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Short communication

Content uniformity studies in tablets by NIR-CI

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ABSTRACT

Near Infrared Chemical Imaging (NIR-CI) is attracting growing interest in pharmaceutical analysis by virtue of its ability to provide a wealth of information from a single sample. Among others, NIR-CI has enabled the determination of the quantitative composition and distribution of acetylsalicylic (ASA) from the analysis of commercial tablets. In this work, we analyzed ASA commercial tablets of four different brands purchased at local chemists. The nominal ASA concentration for the brands was calculated from the nominal content and averaged weight of tablets. The tablets were found to span an ASA concentration range of 71–82%, and to differ in size and composition between brands.

The API content and its homogeneity distribution were determined by applying quantitative algorithm to global hyperspectral image of ten tablets. Multivariate Curve Resolution–Alternating Least Squares (MCR–ALS) is used to quantify each pixel in the images to obtain appropriate concentration maps. No prior calibration or reference data were needed for quantitation and results are close to the nominal content used as reference. Application to an image for 10 tablets and an individual tablet quantitation of the API allowed us to obtain the Accepted Value (AV) as defined by the European Pharmacopoeia. We conclude that all brands meet the pharmacopoeia specifications.

1. Introduction

The NIR-CI technique has been successfully used in a variety of fields ranging from the food industry [1] to legal medicine [2] to cultural heritage [3].

The use of NIR-CI as a new operational methodology in pharmaceutical analysis has grown substantially in recent years and turned the technique become an accurate, robust choice for pharmaceutical control and the determination of quality-related properties of formulations at different production stages. The increasing interest aroused by this technique has been reflected in the development of a huge number of applications including the determination of distribution homogeneity in tablets [4], mixing homogeneity in powder samples [5], content uniformity in tablets [6], coating studies [7] and particle size [8], among others.

The ability of NIR-CI to extract quantitative and distribution-related information about tablet components has turned it into a PAT tool [9] and facilitated quality assurance in the intermediates and end-products [10].

A hyperspectral image consists of a three-dimensional data cube where two dimensions are spatial coordinates (pixels) and the third contains spectral information (wavelengths); therefore, it provides one spectrum per pixel and hence a vast amount of both spatial and spectral information (usually more than 80.000 spectra per sample) that can be acquired in a very short time (1–2 min). Such a vast amount of data obviously requires the use of an efficient chemometric method to extract the physical or chemical information of interest in each case.

Many of the algorithms used with conventional NIR spectroscopy have also been used to process 3D data cube in order to extract qualitative and quantitative information. The actual usefulness of each algorithm for this purpose depends on the particular problem addressed and also on the specific information available.

The algorithms most frequently used in conventional NIR analysis, PLS and PCR, require using a set of calibration samples to construct an appropriate prediction model. The sample preparation is laborious, which constitutes an important limitation for its application. Nevertheless, some applications for PLS have been described for the API determination in pharmaceutical samples and in components of binary samples (lysozyme and trehalose mixtures) [11,12].

Classical Least Squares (CLS) methodology [13] may be an effective, attractive choice here inasmuch as it requires no calibration set, but only the pure spectra for the sample components. This, however, may hinder application of NIR-CI when the sample composition is not accurately known.

Some alternative algorithms requiring no calibration set have proved effective in those cases where the exact composition of the sample is unknown or the spectrum for some component is unavailable. Thus, Multivariate Curve Resolution–Alternating Least Squares (MCR–ALS) methodology [14] and its expanded version

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have been successfully used to extract quantitative information from pharmaceutical samples by use of an imaging technique [15]. Therefore, MCR–ALS can in theory be an effective tool for quantifying pharmaceutical components without the need for a calibration model, but simply to use the spectra for the major components of the product in pure form.

In this article a unique image of 10 tablets has been used to obtain the quantitative information. Only the publication of Lee et al. [6] undertake a similar study, but the proposed quantification method is very simple and its use for API determination can be only applied in restricted cases, but it does not make a study on the distribution of excipients.

The final objective is to define the appropriate method to approach the problem of the quantitation and distribution studies in pharmaceutical tablets, without previous information.

Finally, the content uniformity test is used for proving the uniform distribution of the active content in a production batch; it is performed by measuring the active content of 10 individual dosage units. The determination of content uniformity, defined by the European Pharmacopoeia [16], by using a single hyperspectral image of 10 tablets is studied and it is proposed as an alternative to the traditional methods which can be supposed as an important improvement in the speed and easiness of this test.

2. Materials and methods

2.1. Samples

The samples studied were non-coated commercial aspirin tablets of four different brands that were purchased at local chemists. The acetylsalicylic acid (ASA) concentration in each tablet was calculated by dividing the nominal content into the average weight of 10 tablets. The concentrations thus obtained ranged from 71 to 82 wt%. One additional criterion used in selecting brands for study was tablet size.

The exact composition of each formulation was unknown and the sole information available in this respect (the nature of the major excipient, which was microcrystalline cellulose in the four brands) was obtained from the Vademecum [17]. Table 1 summarizes the characteristics of the studied tablets.

2.2. Instruments

All images were obtained with a Roda-25 focal plane imaging NIR spectrometer from Think Spectrally (Valencia, Spain), equipped with an MCT 320 × 256 pixel detector and an LCTF monochromator spanning the wavelength range 1200–2400 nm with a resolution of 7 nm.

Lighting was provided by four halogen lamps placed around the sample and tilted 45°. Acquiring a reflectance image spanning the whole wavelength range took about 2 min. The distance between the camera lens and sample plate was adjusted in accordance with whether the image to be recorded should contain ten tablets of different size for each brand (see Table 1).

2.3. Software

We used the graphical user interface (GUI) software TS_Capture, bundled with the Hyperspectral TS camera, from Think Spectrally, to control the camera and lighting for the acquisition of images. The hardware was calibrated by using TS_GUI, a graphical user interface for Matlab also bundled with the camera.

All spectral treatments were applied by using customized routines developed in Matlab code (Matlab v. 7.0, The MathWorks, MA) available for download on the Internet [18]. The MCR–ALS software used, which also operates under Matlab, was obtained elsewhere [19].

2.4. Recording of images

The recording of images was preceded by calibration of the instrument with six AP-0200 NIR standards of 99, 80, 40, 20, 10 and 0.2% reflectance from FOSS NIRSystems (Silver Springs, MD). Images were obtained with 7 nm resolution over the wavelength range 1200–2100 nm.

Recording the images for 10 tablets at once (Fig. 1) entailed placing the sample plate at a variable distance from the camera lens subordinate to tablet size and refocusing it to ensure as high optical resolution as possible. This allowed a spatial resolution of around 200 μm × 200 μm and a total image size of 65 mm × 50 mm to be obtained.

Placing the samples farther from the lens in order to widen the focal field alters the lighting angle of the lamps and the amount of light impinging on the sample; this change required recalibrating the camera.

The images for pure acetylsalicylic acid and microcrystalline cellulose (the major excipient in the four formulations) were obtained by placing the powder samples in a cylindrical glass cell about 3.8 cm in diameter and pressing their surface with a metal disc to obtain a flat surface.

2.5. Processing of hyperspectral images

The hyperspectral image for a sample is a 3D data cube represented by the expression $\underline{X} = (M \times N \times \lambda)$, where M and N are the spatial dimensions and λ denotes spectral information. The three-dimensional nature of the image requires the unfolding into a two-dimensional matrix in order to facilitate spectral processing or application of a multivariate model.

The influence of unwanted phenomena frequently affecting NIR spectra was suppressed by using two different spectral treatments, namely: Standard Normal Variate (SNV) and Savitzky–Golay smoothing with a 7-point window and fitting to a second-order polynomial. These treatments were followed by application of the previously cited chemometric algorithm to the data matrix.

The theoretical foundation of the Multivariate Curve Resolution–Alternating Least Squares (MCR–ALS) algorithm and its expanded version, and their application to hyperspectral images, are widely documented; interested readers can find comprehensive information about it elsewhere [10,14,15]. In order to facilitate and improve the chemometric solution, the unfolded matrix obtained from each image was expanded with 10 spectra for the API and 10 for microcrystalline cellulose (MCC).

3. Results and discussion

Each image was processed with the MCR–ALS algorithm on the grounds of the characteristics of the samples and available information. Spectra were subjected to various treatments, and SNV and Savitzky–Golay smoothing with a 7-point window found to provide the best results.

Computations with the MCR–ALS algorithm were subjected to concentration non-negativity and closure restrictions. Although the tablets contained different components, the closure restriction was applied with provision for the active pharmaceutical ingredient (ASA) and major excipient (MCC) alone. Because the original unfolded matrix resulted in poor fitting, it was expanded with 50 spectra for each analyte (ASA and MCC); this expanded matrix afforded better fitting and more accurate determination of both components.

Table 1
Features of commercial Aspirin tablets.

Commercial brand	Nominal ASA (mg)	Diameter (mm)	Mean tablet weight (g)[a]	Calculated concentration (%)	Main excipient
Bayer 500	500	120	0.6037	82.8	MCC
Adiro 100	100	72	0.1368	73.1	
Bioplak 250	250	100	0.3427	73.0	
Bioplak 125	125	70	0.1751	71.4	

[a] 10 tablets per commercial brand.

The API quantitation has been done by two different ways: by applying the quantitative algorithm to the complete image of the 10 tablets (determination of mean batch value) or by selecting the part of the image that corresponds to each individual tablet (study of content uniformity).

The spatial distribution of 10 tablets in the image is shown in Fig. 1; the hyperspectral image contains as well the spectra of each one of the tablets, the spectra corresponding to the background. The application of MCR–ALS requires the use of unfolded data matrices, for this purpose a Matlab routine [19] is applied to the data cube in order to obtain the unfolded matrix spectra of tablets.

The background spectra prevent the correct calculation of concentrations, the elimination of background spectra is performed by a routine which converts the background pixels in non a number data, and a Matlab routine is used to unfold only the spectral data in order to apply MCR–ALS afterwards. The mean concentration value of 10 tablets of the batch is obtained and the values for the four brands are shown in Table 2. The mean value of 10 tables may be considered as a good approximation to the mean batch value; the results show good agreement with the ASA calculated concentrations and the deviations are lower than the variation accepted by the Pharmacopoeia.

An alternative for determining the concentration is the individual analysis of each tablet which concentrations were calculated by cropping the image for each tablet and removing the background. The elimination of background spectra is performed for each cropped image from a tablet in the same way that has been explained before for the global analysis of 10 tablets.

Application of the model to individual commercial tablets of four different brands provided results showed in Table 2.

The ASA concentration values in commercial tablets of four different brands obtained by using the global image and the average of the image of each individual tablet are very similar to each other and next to the value of ASA calculated concentration taken as reference.

3.1. ASA and MCC distribution in the tablets

Fig. 1 shows the concentration map for and ASA and MCC as calculated with the MCR–ALS algorithm, as well as the histograms for a Bayer 500 aspirin tablet, obtained from the image for 10 tablets. As can be seen, both components were evenly distributed throughout the image and no API or excipient clustering zones were observed. So far there has been no reported parameter used to establish the homogeneity of distribution of a component in a mixture, but the histogram of concentrations of each pixel of the image can give a good indication of the distribution. The histograms for both components (ASA and MCC) reveal a very narrow range of concentration values with a maximum close to the average predicted value. These results suggest that ASA and MCC are uniformly distributed in the tablets showing an appropriate homogenization of API and main excipient in the samples. Those observations about homogeneity of distribution can be extended for the rest of brands.

The shape and size of the tablets are important parameters to frame 10 tablets in the visual field of the hyperspectral camera; the different size of tablets require distance changes between the sample plate to the camera lens to get a correct focused image. The superficial characteristics (curvature and surface marks) joined to the tablet size (which influences the lens focus distance and therefore in the lighting intensity) affect the intensity and distribution of incident light on the 10 tablets which can affect the quality of the images and finally their quantitation.

Fig. 2 exposes some deficiencies in distribution at image edges which can be ascribed to inadequate lighting of the 10 tablet samples; this resulted in a poor hyperspectral image that hindered proper resolution of tablet edges with the quantitation algorithms.

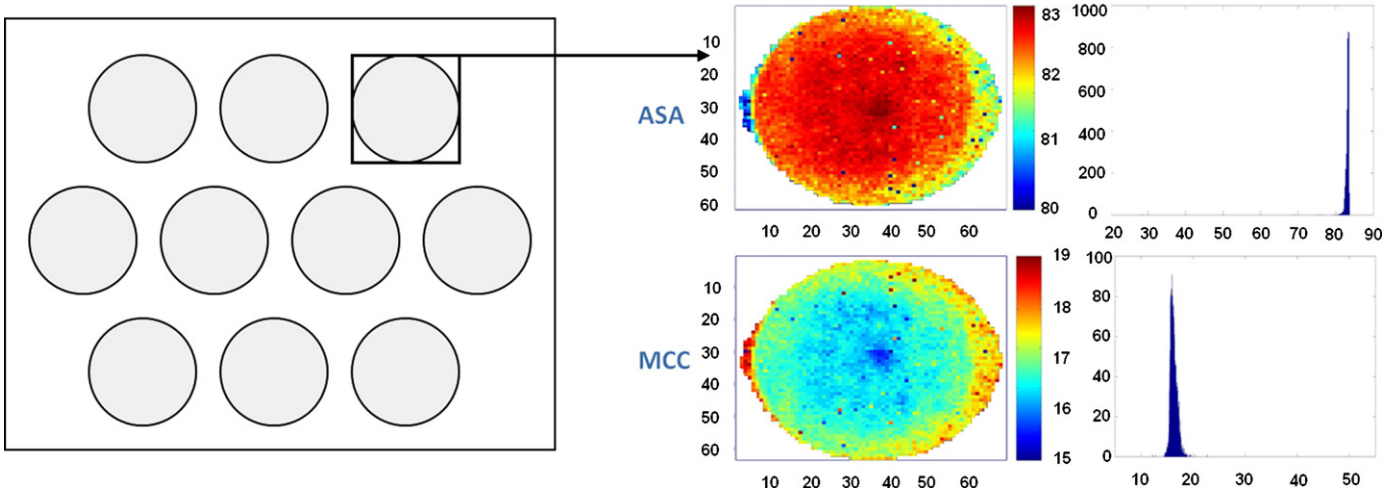

Fig. 1. Concentration maps and histograms for ASA and MCC calculate with MCR–ALS in Bayer 500 tablets.

Table 2
Results for ASA content uniformity test (10 tablets analyzed individually) and for mean batch value (global image analyzed).

Global image analyzed		10 tablets analyzed individually			
Tablets brand	Mean batch value	Range values	Mean value of the individual tablets	Std. deviation	Acceptance Value
Adiro 100	73.9	75.6–68.1	72.6	2.9	5.1
Bioplak 250	72.7	77.9–68.7	72.7	3.3	2.3
Bioplak 125	69.4	74.7–67.2	69.7	2.3	13.5

The results have been obtained by applying of MCR–ALS to the whole hyperspectral image (mean batch value) and by applying the algorithm to each whole tablet individually (content uniformity). Acceptance Value limit for $n = 10$ is 15.

No similar effect was observed on the hyperspectral images for individual tablets [4], which were acquired under better lighting and resolution conditions. These deficiencies are especially marked in the image for the larger tablets (Bayer 500 and Bioplak 250) and less so in that for the smaller tablets (Adiro 100 and Bioplak 125).

Fig. 2 shows the ASA distribution map of 10 tablets of Bioplak 250 and shows deficiencies in the calculation of the concentration of ASA, mainly on the edge of the tablets, confirming the hypothesis of poor lighting (these tablets are flat). Quantification has been tried only into a square of 40 × 40 pixels (equivalent to an area of 8 mm × 8 mm) removing information from the edges of the respective tablets without a significant reduction in amount of analyzed sample.

Table 3 shows the values obtained in the quantification of ASA in each of the tablets by using the two methods: whole tablets and tablet squared central selected. The average values of determination are almost identical, although the standard deviation of the results of the two procedures is very different and improved in the method which selects the central area. Also, the histograms (Fig. 2) show a similar behaviour, the method of selecting the central area shows a histogram narrower than the in the whole tablet's, exhibiting a very narrow concentration distribution with a maximum close to the average predicted value.Similar results are obtained in the processing of images of the other brands.

This shortcoming can be avoided by using an instrument with greater spatial resolution and more uniform lighting of the object.

Table 3
Results for ASA content in Bioplak 250 applying the MCR–ALS algorithm to the whole individual tablet and a square of 40 × 40 pixels for each tablet (10 tablets analyzed individually from the same image).

Bioplak 250		
Tablet	% ASA (square)	% ASA (whole tablet)
1	73.4	69.7
2	72.6	76.1
3	73.9	75.1
4	72.4	69.1
5	73.3	77.9
6	72.6	70.5
7	71.7	74.5
8	72.6	76.3
9	71.7	68.7
10	73.5	69.4
Mean	72.8	72.7
Std. deviation	0.7	3.6

3.2. Content uniformity

Content uniformity was assessed from the 10-tablet image for each brand, using the recommendations of the European Pharmacopoeia. Table 2 shows the average nominal value for the 10 tablets of each brand, the calculated value obtained from the average for the 10 tablets, the standard deviations of the concentrations and the Acceptance Value (AV) calculated as defined by the European Pharmacopoeia.

The European Pharmacopoeia recommends assessing content uniformity by computing AV and comparing it with a previously established limit for the acceptance range [16]. AV is calculated from the following equation:

$$\mathrm{AV} = |M - X| + ks$$

where M is the reference value, X the average value for individual tablets, k a constant equal to 2.4 for $n = 10$ and s the standard deviation. The content uniformity requirement is assumed to be met if AV for the first dosing units examined is equal to or less than 15.

As can be seen from table, the AV values obtained for the four brands were all below 15, so the brands can be deemed compliant with the requirements of the European Pharmacopoeia. Also, the

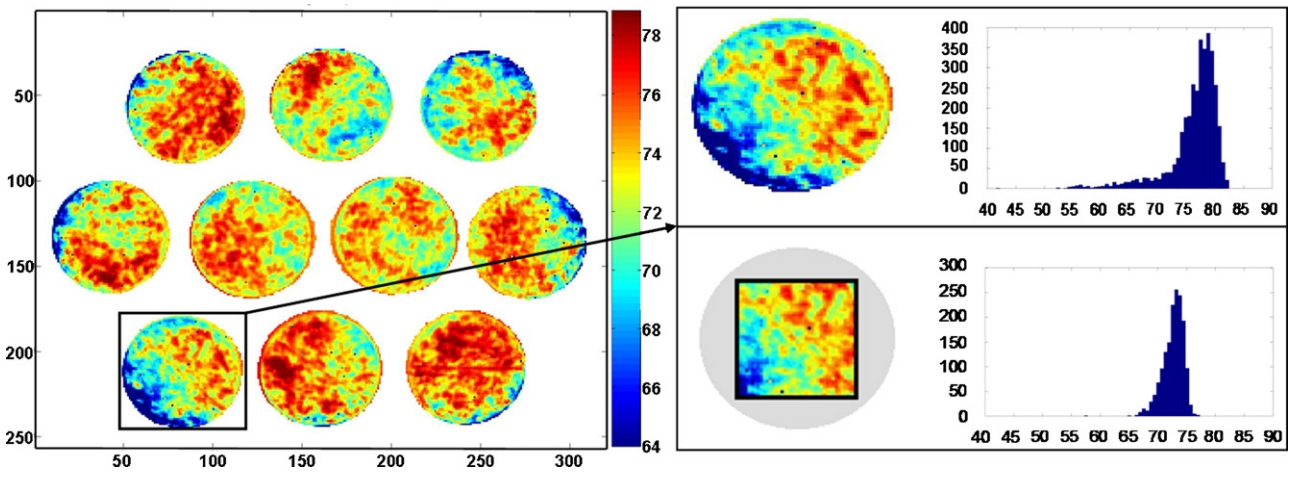

Fig. 2. ASA concentration maps and histograms of the whole tablet and a squared central section of 40 × 40 pixels for Bioplak 250 tablets.

only brand exhibiting a relatively high AV value (13.5) was Bioplak 125 however, was still below the limit (15).

They confirm that a single image allows one to certify content uniformity in a tablet production batch.

4. Conclusions

As shown here, NIR-CI provides the quantitative analysis of intact tablets by using exclusively spectral information of API and its major excipient. The MCR–ALS algorithm is effective with a view to extract quantitative information from images and only requires the spectra for the pure API and major excipient as references. This requirement is no important in pharmaceutical analysis because it is easy to obtain their pure components and hence their spectra.

A single image for 10 tablets allows not only one to determine the API concentration in each, but also to assess content uniformity in a production batch following the recommendations of the European Pharmacopoeia. This makes the proposed procedure more simple and expeditious than available alternatives for the same purpose.

The procedure used in his work allows one to obtain information about the distribution for both components, and hence to assess distribution homogeneity in each individual tablet and confirm it against a concentration histogram.

In summary, the results of this work further testify to the increasingly prominent role of chemical imaging techniques as QbD choices and their significance to PAT.

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