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High performance liquid chromatography of selected alkaloids in ion-exchange systems

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ABSTRACT

A HPLC procedure on strong cation exchange column (SCX) has been developed for the analysis of selected alkaloids from different chemical groups. The retention, separation selectivity, symmetry of peaks and system efficiency were examined in different eluent systems containing different types or concentrations of buffers at various pH and the addition of organic modifiers: methanol (MeOH), acetonitrile (CH_3CN), tetrahydrofuran (THF) or dioxane (Dx). The retention factors as the function of the concentration of buffers, the mobile phase pH and the percentage of modifier in the eluents were investigated. More symmetrical peaks and the highest theoretical plate number were obtained in eluents containing acetonitrile or tetrahydrofuran. In most cases, the increase of buffer concentration caused the decrease of alkaloids' retention, the improvement of peaks' symmetry and the increase of theoretical plate number. The improved peak symmetry and the efficiency of system for most investigated alkaloids were observed in the systems containing buffers at strongly acidic pH. The obtained results also reveal a large influence of salt cation used for buffer preparation. The results obtained on SCX column were compared with those obtained on a C18 column. The most efficient and selective systems were used for the separation of alkaloid standard mixture.

1. Introduction

Alkaloids are a group of compounds containing basic nitrogen atoms, naturally occurring in plants, fungi, bacteria and animals. Many of these compounds are pharmacologically active or strongly toxic substances. For this reason, there is a necessity for the development of new methods for the qualitative and quantitative determination of these analytes. A great variety of analytical techniques have been applied to determine different alkaloids. Nowadays liquid chromatography is the most frequently used method. Most of its procedures are based on reversed-phase (RP) chromatographic separation on chemically bonded stationary phases eluted with aqueous-organic mobile phases. Since alkaloids appear in aqueous solution as ionized and unionized forms which interact differently with the stationary phase (ion-exchange, hydrophobic and H-bond interaction), they are often difficult to separate by commonly used RP HPLC methods [1,2]. RP analysis of basic compounds is still often accompanied by peak tailing and band broadening due to residue silanol groups of the silica matrix. Silanol interaction in RP LC systems can be reduced by the use of low or high pH mobile phase, the addition of ion-pairing or silanol blocker reagents, selecting a proper stationary phase. Recently, polar endcapped and polar embedded RP phases with incorporated amide, carbamate or urea groups have become of great interest [3]. RP LC methods based on silica support with organic solvents and buffers or ionic additives in the mobile phase have been widely and successfully used for separation as well as quantitative or qualitative determination of basic compounds, but often long column equilibration times are necessary [4]. In some cases, RP HPLC system cannot simultaneously and selectively separate a complex mixture of ionizable or ionic compounds. Therefore, an alternative approach to modulate the retention and separation of very polar, ionizable compounds ion exchange chromatography (IEC) is required. Moreover ion-exchange systems can be easily used in multidimensional separations of complex mixtures when column switching method is necessary making use of RP and IEC columns. Different retention mechanism in IEC often leads to quite different separation selectivity of ionic compounds. High efficiency and symmetrical peaks can be obtained by IEC using relatively simple eluents (buffer or organic/buffer mixtures) [5]. The retention of compounds in this method depends primarily on the choice of stationary phases, the ionic strength of eluent (type and concentration of buffer), pH and, in some cases, the addition of organic modifiers [6]. Ion-exchange systems can be used for the extraction from strong acidic aqueous solutions and are often applied for the isolation of basic compounds from the matrix. IEC with aqueous solvents avoids ecologically harmful organic eluents that

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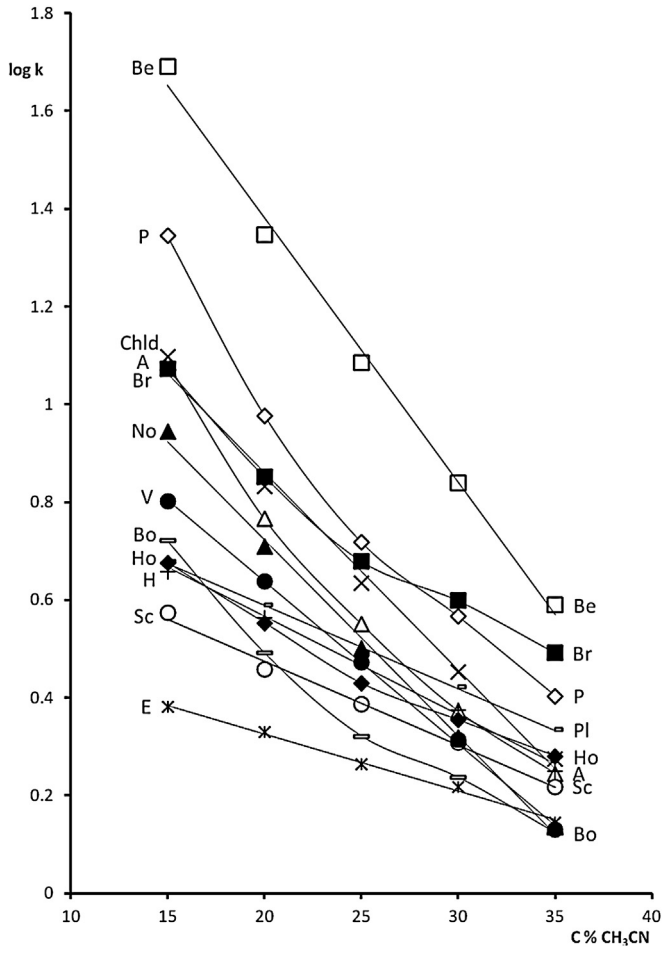

Fig. 1. The dependence of alkaloid log k values versus CH_3CN concentration on the SCX column eluted with a mixture of CH_3CN and buffer containing 50 mM of KCl, 50 mM of KH_2PO_4 adjusted to pH 2.5 by solution of 100 mM of H_3PO_4.

are usually necessary in high concentration for RP HPLC analysis. Neutral and ionic compounds of the opposite charge to the compounds determined are not retained at the functional groups of the ion-exchanger. Therefore, they are eliminated or eluted with the dead time of column. It was applied in sample preparation step by ion-exchange solid phase extraction (IE SPE) for samples of ionic analytes [7–9].

In the case of IEC method, high concentrations of salts in aqueous solutions are often applied, which may cause difficulties when other detectors, such as evaporative light scattering detector (ELSD) are used. The eluent with high concentrations of solid substances may cause salting-out effect and can make the use of such substances impossible in such a chromatographic system.

A strong cation-exchange (SCX) column was used to separate different basic compounds, e.g. amphetamine-type stimulants [10], phenalkylamines [11], alkanolamines [12], amino acids [13], amphetamine [10], peptides and proteins [14], basic drugs such as metformin [15], β-blockers [16], benzodiazepines, tricyclic antidepressants [17], antibiotics [18,19], and antimalarial drug Malarone [6]. Basic drugs were also analyzed on SCX column using eluents containing an ionic modifier at an appropriate pH and organic modifier [20,21]. Cation-exchange chromatography was also used for the determination of some alkaloids e.g. atropine and scopolamine [22], morphine, codeine [23], *Cinchona* alkaloids [24], and psychotropic alkaloids from hallucinogenic fungi (e.g. psilocin, psylocibin) [4].

The aim of this paper was to investigate retention time (t_R), asymmetry factor (A_s) and theoretical plate number per meter (N/m) for alkaloid standards chromatographed on SCX column in various conditions. Such parameters as the effect of eluent composition, type of buffer at different pH, ionic strength, type and concentration of organic modifier on the retention of selected alkaloids were determined.

2. Experimental

The analysis was performed using liquid Shimadzu chromatograph LC-10 AT_{VP} equipped with Luna SCX column of dimension 150 mm × 4.6 mm, 5 μm particle size (Phenomenex, USA) or SUPELCOSIL LC-18-DB column of dimension 150 mm × 4.6 mm 5 μm particle size (Supelco, Bellefonte, PA, USA), Shimadzu detector SPD – 10 AV_{VP} and Rheodyne 20 μl injector. The detection was carried out at 254 nm wavelength. All chromatographic measurements were carried out at 22 °C controlled by CTO-10AS_{VP} thermostat and repeated three times (SD < 2%). Eluent flow rate was 1.0 ml/min. The column pressure during the analysis was in the range of 250–300 Psi. Acetonitrile (CH_3CN), methanol (MeOH), 1,4-dioxane (Dx) and tetrahydrofuran (THF) of chromatographic quality were from E. Merck (Darmstadt, Germany). Potassium dihydrogen phosphate, phosphoric acid (85%), sodium chloride, potassium chloride, ammonium chloride, and lithium chloride were of p.a. quality (Polish Reagents Gliwice, Poland). The pH of buffers used in experiments was measured using pH meter CP-505 (Elmetron, Zabrze, Poland) in aqueous solutions.

Alkaloid standards: homatropine (Ho) and hioscyamine (H) were from Chmos Gmbh (Regenstauf, Germany), other standards: Scopolamine (Sc), Berberine (Be), Allocryptopine (A), Papaverine (P), Boldine (Bo), Brucine (Br), Noscapine (No), Chelidonine (Chld), Ephedrine (E), Vincamine (V) and Pilocarpine (Pl) were purchased from Sigma–Aldrich. The standards were prepared by dissolving in methanol to obtain 0.1% solutions.

2.1. Calculation

All chromatographic parameters such as retention times, retention factors, asymmetry factor (A_S), peak width (calculated by 10% of peak height) were calculated by software CLASS-VP 5.0 controlling the chromatograph. System efficiency – expressed as theoretical plate number (N) was calculated according to the equation for asymmetric non-Gaussian peaks proposed by Foley and Dorsey [25]:

$$N = \frac{41.7(t_R/W_{0.1})^2}{1.25 + (B + A)} \quad (1)$$

where t_R is retention time of the analyte, $W_{0.1}$ is the width at 10% of the peak maximum, B is the width from the center of the peak to the tail of the peak at 10% height, and A is the width from the front of the peak to its center. In the text and tables, system efficiency is expressed as theoretical plate number per meter of column (N/m).

3. Results and discussion

Alkaloid standards were chromatographed on SCX column in eluent systems containing buffers at different pH consisting of salts in various concentrations and organic modifiers and on C18 DB column in eluent system containing acetonitrile, phosphate buffer at pH 3.5 and diethylamine [3]. The retention of the investigated alkaloids in mobile phases containing solely various buffers was very strong, the result of which tailing peaks and low efficiency of the system were obtained. Therefore, the addition of organic modifiers was performed to all eluents. Fig. 1 presents the dependencies