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Investigation of polar stationary phases for the separation of sympathomimetic drugs with nano-liquid chromatography in hydrophilic interaction liquid chromatography mode

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ABSTRACT

In this study, the retention and selectivity of a mixture of basic polar drugs were investigated in hydrophilic interaction chromatographic conditions (HILIC) using nano-liquid chromatography (nano-LC). Six sympathomimetic drugs including ephedrine, norephedrine, synephrine, epinephrine, norepinephrine and norphenylephrine were separated by changing experimental parameters such as stationary phase, acetonitrile (ACN) content, buffer pH and concentration, column temperature. Four polar stationary phases (i.e. cyano-, diol-, aminopropyl-silica and Luna HILIC, a cross-linked diol phase) were selected and packed into fused silica capillary columns of 100 μm internal diameter (i.d.). Among the four stationary phases investigated a complete separation of the all studied compounds was achieved with aminopropyl silica and Luna HILIC stationary phases only. Best chromatographic results were obtained employing a mobile phase composed by ACN/water (92/8, v/v) containing 10 mM ammonium formate buffer pH 3. The influence of the capillary temperature on the resolution of the polar basic drugs was investigated in the range between 10 and 50 °C. Linear correlation of $\ln k$ *vs.* $1/T$ was observed for all the columns; ΔH° values were negative with Luna HILIC and positive with aminopropyl- and diol-silica stationary phases, demonstrating that different mechanisms were involved in the separation.

To compare the chromatographic performance of the different columns, Van Deemter curves were also investigated.

1. Introduction

The retention and separation of polar compounds are challenging in many application fields, especially in the pharmaceutical one, where suitable methods are requested to determine basic highly polar drugs and their metabolites in biological sample matrices.

Reversed-phase liquid chromatography (RPLC) is generally employed for the determination of a large variety of compounds. For the analysis of polar molecules, highly aqueous mobile phases are required to obtain appropriate retention. This condition can cause the collapse of octyl and octadecyl stationary phases and consequently weak retention and poor reproducibility for polar analytes occur. Besides, the use of high contents of water in the mobile phases decreases significantly the sensitivity of mass spectrometer (MS) detectors [1].

In normal phase LC (NPLC), the hydrophilic compounds are more retained. Nevertheless this chromatographic mode presents some drawbacks such as high cost and toxicity of the solvents used, low solubility of polar compounds and incompatibility with MS detector [2].

Hydrophilic interaction liquid chromatography (HILIC), first introduced by Alpert [3], appears to be an interesting alternative approach for the separation of polar and hydrophilic compounds. HILIC is characterized by the use of a polar stationary phase and an aqueous–organic mobile phase.

In HILIC, the retention is mainly caused by partitioning of the analyte between a water-enriched layer of stagnant eluent on a hydrophilic stationary phase surface and the relatively hydrophobic mobile phase [4]. A minimum of 2% of water in the mobile phase is needed to guarantee the presence of the water layer, and to ensure a sufficient hydration of the stationary phase particles [5].

In recent years, HILIC is growing in popularity due to the increasing need to analyze highly polar compounds in all areas of science including bioanalysis, proteomics, pharmaceuticals, food, etc. [6–10]. HILIC has also been used as an orthogonal separation system with RPLC in multi-dimensional chromatography for the analysis of complex matrices [11].

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Usually, silica gel with chemically bonded amino, amido, cyano, diol, carbamate, or polymer matrices are used as stationary phases for HILIC separations [4,12–14]. Recently, new and specific stationary phases, designed for HILIC, have been developed to increase retention and resolution of small polar compounds [15]. Nevertheless, the use of mobile phases with high content of organic solvents leads to further advantages including low back pressures and high compatibility with MS [16].

Finally, HILIC columns packed with sub-2 μm particles and compatible with ultra-high pressures (UHPLC) or superficially porous particles of 2.7 μm were also recently developed to obtain rapid analysis with increased resolution [17–20].

Owing to the high demand of industries for faster, easier, and less costly analysis methods, miniaturization has become one of the current trends of modern analytical chemistry. Among miniaturized techniques, nano-liquid chromatography (nano-LC) can be considered as complementary and/or competitive separation technique to conventional LC. This analytical tool stems from the reduction of both column inner diameter (10–100 μm) and flow rate (200–1000 nL min^{-1}). Compared to HPLC, this technique offers several advantages e.g. good efficiency, shorter analysis time, lower sample dilution, use of low volumes of mobile phases as well as small amounts of packing materials, easy coupling with MS, etc. However due to the relatively low sample volumes injected (20–60 nL), nano-LC presents a drawback concerning the limited sensitivity. Several approaches have been performed to achieve a sensitivity improvement such as the use of appropriate detector systems (MS, UV detection cell with a high path length) and/or on-column focusing. [21,22]. In recent years a large number of publications has proved nano-LC to be very powerful for analytical purposes in different fields such as proteomics, pharmaceutical, food, environmental and enantiomeric analysis. [23–26].

Currently, only few papers dealing with the use of HILIC by nano-LC have been published [27–30].

The purpose of this work was to evaluate the retention behaviour of a mixture of polar and basic sympathomimetic drugs such as ephedrine, norephedrine, synephrine, epinephrine, norepinephrine, norphenylephrine, in HILIC conditions, employing nano-LC. Such drugs are used to treat especially cardiac arrest and low blood pressure and were selected considering their similar structure and physicochemical properties (i.e. polarity).

To our knowledge only pseudoephedrine was separated in HILIC mode employing a silica stationary phase by HPLC [31].

In this study, four different polar packing materials including cyano-, aminopropyl-, diol-silica and Luna HILIC were selected and used in HILIC conditions with ACN concentrations >70% (v/v). The influence of mobile phase composition such as concentration and buffer pH, column temperature on retention and selectivity were investigated.

2. Experimental

2.1. Reagents and samples

All chemicals were of analytical grade and used as received. Acetonitrile (ACN), methanol (MeOH), formic acid, acetic acid, ammonia solution (30%, v/v) were from Carlo Erba (Milan, Italy).

Bidistilled water was obtained using a Milli-Q water purification system (Millipore, Waters Milford, MA, USA).

Stock buffer solutions (100 mM ammonium formate and ammonium acetate) were prepared by diluting in water the appropriate volume of formic and acetic acids and titrating with concentrated ammonia solution at pH 3, 4 and 6 respectively. Mobile phases used for nano-LC experiments were daily prepared by mixing appropriate volumes of stock buffer solutions, water and ACN to obtain a 10 mM aqueous buffer concentration.

The selected sympathomimetic drugs: ephedrine hydrochloride, norephedrine, synephrine, epinephrine, norepinephrine hydrochloride (arterenol), norphenylephrine hydrochloride, were purchased from Sigma–Aldrich (St. Louis, MO, USA). Standard solutions of each drug (1 mg mL^{-1}) were prepared in MeOH, except for epinephrine which was dissolved in an aqueous acidic solution and stored at +4 °C. Further dilutions were performed with ACN to obtain a final concentration of 25 μg mL^{-1}.

2.2. Apparatus

Nano-LC experiments were carried out using a laboratory-assembled instrumentation. The system included a micro-LC pump Rheos 2000 (Flux Instrumentations, Basel, Switzerland) which was controlled by a software system (Janeiro II, Version 2.0, Flux Instrumentations, Basel, Switzerland). A modified injector valve (Sepaserve GmbH, Münster, Germany) with a 50 μL loop and a 100 nL injection valve from (VICI Valco, Houston, TX, USA) were used as a reservoir for the mobile phase and to introduce the sample, respectively. Nano-injector and mobile phase reservoir were coupled using a 50 cm × 20 μm i.d. PEEK capillary and the capillary column was directly connected to the nano-injector.

The splitting device, required to reduce the flow rate from μL min^{-1} to nL min^{-1}, consisted of a stainless steel T piece (VICI Valco, Houston, TX, USA). One entrance of the T piece was connected to the pump through a 50 cm × 130 μm i.d. PEEK capillary; the second entry to the injector valve via a stainless steel tube of 7 cm × 500 μm i.d. and joined to the waste using a 50 cm × 50 μm i.d. fused silica capillary.

The flow rate was estimated connecting the end of the capillary column to a micro-syringe through a Teflon tube and measuring the mobile phase volume after 5 min.

On-column detection was carried out at 205 nm with a Spectra Focus PC 1000 UV–Vis detector, Thermo Separation Products (San Jose, CA, USA).

Nano-LC analyses were performed using packed fused silica capillaries, 100 μm i.d., 375 μm o.d. (Composite Metal Services, Hallow, Worcestershire, UK). Capillary columns contained the following silica based particles: Cogent bidentate C_{18} 4.2 μm, 100 Å, kindly donated by MicroSolv Technology Corporation (Eatontown, NJ, USA), Pinnacle II Cyano 3 μm, 110 Å, and Pinnacle II Amino 5 μm, 110 Å, from Restek (Bellefonte, PA, USA), LiChrospher 100 Diol 5 μm, 100 Å, from Merck (Darmstadt, Germany), Luna HILIC 3 μm, 195 Å, from Phenomenex (Torrance, CA, USA). All the columns were packed for 25 cm and the effective and total lengths were 25.5 cm and 34.0 cm, respectively.

2.3. Capillary column preparation

Fused silica capillaries were packed in our laboratory with the stationary phases according to the procedure previously described in [32]. Briefly, one end of the capillary was connected to a mechanical temporary frit (VICI Valco, Houston, TX, USA) to retain the packing material and the other end to a stainless steel HPLC precolumn (10 cm × 4.1 mm i.d.) which was used as reservoir for the slurry. The suspension was pumped into the capillary by a LC pump (Perkin-Elmer series 10 LC, Palo Alto, CA, USA). The slurry was prepared by adding few milligrams of stationary phase to 1 mL of acetone.

The capillary was packed with the stationary phase for about 35 cm. Afterwards the column was flushed with distilled water at 20 MPa to remove the packing solvent from the capillary. The frits were prepared sintering the particles for 6 s at 700 °C by a heating wire, laboratory made instrumentation, flushing the capillary continuously with water. The packed length was 25 cm. The temporary