Talanta 117 (2013) 492–504

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Blending process modeling and control by multivariate curve resolution

J. Jaumot [a,*], B. Igne [b], C.A. Anderson [b], J.K. Drennen [b], A. de Juan [c]

[a] Department of Environmental Chemistry, IDAEA-CSIC, Jordi Girona, 18-26, 08034 Barcelona, Spain
[b] Duquesne Center for Pharmaceutical Technology, Duquesne University, 600 Forbes Avenue, Pittsburgh, PA 15282, USA
[c] Chemometrics Group, Department of Analytical Chemistry, University of Barcelona, Diagonal 645, 08028 Barcelona, Spain

ARTICLE INFO

Article history:
Received 8 May 2013
Received in revised form
12 September 2013
Accepted 20 September 2013
Available online 27 September 2013

Keywords:
Multivariate Curve Resolution
Processes analysis
Correlation constraint
End-point detection
Homogeneity
Blend trajectories

ABSTRACT

The application of the Multivariate Curve Resolution by Alternating Least Squares (MCR-ALS) method to model and control blend processes of pharmaceutical formulations is assessed. Within the MCR-ALS framework, different data analysis approaches have been tested depending on the objective of the study, *i.e.*, knowing the effect of different factors in the evolution of the blending process (modeling) or detecting the blend end-point and monitoring the concentration of the different species during and at the end of the process (control).

Data analysis has been carried out studying multiple blending runs simultaneously taking advantage of the multiset mode of the MCR-ALS method. During the ALS optimization, natural constraints, such as non-negativity (spectral and concentration directions) have been applied for blend modeling. When blending control is the main purpose, a variant of the MCR-ALS algorithm with correlation constraint in the concentration direction has been additionally used. This constraint incorporates an internal calibration procedure, which relates resolved concentration values (in arbitrary units) with the real reference concentration values in the calibration samples (known references) providing values in real concentration scale in the final MCR-ALS results.

Two systems consisting of pharmaceutical mixtures of an active principle (acetaminophen) with two or four excipients have been investigated. In the first case, MCR results allowed the description of the evolution of the individual compounds and the assessment of some physical effects in the blending process. In the second case, MCR analysis allowed the detection of the end-point of the process and the assessment of the effects linked to variations in the concentration level of the compounds.

1. Introduction

Blending processes are of utmost importance in the industrial production of pharmaceuticals or food commodities [1–4]. The Quality by Design paradigm promotes the use of process analytical technologies to help assess in real time, the state of a unit operation. Pharmaceutical Quality by Design (QbD) is a "systemic approach to pharmaceutical development that begins with predefined objectives and emphasizes product and process understanding and process control" [5,6]. Blending is critical in ensuring uniformity of composition in the final dosage form, as part of the multiple unit-operations involved in the manufacturing process of pharmaceutical solid dosage forms [7,8]. Problems incurred during blending can lead to inadequate tablet quality attributes such as content uniformity, assay, disintegration time, dissolution behavior, *etc.*, all of which can directly impact dosage form efficacy, in-vivo performance, and patient safety. Controlling blend homogeneity is a necessary step in a drug product manufacturing process [9]. When a blend process is analyzed, two main objectives can be of interest: first, optimizing the process parameters to improve the blending operation and, second, detecting when the blend process has reached an homogeneity standard set by external regulations or by the manufacturer [10–13]. The first objective requires modeling the blend process, *i.e.*, describing how the variation of experimental conditions can affect the evolution of the blending profiles (trajectories) of the compounds in the production system, whereas the second calls for blend control, *i.e.*, obtaining quantitative information on the process (concentration values of blended compounds) that allows deciding when the blend end-point, or homogeneity, has been reached.

Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS) is a chemometric method that has been widely used for process analysis in industrial, biological or chemical contexts [14–20]. The term process here must be interpreted as any continuous physical/chemical transformation that can be monitored by an instrumental

* Corresponding author. Tel.: +34 934006140.
E-mail address: joaquim.jaumot@idaea.csic.es (J. Jaumot).

0039-9140/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2013.09.037

response. This includes from classical reaction systems, to chromatographic elutions or to physical changes, such as blending or polymorph transitions [21,22]. Classical MCR-ALS acts as a soft-modeling approach on the raw process (spectroscopic) data to provide concentration profiles, *i.e.*, the evolution of the compounds involved in the process as a function of the control variable (time, pH, temperature …), and their related pure spectra. In this situation, the inclusion of knowledge about the system in the form of natural constraints (non-negativity in the concentration and spectral profiles, unimodality, closure, …) is enough to drive the resolution to optimal solutions from both mathematical and chemical viewpoints [23]. When the underlying physicochemical model driving the process is known, this information can also be included on the hybrid variant of the algorithm incorporating hard-modeling information [24].

Classical MCR provides concentration profiles and pure responses of the compounds involved in a process in arbitrary units. However, in some situations, it is interesting to obtain quantitative information in addition to the qualitative one. In order to obtain this supplementary information, the application of a variant of the MCR-ALS algorithm with a correlation constraint is needed, in which an internal calibration model is built relating the real concentration values of calibration samples to the ones in arbitrary units provided by the constrained least-squares calculated concentration profiles during the MCR optimization procedure [25–28]. The model established helps to rescale the concentration values found by the resolution algorithm (in calibration and test samples) to the real concentration units during the iterative optimization. In this way, the final concentration profiles provided by MCR-ALS with the correlation constraint include quantitative information comparable to that furnished by other multivariate calibration methods.

The quantitative information provided by the new correlation constraint can be especially useful to control processes in industrial environments, in which either the normal operation conditions or the final product quality are very well defined in quantitative terms. An example of this kind is the blending process of pharmaceutical formulation, in which there is a complete knowledge about the individual components that will form the mixture and compliance of nominal composition and homogeneity is required before proceeding to the compression of the sample into the commercial tablet form.

Control of the composition of the mixture was traditionally carried out by sampling from the blender and, then, performing an *ex situ* quantitative analysis until the homogeneity/composition standards was fulfilled [29–31]. Nowadays, continuous *in situ* sampling using NIR probes that allow obtaining immediate knowledge regarding the blending process are being developed [32,33]. However, it is necessary to apply data analysis methods that can work efficiently with these multivariate data sets. In the literature, several methods can be found to monitor the variations that occur in the mixture and to determine the end-point of the blending process according to certain criteria [2,11,31,33].

This paper proposes the use of Multivariate Curve Resolution by Alternating Least Squares method for the double purpose of modeling and controlling the blending of a solid pharmaceutical formulation.

Modeling of the blending trajectories allows assessing the effect of modifying process control variables (filling of the blender, rotation speed …) and can be carried out by classical MCR using natural constraints in a calibration-free mode. This information can be gathered from the evolution and shape of the blending concentration profiles (in arbitrary units).

Control of the blending run (composition along the run and detection of the end-point of the process) is obtained when MCR is used with the correlation constraint that allows recovering quantitative information. In this case, the blend concentration profiles are obtained in real concentration units. Within the quantitative approach, several variants of application will be tested that simulate both *ex situ* (after the run is over) and *in situ* (for ongoing runs) control, or compound-specific and global homogeneity detection that are of interest from an industrial point of view.

2. Materials and methods

2.1. Experimental work

Five components were used in the different formulations: acetaminophen (APAP; Rhodapap, Rhodia Organique, Roussillon, France), lactose (monohydrate NF – product 316/Fast-Flo modified spray-dried; Foremost Farms USA, Rothschild, WI, USA), microcrystalline cellulose (MCC; Avicel PH 200, FMC Biopolymer, Mechanicsburg, PA, USA), croscarmellose sodium (cros, Spectrum, Gardena, CA, USA) and magnesium stearate (MgSt; Mallinckrodt, Hazelwood, MO, USA).

Ingredients were mixed simultaneously in a 3.5 quart, stainless steel, custom-made V-blender. A SpectralProbes Process NIR spectrometer (Thermo Fisher Scientific, Wilmington, MA, USA; serial numbers 1277) was used to monitor the blending process in real-time acquiring one spectrum every 4 s if rotation speed is 15 rpm and every 2.4 s if rotation speed is 25 rpm. Measurements were made through a sapphire window in the top of an arm of the blender. Spectra were sent wirelessly to a computer and imported into a custom-made acquisition and analysis software. NIR spectra were formed by 100 absorption values between 1600 and 2400 nm in reflectance mode and the probe was triggered by a light intensity sensor. For further information of the experimental details, the readers are referred to previous works [11,12].

2.2. Sample sets

Two different data sets have been used in this work. First, a three-component system (one active principle, APAP, and two excipients, MCC and MgSt) has been considered. This system has been used to study the ability of MCR-ALS to model blend processes and to identify physical effects related to the blend evolution. The experimental design, a 4-factor 2-level full factorial design (Table 1) included as factors: the concentration level of the active principle (APAP) in the formulation (3% or 30%); the rotation speed of the blender (15 or 25 rpm), the fill level of the blender (50% or 70%), and the ingredients loading mode (side to side or top to bottom).

Second, a five-component system (one active principle, APAP, and four excipients, lactose, MCC, cros and MgSt) has been studied to assess the ability of MCR-ALS to detect the end-point of the blending process and, in general, to perform quantitative blending control. In this case, Table 2 shows 7 runs with a large range of concentrations for APAP (five concentration levels: 21.0%, 25.5%, 30.0%, 34.5% and 39% w/w) and three lactose:MCC ratio levels (0.4, 1.4 and 2.4). In this case, the amount of croscarmellose sodium and magnesium stearate has been the same in all the batches since they act just as agglutinants. Additionally, two more runs were used as test runs mimicking the concentration level of the target formulation: APAP 30%, lactose 37.3% and MCC 26.7% (lactose:MCC ratio equal to 1.4).

2.3. Chemometric analysis

2.3.1. Data pretreatment

In this work, experimental spectra have been pretreated by means of Multiplicative Scatter Correction (MSC) prior to the chemometric analysis [34].

This pretreatment method gives an estimation of the relation of the scatter of each sample with respect to the scatter of a reference

sample to balance the presence of this phenomenon in the full data set. First, a reference spectrum is selected, usually the mean spectrum ($\mathbf{d}_m$) of the calibration set. Every spectrum ($\boldsymbol{d}_i$) is related to the reference spectrum by the linear equation:

$$\mathbf{d}_i = a_i\mathbf{1} + b_i\,\mathbf{d}_m + \mathbf{e}_i \quad (1)$$

where **1** is a vector of ones of the same length than $\mathbf{d}_i$, a_i (intercept) indicates a constant linear absorption additive effect with respect to the mean spectrum $\mathbf{d}_m$, b_i (slope) indicates the influence of scattering multiplicative effects in the absorption and $\mathbf{e}_i$ is the residual spectrum. MSC of the spectra is performed by correcting the value, d_{ij}, of each spectrum i and wavelength j by:

$$\hat{d}_{ij} = \frac{(d_{ij} - a_i)}{b_i} \quad (2)$$

Fig. 1 shows the effect of the MSC treatment on the spectra of calibration run 4 in Table 2. From the comparison of the raw and the MSC treated data, it can be seen that MSC compensates for the different scattering present in the different NIR recorded spectra.

Table 1
Experimental design for the 3-component dataset.

Run Nr.	APAP (%)	MCC (%)	MgSt (%)	APAP Level[a]	Loading mode[b]	Speed (rpm)	Fill level (%)[c]
1	3.0	96.5	0.5	Low	Top to Bottom	15	50
2	3.0	96.5	0.5	Low	Top to Bottom	15	70
3	3.0	96.5	0.5	Low	Top to Bottom	25	50
4	3.0	96.5	0.5	Low	Top to Bottom	25	70
5	3.0	96.5	0.5	Low	Side to side	15	50
6	3.0	96.5	0.5	Low	Side to side	15	70
7	3.0	96.5	0.5	Low	Side to side	25	50
8	3.0	96.5	0.5	Low	Side to side	25	70
9	30.0	69.5	0.5	High	Top to Bottom	15	50
10	30.0	69.5	0.5	High	Top to Bottom	15	70
11	30.0	69.5	0.5	High	Top to Bottom	25	50
12	30.0	69.5	0.5	High	Top to Bottom	25	70
13	30.0	69.5	0.5	High	Side to side	15	50
14	30.0	69.5	0.5	High	Side to side	15	70
15	30.0	69.5	0.5	High	Side to side	25	50
16	30.0	69.5	0.5	High	Side to side	25	70

[a] Low concentration level (3%, w/w), high level (30% w/w) in global formulation.
[b] Side to side (APAP one side, MgSt the other, MCC at the bottom) or top to bottom (equally distributed).
[c] Fill level (how full is the blender).

Table 2
Concentration of the calibration and test samples for the 5-component dataset.

Run Nr.	APAP (%)	Lactose (%)	MCC (%)	Cros (%)	MgSt (%)
Calibration 1	25.5	48.2	20.3	5.5	0.5
Calibration 2	25.5	20.3	48.2	5.5	0.5
Calibration 3	34.5	17.7	41.8	5.5	0.5
Calibration 4	30.0	37.3	26.7	5.5	0.5
Calibration 5	21.0	42.5	30.5	5.5	0.5
Calibration 6	34.5	34.7	24.8	5.5	0.5
Calibration 7	39.0	32.0	23.0	5.5	0.5
Test 1	30.0	37.3	26.7	5.5	0.5
Test 2	30.0	37.3	26.7	5.5	0.5

2.3.2. MCR analysis

Only some relevant aspects of the MCR-ALS procedure are given here for brevity. For a more detailed description of the method, see previous works and references therein [15,35,36].

MCR-ALS solves iteratively the Eq. (3) by an Alternating Least Squares algorithm which calculates concentration $\boldsymbol{C}$ and pure spectra $\mathbf{S}^{\mathbf{T}}$ matrices that optimally fit the experimental data matrix **D** with non-modeled residuals **E**.

$$\mathbf{D} = \mathbf{C}\mathbf{S}^{\mathbf{T}} + \mathbf{E} \quad (3)$$

This optimization is carried out for a proposed number of components and using initial estimates of either **C** or $\mathbf{S}^{\mathbf{T}}$. In this work, initial estimates were determined using a pure variable detection method based on the SIMPLISMA approach [37].

MCR-ALS multiset analysis of multiple independent experiments run under different experimental conditions is a useful and powerful strategy to improve resolution [17,18,35,38,39]. This strategy implies the analysis of a column-wise augmented data matrix, in which the resolved pure spectra of the same species are common for all experiments and experiment-to-experiment variation is allowed for the resolved concentration profiles. Runs combined in the multiset structure can be of different nature and size, *e.g.*, runs from an experimental design, calibration and/or test runs, spectral information about pure compounds, *etc.*, depending on the purpose of the analysis. Eq. (4) shows the bilinear MCR-ALS model for a multiset system ($\mathbf{D_1}$, $\mathbf{D_2}$,…, $\mathbf{D_n}$), formed by **n** $\mathbf{D_i}$ experiments, which is expressed by a common pure spectra matrix $\mathbf{S}^{\mathbf{T}}$ and submatrices of process profiles $\mathbf{C_1}$, $\mathbf{C_2}$, …, $\mathbf{C_n}$ related to $\mathbf{D_1}$, $\mathbf{D_2}$ … $\mathbf{D_n}$ respectively.

$$\begin{bmatrix}\mathbf{D}_1\\ \mathbf{D}_2\\ \vdots\\ \mathbf{D_n}\end{bmatrix} = \begin{bmatrix}\mathbf{C}_1\\ \mathbf{C}_2\\ \vdots\\ \mathbf{C_n}\end{bmatrix}\mathbf{S}^{\mathbf{T}} + \begin{bmatrix}\mathbf{E}_1\\ \mathbf{E}_2\\ \vdots\\ \mathbf{E_n}\end{bmatrix} \quad (4)$$

Convergence during the optimization is achieved when in two consecutive iterative cycles, relative differences in standard deviations

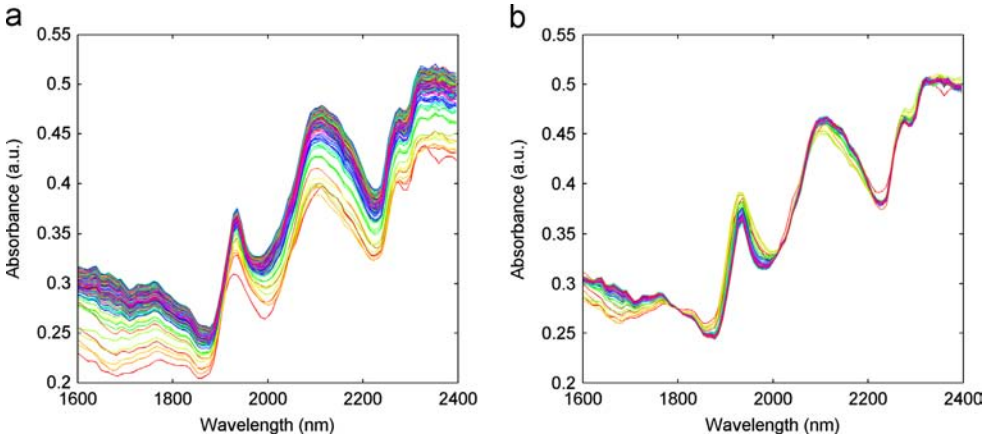


Fig. 1. Experimental NIR spectra (a) and MSC corrected spectra (b) of Run 4 (see composition in Table 2).

of the residuals between experimental and ALS calculated data values are less than a previously selected value, in this case 0.1%. Figures of merit of the optimization procedure are the percent of lack of fit (% LOF) and the percent of variance explained (%R^2). Lack of fit is defined as the difference among the input data **D** and the data reproduced from the $\mathbf{CS^T}$ product obtained by MCR-ALS. This value is calculated according to the expression:

$$\%\,LOF = 100\sqrt{\frac{\sum_{ij} e_{ij}^2}{\sum_{ij} d_{ij}^2}} \tag{5}$$

where d_{ij} designs an element of the input data matrix **D** and e_{ij} is the related residual obtained from the difference between the input element and the MCR-ALS reproduction.

In classical MCR-ALS, several constraints can be applied to model the shapes of the **C** and $\mathbf{S^T}$ profiles during the iterative optimization. In the context of a blending process, non-negativity can be applied to both the blending (concentration) profiles and their related resolved spectra. This strategy is sufficient to study the effect of changing experimental factors in the evolution of the blending process (blending trajectories) and would also be valid to allow for a qualitative detection of the blend homogeneity (*i.e.*, concentration values for each compound would evolve in a realistic way, but the concentration scale would be in arbitrary units). In general, this approach, which we will denominate classical MCR, allows for the qualitative modeling of the blending evolution.

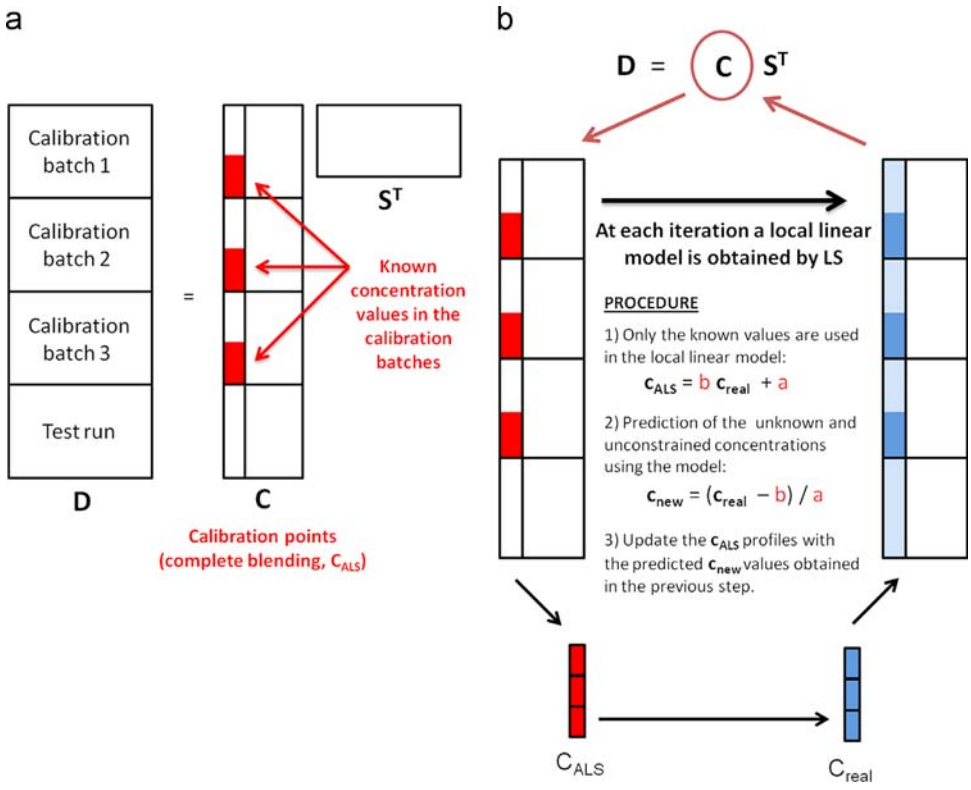
However, a variant of MCR-ALS using a correlation constraint can be used with the aim of obtaining quantitative information, i.e, concentration profiles in real concentration units [25–27]. To do so, within each iterative cycle of the MCR optimization, the correlation constraint works by performing an inner regression model between least-squares calculated MCR-ALS concentration values (in arbitrary units) and reference concentration values that are known for some points in the blending process; in this case, the concentrations from the points in the calibration blending runs that are considered to have reached homogeneity. For these homogeneous blending points, the reference concentration values adopted are those of the nominal composition of the prepared mixture. Once this regression model is established, it is used to predict real concentrations in points of the blending profiles where no reference concentrations are available, *i.e.*, points before blending is achieved in calibration blending runs and full concentration profiles in test blending runs. Updated correlation-constrained concentration profiles in real concentration units are submitted to the MCR-ALS optimization and the procedure continues likewise in following cycles until convergence is achieved. In this way, the final blending profiles can be interpreted in a straightforward way since they are in real concentration units (see Scheme 1).

The correlation constraint acts on one single concentration profile at a time and, therefore, a univariate calibration model is obtained per each compound constrained in the system. As any other constraint, the correlation constraint can be applied to a single compound, *e.g.*, to API if only the concentration of this compound would be controlled, or to some or even all the concentration profiles at once (in all cases, we will have as many univariate calibration models as compounds constrained).

For each of the univariate calibration models, quality parameters such as the correlation coefficient (R) or the Root Mean Square Error of Calibration (RMSEC) calculated following the equation [40]:

$$RMSEC = \sqrt{\frac{\sum_{i=1}^{m} (\hat{y}_{i,cal} - y_{i,cal})^2}{m}} \tag{6}$$

where m is the number of samples used in the building of the univariate model, $y_{i,cal}$ are the known values of the species concentration and $\hat{y}_{i,cal}$ are the predicted values of the species concentration, can be obtained.



Scheme 1. (a) Multiset structure formed by blending calibration and test runs and related MCR model. (b) Application of correlation constraint in blending ***C*** matrix (real values of calibration points are kept constant and blending points in test runs or in non-homogeneous zones of calibration runs are predicted).

It is important to point out some differences among the way of functioning of MCR-ALS with correlation constraint and other multivariate calibration methods, such as CLS or PLS. MCR-ALS and CLS share the same underlying interpretable bilinear model, but the main difference is that CLS performs a single multivariate calibration model relating the full matrix **C** to **D**. As a consequence, the concentrations of all compounds in the samples need to be known. MCR-ALS performs compound-specific individual univariate calibration models, which allows having quantitative information for all compounds or only for some of them, depending on the number of compounds constrained. The possibility to constrain partially the system precludes the need to know the total quantitative composition of the sample and calibration in the presence of unknown or interfering compounds can be carried out. With respect to PLS, the main difference is interpretability (pure spectra are recognizable in MCR-ALS and so are the concentration profiles of compounds not included explicitly in the calibration model); in similarity with PLS, MCR-ALS with correlation constraint can perform quantification of only some of the compounds of the data set in the presence of interferences/unknown compounds. If we compare PLS2 and MCR-ALS to model simultaneously all compounds, the difference is the same as with CLS, MCR-ALS does individual models per each compound, whereas PLS2 builds a global model among **D** and **C**.

2.3.3. MCR approaches to study blending data

In this work, several MCR approaches have been tested in order to compare the performance of the method in different conditions.

2.3.3.1. Classical MCR: Study of the effect of experimental factors on the blending process. Qualitative assessment of homogeneity. Classical MCR analysis consists of the study of a multiset structure by using only natural constraints (non-negativity for concentration blending profiles and spectra) and normalization forcing the pure spectra in matrix $\mathbf{S}^{\mathbf{T}}$ to have equal length. The multiset structure could be formed by blending runs formed by samples with the same or different concentration levels and prepared under different conditions. Whenever is needed and to help in the resolution process, appending spectral information about some compounds in the formulation (e.g, a blending run of pure API if this is the compound to be controlled) is also possible (see Scheme 2a).

The effect of varying experimental conditions (factors) in the blending process can be assessed because interpretable changes in the shape and evolution of the blending trajectories are obtained. In addition, qualitative assessment of the homogeneity will be carried out by finding the point when one (or more) blending profile reaches a stable and invariant plateau.

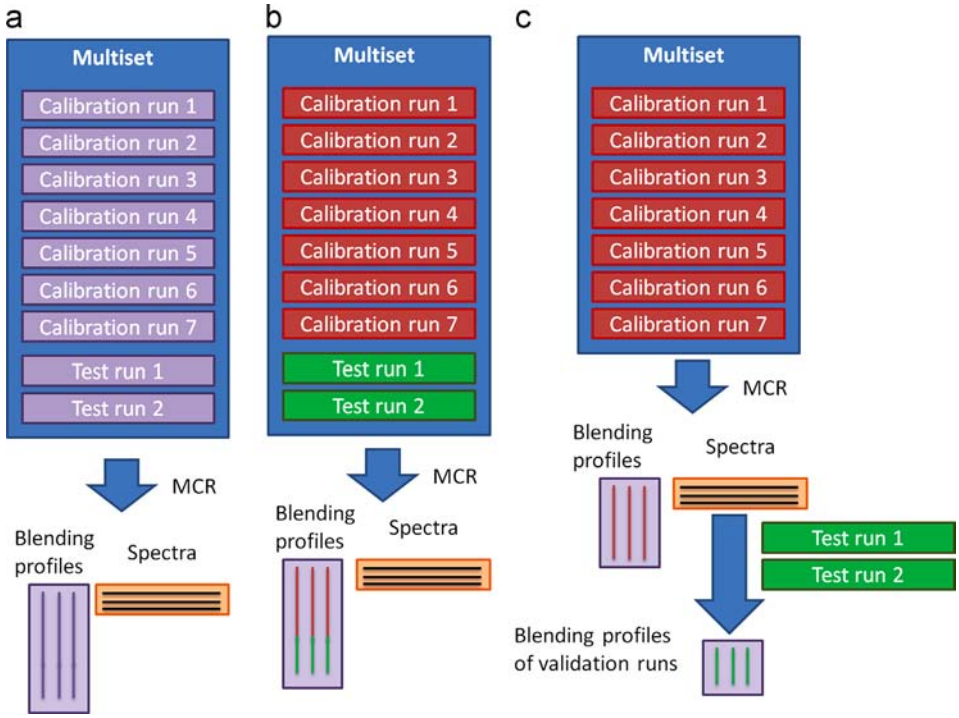
2.3.3.2. MCR-ALS with correlation constraint—Quantitative assessment of homogeneity. As mentioned in Section 2.3.2, correlation constraint allows predicting the evolution of the blending profiles in real concentration units. Constraining one or all blending profiles in a run will allow for controlling individual (i.e. only API), or global homogeneity (i.e. of all compounds), in the formulation studied.

This variant of MCR can be adapted to the blending control *ex* or *in situ*, depending on the configuration of the multiset structure and the steps followed in the resolution analysis.

MCR ex situ process control

This MCR analysis consists of the study of a multiset structure built up including calibration and test runs (Scheme 2b). The correlation constraint is used to obtain concentration blending profiles in real concentration units, based on the calibration models built using reference values linked to homogeneous blending points contained in the calibration runs.

In this approach, the calibration set is built with several blending runs with different concentration levels of compounds. During the ALS optimization, prediction of the concentration levels on test runs (and on the points of all blending runs when homogeneity is not yet achieved) is carried out. To perform this approach, the test runs must be finished before the MCR analysis is carried out since they are included in the resolution analysis. The value of this approach is a final quantitative description of the



Scheme 2. Schematic representation of the building-up the multiset and resolved matrices for (a) Classical MCR, (b) *Ex situ* MCR, and (c) *In situ* MCR.

process carried out. This may be useful when processes are being optimized and real-time control is not the main purpose.

MCR in situ process control

When MCR is meant to be used for *in situ* control of the blending formulation, the multiset structure submitted to MCR-ALS analysis is formed only by the calibration runs (see Scheme 2c). With these runs and the application of the correlation constraint, the calibration model is built and a matrix of pure spectra, $\mathbf{S^T}$, is obtained, which is valid for any future blending run of the same kind of formulation.

This $\mathbf{S^T}$ matrix is later used to predict concentrations on the test runs. For each new spectrum acquired during a test blending run, the two following steps are done:

(a) The new spectrum of the test blending run is MSC-corrected with the reference spectrum of the calibration set ($\mathbf{d_{MSCi}}$).
(b) The concentration values for this blending point are calculated by a single non-negative least-squares step as follows: $\mathbf{c_i}=\mathbf{d_{MSCi}(S^T)}^{+}$. The $\mathbf{S^T}$ matrix is the model previously obtained from the resolution of the calibration set of blending runs.

In this way, *in situ* control can be performed since test runs are not included in the MCR-ALS resolution step and the MSC-correction and single non-negative least-squares step to estimate the concentration in each new point of the batch is instantaneously calculated.

2.3.4. Software

All MCR-ALS calculations were performed using in-house MATLAB (version R2011a; The Mathworks Inc, Natick, MA, USA) routines. Codes are freely available at www.mcrals.info.

2.3.5. Homogeneity indicators

From the information contained in the blending profiles, we can proceed to study the heterogeneity of the different batches. To set the point of the blending run that could be considered homogeneous, two different criteria have been adopted: the first relates to the compliance with a nominal concentration value for the formulation, whereas the second is more related to the invariance of concentration among neighboring blending points. A detailed description of both criteria is presented below.

The concentration value of a particular compound should be within a threshold band around the nominal value (in this work, $\pm$10%). To check for individual compound homogeneity, the nominal value is compared with the concentration of a particular blending point, represented by the mean of the concentration values for that compound using the blending point and some previous neighboring points in the batch analyzed.

To study the global homogeneity, a pooled relative bias around the nominal concentration value is calculated taking into consideration the contribution of each of the compounds in the formulation [41]:

$$global\ bias = \frac{100 \cdot \left(\sum_{i=1,NC} \frac{|\bar{x}_i - \mu_i|}{\mu_i}\right)}{NC} \quad (7)$$

where $\bar{x}_i$ is the mean of the concentration values of a particular batch window and μ_i is the nominal concentration value for a particular constituent. NC means number of compounds. Relative global bias should be under a threshold value (set to 10% in this work).

The relative standard deviation of concentration (RSD) values of neighboring points in the batch should be under a threshold value (set to 10%).

The expression applicable to study the individual homogeneity is [41]:

$$RSD_{compound} = 100 \cdot \sqrt{\frac{s^2_{compound}}{\bar{x}^2_{compound}}} = 100 \cdot \sqrt{\frac{\left(\frac{\sum (x_{i,compound} - \bar{x}_{compound})^2}{n_w}\right)}{\bar{x}^2_{compound}}} \quad (8)$$

where $x_{i,compound}$ is the concentration value of a particular batch point, $\bar{x}_{compound}$ is the mean value of the concentration values of the batch points in the same window and n_w accounts for the number of observations in the moving window of neighboring points.

To study the global homogeneity, a pooled relative standard deviation is used.

$$pooled\ RSD = \sqrt{\frac{\sum_{i=1,\ldots,NC} RSD_i^2}{NC}} \quad (9)$$

For all criteria, a window size of 15 spectra has been considered in order to detect the end-point of the blending runs. Other indicators could be used, always taking as starting point information the values obtained in the blending concentration profiles [11,13,31,33].

3. Results and discussion

3.1. Modeling of the effects of physical factors in the blending process

A multiset structure with the 16 blending runs described in Table 1 was built. After MSC preprocessing, MCR analysis started with the determination of the number of components and only two components could be detected and resolved by MCR-ALS corresponding to APAP and MCC (see resolved spectra in Fig. 2a). The inability to resolve spectra and blending profiles for MgSt is due to the fact that this compound is present in a very low and identical concentration level in all the considered blending runs.

Classical MCR-ALS optimization was carried out by applying only the natural constraints of non-negativity for concentration and spectra profiles and the normalization (equal length) of the pure spectra in $\mathbf{S^T}$. Fig. 2a shows the resolved and the pure spectra of APAP and MCC that agree with those experimentally determined for the pure components. In addition, if the squared correlation coefficients (R^2) between the pure and resolved spectra are calculated it can be seen the good recovery of the spectral shape (R^2_{APAP}=0.9483 and R^2_{MCC}=0.9677).

Fig. 2b shows the APAP resolved blending concentration profiles for the 16 runs. From the comparison of these plots and taking into account the knowledge described in the experimental plan, qualitative interpretation of the effect of the assessed factors in the blending process can be carried out.

The first general consideration is that, when assessing the effect of several factors in a blending process, multiset analysis of all runs is mandatory. The reason is that the complementary information contained in the multiset gives a better model for all runs than if they were considered individually. Besides, the common spectral matrix $\mathbf{S^T}$ ensures consistency in the identity of all compounds modeled in the different blending runs. The higher power and performance of multiset analysis as compared with individual analysis of experiments has been proven since long [23] and it is due to the fact that using simultaneously different experiments, more complementary and diverse information is provided and the ambiguity of the final results decreases. In the context of our multiset analysis of blending runs, this is particularly important for the modeling of the profiles associated with

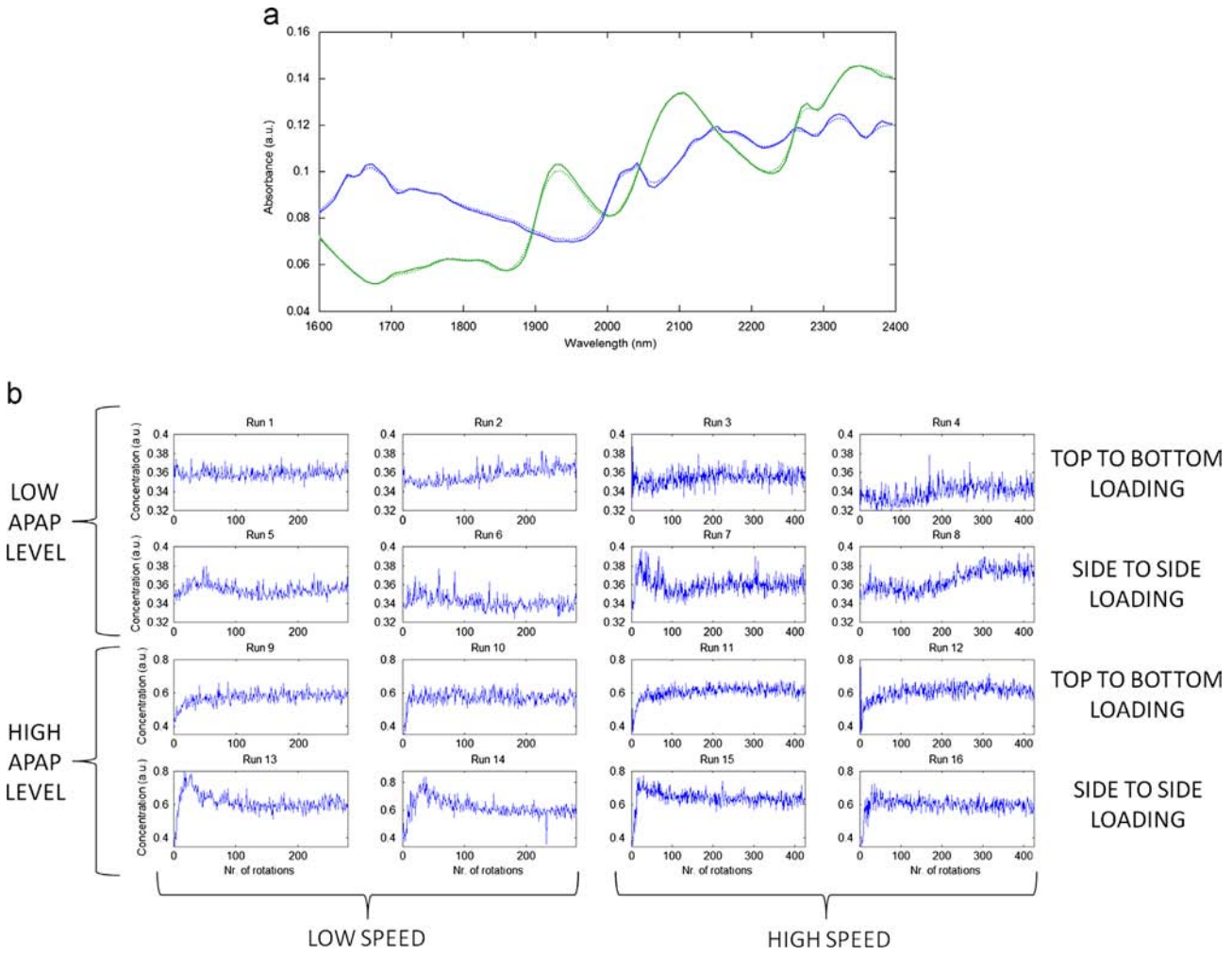


Fig. 2. Analysis of the first data set. (a) Experimental (dotted line) and MCR resolved spectra profiles (solid line) for APAP (blue) and MCC (green) and (b) Resolved MCR blending APAP concentration profiles for the MCR simultaneous analysis of the 16 blending runs. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

APAP in blending runs where the concentration level of this compound is lower (3%), as in the first eight considered runs, which benefit from the better definition of APAP in the blending runs where this compound is more dominant (last eight runs with a concentration of 30%).

From the study of the variations of the blending profiles (trajectories), the effect of the different factors in the blending process is assessed. In this case, the APAP concentration profiles shown in Fig. 2b are used for interpretation.

Thus, the APAP concentration level factor causes a significant change in the achievement of homogeneity. As could be expected, homogeneity is reached earlier and more clearly when the API is at a higher concentration (runs 9–16 in Fig. 2b). The profiles of blending runs with high APAP concentration reach a plateau in a faster way and stay stable, whereas profiles in the low concentration level show wavy forms that take longer to stabilize (runs 1–8 in Fig. 2b).

Second, the loading pattern factor is considered. Again, the MCR resolved blending concentration profiles allow differentiating the sample loading mode into the blender. So, the "top to bottom" runs (runs 1–4 and 9–12 in Fig. 2b) show a trend with a continuous increasing evolution from the beginning until the end of the blending process. On the other hand, the "side to side" runs (runs 5–8 and 13–16 in Fig. 2b) show a different blending trend with an initial fast increase of the APAP concentration followed by a decay until the end of the process. These trends are more easily noticeable in the runs with a higher APAP level (runs 9–16 in Fig. 2b).

Third, the speed of rotation of the blender is considered. In this case, it seems that runs reach homogeneity at the same number of revolutions regardless of the speed of rotation (comparing low and high speed runs at Fig. 2b). However, the time for homogenization will change because of the different mixing speed.

Finally, the filling of the blender is considered (odd runs filling at 50% and even runs filling at 70% at Fig. 2b). In this case, no significant effect can be detected due to this factor.

It can be concluded that classical MCR analysis of blending profiles coming from runs performed in different conditions allows obtaining information about the presence and typology of changes in the blending evolution linked to the physical factors that could control the process.

3.2. Control of the blending process—Homogeneity studies and end-point blending detection

Three different approaches have been tested in order to assess the ability of the MCR methods to detect the end-point of a blending process. For each one of these approaches, a different multiset structure has been built in order to explore the advantages and drawbacks of each method.

3.2.1. Classical MCR

First, the ability of classical MCR (without the correlation constraint) to study the homogeneity of a blending run has been checked. A multiset structure with all blending runs of Table 2 (calibration and test) has been built. Classical MCR-ALS optimization was carried out applying the non-negativity constraints for concentration and spectra profiles and, also, normalizing the spectra in matrix $\mathbf{S}^{\mathbf{T}}$ to equal length. Table 3 shows the figures of merit of the different classical MCR-ALS analyses performed in this study. As can be seen in the first row, explained variance and lack of fit are very satisfactory for the classical MCR analysis of the blending runs with an explained variance higher than 99.99% and a small value of lack of fit. In this case, the mixture has five compounds, but only three of them could be modeled (APAP, lactose and MCC). Both croscarmellose sodium and magnesium stearate cannot be determined because their constant concentration in all the runs.

Resolved blending concentration profiles and spectra are shown in Fig. 3. From the blending concentration profiles obtained, end-point information could also be obtained both for APAP and the entire formulation (Fig. 3a). In this figure, the first row with seven plots corresponds to the recovered blending trajectories of the three resolved components for the calibration runs. The second row of plots corresponds to the resolved blending profiles for the test runs in which it can be seen the different trajectories that follow the species until they reach homogeneity.

Since the concentration of the species obtained by classical MCR is in an arbitrary scale, homogeneity indicators based on the comparison with a real nominal concentration value (such as bias) cannot be used. However, RSD, which shows similarity of neighboring concentration values, is applicable because it is not dependent on the units of the concentration scale. Table 4 displays the homogeneity indicators for individual and global homogeneity showing the time (in seconds) needed to achieve homogeneity.

Table 3
Figures of merit of the MCR-ALS analyses and quality parameters of the calibration models linked to the application of the correlation constraint.

Method	Explained Variance (%)	Lack of fit (%)	Quality parameters of the calibration models					
			APAP		Lactose		MCC	
			R	RMSEC	R	RMSEC	*R*	RMSEC
Classical MCR	99.9993	0.2583	–	–	–	–	–	–
MCR *ex situ* calibration APAP only	99.9989	0.3385	0.9464	1.6982	–	–	–	–
MCR *ex situ* calibration MCC only	99.9988	0.3477	–	–	–	–	0.9867	1.6569
MCR *ex situ* calibration lactose only	99.9993	0.2677	–	–	0.9441	3.8695	–	–
MCR *ex situ* calibration all species	99.9991	0.2978	0.9442	1.7348	0.9557	3.4135	0.9785	2.1170
MCR *in situ* calibration APAP only	99.9987	0.3535	0.9473	1.6817	–	–	–	–
MCR *in situ* calibration all species	99.9991	0.3012	0.9468	1.6906	0.9594	3.2571	0.9794	2.0715

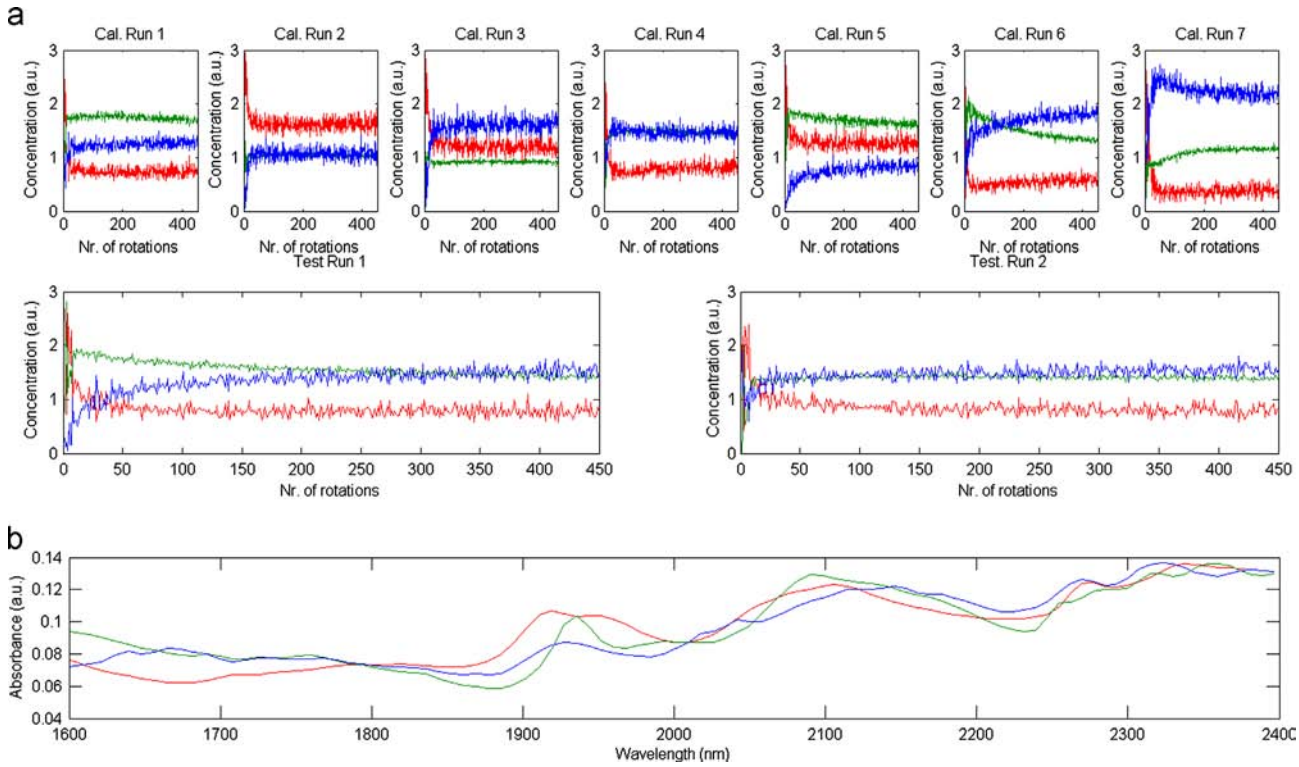


Fig. 3. Analysis of the 9 considered blending runs by means of classical MCR: (a) MCR resolved concentration profiles for the 9 runs (7 calibration and 2 test) and (b) Resolved MCR spectra. APAP (blue), lactose (green) and MCC (red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 4
Blending end-point detection for individual components and global formulation according to bias and RSD criteria in seconds.

Method	Species	Test Run1		Test Run 2	
		Bias[a](s)	RSD[a](s)	Bias[a](s)	RSD[a](s)
Classical MCR	APAP	–	212	–	160
Classical MCR	Global	–	204	–	144
MCR *ex situ* Individual MCR analyses with calibration per each species.	APAP	344	112	92	60
	Lactose	[b]	80	[b]	92
	MCC	92	160	103	92
	Global	96	100	392	92
MCR *ex situ* Single MCR analysis with simultaneous calibration of all species	APAP	356	112	80	68
	Lactose	440	92	392	92
	MCC	92	92	400	96
	Global	100	92	232	92
MCR *in situ* APAP calibration only	APAP	320	108	88	64
MCR *in situ* calibration all species	Global	308	88	232	88

[a] When assessing global homogeneity, bias and RSD should be interpreted as global bias and pooled RSD.
[b] No homogeneity is reached according to the considered criterion.

First, if only APAP homogeneity is considered, Table 4 indicates that the end-point (homogeneity) is detected a bit earlier in test Run 2, compared with test Run 1 (160 s for Run 2 and 212 s for Run 1). Similar conclusions are inferred when pooled RSD is considered to assess global homogeneity. Again, Run 2 (144 s) reaches homogeneity earlier than Run 1 (204 s) and the point of global end-point detection is not very different from that obtained in the individual control of APAP. Pure spectra have shapes similar to the three major compounds despite the fact that two minor compounds were not explicitly modeled (Fig. 3b). In this case, the recovered R^2 values comparing resolved spectra and pure compound spectra were 0.7926 for APAP, 0.8840 for MCC and 0.8979 for lactose. The resolved spectral shapes clearly preserve the identity of the major compounds, particularly from MCC and lactose, taking into consideration that only natural constraints were used in the MCR analysis.

3.2.2. MCR with correlation constraint

Next, the results obtained using two approaches based on the application of MCR with the correlation constraint are presented. In both cases, the correlation constraint has always been applied taking as reference calibration values those related to the spectral blending points 116–125 in the 7 calibration blending runs, which were assessed previously to be homogenous [11,12]. Since the blending runs have different composition (see Table 2), a multilevel concentration calibration model can be easily built. The suitable calibration model is afterwards used to predict real concentration values for the test blending runs and for the points in the calibration blending runs that were not taken as reference to establish the calibration model.

3.2.2.1. MCR ex situ. A multiset structure with all the experiments (7 calibration runs and 2 test runs) shown in Table 2 is built (Scheme 2b). Data pretreatment and analysis is the same as described in previous sections (MSC pre-processing, determination of the number of components and initial estimates, selection of natural constraints), the only difference during the ALS optimization step being the application of the correlation constraint in the concentration profiles. The correlation constraint can be applied to all the species considered in the resolution (APAP, lactose and MCC) to study global homogeneity or to a single species if only the homogeneity of a particular compound had to be controlled.

The figures of merit of the ALS optimization and quality parameters of the calibration models (R and RMSEC) for the three MCR analyses performed to control individually the homogeneity of each of the compounds in the formulation (APAP, lactose and MCC) are presented in Table 3. In all cases, the variance explained exceeds 99.99% and the quality parameters linked to the univariate calibration models provide correlation coefficients larger than 0.94 and acceptable RMSEC values. In all MCR analyses, the pure spectra recovered for the different compounds have a shape in agreement with the expected one (spectral profiles not shown for brevity).

Since in individual homogeneity control, the most important compound is APAP, the results obtained for this compound from the MCR analysis in which only the correlation constraint is applied to APAP concentration are described in more details (analogous output has been obtained for MCC and lactose MCR analyses). Fig. 4 shows only the APAP blending concentration profiles for the 7 runs considered in the calibration set and for the two test runs (Fig. 4a) and the related spectral profile (Fig. 4b). Fig. 4c shows the calibration curve between the concentration values predicted by MCR-ALS and the known values in the calibration sets. As can be seen graphically in the calibration curve (Fig. 4c) and numerically in the quality parameters (Table 3) the model can be considered satisfactory. Finally, the results obtained for the two test runs are zoomed in Fig. 4d and e. If the last 100 time points of the test blending runs are considered, the predicted concentration of APAP with the confidence interval considered in both runs is in agreement with the nominal value expected in the target formulation (Table 5).

The first thing to note is that the APAP spectral shape is much better recovered than in the classical MCR analysis, as indicated by the R^2 value equal to 0.9053, when resolved and pure APAP spectrum are compared. This fact shows the power of the correlation constraint in increasing not only the quantitative information, but in providing a better definition of the identity (spectral shape) of the compound. From the resolved blending concentration profile of

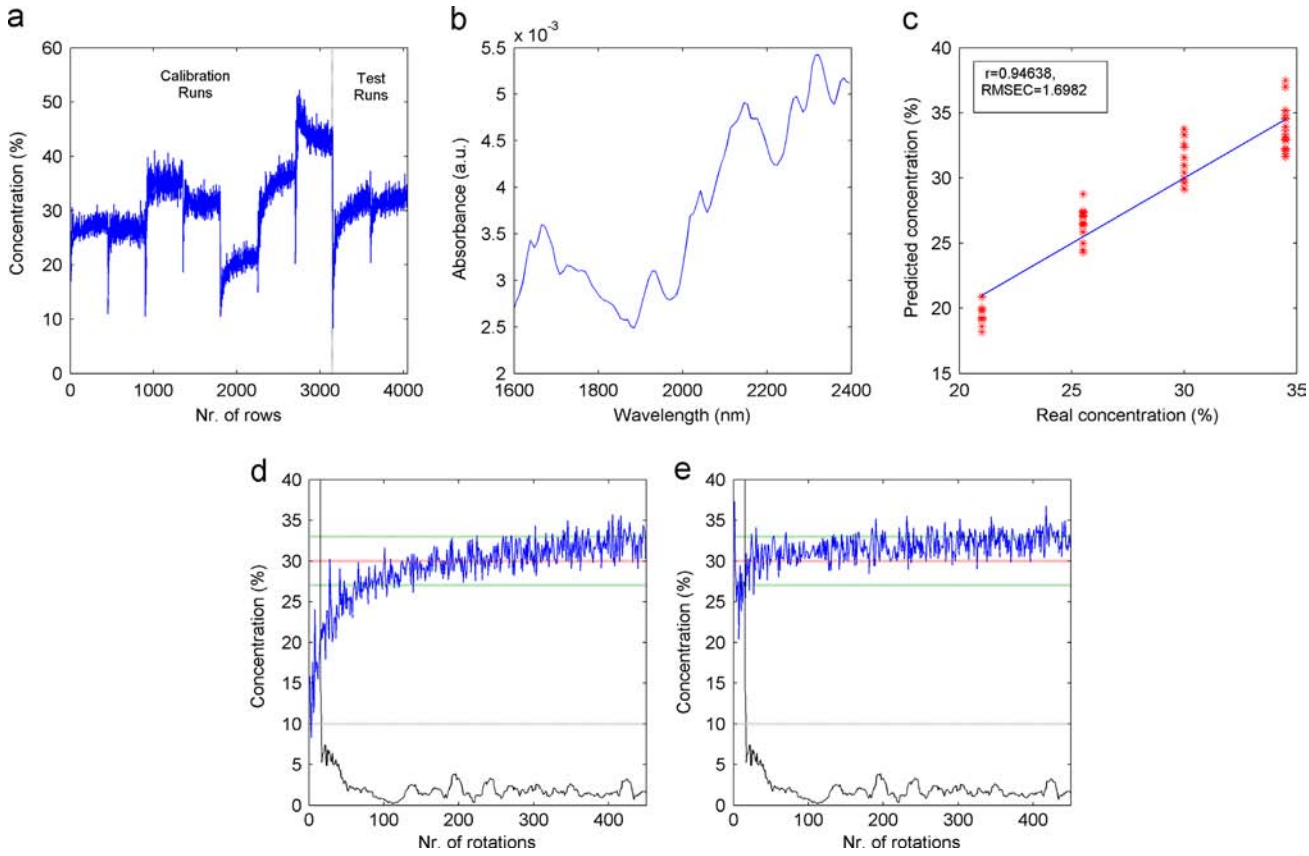


Fig. 4. Analysis of the 9 considered blending runs by means of *ex situ* MCR imposing the correlation constraint only for APAP: (a) Resolved MCR blending concentration profiles for APAP, (b) Resolved MCR spectra for APAP, (c) Calibration curve obtained during the MCR optimization using the correlation constraint, (d) Resolved MCR blending concentration profiles for APAP (blue line) and calculated RSD values from the resolved concentration profile (black line) in test Run 1, (e) Resolved MCR blending concentration profiles for APAP (blue line) and calculated RSD values from the resolved concentration profile (black line) in test Run 2. In these two plots red dotted line indicates the nominal concentration and green dotted lines indicate the 10% threshold value around the nominal concentration for the bias indicator. Gray dotted line indicates the 10% threshold value for the RSD indicator. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 5
Mean concentration values and confidence interval of the concentration of the different species (Calculated with the last 100 values of the MCR resolved blending concentration profiles).

Method	Calibration					
	APAP		Lactose		MCC	
	Run 1 (%)	Run 2 (%)	Run 1 (%)	Run 2 (%)	Run 1 (%)	Run 2 (%)
Target	30.0		37.3		26.7	
MCR *ex situ* calibration APAP only	32.1 ± 3.0	32.6 ± 2.5	–	–	–	–
MCR *ex situ* calibration all species	31.9 ± 2.9	32.5 ± 2.4	35.1 ± 3.1	33.6 ± 2.8	27.0 ± 4.0	27.9 ± 3.8
MCR *in situ* calibration APAP only	32.1 ± 3.0	32.6 ± 2.5	–	–	–	–
MCR *in situ* calibration all species	32.0 ± 3.0	32.6 ± 2.5	34.8 ± 3.1	33.4 ± 2.8	27.1 ± 3.9	27.9 ± 3.8

The confidence interval was calculated assuming a 95% confidence level.

APAP in the test runs (see Fig. 4d for Test run 1 and Fig. 4e for Test run 2), the homogeneity of the blending run can be studied. Table 4 shows the results obtained for the end-point detection using the two criteria previously described, bias and RSD. It can be seen that the results obtained by RSD provide an earlier detection of the end-point for both runs than the bias criterion. This is not necessarily surprising, since both indicators look at different aspects of homogeneity control (bias looks at the similarity with a target concentration value, whereas RSD only looks at the internal stability of the concentration in the blending run). Both criteria are relevant in the quality of a formulation and a wise consideration would be adopting the least optimistic criterion as end-point, since it ensures compliance of nominal concentration and stability in the formulation at the same time. In this particular study and as far as APAP control is concerned, it can also be observed that Run 2 reaches the homogeneity significantly earlier than Run 1, whichever criteria is used. This observation is in agreement with the visual observation of the evolution of blending profiles in Fig. 4d and e, in which the fastest evolution towards homogeneity is observed in Run 2 (Fig. 4e).

In the same manner, individual models for lactose and MCC were built. When the resolved spectral shapes are compared with the pure spectra, R^2 values of 0.9150 for MCC and 0.8518 for lactose are obtained, always similar or higher than when using MCR without correlation constraint. As for the analysis of blending profiles, MCC seems to reach homogeneity earlier in test Run 1 than in test Run 2 in terms of bias, but the opposite conclusion is reached when RSD is

analyzed. In general, the homogeneity RSD criterion seems easier to be fulfilled than the bias condition (Table 4). In the case of lactose, the concentration predicted in the test runs by MCR analysis never reached the expected value in the target formulation. This is the reason why homogeneity according to the bias criterion was not achieved. This fact caused that the determination of the individual homogeneity of lactose could only be determined based on the RSD criterion. At this point, it is difficult to know whether the reason of this low concentration of lactose in the resolved blending profile is caused by a non-optimal resolution due to insufficient information in the MCR analysis (see that there is no improvement in the definition of the spectral shape when the correlation constraint is applied) or to a problem in the preparation of the blending formulation. In order to improve the quality of the general results and, in particular, of the lactose blending profiles, it seems potentially useful to apply the correlation constraint to all the available compounds. To sum up, using the correlation constraint for an individual compound is the option when only this information is available and individual compound homogeneity control is the objective; however, when quantitative information for more than one compound is available, even if those are not of interest from a control point of view, applying the correlation constraint to these additional compounds will help to improve and to stabilize the MCR solution.

Following the last idea above, the results for a single MCR analysis in which the correlation constraint is applied to each of the compounds (APAP, MCC and lactose) are shown. Results are shown in Fig. 5 and Tables 3 and 4. This strategy is valid both to control the individual homogeneity of each compound (using only the compound-specific blending profile) or for global control of the homogeneity of the formulation, taking the blending profiles of all compounds into consideration. A first observation is that the figures of merit of this MCR analysis have the same quality as those found for the MCR analyses with individual application of correlation constraint for each compound (Table 3). Likewise, the three calibration models now obtained within the same MCR analysis have very similar quality parameters to those obtained when calibration models were done one at a time in each separate MCR analysis.

Fig. 5a shows the resolved blending concentration for the calibration runs (first row of plots) and test runs (second row of plots). Comparing the resolved concentration blending profiles for test Run 1 and Run 2, it could be observed again that in the second run the homogeneity is reached before.

The fact of using simultaneously more information results in an improvement of some aspects of the resolution analysis. The pure spectra of all compounds become more similar to the true ones and, hence, the identity of each compound is better defined (Fig. 5b). This can be proven by the increase of the R^2 values (0.9105 for APAP, 0.9874 for MCC and 0.9975 for lactose) compared with the ones obtained with MCR analyses applying the correlation constraint to each compound separately (see above). A consequence of this is that, for this example, now, the concentrations of all compounds at the end of the test runs seem to agree with the expected for the target formulation (Table 5). Even lactose is within limits considering the tolerance of $\pm$ 10%, although in test Run 2 has a slightly lower value than the nominal expected. As for the end-point detection (Table 4),

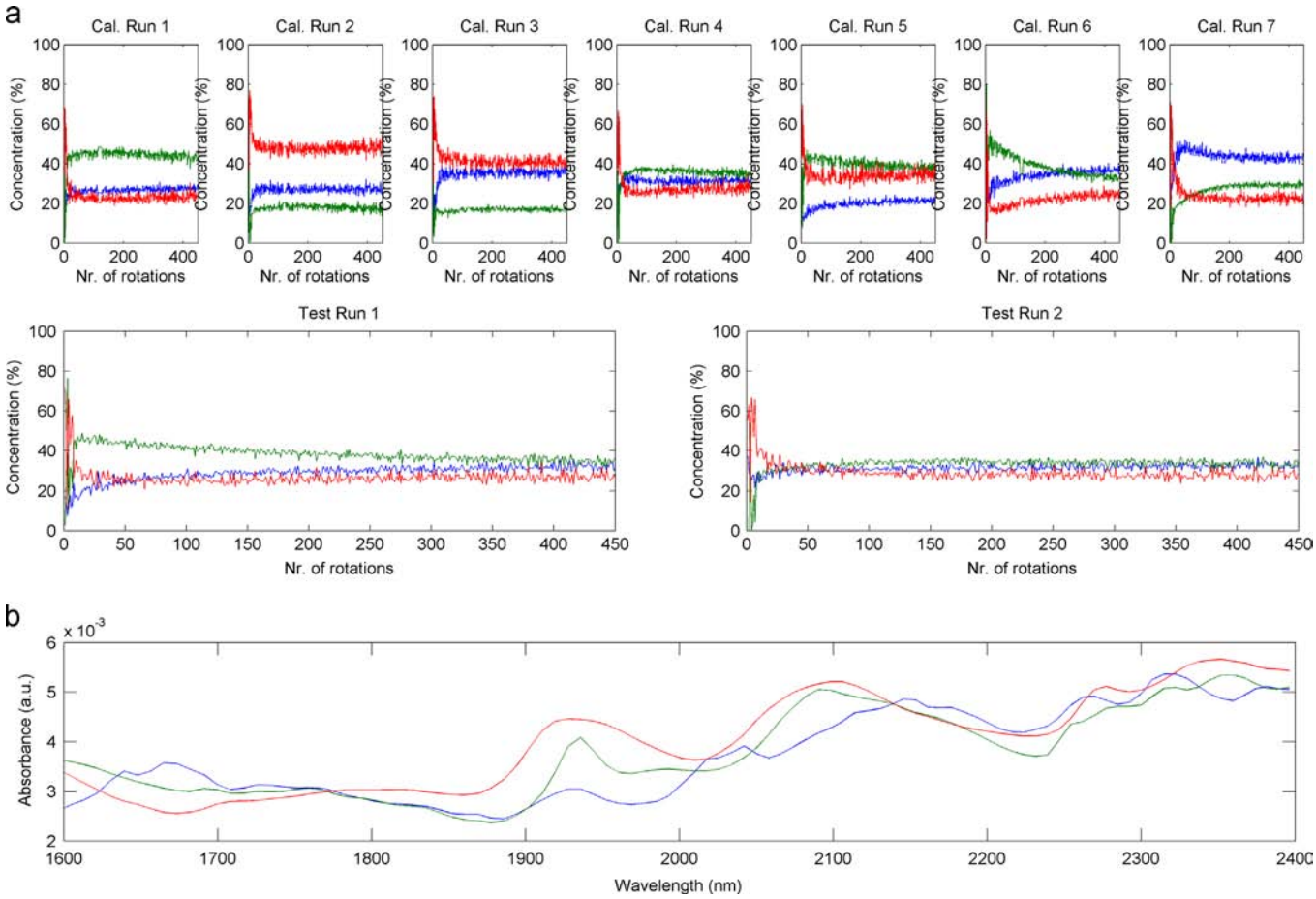


Fig. 5. Analysis of the 9 considered blending runs by means of *ex situ* MCR imposing the correlation constraint for all the species: (a) Resolved MCR blending concentration profiles and (b) Resolved MCR spectra for APAP, lactose and MCC. APAP (blue), lactose (green) and MCC (red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

if we consider first the individual compound homogeneity, the results for APAP and MCC are almost equal to those obtained when these two compounds were the only ones subject to correlation constraint in separate MCR analyses. The gain is that now homogeneity indicators for lactose, both in terms of bias and RSD were improved. Looking at the bias, it can be seen that lactose is the component that generally reaches homogeneity the latest. APAP and MCC species seem to show opposite trends in test Run 1 and Run 2 when this homogeneity indicator is considered: APAP was faster in reaching the nominal concentration in test Run 1 and MCC in test Run 2. If the RSD indicator is considered, all compounds seem to stabilize around the same blending point.

However, it is more interesting to study when the blending run could be considered homogeneous from a global point of view. In this case, global bias and pooled RSD criteria are used in order to estimate the variability due to the three considered components. For the two criteria, the global homogeneity is reached before the expected time point (around 120 rotations) and seems that Run 1 reaches homogeneity according to the global bias criterion in a faster way. As expected from the results of the individual homogeneity control, the pooled RSD-based criterion is fulfilled around the same time for both test runs and seems easier to reach than the global bias-based one.

The control of global homogeneity could also be carried out considering the results of the three MCR analyses with single calibration models (one for APAP, one for lactose and one for MCC). Then, the blending profiles of the constrained compounds in each of the analyses would be combined to calculate the homogeneity indicators. Results obtained with this approach are shown in Table 4. In terms of the pooled RSD criterion, the obtained end-point values are similar to those obtained with the single MCR analysis with all compounds constrained. However, the criterion based on global bias worsens in test Run 2, possibly due to the less satisfactory modeling of the lactose when it was the only compound subject to correlation constraint.

So, whenever information is available, the use of a single MCR analysis with simultaneous application of the correlation constraint to as many species as possible is preferred. This is due to the fact that more information is input in the resolution analysis, the ambiguity of the resolved profiles decreases and, hence, there is an increase in the reliability of the final results.

3.2.2.2. MCR in situ. The second approach using the correlation constraint is the MCR *in situ*. In this case, the multiset structure submitted to MCR analysis is only formed by the blending calibration runs (see Scheme 2c). As in the previous case, the calibration procedure using the correlation constraint could be done only for the known concentration of APAP or for the concentration of all the species in the calibration runs. In this case, the results of the MCR analysis are a matrix of blending concentration profiles for the calibration runs in real concentration units and the related matrix of pure spectra ($\mathbf{S}^T$). Using this matrix of resolved pure spectra ($\mathbf{S}^T$), the real concentrations of each blending point in a newly obtained test run can be calculated in real time by a non-negative least squares step relating the MSC-corrected spectrum acquired of the blending mixture in the test run to the matrix of pure spectra obtained in the previous step (see Section 2.3.3.). It is worth highlighting that this approach allows obtaining the concentration values of the species considered in the calibration step *in situ* during the blending process.

For the sake of brevity, only results related to the *in situ* homogeneity of APAP and to the *in situ* global homogeneity of the formulation are commented.

When modeling only APAP, the *in situ* resolution results obtained using only the blending calibration runs are of similar quality to those of MCR *ex situ* (when both calibration and test runs were used) (see Table 3). After the MCR analysis, the blending concentration profiles for APAP in the test runs can be obtained (shown in Fig. 6a and b). Again, the resulting concentration values of APAP considering the mean of the last 100 time points of the test runs are in good agreement with those of the target formulation (Table 5). In the case of the end-point detection of APAP homogeneity, the results also agree with those obtained by the MCR *ex situ* approach. It can be clearly seen that Run 2 reaches

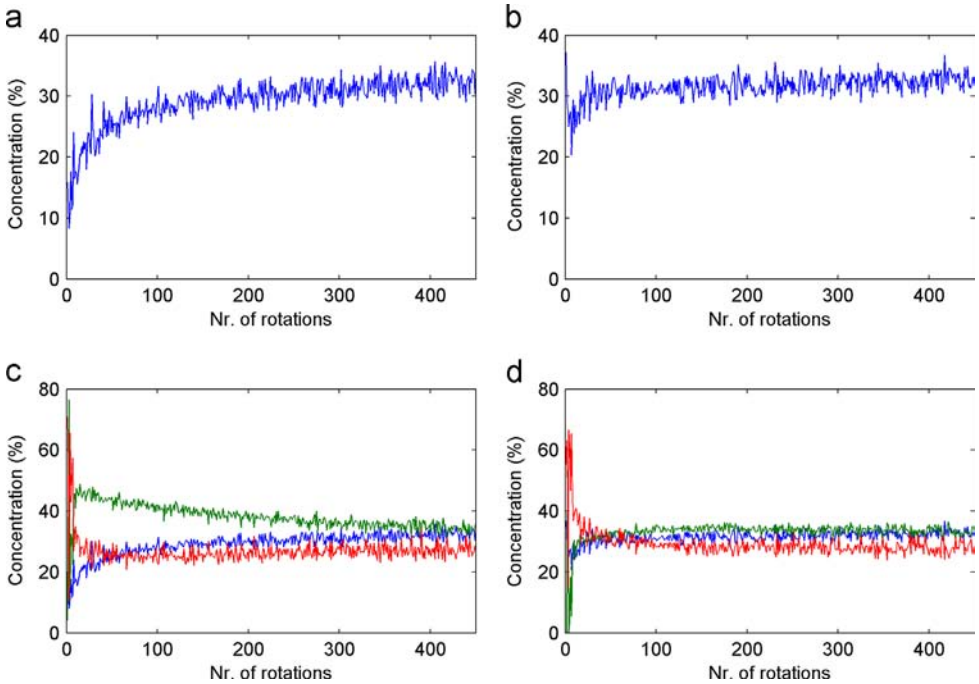


Fig. 6. Results of the MCR *in situ* approach. MCR resolved blending concentration profiles when correlation constraint is only imposed to APAP: (a) Test Run 1 and b) Test Run 2. MCR resolved blending concentration profiles when correlation constraint is imposed to the three species: (c) Test Run 1 and (d) Test Run 2. APAP (blue), lactose (green) and MCC (red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

homogeneity before than Run 1 considering any of the criteria. However, again the RSD criterion is the most optimistic with end-point values significantly smaller than the Bias-based criterion (Table 4).

When the MCR analysis is done on the calibration blending runs applying the calibration constraint to all compounds, again the results of the MCR *in situ* and MCR *ex situ* are extremely similar in terms of quality. Calculated blending profiles for all compounds in the test runs are shown in Fig. 6c and d. The estimated concentration values from the 100 last points in the blending runs agree between the MCR *ex* and *in situ* (see Table 5) and so do the homogeneity indicators (global bias and pooled RSD in Table 4). In this case, it also seems that Run 2 reaches the homogeneity earlier than Run 1 considering the end-point values for the global homogeneity. As in the previous cases, the study of the dispersion of the neighboring points (pooled RSD) provides a faster end-point than the global bias.

The good similarity between the MCR *ex situ* and the MCR *in situ* results is of significant value, since most control tasks are done *in situ* and the MCR method has proven to work equally well in this instance.

4. Conclusions

Multivariate Curve Resolution has been applied to monitor pharmaceutical blending processes. Due to the flexibility in the analysis of augmented data sets and the application of constraints, blending modeling or control can be performed and work in *ex* and *in situ* modes has proven to be possible.

Thus, classical MCR with natural constraints can provide information on the qualitative evolution of blending processes and give information about the effects of modifying process parameters on the blending trajectory. Eventually, a first indication on the homogeneity of the blending run can be obtained with classical MCR, when no external quantitative information is available, by observing when the blending profiles reach a stable and invariant plateau.

When reference quantitative information about calibration blending runs is available, MCR analysis using a correlation constraint allows the determination of quantitative information of the blending profiles of unknown runs in real concentration units, useful to calculate homogeneity indicators (compound-specific or global) to detect the end-point of the blending process. Although the correlation constraint can be applied to one or more compounds in the blending formulation, whenever it is possible, a single MCR analysis constraining as many compounds as possible gives more reliable results, subject to a lower degree of ambiguity.

Varying the multiset structure submitted to MCR analysis (containing calibration and test blending runs or only calibration runs) allows for working in *ex situ* or *in situ* mode, respectively. Interestingly, very similar results are obtained whether working *ex* or *in situ*, which is very promising for the potential use of the MCR methodology for real *in situ* blending control.

Acknowledgments

We acknowledge funding from both the Spanish government (Grant CTQ2012-38616) and the Catalan government (2009SGR45). Joaquim Jaumot acknowledges a CSIC JAE-Doc contract co-funded by the FSE.

References

[1] A.S. El-Hagrasy, H.R. Morris, F. D'Amico, R.A. Lodder, J.K. Drennen III, J. Pharm. Sci. 90 (2001) 1298–1307.
[2] S.S. Sekulic, H.W. Ward II, D.R. Brannegan, E.D. Stanley, C.L. Evans, S.T. Sciavolino, P.A. Hailey, P.K. Aldridge, Anal. Chem. 68 (1996) 509–513.
[3] J. Rantanen, H. Wikström, R. Turner, L.S. Taylor, Anal. Chem. 77 (2005) 556–563.
[4] Y. Roggo, P. Chalus, L. Maurer, C. Lema-Martinez, A. Edmond, N. Jent, J. Pharm. Biomed. Anal. 44 (2007) 683–700.
[5] L.X. Yu, Pharm. Res. 25 (2008) 781–791.
[6] H. Wu, M. Tawakkul, M. White, M.A. Khan, Int. J. Pharm. 372 (2009) 39–48.
[7] A.J. Hickey, D. Ganderton, Pharmaceutical Process Engineering, Informa Healthcare, New York, 2010.
[8] A.U. Vanarase, M. Alcalà, J.I. Jerez Rozo, F.J. Muzzio, R.J. Romañach, Chem. Eng. Sci. 65 (2010) 5728–5733.
[9] Z. Shi, R.P. Cogdill, S.M. Short, C.A. Anderson, J. Pharm. Biomed. Anal. 47 (2008) 738–745.
[10] P.A. Hailey, P. Doherty, P. Tapsell, T. Oliver, P.K. Aldridge, J. Pharm. Biomed. Anal. 14 (1996) 551–559.
[11] B. Igne, S. Talwar, J.K. Drennen III, C.A. Anderson, J. Pharm. Innov. 8 (2013) 45–55.
[12] B. Igne, B.M. Zacour, Z. Shi, S. Talwar, C.A. Anderson, J.K. Drennen III, J. Pharm. Innov. 6 (2011) 47–59.
[13] S.S. Sekulic, J. Wakeman, P. Doherty, P.A. Hailey, J. Pharm. Biomed. Anal. 17 (1998) 1285–1309.
[14] A. De Juan, R. Tauler, Anal. Chim. Acta 500 (2003) 195–210.
[15] A. De Juan, R. Tauler, Crit. Rev. Anal. Chem. 36 (2006) 163–176.
[16] J. Jaumot, M. Vives, R. Gargallo, Anal. Biochem. 327 (2004) 1–13.
[17] R. Tauler, B. Kowalski, S. Fleming, Anal. Chem. 65 (1993) 2040–2047.
[18] R. Tauler, Chemom. Intell. Lab. Syst. 30 (1995) 133–146.
[19] S. Mas, R. Tauler, A. de Juan, J. Chromatogr. A 1218 (2011) 9260–9268.
[20] S. Piqueras, J. Burger, R. Tauler, A. de Juan, Chemom. Intell. Lab. Syst. 117 (2012) 169–182.
[21] K. De Braekeleer, A. De Juan, F. Cuesta Sánchez, P.A. Hailey, D.C.A. Sharp, P. Dunn, D.L. Massart, Appl. Spectrosc. 54 (2000) 601–607.
[22] J. Jaumot, J.C. Menezes, R. Tauler, J. Chemom. 20 (2006) 54–67.
[23] R. Tauler, A. Smilde, B. Kowalski, J. Chemom. 9 (1995) 31–58.
[24] A. De Juan, M. Maeder, M. Martínez, R. Tauler, Chemom. Intell. Lab. Syst. 54 (2000) 123–141.
[25] T. Azzouz, R. Tauler, Talanta 74 (2008) 1201–1210.
[26] H.C. Goicoechea, A.C. Olivieri, R. Tauler, Analyst 135 (2010) 636–642.
[27] M.C. Antunes, J.E.J. Simão, A.C. Duarte, R. Tauler, Analyst 127 (2002) 809–817.
[28] L.B. Lyndgaard, F. Van den Berg, A.De Juan, Chemom. Intell. Lab. Syst. 125 (2013) 58–66.
[29] A.S. El-Hagrasy, F. D'Amico, J.K. Drennen III, J. Pharm. Sci. 95 (2006) 392–406.
[30] A.S. El-Hagrasy, M. Delgado-Lopez, J.K. Drennen III, J. Pharm. Sci. 95 (2006) 407–421.
[31] A.S. El-Hagrasy, J.K. Drennen III, J. Pharm. Sci. 95 (2006) 422–434.
[32] F. Cuesta Sánchez, J. Toft, B. van den Bogaert, D.L. Massart, S.S. Dive, P. Hailey, Fresenius J. Anal. Chem. 352 (1995) 771–778.
[33] M. Blanco, R. González Banó, E. Bertran, Talanta 56 (2002) 203–212.
[34] P. Geladi, D. MacDougall, H. Martens, Appl. Spectrosc. 39 (1985) 491–500.
[35] J. Jaumot, R. Gargallo, A. De Juan, R. Tauler, Chemom. Intell. Lab. Syst. 76 (2005) 101–110.
[36] A. de Juan, S.C. Rutan, R. Tauler, 2.19—Two-Way Data Analysis: Multivariate Curve Resolution – Iterative Resolution Methods, in: S.D. Brown, R. Tauler, B. Walczak (Eds.), Comprehensive Chemometrics, Elsevier, Oxford, 2009, pp. 325–344.
[37] W. Windig, J. Guilment, Anal. Chem. 63 (1991) 1425–1432.
[38] R. Tauler, A. Izquierdo-Ridorsa, E. Casassas, Chemom. Intell. Lab. Syst. 18 (1993) 293–300.
[39] R. Tauler, M. Maeder, A. de Juan, 2.24-Multiset data analysis: extended multivariate curve resolution, in: S.D. Brown, R. Tauler, B. Walczak (Eds.), Comprehensive Chemometrics, Elsevier, Oxford, 2009, pp. 473–505.
[40] P.J. Gemperline, Practical Guide To Chemometrics, 2nd Edition, CRC Press, Boca Raton, FL, US, 2006.
[41] S.L.R. Ellison, V.J. Barwick, T.J.D. Farrant, Practical Statistics for the Analytical Scientist. A Bench Guide., 2nd ed., Royal Society of Chemistry, Cambridge, UK, 2010.