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# Orthogonal screening system of columns for supercritical fluid chromatography

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ABSTRACT

The application of supercritical fluid chromatography is expanding nowadays, particularly in the pharmaceutical industry and for natural extracts. In order to select appropriate columns from the continuously increasing number of potentially suitable ones, a test that evaluates stationary phases properties, based on the solvation parameter model, was performed earlier. In this study, it is investigated whether the number of stationary phases can be reduced to an optimized set with only highly orthogonal systems. Such a set of orthogonal chromatographic systems having different selectivities may provide the initial separation for method development. Moreover, it was considered important to include systems with good overall separation performance in our final set. Thus the columns are also selected based on their ability to provide sharp and symmetric peaks. An example application based on the analysis of seven sunscreen molecules is presented as a validation of the suggested column set.

## 1. Introduction

The retention rules of packed column supercritical fluid chromatography (pSFC) separation have long received only little attention. The primary goal of most pSFC studies was to provide an understanding of the role of mobile phase composition, pressure and temperature on retention. As a matter of fact, despite the current widespread application of supercritical fluids, particularly in the pharmaceutical industry [1–6], no standardized method has been adopted for the characterization and comparison of the various stationary phases available to the chromatographer. The stationary phases in common use in pSFC are generally HPLC stationary phases, thus based on silica with different bonding chemistries to introduce a wide number of functional groups (alkyl, phenyl, cyano, amino, propane-diol, etc.) or coated with polymers [poly(ethylene glycol), poly(vinyl alcohol)]. It is a big advantage for pSFC that a large number of different types of stationary phases are available for LC and one can use those with supercritical fluids without any specific care. However, due to the great difference in the mobile phase nature (with or without water), the knowledge acquired on those phases in LC is generally of little help when working with supercritical fluids. Besides, different bonded ligands sometimes provide highly similar selectivities (for instance, cyanopropyl and ethyl-pyridine bonded phases), while in other cases, small differences in the bonded ligand provide significantly different selectivities (for instance, phenyl-propyl and phenyl-hexyl bonded phases) [7].

In our laboratory a number of columns containing various stationary phases and obtained from different manufacturers were investigated [7]. Retention data for a large number of test substances covering a wide range of chemical properties were evaluated, all in identical chromatographic conditions. Using the retention data acquired, the interaction capabilities of the different columns were evaluated with the solvation parameter model [8–10], following the equation:

$$\log k = c + eE + sS + aA + bB + vV \quad (1)$$

In this equation, capital letters represent the solute descriptors, related to particular interaction properties, while lower case letters represent the system constants, related to the complementary effect of the phases on these interactions. $c$ is the model intercept term and is dominated by the phase ratio. $E$ is the excess molar refraction (calculated from the refractive index of the molecule) and models polarizability contributions from $n$ and $\pi$ electrons; $S$ is the solute dipolarity/polarizability; $A$ and $B$ are the solute overall hydrogen-bond acidity and basicity; $V$ is the McGowan characteristic volume in units of $cm^3\ mol^{-1}/100$. The system constants ($e$, $s$, $a$, $b$, $v$), obtained through a multilinear regression of the retention data for a certain number of solutes with known descriptors, reflect the magnitude of difference for that particular property between the mobile and stationary phases. Thus, if a particular coefficient is numerically large, then any solute having the complementary prop-

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erty will interact very strongly with either the mobile phase (if the coefficient is negative) or the stationary phase (if the coefficient is positive).

Thus, a database of system constants was established, which currently comprises data for 28 columns determined with a subcritical carbon dioxide–methanol mixture. Among all possible stationary phase chemistries, we have investigated widely different columns in order to demonstrate the differences in column characteristics. Complete discussion on the solvation coefficients obtained on each column, together with comparison of the columns can be found in our previous studies [11–13].

The system constants facilitate the comparison of the separation characteristics of different stationary phases and enable the identification of stationary phases of similar or dissimilar selectivity. Indeed, for the selection of columns, selectivity is more important than retention. Regarding the solvation parameter model, comparing the columns on the basis of the interactions contributing to retention is identical to comparing them on the basis of interactions contributing to separation as Eq. (2) can be deduced from Eq. (1):

$$\log\alpha = e\Delta E + s\Delta S + a\Delta A + b\Delta B + v\Delta V \qquad (2)$$

where $\alpha$ is the separation factor between two solutes and $\Delta X$ represents the difference in the $X$ coefficient between these two solutes.

If the selectivity of a stationary phase is defined as its relative capacity for specific molecular interactions, then the comparison of columns based on the solvation coefficients is perfectly sound to compare column selectivity. However, at this time, the use of the solvation parameter model for systematic selectivity optimisation is only poorly developed.

We have also designed a particular plot of the results issued from the solvation parameter model, which we call a spider diagram [14]. It is a useful tool to visualise how individual columns occupy the selectivity space [7]. For general method development, it would be reasonable to choose a single stationary phase from each region of the diagram. After identifying the most suitable type of stationary phase for a separation, when optimization of the mobile phase and temperature would not be sufficient to achieve perfect resolution, near neighbors could be investigated for optimization.

In this paper, we present a selection of possible stationary phases for method development, based on columns with different selectivities and sharp, symmetrical peaks for different classes of solutes. Indeed, the large database of chromatograms of individual solutes analyzed on all columns provides information on efficiency and peak asymmetry that is not taken into account by the solvation parameter model but that is essential to the chromatographer. An example application with the analysis of sunscreen molecules will illustrate the applicability of the column set.

## 2. Experimental

### 2.1. Stationary phases

All the stationary phases used in this study are commercially available and were kindly offered by the manufacturers. The names and known properties of the columns used are presented in Table 1. All columns were 250 mm × 4.6 mm, apart from PGC (100 mm × 4.6 mm) and PS (150 mm × 4.6 mm). All columns were 5 μm, apart from C12 and OPHE (4 μm).

### 2.2. Chemicals

Solvent used was HPLC grade methanol (MeOH) provided by Carlo Erba (Milan, Italy). Carbon dioxide was provided by l'Air Liquide (Paris, France). The sunscreen molecules were kindly provided by L'Oreal (Chevilly Larue, France). Solutions of these compounds were prepared in MeOH.

### 2.3. Chromatographic system and conditions

Chromatographic separations were carried out using equipment manufactured by Jasco (Tokyo, Japan, supplied by Prolabo, Fontenay-sous-Bois, France). Two model 980-PU pumps were used,

**Table 1**
Stationary phases compared is this study

| Abbreviation | Nature of the stationary phase | Trade name | Manufacturer |
|---|---|---|---|
| C4 | Butylsiloxane-bonded silica | Uptisphere C4 | Interchim |
| C8 | Octylsiloxane-bonded silica | Uptisphere C8 | Interchim |
| C12 | Dodecylsiloxane-bonded silica | Synergi Max RP | Phenomenex |
| C18 | Octadecylsiloxane-bonded silica | Kromasil C18 100 | Eka Nobel |
| C18-C | Octadecylsiloxane-bonded type-C silica | Cogent Bidentate C18 | MicroSolv Technologies |
| RPH | Octadecyl- and phenylsiloxane-bonded silica | Uptisphere RPH | Interchim |
| MIX | Octadecyl- and phenylpropyl-bonded silica | Nucleodur Sphinx RP | Macherey-Nagel |
| PE1 | Amide-embedded hexadecylsiloxane-bonded silica | Supelcosil ABZ + Plus | Supelco |
| PE2 | Ether-sulfonamide-embedded hexadecylsiloxane-bonded silica | Acclaim Polar Advantage | Dionex |
| FD | Fluorodecylsiloxane-bonded silica | Chromegabond Fluorodecyl | ES Industry |
| SI | Silica gel | Kromasil SIL 100 | Kromasil |
| PEG | Polyethylene glycol bonded on silica | Discovery HS PEG | Supelco |
| PVA | Polyvinyl alcohol bonded on silica | YMC-Pack PVA-Sil | YMC |
| DIOL | Propanediol-bonded silica | Diol | Princeton Chromatography |
| NH2 | Aminopropyl-bonded silica | Amino | Princeton Chromatography |
| CN | Cyanopropyl-bonded silica | Cyano | Princeton Chromatography |
| EP | 2-Ethylpyridine bonded silica | Ethylpyridine | Princeton Chromatography |
| PGC | Porous graphitic carbon | Hypercarb | Thermo-Hypersil Keystone |
| PS | Polystyrene-divinylbenzene | PLRP-S | Polymer Lab |
| OPHE | Phenyl-oxypropyl-bonded silica | Synergi Polar RP | Phenomenex |
| DP | Diphenyl-propyl-bonded silica | Pursuit Diphenyl | Varian |
| DP-X | Diphenyl-propyl-bonded silica | Pursuit XRs Diphenyl | Varian |
| C3P | Phenyl-propyl-bonded silica | Uptisphere PH | Interchim |
| C6P-L | Phenyl-hexyl-bonded silica | Luna Phenylhexyl | Phenomenex |
| C6P-G | Phenyl-hexyl-bonded silica | Gemini Phenylhexyl | Phenomenex |
| PYE | 2-Pyrenyl-ethyl-bonded silica | Cosmosil 5PYE | Nacalai Tesque |
| PFP | Pentafluorophenyl-propyl-bonded silica | Discovery HS F5 | Supelco |
| PBB | Pentabromobenzyl-oxypropyl-bonded silica | Cosmosil 5PBB | Nacalai Tesque |