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Classification and replacement test of HPLC systems using principal component analysis

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Abstract

Theoretical plate numbers and symmetry factor values have been measured for three solutes (benzoic acid, *N*,*N*-dimethyl-aniline and Vancomycin) in various chromatographic systems (stationary phases and different mobile phase compositions) with the aim of grouping the systems and studying the possibilities for replacement of columns and eluent compositions. The mobile phases consisted of acetonitrile or methanol modifier and KH_2PO_4–H_3PO_4 or triethylamine–H_3PO_4 aqueous buffer. The organic content of mobile phases was adjusted so that the $\log k$s (logarithms of retention factor) of tested compounds vary in the range of 0.1–1.3 in case of all chromatographic systems and test compounds. Plate numbers and symmetry factors at all retention factor values (between $\log k = 0.1$–1.3 with 0.1 units) were subjected to principal component analysis (PCA). The input data were arranged in 13×28 data-matrices in case of all compounds separately for plate numbers and symmetry factors. Two principal components were retained in the model, which carry not less than 98% of the total variance. Principal component analysis showed which chromatographic systems (stationary and mobile phases) gave the same (similar) chromatographic parameters using three different kinds of compounds.

Replacement of chromatographic systems is possible on the basis of measured chromatographic parameters using principal component analysis. The monofunctional test compounds provide only approximate information on the usefulness, classification and replacement of HPLC systems, if the aim is the separation of multifunctional solutes. Although the theoretical plate number (column efficiency) is negatively correlated with the symmetry factor, both are necessary for proper classification and characterization of stationary phases.

Keywords: Stationary phases; Principal component analysis; Classification; Reversed phase HPLC; Vancomycin; Chemometrics; Symmetry factor; Theoretical plate number

1. Introduction

Many liquid chromatographic methods are described in the United States Pharmacopoeia (USP) [1], the European Pharmacopoeia (EP) [2] and other national pharmacopoeias (see e.g. [3]). These methods prescribe the precise mobile phase compositions, flow rates, injection volumes and so on to be used for separation of active pharmaceutical ingredients (API). But the Refs. [1–3] do not mention the trademark of the stationary phases that should be used in order to obtain the required chromatographic parameters for components to be tested (selectivity, theoretical plate number, symmetry factor, resolution, etc.). The given information about stationary phases (for example: C_{18} end-capped stationary phase or C_{18} base deactivated stationary phase) in the monographs is insufficient to make the proper choice from among commercially available columns.

The differences that exist among commercially available reversed-phase high-performance liquid chromatographic stationary phases are considerable and have attracted our interest from both theoretical and practical point of view. The

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observed chromatographic differences between similarly prepared columns are due to differences in the characteristics of the silica material used as a support and in the technique used to form the bounding phase. The surface coverage, end-capping, surface area, carbon content, pore size and volume, particle shape and size, the presence of trace elements in the silica matrix, etc. affect the chromatographic parameters (retention time, plate number, symmetry factor, selectivity and resolution) considerably [4–8].

The column selection in reversed-phase liquid chromatography (RPLC) is still not a straightforward process. Several chromatographic tests can be found in the literature to characterize reversed-phase columns [9–25]. These methods, which generally use monofunctional test compounds, afford opportunity to examine the properties of stationary phases, such as column efficiency, hydrophobicity, silanol activity, ion-exchange capacity, steric selectivity and the amount of metal impurities.

Claessens and co-workers [14] studied and compared several tests for RP-HPLC columns: the tests of Engelhardt, Tanaka, Galushko and Walters. 18 silica, alumina and polymer based C_8- and C_{18}-columns were tested. They found that the hydrophobicity data were generally good and interchangeable between the tests resulting in a column classification that is independent from the applied test. The buffering of the eluent was mandatory for adequate testing of column silanol activity. The silanol activity from various applied test methods differed significantly in contrast with the hydrophobicity data.

The trend and progress in the characterization of stationary phases for RPLC were published by Claessens in 2001 [15]. In this study, he summarized the state-of-the-art and progress in the characterization of RPLC columns and compared the existing column tests with the ones under development. He also mentioned chemometrical and statistical methods for evaluation of chromatographic data besides commonly used RPLC column tests.

Sander and Wise [20] have determined the column void volume, hydrophobicity, methylene selectivity, activity toward organic bases and activity toward metal chelators using a special test mixture of five components: uracil, toluene, ethylbenzene, quinizarin and amitriptyline. The measurements of retention and peak symmetry factors for these components were found to be useful in column classification.

The stationary phases can be compared and classified on the basis of these test methods. However, the usage of stationary phases depends on the specific application (on the solutes that are intended to be separated) as well, so the given classification of phases based on chromatographic properties will not reflect the reality completely. Hence, researchers are elaborating more and more new test methods with novel test compounds to classify the columns.

Handling (processing) of numerous data and the extraction of useful information are difficult using common processing methods. Multivariate techniques (principal component analysis, factor analysis, cluster analysis, etc.) are suitable to analyze and to evaluate reversed-phase high-performance liquid chromatographic data [26–35]. The main goal of these investigations was to classify and select proper stationary phases, recommend preferred solvents to a given separation, identify selectivity measures, etc. It should be mentioned that the results and conclusions of above investigations are often contradictory.

Brereton and McCalley [26] analyzed the performance of eight high-purity silica-based reversed-phases using chemometric pattern recognition techniques. The tests were performed at pH 3.0 and 7.0 using 10 compounds, three mobile phase modifiers (methanol, acetonitrile and tetrahydrofuran) and four parameters (retention factor, plate number, Dorsey–Foley efficiency and asymmetry factor). Data sets were evaluated using principal component analysis (PCA). Methanol and acetonitrile exhibited similar properties; hence, the usage of one modifier is sufficient for characterization of columns. As the relative performance of columns is significantly different at low and high pH, the evaluation of columns must be performed at least at two different pH. It has been proven that the number of compounds used in the test could be decreased to the recommended five compounds.

Vervoort et al. [27] measured chromatographic data (retention, peak symmetry and plate height) of five basic test compounds in case of 14 stationary phases. They also used PCA for data evaluation. The evaluation of all three measured chromatographic parameters was carried out without their separation. Stationary phases with similar chromatographic behavior clustered in the score plots and the relationships between the variables were depicted in the loading plots.

Euerby and Petersson [28] characterized 135 commercially available liquid chromatographic stationary phases in terms of their surface coverage, hydrophobic selectivity, shape selectivity, hydrogen bonding capacity and ion-exchange capacity at pH 2.7 and 7.6 using acceptable test methods and compounds. The columns and their chromatographic parameters have been analyzed by PCA in order to identify equivalent phases and to assist in the rational selection of suitable stationary phases.

Visky et al. [30] performed the classification of 69 reversed-phase liquid chromatography columns on the basis of 24 measured chromatographic parameters. Eight different chromatographic methods were used to determine these parameters. The evaluation of data and the classification of stationary phases were accomplished by PCA. Principal component analysis was used to estimate the minimal number of parameters necessary for a rational classification. The best parameter set, which gave similar results to column groupings based on all 24 parameters, utilized four parameters only. These four parameters can be determined using three reproducible chromatographic methods. The next features can be distinguished: stationary phases made of silica gel type A and B with different pore size, end-capped/non-end-capped, base deactivated/not based deactivated and polar embedded columns.