BUILDING QSRR MODEL FOR RP/WCX HPLC METHOD DEVELOPMNET

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Reverse-phase high-performance liquid chromatography (RP-HPLC) is most common in pharmaceutical analysis and requires significant consumption of toxic mobile phase. Therefore, more eco-friendly solutions are preferred (1). When it comes to complex samples, RP-HPLC, due to inability to separate highly polar and charged analytes, often requires multiple unimodal or two-dimensional HPLC analyzes. The development of mixed-mode liquid chromatography (MMLC), where multiple separation modalities are incorporated into a single stationary phase, allows the separation of complex samples in a single run. Numerous factors affect MMLC separation, which makes method development demanding and limits their practical application (2). Building predictive mathematical models, such as Quantitative structure-retention relationship (QSRR), could improve method development. QSRR links the molecules' retention behavior with their physicochemical properties (molecular descriptors (MD)), which allows retention behavior prediction of untested analytes. Including experimental parameter values in the QSRR extends the predictability of the model to entire experimental space (3). For model development purposes, experiments were performed on Thermo's Acclaim Mixed-Mode WCX-1 (3 µm, 2.1 x 150 mm) column which combines hydrophobic and weak cation exchange (WCX) interactions. Small diameter column agrees with low mobile phase flow rate (400 µl/min). Mobile phase composition (acetonitrile content (30 – 50 % (v/v)), pH (3.8 - 5.6) and ionic strength (20 - 40 mM) of acetic buffer) and column temperature (30 – 38 °C) were varied according to face-centered central composite design. Retention factor of 33 pharmaceuticals of different pharmacological and ionization properties were monitored. MDs were calculated using AlvaDesc software. RapidMiner software was used for obtaining QSRR models. Several machine learning algorithms were considered and the most informative (gradient boosted trees (GBT) and bagging neural network (BNN)) were selected. Models were built upon data of 30 analytes, and the remaining three (anion, cation, neutral) were used as a test set. The most influential MDs for BNN were chosen by forward selection, contrary to GBT which did not require preselection. For internal model evaluation 10-fold cross-validation was applied, while external was performed with a test set. Models were compared based on the relative mean square error (RMSE) of the test set. The BNN (RMSE = 0.104; R^2 = 0.976) model outperformed GBT (RMSE = 0.122; R^2 = 0.963). The obtained QSRR models showed good potential to predict the retention behavior of molecules of different ionization abilities in the RP/WCX system. This could improve the development of MMLC methods and make them more accessible for practical use.

References

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IZGRADNJA QSRR MODELA ZA RAZVOJ RP/WCX HPLC METODE

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U farmaceutskim analizama najzastupljenija je reverzno-fazna tečna hromatografija visokih performansi (*reverse-phase high-performance liquid chromatography* (RP-HPLC)) koja iziskuje značajnu potrošnju mobilne faze toksične prirode. Iz tog razloga, teži se ekološki prihvatljivijim rešenjima (1). Kada su u pitanju kompleksne smeše uzorka, RP-HPLC zbog nemogućnosti sparacije visoko polarnih i naelektrisanih analita, često zahteva više unimodalnih ili dvodimenzionalne HPLC analize. Razvoj multimodalne tečne hromatografije (*mixed-mode liquid chromatography* (MMLC)) koja podrazumeva više separacionih modaliteta inkorporiranih u jednu stacionarnu fazu, omogućava razdvajanje složenih uzorka jedinstvenom analizom. Brojni faktori utiču na MMLC separaciju, što razvoj metoda čini zahtevnim i ograničava im praktičnu primenu (2). Izgradnja prediktivnih matematičkih modela, kao što su modeli kvantitativnog odnos strukture i retencionog ponašanja (*Quantitative structure-retention relationship* (QSRR)), može ubrzati razvoj metode. QSRR povezuje fizičko-hemijska svojstva (molekulski deskriptori (MD)) sa retencionim ponašanjem molekula, što omogućava predviđanje retencionog ponašanja neispitanih analita. Uključivanje vrednosti eksperimentalnih parametara u QSRR, proširuje prediktivnost modela na ceo eksperimentalni prostor (3). Podaci o retencionom ponašanju za potrebe razvoja QSRR modela, dobijeni su upotrebom *Thermo Acclaim Mixed-Mode WCX-1* (3 μm; 2,1x150 mm) kolone koja uključuje hirdrofobne i interakcije slabe katjonske izmene (*weak cation exchange* (WCX)). Malim prečnikom kolone omogućen je nizak protok i utrošak mobilne faze (400 μl/min). Sastav mobilne faze (udeo ACN (30 – 50 % (v/v)), pH (3,8 – 5,6) i jonska jačina (20 – 40 mM) acetatnog pufera) i temperatura kolone (30 – 38 °C) menjani su u skladu sa centralnim kompozicionim dizajnom – ka centru orijentisanim. Praćen je retencioni faktor 33 farmaceutska jedinjenja različitih farmakoloških i jonizacionih osobina. MD su računati AlvaDesc softverom. Za izgradnju QSRR modela RapidMiner softverom razmatrana je nekolicina algoritama mašinskog učenja, a odabrani su najinformativniji (*gradient boosted trees* (GBT) i *bagging neural networks* (BNN)). Modeli su građeni na osnovu podataka za 30 analita, dok su preostala tri (anjon, katjon, neutralni) odabrani za test set. Selekcijom unapred odabrani su najznačajniji MD za izgradnju BNN, za razliku od GBT koji ne zahteva preselekciju MD. Interna procena modela vršena je desetostrukom unakrsnom validacijom (*10-fold cross-validation*), dok je eksterna vršena test setom podataka. Modeli su upoređeni na osnovu relativne srednje kvadratne greške (RMSE) test seta. BNN (RMSE = 0,104; R^2 = 0,976) se pokazao boljim u poređenju sa GBT (RMSE = 0,112; R^2 = 0,963). Dobijeni QSRR modeli pokazali su dobru sposobnost predviđanja retencionog ponašanja molekula različitih jonizacionih sposobnosti u RP/WCX sistemu. Tako bi mogao da se unapredi razvoj MMLC metoda i učini ih pristupačnijim za praktičnu upotrebu.

Literatura

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