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Application of chemometric algorithms to MALDI mass spectrometry imaging of pharmaceutical tablets

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ABSTRACT

During drug product development, the nature and distribution of the active substance have to be controlled to ensure the correct activity and the safety of the final medication. Matrix assisted laser desorption/ionization mass spectrometry imaging (MALDI–MSI), due to its structural and spatial specificities, provides an excellent way to analyze these two critical parameters in the same acquisition. The aim of this work is to demonstrate that MALDI–MSI, coupled with four well known multivariate statistical analysis algorithms (PCA, ICA, MCR–ALS and NMF), is a powerful technique to extract spatial and spectral information about chemical compounds from known or unknown solid drug product formulations. To test this methodology, an in-house manufactured tablet and a commercialized Coversyl® tablet were studied. The statistical analysis was decomposed into three steps: preprocessing, estimation of the number of statistical components (manually or using singular value decomposition), and multivariate statistical analysis. The results obtained showed that while principal component analysis (PCA) was efficient in searching for sources of variation in the matrix, it was not the best technique to estimate an unmixing model of a tablet. Independent component analysis (ICA) was able to extract appropriate contributions of chemical information in homogeneous and heterogeneous datasets. Non-negative matrix factorization (NMF) and multivariate curve resolution–alternating least squares (MCR–ALS) were less accurate in obtaining the right contribution in a homogeneous sample but they were better at distinguishing the semi-quantitative information in a heterogeneous MALDI dataset.

1. Introduction

European Medicines Agency (EMA) recommendations stipulate that pharmaceutical companies have to continually improve manufacturing efficiency to ensure drug product quality [1]. The commonly used analytical tools (such as liquid chromatography or dissolution) provide information about drug substance quality and dosage or the drug release profile by dissolving the whole tablet. A few years ago, chemical imaging, also called hyperspectral imaging, began to be used in the environmental and remote sensing fields in order to combine the spatial and spectral information of a chosen area [2]. This technique is now commonly used in a variety of domains such as material inspection, the food industry, or biological and pharmaceutical analysis [3–5]. During drug product development, the distribution of the active substance within the drug product is considered a critical parameter since the heterogeneity of active pharmaceutical ingredients (APIs) or excipients may influence the activity and safety of the drug product. The spatial information provided by using magnetic resonance imaging (MRI) [6], or Raman and near infrared (NIR) chemical imaging [7,8] is widely appreciated for the investigation of pharmaceutical products. Vibrational spectroscopies (Raman or near infrared) are particularly useful to obtain well-defined maps of APIs and excipients [9] as they do not require sample preparation and as they are relatively fast, non-invasive and non-destructive. Although existing techniques can provide images of APIs or excipient distributions, the poor structural information contained in the spectra does not allow the identification of an unknown or unidentified compound within a drug product, which could be necessary for tablet analysis without prior knowledge.

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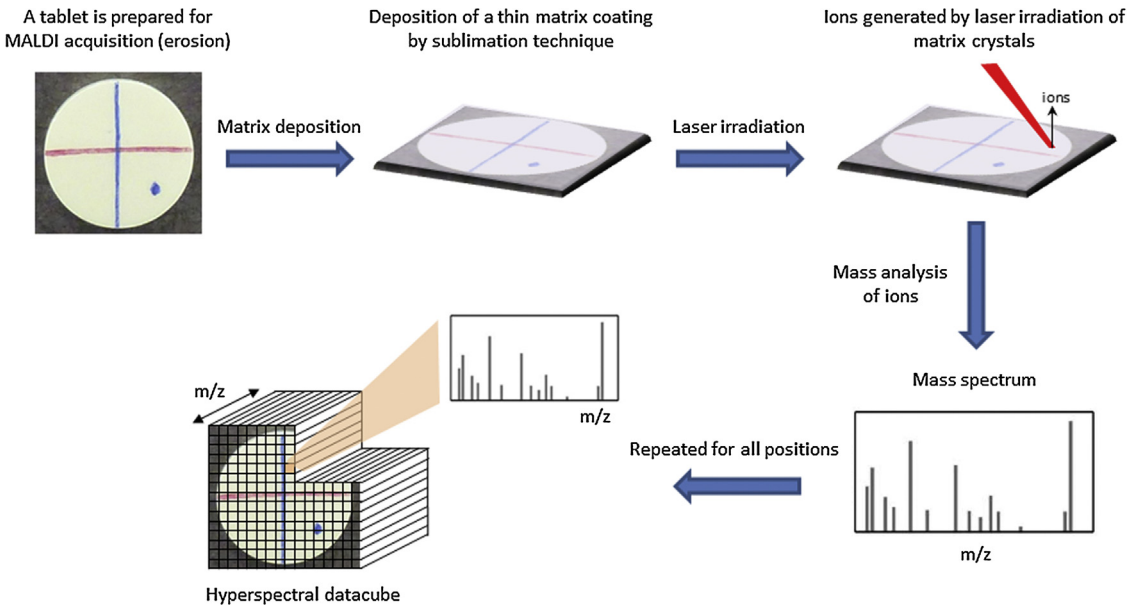

Fig. 1. Representative scheme of the MALDI–MSI principle.

Mass spectrometry, due to its structural specificity, is an essential analytical tool during drug product development. Depending on the product and objectives, several techniques are available. Desorption electrospray ionization mass spectrometry (DESI-MS) was used to extract chemical structural patterns in illicit ecstasy powders and tablet screening [10]. In the forensic field, matrix assisted laser desorption/ionization–time of flight (MALDI–TOF) mass spectrometry made it possible to analyze amphetamine and methamphetamine in clandestine tablets [11]. Hot cell membrane inlet mass spectrometry (hot cell MIMS) coupled with an electron ionization mass spectrometer (EI-MS) can recognize active ingredients in many common tablets without any preprocessing. Using this mass spectrometry technique, the possibility of creating a small portable mass spectrometer for the identification of medications in paramedical vehicles was studied [12].

In the last decade, mass spectrometry imaging systems started to appear in analytical laboratories. Coupled with spatial information, the structural aspect is a real advantage compared with the previously described imaging technologies. The first applications were in the biological domain [13–18] followed by the pharmaceutical field. For example, secondary ion mass spectrometry (SIMS) was applied to highlight leuprorelin peptide drug distribution within a matrix of hydroxypropyl-cellulose, thus providing an insight to the solid-state stability of peptides, proteins and biopolymers [19].

The earliest applications of MALDI–MSI were on tissues [20]. This technique needs specific sample preparation. The acquisition procedure is divided in two main steps. First, the sample is coated with a chemical component called the matrix. Second, the coated surface is irradiated with a pulsed laser to produce gas phase ions. The sample (i.e. the tablet) is positioned on a motorized plate which is moved with a specific spatial resolution under the pulsed laser beam to obtain a mass spectrum on each pixel. Data can be represented as a hyperspectral datacube where each pixel contains a mass spectrum with intensity values for each m/z variable [Fig. 1]. The dataset contains a huge amount of data which makes visual extraction of information (i.e. observation of data at a specific m/z value) extremely difficult or impossible. The huge datasets generated by the acquisition system justify the application of multivariate statistical analysis algorithms [21,22].

In the pharmaceutical field, the application of MALDI–MSI on solid drug products is still uncommon. The feasibility of tablet imaging using MALDI–MSI was demonstrated on commercial drug formulations including aspirin, paracetamol or sildenafil citrate (Viagra® 25 mg) [23]. In the present study, specific m/z ratios of each constituent were manually extracted from the mass spectra dataset after applying principal component analysis (PCA) to reduce the data dimensionality. The discrimination between placebo and solid drug product was performed using a supervised linear discriminant analysis (group information was given by the user) based on PCA scores. As in most of publications concerning MALDI MSI, compound distributions were displayed by studying specific m/z values.

In the present study, MALDI–MSI analysis was carried out to investigate the distribution of constituents (APIs and excipients) within two drug product formulations. Two different formulations were analyzed: a specially manufactured in-house product, and a commercialized tablet. Four different well-known multivariate data analysis algorithms were compared: PCA, independent component analysis (ICA), non-negative matrix factorization (NMF) and multivariate curve resolution – alternating least squares (MCR–ALS). The novelty of this study is that, despite the difficulty of obtaining the optimal number of "pure" components of a complex and relatively homogeneous mixture, statistical analysis algorithms were able to extract significant signals and to display the distribution of tablet compounds without prior knowledge.

2. Materials and methods

This section describes the data that were used in the two experiments. Preparation of the tablets, their acquisition and the preprocessing methods are also presented.

2.1. Samples

An in-house tablet composed of aspartame, lactose and inks [Fig. 2a] was first studied. Lactose and aspartame were compressed with a SPECAC (Slough, England) press system at 5 t. A horizontal red line, a vertical blue line, and a blue dot were manually drawn on the tablet surface. Each quarter of the tablet was manufactured