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Serial coupling of reversed-phase and hydrophilic interaction liquid chromatography to broaden the elution window for the analysis of pharmaceutical compounds

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ABSTRACT

It is presently a common practice in drug discovery to analyse samples by reversed-phase liquid chromatography (RPLC) and hydrophilic interaction chromatography (HILIC). To increase throughput, HILIC was connected in series to RPLC by means of a T-piece with make-up flow. The first column is a 2 mm I.D. column having an optimal flow between 0.1 and 0.2 mL/min. Via the T-piece, the flow for the second dimension column with an I.D. of 4.6 mm is adjusted to 1.5–2.0 mL/min with a high acetonitrile content (i.e. ≥80%) mobile phase. Therefore, even in gradient RPLC analysis starting with a mobile phase with high water content, the HILIC column is always operated at high acetonitrile concentration which is required to obtain retention on the HILIC column. The performance of the hyphenated RPLC/HILIC set-up is illustrated with the analysis of two model samples of pharmaceutical interest. Optimization of the conditions in the HILIC dimension is discussed.

1. Introduction

In the pharmaceutical development lifecycle, achieving adequate separation of the typically diverse range of synthetic route starting materials, impurities, intermediates and excipients from the active pharmaceutical ingredient (API) is a task which often demands a combination of orthogonal analytical measurement/generic screening tools. The nature of the impurities is, however, difficult to predict and often the analytical screening systems used in early development stages must be generic enough to encompass a wide range of solute physico-chemical properties as structural confirmation may not be available.

The typical pharmaceutical approach is to analyse the samples separately by reversed-phase liquid chromatography (RPLC) and normal-phase liquid chromatography (NPLC) or supercritical fluid chromatography (SFC). Recently the latter techniques are more and more replaced by hydrophilic interaction chromatography (HILIC) [1]. The term HILIC was introduced by Alpert in 1990 [2] although the principle was already known in the 1970s, e.g. for the analysis of sugars on aminopropyl silica with mobile phases rich in acetonitrile content [3]. HILIC is ideally suited to separate polar and ionisable solutes and we refer the reader to reviews covering HILIC [4,5], HILIC/MS [6] and HILIC in proteomics [7], a special issue [8] and some recent articles [9–13] for in-depth information.

The objective of this work was to develop a generic and simple HPLC method that is capable of simultaneously retaining and separating hydrophilic and hydrophobic solutes within a single chromatographic analysis.

A simple approach is to serially connect two or more columns with different polarities. In the so-called stationary phase optimised LC (SOSLC) strategy [14,15] (commercialised as POPLC, Bischoff, Leonberg, Germany), a software assisted package enables the most appropriate serial combination of different stationary phases to achieve optimal selectivity for a specific mixture of compounds with a chosen mobile phase. Disadvantages of the system are that the separation mechanisms (e.g. RP) must be similar because the same mobile phase is used for all column fragments and that the software only covers isocratic analysis. Another solution can be found in the use of mixed-mode stationary phases. Several mixed-mode phases containing hydrophilic, hydrophobic and ion exchange domains have been described in the literature for the analysis of hydrophobic and acidic [16,17] or basic [18] solutes. In aqueous normal phase (ANP), term coined by Pesek and Matyska [19], a hydrophobic/hydrophilic separation is obtained by using a

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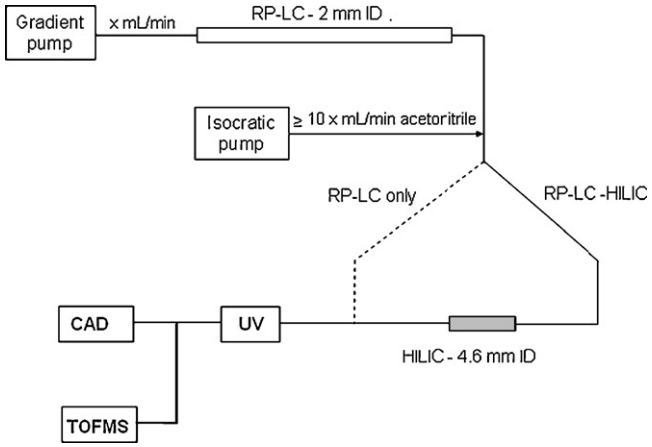

Fig. 1. Diagram of the instrumental set-up.

silicon-hydride stationary phase in combination with HILIC-type mobile phases. Although mixed-mode phases have great potential as an alternative concept to alter retention and selectivity, the contribution of the individual separation principles, i.e. hydrophilic, hydrophobic and/or ionic are not easy to elucidate for a given sample. Serial coupling of columns without any form of interface like the 'bi-dimensional (2D) HILIC' method, where an anion-exchange column is directly connected to a HILIC column, suffers from the same shortcoming [20]. Decoupling the orthogonality can be performed by two-dimensional chromatographic techniques. Off-line HILIC/RPLC for entire component analysis of biofluids has been described [21,22]. An on-line RPLC/HILIC system based on valve switching was recently developed by the group of Xu [23]. The same group also described comprehensive HILIC (HILIC × HILC) but, as expected, orthogonality was poor [24]. An SPE-HILIC–SPE–RPLC system has been described to solve the problem of solvent incompatibility between HILIC and RPLC and at the same time to allow injection of large aqueous samples [11]. Two-dimensional and comprehensive HPLC using valve switching might provide a solution in the future, but currently these techniques are too sophisticated and lack robustness for routine use in a regulated development or manufacturing laboratory. Note that in published combinations, RPLC is coupled to HILIC and dedicated interfaces had to be constructed to cope with claimed solvent incompatibilities, i.e. the HILIC mobile phase is too strong for RPLC.

A mere widening or broadening of the retention window in RPLC for poorly retained compounds is actually the most efficient solution, i.e. by coupling HILIC to RPLC.

In this contribution a simple approach is described to couple HILIC to RPLC. The key aspect of the approach is the use of a 2 mm I.D. column in the first dimension and a 4.6 mm I.D. in the second dimension combined with the addition of an excess of mobile phase containing high acetonitrile content (i.e. ≥80%) to the mobile phase eluting from the RPLC column via a T-piece. The performance of coupling RPLC and HILIC is illustrated with two model samples of pharmaceutical interest, i.e. a mixture of sugars and sulfonamides and a mixture of arylamines and aminopyridines. The analysis of real pharmaceutical sample sets containing parent compounds and polar metabolites, will be published elsewhere.

2. Experimental

2.1. Chemicals and reagents

HPLC gradient grade acetonitrile and water (Chromasolv) were purchased from Sigma–Aldrich (Bornem, Belgium) and formic acid was obtained from Acros Organics (Geel, Belgium). Ammonium formate, sulfamerazine, ribose, sucrose, lactose and all the arylamines and aminopyridines were from Sigma–Aldrich Chemie (Steinheim, Germany). Sulfamethizole, sulfamerazine, sulfamethoxazole and sulfaquinoxaline were obtained from Riedel-de Haën (Seelze, Germany). Glucose was purchased from Janssen Chimica (Geel, Belgium) and raffinose was from Merck (Darmstadt, Germany).

Stock solutions of the sugars and the sulphonamides, except for sulfaquinoxaline were prepared at ca. 5 mg/mL in 100% water and at ca. 7 mg/mL in 100% acetonitrile, respectively. Sulfaquinoxaline was dissolved in water/acetonitrile (10/90; v/v) at the same concentration level as the other sulfonamides. A mixture of sugars and sulfonamides was prepared in water/acetonitrile (80/20; v/v) at ca. 0.1 mg/mL. Stock solutions for all the arylamines and

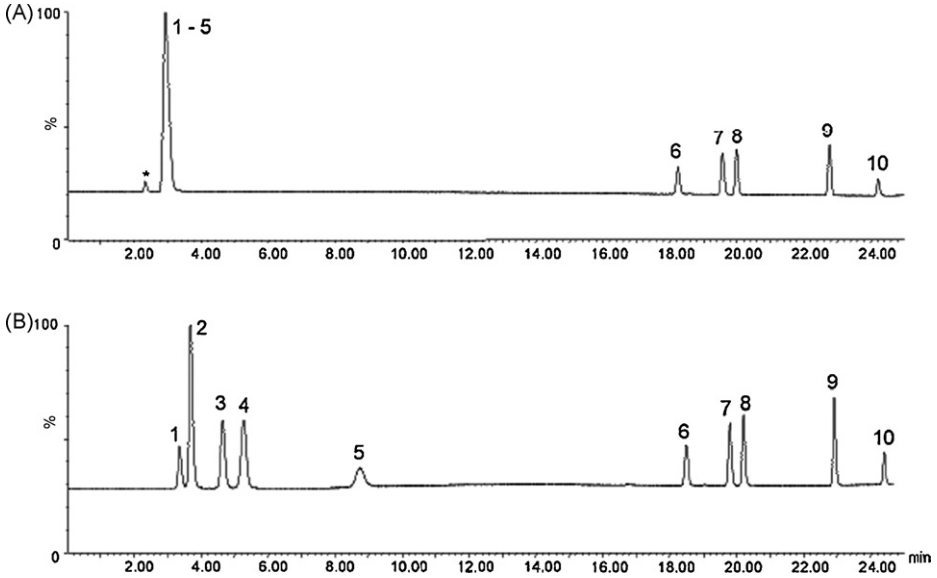

Fig. 2. Separation of sugars and sulfonamides using (A) RPLC gradient analysis and (B) gradient RPLC/isocratic HILIC analysis. Injection volume: 5 μL; detection: CAD. Compounds: (1) ribose, (2) glucose, (3) sucrose, (4) lactose, (5) raffinose, (6) sulfamerazine, (7) sulfamethizole, (8) sulfamethazine, (9) sulfamethoxazole, and (10) sulfaquinoxaline (*Baseline disturbance caused by injection).