correlation with chromatographic parameters for column

IC, with regard to most classes of molecular descriptors. The only occurrence of lower negative correlation compared to the other three columns was seen for connectivity index descriptors, where column ID registered a percentage of 5.97, while for column IC it was 1.87 and the other two 0%.

CONCLUSIONS

Several approaches were tested in order to obtain useful information from the data obtained after descriptor calculation with MOE. The models obtained were tested and further refined by splitting the observations into calibration and validation sets. In the case of the external validation set, relatively close values of predicted retention times were obtained compared to the experimental ones. After PLS-Tree®, two main clusters of b-blockers were distinguished: the 1st containing oxprenolol, metoprolol, alprenolol, propranolol, pindolol and the 2nd group containing the remaining β-blocker representatives. Two classes of descriptors exhibit by far the strongest contributions: the class of 3D descriptors that depend on surface area, volume and shape and the class of 2D partial charge descriptors.A preferential behaviour of descriptors is also observed in case of MOPAC MDs, which for columns with cellulose-based polymer backbone correlate positively with the chromatographic parameters, while for amylose-based columns the correlation is insignificant.

Descriptors that exhibited significant contributions to the models, either positive or negative were H-bond donor and acceptor atoms, total potential energy, heat of formation, ionization potential, van der Waals surface area and volume, molecular weight, molecular refractivity and polarizability, total polar surface area.

It is estimated that the predictive power of the models could be further improved by expanding the number of observations on the entire range of existing β-blockers as well as by feeding into the model 3D molecular descriptors derived from the used CSPs. Developing models able to correlate molecular descriptors of CSPs and of various chiral molecules of interest with their corresponding chromatographic parameters could rationalize the selection procedure of CSP during method development as well as further contribute towards the elucidation of the molecular interactions responsible for chiral separation.

EXPERIMENTAL SECTION

HPLC experiments

All HPLC experiments were performed as described by Moldovan et al. [36]. The chromatographic behaviour (retention times, separation factors, capacity factors, resolutions) of the racemic mixtures of 14 β- blockers was studied by

gradient elution HPLC [36], with a mobile phase made up of 2-Propanol/n-Hexane with their ratio varying from 80/20 to 50/50 (v/v)) with 0.1% (v/v) additive (EA, DEA, EDA) added to 2-Propanol.

Molecular Modeling and Geometry Optimization

The 2D chemical structures of the β- blockers (14 molecules with one stereogenic centre) were downloaded from ChemicalBook, the chemistry of the structures was verified and then they were cleaned in 3D, using MarvinView Chemaxon). The chirality at the stereogenic centres was verified by applying the Cahn-Ingold-Prelog priority rules. Then the structures were preoptimized using MOPAC2012, by PM6 method. The resulting geometries were further refined by means of low mode dynamics (LMD) conformational search using the standard settings in MOE and MMFF94x force field to enforce low energy conformations of the molecules. The lowest energy conformer of all the compounds was transferred to database viewer and different classes of 2D and 3D descriptors were calculated.

Generation of Descriptors

A total of 340 2D and 3D descriptors were calculated for each conformation of each enantiomer with MOE and then the values corresponding to the conformation with the lowest energy were chosen. Calculations were performed with MOE (Molecular Operating Environment, v. 2014.09 on an Intel ® Core(TM) i3-4005U CPU @ 1.7 GHz personal computer with 12 GB of RAM running under Microsoft Windows 8.1). These data served as a basis for a further statistical analysis.

Statistical Analysis

The obtained chromatographic data and the calculated molecular descriptors were subjected to orthogonal partial least-squares (O2PLS) multivariate analysis using Simca-p+ v.13 software (MKS Data Analytics Solution, Sweden). The variables (K=340) building the X-block of data represented the molecular descriptors generated by MOE, derived from MOPAC calculation output and 2 qualitative variables/class identifiers (nature of column and type of additive), whereas the Y-block of data was made up by the chromatographic parameters (separation factor (α), retention time (t_r), capacity factor (k') and resolution (R_s)).

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