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A new criterion to assess distributional homogeneity in hyperspectral images of solid pharmaceutical dosage forms

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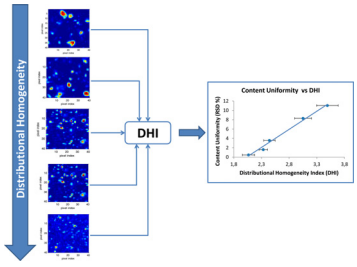
HIGHLIGHTS

- DHI has been developed to assess distributional homogeneity in hyperspectral maps.
- This criterion has been tested with simulated maps of different homogeneity.
- A linear relationship is observed between homogeneity and DHI value.
- DHI methodology has been applied on real samples.
- A linear relationship is observed between DHI and content uniformity values.

GRAPHICAL ABSTRACT



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ABSTRACT

During galenic formulation development, homogeneity of distribution is a critical parameter to check since it may influence activity and safety of the drug. Raman hyperspectral imaging is a technique of choice for assessing the distributional homogeneity of compounds of interest. Indeed, the combination of both spectroscopic and spatial information provides a detailed knowledge of chemical composition and component distribution.

Actually, most authors assess homogeneity using parameters of the histogram of intensities (e.g. mean, skewness and kurtosis). However, this approach does not take into account spatial information and loses the main advantage of imaging. To overcome this limitation, we propose a new criterion: Distributional Homogeneity Index (DHI). DHI has been tested on simulated maps and formulation development samples. The distribution maps of the samples were obtained without validated calibration model since different formulations were under investigation. The results obtained showed a linear relationship between content uniformity values and DHI values of distribution maps. Therefore, DHI methodology appears to be a suitable tool for the analysis of homogeneity of distribution maps even without calibration during formulation development.

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1. Introduction

During pharmaceutical development, assessment of the homogeneity of powder blends is a critical step that will impact both medicine safety and efficacy. Actually, HPLC is the commonly used technique consuming time and requiring a lot of resources. This is the reason why NIR and Raman spectroscopies have been more and more used to study powder blend processes [1–3]. However, none of these techniques can determine the spatial distribution of the components in the final product.

Hyperspectral imaging combines spectral and spatial information. Therefore, it has gained importance in pharmaceutical analysis during the last decade. Indeed, it allows obtaining simultaneously the API (Active Pharmaceutical Ingredient) concentration and its corresponding distribution map [4].

In the pharmaceutical field, hyperspectral techniques are mainly based on Raman, near-infrared (NIR-CI) or mid-infrared (MIR-CI) spectroscopies and have been used to obtain quantitative distribution maps of pharmaceutical ingredients [5–7], to detect and quantify polymorphs [8,9], to characterize particle size [10], to detect counterfeit medicines [11] and to characterize blending conditions [12,13].

Several approaches have been used to assess the distributional homogeneity in an objective way. Most of them used a quantitative model to obtain distribution maps and then analyzed the histogram of pixel concentrations [14–17]. Histogram parameters (mean, standard deviation, skewness and kurtosis) are useful to assess the "constitutional homogeneity", that is, the dispersion of pixel concentration values [6]. However, two maps may have exactly the same constitutional homogeneity while being spatially totally different. This is why it is also important to assess the distributional homogeneity.

Usually, distributional homogeneity is assessed by visual inspection of distribution maps. This approach clearly lacks objectivity and if the difference between the two maps is tight, it is impossible to unequivocally declare which one is the most homogeneous.

Therefore, Rosas et al. [18–20] developed a criterion to obtain an objective value of distributional homogeneity. This criterion is based on the analysis of the Poole index of non-overlapping macropixels. However, this approach has several limitations. As it works with non-overlapping macropixels, it is quickly limited for the analysis of small distribution maps. Furthermore, the studied map must be binarized. This binarization step is inevitably a source of error. Therefore, it appears that it could be advantageous to develop a new criterion which could analyze small maps and which would need as few input and pre-processing as possible to avoid as much error as possible.

In this paper, we describe a new criterion called Distributional Homogeneity Index (DHI). This index can be performed on small maps with continuous values. Relevance of the developed DHI has been tested on simulated distribution maps of controlled increasing homogeneity.

Secondly, DHI has been applied on several developed formulations with different content uniformity values. As these formulations were under investigation, no quantitative model (e.g. partial least square model) should be built. DHI was then tested on distribution maps obtained by semi-quantitative methods.

2. Material and methods

2.1. Samples

Several pilot blends of 8 kg were produced with different blending conditions, API particle size and excipients grade. Final concentration of API was of 8.4% (w/w). These blends were then pressed in tablets of 80 mg and of 6 mm of diameter.

Tablets were collected in a stratified way (begin, middle and end of the tableting) for several blends. For each blend, begin, middle and end samples were considered as different batches. Batch selection for hyperspectral analysis was performed choosing a specific blend and a specific tableting time. To do so, 10 tablets per batch were randomly chosen, assayed by HPLC and the content uniformity (expressed as relative standard deviation, RSD%) and the European Pharmacopoeia's acceptance value [21] were calculated.

Batches with different content uniformity and acceptance values ranging from 0.46% to 11.04% and from 1.10 to 29.41 respectively were selected. Once the batch selected, 10 other tablets were randomly chosen and analyzed by hyperspectral Raman imaging.

For confidentiality reasons, neither HPLC method nor information of tablet's qualitative composition and blending conditions can be presented. Tablet's quantitative composition is presented in supplementary Table S1. Spectral similarities between tablet's components are presented as correlation coefficient values in supplementary Table S2.

2.2. Instrumentation

Raman hyperspectral images were collected with a dispersive Raman spectrometer RamanStation 400 F (Perkin Elmer, MA, USA) equipped with a two-dimensional CCD detector (1024 × 256 pixel sensor). The laser excitation wavelength used was 785 nm with a power of 100 mW.

The measured spectral region was 1622–90 cm^{-1} and the spectral resolution was equal to 2 cm^{-1}. One accumulation with a 1 s exposure time was performed per sample mapping point. The distance between two consecutives mapping measurements was fixed at 100 μm. Background acquisition during mapping was repeated each 20 min. The spectra were collected with the Spectrum 6.3.2.0151 (Perkin Elmer) software.

The analyzed tablet surface was prepared beforehand with a Leica EM Rapid milling system equipped with a tungsten carbide miller (Leica Microsystems GmbH, Wetzlar, Germany).

Tablets were circular with a diameter of 6 mm (area of 28 mm^2). Measured maps represented the greatest square possible with a map size of 40 × 40 and a step size of 100 μm. A total surface of 16 mm^2 was covered.

Ten tablets per batch were analyzed.

2.3. Data processing

Once acquired, the hyperspectral images underwent preprocessing and multivariate analysis to extract useful information.

First, hyperspectral data cubes ($M \times N \times \lambda$) were unfolded into a two-dimensional array ($MN \times \lambda$), where M and N are the spatial information and λ the spectral information. Once unfolded, Raman spectra were baseline corrected using the Asymmetric Least Squares (AsLS) algorithm [22] with a λ value of 10^5 and a p value of 0.001.

Then, cosmic rays have been removed using the algorithm developed by Sabin et al. [23] with a parameter k set at 15.

Two multivariate data analysis approaches were used.

2.3.1. Classical Least Squares (CLS) regression

Distribution maps were obtained using CLS regression. This method assumes that Beer–Lambert's law is respected and that the sum of the individual absorbance for each component equals the total absorbance for each pixel. Therefore, it computes the concentration of each component by direct regression of the hyperspectral data cube by using the pure spectra.