INFLUENCE OF CHARGED AEROSOL DETECTOR PARAMETERS ON THE RESPONSE OF CHOSEN ANALYTES IN THE MIXED-MODE CHROMATOGRAPHY SYSTEM

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With respect to significant market presence (50%), the analysis of pharmaceuticals containing counterions is a crucial component of the drug development life cycle, quality control and lot release processes (1). Mixed-Mode Chromatography (MMC) offers the ability to simultaneously separate cationic and anionic species within a single run, streamlining the laboratory processes (2). However, the detection of typical counterions leaves analysts little room for maneuver. In recent years, the counterions are efficiently detected with the help of Charged Aerosol Detector (CAD) that generates a signal independent of the chemical structure (3). In this regard, the use of MMC-CAD hyphenated technique rationalizes the number of individual analytical activities required for analyte and counterion testing, causes no resource depletion and ultimately supports the concept of sustainability in contemporary drug analysis. In this paper, the influence of the CAD parameters on the response of diclofenac potassium and tramadol hydrochloride was studied performing systematic variations. The analyses were carried out on Acclaim™ Mixed-mode WCX-1 (2.1 x 150 nm, 3 μm) column that provides both hydrophobic reversed-phase and weak cation-exchange properties. Satisfactory separation of the active pharmaceutical ingredients and their counterions was achieved using a mixture of 90 mM acetate buffer at pH 5 (A) and acetonitrile (B). The isocratic elution (40% B) was performed at a flow rate of 0.4 mL/min. Based on preliminary experiments, the following variables were identified as significant and, thus, tested at the listed values: evaporation temperature (35ºC and 50ºC), filter constant (1 s, 5 s and 10 s) and power function value (0.8, 1.0 and 1.2). The results showed that the evaporation temperature had a positive impact on the signal-to-noise (S/N) ratios. On the other hand, the peak area and the peak height decreased significantly upon raising the temperature. This finding pointed out a strong need for fine tuning of mentioned parameter with respect to the analytes' volatility. The increase in the value of the filter constant led to a baseline smoothing as well as peak broadening. The filter constant set at 5 s resulted in the largest S/N ratios. The power function value, which directly modified the CAD signal, exhibited an obvious negative effect toward the peak area. In light of the above, optimal CAD detection of diclofenac, tramadol, potassium and chlorine was achieved at the following settings: filter constant 5 s, power function value 0.8 and evaporation temperature 35ºC.

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UTICAJ PARAMETARA DETEKTORA NAELEKTIRSANJA U AEROSOLU NA ODGOVORE ODABRANIH ANALITA U SISTEMU MULTIMODALNE HROMATOGRAFIJE

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S obzirom na veliku tržišnu zastupljenost farmaceutskih proizvoda koji sadrže kontra-jone (50%), analiza istih ključna je komponenta procesa razvoja leka, kontrole kvaliteta i puštanje serije leka u promet (1). Multimodalna hromatografija (eng. *Mixed-Mode Chromatography*, MMC) nudi mogućnost istovremene separacije katjonskih i anjonskih vrsta na jednoj koloni, pojednostavljujući time laboratorijske procese (2). Međutim, izbor tehnika za detekciju tipičnih kontra-jona veoma je sužen. Poslednjih godina, kontra-joni efikasno se detektuju uz pomoć detektora naelektrisanja u aerosolu (eng. *Charged Aerosol Detector*, CAD) koji generiše signal nezavisan od hemijske strukture (3). S navedenim u vezi, smatra se da upotreba MMC-CAD tehnike racionalizuje broj pojedinačnih analitičkih aktivnosti potrebnih za kontrolu kvaliteta analita i kontra-jona, smanjuje iscrpljivanje resursa i time podržava koncept održivosti u domenu savremene analitike lekova. U ovom radu proučavan je uticaj sistemskog variranja parametara CAD detektora na odgovor diklofenak-kalijuma i tramadol-hidrohlorida. Hromatografkse analize izvedene su na *Acclaim ™ Mixed-mode WCX-1* (2,1 x 150 nm, 3 μm) koloni koja kombinuje hidrofobne reverzno-fazne i mehanizme slabe katjonske izmene. Zadovoljavajuće razdvajanje aktivnih farmaceutskih supstanci i njihovih kontra-jona postignuto je upotrebom 90 mM acetatnog pufera pri pH 5 (A) i acetonitrila (B). Korišćeno je izokratsko eluiranje mobilne faze (40% B), pri protoku od 0,4 mL/min. Na osnovu preliminarnih eksperimenata, sledeći faktori identifikovani su kao značajni, te su testirani pri navedenim vrednostima: temperatura isparavanja (35ºC i 50ºC), konstanta filtera (1 s, 5 s i 10 s) i vrednost stepene funkcije (0,8, 1,0 i 1,2). Rezultati su pokazali da je temperatura isparavanja imala pozitivan efekat na odnos signal/šum (eng. *signal-to-noise*, S/N). S druge strane, visina i površina pika značajno su se smanjili pri povećanju temperature. Ovaj nalaz ukazao je na snažnu potrebu za finim podešavanjem pomenutog parametra imajući u vidu isparljivost analita. Povećanje vrednosti konstante filtera dovelo je do boljeg izgleda bazne linije, ali i do širenja pikova. Konstanta filtera postavljena na 5 s rezultirala je najvećim vrednostima odnosa S/N. Vrednost stepene funkcije, koja je direktno modifikovala CAD signal, pokazala je očigledan negativan efekat na površinu pika. U svetlu eksperimentalnih nalaza, optimalna CAD detekcija diklofenaka, tramadola, kalijuma i hlora postignuta je pri postavkama: konstanta filtera 5 s, vrednost funkcije snage 0,8 i temperatura isparavanja 35ºC.

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