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Some factors that can lead to poor peak shape in hydrophilic interaction chromatography, and possibilities for their remediation☆

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ABSTRACT

Some factors which present difficulties for obtaining good peak shape in hydrophilic interaction chromatography (HILIC) were studied. The effect of injection solvent composition and volume was systematically investigated using a selection of weak and stronger basic compounds on a hybrid bare silica phase. Increasing the mismatch between the injection solvent (range 95–0% ACN v/v) and the mobile phase (maintained at 95% ACN v/v) gave increasing deterioration in peak shape. With the 2.1 mm ID columns used, injections in the mobile phase of increasing volume (1–20 μL) gave poorer peak shape, but the magnitude of the effect was considerably smaller than that of solvent mismatch over this range. Some solute structural features such as galloyl (trihydroxy benzene), catechol (benzene diol) and phosphate (in nucleotides) gave serious peak tailing, attributed to interactions with metals in the stationary phase or the chromatographic hardware. These undesirable effects can be moderated by including complexing agents in the mobile phase, by changing the stationary phase chemistry, or by altering the mobile phase pH.

1. Introduction

Hydrophilic interaction liquid chromatography (HILIC) is fast becoming an established tool for the separation of polar and/or ionised compounds. This technique offers an alternative approach for compounds difficult to retain by reversed-phase (RP) methods. Stationary phases in HILIC include bare silica and polar bonded phases. Retention occurs through solute partitioning between a pseudo-immobilised water layer and the bulk mobile phase, as well as by adsorption and ionic interactions. There are important applications of the technique in biomedical and biological science, including in pharmaceutical analysis [1], metabolomic fingerprinting [2,3], anti-doping investigations [4,5] and clinical applications [6]. The mobile phases used in HILIC are typically hydro-organic mixtures of an aprotic solvent such as acetonitrile (>70% ACN v/v) in the presence of a soluble buffer (e.g. ammonium-formate/acetate/bicarbonate). These buffers are also volatile and thus useful for electrospray ionisation (ESI) mass spectrometry. Buffers are required at sufficient ionic strength to improve peak shape and control retention, particularly for ionogenic compounds [7].

Method development in HILIC can sometimes be more challenging compared with RPLC. For instance, the diverse chemical functionality of many hydrophilic compounds (e.g. the presence of a combination of one or more ionisable amino, carboxyl, diol, phosphate groups) can lead to complex interactions. Certain solute structural features may lead to unwanted, strong secondary interactions with the column and/or with the instrumentation, that can lead to deterioration of chromatographic performance [8]. These effects are clearly of concern in the LC–MS analysis of complex biological matrices where high separation efficiency is required [9]. Some recent articles have outlined approaches for overcoming poor peak shapes in metabolite profiling, notably by using either polymeric zwitterionic columns combined with high pH [10] or by adopting ion-pair chromatography [11]. Another factor is the influence of injection solvent composition on peak efficiency/capacity with different solutes and stationary phases during HILIC method development. In general, injection solvent mismatch should be minimised to obtain optimum chromatographic efficiency. However, problems may occur when poor sample solubility is encountered with a given mobile phase. For instance, highly polar compounds may have limited solubility in ACN-rich (>90% v/v) mobile phases. This limitation may be problematic in scaling up HILIC separations for preparative work. Previous studies have studied substituting amounts of methanol [12] and/or isopropyl alcohol

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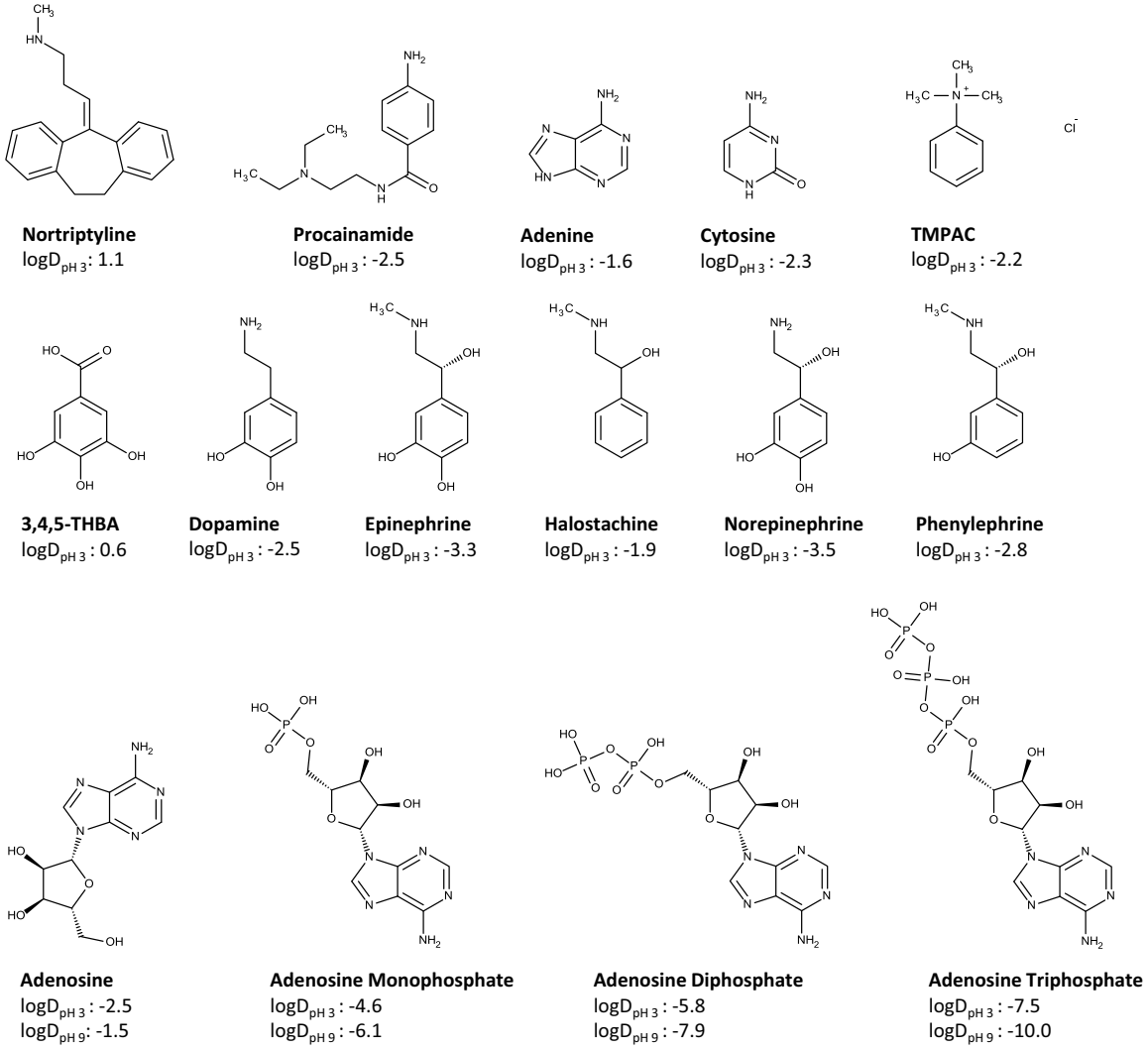

Fig. 1. Structures and predicted log *D* values at $_{w}^{w}$pH 3 and $_{w}^{w}$pH 9 for the probe solutes.

[13] in place of acetonitrile while including or excluding some of the water content in the injection solvent. However, no detailed measurements of the effects on column efficiency were made, or on the solution properties of different compounds. In the present study, we systematically investigated the influence of sample diluent from 0 to 95% ACN using a bare silica stationary phase (BEH HILIC) and a selection of small solutes. The influence of increasing injection volume on column efficiency was also evaluated using the flow-through needle autosampler design in the instrument used.

A further problem in HILIC is the unexplained poor peak shapes of some compounds that are unrelated to injection effects. For example, a previous investigation showed very poor peak shapes in the analysis of catecholamines by HILIC on a bare silica column [14]. In the present work, we have investigated the possible role of metals in the stationary phase or in the chromatographic hardware. Throughout all of these studies, we exclusively used isocratic elution to minimise the influence of gradient peak compression, which may otherwise complicate the interpretation of results [13].

2. Experimental

All experiments were performed using a 1290 ultra-high pressure liquid chromatograph (UHPLC) (Agilent, Waldbronn, Germany) consisting of a binary pump, autosampler and photodiode array UV detector (0.6 μL flow cell). ChemStation software was

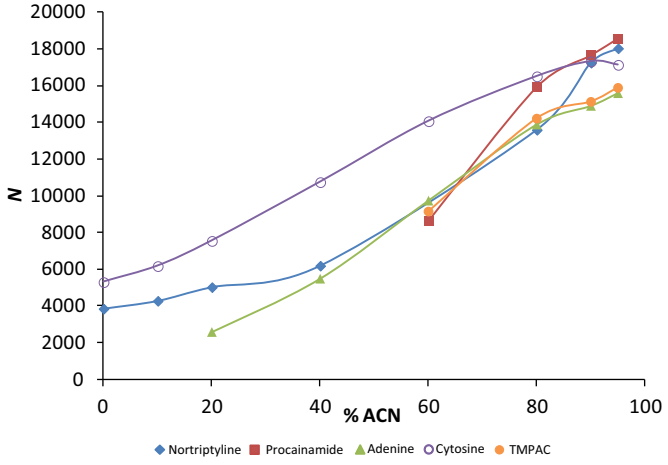

Fig. 2. The effect of decreasing acetonitrile (increasing water content) in the injection solvent on column efficienty ($N_{5\sigma}$) using BEH HILIC for a selection of basic solutes. Column dimensions 100 × 2.1 mm, 1.7 μm particles. Adenine, cytosine, nortriptyline and procainamide were at 20 mg/L whereas TMPAC was at 50 mg/L. Injection volume 1 μL. Conditions: Mobile phase 95% ACN containing 5 mM overall ammonium formate $_{w}^{w}$pH 3.