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How to select equivalent and complimentary reversed phase liquid chromatography columns from column characterization databases

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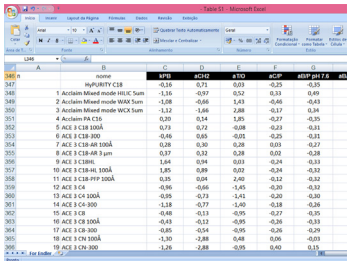
HIGHLIGHTS

- The SRM 870, Tanaka and PQRI tests were compared.
- Special attentions to different manners to interpret the data were given.
- Correlations between the Tanaka and PQRI tests were reported.
- An interactive tool is given, where the data was afforded in eight Microsoft Excel tables.
- Disadvantages of PCA use are highlighted.

GRAPHICAL ABSTRACT



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ABSTRACT

Three RP-LC column characterization protocols [Tanaka et al. (1989), Snyder et al. (PQRI, 2002), and NIST SRM 870 (2000)] were evaluated using both Euclidian distance and Principal Components Analysis to evaluate effectiveness at identifying equivalent columns. These databases utilize specific chromatographic properties such as hydrophobicity, hydrogen bonding, shape/steric selectivity, and ion exchange capacity of stationary phases. The chromatographic parameters of each test were shown to be uncorrelated. Despite this, the three protocols were equally successful in identifying similar and/or dissimilar stationary phases.

The veracity of the results has been supported by some real life pharmaceutical separations. The use of Principal Component Analysis to identify similar/dissimilar phases appears to have some limitations in terms of loss of information. In contrast, the use of Euclidian distances is a much more convenient and reliable approach. The use of auto scaled data is favoured over the use of weighted factors as the former data transformation is less affected by the addition or removal of columns from the database. The use of these free databases and their corresponding software tools shown to be valid for identifying similar columns with equivalent chromatographic selectivity and retention as a "backup column". In addition, dissimilar columns with complimentary chromatographic selectivity can be identified for method development screening strategies.

1. Introduction

Mobile phase composition (i.e. pH, type and proportion of the organic modifier), the operating HPLC parameters (i.e. temperature, gradient time and slope) and the stationary phase used can dramatically influence the chromatographic performance and selectivity of the separation [1–6]. While the influence of the former parameters can be predicted and modelled successfully, the influence of using differing stationary phases is less well understood. Even changing from the same nominal type of stationary phase chemistry from differing manufacturers can give rise to differing chromatographic selectivities [7–9]. By 2005, there were approximately 220 C18 columns (i.e. USP L1 classification) commercially available and, every year, a plethora of new L1 columns are launched.

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Several attempts have been made to produce a definitive set of chemical probes and test conditions to best characterize the huge number of stationary phases available. As of 2013, it is believed that there are over 1000 reversed-phase stationary phases commercially available [1–3]. These chromatographic characterization approaches have been reviewed in several manuscripts [10–27]) including that of Lesellier and West [17] who discussed the main stationary phase characterization procedures that have been developed over last 20 years.

To date, a unified column characterization approach has not been agreed upon by chromatographers or the stationary phase manufacturers. An early attempt to do so was made by Tanaka and co-workers [28]. Since then, the USP Working Group on HPLC Columns, the Impurities Working Group of the PQRI Drug Substance Technical Committee in collaboration with Lloyd Snyder [29–38], the NIST Standard Reference Material (SRM) 870 [39–41] and the group of Euerby and Petersson [42–48] have expanded this type of work. These groups have all attempted to create a testing protocol and rationale that can assess the most important chromatographic properties of a stationary phase.

Most of these approaches have utilized various chemometric and statistical approaches to visualize the similarities/dissimilarities of the stationary phases in the databases based on Principal Component Analysis (PCA) or computing a numerical "similarity" factor, as a measure of a stationary phase's equivalent or complementary chromatographic selectivity. The column characterization databases developed by these three groups (which are now freely available [49,50]) can be used to rapidly identify similar/dissimilar stationary phases as a "backup column" or for method development screening strategies respectively.

This paper evaluates how stationary phase characterization databases can assist in the selection of similar/dissimilar stationary phases. The supplementary material includes all the Microsoft Excel spreadsheets which can be used by the reader to perform their own evaluations.

2. Experimental

All calculations were performed using Microsoft Excel 2007. Correlation coefficients, standard deviations (s) and averages were obtained using the Microsoft Excel functions CORREL, STDEV and AVERAGE respectively. The percentage relative standard deviations (%RSD) were calculated dividing the s by the average and multiplying by 100.

PCA was performed using STATISTICA 7.1 StatSoft. Inc. (Tulsa, OK, USA). The data was auto scaled before performing the PCA.

The SRM 870 and PQRI databases were accessed via the USP web site [49] and the Tanaka database by the Analytical Chemistry Development web site [50].

2.1. Description of the column characterization parameters

2.1.1. Tanaka column characterization approach

In 1989, Tanaka [28] reported a simple, rapid and very useful approach for the evaluation the chromatographic properties of various stationary phases intended for reversed phase (RP) use. The protocol determines six chromatographically relevant variables which are briefly described below [42–48,50].

Hydrophobicity: Retention factor for pentylbenzene, k_{PB}: which reflects the surface area and surface coverage (ligand density). Chromatographic conditions: methanol–water (80:20, v/v).

Hydrophobic selectivity, α_{CH2}: retention factor ratio between pentylbenzene and butylbenzene, $\alpha_{CH2} = k_{PB}/k_{BB}$, this is a measure of the surface coverage of the phase as expressed by the selectivity between alkylbenzenes differentiated by one methylene group which is dependent on the ligand density. Chromatographic conditions: as for hydrophobicity.

Shape selectivity, $\alpha_{T/O}$: retention factor ratio between triphenylene and *o*-terphenyl, $\alpha_{T/O} = k_T/k_O$, this descriptor is a measure of the shape selectivity, which is influenced by the spacing of the ligands and probably also the shape/functionality of the silylating reagent. Chromatographic conditions: as for hydrophobicity.

Hydrogen bonding capacity, $\alpha_{C/P}$: retention factor ratio between caffeine and phenol, $\alpha_{C/P} = k_C/k_P$, this descriptor is an empirical descriptor relating to the concentration and accessibility of silanol groups in the stationary phase. Chromatographic conditions: methanol–water (30:70, v/v).

Total ion-exchange capacity, $\alpha_{B/P}$ pH 7.6: the retention factor ratio between benzylamine and phenol, $\alpha_{B/P}$ pH 7.6 = k_B/k_P, this is an estimate of the total silanol activity. Chromatographic conditions: methanol–phosphate buffer (pH 7.6; 20 mM) (30:70, v/v).

Acidic ion-exchange capacity, $\alpha_{B/P}$ pH 2.7: The retention factor ratio between benzylamine and phenol, $\alpha_{B/P}$ pH 2.7 = k_B/k_P, this is a measure of the acidic activity of the silanol groups. Chromatographic conditions: methanol–phosphate buffer (pH 2.7; 20 mM) (30:70, v/v).

Euerby et al. [42–48,50] evaluated 322 columns using the mentioned conditions above, this database is freely available in the web [50] and in Table S1. These columns are, in most part, chemically bonded type B silica having (150 mm × 4.6 mm, dp 5 μm), but there are some exotic columns like polymer coated zirconium oxide, polymer coated alumina and type A silica chemically bonded. Chromatographic testes were carried out at 40 °C, 1 mL min^{-1} and detection at 220 nm. Flow rates were adjusted using Eq. (1), where F is the flow rate and d the column internal diameter of the new or original method.

$$F_{new} = F_{original}\frac{d^2_{new}}{d^2_{original}} \quad (1)$$

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.aca.2013.11.010.

2.1.2. SRM 870 column characterization approach

The NIST SRM 870 test, developed in 2000 ([39–41,49]), consists of a mixture of five test solutes (uracil, toluene, ethylbenzene, quinizarin and amitriptyline) which are analyzed using a methanol–phosphate buffer (pH 7; 5 mM) (80:20, v/v) mobile phase at 23 °C to evaluate the following parameters:

Hydrophobicity: retention factor for ethylbenzene, k_E: reflects the surface area and surface coverage (ligand density).

Silanol activity: retention factor and tailing factor for amitriptyline, k_{ami} and Tf_{ami}. Tf_{ami}, reflects how good a stationary phase should be to analyze basic solutes and k_{ami} reflects the participation of ion-exchange interactions in the retention process of basic solutes.*The activity towards chelators*: tailing factor for quinizarin, Tf_Q, reflects the metal content in the stationary phase.

This database is freely available in the web [49] and in Table S2. These columns are, in most part, chemically bonded type B silica having (250–150 × 4.6 mm, dp 10–5 μm), but there are some type A silica chemically bonded columns. Chromatographic testes were carried out at 23 °C, 2 mL min^{-1} and detection could be carried out at detection at 254 nm, 210 nm, and 480 nm. In the event of coelution of quinizarin and amitriptyline, data for each component can be obtained by selective detection at 210 nm and 480 nm. At 210 nm, the area of quinizarin is approximately 2% of the area of amitriptyline, making the interference to amitriptyline small [40].

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