MODELING RETENTION BEHAVIOUR OF IMIDAZOLINE AND SEROTONIN RECEPTOR LIGANDS UNDER CONDITIONS OF GREEN SFC TECHIQUE

Darija Obradović[1], Andrey Nikolaevich Stavrianidi[2],
Oleg Alekseevich Shpigun[2], Danica Agbaba[1]

[1] University of Belgrade- Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Belgrade, Serbia
[2] University M.V. Lomonosov - Faculty of Chemistry, Department of Analytical Chemistry, Moskow State Moskow, Russia

A possibility of predicting retention behaviour without a large number of preliminary experiments is a significant segment of theoretical and experimental investigations. By using an appropriate design to define experimental conditions (Design of Experiments, DoE), the retention behaviour of compounds can be described as a function of the most important parameters of chromatographic system. Further, the DoE methodology has shown successful applicability to modeling retention under the environmentally friendly Supercritical Fluid Chromatography (SFC) conditions (1). In the last decades, robustness of the SFC instruments has been improved in order to minimize ecological risks, and routine application of the SFC technique has been introduced into many pharmaceutical strategies (e.g., the Good Manufacturing Practice, GMP) (2). Therefore, the aim of our study was to define the influence of the main chromatographic factors on the retention behaviour of fourteen imidazoline and serotonin receptor ligands under the SFC conditions. Using the Central Composite Design (CCD) approach, retention characteristics (k) of the test compounds were examined on the mixed-mode stationary phase, with the mixture of supercritical CO_2 and methanolic ammonium-formate (with an addition of 0.1% formic acid) used as mobile phase. We took into the consideration the influence of the following factors: volume fraction of methanol in mobile phase (20-30 %), ammonium-formate concentration (15-25 mM), and temperature deviation (20-30 °C). The most important chromatographic factors were selected by the step-wise multilinear regression (MLR), and their statistical significance was assessed using the ANOVA analysis. Based on the results obtained, it was established that the retention characteristics were significantly influenced by changing the methanol and ammonium-formate concentrations in mobile phase ($r > 0.90$, $p < 0.05$), Figure 1. High degree of agreement ($r > 0.98$) was observed, when the theoretically predicted logk values for 35% and 15% volume fraction of methanol in mobile phase were compared with the experimental ones. The obtained results confirm successful applicability of the experimental design methodology in order to perform a minimum number of experiments, as demonstrated upon an example of modeling and predicting the retention behaviour of imidazoline and serotonin receptor ligands under the SFC conditions.
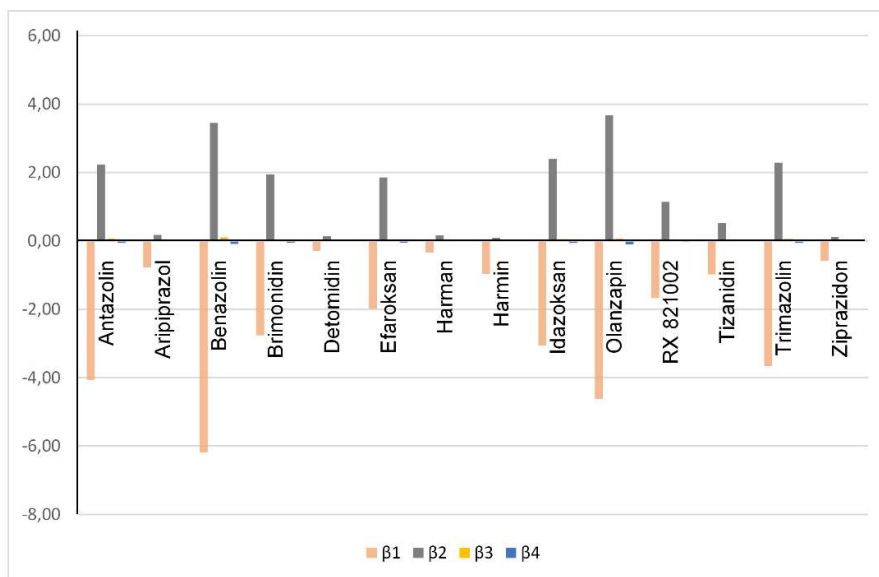
Figure 1. The influence of methanol fraction in mobile phase (β_1), concentration of ammonium-formate (β_2), and their quadratic values (β_3, β_4) on the retention behaviour of imidazoline and serotonin receptor ligands.

References

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2. Hicks MB, Regalado EL, Tan F, Gong X, Welch CJ. Supercritical fluid chromatography for GMP analysis in support of pharmaceutical development and manufacturing activities. J. Pharm. Biomed. Anal. 2016; 117: 316-324.
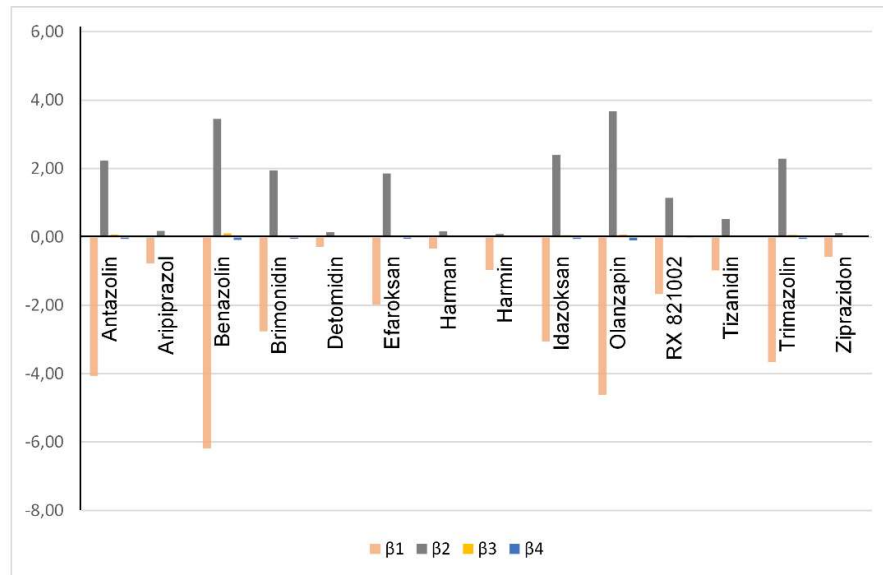
Acknowledgements

MODELOVANJE RETENCIONOG PONAŠANJA LIGANADA IMIDAZOLINSKIH I SEROTONINSKIH RECEPTORA U USLOVIMA EKOLOŠKI BEZBEDNE SFC TEHNIKE

Darija Obradović[1], Andrey Nikolaevich Stavrianidi[2], Oleg Alekseevich Shpigun[2], Danica Agbaba[1]

1 Univerzitet u Beogradu - Farmaceutski fakultet, Katedra za Farmaceutsku hemiju, Beograd, Srbija
2 Moskovski državnog univerzitet M.V. Lomonosov - Hemijski fakultet, Katedra za analitičku hemiju, Moskva, Rusija

Mogućnost predviđanja retencije u odsustvu velikog broja prethodnih eksperimenata, značajan je segment teorijskih i eksperimentalnih ispitivanja. Odgovarajućim dizajnom eksperimentalnih uslova (*Design of Experiments*, DoE), a na osnovu minimalnog broja eksperimenata, može se definisati retenciono ponašanje jedinjenja u funkciji najznačajnijih parametara hromatografskog sistema. Primena DoE metodologije je takođe zabeležena i prilikom modelovanja retencije u uslovima ekološki bezbedne, superkritične fluidne hromatografije (*Supercritical Fluid Chromatography*, SFC) (1). Zbog minimalnih ekoloških rizika, robusnost SFC instrumenata je poboljšana, a rutinska primena SFC tehnike je uvedena i u regulatorne farmaceutske propise (npr. dobra proizvođačka praksa; *Good Manufacturing Practice*-GMP) (2). Na osnovu toga, cilj ovog istraživanja obuhvatio je definisanje uticaja najznačajnijih faktora hromatografskog sistema na retenciono ponašanje 14 odabranih liganada imidazolinskih i serotoninskih receptora na *mixed-mode* stacionarnoj fazi u SFC uslovima. Uticaj zapreminskog udela metanola (20-30 %), koncentracije amonijum-formijata (15-25 mM) i temperature (20-30 °C) na vrednosti retencionih faktora (k) odabranih liganada imidazolinskih i serotoninskih receptora je ispitana primenom centralnog kompozitnog dizajna (*Central Composite Design*, CCD) na *mixed-mode* stacionarnoj fazi. Kao mobilna faza korišćena je smeša superkritičnog CO_2 i metanolnog rastvora amonijum-formijata uz dodatak 0,1% mravlje kiseline. Najznačajniji hromatografski faktori su izdvojeni *step-wise* postupkom u višestrukoj linearnoj regresionoj analizi (*Multiple Linear Regression,* MLR), a njihova statistička značajnost je procenjena primenom ANOVA testa. U konstruisanim retencionim modelima, zapremina metanola i koncentracija pufera su pokazale najveći uticaj na retenciono ponašanje testiranih jedinjenja ($r > 0,90$; $p < 0,05$), Slika 1. U koliko se teorijski predviđene log$k$ vrednosti uporede sa eksperimentalno dobijenim vrednostima na 35% i 15% zapreminskim udelima metanola u mobilnoj fazi, uočava se da je prisutan visok stepen slaganja ($r > 0,98$). Dobijeni rezultati potvrđuju uspešnu primenljivost metodologije eksperimentalnog dizajna u cilju izvođenja minimalnog broja eksperimenata, prilikom modelovanja i predikcije retencionog ponašanja liganda imidazolinskih i serotoninskih receptora u SFC sistemima.

Slika 1. Uticaj zapreminskog udela metanola (β_1), koncentracije pufera (β_2) i njihovih kvadratnih vrednosti (β_3, β_4) na retenciono ponašanje liganada imidazolinskih i serotoninskih receptora.

Literatura

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Zahvalnica