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Simultaneous acquisition of the dissolution curves of two active ingredients in a binary pharmaceutical association, employing an on-line circulation system and chemometrics-assistance

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ABSTRACT

The association of an on-line circulation system with a chemometrics-assisted UV detection strategy is described as a useful system to continuously monitor the dissolution of a pharmaceutical preparation containing two active ingredients, and as a tool for the simultaneous determination of the dissolution curves and dissolution profiles of the latter. Multivariate curve resolution with alternating least squares (MCR-ALS) was used as the chemometric tool to quantitate the analytes, while the hydrochlorothiazide–bisoprolol fumarate (HCT–BIS) association was employed as a model for method development. The experiments were carried out with a dissolution tester configured as apparatus II (paddles), under USP 32 official conditions. The suitability of the calibration procedure for quantitating the dissolved drugs was assessed according to ICH guidelines, with regards to linearity in the working range, specificity, accuracy and precision. Figures of merit, including limits of detection and quantitation were also determined, as well as the method's robustness with regards to detection wavelength range. The system was able to consistently provide very reproducible dissolution curves of commercial tablets, which were statistically similar to those furnished by a manual sampling technique, followed by HPLC analysis. To demonstrate the usefulness of the proposed system, the dissolution profiles of different lots of HCT-BIS tablets were acquired and three of them were conveniently compared at a 31 data point level, employing the f_1 and f_2 ("difference" and "similarity") indexes. Use of multiple data points for comparison ensured reliability of the results.

1. Introduction

The quality of pharmaceutical products is currently controlled on the basis of a scientific understanding of their formulation and manufacturing processes, and the knowledge of their quality attributes. Proper drug dissolution is important among the quality features which ensure safety and proper performance of oral solid dosage forms, because release of the active ingredients is crucial to gastrointestinal drug absorption [1]. Nowadays, the in vitro dissolution test is the most widely used method of evaluating this property [2].

Application areas of the dissolution test include pharmaceutical manufacturing, the development of new dosage forms and routine quality control, where it is employed to predict drug bioavailability, to understand the influence of different excipients on drug dissolution rate, and to provide evidence about the "similarity" or "sameness" between lots produced by the same or different manufacturers. The regulatory interest in knowing how similar two dissolutions are is identified with demonstrating manufacturing consistency and ensuring the suitability for exchange of the lots being compared [3].

Official dissolution testing procedures involve sampling at a few pre-determined times and evaluating the amount of the drug that has entered into the dissolution medium. Often, only a single sampling point is prescribed. A batch is approved when the amount of dissolved drug at the pre-established times satisfy the minimum requirement [4,5].

Biorelevance and technique variability are two of the main challenges of modern dissolution testing [6]. Therefore, regardless of the quantitation method, the results provided by one or two data points are clearly insufficient to precisely describe the entire kinetics of the dissolution. In these cases, dissolution curves and dissolution profiles (mean of 12 curves) are required [7].

The dissolution profiles contain information which is very important for understanding the importance of the dissolution process with regards to the overall drug action, as well as for designing

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new dosage forms, assessing manufacture consistency and establishing performance similarity between lots of the same or different brands.

The official dissolution tests make use of single wavelength spectroscopic (UV) and chromatographic (HPLC) methods for carrying out the quantitation of the dissolved drugs [4,5]. Ultraviolet spectroscopy is fairly inexpensive and quantitative analyses can be carried out with minimum sample preparation; however, single wavelength UV-spectroscopy lacks selectivity, usually being unsuitable for testing complex pharmaceutical preparations. On the contrary, HPLC-based methods can be highly selective; however, this approach requires special solvents and care in sample preparation, being also laborious and highly time-consuming.

However, the construction of dissolution profiles employing single wavelength UV-detection is frequently impeded in case of pharmaceutical associations containing more than one absorbing species (active principles or interfering excipients), while the use of chromatographic methods becomes highly demanding in terms of required time, manpower and solvent consumption, being therefore not sustainable. As a result, often only a handful of data points per curve can be determined with reasonable effort in a given timeframe.

Chemometric strategies are playing an increasingly important role in the analysis of multi-component mixtures. Different alternatives have been recently proposed for the evaluation of drug dissolution in pharmaceutical associations; accordingly, dissolution curves containing from 6 to 10 points have been constructed, on a point by point basis, resorting to manual sampling and to the aid of first-order chemometric procedures [8–12].

Fiber optic probes and on-line circulation systems constitute suitable dissolution testing alternatives which enable continuous analyte monitoring; they facilitate the acquisition of many data points per curve with minimum sample manipulation [13]. On-line circulation systems that use the peristaltic pump movement of the dissolution fluid through a UV flow cell have also been coupled to first-order chemometric procedures for the evaluation of drug dissolution, mainly for single active principle formulations [14]; scattered examples of the study of mixtures of active components have also been recorded [15,16]. However, in all cases dissolution curves were constructed on a point-by-point basis.

The development of new analytical techniques for the rapid and efficient construction of dissolution curves of several active principles concomitantly being dissolved from their combined formulations, under cost-effective and eco-friendly conditions, constitutes an important goal in current pharmaceutical analysis. In order to fulfill this purpose, we decided to develop and evaluate a second-order multivariate calibration strategy for dissolution analysis in which a closed circulation system, capable of performing on-line UV spectral measurements, was employed for data acquisition. To the best of our knowledge, second-order chemometric methods have been reported only twice as tools to evaluate drug dissolution; however, in both times a single active principle was determined [17,18].

The pharmaceutical association between hydrochlorothiazide (HCT) and bisoprolol fumarate (BIS) was selected as a model (Fig. 1). This combination is widely employed for the treatment of hypertension and chronic heart failure [19]; in addition, its active principles have different official dissolution rate requirements [20] and their UV spectra are strongly overlapped along most of the useful spectral range. On the other hand, multivariate curve resolution with alternating least squares (MCR-ALS) was selected as the chemometric tool [21], for its ability to resolve time-evolving processes. The results of this study are disclosed herein.

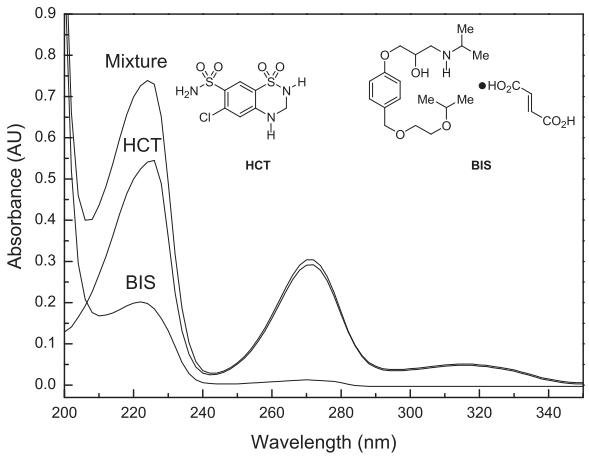

Fig. 1. UV spectra of BIS (4 mg l^{-1}), HCT (5 mg l^{-1}), and a mixture of both at the same concentrations, in 0.1 N HCl, between 200 and 350 nm.

2. Materials and methods

2.1. Instrumentation and software

The UV spectroscopic determinations were carried out with a Shimadzu UV-1601PC double beam spectrophotometer (Shimadzu Corp., Kyoto, Japan), fitted with a 80 μl flow cell (Hellma, Müllheim, Germany; optical path length = 10 mm) and controlled by Shimadzu's UV-Probe (version 2.00) software.

The dissolution tests were performed with a Hanson SR8-Plus dissolution test station (Hanson Research, Chatsworth, USA), configured as USP apparatus II (paddles). The pH of the dissolution medium was determined with a Corning 125 digital pH-meter (Corning, Inc., New York, USA) fitted with a Corning combined glass electrode.

For acquisition of the dissolution data, samples were continuously withdrawn at 1.0 ml min^{-1} from the dissolution vessel through the sampling probe, by means of a Gilson Minipuls 3 peristaltic pump, passed through the flow cell and returned to the dissolution vessel. In order to avoid potential interferences by undissolved particles and bubbles, the dissolution solution was passed through a filter held at the bottom end of the sampling probe and the dissolution medium was degassed by sonication in a water bath before use. Spectra were acquired every 30 s. Data acquisition started 1.5 min (dead volume of tubing) after triggering the dissolution process. Spectra were collected for 35 min at 2 nm intervals in the range 210–350 nm (71 data points/spectrum), against a blank of dissolution medium and saved as a matrix in CSV format.

A Varian Prostar liquid chromatograph (Varian Inc., Palo Alto, USA) equipped with two isocratic pumps, a 20 μl injection loop and a variable dual-wavelength UV-detector, was used for HPLC analyses. The chromatograms were acquired and processed with Varian's Galaxie software (version 6.41).

The computing routines involving spectral data manipulation and the MCR-ALS method were processed in Matlab R2010a (Mathworks, Natick, USA). Statistical data analyses were performed with Origin 7.5 (OriginLab Co., Northampton, USA).

2.2. Reagents

2.2.1. Chemicals

Pharmaceutical-grade bisoprolol fumarate (BIS) and hydrochlorothiazide (HCT) were used throughout the experiments. HPLC-grade methanol and acetonitrile were purchased from Fischer Scientific (Pittsburgh, USA). HPLC-grade water was obtained from a Milli-Q system (Millipore, Bedford, USA).

Double-distilled water was used for the preparation of the samples and the dissolution medium. All other chemicals were of analytical grade.

2.2.2. Commercial tablets

Four lots of tablets, corresponding to three different commercial brands (A, B and C) were employed. They were acquired in a local pharmacy and declared to contain 10 mg BIS and 6.25 mg HCT/tablet (Lot A) and 5 mg BIS and 12.5 mg HCT/tablet (Lots B_1, B_2 and C). All the lots complied with official specifications for weight variation, content uniformity and assay.

2.2.3. Stock standard and working solutions

Stock standard solutions of BIS (1012 mg l^{-1}) and HCT (596 mg l^{-1}) were independently prepared in 25 ml volumetric flasks, by dissolving accurately weighed amounts of the drugs in MeOH. Working solutions (50.0 mg l^{-1} for BIS and HCT) were prepared by transferring appropriate volumes of the stock solutions to separate 10 ml volumetric flasks and diluting to their marks with 0.1 N HCl.

2.2.4. Calibration samples

Calibration samples were prepared in 10 ml volumetric flasks, by mixing appropriate volumes of the working solutions of the drugs and diluting to their marks with 0.1 N HCl, to obtain concentration levels of BIS and HCT in the ranges 2.0–13.8 mg l^{-1} and 2.0–16.0 mg l^{-1}, respectively.

For HPLC analyses, the samples were filtered through a 0.47 μm nylon membrane filter before injection; the calibration equations were obtained by linear regression of the peak areas against the corresponding analyte concentrations.

2.3. Chromatographic conditions

Chromatographic analyses of the dissolution samples were carried out with a 1:4 mixture of acetonitrile and 0.2% triethylammonium phosphate solution, pH 3.0 [20] as mobile phase, pumped at 1.5 ml min^{-1} through a 4.6 mm × 25 cm C18 column (Luna, Phenomenex, 5 μm particle size). The separations were performed at 30.0 ± 0.2 °C and detection was carried out at 260 nm. Retention times for HCT and BIS were 5.6 and 9.0 min, respectively. The concentrations of the analytes were obtained from the corresponding calibration equations.

2.4. Dissolution conditions

Dissolutions were carried out according to the USP 32 [4,20], employing 0.1 N HCl as dissolution medium (900 ml). Paddle rotation rate was 75 rpm. Each test comprised the dissolution of 12 tablets. For HPLC analysis, aliquots (3 ml) were withdrawn at preestablished times, filtered and injected in the chromatograph. Each dissolution curve contained 13 time points.

3. Theoretical background

Boldface capital letters are used for matrices, boldface lowercase characters are employed for vectors and lowercase italics are used for scalars. A transposed matrix is indicated by a superscript "T" and $||\mathbf{X}||$ stands for the norm of matrix **X**.

3.1. MCR

Assuming an additive linear model, the matrix form (**X**) of a set of *m* spectra corresponding to mixtures containing contributions from *a* components acquired at *n* different wavelengths, can be described as in Eq. (1):

$$\mathbf{X}_{(m\times n)} = \mathbf{C}_{(m\times a)}\,\mathbf{S}^{\mathrm{T}}_{(n\times a)} + \mathbf{E}_{(m\times n)} \tag{1}$$

$$\mathbf{C} = \begin{bmatrix} \mathbf{c}_1 \\ \mathbf{c}_2 \\ \vdots \\ \mathbf{c}_m \end{bmatrix} = \begin{bmatrix} c_{11} & c_{12} & \dots & c_{1a} \\ c_{21} & c_{22} & \dots & c_{2a} \\ \vdots & \vdots & & \vdots \\ c_{ml} & c_{m2} & \dots & c_{ma} \end{bmatrix} \tag{2}$$

$$\mathbf{S}^{\mathrm{T}} = \begin{bmatrix} \mathbf{s}_1 \\ \mathbf{s}_2 \\ \vdots \\ \mathbf{s}_m \end{bmatrix} = \begin{bmatrix} s_{11} & s_{12} & \dots & s_{1n} \\ s_{21} & s_{22} & \dots & s_{2n} \\ \vdots & \vdots & & \vdots \\ s_{al} & s_{a2} & \dots & s_{an} \end{bmatrix} \tag{3}$$

$$rss = ||\mathbf{E}|| = ||\mathbf{X} - \mathbf{C}\mathbf{S}^{\mathrm{T}}|| \tag{4}$$

where **C** is the matrix of the concentration profiles of the pure components, **S** is the matrix of their pure spectra [Eqs. (2) and (3)], and **E** is a matrix containing the model error. The aim of MCR as a resolution method is to achieve the optimal decomposition of the data matrix **X** into the product of matrices **C** and $\mathbf{S}^{\mathrm{T}}$, from initial estimates of either one of them. Therefore, once started the MCR algorithm will iteratively try to find a model able to minimize the residual sum of squares (*rss*) of Eq. (4), employed as an error criterion.

An initial guess of **C** can be obtained by different methods, including evolving factor analysis [22,23], while initial estimates of **S** can be accessed by SIMPLISMA [24,25] or employing the spectra of standards of the components, when they are known.

3.2. ALS

Given initial estimates of matrices **C** or **S**, the ALS algorithm is used to carry out the decomposition of matrix **X** given in Eq. (1) [26,27]. ALS iteratively solves two alternating least squares problems, minimization of *rss* over **C** for fixed **S** and minimization of *rss* over **S** for fixed **C**, until *rss* reaches a minimum value, according to Eqs. (5) and (6). Because of rotational ambiguities, constraints must be added in order to ensure that the mathematical solutions found by the iteration process also make chemical sense [28].

$$\mathrm{Min}(\mathbf{C})||\mathbf{X} - \mathbf{C}\mathbf{S}^{\mathrm{T}}|| \rightarrow \mathbf{C} = \mathbf{X}\mathbf{S}(\mathbf{S}^{\mathrm{T}}\mathbf{S}) \tag{5}$$

$$\mathrm{Min}(\mathbf{S}^{\mathrm{T}})||\mathbf{X} - \mathbf{C}\mathbf{S}^{\mathrm{T}}|| \rightarrow \mathbf{S} = \mathbf{X}^{\mathrm{T}}\mathbf{C}(\mathbf{C}^{\mathrm{T}}\mathbf{C})^{-1} \tag{6}$$

4. Results and discussion

4.1. Data acquisition and MCR-ALS initial requirements

The dissolution test can be regarded as a time-evolving process, in which the resulting dissolution curves and profiles are time-dependent drug concentration profiles. The MCR-ALS algorithm [29], which has been successfully employed to solve various dynamic processes and to develop kinetic models with or without an a priori knowledge, was chosen as a chemometric aid for building dissolution curves from spectral data of the dissolving species.

In the conventional setup, the dissolution step is carried out at first and during this stage samples are periodically taken from the dissolution medium. Subsequent quantitative analyses of the samples against external standards of the dissolved analytes provide drug concentration results, which are used in a third step to construct, point by point, the corresponding concentration vs. time graphs. Unlike the conventional approach, in the proposed system

sequentially acquired calibration and dissolution data were simultaneously fed to the MCR-ALS algorithm, with the aim of employing the whole data package to provide calibration graphs and concentration vs time curves of the simultaneously dissolving drugs.

However, in order to obtain meaningful results with MCR-ALS, data require proper arrangement and accurately defined, sound working conditions. These include selection of the operational spectral region, the number of components and the constraints.

4.1.1. Selection of the operational spectral region

The absorption spectra between 200 and 350 nm of pure BIS and HCT, and their mixture, are shown in Fig. 1. The spectra exhibit strong overlapping, both analytes exhibit a strong absorptivity variation between 200 and 210 nm, displaying similar absorption profiles in the region between 210 and 240 nm; however, at longer wavelengths (240–290 nm) the absorbance of HCT becomes 2–15 times that of BIS, and the latter does not absorb above 290 nm, while HCT still exhibits a small absorption up to 350 nm. Both analytes are transparent beyond 350 nm. Therefore, the interval between 210 and 350 nm ($n = 71$ data points) was selected as the operational spectral region.

4.1.2. Data input. Matrix augmentation

Separation of the contribution of each drug during the dissolution process with subsequent acquisition of quantitative dissolution data was achieved by use of a matrix augmentation strategy. This was carried out by appending tablet dissolution data to the quantitative information provided by the BIS and HCT calibration samples.

To that end, the spectral matrices [$\mathbf{BIS}_{(j\times n)}$ and $\mathbf{HCT}_{(j\times n)}$] obtained from j determinations of the analytes ($j = 20$) at n wavelengths and at each of the h levels of the calibration solutions ($h = 5$ for both analytes) were coupled in the direction of time (column-wise), to yield a single augmented calibration matrix ($\mathbf{M}_{BIS}$ and $\mathbf{M}_{HCT}$) per calibration set [Eqs. (7) and (8)].

$$\mathbf{M}_{BIS(hj\times n)} = [\mathbf{BIS}_{1(j\times n)}; \mathbf{BIS}_{2(j\times n)}; \ldots; \mathbf{BIS}_{h(j\times n)}] \quad (7)$$

$$\mathbf{M}_{HCT(hj\times n)} = [\mathbf{HCT}_{1(j\times n)}; \mathbf{HCT}_{2(j\times n)}; \ldots; \mathbf{HCT}_{h(j\times n)}] \quad (8)$$

Analogously, the matrices obtained from the dissolution of each lot of tablets ($q = 12$) at k times (monitoring time = 35 min; $k = 71$) were column-wise coupled, to yield an augmented sample data matrix $\mathbf{M}_{SPL}$ [Eq. (9)]. Finally, the augmented sample data matrix was adjoined column-wise to both calibration augmented matrices, yielding the $\mathbf{M}_{DIS(m\times n)}$ input supermatrix [Eq. (10)], where $m = (hj + hj + 2k)$.

$$\mathbf{M}_{SPL(qk\times n)} = [\mathbf{SPL}_{1(k\times n)}; \mathbf{SPL}_{2(k\times n)}; \ldots; \mathbf{SPL}_{q(k\times n)}] \quad (9)$$

$$\mathbf{M}_{DIS[(hj+hj+2k)\times n]} = [\mathbf{M}_{BIS(hj\times n)}; \mathbf{M}_{HCT(hj\times n)}; \mathbf{M}_{SPL(qk\times n)}] \quad (10)$$

Fig. 2 illustrates details of the architecture of the data. Part of the structure of the input supermatrix time vectors is depicted in Fig. 2A, while data of a dissolution matrix corresponding to a single tablet sample are shown in Fig. 2B.

4.1.3. MCR-ALS initialization. Number of components and initial estimation of their spectra

Based on knowledge of the system, the number of the relevant spectral components in the dissolution data was set to 2. This was confirmed by a principal component analysis check of the $\mathbf{M}_{DIS}$ supermatrix, which revealed that two components accounted for almost 99% of the cumulative explained variance. Because excipients may act as unexpected interferents, affecting the number of sample components, this procedure was repeated for every commercial lot under analysis. However, the presence of two relevant analytes was confirmed in each case.

On the other hand, calibration augmented matrices $\mathbf{M}_{HCT}$ and $\mathbf{M}_{BIS}$ provided the mean spectra of the analytes, which were employed for the initial estimation of the spectra of the pure components.

4.1.4. MCR-ALS restrictions

In order to convey physical sense to the results, the non-negativity restriction was applied to the spectral and time dimensions. This was based on the fact that in every dissolution experiment the spectral intensities, the analytes' concentrations and the dissolution times are all non-negative values.

4.1.5. Resolution of pure spectra and dissolution curves

The input supermatrix (Fig. 2A) provided all the information required by the MCR-ALS algorithm to iteratively solve the contribution of each individual analyte to the dissolution solution and its corresponding spectrum.

Unambiguous identification of the dissolved analytes (MCR components) was carried out by comparison of the MCR output spectra with the reference spectra of BIS and HCT employed for calibration purposes (Fig. 3B). The high similarity between the reference spectra and those provided by MCR confirmed that the determination was devoid of interferences from excipients and bubbles.

4.2. Method validation

The suitability of the calibration procedure for providing quantitative information about the dissolved analytes was assessed, following ICH guidelines [30]. Linearity in the working range, as well as precision, specificity and accuracy were assessed. Method robustness with regards to the selected wavelength range was also verified and figures of merit were obtained.

4.2.1. Range and linearity

Calibration was performed in the ranges 2.0–13.8 mg l^{-1} and 2.0–16.0 mg l^{-1} for BIS and HCT, respectively in order to take into account the different amounts of the active principles in their pharmaceutical formulations.

Pseudo-univariate calibration curves were obtained by plotting the amplitudes of the MCR concentration components (Fig. 3A, data points 1–100 for BIS and data points 101–200 for HCT) against their corresponding concentrations. The squares of the correlation coefficients (R^2, an indication of the quality of fit of the straight lines) of the curves exceeded 0.99 and their intercepts were close to 0, confirming the absence of systematic errors (Table 1). These results confirmed method linearity in the working ranges of the analytes.

4.2.2. Precision

The precision of the calibration was assessed at the repeatability level, by evaluation of the relative standard deviations of multiple determinations ($N = 20$) of the standard solutions (Fig. 3A) at three different concentration levels. The observed RSD values were less than 2% (Table 1) confirming that the calibration furnished precise determinations of the analytes.

4.2.3. Specificity

Small values of the residual standard deviations of the fit (s_{fit}) between normalized spectra (from MCR-ALS and the corresponding reference) for both analytes (s_{fit} = 0.01 and 0.009 AU for BIS and HCT, respectively), ensured specificity of the determinations.

4.2.4. Accuracy

Drug recoveries after addition of known amounts of the drug standards to a dissolution vessel continuously monitored by the

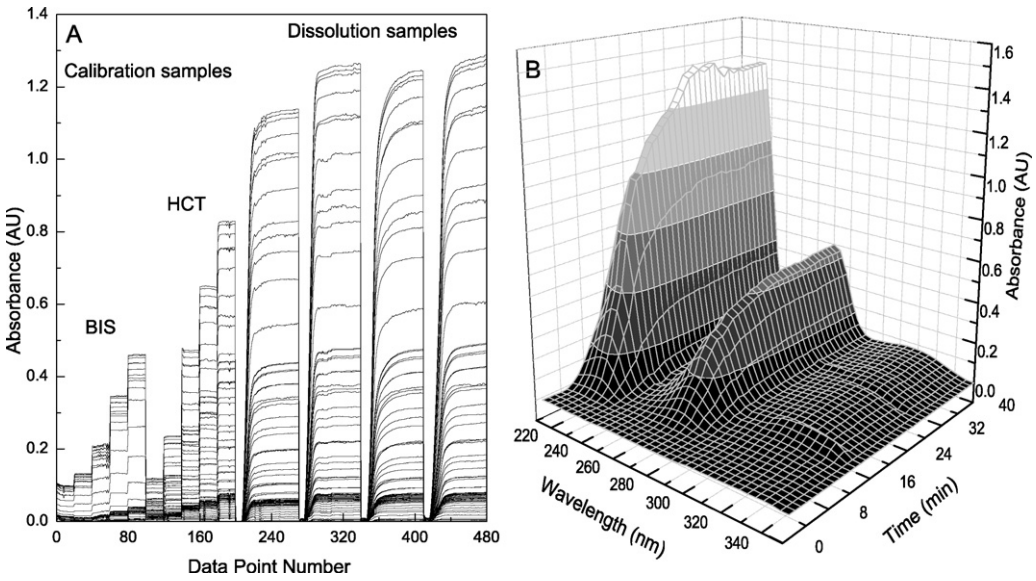

Fig. 2. (A) Input supermatrix for the MCR-ALS algorithm (overlapped time vectors acquired from 210 to 350 nm). Calibration (data points 1–100: BIS calibration matrix; data points 101–200: HCT calibration matrix) and dissolution data of four tablets of Lot A [71 time data points/tablet (monitoring time = 35 min); for the sake of clarity, data shown are from only four tablet samples]. (B) Dissolution data of a single tablet sample of Lot A.

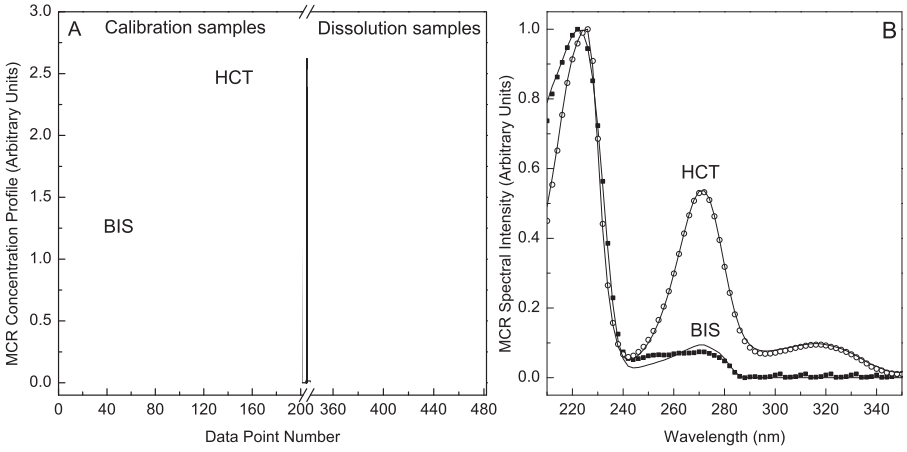

Fig. 3. (A) Concentration profiles of BIS (- - -) and HCT (––) in the calibration and dissolution samples, provided by MCR. (B) Comparison between the spectral components provided by MCR for BIS (■) and HCT (○) and their corresponding normalized spectra.

UV-detection system were evaluated. Analysis of the resulting matrix by MCR-ALS under the conditions employed for the tablets (Fig. 4A) furnished data which exhibited low bias from the expected results (Table 1). These were indicative of the almost quantitative recoveries of the added analytes, confirming the accuracy of the determinations.

4.2.5. Robustness

The ability of the method to provide results unaffected by the operational spectral region (210 and 350 nm) was verified by subjecting the initial and final data acquisition wavelengths to slight modifications (±10 nm). As shown in Fig. 4B, the resulting dissolution curves evidenced insensitivity to these changes, confirming the robustness of the determinations.

4.2.6. Figures of merit

Figures of merit, such as the LOQ, LOD [31], sensitivity, analytical sensitivity (γ) and γ^{-1}, the minimum concentration difference between two samples that could be determined across the linear dynamic range of the employed technique, were obtained from the pseudo-univariate calibration curves, built with the net analyte signals (estimated as the portion of the total signal uniquely ascribed to the analyte of interest) of the corresponding analytes (Table 1) [32]. All of them were considered satisfactory, confirming the suitability of the determination for the intended purpose.

4.3. Application. Dissolution profiles of commercial tablets

The proposed methodology was employed for the evaluation of four lots (A, B_1, B_2 and C) of commercial tablets of the HCT-BIS association. Despite the fact that the doses of the drugs in lot A (Fig. 5A) were different from those in the others, the method demonstrated its versatility by successfully providing dissolution profiles for all the lots.

Good statistical agreements ($p < 0.05$) were observed when the dissolution curves provided by MCR-ALS for both analytes of lot A were compared (Fig. 5A) with the curves obtained with the officially prescribed USP 32 method (manual sampling followed by HPLC analysis) for HCT and BIS in tablets [20]. On the other hand, all the lots were shown to comply with the official requirements regarding the amount of dissolved drug (Fig. 5B) with respect to the label claim (no less than 80% for BIS at 20 min and no less than 80% for HCT at 30 min).

Table 1
Results of method validation.

Parameter	BIS	HCT
Concentration levels (mg l^{-1})	2.0, 5.0, 8.0, 10.6, 13.8	2.0, 5.0, 9.0, 12.0, 16.0
Number of replicates/level (N)	20	20
Range (mg l^{-1})	2.0–13.8	2.0–16.0
Linearity		
Slope ± SD (mg l^{-1} AU^{-1})	0.132 ± 0.001	0.378 ± 0.002
Intercept ± SD (mg l^{-1})	−0.02 ± 0.01	−0.03 ± 0.01
R^2 (n = 100)	0.9991	0.9993
SD of the residues ($s_{y/x}$)	0.005	0.02
Precision (repeatability, RSD, %)[a]		
Low concentration level	2	0.5
Medium concentration level	1	0.4
High concentration level	0.2	0.9
Accuracy (bias, %)[a]		
Low concentration level	+6.9	+6.4
Medium concentration level	+2.5	+0.2
High concentration level	+3.0	−0.01
Specificity (s_{fit}, AU)	0.01	0.009
Figures of merit		
Sensitivity (mg l^{-1} AU^{-1})	0.13	0.38
Analytical sensitivity (γ, l mg^{-1})	24.9	18.9
Minimum concentration difference (γ^{-1}; mg l^{-1})	0.04	0.05
LOD (mg l^{-1})	0.09	0.10
LOQ (mg l^{-1})	0.25	0.32

[a] The lowest, medium and highest concentration levels of the calibrator solutions were used.

In addition, the easy availability of more detailed dissolution profiles provided by the continuous monitoring system facilitated carrying out comparisons among the dissolution profiles of lots B_1, B_2 and C. These operations were performed employing the Moore and Flanner's difference (f_1) and similarity (f_2) indexes [33], and lot B_1 as a reference, according to Eqs. (11) and (12),

$$f_1 = 100 \times \left(\sum_{t=1}^{k} |R_t - T_t| / \sum_{t=1}^{k} R_t \right) \quad (11)$$

$$f_2 = 50 \log \left\{ 100 \times \left[1 + 1/k \sum_{t=1}^{k} (R_t - T_t)^2 \right]^{-0.5} \right\} \quad (12)$$

where R_t and T_t are the dissolution measurements corresponding to the reference and test drug dosage formulation, respectively, at the tth time point, from a total of k time points of dissolution measurements. According to regulatory guidelines, the f_1/f_2 dissolution profile comparisons can be performed when three, four or more dissolution time points are available [7].

Table 2 records the inter-lot dissolution profile comparisons at various data point levels, using lot B_1 as reference. When these were performed employing three or four data points, a conclusion of sameness of the dissolution profiles was obtained for both drugs ($0 < f_1 < 15$; $50 < f_2 < 100$) in both lots. On the other hand, lot B_2 demonstrated to comply with the sameness criteria ($f_1 = 2.3$ and 12.7 and $f_2 = 99.1$ and 97.7 for BIS and HCT, respectively) for

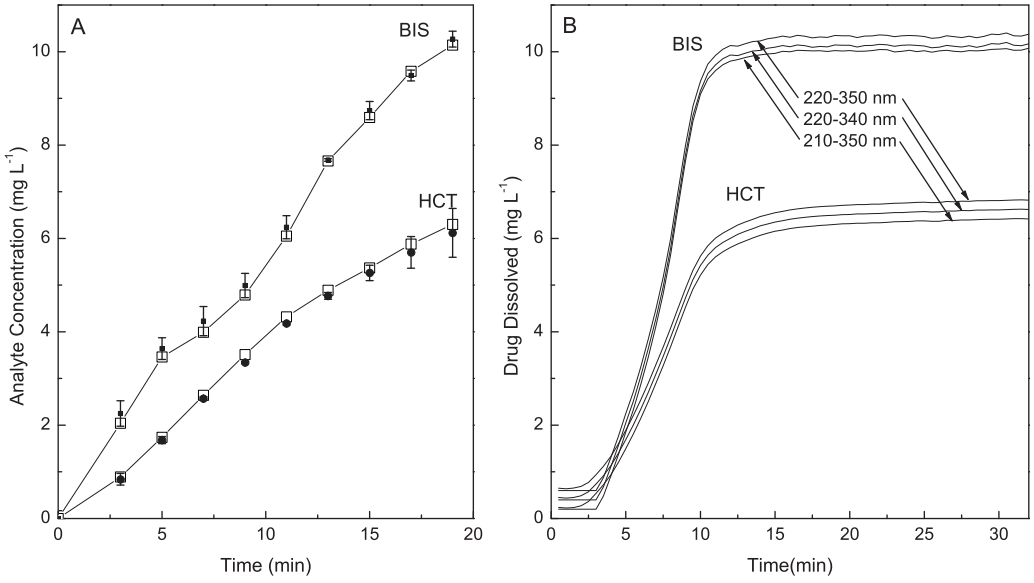

Fig. 4. (A) Assessment of the accuracy of analyte determinations through a simulated dissolution curve. Comparison between MCR-ALS results (□) and manual sampling followed by HPLC analysis (●). (B) Stacked dissolution curves for assessment of method robustness against variations of the operational wavelength range.

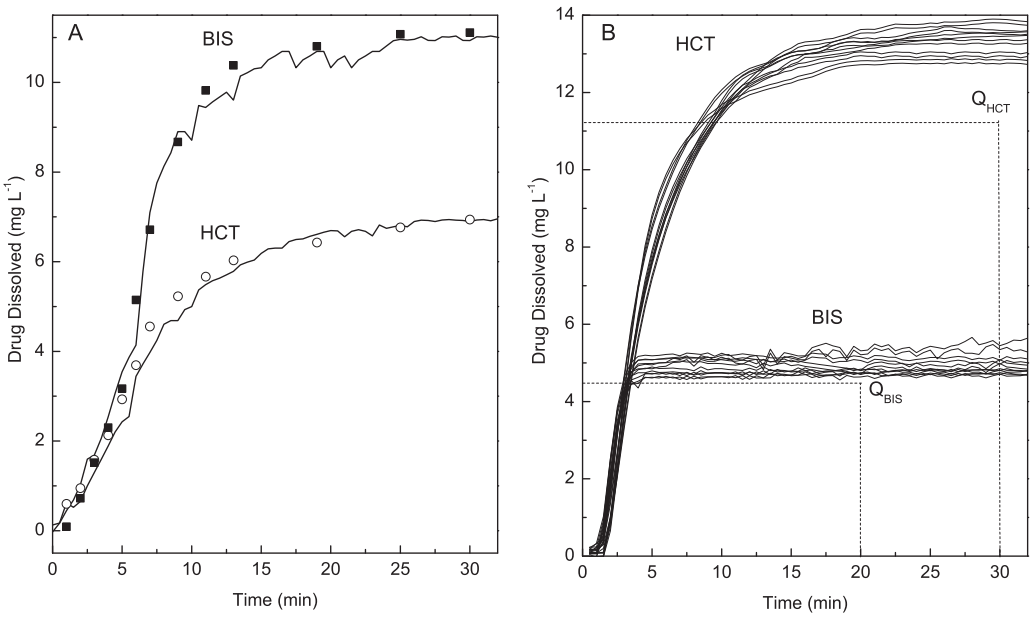

Fig. 5. (A) Comparison between the data acquired with manual sampling and HPLC for HCT (■) and BIS (○) in their combined tablet (lot A), and the corresponding dissolution curves obtained with the on-line UV-MCR-ALS continuous monitoring system. (B) Dissolution curves of 12 tablets of Brand B. Q_{HCT} and Q_{BIS} indicate the officially required minimum amounts of dissolved drugs for HCT (at 30 min) and BIS (at 20 min), respectively.

Table 2
Comparison between the dissolution profiles of lot B_1 with those of lots B_2 and C, employing f_1 and f_2 statistical indicators and various number of time points.

Number of time points		3		4		5		6		16		31	
Lot	Analyte	f_1	f_2	f_1	f_2	f_1	f_2	f_1	f_2	f_1	f_2	f_1	f_2
B_2	HCT	2.3	99.5	1.9	99.5	2.9	99.1	2.3	99.2	2.3	99.2	2.4	99.2
	BIS	12.8	98.1	11.7	98.1	12.6	97.9	12.7	97.9	13.0	97.7	12.7	97.7
C	HCT	6.9	96.3	6.9	95.4	9.1	93.0	9.0	91.8	9.0	92.3	9.2	92.2
	BIS	9.6	98.7	10.1	98.3	13.6	96.3	16.0[a]	93.3	16.4[a]	93.3	16.5[a]	93.2

[a] Non-compliance with the statistical criterion.

both drugs when 31 data points (15 min. of dissolution time) were employed, thus being also considered similar to B_1 under these conditions.

With regards to lot C, it was observed compliance with the similarity criterion (f_2 = 92.2 and 93.2 for BIS and HCT, respectively). However, and contradicting the comparison results obtained with the aid of three and four points, the profile of BIS failed to comply with the difference factor (f_1 = 9.2 and 16.5 for BIS and HCT, respectively), indicating the non-similarity between lost B_1 and C.

Moore and Flanner's statistical indexes have been criticized for being sensitive to large differences at a single time point and to the part of the profiles from where the dissolution data were obtained [34,35]. Taking into account that data variance decreases with an increase in sampling time points [36], the use of more data points to perform the comparison is likely to diminish the effect caused by large differences at a single time point. Multipoint data comparisons are also likely to reflect more accurately the dissolution differences along the curves, providing better assurance of the reliability of the results and diminishing in this way the chances of lot misclassification.

5. Conclusions

This study demonstrated that coupling an on-line circulation device with chemometrics-assisted UV detection (UV-MCR-ALS) results in a system capable of performing the simultaneous quantitation of two active principles being concomitantly dissolved from a combined pharmaceutical formulation, which also enables the construction of the corresponding dissolution curves, from their time-evolution data along the dissolution test.

The dissolution curves acquired from tablets of the hydrochlorothiazide–bisoprolol association exhibited excellent statistical agreement with those obtained through manual sampling followed by HPLC analysis under official conditions.

The quantitative aspects of the proposed method were properly validated. Compared to the traditional manual sampling approach, the system is faster and has less operational errors. The method provides full results immediately after the dissolution stage and conveniently allows acquisition of a high number of time points. As a result, better dissolution curves are obtained and more accurate and precise dissolution profile comparisons can be achieved.

Therefore, the proposed approach is a useful tool for investigating the behavior of many active components, when they are simultaneously being dissolved from their complex multidrug pharmaceutical formulations.

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