Complementary classification of solid oral dosage forms in ambient conditions by desorption electrospray ionization mass spectrometry and transmission Raman spectroscopy

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Abstract

Discrepancies or defects in active ingredients, excipients and coatings that form solid oral dosage forms can both impact product quality and provide hallmarks of off-brand or counterfeit products. There is therefore a need for rapid and continuous analytical techniques that can assess and classify product differences of intact samples at- or near the production line, or in analytical labs, ideally without resorting to product dissolution.

Here we test the ability of two rapid ambient chemical characterization methods to discriminate between solid dosage forms: desorption electrospray ionization mass spectrometry and transmission Raman spectroscopy. These two techniques are highly complementary, offering greater sensitivity to the analysis of the surface and the tablet bulk, respectively. The data sets generated were then used to test a variety of classification algorithms including linear discriminate analysis, tree-based methods, a simple neural network, and support vector machines (SVM). The highest performing algorithms for DESI-MSI were the SVM, with an additional performance boost when used with a polynomial kernel. For transmission Raman data, a linear discriminant analysis (LDA) model was found to be the most effective.

Introduction

Inconsistencies in active ingredients, excipients, the thickness and integrity of coatings and the presence of impurities in solid oral dosage forms all negatively affect their performance. Inferior quality attributes can be useful to identify off-brand or counterfeit products. There is a need for rapid and continuous analytical techniques that can assess and classify product differences of intact samples at- or near the production line, or in analytical labs, ideally without resorting to product dissolution [1]. Rapid measurement tools are particularly important to enable continuous monitoring, necessary to support the change from batch to continuous manufacturing. Analytical methods are required to monitor both the actives, coatings and consistency of the product: For example, in addition to the total API content, insight is also needed on degradation products, impurities, (co-) crystallinity/presence of polymorphs, and content uniformity. The ability to monitor tablet coating thickness and integrity is of great importance, particularly for functional coatings, such gastro-resistance, which would be compromised by insufficient thickness, or the occurrence of cracks in the film [2].

Quantitative analysis of pharmaceutical tablets is routinely performed by HPLC which offers accurate and sensitive measurements of the active ingredient(s) and excipients, in addition to the presence of any contaminants. However, solution-based analytical methods are destructive and labor-intensive.

Mass spectrometric methods can provide unlabeled identification, both of expected ingredients in known samples and of contaminants or components of unknown formulations. Ambient ionization mass spectrometry approaches including DESI (desorption electrospray ionisation) and DART (direct analysis in real time) facilitate the desorption and ionization from the surface of samples at atmospheric conditions, without dissolution or additional sample preparation. They are therefore potentially useful tools for rapid assessment of solid oral dosage forms

Optical spectroscopy techniques offer rapid, non-destructive analysis, including polymorphic identification [3], and are also able to measure insoluble ingredients. They have consequently been exploited for in-line process analytical testing and as quality control tools [1]. For example, infra-red-based techniques (FTIR and DESI [4]; and NIR and Mid-IR spectroscopy classification of MDMA containing tablets [5]. Near infrared is the most commonly used process analytical tool [1], however Raman spectroscopy provides complementary information and has grown in popularity in recent

years, since it provides more distinct spectral features, and is better-suited to analysis in aqueous environments owing to the relatively weak strength of the Raman O-H band. Technological advancements have facilitated miniaturization, increased speed and reduced cost, resulting in more widespread implementation. [6]

Ambient ionisation mass spectrometry of tablets

Desorption electrospray ionization uses a charged electrospray of organic solvent which, when directed at the sample surface in proximity to the mass spectrometry inlet, desorbs ions from the sample which may be taken up into a mass spectrometer [7]. As this process takes place at ambient pressure and with a flexible geometry, the technique is suited for the analysis of a wide range of samples including explosives on surfaces [8], fingerprints [9], plants [10] and tissues [11,12]

One of the early descriptions of DESI-MSI was in the profiling of tablets [13]. Chen et al demonstrated the use of DESI-MS to profile tablets containing loratadine, folic acid, acetaminophen (paracetamol), aspirin, melatonin or caffeine. Optimization of DESI parameters including voltage, solvent delivery and capillary temperature facilitated analysis at up to three scans per second.
Subsequent studies using DESI-MS of tablets have focused on targeted analysis for active ingredients. For example, the identification MDMA and amphetamine derivatives in ecstasy tablets [14], counterfeit artesunate antimalarial tablets [15,16] and antiviral capsules [17].

For ambient mass spectrometry to be deployable in the field for counterfeiting applications, or in manufacturing environments for QA/QC, the mass spectrometer must be compact. Several designs for small field-deployable mass spectrometers have been demonstrated with DESI MS sources [18,19].

Each of these applications has targeted expected components of the tablet of interest, predominantly active ingredients or excipients. However, in manufacturing QA/QC and counterfeit-detection applications, additional information on unexpected changes in active or excipient source or quality, as well as the introduction of contaminants may be of importance. Untargeted multivariate and machine learning approaches are therefore of interest to determine differences between samples using all spectral information.

Classification approaches for mass spectrometry applications are proving powerful in a range of applications. The two most widespread applications of classification in mass spectrometry are in disease diagnosis and determination of bacterial type [20]. A range of classification algorithms have been applied to mass spectrometry and spectroscopy data. PLS-DA is most commonly reported, although a range of algorithms including neural networks, and support vector machines [21] have been reported. Several publications have evaluated different classification algorithms but unsurprisingly the optimal algorithm depends greatly on the nature of the input data. A summary of classification and other data analysis for proteomics can be found here [22] and for metabolomics here [23]. Classification approaches are becoming more accessible through modeling tools with consistent grammar and data structure, and their integration into mass spectrometry software [ScilsLab,Waters software] [23].

Notably, classification of rapid evaporative ionization MS enables real-time classification of tissue types during surgery [24]. Classification of REIMS data has also found applications in food security [25] and bacterial speciation [26]. Classification approaches have also been widely employed in mass spectrometry imaging data, particularly in the classification of cancerous tissue [27]

Raman spectroscopy analysis of tablets

Raman spectroscopy exploits the inelastic scattering of light by the sample to reveal valuable chemical and structural information. Information can be obtained from the sample in a non-destructive manner, making it a popular process analytical technology tool. Raman spectroscopy can be performed in a variety of sampling configurations/geometries, the most appropriate depending on the application. Confocal Raman microscopy can provide detailed chemical mapping with high spatial resolution, however this is generally reserved for forensic investigation rather than continuous monitoring, since it requires lengthy acquisition times. Sub-sampling issues associated with conventional backscattered Raman can be overcome by strategies such as sample rotation in conjunction with spectral averaging, or simultaneous wide angle illumination [28].

Matousek et al demonstrated the ability of transmission Raman spectroscopy to probe deep into turbid materials such as pharmaceutical tablets and provide information on their bulk properties [29,30]. In contrast to conventional backscattered Raman, in transmission Raman spectroscopy the beam passes through the full thickness of the tablet, sampling a much larger volume of the material, and consequently provides more representative sampling [31]. Although Raman scattering intensity is linear with concentration within the same confocal plane, transmission Raman signal intensity is slightly biased towards the bulk of the tablet relative to the exterior due to internal scattering [32]. In contrast, DESI-MSI sampling is biased towards the surface/coating composition. Therefore, in combination, these two techniques should provide a powerful toolkit with which to assess compositional differences between pharmaceutical tablet formulations.

Raman spectra of complex mixtures such as solid dosage forms often have complicated spectra with overlapping peaks. For this reason, multivariate techniques are often applied to help identify the components of interest and changes in chemistry. The selection and use of unsupervised and/or supervised techniques on Raman spectra rely on factors such as prior knowledge of the raw component spectra, and the quantity and complexity of the spectra [33].

As with mass spectrometry, classification of Raman spectroscopy data has been primarily focused on disease diagnostics [34,35,36] and bacterial analysis [37,38]. Other noteworthy examples of the use of classification in Raman spectroscopy include differentiation of narcotics [39], pharmaceuticals [40], [41], and counterfeit tablets [42].

There have been relatively few comparisons of different classification methods for Raman spectroscopy data. Zheng et al. compared SVM, LDA and k-nearest neighbours (KNN) methods to classify renin hypertension from Raman data from serum [43]. They found that SVM and LDA performed similarly, and both outperformed the KNN algorithm. Partial least squares (PLS) and PLS discriminant analysis are also commonly used methods in characterizing tablets, however care is required depending on the data quantity and the pre-preprocessing performed [44]. Qun et al. tested the classification of expired drugs using PLS-DA, SVM and KNN, and reported that SVM gave the strongest performance [45]. F ransson et al tested the performance of multivariate methods including PLS, classical least squares (CLS) and multivariate curve resolution (MCR) for classification of pharmaceutical tablets [46].

Objective

In this study we set out to explore the potential of DESI-MSI and transmission Raman spectroscopy to distinguish commercially available pharmaceutical tablets with similar or different formulations. Pairing DESI with transmission Raman spectroscopy was of particular interest due to their complementarity and relative abilities to sensitively probe the surface vs the bulk of the tablets. Classification of tablets based on both active ingredients and excipients has the potential to be used for in-line quality control measures during pharmaceutical manufacturing, and for rapid counterfeit testing. As such we have tested a range of classification algorithms on their capability to differentiate

these tablets using a range of pre-processing methods to determine the best approaches to use in different applications.

Experimnental

Samples

Samples were selected from commercially available of-the-shelf products and purchased from a local supplier. Their names, active ingredients, listed excipients and MHRA product license numbers are included in Table 1

Table 1: Details of the tablet types analysed. Letter codes for each type are used throughout

Type	Product name	Active ingredients	Listed excipients	MHRA licence
A	Anadin Extra Tablets	300 mg Aspirin, 200 mg Paracetamol, 45 mg Caffeine	Maize starch, microcrystalline cellulose (E460), hydrogenated vegetable oil, hydroxypropyl methylcellulose (E464), polyethylene glycol, pregelatinised starch and povidone	PL 00165/5013R
B	Tesco Paracetamol Extra Tablets	500 mg Paracetamol, 65 mg Caffeine	tarch, povidone k-30, povidone k-90, croscarmellose sodium, talc, stearic acid and magnesium stearate	PL 08977/0025
C	Tesco Paracetamol Tablets	500 mg Paracetamol	Potato Starch, pregelatinised starch, magnesium stearate, povidone, stearic acid and talc	PL 08977/0014
D	Tesco Extra Power Pain Control Tablets	300 mg Aspirin, 200 mg Paracetamol, 45 mg Caffeine	Povidone, hydroxypropylcellulose, stearic acid, microcrystalline cellulose, maize starch, pregelatinised starch, hydroxypropyl methylcellulose 5cPs, hydroxypropyl methylcellulose 15cPs, macrogol 4000	PL 29831/0164

DESI MS

DESI-MS measurements were performed on a Synapt G2-Si Q-IM-ToF mass spectrometer (Waters Corp). The instrument was operated in ‘resolution mode’. The ion mobility cell was not used. Positive ion mode spectra were collected with a scan time of 1 second across a mass range of m/z 50 to m/z 1200. The instrument was fitted with a prototype DESI source (Waters Corp), with the sprayer configured for electroflow focusing with a fused silica capillary sitting approximately 1 mm behind a 200 µm steel orifice. Methanol with 5% water by volume was delivered at 2 µl/m by a pressure pump (Dolomite). Nitrogen gas was delivered at 0.2 MPa. The spray voltage was set at 5 kV. A heated inlet capillary was set to a calibrated temperature of 400°C using a PID (Waters Research Centre, Hungary). Tablets were sampled by holding the tablet 1-2 mm away from the DESI spray head using plastic

tweezers. For training data, acquisition was started with the tablet already under the spray head, such that only data from the tablet surface was acquired, while validation data was collected continuously.

Transmission Raman spectroscopy

Transmission Raman spectra were acquired using a Renishaw InVia Qontor Raman microscope equipped with a 830 nm excitation source fibre-coupled to an InVia transmission Raman accessory (Renishaw plc, Wotton-under-Edge, Gloucestershire), in a temperature controlled environment. Light was collected in transmission with the x5 air objective lens (0.12 NA, N-PLAN, Leica). Tablets were carefully placed onto a flat silicon sample support with a hole just smaller than the tablet dimensions, so that the excitation beam was able to pass through the tablet but not the sample support. Six tablets were analysed of each type in pseudo-random order. Three measurement replicates were acquired, each complete data set was collected on three separate days.

For all tablets, extended spectra were acquired using Renishaw Wire (version 5.3) software for the spectral range of 50 to 1800 cm^{-1}, with an acquisition time of 30 seconds, and 5 accumulations. Laser power was set to 100% which has been measured at the sample to be approximately 117 mW. An internal silicon calibration reference spectrum was acquired each day to correct the Raman shift of the data.

Data analysis

All data were analyzed in R version 3.6.2 (2019-12-12) “Dark and Stormy Night” and RStudio Server version 1.2.5019. Analysis was conducted using the tidyverse [47] and tidymodels [48] metapackages. Raman data preprocessing was conducted in MATLAB 2020a. All analysis was performed on a Linux workstation (Intel Core i9-7900X CPU with 10 cores @ 3.30 GHz, 128G RAM, Ubuntu 16.04.6 LTS.

DESI preprocessing

For model development and comparison, data were converted from Waters raw format to mzML format using ProteoWizard MSConvert version 3.0.19239-0ae547798. These were read into R using the mzR package. All spectra were re-binned onto the same mass axis with a bin width of m/z 0.01. A mean spectrum of all training data was peak picked using the findPeaks function from the prcma package with a peak intensity threshold of three times the median intensity of the spectrum. 1217 peaks were found. Each spectrum was then individually integrated across the found peak widths to form a datacube. Each scan of the validation dataset was similarly integrated across the peak widths from the training dataset. Reduced peak datacubes were generated by filtering for the top n most intense peaks. Down-binning to simulate reduced mass resolving power was performed by rounding m/z values and summing intensities within each rounded m/z bin.

Transmission Raman spectroscopy preprocessing

Cosmic ray removal was performed automatically by Renishaw Wire (version 5.3) and spectra exported to .txt format. The Raman spectra were baseline corrected using the msbackadj() Matlab function. The baseline was estimated within multiple shifted windows of width 20 separation units, then a spline approximation was used to regress the varying baseline to the window points. The estimated baseline for each spectrum was then subtracted from the corresponding original. The background subtracted spectra were read in R for subsequent processing and analysis. The data were normalized to total spectrum intensity and the Raman shift recalibrated using the weighted-mean centroid to the 520.7 cm-1 peak from the daily Si wafer sample spectrum as a reference. Extended spectra were truncated to a wavenumber range between 250 cm-1 and 1700 cm-1. Due to the limited

number of wavenumber bins and the challenges of peak-picking Raman data, the continuous data were taken forward for classification.

Classification

Spectra were collated into a 10-fold cross validation 1 with 10 repeats in a 4/1/5 (train/test/total) split. To remove highly co-variate polymer peak sequences leading to overfitting, highly correlating variables (Pearson correlation > 0.9) were removed from DESI MSI data. Data were centered around the arithmetic mean and scaled to have a standard deviation of one. Underrepresented classes (for DESI MSI, the background class) were up sampled. Each training fold was applied to a range of classification algorithms using the tidymodels package. All models were implemented with their default parameters beyond setting to classification mode. The functions, engines and default parameters used for each model are provided in supplementary table 1. These models were then used to predict each testing fold. For each fold the F1 score was calculated. For DESI MSI the algorithm with the highest F1 score, a support vector machine with a polynomial kernel was selected for further model tuning on a single 4/1/5 validation split of the training data. For transmission Raman data a LDA model was selected. A final model was fitted on all the training data. These models were used to predict the test independent test sets. Cosine similarity between spectra were calculated using the cosine function from the coop package [49, =coop]. Considering the angle between vectors, rather than magnitude, cosine similarity provides a useful and robust measure of spectral similarity for highly multivariate datasets [50]

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