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Monitoring aged reversed-phase high performance liquid chromatography columns

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Abstract

In this paper, a new approach for the quality assessment of routinely used reversed-phase high performance liquid chromatography columns is presented. A used column is not directly considered deteriorated when changes in retention occur. If attention is paid to the type and magnitude of the changes, columns often can still be used. Therefore, columns have to be monitored at regular time points. This means that, in the first place, a few well chosen measurements have to be done on the used column. With statistical techniques, Hotelling's T^2 statistic in combination with three-way analysis, the type and magnitude of changes in retention then can be detected. The type of changes can be divided in hydrophobicity changes, selectivity changes and both hydrophobicity and selectivity changes. This paper describes the approach in theory, completed with examples. At the end, a strategy for monitoring during routine use is proposed, which is visualized in a monitoring scheme.

Keywords: Reversed-phase high performance liquid chromatography; Hydrophobicity; Chromatography

Contents

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1. Introduction

One of the problems in modern chromatography, especially in reversed-phase high performance liquid chromatography (RP-HPLC), is the change (ageing) of stationary phases during routine use. The inner structure of the stationary phase changes, which results in a change of chromatographic properties such as retention, selectivity and efficiency [1]. This does not necessarily make an aged column worthless. Often a column can still be used if attention is paid to changed chromatographic properties. It is important to know what properties are changed and how much they are changed. Then, the aged stationary phase can be calibrated. On the calibrated column new optimal circumstances can be found such that the column can still be used.

Of course, an aged column can also be replaced by a new one. This can however be more costly, because more measurements have to be done to optimize the column than on an aged column. On an aged column only a few new measurements, according to the approach which will be presented in this paper, have to be done to find new optimal conditions. In all cases it is important to assess the quality of a column to detect whether a column is changed.

In practice, the quality assessment of columns during routine use mostly is done visually. Usually, a few standard solutes are injected. If their chromatographic peaks are too skewed, tailed, too broad and/or the resolution between two solutes is too low it is decided that the column is deteriorated. In this cases the column is replaced. Besides the fact that it is difficult to decide what is too broad and what resolution is too low, updating, to be able to continue the use of the column, is not often considered.

The approach for the quality assessment of used columns presented in this paper incorporates the possibility of continuation of use with some kind of updating. A first screening is still done visually. If bad separations are caused by visual large changes in peak shape this can be solved best by replacing the aged column. If the bad separations, however, are caused by changes in retention an appeal is made on monitoring with statistical quality control based on three-way analysis. This approach can easily be combined with calibration models for updating mobile phases (that is, the data needed for monitoring can also be used for calibration). This can be valuable for the construction of integrated software for quality assessment, calibration and updating.

Monitoring is not performed on all solutes, but on a small selection of solutes and mobile phases. Measuring and analysing a large number of variables (solutes at different mobile phases), such as needed for the optimization of a new column, is too time-consuming for monitoring in practice. With the use of statistical variable selection techniques, solutes and mobile phases will be selected in such a way that they represent the structure in retention data. This means that these ‘markers’ are chosen in such a way that they explain most of the variation in the data.

To detect changes in retention, in former research Bolck2, monitoring based on two-way analysis was used. In this paper, it will be shown how the *type* and *magnitude* of changes can be detected with three-way analysis. Different types of changes in retention will be distinguished. In the first place, a change in hydrophobicity causing only a shift in retention which is the same for all solutes. Furthermore, a change in selectivity causing retentions of all solutes to change differently. Finally a combination of these two changes will be considered.

Three-way analysis will be used to find underlying variables that represent structure in retention data of the markers. Often two underlying variables can represent as much as 4 (or more) markers. The difference between the values of the underlying variables of fresh and that of used columns, corrected for the random variation, all denoted in the so called

Hotelling's T^2 statistic, gives information on the most important aspects of the differences between fresh and aged columns. This can be visualized in control charts, which can give an idea about the type and magnitude of the changes at different time points during use of a column. Depending on the type and magnitude of changes different calibration models are needed for continuation of use of the changed column.

The paper starts with Section 2 describing the experiments done for the examples, followed by a brief description of the statistical theory behind three-way analysis and Hotelling's T^2. After that, a description of the three phases, that will be distinguished in monitoring, is given. This is illustrated with simulations. The reference data used in the simulations are based on real data, while various types and magnitude of changes are simulated. This, to get a clear picture how magnitude and type of changes can be detected. In addition, an example with artificially aged columns is given. Finally, a scheme is provided for the monitoring of columns during routine use.

2. Experimental

Theory in this paper is illustrated with real data and half real data half simulation examples. These examples make use of stationary phases of difunctional octylsilane synthesized on LiChrospher Si 100 silica packed in two columns (125 mm × 4 mm I.D.) according to a standard packing procedure. The stationary phase was prepared at the R & D chromatography department of E. Merck (Darmstadt, Germany).

Retention values of 4 marker solutes *n*-butyl *p*-aminobenzoate (BAB), ethylbenzene (ETB), *p*-nitrobenzaldehyde (NBA), and prednisone (PRE) were measured at 3 (marker) mobile phases. On the first of the two columns these measurements were repeated 8 times, to get an idea of the repeatability. On the second column measurements were only duplicated. The mobile phases are coded: wa3 (46% acetonitrile and 54% water), wam2 (19% acetonitrile, 24% methanol and 57% water), and wm1 (40% methanol and 60% water). The marker solutes and mobile phases are a selection from a larger number of solutes and mobile phases used in former research [2,3]. Measurements were performed at a flow rate of 1 ml/min. The column was thermostated at 30° (±0.1). Sodium nitrate was used as the dead time marker.

The 8 repeated measurements on the first column of, for instance, ln k values of the 4 test solutes measured at 3 mobile phases are stored in a $(8 \times 4 \times 3)$ data cube of reference measurements (Fig. 1). The second column only has a $(2 \times 4 \times 3)$ data cube of reference measurements.

After measuring the fresh columns, a 0.05 M phosphate buffer solution was prepared by mixing 0.05 M solutions of analytical grade (Merck) potassium dihydrogen phosphate and disodium hydrogen phosphate. For the real data example, the columns were aged for one day with an eluent containing this phosphate buffer of pH 8.0 in 100% water at a flow rate of 0.5 ml/min at 20°C. After ageing the column were rinsed with water, water/methanol and methanol. Then the 4 solutes were measured 2 times at the 3 mobile phases. The columns were aged for another day, followed by the rinsing and measuring, and so on. The intention was to age both columns for 8 days.

The first column showed a hole of 6 mm in the packing after 4 days of ageing. While the second column, which had only duplicated measurements before ageing instead of 8 replicated measurements, could be aged for the intended 8 days. The holed column could not be used any more because the chromatographic peaks would become distorted and besides that the pump simply could not deliver the necessary pressure to maintain the flow rate of 1 ml/min as used for the measurements. It appeared that the silica particles were cracked. We could not find a reasonable explanation for this premature collapse of the first column.

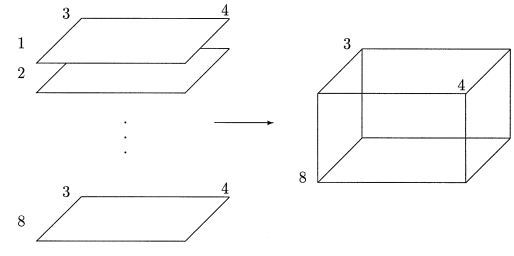

Fig. 1. A (8×4×3) data cube of ln k values on 8 fresh columns of one stationary phase type.

3. Theory

3.1. Three-way analysis

Three-way analysis is an extension of PCA [4]. Instead of a decomposition of a two-way data matrix in two directions a three-way data matrix is decomposed in three directions. Investigations on three-way analysis and three-way data tables are done by Law et al. [5], Caroll et al. [6], Kroonenberg and de Leeuw [7], Harshman [8], and Tucker [9]. Applications in chemistry are given by Wold et al. [10], de Ligny et al. [11], Öhman et al. [12,13], Smilde et al. [14,15].

Let X be a $(n \times p \times m)$ three-way data matrix (see for instance the cube in Fig. 1). This three-way data matrix can be decomposed in three directions. The difference between object and variable is not always clear, that is why the three directions are all called modes. The three modes can, for instance, correspond to the solutes, the mobile phases and the stationary phases. X is often centred in the direction of the mode in which the differences which are of primary interest.

In order to analyze a three-way data matrix, a generalization of the PCA model is needed. Such a generalization is obtained by applying the principle of unfolding [10]. This is shown in Fig. 2. The unfolded form of an $(n \times p \times m)$ three-way matrix X (left part of Fig. 2) is the $(n \times pm)$ two-way data matrix $\mathbf{X}$ (right part of Fig. 2).

A three-way data matrix can be unfolded in three directions. Usually the matrix will be unfolded in such a direction that the mode of primary interest will form the objects in the unfolded matrix. PCA is performed on the unfolded two-way data matrix (U-PCA). The generalization can be written as:

$$x_{ijk} = \sum_{s=1}^{g} t_{is} p_{sjk} + e_{ijk}, \qquad (1)$$

where t_{ig} is an element of the $(n \times g)$ score matrix

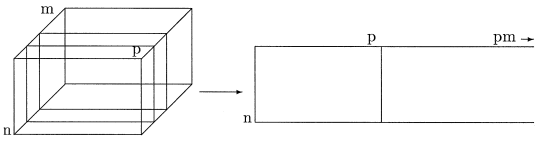

Fig. 2. The principle of unfolding.

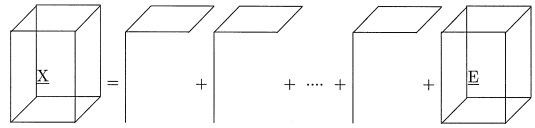

Fig. 3. Decomposition of a three-way data matrix; U-PCA.

$\mathbf{T}$, containing the first g factors or Principal Components (PCs) and p_{sjk} an element of the three-way $(g \times p \times m)$ loading matrix P (or the two-way $(g \times pm)$ loading matrix $\mathbf{P}_g$). Here, $\mathbf{T}$ and P are calculated in such a way that the residual sum of squares is minimized. The solution is not unique. Eq. (1) is visualized in Fig. 3.

Another generalization is given by the parallel factor analysis (PARAFAC) model [5,8]

$$x_{ijk} = \sum_{s=1}^{g} a_{is} b_{js} c_{ks} + e_{ijk}, \qquad (2)$$

with x_{ijk} an element of X, e_{ijk} an element of the residual matrix E and a_{is}, b_{js} and c_{ks} are elements of the loading matrices $\mathbf{A}$ $(n \times g)$ of the first mode, $\mathbf{B}$ $(p \times g)$ of the second mode and $\mathbf{C}$ $(m \times g)$ of the third mode. $\mathbf{A}$, $\mathbf{B}$ and $\mathbf{C}$ are chosen such that the sum of the squared residuals is minimized. Like with PCA, this means that the 'factors' are chosen such that they explain as much of the variation of X as possible. Contrary to PCA the solutions are unique.

Other generalization do exist, but are not described in this paper.

3.2. Monitoring with three-way analysis

Monitoring with three-way analysis is described for the three-way analysis technique U-PCA. Let $\mathbf{x}_i^{(0)}$, arrayed as $(\ln k_1, \ln k_2, \ldots \ln k_{pm})$, denote a reference vector of $\ln k$ values of p solutes measured at m mobile phases on a fresh column. The vector $\mathbf{x}_i^{(0)}$ can be considered as the ith row from the unfolded $(n \times pm)$ data matrix, with n the number of measurements. In the simulations and examples measurements (on the first column) were replicated 8 times, therefore $n = 8$. It is assumed that $\mathbf{x}_i^{(0)} \sim N_{pm}(\boldsymbol{\mu}^{(0)}, \boldsymbol{\Sigma})$. The mean of the reference vectors, $\boldsymbol{\mu}^{(0)}$, is estimated by the sample mean of the 8 measurements, $\bar{\mathbf{x}}^{(0)}$, and the covariance matrix, $\boldsymbol{\Sigma}$, by the sample covariance of the 8 measurements, $\mathbf{S}$. Let $\mathbf{x}^{(j)}$ be a vector of $\ln k$ values of p solutes measured at

m mobile phases on an used column. It is assumed that $\mathbf{x}^{(j)} \sim N_{pm}(\boldsymbol{\mu}^{(j)}, \Sigma)$, with the same Σ as $\mathbf{x}_i^{(0)}$, which is estimated by $\mathbf{S}$. Hotelling's T^2 [16] statistic can now be used to compare the ln k values measured on an used column with mean ln k values measured on reference columns. In this case it is written as

$$T^2 = \left(\mathbf{x}^{(j)} - \bar{\mathbf{x}}^{(0)}\right)' \mathbf{S}^{-1} \left(\mathbf{x}^{(j)} - \bar{\mathbf{x}}^{(0)}\right) \quad (3)$$

This Hotelling's T^2 is the multivariate analogue of the Student t-statistic [17]. It is used in the same way to compare (mean) values with reference (mean) values when the variance is unknown and has to be estimated.

Hotelling's T^2 statistic can be applied on the ln k values of the markers, but also on their underlying factors obtained by U-PCA 2, 18–21]. In that case, not the original variables $\mathbf{x}^{(j)}$, but principal components, $\mathbf{t}_g^j$, that represent the original data are used. Hotelling's T^2 statistic can now be written as

$$T^2 = \left(\mathbf{t}_g^{(j)}\right)' \mathbf{S}_t^{-1} \left(\mathbf{t}_g^{(j)}\right) \quad (4)$$

with $\mathbf{t}_g^j$ the vector of the first g principal components (PCs) of the measurements on the used column after mean centring, and $\mathbf{S}_t$ the covariance matrix corresponding to the PCs. Because of the mean centring the mean of the reference scores (on the fresh column) is zero.

The T^2 values can be compared with a probability limit with, for instance $\alpha = 0.05$. This means that the probability is 5% that observations on the reference column fall outside the probability limit. For two PC's the limit is an ellipse. The ellipse can be calculated with the distribution of Hotelling's T^2 statistic. If $\mathbf{x}_i^{(0)} \sim N_{pm}(\boldsymbol{\mu}^{(0)}, \Sigma)$ and $\mathbf{x}^{(j)} \sim N_{pm}(\boldsymbol{\mu}^{(j)}, \Sigma)$ Hotelling's T^2 statistic is distributed according to

$$T^2 \sim (n+1)(n-1)\, pm/n(n-pm)\, F_{n-pm}^{pm} \quad (5)$$

The Hotelling T^2 statistic based on principal components is distributed according to [2,18–21]

$$T^2 \sim (n-1)\, g/(n-g)\, F_{n-g}^{g} \quad (6)$$

Monitoring T^2 on the first g PCs will only detect whether or not the variation in the plane of the first g PCs is greater than can be explained by random variation. When a totally new type of variation occurs corresponding to new underlying factors this can be detected with the squared prediction error (SPE).

$$\mathrm{SPE} = \sum \left(x_i^{(j)} - \hat{x}_i^{(j)}\right)^2, \quad (7)$$

with

$$\hat{\mathbf{x}}^{(j)} = \mathbf{t}_g^{(j)} \mathbf{P}_g'.$$

The SPE is often referred to as the Q-statistic [19], and again this can be used in combination with a control chart with a control limit based on a chosen level of significance.

4. A strategy

The strategy presented in this paper for the monitoring of columns during routine use consists of three phases. The *first phase* is the creation of a reference data set. If only a few analytes are used, these can be used as reference data when measured at various mobile phases. If more analytes are used a selection of solute/mobile phase combinations has to be made. Ageing is a difficult process to describe, because it has several systematic effects on the retention times. Therefore, a number of selected marker solutes have to be measured at various mobile phases to represent all effects. These so-called markers can be selected with variable selection techniques [14,15,22]. The markers have to represent the other solutes with respect to the different kind of changes. Some solute/mobile phase combinations should represent the hydrophobic changes and other combinations should represent selectivity changes or both changes.

The *second phase* is the construction of a reference situation. This concerns the construction of a reference model with three-way analysis. It also includes the notation of the reference scores, based on this model, in a plot of the first (2) components. With these scores, values on used columns can be compared.

The *third phase* is the monitoring during routine use itself. The markers are measured on a used column, at different time points, to investigate if it is aged or not. It is assumed that the peak shapes of the chromatograms of columns are first checked visually. Subsequently, three-way scores for the used column are calculated with the reference model and

the measurements on the used column. Hotelling's T^2 statistic, that gives an indication of the differences between the used column and the reference column, is calculated and denoted in the reference plots to detect if the differences with the reference situations are such that can be concluded that the column did age.

5. Results

5.1. Introduction

The theory in the previous sections can be used for monitoring columns during routine use. This is illustrated with the data obtained from the two columns described in Section 2. The *first phase* of constructing the reference data, including marker selection, was already performed in former research [2,3]. The resulting reference data are described in Section 2. Summarized, on the first column 8 replicated measurements of the marker solutes (BAB, PRE, ETB, NBA) at 3 mobile phases (wm1, wam2, wa3) were performed before ageing. On the second column duplicated measurements of the marker solutes at 3 mobile phases were performed before ageing.

The *second phase* concerns three-way analysis on the reference data. In the U-PCA case, this results in a score and loading plot for the reference data. The score plot will be used in the monitoring. Examples of score plots of reference data can be found in the figures of the next sections. For instance, Fig. 4 contains in the lower left a plot of the 8 reference scores, denoted by $*$, on the first column. Subsequently, SPE plots can be constructed. Examples can be found in Figs. 4–9, with $*$ representing the scores on the fresh column.

The *third phase*, concerning monitoring of used columns itself is illustrated in the sections below by some simulation experiments and a practical example with artificial aged columns. They are provided to

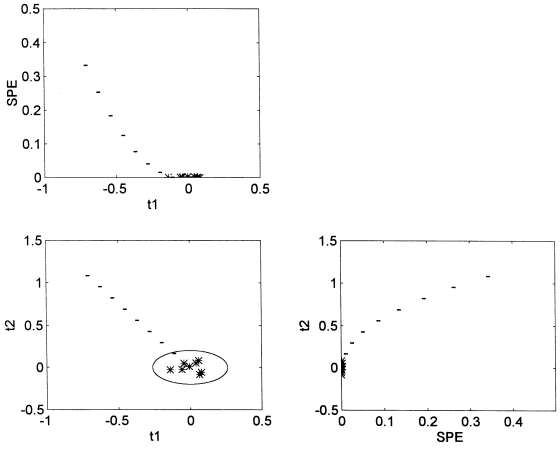

Fig. 4. Score plot based on the first two PCs (t1 and t2) and SPE plots, relative to t1 and t2, of a column with simulated hydrophobicity changes (−) compared to measurements when the column was still fresh (∗).

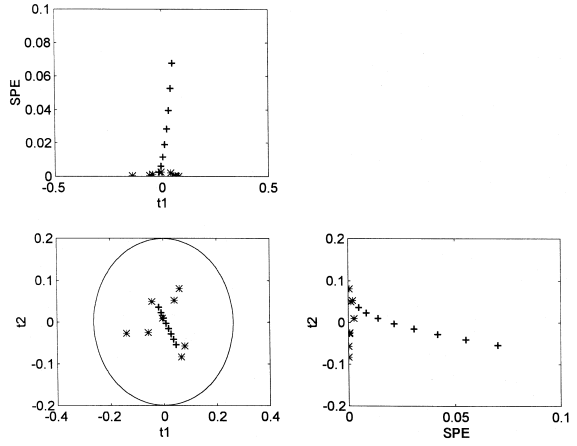

Fig. 5. Score plot based on the first two PCs (t1 and t2) and SPE plots, relative to t1 and t2, of a column with simulated selectivity changes (+) compared to measurements when the column was still fresh (∗).

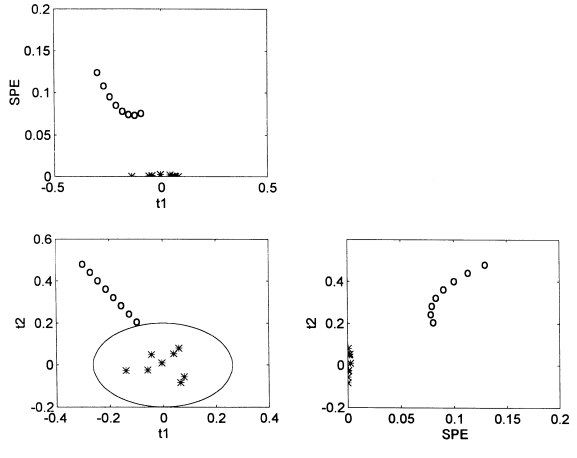

Fig. 6. Score plot and SPE plot of a column with simulated hydrophobicity and selectivity changes compared to measurements of the column before ageing.

show how various types and magnitudes of changes in retention can be detected during routine use with the use of plots based on three-way analysis and Hotelling's T^2 statistic. On certain time points, for instance every week before the column is used, the marker solutes are measured at the selected mobile phases. First, the chromatograms are checked visually. Here it is assumed, that the columns are not considered deteriorated based on this check. After that, scores and SPE values are calculated with the reference model for the measurements on the used column. The scores are denoted in a score plot and the SPE values in a SPE plot. These plots contain already the reference values with, eventually, control limits (phase 1 and 2). The position of the values in the plots of the used column tells something about the state of the columns. An idea can be formed of the type and magnitude of the changes in retention.

5.2. Simulation experiments

The simulation experiments are only partly simulated. The reference data set for the simulations contains real data. It consists of ln k values of the 8 observations of 4 solutes at 3 mobile phases (wm1, wam2, wa3) on the first column. For U-PCA this data cube is unfolded such that the direction of the 8 measurements stays the same. The aged ln k values are simulated. The height of the simulated aged ln k is of the same order as the values of real measurements, found in former research [2,3]. For the simulations a distinction is made between i) a situation in which the column only shows hydrophobicity changes (a shift in retention which is the same for all solutes), ii) a situation in which the column only shows selectivity changes (causing retentions of all solutes to change differently) and iii) a situation in which a column shows both hydrophobicity and selectivity changes.

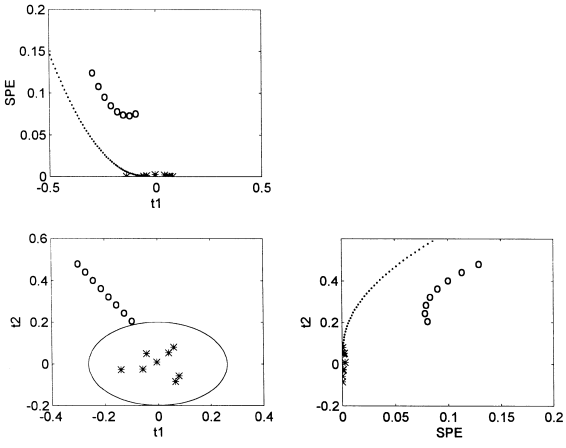

Fig. 7. Score plot and SPE plot of a column with simulated hydrophobicity changes compared to measurements on the column before ageing; A hydrophobicity line representing growing hydrophobicity changes is drawn in the SPE plots.

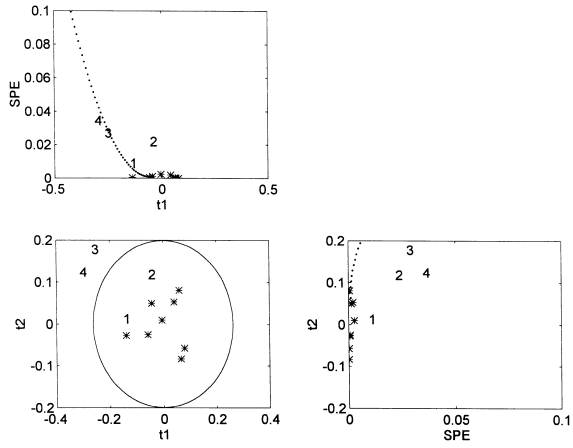

Fig. 8. U-PCA score plot and SPE plot of a column before and after ageing for 1, 2, 3 and 4 days with an eluent consisting of a phosphate buffer of pH 8 in 100% water.

The first situation concerns a column which shows a simulated increase in *hydrophobicity* changes. Starting point is the observation out of the 8 observations of the reference data set that is closest to the

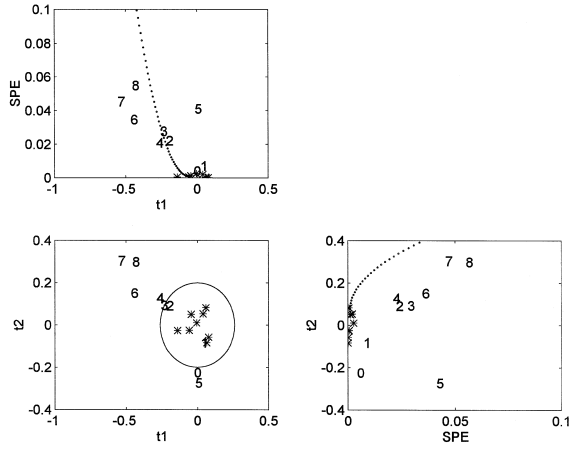

Fig. 9. U-PCA score plot and SPE plot of a fresh column and another column after 1 to 8 days of ageing.

average. From this (multivariate) observation of 4 solutes at the 3 mobile phases constant values are subtracted, each representing the columns after some time of ageing. In this way new, observations on the 'aged' column with the same shift (decrease) in retention for all solutes, are created. The subtracting constants vary from 0.05 to 0.4 by steps of 0.05. This means that 8 'aged' observations are created. As stated before, the height of the substraction constants is based on the results from former research [2,3]. The lowest subtraction constant is realistic when comparing with artificially aged columns in former research. The highest one (0.4) is quite large, but is used to give a good impression of the trend if retentions keep on decreasing with the same speed.

The lower left of Fig. 4, shows the PCA score plot with the scores of the reference data (∗) and the scores of the column with simulated hydrophobicity changes (−). A probability ellipse for $\alpha = 0.05$ around the reference data is also presented. The distance of the scores of the simulated aged column to the reference data is larger when the subtraction constant (change in hydrophobicity) is increased. A subtraction constant of 0.1 already has a score that falls outside the probability limit, indicating a significant change at the 5% level.

Fig. 4, shows two other plots, containing prediction errors (SPE values) of the reference data (∗) and the simulated aged columns (−) presented relative to the first PC (upper left) and second PC (lower right). Probability limits could have been given, but are not considered informative. All the presented aged columns would fall outside it. A clear deviation from the fresh scores shows that hydrophobicity changes cause changes in SPE values.

The second situation concerns a column that shows an increase in *selectivity* changes. This is simulated in a simple way by taking the same reference observation as in the first situation and add a constant value to the measured value of one of the markers (e.g., PRE). In this way, 8 measurements on an 'aged' column are simulated, with an increase in selectivity changes. The summation constant varies from 0.02 to 0.16. These are realistic constants, when compared with former research and when compared to the size of the hydrophobicity changes.

Fig. 5, shows the score plot with the scores of the reference data (∗) and the scores of the columns with simulated selectivity changes (+) in the lower left. A probability ellipse for $\alpha = 0.05$ around the reference data is also presented. The scores of the column with simulated selectivity lie between the scores of the reference data measured, in contrast to the simulated hydrophobicity changes. At a first glance, one might think that a reason for this lies in the fact that the substraction constants for the simulation of hydrophobicity changes are larger than the summation constants for the simulation of selectivity changes and that the substraction concerns all solutes instead of one. However, if score plots are created of simulated aged columns with different summation constants (positive or negative) for each solute, the scores still will lie between the reference data. Only when the summation constants are increased more than the size of the hydrophobicity changes the scores start to fall outside t he probability ellipse. These latter constants are not realistic. In reality, the hydrophobicity changes are larger in absolute values than the subtle changes in selectivity. Therefore, it seems that if scores fall outside the probability ellipse this is most likely due to hydrophobicity changes.

Fig. 5, also shows two SPE plots of the reference data (∗) and the columns with simulated selectivity changes (+), presented relative to the first PC (upper left) and second PC (lower right). In these plots it can easily be seen that the SPE values of the column with simulated selectivity changes behave different from the values on the fresh column. The SPE values are lower than the SPE values of the simulated hydrophobicity changes, but they can be distinguished from the values on the fresh column and therefore detect changes.

The third situation concerns a column that shows an increase in both *hydrophobicity and selectivity* changes. This is simulated by subtracting a constant of all the fresh measurements used in the first two situations and adding a positive constant on one of the markers (e.g., PRE) and a negative constant on another marker (e.g., NBA). The measurements on the column are simulated, with an increase of the subtraction constant for all measurements from 0.05 to 0.4 and a positive summation constant for PRE from 0.02 to 0.16, and a decrease from the negative summation constant for NBA from -0.16 to -0.02.

Fig. 6, shows the score plot with the scores of the reference data (∗) and the scores of the column with

simulated hydrophobicity and selectivity changes (o) in the lower left. A probability ellipse for $\alpha = 0.05$ around the reference data is also presented. The scores of the columns with simulated changes fall outside the probability limit. This indicates that, according to situation 1, there are hydrophobicity changes.

Fig. 6, shows also two SPE plots of the reference data (∗) and the columns with simulated changes (o) presented relative to the first PC (upper left) and second PC (lower right). In these plots it can easily be seen that the SPE values of the columns with simulated hydrophobicity and selectivity changes behave different compared to the fresh column. The curvature in the trend can be explained by opposite increase (decrease) of the positive and negative summation constant. The difference between reference SPE values and SPE values corresponding to the aged column can possibly be explained by hydrophobicity changes as well as selectivity changes.

Plots like Fig. 6 in practice, can point to columns that only show hydrophobicity changes or columns that show both hydrophobicity and selectivity changes. To detect, in practice, if there are only hydrophobicity changes or also selectivity changes, a 'hydrophobicity line' can be drawn in the SPE plots. Fig. 7, is the same as Fig. 6 except for a so called 'h(ydrophobicity)-line', denoted by dots. This line represents all possible SPE values that can be calculated if an arbitrary constant between 0 and 1 is subtracted from the reference value. An aged column that only shows a shift in retention or in other words that only shows hydrophobicity changes, will in theory have SPE values on this line. Therefore, if a SPE value of a real aged column is far from this line it probably shows selectivity changes. If one SPE value of a column is near to this line not much can be said of the column, unless the course of SPE values of a column during routine use is about the same as this h-line. In this last case, there are probably only hydrophobicity changes. If the trend is completely different there are probably also selectivity changes.

Some remarks have to be made with respect to the simulations. No simulations were shown with subtraction or summation constants that varied over the mobile phases. This aspect, however, was studied and it was found that results did not differ much if different effects at various mobile phases were incorporated, as long as the effects were not too large. However, large differences in effects at various mobile phases are not realistic in practice. A decrease in overall retention at one mobile phase is often accompanied by a comparable decrease at another mobile phase. With respect to above mentioned remarks it has to be noted that the h-line should not be considered as an exact line; it can only be used as a good indicator.

5.3. Practical example

In this example artificially aged columns are monitored. The reference data are the same as in the simulation experiments described in Section 2. The first column with 8 replicated measurements before ageing was artificially aged for 1, 2, 3 and 4 days, with an eluent containing a phosphate buffer of pH 8 in 100% water. After each ageing day, duplicated measurements were done on the 4 marker solutes at the 3 marker mobile phases. The reference measurements and the measurements after ageing are denoted in Fig. 8. The reference model, including the probability ellipse and the scores (∗) for the reference data, are the same as in Figs. 4–7. The scores of the columns after ageing are denoted by a number referring to the days of ageing.

In former research [2,3] the above mentioned and other columns were investigated, after ageing, based on a large number of measurements (14 solutes at 9 mobile phases). The investigation resulted in a categorization of the columns in 5 categories: not aged, only hydrophobicity changes, only selectivity changes, both selectivity changes and hydrophobicity changes, and aged too much. The above mentioned column was after 1 and 2 days categorized as showing only selectivity changes, and after 3 and 4 days as showing both selectivity and hydrophobicity changes. In Fig. 8 it can be seen very clear that only when the column shows large hydrophobicity changes the T^2 value falls outside the probability ellipse, while all columns have high SPE values. In the SPE plot relative to the second PC it can be seen that the course of the column is not the same as the course of a column that only shows hydrophobicity changes, because this column would have a course similar to the dotted h-line. These results corroborate the simulations.

Unfortunately, the column showed a large hole after 4 days of ageing and could not be used any more. Therefore, another column, packed with the same stationary phase, was also aged for several days. Fig. 9 shows the score plot and SPE plots of this column. The reference data are the data measured on the first column and thus the same as in Fig. 8 and the same as used in the simulations, but the data representing ageing are measured on the second column. Because, only duplicated measurements were performed on the second fresh column, the fresh measurements on the first column are used as reference data. One of the measurements in Fig. 9 is denoted by the number 0. This represents the fresh measurement on the second column. The (second) column was aged for 8 days and measured after every day of ageing. The numbers refer to the days of ageing.

The measurement before ageing lies just under the probability ellipse of the fresh measurements on the first column. This indicates that the probability ellipse for the second column is probably too small. There is extra variation, because two columns were used; one as reference column and one in the ageing experiments. Till 5 days of ageing the T^2 values lie however in the probability ellipse. After 5 days the values start falling outside the ellipse. The SPE values also grow from 0 to 8 days of ageing. A nice course through time can be noted. After 5 days of ageing there were some problems. Probably there were some air bubbles in the pump. The chromatograms showed a lot of noise. It can be seen that the T^2 and SPE values are deviant from the other measurements. From Fig. 9 it can be concluded that in practice the state of a column can be followed very well through time. This can be used to decide if and what kind of calibration models are needed for optimal mobile phase updating. Suggestions when updating is needed with what kind of models, are presented in Section 6.

6. Conclusions and proposed strategy

In this paper a new approach for the quality assessment of aged columns in practice is presented. The approach is based on a first visual check of chromatograms followed by a monitoring with statistical quality control charts based on three-way analysis. At the end of this section, a proposed strategy for dealing with this in practice, visualized in a monitoring scheme, will be presented.

Important in the strategy are three phases. The first phase concerns the construction of the reference data. These are retention values of a well chosen selection of all analytes (markers) measured on the fresh (not used) column. The second phase concerns the construction of the reference situation including the reference model. Scores and prediction errors (of the reference data) of a three-way analysis are denoted in charts, and a Hotelling's T^2 probability ellipse is calculated and also denoted in the score chart. The third phase concerns the measuring of the markers on the used column at certain time points, the calculation of the scores and predictions errors (SPE values) of the used columns with the reference model, and the notation of these in the charts. When this is done, changes in retention can be detected by use of the values of Hotelling's T^2 and the SPE values.

If the value of Hotelling's T^2 statistic and the prediction error for an aged column do not deviate much from these values for the fresh column, the aged column can be considered as showing only minor changes.

If the value of the prediction error for an aged column deviates much from the values for the fresh column and Hotelling's T^2 does not deviate, the aged column can be considered as showing mainly selectivity changes.

If the value of Hotelling's T^2 statistic deviates from the values for the fresh column independent of the prediction error, the aged column can be considered as showing at least (large) hydrophobicity changes.

If the value of Hotelling's T^2 statistic and the prediction error for an aged column deviate both from the values of the fresh column, the aged column can be considered as showing only hydrophobicity changes or showing hydrophobicity and selectivity changes. An 'h-line', representing a same shift in retention for all solutes and mobile phases of different size, can give an indication of the trend of a column that only shows hydrophobicity changes. If the course of the SPE values on an aged column deviates much from this line, probably some selectivity changes exist.

With the rules above it is shown that it is more or less possible to make a distinction between large hy-

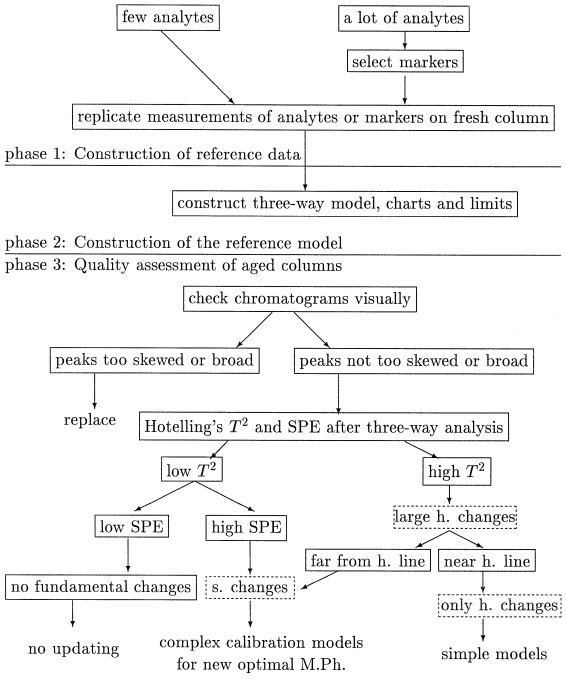

Fig. 10. Monitoring scheme for the quality assessment of columns during routine use.

drophobicity changes, selectivity changes and no changes at all. These different types of changes have to be handled differently in further usage of the columns. Columns that did hardly change compared to the fresh columns can be considered as fresh columns. Columns with only hydrophobicity changes can do with a simple linear updating. Columns with changes in selectivity need calibration models (e.g., PLS models) to predict retention values and update mobile phase compositions [3].

For the calibration and mobile phase optimization the same measurements can be used as for the monitoring. Besides that this saves a lot of work, it makes it easy, in future, to construct a computer program for monitoring used columns as well as for their calibration and updating (mobile phase optimization).

In this paper, theory and examples were based on U-PCA. In practice, however, other three-way techniques, such as PARAFAC are as easy to work with as U-PCA [1] In fact PARAFAC has some advantages above U-PCA. If the models are also used to get an overview of the structure in the data (before monitoring), PARAFAC gives a more clear overview than U-PCA. PARAFAC can show the effects in all directions separately, while U-PCA combines directions. Moreover, PARAFAC gives unique solutions and therefore does not show rotation problems as U-PCA plots do. Finally, PARAFAC is more efficient, because less parameters have to be estimated. However, the PARAFAC pictures for the examples used in this paper do not differ that much from U-PCA pictures that other conclusions could be drawn. Therefore, the U-PCA pictures are here as illustrative as PARAFAC pictures would be.

Based on the foregoing a strategy is proposed for monitoring used columns in practice. This proposed strategy is visualized in Fig. 10.

[1] In the PARAFAC model, the factors $\mathbf{a}_g$ are used instead of the factors $\mathbf{t}_g$ in U-PCA.

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References

[1] A. Houben-Michalkova, Study of ageing processes of chemically modified silica substrates in liquid chromatography, Internal report Eindhoven University of Technology, December.
[2] A. Bolck, A.K. Smilde, C.A.A. Duineveld, P.M.J. Coenegracht, Multivariate quality assessment of aged RP-HPLC columns, J. Chemometrics 10 (1996) 351–370.
[3] A. Bolck, Chemometric analysis of aged RP-HPLC stationary phases, PhD thesis, Rijks Universiteit Groningen, Groningen, the Netherlands, May 1996.
[4] T. Jolliffe (Ed.), Principal Component Analysis, Springer Verlag, New York, 1986.
[5] H.G. Law, C.W. Snyder, J.A. Hattie, R.P. McDonald, Research Methods for Multimode Data Analysis, Praeger, New York, 1984.

[6] J.D. Caroll, S. Pruzansky, J.B. Kruskall, Candelinc: a general approach to multidimensional analysis of many-arrays with linear constraints on parameters, Psychometrika 45 (1980) 3–24.
[7] P.M. Kloonenberg, J. de Leeuw, Principal component analysis of three-mode data by means of alternating least squares algorithms, Psychometrika 45 (1984) 69–97.
[8] R.A. Harshman, Foundations of the PARAFAC procedure: models and conditions for exploratory multi-mode factor analysis, UCLA Working Phonetics 16 (1970) 1–84.
[9] L.R. Tucker, Implications of factor analysis of three-way matrices for measurement change, in: C. Harris (Ed.), Problems of Measuring Change, Madison, Univ. of Wisconsin Press, 1963, pp. 122–137.
[10] S. Wold, P. Geladi, K. Esbensen, J. Öhman, Multi-way principal components and PLS analysis, J. Chemometrics 1 (1987) 41–56.
[11] C.L. de Ligny, M.C. Spanjer, J.C. van Houwelingen, H.M. Weesie, Three-mode factor analysis of data as retention in normal-phase high-performance liquid chromatography, J. Chromatogr. 301 (1984) 311–324.
[12] J. Öhman, P. Geladi, S. Wold, J. Chemometrics 4 (1990) 79–88.
[13] J. Öhman, P. Geladi, S. Wold, J. Chemometrics 4 (1990) 135–146.
[14] A.K. Smilde, C.H.P. Bruins, P.M.J. Coenegracht, D.A. Doornbos, Characterization of stationary-mobile phase combinations by using markers: Part 1. preliminary results for the prediction of rp retention on various stationary phases, Anal. Chim. Acta 212 (1988) 95–104.
[15] A.K. Smilde, P.M.J. Coenegracht, C.H.P. Bruins, D.A. Doornbos, Multivariate calibration strategy for reversed phase chromatographic systems based on the characterization of stationary phase-mobile phase combination with markers, J. Chromatogr. 485 (1989) 169–181.
[16] H. Hotelling, Multivariate quality control, illustrated by the air testing of sample bombrights, in: C. Eisenhart, M.W. Hastay, W.A. Wallis (Eds.), Techniques of Statistical Analysis, New York, Academic Press, 1947, pp. 113–184.
[17] T.W. Anderson, An Introduction to Multivariate Statistical Analysis, Wiley, New York, 1984.
[18] S.J. Wierda, Multivariate statistical process control; recent results and directions for future research, Statistica Neerlandica 48 (1994) 147–168.
[19] J.E. Jackson, G.S. Mudholkar, Control procedures for residuals associated with principal component analysis, Technometrics 21 (1979) 341–349.
[20] P. Nomikos, J.F. MacGregor, Multivariate spc charts for monotoring batch processes, Technometrics, January 1994, pp. 1–29.
[21] J.V. Kresta, J.F. MacGregor, T.E. Marlin, Multivariate statistical monotoring of process operating performances, Can. J. Chem. Eng. 69 (1991) 35–47.
[22] G.P. McCabe, Principal variables, Technometrics 26 (1984) 137–144.