Trends in Analytical Chemistry 108 (2018) 74–87

Contents lists available at ScienceDirect

Trends in Analytical Chemistry

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

Chemometrics coupled to vibrational spectroscopy and spectroscopic imaging for the analysis of solid-phase pharmaceutical products: A brief review on non-destructive analytical methods

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ARTICLE INFO

Article history:
Available online 30 August 2018

Keywords:
Counterfeit pharmaceutical products
Distribution of API and excipients
API polymorphic forms
Pharmaceutical cocrystallization process
Chemometrics and spectroscopy/imaging

ABSTRACT

This brief review reports on selected case studies aimed at verifying the authenticity of medicinal products, content uniformity of tablets and polymorphic forms in final products, and monitoring pharmaceutical cocrystallization processes. The studies combine chemometrics with vibrational spectroscopy or spectroscopic imaging, leading to non-destructive analytical methods. These methodologies allow one to analyze intact pharmaceutical formulations. Emphasis is directed to: (a) fighting against counterfeit pharmaceutical products, (b) spatial distribution of active pharmaceutical ingredients (API) in final products, (c) occurrence of polymorphic transitions in commercial tablets due to unsuitable storage conditions or excipient moisture, which could affect the apparent solubility, and (d) solubility enhancement of API polymorphic forms through a cocrystallization process.

1. Introduction

Non-destructive analytical methods based on chemometrics coupled to vibrational spectroscopy and spectroscopic imaging are useful for pharmaceutical product analysis in intact form. This makes these methods attractive to forensic science evidence because the samples still maintain their intact forms after analysis [1].

Multivariate models have been employed to extract chemical information of medicines, by processing data generated by vibrational spectroscopy, e.g. mid-infrared (MIR), near-infrared (NIR) and Raman spectroscopies [2], as well as by spectroscopic imaging, also called as hyperspectral imaging [3]. Particularly, in the case of first- and second-order multivariate models coupled to vibrational spectroscopy and spectroscopic imaging, recent reviews have highlighting their potentialities as non-destructive analytical methods for identification of counterfeit pharmaceuticals [4], verification of the authenticity of herbal medicines [5] and solid-state characterization of pharmaceutically relevant materials [6].

In the present report, a review is presented on the use of chemometrics coupled to vibrational spectroscopy and spectroscopic imaging as non-destructive analytical methods with the potentiality of verifying the authenticity of medicine products, the content uniformity of tablets, the characterization of polymorphic forms in final medicinal products, and the monitoring of pharmaceutical cocrystallization processes. They represent exciting examples of practical applications of chemometrics and vibrational spectroscopy and spectroscopic imaging in pharmaceutical analysis.

This brief review discusses the coupling of emerging technologies associated to vibrational spectroscopy and spectroscopic imaging, as recently reviewed in detail by Ewing and Kazarian [7], with chemometrics tools, which can be defined as “application of mathematics, statistics, and formal logic to design experimental procedures and extract relevant chemical information by analyzing chemical data” [8].

Chemometrics coupled to vibrational spectroscopy and spectroscopic imaging as non-destructive analytical method have been employed to: (a) fight against counterfeit pharmaceutical products [9,10,11], (b) assess content uniformity through the active pharmaceutical ingredient (API) distribution in final products in order to visualize the spatial distribution of each ingredient in the layers [12,13], (c) monitor the occurrence of polymorphic transitions, which

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https://doi.org/10.1016/j.trac.2018.08.013
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makes it necessary to identify the profile and quantify API crystalline forms which may change its crystalline structure through storage conditions or humidity present in the excipients, a phenomenon of huge importance due to the possibility of such polymorphic transitions to affect some properties of the API such as apparent solubility [14], and (d) monitor in-line the API solubility enhancement of polymorphic forms with low solubility, by adding of soluble molecules in its crystalline structure to improve its bioavailability through a pharmaceutical cocrystallization process [15]. Notably, these topics have yet to have been reviewed in detail to show the potentialities of first- and second-order multivariate calibration models coupled to vibrational spectroscopy and spectroscopic imaging in the analysis of medicinal products in their intact forms.

2. Chemometric models

Regression models for quantitative analysis such as partial least-squares (PLS) are well-known. For spectral imaging analysis, the most employed model is multivariate curve resolution – alternating least-squares (MCR-ALS), which will be explained below in connection with a specific example. Thus in this section we briefly summarize the usual chemometric models employed for discrimination and classification.

It may be worth to discuss the multivariate tools employed for qualitative data modeling and class assignment. When class memberships are not available, the models belong to the category of unsupervised. Although sometimes referred as classification models, principal components analysis (PCA) and hierarchical cluster analysis (HCA) are unsupervised models, mainly used for exploratory purposes, to identify trends, similarities and differences between samples, by reducing the dimensionality of the data and/or extracting dominant patterns in complex matrices.

On the other hand, supervised models employ both the instrumental data and the known class memberships to build classification models. They provide a rule to assign classes to new samples, by first training a model using a set of samples whose classes are already known. From an operational point of view, classification models can be subdivided in discriminant and class-modeling [16]. Discriminant models, such as PLS-Discriminant Analysis (PLS-DA), K-Nearest Neighbors (K-NN) among others are focused on the differences between samples belonging to different groups, whereas class-modeling tools, such as soft independent modeling of class analogy (SIMCA) and unequal class-modeling (UNEQ) are rather focused on the similarities between samples of the same group, that is, these models decide whether a new sample belongs to a certain group or not.

Different exploratory or classification models can be selected according to their characteristics, the data structure and the specific applications. It is difficult to provide decision rules for selecting particular models, an activity that has become a matter of trial and error. Some advices may be given based on the knowledge of the data structure. For example, if the relationship between experimental variables and class indexes is linear, PCA and linear models will be useful. Linear PLS-DA is an ideal classification model to discriminate samples, when the variability within a group is greater than the variability between several groups [17]. On the other hand, in the presence of substantial non-linearity, quadratic or universal non-linear models should be applied, such as artificial neural networks.

3. Counterfeit pharmaceutical products: tablet analysis in the solid-phase

Counterfeit pharmaceuticals and medicines may be harmful for patients that consume them because they may not contain the API or may contain less or more than the required amount of API used in the authentic version [18]. According to World Health Organization (WHO), they are defined as “products deliberately and fraudulently manufactured or mislabeled with respect to identity and/or source to make them appear to be authentic products” [19].

Counterfeit medical products represent about 10% of commercialized pharmaceutical tablets [20]. To fight against counterfeiting, efforts are required for developing non-destructive analytical methods able to analyze intact sample forms. Pharmaceutical products analysis is challenging due to the existence of genuine formulations of the same product family containing: (1) the same API content, but different excipients, leading to a different API spectral profile, or (2) lower API content than the recommended one, with similar API spectra if they have the same excipients. In this context, literature non-destructive analytical methods have been reported, based on first- and second-order multivariate models combined with vibrational spectroscopy and spectroscopic imaging, allowing for non-invasive analysis in intact pharmaceutical products [21,22,23,24].

For example, Roggo et al. [21] faced different challenges in the analysis of counterfeit pharmaceutical products using Raman spectroscopy to study real samples of genuine and counterfeit tablets. From the analysis of solid-phase pharmaceutical products, Raman spectroscopic data revealed the following relevant results: (1) various genuine tablets formulated with the same pharmaceutical product, but containing different excipients, generated different spectral profiles (Fig. 1), (2) formulations of the same pharmaceutical product with a low API content generated a similar spectral profile when compared with those containing the right API amount, provided they have the same excipients (Fig. 2) [21]. These facts demonstrate that the analysis based on API correlations is insufficient to confirm authenticity, making it necessary the application of non-destructive analytical methods based on different multivariate tools coupled to vibrational spectroscopy and spectroscopic imaging.

Counterfeit antimalarial tablets constitute a major public health concern, especially in developing countries such as those in Southeast Asia and Sub-Saharan Africa, where the pharmaceutical market is poorly regulated and controlled [25]. The use of these counterfeit products may lead to therapeutic failure, death and reinforced drug resistance [26] because they could contain no API, or an incorrect dosage [20]. Dowell et al. [27] processed NIR spectroscopic data with a first-order multivariate calibration model based on PLS-DA for solid-phase antimalarial tablet analysis. PLS-DA achieved 100% accuracy in the discrimination between authentic samples containing the API artesunate in the pharmaceutical formulations, and counterfeit antimalarial tablets where artesunate was absent. In a similar way, de Veij et al. [28] developed a model to fight against counterfeiting of artesunate tablets in Southeast Asia using Raman spectroscopy and first-order multivariate models based on PCA and HCA. They showed the potential of the developed methods by identifying 50 spectra of samples for each group, i.e. genuine artesunate and three different groups of counterfeits.

On the other hand, counterfeit pharmaceutical tablets are well-known in wealthier countries, such as sildenafil citrate commercialized as Viagra® and tadalafil commercialized as Cialis® and used against erectile dysfunction [29]. Sacré [30] studied 26 counterfeits and imitations of Viagra® tablets and 8 genuine tablets using Raman micro-spectroscopy imaging. The collected data were unfolded into a matrix to be processed by PCA and K-NN. After a preliminary feature extraction stage to compress the relevant information using PCA, the authors were able to detect counterfeit products based on different spectral ranges, according to the presence of lactose (830-880 cm^{-1}) and the spatial distribution of Viagra® (1200-1290 cm^{-1}). Subsequently, K-NN models were used

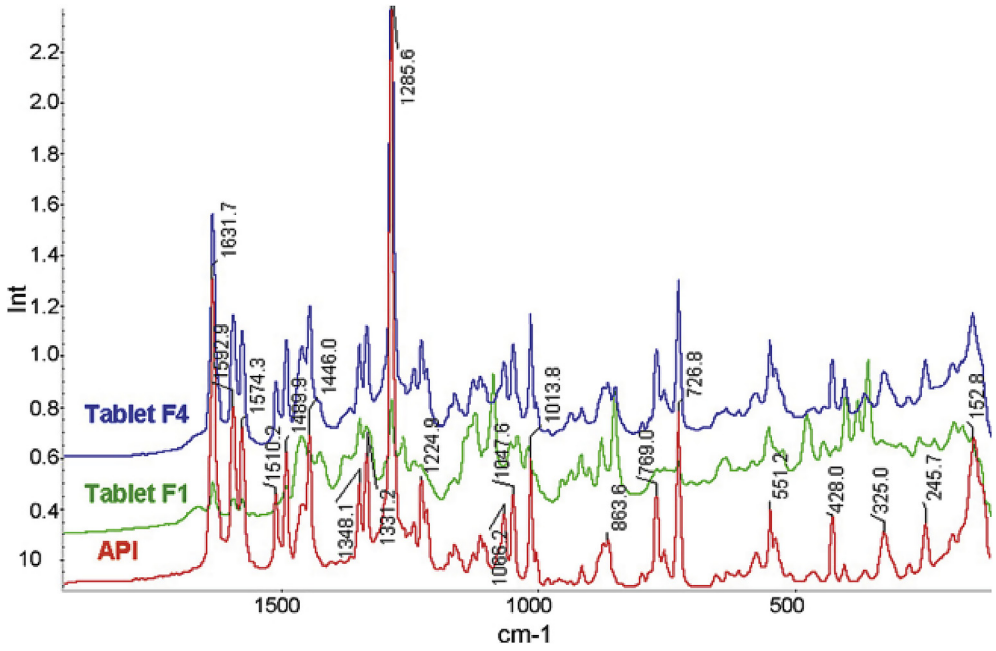

Fig. 1. Comparison of spectra for two pharmaceutical formulations of the same medicinal product. Differences in the excipients composition lead to different spectra for tablets of the same product. Reprinted from Ref. [21]. Copyright 2010 Elsevier.

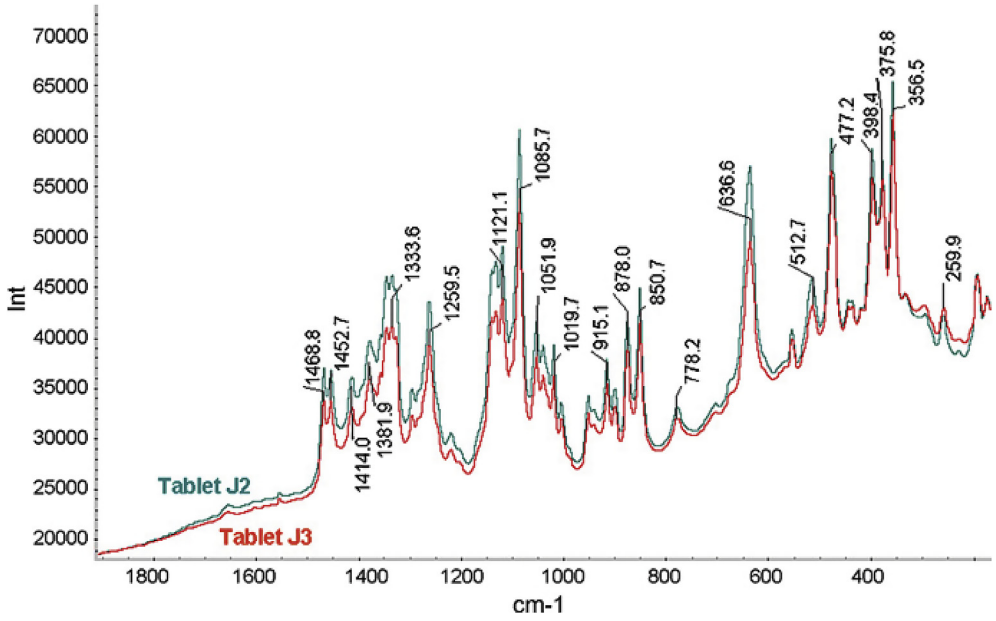

Fig. 2. Similar spectra of two pharmaceutical formulations of the same medicinal product. The low API concentration (0.8% for tablet J2 and 1.3% for tablet J3) and the presence of the same excipients prevent the differentiation of both formulations. Reprinted from Ref. [21]. Copyright 2010 Elsevier.

to predict a class membership for new observations inserted in the training set, with 100% correct discrimination between illegal samples from genuine ones.

de Veij et al. [31] coupled Raman spectral data with PCA and HCA to differentiate genuine from counterfeit Viagra® tablets without the need of sample preparation. The HCA results are shown in Fig. 3, where the dendrogram displays a notable cluster formation. One cluster contains genuine Viagra® tablets, whereas the counterfeit tablets are at a clear distance, indicating that HCA was able to identify them correctly. However, the dendrogram was obtained using a nearest neighbors algorithm, which tends to form rather scattered clusters, and is not sensitive to outliers. To circumvent this issue a furthest neighbor algorithm can be employed. The latter one produces compact clusters of similar size, and is more sensitive to outlying samples.

Deconinck et al. [32] employed MIR, NIR and Raman spectroscopies associated with classification and regression trees (CART) to discriminate between genuine and counterfeit Cialis® and Viagra® tablets, with correct classification rates of 83.3% and 100% respectively. The data were computed by first growing a binary tree during the CART modeling, where the maximum tree was overgrown and closely described the training samples.

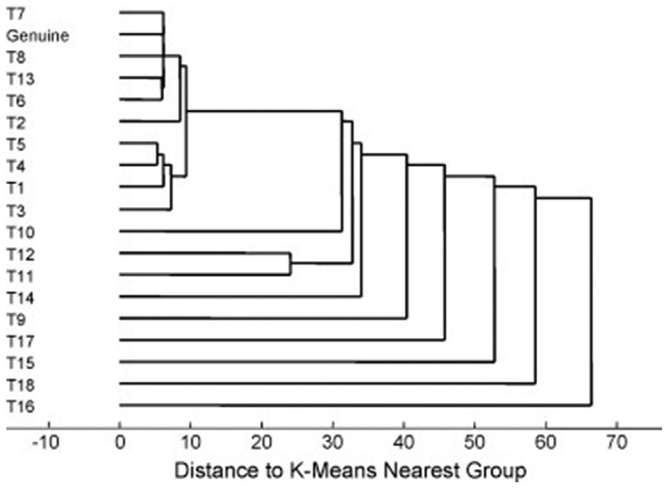

Fig. 3. Dendrogram for 18 Viagra® tablets, a genuine Viagra® tablet, and counterfeit Viagra tablets. Reprinted from Ref. [31]. Copyright 2007 Elsevier.

Maltaş et al. [33] generated Raman spectroscopic data based on liquid crystal spatial light modulator compressive detection (LC-SLM-CD). A PLS-DA model was applied to process the data, to discriminate between the API tadalafil, commercialized as Cialis®, and tablets containing only different excipients or placebos, such as microcrystalline cellulose, magnesium stearate, titanium (IV) oxide, talc, sodium lauryl sulfate and hydroxypropyl cellulose, with good separation between the clusters. The study proved that the developed non-destructive analytical method can be useful to detect counterfeit Cialis® which do not contain API. LC-SLM-CD Raman spectroscopy shows some advantages over the traditional technique, because a single channel detector is used to collect all the transmitted light (reflected) by each programmable optical filter. This provides higher signal-to-noise than when the same light is distributed over the many channels of an optical array detector such as the traditional charge-coupled-device (CCD) used as Raman detector [33].

Custers et al. [34] have reported a work based on NIR spectroscopy coupled to CART modeling of Viagra® and Cialis® spectra or tablets. They achieved good discrimination between genuine and illegal medicines with 98.93% correct classification for Viagra® and 99.42% for Cialis®.

Another area of interest in counterfeit pharmaceuticals includes antiretroviral tablets, specifically those for treating human immunodeficiency virus (HIV) infection [35,36]. Lopes [35] generated second-order data for antiretroviral lamivudine (commercialized as Heptodin®) by NIR chemical imaging (NIR-CI). The data were then unfolded to an augmented data matrix to be analyzed by PCA. The proposed fast and non-destructive analytical method showed that, from 55 counterfeit Heptodin® tablets obtained from local pharmacies, only 18% contained the correct API, while 82% counterfeit tablets were placebos because they only contained excipients, mainly talc and starch. This methodology can help researchers and authorities to better combat pharmaceutical counterfeiting, to prevent the emergence of drug-resistant HIV variants, which can result in treatment failure and in the risk for HIV progression in the ca. 37 million globally affected people [37].

Table 1 shows additional selected examples of non-destructive analytical methods based on chemometric models and vibrational spectroscopy and imaging to detect counterfeit tablets. Various other diseases are covered, such as high cholesterol level [38], hypertension [39], and diabetes [40].

Most of the studies summarized in Table 1 are based on first-order multivariate models such as PCA or PLS-DA, which are not able to estimate the pure spectral profiles of each constituent used to pharmaceutical counterfeiting, but only linear combinations of them. However, some studies allow for the identification of counterfeits in unknown tablet samples by recovering the spectral profiles of each constituent, using MCR-ALS [24]. The latter resorts to the bilinear decomposition of a data matrix, which can be generated by placing first-order vibrational spectra for a group of samples adjacent to each other [41,42]. The model can also be applied to second-order spectroscopic imaging data [43] contained in an augmented data matrix, created by placing the matrices for a set of samples adjacent to each other in the direction of the columns or rows. The augmentation direction is selected so that bilinearity is preserved [44]. MCR-ALS has some advantages over first-order models, such as: (a) the second-order advantage can be achieved, meaning that the analytes can be quantitated in the presence of uncalibrated interferents and (b) only small, pure-analyte calibration sets are required, instead of large and diverse calibration sets containing all possible interferents. Fig. 4 depicts the MCR-ALS process of modeling first-order vibrational spectroscopic data and second-order spectroscopic imaging data for the identification pure components of counterfeit spectral profiles.

From the practical point of view, Fig. 4 shows the steps to carry out a typical MCR-ALS analysis [41,42,43]. Prior information on the number of chemical components is required to perform the bilinear decomposition of the data matrix **D** (in the present case, **D** is the vibrational spectroscopic or augmented data matrix for spectroscopic imaging). MCR-ALS estimates the number of pure components present in the system using different strategies, such as singular value decomposition (SVD) or previous chemical knowledge of the studied system. Initial estimates of pure concentration (**C**) and spectral profiles ($\mathbf{S}^{\mathbf{T}}$) are required to start the alternating least-squares (ALS) optimization. These estimations may come from: the so-called purest variables in the spectral domain [41,42,43] or from the experimental knowledge of the pure component spectra. The ALS phase is iterative: the cycles continue until convergence is achieved, under the application of various restrictions (non-negativity for concentration and most spectral profiles, unimodality for chromatographic or kinetic time profiles, correspondence between species and samples in augmented data matrices, selectivity, etc.). The applied constraints help to limit the number of possible bilinear solutions and to drastically reduce the degree of ambiguity in the decomposition [41,42,43]. Once the ALS phase finishes, the output consists of pure spectral (**S**) and concentration profiles (**C**). For quantitative purposes using first-order data, integration of the concentration profiles provides the concentration scores, which can subsequently be employed for quantitative prediction. In the case of spectroscopic imaging, each column of the **C** matrix, corresponding to each chemical component, is refolded into a matrix whose size is equal to the original image. This allows one to build chemical pure-component images of the surface.

Kwok and Taylor [24] reported the generation of second-order spectroscopic imaging data based on Raman microscopy, and their application to collect chemical images of counterfeit Cialis® tablets. MCR-ALS was used for exploiting the second-order advantage and retrieving the pure spectral profiles of each component present in the tablets. For unknown tablet samples, they recommended building an augmented data matrix containing pure-analyte calibration matrices, and each of the test samples in turn, because the latter may contain different constituents in the counterfeit process. Fig. 5 shows the recovered pure spectral profiles from counterfeit Cialis® tablets and compares the resolved genuine spectra. The analysis revealed similarities and dissimilarities which are useful to discriminate genuine and counterfeit Cialis® tablets using the identities of the API and excipients.

Table 1
Non-destructive analytical methods to detecting counterfeit pharmaceutical products in the intact form.

Type of analysis in pharmaceutical products	Action/function	Non-destructive analytical methods	Ref.
Detecting counterfeit tablets	Antimalarial artesunate	NIR spectroscopy and PLS-DA	[25]
	Antimalarial artesunate	Raman spectroscopy with PCA and HCA	[26]
	Inhibitor Viagra® for erectile dysfunction	Near-infrared chemical imaging with PCA and K-NN	[30]
	Inhibitor Viagra® for erectile dysfunction	Raman spectroscopy with PCA and HCA	[31]
	Inhibitor Cialis® for erectile dysfunction	Raman and NIR spectroscopy with CART	[32]
	Inhibitor Cialis® for erectile dysfunction	Raman spectroscopy and PLS-DA	[33]
	Inhibitor Viagra® and Cialis®	NIR spectroscopy and CART	[34]
	antiretroviral Heptodin®	Near-infrared chemical imaging and PCA	[35]
	Lipitor® used for high levels of cholesterol	Raman and NIR spectroscopy with PLS-DA and PCA	[38]
	Concor® used for hypertension disease	Near-infrared chemical imaging and PCA	[39]
	anti-diabetic glibenclamide (Daonil®)	NIR spectroscopy with SIMCA and PLS-DA	[40]
	Inhibitor Cialis® for erectile dysfunction	Raman microscopy and MCR-ALS	[24]
	Inhibitor Viagra® for erectile dysfunction	Hyperspectral imaging instrument and PCA	[22]

Ref.: reference; NIR: near-infrared; PLS-DA: partial least-squares to discriminant analysis; PCA: principal component analysis; HCA: hierarchical cluster analysis; K-NN: k-nearest neighbors; CART: classification and regression tree; MCR-ALS: multivariate curve resolution - alternating least-squares; SIMCA: soft independent modeling of class analogy.

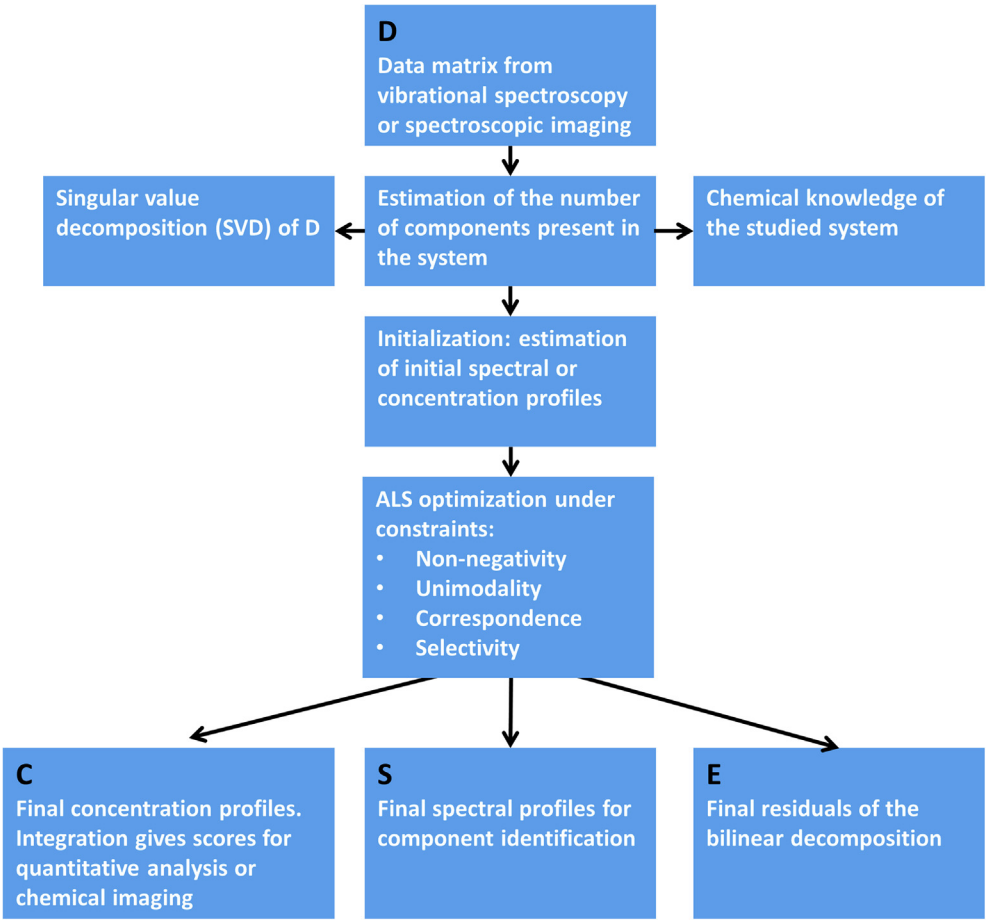

Fig. 4. Flow chart of MCR-ALS, as applied to modeling vibrational and imaging spectroscopy data.

4. Analysis of the API distribution and excipients in final medicinal products

Pharmaceutical formulations are usually composed of multicomponent blends, because formulated dosage forms contain other components along with the single/multiple API. The distribution of API and excipients within a pharmaceutical formulation can affect the stability and functionality of the final pharmaceutical products, because the pure API may not retain its stability for a long time. This can result in changes in its polymorphic form. Additionally, the excipients may improve the bioavailability of the API, improving the patient acceptance. For this reason, blend and content uniformity are important aspects of pharmaceutical formulations. Spectroscopic imaging enables visualization of the chemical, spatial and spectral information of tablet components in dosage or intact forms [2]. Different multivariate calibration models are useful to extract the detailed chemical information which is collected by mapping the tablets.

Raman hyperspectral imaging has been applied for assessing the distributional homogeneity index (DHI) of compounds of interest [45]. Sacré et al. [45] reported for the first time an interesting

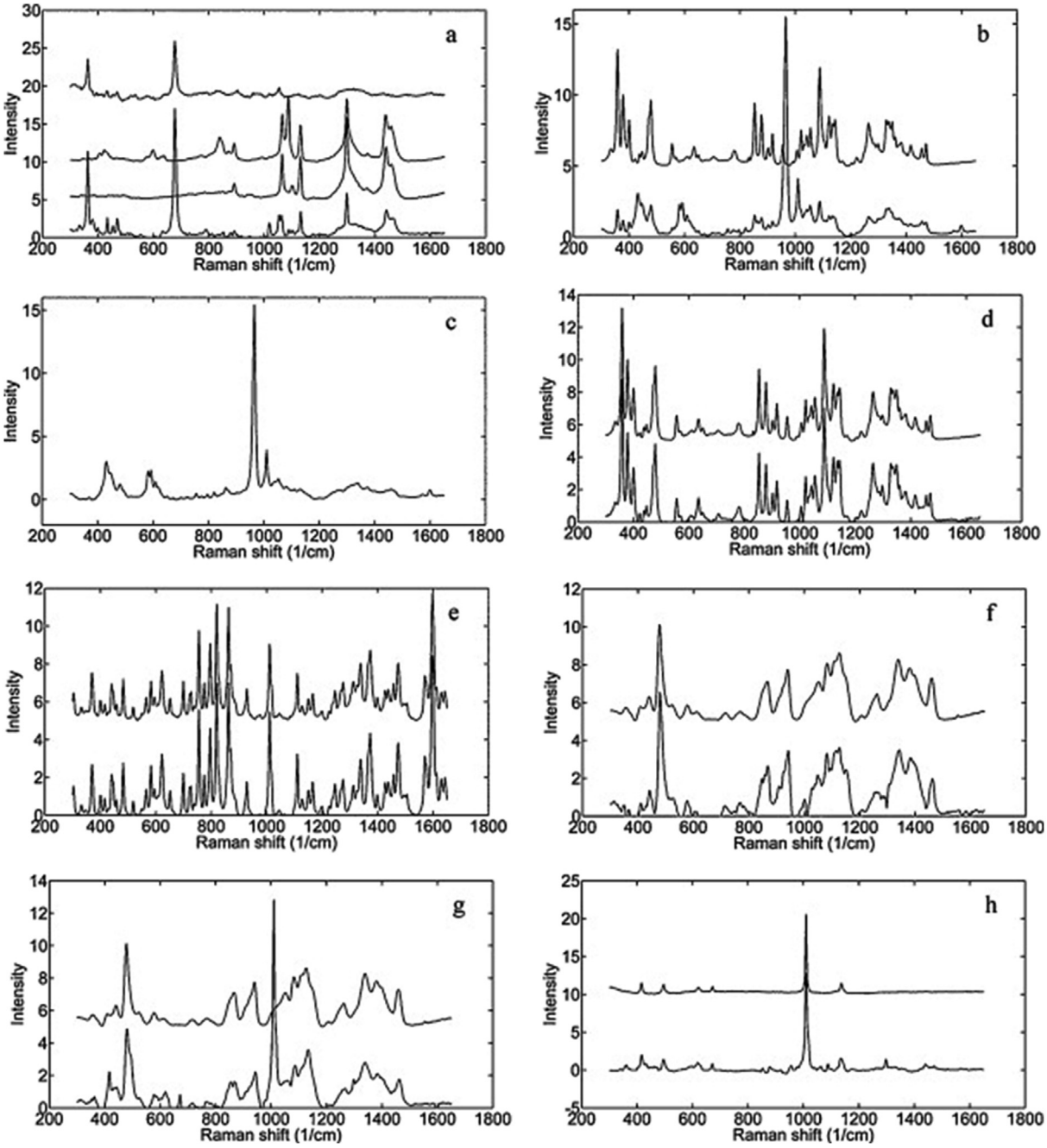

Fig. 5. Retrieved spectral profiles for counterfeit sample A. (a) First component compared to talc (top), sodium lauryl sulfate (second from top), and magnesium stearate (second from bottom). (b) Second component compared to lactose monohydrate (top). (c) Corrected second component. (d) Third component compared to lactose monohydrate (top). (e) Fifth component compared to tadalafil (top). (f) Sixth component compared to corn starch (top). (g) Fourth component compared to corn starch (top). (h) Corrected fourth component compared to calcium sulfate (top). Reprinted from Ref. [24]. Copyright 2012 Elsevier.

methodology for content uniformity assessment of blends in tablets, based on DHI tests. In their approach, classical least-squares (CLS) and PCA were applied to second-order hyperspectral image data to extract distribution maps, from which DHI values were calculated. Fig. 6 shows the relationship between the content uniformity values and the measured DHI values. It should be noticed that the CLS model is not useful for unknown tablet analysis, e.g. commercialized tablets, because it estimates the component concentrations by direct regression of the hyperspectral data using pure spectra as necessary information. In addition, even when pure spectra are known, CLS may not work properly in the presence of high noise levels and several sample components to be modeled with highly overlapped spectra. To circumvent the lack the pure spectra, the MCR-ALS procedure can be adopted, as depicted in Fig. 4. MCR-ALS with constraints can recover estimates to the pure spectral profiles of all components present in unknown tablets. The non-destructive analytical method reported by Sacré et al. [45] can be employed in the pharmaceutical industry for intact tablet analysis while developing new formulations. This meets the current good manufacturing practice [46], since DHI represents the spatial heterogeneity in API distribution, and was validated using content uniformity test.

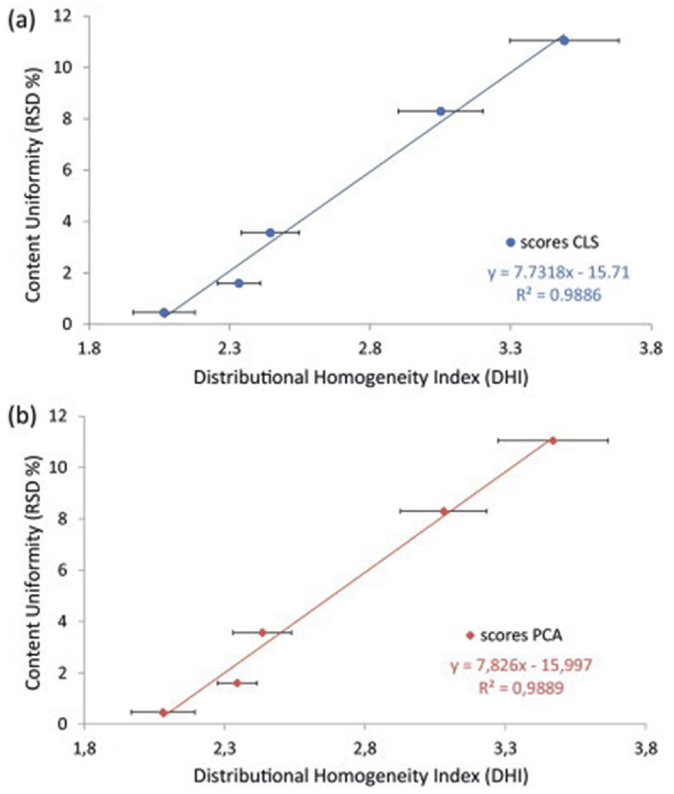

Fig. 6. Relationship between the content uniformity values (RSD %, relative standard deviation) and DHI values computed from: (a) distribution maps achieved by CLS analysis of the hyperspectral images of the tablet samples, and (b) PCA. Each data point represents the mean DHI value of 10 tablets with the corresponding standard error. Reprinted from Ref. [45]. Copyright 2014 Elsevier.

In 2007, Gendrin et al. [47] applied near-infrared imaging to obtain spectral and spatial information, with the aim of assessing content uniformity in pharmaceutical solid dosage forms. Partial least-squares 2 (PLS-2) was employed to simultaneously model several compounds from data collected in two pixel sizes: (1) 10 μm/pixel and (2) 40 μm/pixel. The results suggest that the best content predictions were obtained using PLS-2 with a pixel resolution of 40 μm. These results are important for the development of a non-destructive analytical method to assess content uniformity with the aim of providing the API and excipient distributions.

Wahl et al. [48] have recently reported near-infrared chemical imaging with high-speed cameras, based on the push-broom acquisition principle and different first-order multivariate calibration models. The aim was the continuous monitoring of API content and distribution in the entire production stream. The DHI criteria was applied as reported by Sacré et al. [45] to assess API distribution. Initially, all macropixels of size 2 × 2 pixels were searched and evaluated, and then the macropixel size was increased until the total map size was reached. Wahl et al. [48] introduced an approach based on the standard deviation (SD) to circumvent the issues observed with the performance of the DHI test.

Sabin et al. [49] performed a forensic analysis for the characterization of table formulations of sildenafil citrate from six different sources, to recognize the patterns of distribution maps of sildenafil citrate concentration, distinguishing the true formulation of Viagra®. A non-destructive analytical method was used, based on near-infrared chemical imaging coupled to both first- and second-order multivariate calibration models. The MCR-ALS model was applied to obtain the spectrum and concentration of sildenafil in each pixel of five pharmaceutical formulations provided by Brazilian Federal Police. Several samples corresponded to adulterated drug products (named as samples A-E) and one was an authentic Viagra sample, denoted as sample F. The employed MCR-ALS strategy estimates the distribution concentration map of the API in tablets from different sources, even when the chemical composition of all excipients was not known. A sildenafil chemical concentration image for each sample was produced and translated into histograms of frequency distribution for the API concentrations. This analysis removes the spatial components of the acquired information, retaining the ability to study the distribution profile, i.e. the API homogeneity. Fig. 7 shows the clusters for samples A-F according to the similarities of the histograms. HCA dendrograms analysis showed a better homogeneity for the genuine product F (samples No. 21 to 24) whereas other samples showed poor homogeneity.

Table 2 summarizes selected examples reporting different applications of spectroscopic imaging coupled with first- and second-order multivariate calibration models for content uniformity assessment of pharmaceutical formulations in intact form.

5. Quantification and identification of API polymorphic forms in pharmaceutical products

This section presents a brief discussion about polymorphic forms in pharmaceutical products. The motivation is the possibility of changes in API properties associated to the fact that polymorphs usually have different physical and thermodynamic properties in drug product development and delivery. They may cause some limitations related to instability, low solubility, different dissolution behavior, among others. It should be noticed that the definition of polymorphism is still discussed in the scientific literature. A reasonable one is “a reversible transition of a solid crystalline phase at a certain temperature and pressure to another phase of API” [55]. Sometimes amorphous solid forms have been selected for tablet formulations; however to meet specifications in terms of stability of tablets, it is usually preferred to employ crystalline solids rather than amorphous solids [56,57]. This is probably the reason for the conflicts arising from the previous definition, which clearly

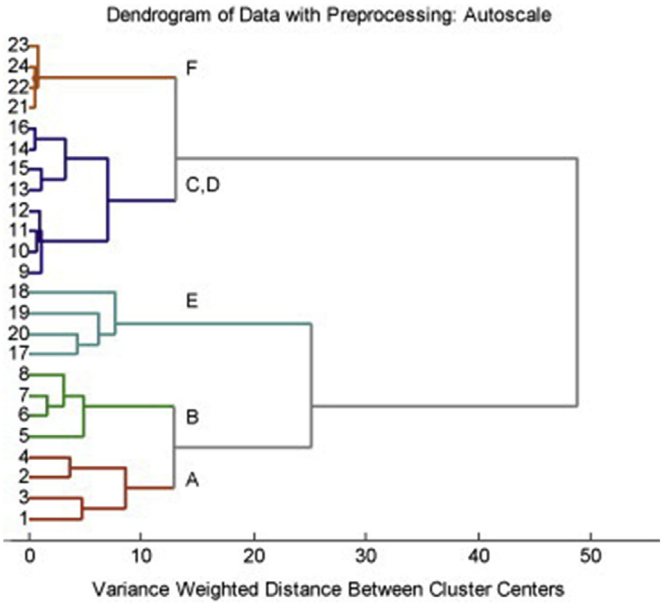

Fig. 7. HCA dendrograms for tablets A-F. Reprinted from Ref. [49]. Copyright 2013 Elsevier.

Table 2
Non-destructive analytical methods for content uniformity assessment of the pharmaceutical formulations in intact form.

Type of analysis in pharmaceutical products	Analyzed samples	Non-destructive analytical methods	Ref.
Assessing the homogeneity	Tablets of different batches	Raman chemical imaging with CLS and PCA	[45]
	Batches of about 500 tablets	near-infrared imaging systems and PLS2	[47]
	Tablets during the manufacturing process	Near-infrared chemical imaging with PCA and PLS	[48]
	Six different Viagra® formulations	Near-infrared chemical imaging with MCR-ALS and HCA	[49]
	Glibenclamide (Daonil®) formulations	UV imaging systems and MCR-ALS	[50]
	Lorazepam Normon tablets (same batch)	Near-infrared chemical imaging and MCR-ALS	[51]
	Tablets of different batches	Raman chemical imaging and MCR-ALS	[52]
	Batch of Bipreterax® tablets	Raman chemical imaging with MCR-ALS and PCA	[53]
	Multiple unit pellet system tablets	UV imaging systems with PLS and PCA	[54]

Ref.: reference; CLS: classical least-squares; PCA: principal component analysis; HCA: hierarchical cluster analysis; PLS: partial least-squares; MCR-ALS: multivariate curve resolution - alternating least-squares; UV: Ultraviolent.

excludes amorphous solid forms and the pseudo-polymorphs (i.e. solvation or hydration products). However, Aitipamula et al. [58] have provided a broad definition of polymorphism as "different crystalline forms of API, including solvation or hydration products and amorphous forms". We will employ the latter definition in this section, because various literature reports are based on the latter point of view.

Piqueras et al. [59] reported a new polymorphic form of carbamazepine denoted in their study as polymorph C after MCR-ALS analysis of Raman image data collected along the thermal degradation at different temperatures: 25, 50, 100, 130, 152, 154, 156, 158 and 160 °C. As shown in Fig. 8a polymorph A corresponds to polymorphic form I based on reference spectrum, whereas polymorph B corresponds to polymorphic form III. Carbamazepine tablets are marketed as polymorphic form III due to its high stability at room temperature, which in contact with atmospheric moisture can be hydrated. This hydrated form of carbamazepine, however, shows weak solubility and low stability [60]. Promising results were obtained by MCR-ALS in terms of retrieved spectral profiles for different polymorphic forms in Raman image data, making it a non-destructive analytical method to identify API polymorphic forms in commercialized tablets. Incidentally, labeling does not follow a definite terminology rule, and polymorphic forms are usually differentiated as: (1) A, B, C, (2) I, II, III or (3) α, β, γ [55].

Recently, Farias et al. [61] showed the application of Raman spectroscopy combined with chemometric models for the monitoring and quantitation of two polymorphic transitions of ezetimibe in final tablets, promoted by the humidity of excipients. Since ezetimibe is marketed in its anhydrous form, ten tablets containing API anhydrous form and excipients were packed using a polyethylene film. The MCR-ALS model showed that after 24 h anhydrous ezetimibe changed to the hydrate, and both forms were quantified by a PLS model, with good correlation (R higher than 0.96 for both crystalline forms). The humidity of excipients promoted the crystalline transitions shown in Fig. 9. The spectral profiles were recovered by MCR-ALS after modeling Raman spectroscopy data along the phase transition of anhydrous ezetimibe to the hydrate. Therefore, the hydrate should not be welcome in marketed tablets, because it may change the solubility of the API, causing a decrease in performance from the therapeutic point of view.

Simone et al. [62] showed the potential of non-destructive analytical based on Raman Spectroscopy and different first-order multivariate calibration models for the quantitative monitoring of the polymorphic transformation of *ortho*-aminobenzoic acid from polymorphic form II to polymorphic form I during a cooling crystallization. Recently, Netchacovitch et al. [63] monitored the transformation of crystalline itraconazole in its amorphous state in order to minimize the lack of solubility and bioavailability observed in final tablets. They used PCA and PLS to model Raman spectroscopic data. The PCA model provided a satisfactory classification between tablets with different crystalline percentages, whereas PLS was able to quantify crystalline percentages, with good correlation in comparison with the reference values ($R = 0.99$), and root mean square error of the prediction (RMSEP) of 2.8%.

Kachrimanis et al. [64] reported a non-destructive analytical method based on PLS and Raman spectroscopy for the quantitative analysis of paracetamol polymorphic forms I and II in powder mixtures. The aim was the prediction of the polymorphic stability upon prolonged storage, because solution-grown polymorphic form II is usually contaminated with crystals of polymorphic form I, depending on harvesting time and drying conditions.

Xie et al. [65] reported promising results using MCR-ALS, applied to Raman spectroscopy data to recover spectral profiles of different solid-sate forms (amorphous, form B and form C) of a development pharmaceutical in tablets (the structure was undisclosed). The different polymorphic forms of the API exhibit different stability. The long-term stability of polymorphic form B as API in a tablet formulation was quantitatively monitored under various conditions of temperature and moisture, showing the potential of non-destructive analytical based on multivariate calibration models and Raman spectroscopy in tablets analysis.

Calvo et al. [66] employed MIR spectroscopy and PCA to classify different cimetidine polymorphic forms present in commercial tablets, namely the authentic forms A, B, D and the monohydrate form denoted as M1. The classification polymorphic is important because each form presents specific physical and chemical properties, which lead to different biological activities. For example, form A is used for tablets, whereas form B is preferred for suspensions. However, cimetidine polymorphic forms can undergo transformations in the dry state upon milling, which supports the need of classifying the polymorphic forms in the final medicinal products. In the same way, Alexandrino et al. [67] showed that near-infrared hyperspectral imaging and MCR-ALS were suitable for monitoring the solid-state transformations of piroxicam hydrates in tablet surfaces exposed to different temperatures. MCR-ALS resolved the spectral profiles of piroxicam tablets during the transition phases; under certain conditions the drug may present at least three anhydrous forms I, II and III, as well as a monohydrate, all with different clinical properties.

Heinz et al. [68] assessed the ability of vibrational spectroscopy (Raman and NIR) coupled with PLS to extract and quantify ternary mixtures of solid-state indomethacin forms α and γ, as well as the amorphous form. PLS with four latent variables showed RMSEP values ranging from 5.3 to 6.5% for Raman spectroscopy and 4.0–5.9% for NIR spectroscopy. In the same perspective, Guo et al. [69], evaluated the ability of three vibrational spectroscopies (diffuse reflectance MIR, diffuse reflectance NIR and Raman)

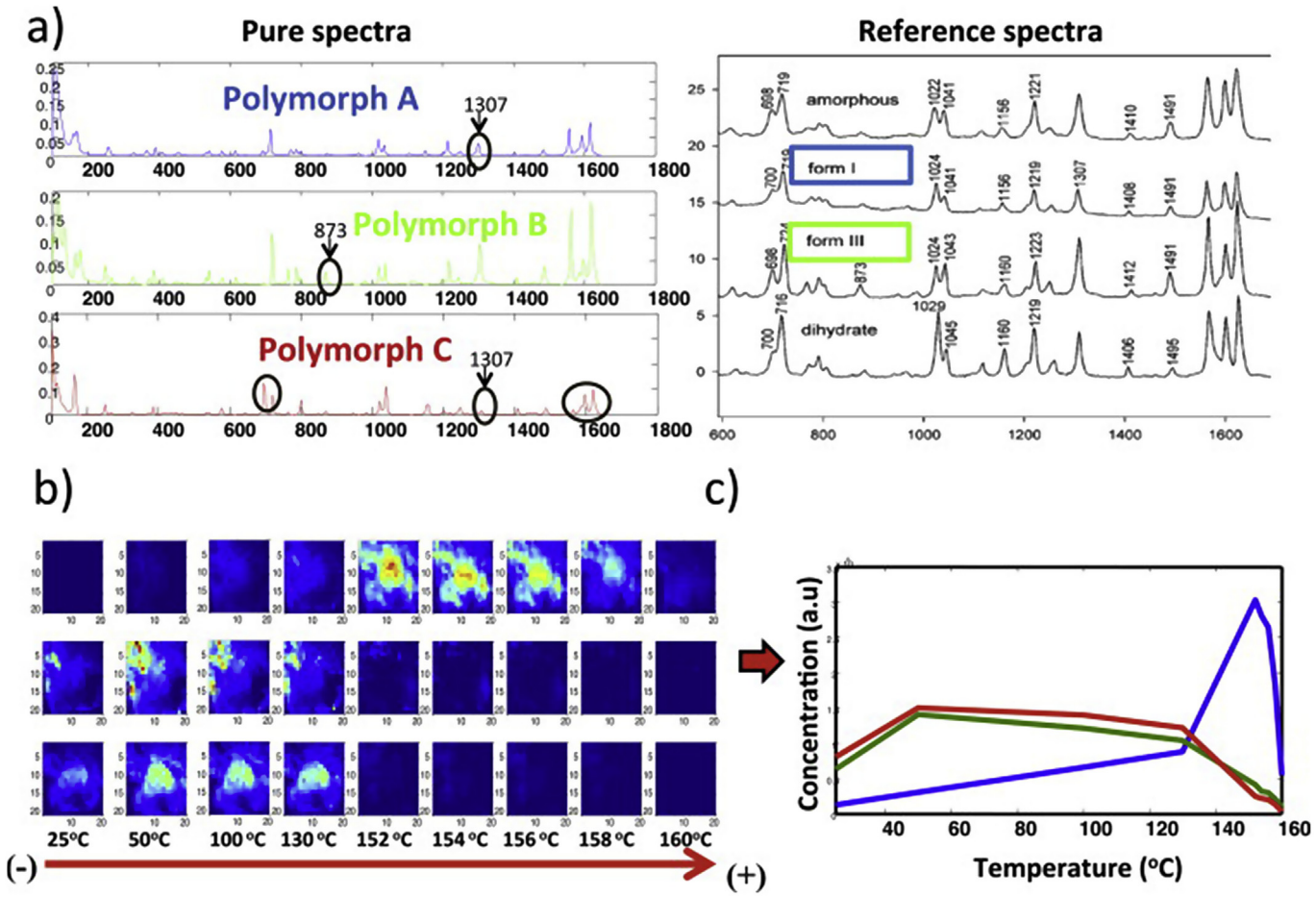

Fig. 8. (a) Left: spectral profiles retrieved by MCR-ALS after modeling Raman imaging data for carbamazepine polymorphic transitions. Right: reference spectra for carbamazepine polymorphs. (b) Distributions maps for the polymorphic forms involved in the thermally induced phase transformations. Reprinted from Ref. [59]. Copyright 2014 Elsevier.

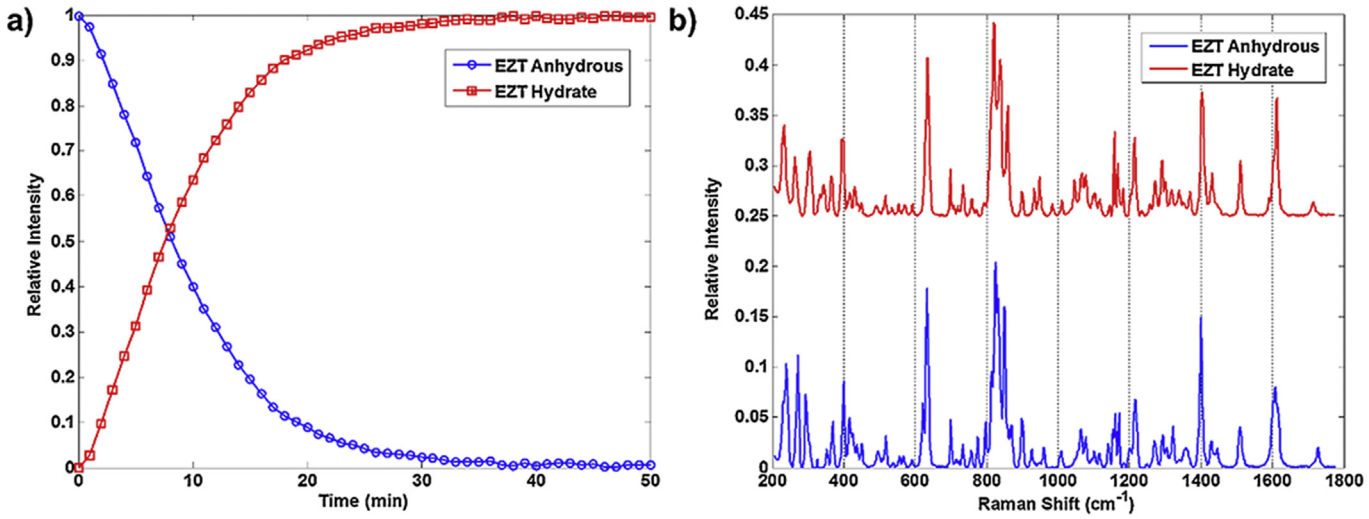

Fig. 9. (a) Relative concentration and (b) spectral profiles retrieved by MCR-ALS for ezetimibe (EZT) (anhydrous and hydrate forms), observed during the phase transition. Reprinted from Ref. [61]. Copyright 2016 Elsevier.

coupled with PLS and support vector machines (SVM) to quantify a polymorphic impurity (form I) in commercial fusidic acid (form III). Promising results were shown by PLS after modeling diffuse reflectance MIR data for determining the polymorphic content in fusidic acid binary mixtures, with RMSEP values ranging from 0.48% to 1.17%.

Recently, da Silva et al. [70] reported the performance of three different portable NIR instruments coupled with PLS for

quantifying mebendazole polymorphic forms A, B and C in pharmaceutical feedstock. The calibration was transferred from a benchtop instrument. Favorable results were achieved by one of the three portable instruments, denoted as Port. 1, with results similar to those obtained with the benchtop instrument. This suggests that the developed non-destructive analytical method is a good calibration strategy for monitoring mebendazole polymorphic forms *in-field*, at points of sale, since the selected equipment it is compact, robust, fast, and less expensive.

Griffen et al. [71] developed a non-destructive analytical method using transmission Raman spectroscopy and PLS for the rapid quantitation of low levels (in the range 0.62–1.32% w/w) of flufenamic acid polymorphic forms I and III in intact tablets. The PLS model showed a good with RMSEP, prediction uncertainties of ±0.04–0.05% w/w, and a limit of detection of 0.1–0.2% w/w. From the latter value, they concluded that PLS could detect API polymorphic forms in intact tablets samples above 0.1% w/w, whereas for quantification the models required values larger than 0.62% w/w.

The above reviewed papers, and additional selected examples are summarized in Table 3 [59,61,62,63,64,66,67,68,69,70,71,72,73,74,75,76]. The relevant question to be answered is how to circumvent the issues related to polymorphic transitions under certain conditions, once commercial tablets are produced. Although the commercial forms are in general stable, during storage and subsequent commercialization, e.g. in contact of humidity, stable polymorphic forms may change to unstable forms with poor solubility. Pharmaceutical cocrystals may help in these situations, improving the physical properties of API polymorphic forms, such as solubility, hygroscopicity and dissolution behavior, among others [77]. Pharmaceutical cocrystals will be addressed in the next section.

6. Pharmaceutical cocrystals

Pharmaceutical cocrystals are used in the pharmaceutical industry to improve the clinical performance of medicines because they can alter the physical properties of tablets, and improve the solubility, stability and bioavailability of the API without changing its chemical composition [78]. A pharmaceutical cocrystal can be defined as “a single crystalline solid that incorporates two or more different molecules, of which at least one is an API, and/or ionic compounds, usually in a stoichiometric ratio, which are neither solvates nor simple salts” [78,79]. However, in the scientific literature there is no general consensus concerning the definition of cocrystals or pharmaceutical cocrystals [58]. Cocrystals can be classified into: (1) molecular cocrystals, which contain only neutral components (coformers) and (2) ionic cocrystals, which comprise at least one ionic coformer is a salt [80]. Coformers may include solvates, hydrates, salts [80], excipients such as cyclodextrin complexes, and polymer dispersions [58] which are used for enhancing the bioavailability of tablets with low aqueous solubility.

Soares and Carneiro [81] reported a novel synthesis of carbamazepine-nicotinamide cocrystals in aqueous media, circumventing one of the biggest issues regarding carbamazepine polymorphic transition to its hydrated forms in the presence of moisture [60]. The carbamazepine hydrated form presents a reduced solubility, which is an apparent clinical problem. Therefore, the cocrystallization process was monitored by Raman spectroscopy and MCR-ALS, and the quantification of the final product among its coformers was performed using Raman spectroscopy and PLS. According to Fig. 10 (bottom), the relative concentration of stable carbamazepine polymorphic form III, obtained by MCR-ALS at near 80 min of reaction, started to decrease. Simultaneously the intensity of the pharmaceutical carbamazepine-nicotinamide cocrystal spectrum increased. The PLS model, on the other hand, showed analytical results in good agreement with the experimental data, with a percentage of explained variance of 96.62% and lack of fit of 14.88%. The reaction at 60 °C enabled a high conversion of the initial reactants to cocrystals (see Fig. 10 top).

Recently, Wood et al. [82] have demonstrated that NIR spectroscopy coupled with PLS can be employed as a non-destructive analytical method to predict the concentration of the API in a powder blend and in two cocrystals synthesized in the proportion of 1:1 for ibuprofen-nicotinamide and carbamazepine-nicotinamide. Promising results were achieved by PLS modeling

Table 3
Non-destructive analytical methods for identification and quantification of polymorphic forms of an API in pharmaceutical products.

Type of analysis in pharmaceutical products	Action/function	Non-destructive analytical methods	Ref.
Polymorphic analysis	Carbamazepine® used for treatment of epilepsy	Raman hyperspectral imaging and MCR-ALS	[59]
	Ezetimibe® used for high levels of cholesterol	Raman spectroscopy with MCR-ALS and PLS	[61]
	Ortho-aminobenzoic acid® used as a drug against fibrotic skin disorders	Raman spectroscopy and PLS	[62]
	Antifungal itraconazole®	Raman spectroscopy with PCA and PLS	[63]
	Paracetamol® used for treatment of headache and fevers	Raman spectroscopy and PLS	[64]
	Cimetidine® used as an anti-ulcer agent	MIR spectroscopy and PCA	[66]
	Anti-inflammatory piroxicam®	Near-infrared hyperspectral imaging and MCR-ALS	[67]
	Anti-inflammatory indomethacin®	Raman and NIR spectroscopy with PLS	[68]
	Antibiotic fusidic acid® used for treatment of infectious diseases	Raman, MIR and NIR spectroscopy with PLS and SVM	[69]
	Mebendazole® used for treatment of helminthic infection	Portable NIR instruments and PLS	[70]
	Anti-inflammatory flufenamic acid®	Raman spectroscopy and PLS	[71]
	Ranitidine hydrochloride® used for treatment of duodenal-gastric ulceration	NIR spectroscopy with PCR and PLS	[72]
	Piracetam® used in combination with other medicines for treatment of myoclonus	Raman and NIR spectroscopy with PLS	[73]
	Carbamazepine® used for treatment of epilepsy	Raman spectroscopy and PLS	[74]
	Anti-inflammatory flufenamic acid®	Raman spectroscopy and PLS	[75]
	Ranitidine hydrochloride® used for treatment of duodenal-gastric ulceration	Raman spectroscopy and PLS	[76]

Ref.: reference; PCA: principal component analysis; PCR: principal components regression; PLS: partial least-squares; MCR-ALS: multivariate curve resolution - alternating least-squares; SVM: support vector machine; NIR: near-infrared; MIR: mid-infrared.

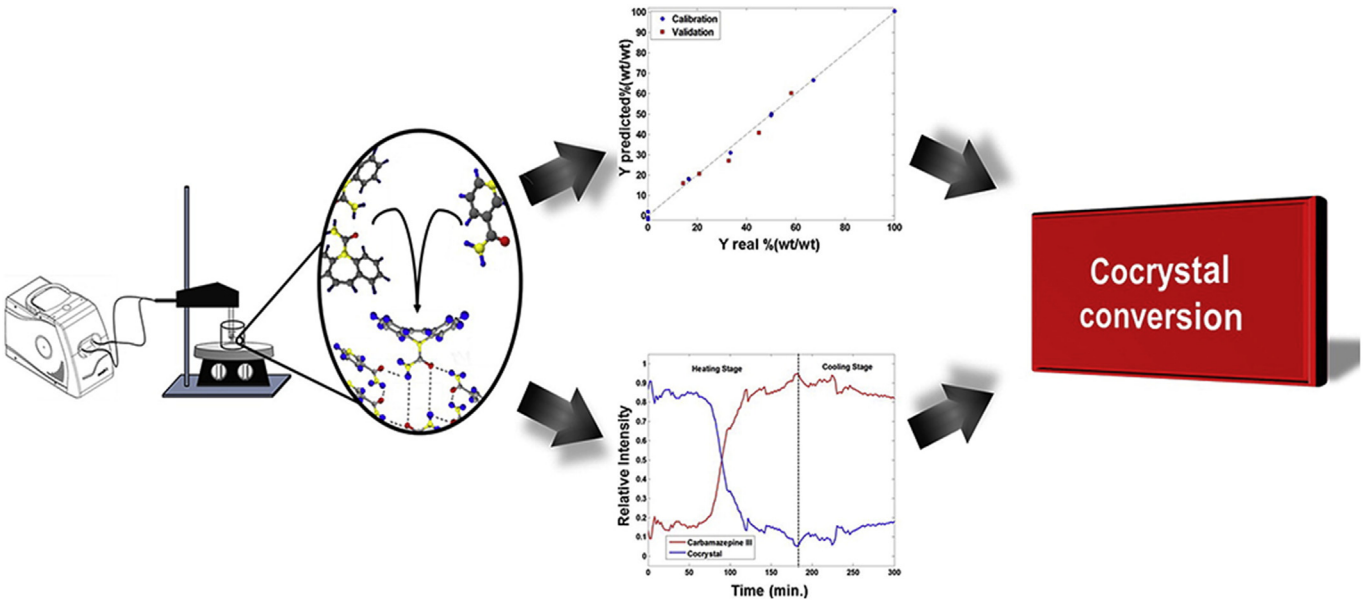

Fig. 10. Flow chart of the proposed methodology for monitoring a cocrystallization process and quantifying a pharmaceutical carbamazepine-nicotinamide cocrystal. Reprinted from Ref. [81]. Copyright 2017 Elsevier.

of NIR spectroscopy data, with RMSEP values which are smaller for ibuprofen-nicotinamide cocrystals than for carbamazepine-nicotinamide cocrystals.

In the same way, Soares and Carneiro [83] reported the pioneering quantification of ibuprofen-nicotinamide cocrystals, to determine the co-crystallization yield during the synthesis of 1:1 cocrystals. Raman spectroscopy coupled with interval PLS (iPLS) for variable selection showed good results, showing an average error lower than 5% for all mixture components.

Sarraguça et al. [84] employed PCA and NIR spectroscopy for in-line monitoring the formation of pharmaceutical furosemide-adenine cocrystals. Methanol was used as solvent to improve the poor solubility (6 mg L^{-1}) and bioavailability (60–65%) of furosemide [85,86]. Due to the similarity of spectral profiles, a PCA model was built with NIR spectra data from pure and recrystallized furosemide and adenine, and with the furosemide-adenine cocrystal obtained during six cocrystallization experiments. Good classification results were obtained between furosemide, adenine, and the pharmaceutical cocrystal. The separation indicated that the final product is indeed different form the two initial components. This means that a new tablet formulation was formed during the cocrystallization process, with different characteristics than their precursors, as shown by the clear separation in the obtained clusters.

As concluding remarks, this section highlights the non-destructive analytical methods based on vibrational spectroscopy such as Raman and NIR spectroscopies coupled to chemometrics for monitoring pharmaceutical cocrystals (Table 4). The latter improve medicines bioavailability through changes in API physical properties during the pharmaceutical cocrystallization process. Pharmaceutical cocrystals represent an innovative approach to improve tablet solubility. For example, ibuprofen presents a low water solubility [87], however, adding soluble molecules to the crystalline structure of ibuprofen is an alternative to improve its solubility and its subsequent bioavailability [88]. This has been demonstrated by Soares and Carneiro, when monitoring the synthesis of ibuprofen-nicotinamide cocrystals in aqueous media using Raman spectroscopy and MCR-ALS [89]. Unfortunately, no articles have been published yet reporting the application of multivariate calibration models to commercial pharmaceutical cocrystals, such as e.g. Entresto ™, a pharmaceutical cocrystal composed of monosodium sacubitril, disodium valsartam and water (CSD refcode: NAQLAU) [90], approved by the United States Food and Drug Administration [91] and the European Union [92]. Pharmaceutical

Table 4
Non-destructive analytical methods for in-line monitoring during the synthesis of pharmaceutical cocrystals.

Type of analysis in pharmaceutical products	Action/function	Non-destructive analytical methods	Ref.
Monitoring of pharmaceutical cocrystallization process	Pharmaceutical carbamazepine-nicotinamide cocrystal with high stability in contact of humidity, presenting high solubility and bioavailability for treatment of epilepsy	Raman spectroscopy with MCR-ALS and PLS	[81]
	Pharmaceutical ibuprofen-nicotinamide cocrystal that improved the poor solubility and bioavailability of ibuprofen and the cocrystal can be used for treatment of high levels of cholesterol	NIR spectroscopy and PLS Raman spectroscopy and iPLS Raman spectroscopy and MCR-ALS	[82] [83] [89]
	Pharmaceutical furosemide-adenine cocrystal that improved the poor solubility and bioavailability of furosemide, which the diuretic furosemide can be applied in the treatment of edematous states associated with hypertension, heart failure, renal failure, nephritic syndrome	NIR spectroscopy and PCA	[84]

Ref.: reference; PCA: principal component analysis; PLS: partial least-squares; MCR-ALS: multivariate curve resolution - alternating least-squares; NIR: near-infrared; iPLS: interval partial least-squares.

cocrystals are a modern trend in the pharmaceutical industry and constitute a challenge to non-destructive analytical methods.

7. Conclusion

This brief review highlights the potentiality of first- and second-order multivariate calibration models, coupled to vibrational and imaging spectroscopic techniques for the analysis of medicinal products in their intact forms. This combination of experimental and theoretical techniques, with special emphasis on multivariate curve resolution, constitutes a powerful non-destructive analytical strategy to deal with some current challenges in pharmaceutical analysis, such as: (a) fighting against counterfeit pharmaceutical products, (b) assessing content uniformity through API distribution in final products, by visualizing the spatial distribution of each ingredients in the layers, (c) monitoring the occurrence of polymorphic transitions, identify profiles and quantify crystalline API forms, because unsuitable storage conditions or excipient moisture may change the crystalline structure to polymorphic forms, affecting some of the API properties, and (d) monitoring in-line the solubility enhancement of API polymorphs with low solubility by adding soluble molecules in the crystalline structure by cocrystallization. Finally, this review provides the future trend of non-destructive analytical methods in pharmaceutical analysis related to four topics covered. The future trend is directed to MCR-ALS as an appropriate multivariate tool to modeling pharmaceutical data provided by vibrational spectroscopy and spectroscopic imaging. Because, pure spectral profiles and pure concentration profiles can be recovered using MCR-ALS as shown in Fig. 4. Those qualitative and quantitative analytical results are required to carry out analysis effectively in each of the four topics addressed for pharmaceutical field.

Acknowledgements

S.J. Mazivila thanks the Camões-Instituto da Cooperação e da Língua, gratefully acknowledges the scholarship granted to his Ph.D. A. C. Olivieri thanks Universidad Nacional de Rosario (Project 19/B487), CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas, Project PIP 0163) and ANPCyT (Agencia Nacional de Promoción Científica y Tecnológica, Project PICT 2016–1122), are gratefully acknowledged for financial support.

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