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Simultaneous determination of cations, zwitterions and neutral compounds using mixed-mode reversed-phase and cation-exchange high-performance liquid chromatography

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Abstract

A novel mixed-mode reversed-phase and cation-exchange high-performance liquid chromatography (HPLC) method is described to simultaneously determine four related impurities of cations, zwitterions and neutral compounds in developmental Drug A. The commercial column is Primesep 200 containing hydrophobic alkyl chains with embedded acidic groups in H^+ form on a silica support. The mobile phase variables of acid additives, contents of acetonitrile and concentrations of potassium chloride have been thoroughly investigated to optimize the separation. The retention factors as a function of the concentrations of potassium chloride and the percentages of acetonitrile in the mobile phases are investigated to get an insight into the retention and separation mechanisms of each related impurity and Drug A. Furthermore, the elution orders of the related impurities and Drug A in an ion-pair chromatography (IPC) are compared to those in the mixed-mode HPLC to further understand the chromatographic retention behaviors of each related impurity and Drug A. The study found that the positively charged Degradant 1, Degradant 2 and Drug A were retained by both ion-exchange and reversed-phase partitioning mechanisms. RI2, a small ionic compound, was primarily retained by ion-exchange. RI4, a neutral compound, was retained through reversed-phase partitioning without ion-exchange. Moreover, the method performance characteristics of selectivity, sensitivity and accuracy have been demonstrated to be suitable to determine the related impurities in the capsules of Drug A.

Keywords: Mixed-mode chromatography; Ion-pair chromatography; Ion-exchange chromatography; Reversed-phase chromatography; Method validation

1. Introduction

Reversed-phase high-performance liquid chromatography (RP-HPLC) based on a silica support with organic solvents and buffers or acid and base additives in a mobile phase have been widely and successfully used to retain and separate moderate hydrophilic and hydrophobic compounds. However, separation selectivity provided by the RP-HPLC has been limited to the hydrophobic based resolution of relatively non-polar compounds. In some cases, RP-HPLC cannot simultaneously and selectively retain and separate a complex mixture of very polar, ionizable or ionic and hydrophobic organic compounds when the manipulation of the pH of the mobile phases fails. Therefore, other alterative approaches to modulate the retention and separation of a mixture of very polar, ionizable or ionic and hydrophobic organic compounds have been explored. Ion-pair chromatography (IPC) is one of the most popular approaches to selectively retain and separate a mixture of very polar, ionizable or ionic and neutral organic compounds. IPC can be easily achieved by slightly modifying the mobile phases used in the RP-HPLC and directly adapting the RP-HPLC columns. In the IPC, an amphiphilic anion or cation, usually an alkyl sulfonic acid or salt and alkyl quaternary amine, respectively, is added to the mobile phases to augment the retention of samples bearing opposite charges [1]. Various retention mechanisms of IPC have been reviewed thoroughly [2]. Ion-pair formation may only occur in the mobile phases and the ion-pair is subsequently subjected to the chromatographic phase distribution process. In other cases, only ion-pairing reagents adsorbed to the stationary phase surface will interact with either the ana-

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lyte molecules or with the ion-pairs of the analyte in the mobile phases. Also, a nonstoichiometric theory assumes the interaction of analytes with the electric field produced by the bound ionic reagents. Regardless of the retention mechanisms, IPC has been extensively used to retain and selectively separate ionizable and ionic organic compounds in food supplement [3], antihistamine drug [4] and metabolic profiling of mono and polyglutamated foliates and their precursors in animal and plant tissues [5]. Additionally, IPC has been used to separate enantiomers when the counter-ions are single enantiomers [6]. Also, IPC has been applied to separate and detect ionizable organic compounds without chromophores using indirect ultraviolet (UV) detection when the counter-ions in the mobile phases have UV absorbance [7,8].

However, IPC can encounter some practical problems in routine pharmaceutical analysis, such as, artifact peaks, system peaks, longer column equilibrium time, potential impurities from ion-pair reagents, non-compatible with a mass spectrometer for non-volatile ion-pair reagents and the cost of an ultra pure ion-pair reagent.

Another often used strategy to simultaneously retain and selectively separate a complex mixture is to modify the silica based stationary phases to accommodate the hydrophobic alkyl chains and ionic functional groups in a single column or a combination of two columns in series. Mixed-mode HPLC has mainly involved alkyl chains with embedded ion-exchangers on a silica based support. The mobile phase compositions are similar to those in the RP-HPLC. Mixed-mode HPLC can be achieved by connecting two columns physically with similar or dissimilar stationary phases [9–12], by mixing two packing materials of reversed-phase and ion-exchange stationary phases [13] in a column, and by chemically binding an ionizable group in an alkyl chain on silica based support [9,14,15]. Mix-mode of cation-exchange and reversed-phase interaction is even observed on classical silica based ODS and organic-inorganic hybrid silica ODS without intentionally introducing an ion-exchanger [16].

Silica based mixed-mode HPLC may provide better column efficiency for ionizable and ionic organic compounds than the polymer resin based ion-exchange chromatography (IEC). The relatively slow kinetics between the hydrophobic portions of organic compounds and the relatively hydrophobic backbones of the polymer resins is known to cause excess band broadening and tailing of organic compounds in the IEC [17].

Mixed-mode HPLC appears to combine the advantages of the reversed-phase hydrophobic alkyl chains with the hydrophilic ion-exchangers on a silica based support. It has been mainly used to separate biologic molecules of amino acids [18], nucleic acids [14], peptides and proteins [19], and tRNA and tRNA derivatives [20,21]. Relatively few of the mixed-mode HPLC has been used in pharmaceutical dosage analysis.

Recently, a mixture of six pharmaceutical active ingredients with diverse chemical structures in an ophthalmic solution was separated and quantitated with a run time of 60 min on two columns connected in tandem to achieve reversed-phase and weak anion-exchange separation [9]. Obviously, the combination of two individual columns in series is more eluent and time consuming compared to single column chromatography. It was also reported that one hydrophobic and one very polar pharmaceutical compound and their two major metabolites in an antimalarial combination drug were successfully determined on a single commercial column (Hypersil cation Duet) containing 50% (w/w) reversed-phase C18 and 50% (w/w) strong cation-exchanger [22].

Although the mixed-mode HPLC has been successfully used to separate complex analytes, the retention mechanisms are rarely studied. Early, mixed-mode retention has been observed on silica based ODS and ODS-2 [23], polymeric cation-exchanger [24] and polybutadiene coated zirconia (PBD-ZrO_2) columns [25]. The dependence of the retention factors on either the volume fraction of organic solvents or on the concentrations of counter-ions in the mobile phases are attributed to reversed-phase (hydrophobic) and ion-exchange (or silanophilic) interaction. The retention factors are quantitatively described by different models for each individual chromatographic system. Recently, the retention mechanisms of a tetra peptide and its main impurity on silica based RP and weak anion-exchange column were investigated quantitatively. The reversed-phase interactions were observed for both the peptide and its impurity. Weak anion-exchange interactions were only found for the peptide when the counter-ion was acetate but not formate [15].

In this paper, a mixed-mode HPLC column Primesep 200 containing cation-exchanger in H^+ form embedded in a hydrophobic alkyl chain on a silica support is evaluated to simultaneously retain and separate four structurally diverse related impurities (RIs) in a developmental Drug A. RI2, a small ionic compound, is α-aminoglutarimide hydrochloride. RI4, a neutral compound, contains aromatic nitro and glutarimide functional groups. Degradant 1 (D1), a zwitterion, contains aromatic amine and glutamine, and Degradant 2 (D2), a zwitterion, has aromatic amine and isoglutamine functional groups. D1 and D2 are also geometric isomers with the same molecular weights and functional groups. Drug A consists of aromatic amine and glutarimide.

The similarity and difference among these molecules are clearly seen in Fig. 1. The R group is a heterocycle and relatively hydrophobic.

In addition, the retention mechanisms of each related impurity and Drug A are elucidated quantitatively by varying the concentrations of potassium chloride and the percentages of acetonitrile in the mobile phases. The initial attempt to simultaneously retain and separate these related impurities and Drug A on a conventional RP-HPLC by adjusting the pH of the mobile phases has also been discussed. Furthermore, the elution orders in an IPC have been compared to those on the mixed-mode HPLC to further illustrate the chromatographic behaviors of these related impurities and Drug A in the mixed-mode HPLC. Moreover, the method performance characteristics of selectivity, linearity, limits of detection and quantitation as well as method accuracy are determined. Finally, the mixed-mode HPLC method is applied to determine the related impurities in the capsules of Drug A.