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Facilitated column selection in pharmaceutical analyses using a simple column classification system

Dóra Visky [a,b], Erik Haghedooren [a], Pieter Dehouck [a], Zsuzsanna Kovács [a,b], Kristof Kóczián [a,b], Béla Noszál [b], Jos Hoogmartens [a], Erwin Adams [a,*]

[a] *Katholieke Universiteit Leuven, Laboratorium voor Farmaceutische Chemie en Analyse van Geneesmiddelen, Van Evenstraat 4, B-3000 Leuven, Belgium*
[b] *Semmelweis University, Department of Pharmaceutical Chemistry, Hőgyes E. u. 9. H-1092 Budapest, Hungary*

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Abstract

In this paper, the performance of a previously developed classification system applied to pharmaceutical chromatographic analyses, is investigated. The separation of seven different drug substances from their respective impurities was studied. The chromatographic procedure for acetylsalicylic acid, clindamycin hydrochloride, buflomedil hydrochloride, chloramphenicol sodium succinate, nimesulide and phenoxymethylpenicillin was performed according to the corresponding European Pharmacopoeia (Ph. Eur.) monograph. The separation of dihydrostreptomycin sulphate was performed according to the literature. It is shown that the column ranking system is a helpful tool in the selection of a suitable column in these analyses.

Keywords: Column characterisation; Chromatographic tests; Column classification

1. Introduction

Nowadays, hundreds of different brands of reversed phase (RP) liquid chromatographic (LC) C18 columns are available on the market. Therefore, the selection of a suitable RP-LC C18 column is difficult. This explains why analysts have much interest in the characterisation and classification of C18 columns. Many papers have been published since the 1970s, but only those, which appeared in the last 15 years, are cited here [1–19]. The aim of these studies was: (i) to examine properties of RP-LC supports like efficiency, hydrophobicity, silanol activity, ion-exchange capacity, metal impurity level and steric selectivity with simple chromatographic methods and (ii) to characterize and classify the different brands of stationary phases. Chemometric tools were often used to facilitate the data evaluation [4,8–10,15,16].

The availability of a good characterisation and classification system is important for several reasons. Often, one has to find a column similar to one that is described in an existing method or in a paper because the prescribed column is not available in the laboratory. Sometimes, the column that was used for method development is simply not available anymore on the market. It is also possible that column properties differ between batches or that previous use of a column changed its properties. Many laboratories, e.g. control laboratories, do not use a new column for each separation they have to perform. In each of these cases, it is desirable to be able to identify an alternative column of similar selectivity, so that the replacement column will provide an "equivalent" separation as the original column.

Since 1998, column selectivity in RP-LC has been extensively studied by the group of Snyder. This leads to the hydrophobic-subtraction model describing five various solute-column interactions [20–30]. Recently, a procedure to characterise the selectivity of RP-LC columns was presented and evaluated. The selection and comparison of equivalent columns were examined for 12 routine separations performed in five different laboratories [26,27].

Interesting work has also been published by the group of Kaliszan. Their approach is based on quantitative structure–retention relationships (QSRR) where retention is evaluated in terms of the chemical structure of the analytes and of the physicochemical properties of both the stationary and mobile phase [31–35].

In the same time period, a simple chromatographic test procedure to characterise RP-LC C18 columns was derived from a

* Corresponding author. Tel.: +32 16 323443; fax: +32 16 323448.
E-mail address: erwin.adams@pharm.kuleuven.be (E. Adams).

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series of published tests by Hoogmartens and co-workers. The system allows to rank C18 columns, which are each characterised by four parameters: the retention factor of amylbenzene, $k'_{\text{amylbenzene}}$ (k'_{amb}), the relative retention factor benzylamine/phenol at pH 2.7, $rk'_{\text{benzylamine/phenol}}$ ($rk'_{\text{ba/ph 2.7}}$), the relative retention factor triphenylene/*o*-terphenyl, $rk'_{\text{triphenylene/o-terphenyl}}$ ($rk'_{\text{tri/ter}}$) and the retention factor of 2,2′-dipyridyl, $k'_{2,2'\text{-dipyridyl}}$ ($k'_{2,2'\text{-d}}$) [36–41]. This approach starts with the selection of a reference column or 4 reference parameter values. A *F*-value for a column *i* is calculated as:

$$F = (k'_{\text{amb, ref}} - k'_{\text{amb, }i})^2 + (rk'_{\text{ba/pH 2.7, ref}} - rk'_{\text{ba/pH 2.7, }i})^2 + (rk'_{\text{tri/ter, ref}} - rk'_{\text{tri/ter, }i})^2 + (k'_{2,2'-d\text{, ref}} - k'_{2,2'-d,\,i})^2 \quad (1)$$

The *F*-value of a column *i* equals the sum of squares of the differences between each parameter value of the reference column and of a column *i*. The smaller the *F*-value, the more similar is column *i* to the reference column and the higher is column *i* found in the ranking (high ranked columns). Before being introduced in Eq. (1), the parameters are autoscaled:

$$\frac{x_{ij} - \bar{x}_j}{s_j} \quad (2)$$

where x_{ij} is the value of parameter *j* on column *i*, $\bar{x}_j$ is the mean value of parameter *j* on all tested columns and s_j is the standard deviation for parameter *j*.

The calculation of *F*-values results in a single parameter that is a function of four different contributions to column selectivity. Furthermore, all values of *F* are relative to a single column. While this is a convenient and useful simplification, it implies that all columns can be arranged in order of relative selectivity. However, the accuracy of *F* as a measure of similar column selectivity will be decreased for two columns, each of which has $F \gg 0$. An alternative approach is to define the value of *F* in terms of the two columns being compared [23]. Since it was our intention to keep the system as simple as possible, no weighting factors that would introduce complexity, were used.

Of course, one also has to check whether columns having similar parameters give similar separations in practice. In this context, it should be remarked that although the four column parameters are each independent of column size, the latter will have an influence on the real separations performed. However, this is of minor importance, as method descriptions always specify the column length to be used.

In a previous study, the separation of acetylsalicylic acid (ASA) and its impurities was selected as a case study and carried out as prescribed by the European Pharmacopoeia (Ph. Eur.) monograph on the columns tested earlier [40,41]. In general, monographs in the Ph. Eur. only give very general information about the stationary phase to be used in terms of chain length, end-capping, base-deactivation, particle size and sometimes pore size and specific surface area [42]. For more recent monographs, the brand name of the column that was used for method development can be found on the Ph. Eur. website or in Pharmeuropa. After testing 69 columns, a relationship between the ranking of the columns and the selectivity in the separation of ASA was demonstrated and it was concluded that the column classification system can help analysts in the selection of a suitable RP-LC C18 column for the analysis of ASA. In the mean time, a freely accessible website was constructed [41,43]. In the ASA study, it was also examined whether the Ph. Eur. system suitability test (SST) was able to differentiate between suitable and non-suitable columns. It was found that meeting the SST requirements does not always predict the suitability of a column. In order to evaluate the separation on the stationary phases, the chromatographic response function (CRF), which is a measure for the overall selectivity, was used [44]. The CRF was calculated as:

$$\text{CRF} = \prod_{i=1}^{n-1} \frac{f_i}{g_i} \quad (3)$$

where *n* is the total number of solutes, *g* the interpolated peak height, i.e. the distance between the baseline and the line connecting the two peak tops, at the location of the valley and *f* the depth of the valley, measured from the line connecting the two peak tops [40,45]. This means that a baseline separated peak pair has an *f*/*g* ratio of 1, a non-separated pair has a value of 0, while a partly separated peak pair has an intermediate value. The use of these values has been described for thin-layer chromatographic methods [44], but they can be used in LC as well [46].

In this paper, the ASA study will be evaluated using a procedure without principal component analysis (PCA). In order to become a general chromatographic column test procedure, more case studies are needed to evaluate the correlation between column test parameters and separation characteristics. So, this paper describes six other representative separations, which were performed on the stationary phases already tested earlier. The selected methods are used for impurity profiling of drug substances. They are all isocratic and reference substances were available in order to enable calculation of CRF. A majority of the methods are tests for related substances as prescribed in the Ph. Eur. [42] (ASA, clindamycin hydrochloride, buflomedil hydrochloride, chloramphenicol sodium succinate, nimesulide and phenoxymethylpenicillin), while one other was chosen from the literature (dihydrostreptomycin sulphate [47]). For the 6 separations from the Ph. Eur., a SST was available. It was tested whether the SST could predict the suitability of the stationary phases. Also the correlation between the column ranking and the selectivity in each separation was examined in order to investigate whether the column ranking system based on the developed test procedure could be used to facilitate RP-LC C18 column selection.

2. Experimental

2.1. Chromatographic tests and columns tested

General information concerning the test methods, the chromatographic conditions applied, the measured parameters and column properties were published earlier [36,38]. Columns that did not meet the requirements of the monographs were removed