



An eco-friendly strategy, using on-line monitoring and dilution coupled to a second-order chemometric method, for the construction of dissolution curves of combined pharmaceutical associations



Natalia L. Calvo, Rubén M. Maggio, Teodoro S. Kaufman*

Pharmaceutical Analysis, Department of Organic Chemistry, School of Pharmaceutical and Biochemical Sciences, National University of Rosario and Institute of Chemistry of Rosario (IQUIR, CONICET-UNR), Suipacha 531, Rosario S2002LRK, Argentina

ARTICLE INFO

Article history:

Received 25 August 2013
Received in revised form 24 October 2013
Accepted 26 October 2013
Available online 9 November 2013

Keywords:

MCR-ALS
Green analytical chemistry
Simultaneous dissolution profiles

ABSTRACT

A simple, precise, economic and minimally operator-dependent method was developed under green analytical chemistry principles, for the simultaneous construction of the dissolution curves of a pharmaceutical association in short time and without employing organic solvents, allowing important savings of labor and resources. The carvedilol (CAR) and hydrochlorothiazide (HCT) combined formulation was employed as a model. The method (OD/UV-MCR) involves on-line sample dilution (OD) and UV detection of the analytes, coupled to multivariate curve resolution with alternating least squares (MCR-ALS).

OD/UV-MCR proved to be robust and was successfully validated in accordance to ICH guidelines, fulfilling acceptance criteria for specificity (r^2 of spectral correlation > 0.950), linearity [$r > 0.999$ ($N = 25$) in the ranges 1.00–31.1 mg l⁻¹ and 0.51–15.2 mg l⁻¹ for CAR and HCT, respectively] and precision (RSD < 2%). Accuracy was assessed by point-to-point comparison between the dissolution profiles furnished by the proposed method with those provided by HPLC analysis, evidencing the usefulness of this monitoring system.

In addition, OD/UV-MCR was successfully employed for the comparative analysis of three lots of commercial formulations of the CAR–HCT pharmaceutical association, belonging to a couple of different brands, employing Moore and Flanner's f_2 similarity indicator.

© 2013 Published by Elsevier B.V.

1. Introduction

In vitro dissolution testing has become a relevant strategy for assessing the performance of solid oral dosage forms. Construction of dissolution profiles of pharmaceutical formulations is currently an established operation, contained in modern pharmaceutical regulations [1–4].

The dissolution profiles gain significance because they are suitable for estimating the availability of the active ingredients and as critical means of assessing the similarity between innovator and generic products for exchangeability purposes [5]. They are also useful guides during the development of new formulations, being employed to control the lot-to-lot consistency during the manufacturing process [6], and serving to confirm its reproducibility after changes in location, equipment, lot size and other key manufacturing parameters [1,7,8].

Green chemistry is an established working paradigm which pursues eco-friendliness of chemical activities, by focusing on preventing pollution caused by chemicals. Its principles include diminishing contaminating wastes, using safer solvents and performing analysis in real time. Accordingly, green analytical chemistry strategies involve direct measurement of untreated samples, replacement of toxic reagents and automation [9].

Fixed-dose pharmaceutical associations are a special case of combination products, which are advantageous in terms of better therapeutic efficiency, reduced adverse effects, convenience of dose and improved patient compliance. However, their dissolution profiling for quality control purposes faces some practical challenges [10], including the need of carrying out the simultaneous quantification of their active principles in several samples, in a wide range of analyte concentrations and under cost-effective conditions. Furthermore, performing this operation without any physical separation step entails an additional hurdle, due to the possibility of mutual interference of the formulation ingredients.

Different chromatographic, electrophoretic and spectroscopic approaches have been developed in recent times toward the elaboration of dissolution profiles of drug associations [11,12]. The

* Corresponding author. Tel.: +54 341 4370477x118; fax: +54 341 4370477x112.
E-mail address: kaufman@iquir-conicet.gov.ar (T.S. Kaufman).

first group is based mainly on HPLC; however, this methodology requires skilled manpower and demands the use of significant amounts of organic solvents, being also time-consuming, poorly eco-friendly and not very cost-effective.

The second alternative is represented by capillary electrophoresis (CE) [13]; however, it also entails sample conditioning and the use of expensive equipment. In addition, CE methods often suffer from problems of reproducibility and, as for the HPLC determinations, measurements cannot be carried out on-line.

The third choice involves using spectroscopic means. Despite being fast and their use inexpensive, they are sensitive to interferences due to the presence, in the dissolution medium, of other absorbing active ingredients or excipients from the dosage form under test.

Some of these drawbacks can be overcome by chemometrics processing of the spectroscopic signals. Although NIR, Raman and IR spectroscopies have been employed for monitoring drug dissolution [14–16], UV–vis is the most used spectroscopic approach. We and others have developed UV-chemometric strategies to the point-by-point acquisition of dissolution curves and dissolution profiles from pharmaceutical associations [17].

However, in order to deal with the wide range of analyte concentrations in the dissolution samples, different treatments were employed, including sample dilutions [18], use of multiple calibrators [19] and asymmetric calibration designs [20], rendering the overall strategy too laborious, hence time-consuming, less user-friendly and more prone to errors.

The association between fiber optics and UV–vis detection [21,22], an alternative which became an area of interest in dissolution testing technology since it enables on-line monitoring of the process, has attracted attention among the pharmaceutical analysts and has found some use for the elaboration of dissolution curves of drug associations [23]. However, the fiber-optics setups demonstrated not to be versatile enough to deal with the scatter produced by undissolved materials and with detector saturation due to high analyte concentrations.

Recently, we have also reported a simple and efficient on-line monitoring strategy for the simultaneous construction of two dissolution curves from a binary drug association, entailing a closed circulation system with a flow cell associated to a UV–vis detection system, and a chemometrics application for data analysis (UV-MCR-ALS) [24]. However, we have observed that this experimental setup is not general. One of its important practical limitations becomes evident when high concentrations of the analytes must be quantified; in these cases, the direct determination of the active principles in the dissolution media is hindered by detector saturation or impeded by lack of linearity of the detector response. In these cases, signal adjustment becomes mandatory.

One of such scenarios is the dissolution testing of the carvedilol (CAR) and hydrochlorothiazide (HCT) association (Fig. 1), where loss of linearity followed by detector saturation, only allows unattended monitoring of the initial stages of the dissolution.

The CAR–HCT association, which combines a non-cardioselective β -adrenergic blocker [25] and a benzothiadiazine diuretic, respectively, has been patented for the treatment of cardiac and cardiovascular disorders, such as hypertension, angina pectoris and cardiac insufficiency [26]. The combination is indicated when the monotherapy fails to achieve arterial blood pressure normalization. However, CAR is poorly soluble (0.02 mg ml^{-1} at pH 7.4) and the association must be administered in very carefully adjusted doses for each patient; hence, dissolution studies are needed to guarantee manufacturing consistency and that proper amounts of the drugs are made bioavailable.

The simultaneous quantification of CAR and HCT has received much attention, and several spectrophotometric [27–29] and chromatographic [30,31] methods have been reported for that purpose

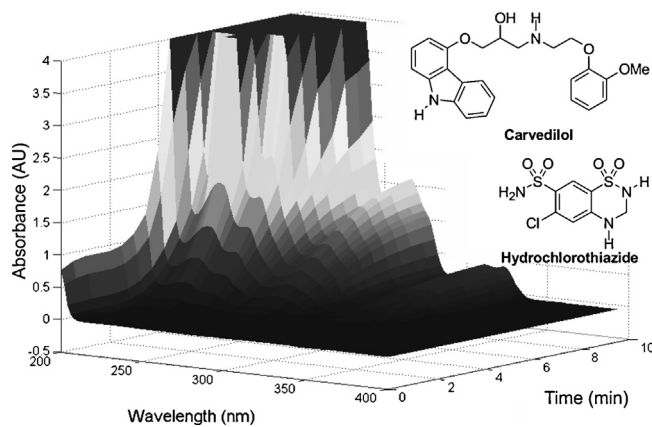


Fig. 1. Chemical structures and 3D graphical representation of the dissolution process of tablets containing the CAR–HCT association employing the system described in Ref. [21].

in combined dosage forms or in biological fluids [32,33]; however, while the former are not very well suited for dissolution testing purposes, the latter require sophisticated equipment and were designed for aims other than routine pharmaceutical quality control.

Therefore, as a natural evolution of our previous study, herein we report the development and validation of a simple and efficient chemometrics-assisted alternative for the on-line monitoring of the pharmaceutical dissolution of a drug association, especially when the amounts of the dissolved drugs saturate the detector.

The strategy (OD/UV-MCR), which complies with green analytical chemistry principles and strategies, and allows the simultaneous construction of the dissolution curves of the active components, is based on performing on-line sample dilution and requires minimal operator intervention. The association between CAR and HCT was employed as an example.

2. Materials and methods

2.1. Reagents

2.1.1. Chemicals

All the experiments were performed with pharmaceutical-grade CAR and HCT. HPLC-grade acetonitrile (ACN) was acquired from Panreac (Barcelona, Spain). Water was obtained from a Milli-Q system (Millipore, Bedford, USA) and was employed for HPLC experiments and for preparing the samples and dissolution media. All other chemicals were of analytical grade and were used as received. Three lots of tablets (A_1 , A_2 and B) belonging to two different brands of the commercial CAR–HCT association (25 mg CAR and 12.5 mg HCT) were acquired in a local pharmacy.

2.1.2. Sample preparation

Stock standard solutions of CAR (5020 mg l^{-1}) and HCT (2530 mg l^{-1}) were independently prepared in 10 ml volumetric flasks, by dissolving accurately weighed amounts of the drugs in methanol. Working solutions (100.4 mg l^{-1} for CAR and 50.6 mg l^{-1} for HCT) were prepared by transferring appropriate volumes of the stock solutions to separate 50 ml volumetric flasks and diluting to their marks with 0.1 N HCl.

Two sets of five calibration samples each were prepared in volumetric flasks, by mixing appropriate volumes of the working solutions of the drugs and diluting to the mark with 0.1 N HCl. The concentration levels obtained for CAR were 1.00, 8.03, 16.1, 24.1 and 31.1 mg l^{-1} and those for HCT were 0.51, 2.02, 8.10, 12.1 and 15.2 mg l^{-1} .

2.2. Dissolution testing

The dissolution tests were carried out with a Hanson SR8-Plus dissolution test station (Hanson Research, Chatsworth, USA), configured with paddles (USP apparatus II) [3]. The dissolutions were performed in 900 ml of 0.1 N HCl as dissolution medium, thermostated at 37 °C, and at a paddle rotation rate of 75 rpm. The dissolution medium was degassed before use by sonication in a Cole Parmer 8891 ultrasonic bath (Cole Parmer, IL, USA). Each test comprised the dissolution of a lot of 12 tablets.

For sample analysis by HPLC, 3 ml aliquots were withdrawn from the dissolution medium at eight pre-established times (2, 5, 7, 10, 12, 15, 20 and 25 min), filtered and injected in the chromatograph.

2.3. On-line dilution system and UV measurements

The spectroscopic determinations in the UV were carried out with a Shimadzu UV-1601PC double beam spectrophotometer (Shimadzu Corp., Kyoto, Japan) controlled by Shimadzu's UV-Probe (version 2.00) software and fitted with an 80 μ l flow cell of 10 mm optical path length (Hellma, Müllheim, Germany). The determinations were performed against a blank of dissolution medium contained in a 10 mm optical path length quartz cuvette placed in the reference cell holder.

The dissolution medium was continuously withdrawn from the dissolution vessel through the sampling probe at a 1.10 ml min⁻¹ flow-rate, by means of a Gilson Minipuls 3 peristaltic pump. Concomitantly, fresh dissolution medium contained in an external reservoir was pumped at the same rate and mixed with the dissolution solution coming from the vessel through a three way switch valve. The mixed solution was discarded after passing through the flow cell [34]. Degassing of the dissolution media and on-line sample filtration avoided potential interferences due to bubbles or undissolved particles.

Spectra were collected after a standard 3.1 min pre-established delay time, corresponding to the tubing dead volume. The dissolution experiments were monitored every 0.5 min during 30 min and spectra were acquired at 2 nm intervals in the 238–400 nm range (82 data-points per spectrum). The acquired data were saved as a matrix in CSV format.

2.4. Calibration and dissolution procedures: input data arrangement

The calibration solutions of each analyte were sequentially run through the system for periods of 5 min each. Ten spectra per concentration level were collected. The full calibration data of each analyte were saved in CSV format, read into Matlab and stored as matrices ($D_{\text{CAR}(50 \times 82)}$ and $D_{\text{HCT}(50 \times 82)}$). Analogously, the dissolution of 12 tablets per lot was monitored. The acquired data were stored in CSV format and read into Matlab as a matrix ($D_{\text{t}(60 \times 82)}$) for each dosage unit.

2.5. Chromatographic analysis

A Varian Prostar 210 liquid chromatograph (Varian Inc., Palo Alto, USA) equipped with two isocratic pumps, a 20 μ l injection loop and a variable dual-wavelength UV-detector, was used for HPLC analysis. The chromatograms were acquired and processed with Varian's Galaxie software (version 6.41).

The dissolution samples were filtered through a 0.47 μ m membrane filter before injection and their determination was carried out according to Haggag and co-workers [31]. The separation was performed with a C-8 column thermostated at 30.0 \pm 0.2 °C, employing a 50:50 (v/v) mixture of ACN:phosphate buffer (0.05 M, pH = 4.0) as mobile phase, pumped at 1.0 ml min⁻¹.

The approximate retention times for HCT and CAR were 3.2 and 7.3 min, respectively. The calibration equations were obtained by linear regression of the peak areas against the analyte concentrations in the corresponding standard solutions.

2.6. Computational methods

Chemometrics computations were carried out using MCR-ALS routines [35] written for Matlab R2010a (Mathworks, Natick, USA). Statistical data analyses were performed with Origin 8.5 (OriginLab Co., Northampton, USA).

3. Results and discussion

3.1. Theoretical background

MCR is a bilinear second order calibration method. Detailed theoretical background information regarding its operation has been given elsewhere [36]. The following is a brief rationale of its application to the elaboration of dissolution curves and profiles.

The dissolution test can be regarded as an evolving process, where the dissolving analytes maintain their spectral characteristics over time while changing their concentrations up to a maximum. Monitoring of the dissolution test of a pharmaceutical dosage form containing n relevant analytes, and collecting i spectra over time at j wavelengths per sample, yields a series of data that can be arranged as a matrix ($D_{(i \times j)}$). The rows of this matrix represent the overall contribution of the dissolving species to the absorbance of the dissolution medium recorded at different times, while its columns contain the combined dissolution curves acquired at different wavelengths. This chemical system can be described by a bilinear model based on the multi-wavelength extension of Lambert–Beer's absorption law Eq. (1).

$$D_{(i \times j)} = C_{(i \times n)} S_{(n \times j)}^T + E_{(i \times j)} \quad (1)$$

MCR can perform the decomposition of the above matrix into the product of two smaller matrices. These are the scores matrix $C_{(i \times n)}$, which contains information on the evolution of the concentration (dissolution curves) of the active principles and the loadings matrix $S_{(j \times n)}$, which hosts the pure spectra of the active pharmaceutical ingredients of the drug association. Every vector of C is associated with a vector of S through a product which represents a system component. Matrix $E_{(i \times j)}$ contains the error of the model.

During the MCR operation, C and S are iteratively optimized by an alternating least square regression procedure, based on initial estimations [37] of C or S and some mathematical constraints. New estimations of the dissolution curves and the pure spectra are obtained after every iteration, by solving Eqs. (2) and (3),

$$C = D(S^T)^+ \quad (2)$$

$$S^T = C^+ D \quad (3)$$

where C^+ and $(S^T)^+$ are the pseudoinverses of matrices S^T and C , respectively. Computation of C and S^T is carried out until a convergence criterion is reached.

The augmented experimental data matrix (D_{aug}), shown in Eq. (4), is a multiset structure obtained by organizing the data of the m calibration ($D_{\text{c1} \dots \text{cm}}$) standards and all the dissolution ($D_{\text{t1} \dots \text{t12}}$) runs in such a way that the data matrices are placed one aside each other [24].

$$D_{\text{aug}} = [D_{\text{c1}}; D_{\text{c2}}; \dots; D_{\text{cm}}; D_{\text{t1}}; D_{\text{t2}}; \dots; D_{\text{t12}}] = C_{\text{aug}} S^T + E \quad (4)$$

By analogy with Eq. (1), the MCR-ALS algorithm is used to decompose D_{aug} into the product of matrices C_{aug} , and S^T . C_{aug} is the matrix which contains the concentration contributions of

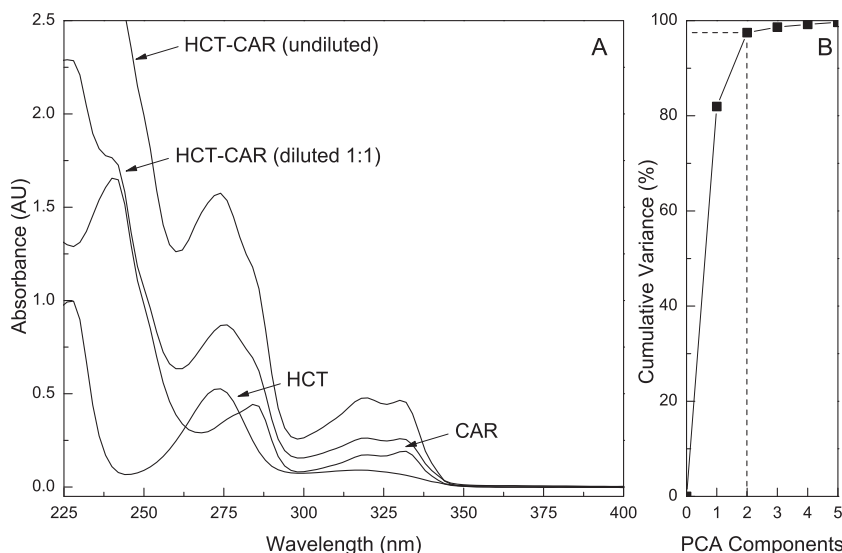


Fig. 2. (A) UV-vis spectra of CAR (31.1 mg l⁻¹), HCT (15.2 mg l⁻¹) and their mixture in the 225–400 nm region, before and after a 1:1 dilution. (B) PCA-based determination of the number of components for initialization of the MCR-ALS algorithm.

the analytes for all sub-matrices (m calibration standard sets and 12 dissolution curves), while S houses the spectra of the analytes present in all test matrices.

3.2. System configuration for monitoring the dissolution process

Quantitative analysis by UV-vis spectroscopy requires fulfilling two main requirements. These include that the detector response

must follow the Lambert-Beer law and that the analyte's signal should not be interfered.

When the dissolution test of CAR-HCT tablets was performed employing the original setup of the on-line monitoring system [24], it was observed that high absorbances quickly caused detector saturation, hindering the correct determination of the dissolved species (Fig. 1). Therefore, two alternative solutions were considered, including the use of a narrower part of the

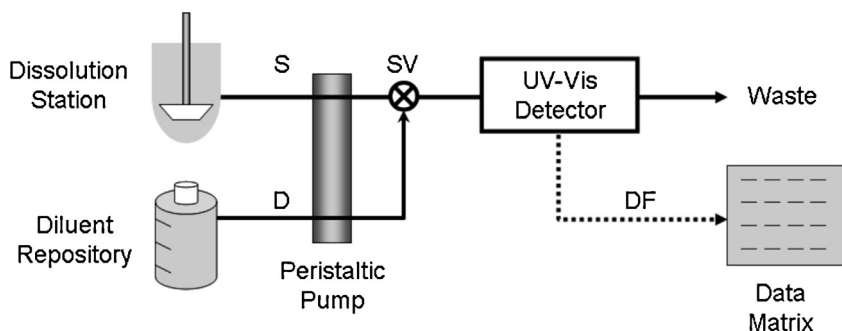


Fig. 3. Diagram of the flow system and coupled data acquisition setup employed for the chemometrics-assisted simultaneous evaluation of the dissolution of CAR and HCT. S = sample; D = diluent; SV = switch valve and DF = data flow.

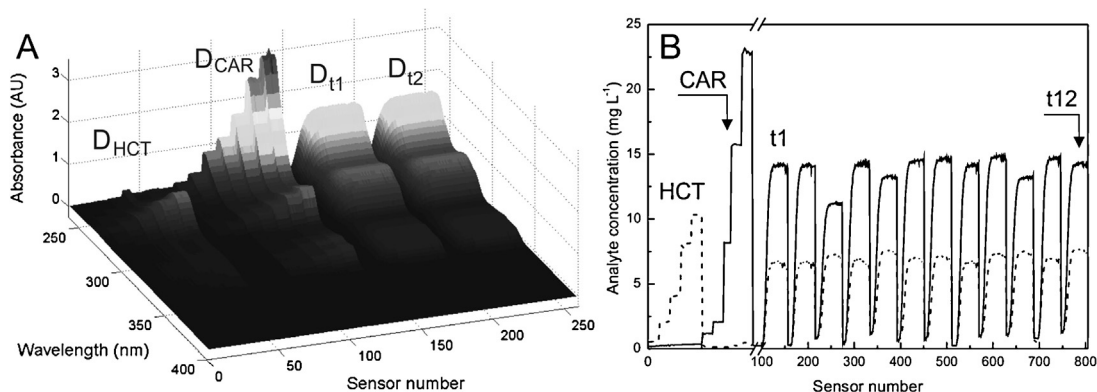


Fig. 4. (A) Data arrangement in the input supermatrix (sensors 1–50 for HCT and sensors 51–100 for CAR). Detector responses corresponding to the calibrators and two dissolution curves (data arranged time-wise). (B) Representation of the concentration profiles of HCT (---) and CAR (—) in the calibrators and the deconvoluted dissolution curves of the test tablets.

spectrum and performing a continuous on-line sample dilution strategy.

Since the first choice invariably reduces the amount of information and may not be a general solution, the second option was considered more appropriate and was investigated, with the dissolution medium as diluent. A 1:1 dilution was estimated convenient for achieving the analytical goal. Under these conditions it was demonstrated that the resulting concentrations of the analytes obeyed Beer's law in the region of interest (Fig. 2A).

3.3. Data acquisition and resolution of the dissolution curves

Fig. 3 outlines the methodology employed for data acquisition. Mixing of the dissolution medium with the diluent after the pump ensured better robustness and reproducibility of the results. The process did not require organic solvents, thus minimizing waste disposal costs.

MCR-ALS was chosen as the chemometrics algorithm for deconvolution of the dissolution curves based on its proven ability to extract information about the components involved in evolving mixtures, attribute the resulting spectra to chemical compounds, quantify their individual contributions, and use these data to develop dynamic models of the process with or without an a priori knowledge of the system [36]. In order to convey physical meaning to the results, working conditions including the preferred spectral region, number of components and operating restrictions were defined.

3.3.1. Selection of the operational range

Fig. 2A depicts the UV–vis spectra of pure CAR and HCT and their mixture in the proportion found in their combined commercial tablets, between 225 and 400 nm. The spectra are strongly overlapped, and large differences were observed at lower wavelengths (225–250 nm). However, both spectra exhibit absorption profiles of the same order of magnitude between 250 and 350 nm, and the drugs become transparent beyond 350 nm. Therefore, the spectral interval between 238 and 400 nm, where $Abs_{max} < 1.80$, was selected as the operational zone. The 350–400 nm region, where none of the analytes exhibits relevant absorbance, was included in order to have a proper baseline reference.

3.3.2. Input data matrix and constraints

A correct arrangement of the input data is crucial for meaningful results. The signals of the calibration samples (sensors 1–50 for HCT and 51–100 for CAR) were placed together with the data of the dissolution samples in the D_{aug} matrix; the latter were accommodated along the time-wise direction (60 sensors per sample). Fig. 4A represents the structure of the MCR-input supermatrix, exhibiting the absorbances of the calibration solutions and those of two tablets.

The dissolution analysis was performed in the presence of several constraints, which were put in place in order to avoid rotational ambiguities, thus ensuring the proper physicochemical sense of the resulting dissolution curves. These included 'non-negativity', which requires that spectra and analyte concentrations must be non-negative, taking into account that the dissolution curves exhibit a monotonously growing, S-shaped behavior until they reach a plateau and that the concentrations of the analytes involved are always positive.

In agreement with previous knowledge of the chemical system, PCA of the input supermatrix of lot A₁ revealed that two components explained more than 95% of the overall variance (Fig. 2B); therefore, this information was employed for system modeling.

Finally, the mean spectra ($N=10$) of the central concentration levels of the calibration curves of CAR and HCT (16.1 and

Table 1
Results of method validation. Range, linearity and figures of merit.

Parameter	Analyte	
	CAR	HCT
Range		
Concentration levels ($mg\ l^{-1}$)	1.00, 8.03, 16.1, 24.1, 31.1	0.51, 2.02, 8.10, 12.1, 15.2
Range ($mg\ l^{-1}$)	1.00–31.1	0.51–15.2
Number of replicates/level (N)	5	5
Linearity (pseudo-univariate regression)		
Slope \pm SD ($mg\ l^{-1}$ AU)	0.136 \pm 0.002	0.1009 \pm 0.0007
Intercept \pm SD ($mg\ l^{-1}$)	0.14 \pm 0.04	0.071 \pm 0.007
r ($N=50$)	>0.999	>0.999
sR	0.051	0.012
Figures of merit		
Sensitivity ($mg\ l^{-1}$ AU)	0.136	0.101
Analytical sensitivity (γ , $l\ mg^{-1}$)	2.59	8.60
MCD (γ^{-1} , $mg\ l^{-1}$)	0.39	0.12
LOQ ($mg\ l^{-1}$)	0.90	0.27

$8.10\ mg\ l^{-1}$, respectively) were also feed as initial estimations of the spectra before triggering the MCR algorithm.

3.3.3. Resolution of pure spectra and dissolution curves by MCR-ALS

Once the initial conditions were established, the MCR-ALS algorithm was used to deconvolute D_{aug} into the set of spectra of the analytes and solve their individual contributions over time to the whole dissolution process. Comparisons between the reference spectra of CAR and HCT and the spectra resulting from MCR analysis of the input supermatrix allowed the unequivocal identification of the corresponding analytes and assignment of their contributions. Interferences were not observed, since no additional components were found when MCR was initialized with 3 components. Nevertheless, being a second order method, it is expected that MCR-ALS could be able to selectively determine the analytes even in the presence of unknown interferences, exploiting its second order advantage [38].

Pseudo-univariate calibration curves were obtained by plotting the amplitude of the MCR-concentration component of each analyte (sensors 1–50 for HCT and sensors 51–100 for CAR) against their actual concentrations. In turn, these were employed to estimate the dissolution curves of the analytes (Fig. 4B). Finally, the dissolution profiles were computed as the means of 12 dissolution curves.

3.4. Method validation

In order to guarantee the suitability of the entire procedure, the method was validated in agreement with ICH guidelines [39]. Pseudo-univariate calibration curves were built from the responses of the standards, in the ranges 1.00 – $31.1\ mg\ l^{-1}$ and 0.51 – $15.2\ mg\ l^{-1}$ for CAR and HCT, respectively [40]. These ensured coverage of sample concentrations from a minimum of 5% up to 110% with regards to complete tablet dissolution (approximately $27.8\ mg\ l^{-1}$ and $13.9\ mg\ l^{-1}$ for CAR and HCT, respectively).

The regression curves exhibited correlation coefficients (r) exceeding 0.990 ($N=50$); in addition, the residues were distributed at random and the intercepts were close to zero, confirming method linearity (Table 1).

Additionally, figures of merit such as limit of quantification (LOQ), sensitivity, analytical sensitivity (γ) and the minimum concentration difference (MCD, γ^{-1}), were obtained from the pseudo-univariate calibration curves [41]. These were considered satisfactory, confirming method suitability for the intended purpose across the linear range (Table 1).

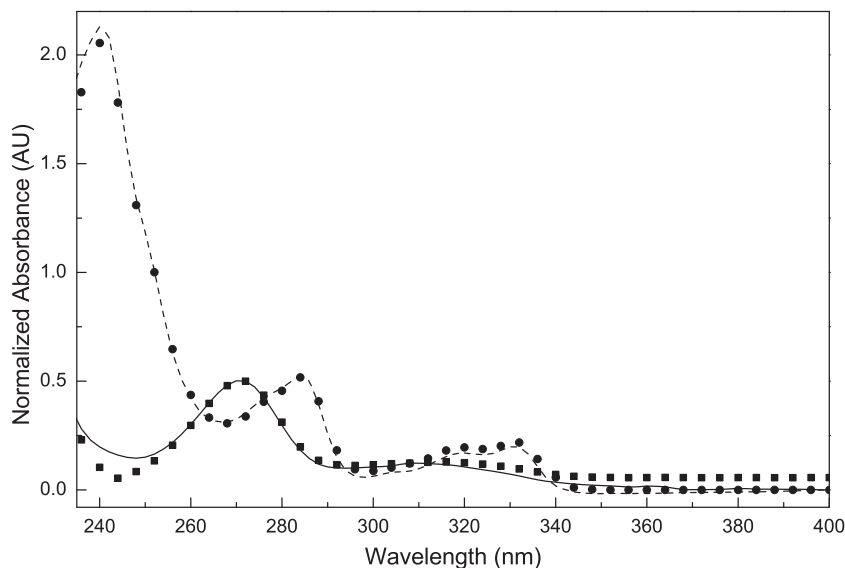


Fig. 5. Assessment of method specificity. Comparison among normalized UV–vis spectra. Spectra obtained with solutions of pure CAR (---) and HCT (—) and by MCR-ALS analysis of the dissolution curves for CAR (●) and HCT (■).

Method specificity refers to its ability to properly resolve each analyte, free from interferences of other analytes and the matrix. This was confirmed by statistical comparison between the spectra obtained by MCR-ALS and those of the corresponding standards, through the squared correlation coefficient (r^2), a numerical value used to characterize the degree of spectral dissimilarity [42]. Linear regression analysis furnished r^2 values of 0.991 and 0.966 ($N=82$) for CAR and HCT, respectively. The excellent agreement ($r^2 > 0.950$) between the pairs of curves (Fig. 5) evidenced that the determination was specific.

Method precision at the repeatability level was assessed by evaluating the relative standard deviations of 20 determinations of solutions containing mixtures of the standards of CAR and HCT at three different concentration levels (low, medium and high). The observed RSD values (Table 2) were lower than 2%, being considered satisfactory. In addition, at the same levels analyte recoveries were almost quantitative, yielding low bias results.

In order to further assess method accuracy, a batch of commercial tablets was simultaneously evaluated by OD/UV-MCR and HPLC as a reference [39], at eight time-points, and the resulting dissolution profiles (Fig. 6) were subjected to Student t -tests on a point by point basis. No statistically significant differences were observed along the curves, demonstrating the accuracy of the chemometrics-based method.

Method robustness, referred to its ability to remain immune to small variations in critical parameters, was assessed by evaluation of the effect of modifying several operational variables, including the spectral region of interest (± 5 nm), the manifold length (± 10 cm), the flow rate (± 0.1 ml min $^{-1}$) and the spectral acquisition speed (± 5 s). To that end, a solution of dissolution medium containing a fully dissolved tablet was monitored under the different conditions, changing one variable at a time, and the analyte recoveries were evaluated by a one way ANOVA test. The results ($F=0.51$ and 0.69 for CAR and HCT, respectively; $F_{\text{crit}}=3.87$) confirmed method robustness.

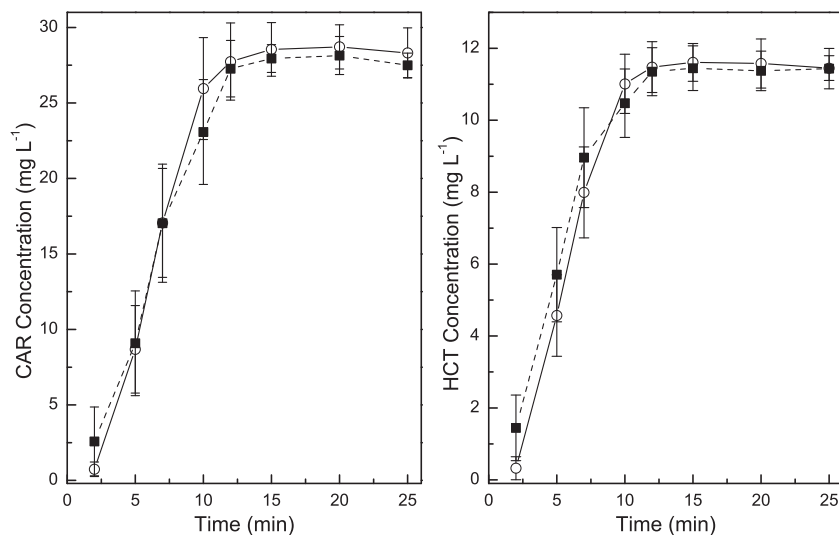


Fig. 6. Verification of method accuracy. Concentration profiles obtained by HPLC (—■—) for the dissolution of a commercial HCT-CAR tablet. Comparison with those furnished by MCR-ALS (—○—).

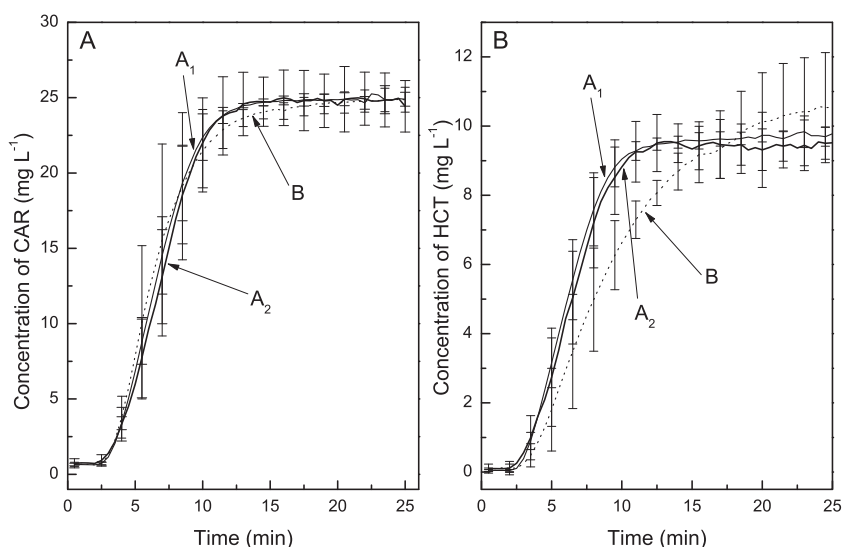


Fig. 7. Dissolution profiles comparison among brands and lots of CAR–HCT tablets. Profiles obtained by MCR-ALS method for CAR (A) and HCT (B).

Table 2

Results of method validation. Assessment of precision (repeatability).^a

Analyte	Low level			Medium level			High level		
	Rec (%)	RSD (%)	Bias (%)	Rec (%)	RSD (%)	Bias (%)	Rec (%)	RSD (%)	Bias (%)
CAR	98.5	0.1	−1.5	101.3	0.3	+1.3	101.8	0.6	+1.8
HCT	97.8	0.4	−2.2	99.1	0.2	−0.9	102.0	0.5	+2.0

^a The lowest, medium and highest concentration levels were 5.02, 16.1 and 24.1 mg l^{−1} for CAR and 2.53, 8.10 and 12.1 mg l^{−1} for HCT, respectively.

3.5. Evaluation of commercial samples

According to the WHO, a comparative *in vitro* dissolution profile similarity can be used to document equivalence of a multisource (generic) with a comparator (ethical) product [5]. The ability of the proposed OD/UV-MCR monitoring scheme to acquire an important number of dissolution data-points, is advantageous for comparing dissolution profiles, since the official Moore and Flanner's f_2 parameter [43] is sensitive to the number of data-points [44]. Therefore, it was applied for the evaluation of three lots (A_1 , A_2 and B) of commercial tablets of the CAR–HCT association (Fig. 7), corresponding to two commercial brands, using f_2 .

OD/UV-MCR was run as before, with the same initialization data and restrictions, and PCA analysis confirmed the presence of only two components in all of the tested lots, ensuring the absence of unexpected interferences that may affect the results.

Analysis of the dissolution profiles revealed similarity between lots A_1 and A_2 ($f_2 = 84$ and 75 for CAR and HCT, respectively), since their f_2 values exceed the threshold of 50. On the other hand, lot B exhibited $f_2 = 71$ for CAR and $f_2 = 37$ for HCT, suggesting that it should not be considered as similar.

4. Conclusions

A rapid, inexpensive and general chemometrics strategy (OD/UV-MCR) for the simultaneous construction of dissolution curves of a pharmaceutical association was developed under green analytical chemistry principles. The strategy, which hinges on on-line sample dilution, avoids drug quantification errors stemming from saturation of the detector and lack of linearity due to high analyte concentrations. Tablets of the carvedilol–hydrochlorothiazide association (CAR–HCT) were employed as a model.

The absorbances of the on-line diluted samples were acquired in a pre-selected region of the UV–vis spectrum. Their chemometrics

processing with MCR-ALS furnished qualitative and quantitative information of the pure components, including their spectra and time–concentration profiles (dissolution curves). The so obtained pure component spectra were in excellent relation with those of standard samples, while the dissolution curves showed not statistical differences with those obtained by HPLC analysis.

On-line dilution avoided lack of linearity or detector saturation due to high concentration of the dissolved analytes, while eliminating the need to further handling process samples. With the exception of system calibration, the whole operation did not require for laboratory personnel to come in contact with samples. Furthermore, the quantification of the dissolved drugs over the entire dissolution range was accomplished with a few external standard solutions covering the expected concentration span of the analytes.

The dissolution profiles of three different lots of commercial CAR–HCT tablets were built with the OD/UV-MCR approach and compared, confirming that it is a powerful and versatile tool for gaining better understanding of the quality of multicomponent pharmaceutical products.

Being amenable to be carried out without the need of sophisticated instrumentation or complex sample pre-treatments, the proposed alternative can be easily implemented in a routine quality control laboratory, suggesting that it may find use within the current pharmaceutical quality assurance paradigm.

Acknowledgments

The authors gratefully acknowledge financial support provided by the Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT), Secretaría de Ciencia y Técnica de la UNR (SECyT-UNR) and Secretaría de Ciencia, Tecnología en Innovación

de la Provincia de Santa Fe (SECTel). N.L.C. is also thankful to CON-ICET for her fellowship.

References

- [1] FDA Guidance for Industry. Dissolution Testing of Immediate Release Solid Oral Dosage Forms, FDA, Rockville, MD, 1997, Available from: <http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070237.pdf>
- [2] O. Anand, L.X. Yu, D.P. Conner, B.M. Davit, Dissolution testing for generic drugs: an FDA perspective, *AAPS J.* 13 (2011) 328–335.
- [3] The United States Pharmacopeia, USP35-NF30, Chapter 1092. Dissolution Procedure: Development and Validation, U.S. Pharmacopeial Convention Inc., Rockville, MD, USA, 2011.
- [4] Note for Guidance on the Investigation of Bioavailability and Bioequivalence, EMEA, London, 2001.
- [5] World Health Organization, WHO Technical Report Series, No. 937, 2006, Annex 7. Available from: http://apps.who.int/prequal/info_general/documents/TRS937/WHO_TRS_937_annex7_eng.pdf
- [6] J.J. Dressman, J. Kramer, *Pharmaceutical Dissolution Testing*, Marcel Dekker, New York, USA, 2005.
- [7] V.P. Shah, L.J. Lesko, J. Fan, N. Fleischer, J. Handerson, H. Malinowski, M. Makary, L. Oudekirk, S. Roy, P. Sathe, G.J.P. Singh, L. Tillman, Y. Tsong, R.L. Williams, FDA guidance for industry: dissolution testing of immediate release solid oral dosage forms, *Dissol. Technol.* 4 (1997) 15–22.
- [8] FDA Guidance for Industry: Immediate Release Solid Oral Dosage Forms. Scale-Up and Post-Approval Changes: Chemistry, Manufacturing and Controls, In Vitro Dissolution Testing and In Vivo Bioequivalence Documentation, FDA, Rockville, MD, 1995.
- [9] M. de la Guardia, S. Garrigues, *Handbook of Green Analytical Chemistry*, Wiley, NY, 2012.
- [10] N. Pourkavoos, Unique risks, benefits, and challenges of developing drug–drug combination products in a pharmaceutical industrial setting, *Comb. Prod. Ther.* 2 (2012) 2–31.
- [11] Z.-L. Fang, Q. Fang, X.-Z. Liu, H.-W. Chen, C.-L. Liu, Continuous monitoring in drug dissolution testing using flow injection systems, *Trends Anal. Chem.* 18 (1999) 261–271.
- [12] Q. Fang, S.S. Liu, J.F. Wu, Y.Q. Sun, Z.L. Fang, A stopped-flow microdialysis sampling-flow injection system for automated multi-vessel high resolution drug dissolution testing, *Talanta* 49 (1999) 403–414.
- [13] H.-W. Chen, Z.-L. Fang, Combination of flow injection with capillary electrophoresis: Part 4. Automated multicomponent monitoring of drug dissolution, *Anal. Chim. Acta* 376 (1998) 209–220.
- [14] A.C. de Oliveira Neves, G. Mesquita Soares, S. Cavalcante de Morais, F.S. Lopes da Costa, D. Lopes Porto, K.M. Gomes de Lima, Dissolution testing of isoniazid, rifampicin, pyrazinamide and ethambutol tablets using near-infrared spectroscopy (NIRS) and multivariate calibration, *J. Pharm. Biomed. Anal.* 57 (2012) 115–119.
- [15] A. Fussell, E. Garbacik, H. Offerhaus, P. Kleinebudde, C. Strachan, In situ dissolution analysis using coherent anti-Stokes Raman scattering (CARS) and hyperspectral CARS microscopy, *Eur. J. Pharm. Biopharm.* (2013), <http://dx.doi.org/10.1016/j.ejpb.2013.08.012>.
- [16] A. Kassis, V.M. Bhawtanka, J.R. Sowa Jr., Attenuated total reflection infrared spectroscopy (ATR-IR) as an in situ technique for dissolution studies, *J. Pharm. Biomed. Anal.* 53 (2010) 269–273.
- [17] E. Dinc, C. Serin, F. Tugcu-Demiröz, T. Doganay, Dissolution and assaying of multicomponent tablets by chemometric methods using computer-aided spectrophotometer, *Int. J. Pharm.* 250 (2003) 339–350.
- [18] M.C.F. Ferraro, P.M. Castellano, T.S. Kaufman, Simultaneous determination of amiloride hydrochloride and hydrochlorothiazide in synthetic samples and pharmaceutical formulations by multivariate analysis of spectrophotometric data, *J. Pharm. Biomed. Anal.* 30 (2002) 1121–1131.
- [19] P.M. Castellano, S.E. Vignaduzzo, R.M. Maggio, T.S. Kaufman, Application of a chemometric method for simultaneous determination of acetaminophen and diclofenac in content-uniformity and drug-dissolution studies, *Anal. Bioanal. Chem.* 382 (2005) 1711–1714.
- [20] R.M. Maggio, P.M. Castellano, T.S. Kaufman, A multivariate approach for the simultaneous determination of losartan potassium and hydrochlorothiazide in a combined pharmaceutical tablet formulation, *Anal. Bioanal. Chem.* 391 (2008) 2949–2955.
- [21] T. Mirza, Q. Liu, R. Vivilecchia, Y. Joshi, Comprehensive validation scheme for in situ fiber optics dissolution method for pharmaceutical drug product testing, *J. Pharm. Sci.* 98 (2009) 1086–1094.
- [22] V.A. Gray, Dissolution testing using fiber optics – a regulatory perspective, *Dissol. Technol.* 10 (2003) 33–36.
- [23] W. Li, J. Chen, B. Xiang, D. An, Simultaneous on-line dissolution monitoring of multicomponent solid preparations containing vitamins B1, B2 and B6 by a fiber-optic sensor system, *Anal. Chim. Acta* 408 (2000) 39–47.
- [24] R.M. Maggio, M.A. Rivero, T.S. Kaufman, Simultaneous acquisition of the dissolution curves of two active ingredients in a binary pharmaceutical association, employing an on-line circulation system and chemometrics-assistance, *J. Pharm. Biomed. Anal.* 72 (2013) 51–58.
- [25] S. Chakraborty, D. Shukla, B. Mishra, S. Singh, Clinical updates on carvedilol: a first choice beta-blocker in the treatment of cardiovascular diseases, *Expert Opin. Drug Metab. Toxicol.* 6 (2010) 237–250.
- [26] R. Heller, *Pharmaceutical compositions containing carvedilol and hydrochlorothiazide*, US Patent 6,403,579 (2002).
- [27] N.S. Abdelwahab, Spectrophotometric methods for simultaneous determination of carvedilol and hydrochlorothiazide in combined dosage form, *Arab. J. Chem.* (2011), <http://dx.doi.org/10.1016/j.arabc.2011.05.002>.
- [28] A. Ansary, M.M. Abdel-Moety, F.M. Abdel-Gawad, E.A. Mohamed, M.M. Khater, Simultaneous determination of carvedilol and hydrochlorothiazide in tablets and spiked human plasma using derivative spectrophotometry, *Pharm. Anal. Acta* 3 (9) (2012) 1–6.
- [29] H.M. Patel, S.S. Pancholi, N.P. Jivani, UV spectrophotometric method for simultaneous estimation of carvedilol and hydrochlorothiazide in bulk and pharmaceutical dosage form by simultaneous equation method, *Pharm. Anal. Qual. Assur.* (2013), Article ID: Inventi:ppaqj/602/12, Inventi Rapid, India.
- [30] M.A. Sultan, Simultaneous determination of carvedilol and hydrochlorothiazide in tablets by derivative spectrophotometric and HPLC methods, *Asian J. Chem.* 20 (2008) 2283–2292.
- [31] R.S. Haggag, R.A. Shaalan, T.S. Belal, Validated HPLC determination of the two fixed dose combinations (chlordiazepoxide hydrochloride and mebeverine hydrochloride; carvedilol and hydrochlorothiazide) in their tablets, *J. AOAC Int.* 93 (2010) 1192–1200.
- [32] K. Deventer, P. van-Eenoo, F.T. Delbeke, Simultaneous determination of beta-blocking agents and diuretics in doping analysis by liquid chromatography/mass spectrometry with scan-to-scan polarity switching, *Rapid Commun. Mass Spectrom.* 19 (2005) 90–98.
- [33] G.J. Murray, J.P. Danaceau, Simultaneous extraction and screening of diuretics, beta-blockers, selected stimulants and steroids in human urine by HPLC-MS/MS and UPLC-MS/MS, *J. Chromatogr. B* 877 (2009) 3857–3864.
- [34] B. Singh, T. Kaur, S. Singh, Correction of raw dissolution data for loss of drug and volume during sampling, *Indian J. Pharm. Sci.* 59 (1997) 196–199.
- [35] J. Jaumot, R. Gargallo, A. de Juan, R. Tauler, A graphical user-friendly interface for MCR-ALS: a new tool for multivariate curve resolution in MATLAB, *Chemom. Intell. Lab. Syst.* 76 (2005) 101–110.
- [36] A. de Juan, R. Tauler, MCR from 2000: progress in concepts and applications, *Crit. Rev. Anal. Chem.* 36 (2006) 163–176.
- [37] W. Windig, Spectral data files for self-modeling curve resolution with examples using the SIMPLISMA approach, *Chemom. Intell. Lab. Syst.* 36 (1997) 3–16.
- [38] N.E. Llamas, M. Garrido, M.S. Di Nezio, B.S. Fernández Band, Second order advantage in the determination of amaranth, sunset yellow FCF and tartrazine by UV–vis and multivariate curve resolution-alternating least squares, *Anal. Chim. Acta* 655 (2009) 38–42.
- [39] International Conference on Harmonisation (ICH) Guidelines, Q2(R1): Validation of Analytical Procedures: Text and Methodology, Geneva, Switzerland, 2005.
- [40] M.B. Mamián-López, R.J. Poppi, Standard addition method applied to the urinary quantification of nicotine in the presence of cotinine and anabasine using surface enhanced Raman spectroscopy and multivariate curve resolution, *Anal. Chim. Acta* 760 (2013) 53–59.
- [41] J. Saurina, C. Leal, R. Compañó, M. Granados, M.D. Prat, R. Tauler, Estimation of figures of merit using univariate statistics for quantitative second-order multivariate curve resolution, *Anal. Chim. Acta* 432 (2001) 241–251.
- [42] J. Li, D.B. Hibbert, S. Fuller, G. Vaughn, A comparative study of point-to-point algorithms for matching spectra, *Chemom. Intell. Lab. Syst.* 82 (2006) 50–58.
- [43] J.W. Moore, H.H. Flanner, Mathematical comparison of dissolution profiles, *Pharm. Technol.* 20 (1996) 64–74.
- [44] V.P. Shah, Y. Tsong, P. Sathe, R.L. Williams, Dissolution profile comparison using similarity factor, f_2 , *Dissol. Technol.* 6 (1999) 15–16, Available from: <http://www.dissolutiontech.com/DTresour/899Art/DissProfile.html>