A Survey of Multispectral High Resolution Imaging Based Drug Surface Morphology Validation Techniques

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Abstract: According to World Health Organization (WHO) low quality, expired and counterfeit medicines are real threat to the health of patients starting from minor allergies to the painful death. Such medicines are affecting patients in developing as well as developed countries. In pharmaceutical industry different techniques are being used for the validation of drugs either at the time of manufacturing or after production. Traditional quality control techniques are time-consuming, destructive and expensive. These techniques also require sample preparation before testing. There is an immense need of a drug validation method that does not require sample preparation and can evaluate quality of medicines nondestructively in real time. The use of Multispectral Imaging (MSI) in the pharmaceutics for quality validation can be very helpful. MSI uses different wavelengths of the electromagnetic spectrum by dividing it into multiple bands. In this paper we are proposing a novel method for nondestructive validation of solid medicines from their surface morphology using MSI. The surface structure captured from MSI is further evaluated using digital image processing techniques and is helpful for the qualitative and quantitative analysis of chemical composition, surface features and interference effects. Different pattern recognition techniques can be used for the classification of this spectral and spatial information of drugs between substandard and genuine drugs. Fast, easy and nondestructive quality control and process monitoring can be achieved using the proposed method. This proposed method will be a contribution to the pharmaceutical industry and will be beneficial for quick, cost-effective and nondestructive quality assessment of the end products.

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1. Introduction

In pharmacology, drugs are such chemical substances that are used to change the physical condition of the patients for the treatment of different diseases. These drugs are prescribed by the physician either for a short period of time in case of acute diseases or on regular basis for chronic disorders. According to WHO substandard drugs like low quality, expired and counterfeits are real threat to the health of patients. They are common problem of developing and developed countries. According to US Food and Drug Administration (FDA), up to 25% of all drugs consumed in poor countries are thought to be counterfeit or substandard. Many reports describe; drugs that treat serious diseases such as malaria, tuberculosis, AIDS or other infections are more often the object of counterfeits.

For a particular disease, the decision for the drug delivery system and accurate required amount is very important. The physical type and amount of medication is known as Dosage Form. The dosage forms also describe the rout of the drug administration; rout of administration means the path through which a drug is delivered to the site of action in the body. Dosage forms are required for accurate dosage for patient. With the passage of time, the evolution in the medical therapies and drugs also result in new dosage forms. Drugs are available in different dosage forms e.g., Solid Dosage Forms, Liquid Dosage Forms and Semi-solid Dosage Forms.

The solid dosage forms are medicines that contain accurate dosage and can be given to the patients as a single unit (dose). They are administered orally in the form of tablets, capsules or powders (Sahoo, 2007). Solid medicines are mixture of Active Pharmaceutical Ingredients (APIs), with a combination of different diluents, binders, lubricants, glidants and many other excipients. Manufacturing of solid medicines require complicated and costly machines. Capsules and tablets are the most common forms that are widely used in the industry and have similar manufacturing procedure. Solid dosage forms are easy for shipment and more stable as compared to liquid drugs. Mostly they have longer expiration dates.

On the other hand liquid dosage forms cover solutions, syrups, emulsions, suspensions and many more. Homogeneous mixtures of one solute dispersed in a solvent are known as solutions. Syrups are the aqueous solutions having sugar or any substitute for sugar with a combination of different flavoring agents. Physicochemical stability of the drug can be maintained by using stabilizers in syrups and solutions.

The combinations of two or more liquids which are immiscible are known as emulsions. Emulsions range from low viscosity lotions to ointments and creams which come under the category of semi-solid dosage forms. Insoluble fine solid particles of a drug substance form suspension when dispersed in a liquid medium (Mahato, 2005). Some of the suspending agents are used to increase the viscosity of the drug which results in slow dissolution of drug.

Ointments, creams, gels etc. come under the category of Semi-solid dosage forms. The greasy medications used for skin, rectum or nasal mucosa which can dissolve to the skin are known as Ointments. Creams are mixtures of oil and water. Oil-in-water (O/W) creams are more effective and comfortable as they are less greasy. They are easy to washout so as to be cosmetically acceptable. Another category is water-in-oil (W/O) creams. They are reverse of O/W creams, more greasy and difficult to handle.

In pharmaceutical industry, every medicine that is produced by manufacturers should be according to the defined quality metrics. In market, along with genuine drugs, customers can also find substandard medicines. According to WHO, Substandard Medicines are genuine products but do not fulfill the quality standards. They are also known as OOS (Out Of Specification) products. They are developed by the licensed manufacturers who are working under National Pharmaceutical Regulatory Authority (NPRA) (Christian, et al., 2012). Substandard medicines are such medicines that are (Clift, 2010) either sold after expiration date or affected due to improper supply and storage. They can have either too much or too low amount of API. Their ingredients can be contaminated. Sometimes they can have fake packaging or any other kind of quality negligence.

These medicines may be produced either by carelessness of the pharmacists, insufficient financial and human resources, by the use of obsolete or malfunctioning of the laboratory equipment or counterfeiting. Substandard medicines are harmful for patient's health or sometime even cause death (Christian, et al., 2012). Substandard medicines can be further categorized into Counterfeit Drugs, Expired Drugs and Environment Effected Drugs.

Counterfeit drugs are subsets of substandard medicines but they are not developed by the authorized or licensed manufacturers. According to World Health Organization (WHO, 2012), they are also known as SFFC (Spurious/Falsely-labeled/Falsified/Counterfeit) medicines. They are fake medicines that look like genuine but in reality they are not. The difference between other substandard medicines and counterfeiting is that they are illegally or intentionally created false medicines (Christian, et al., 2012). Sometimes they have fake packaging or there can be absence of API or presence of API in an inappropriate amount (WHO, 1999). There are multiple different factors that contribute to the proliferation of the counterfeit drugs. They should be identified accurately so that government can take appropriate actions to eradicate them.

Another category of substandard drugs is the expired ones. Every medicine has an expiry date determined by its manufacturers. Expired medicines are unpredictable in their effectiveness level. Due to the loss of potency level chemical compounds may change their composition with the passage of time; these changes can be either in their color, smell, or texture.

The third category of substandard drugs can be environment effected drugs. The drugs which were genuine and according to the quality measures at the time of manufacturing but with the passage of time due to some environmental factors turned into substandard drugs. These environmental factors can be oxidation and reduction, light, moisture and temperature. Oxidation and reduction occur due to the exposure of drug formulation with oxygen, which results in instable or substandard drug. Similarly high temperature and light (especially ultraviolet light) may also fasten the oxidation and reduction within a drug. Moisture and humidity may also damage the stability of any drug.

In pharmaceutical industry 70% of the medicines are manufactured in the form of tablets. This instability of tablets may result in unpredictable behavior (like their disintegration and dissolution time) or change in their physical appearance (like harness, shape, color etc.). The use of such substandard medicines is harmful and real threat to the health of patients, starting from minor allergies to painful death.

In this paper we have proposed a new methodology for the nondestructive validation of solid drug surface morphology. Section 2 provides the literature review and comparison of different quality assessment techniques. Section 3 describes proposed methodology while section 4 concludes the paper.

2. Literature Review

The assessment for the formulation, efficiency, correctness and stability of a medicine is very important. In pharmaceutical industries, different methods are used for quality assessment of drugs. These tests can give information about the active ingredients and structural information of the drug. They can be divided into three major categories: Traditional Assessment Techniques, Spectrum Based Assessment Techniques and Spectral Image Based Assessment Techniques.

Traditional assessment techniques may include tests that are mostly being used in pharmaceutical industry. Traditional techniques are destructive, time consuming, expensive and require

sample preparation. Spectral techniques use spectrometers which provide spatial information in the form of spectrums for every sample being studied. All of these spectral methods are non-destructive, less time-consuming and require less or even no sample preparation except Mass Spectrometry. Chemical Imaging is a newly emerging technique in pharmaceutics. Image based assessment techniques are combination of spectroscopic techniques and traditional imaging which provide both spatial as well as spectral information of the given sample (Gowen, et al., 2008). Our main focus will be on image based techniques. The detail of some of these techniques is discussed below.

2.1 Traditional Assessment Techniques

Thin Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC), Test-tube color reaction and melting point determination (WHO, 1999) are most common techniques used for drug quality testing. The brief description of some of them is given below.

TLC procedures can be used for the detection of counterfeit drugs. These procedures can be used for the identification and estimation of APIs from the drug (Deisingh, 2005). Impure drug substances can also be identified using TLC (WHO, 1999).

Most of the manufacturers use HPLC in pharmaceutical industry to test the end products (medicines) and their raw material or ingredients. Manufacturers assign skilled analysts for this test. They pass raw materials or prepared medicines through the HPLC machine and then analyze their results. These machines required sample preparation for testing which destroys the sample. So HPLC is a destructive, slow, expensive and time consuming method (Perkinelmer, 2003). HPLC provides less information e.g., can only measure API and provide no information about the distribution of chemical components of the sample (Perkinelmer, 2003). This test is more suitable for herbal products and for the detection of organic residues (Deisingh, 2005).

2.2 Spectrum Based Assessment Techniques

The interaction of light (ER) with molecules and atoms of the natural product (like drugs) can provide information about their structures. This interaction may result in spectrums which lie under different regions of the ES and provide information about surface structure and ingredients. These spectrums are further processed by the computers (Gowen, et al., 2008). Along with traditional tests manufacturers may also use such techniques for the analysis of drugs composition and for the detection of counterfeit and substandard medicines (WHO, 1999). These techniques just provide some characteristics of the molecules but do not provide its three dimensional image. Spectral techniques which are mostly used in literature for the analysis of drugs may include Mass Spectrometry (MS), Nuclear Magnetic Resonance Spectroscopy (NMR), X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM) and Vibrational Spectroscopic Techniques.

MS can be widely used to characterize pharmaceutical products. Drug profiling can be done using Time of Flight (TOF) and electrospray ionization (Deisingh, 2005). MS primarily LC-MS/MS can be used in all stages of drug development. It can be used to elucidate the structure of pharmaceutical drug mixtures through mass determination. Pharmacokinetics of the newly created drug can be investigated through this technique (Vogeser, et al., 2007). MS is a destructive and time consuming method.

In pharmaceutical industry NMR is widely being used for the confirmation and elucidation of the drug structures. Analysis of synthetic or natural products can be performed through NMR spectroscopy. They can also be used to characterize the composition and to find impurity profile of the drugs. NMR measurement can also provide information about the conformations of drugs especially in tablets. This can be used by European Pharmacopoeia for the identification of drugs and reagents. Measurement of NMR spectra for liquid drugs is easier than the measurement that is done for the solid drugs (Holzgrabe, et al., 1998).

XRD is one of the spectroscopic techniques, mainly used for the analysis and identification of polymorphic and solvated forms. It is used to measure the degree of crystallinity but has lower sensitivity as compared to IR Spectroscopy. This technique can also be used to determine the quantitative amount of API from multicomponent tablets.

In SEM surface information of a sample can be traced using electron beam in a raster pattern which produces three dimensional black and white images. These images can be further converted into color images using image processing techniques. Surface fractures, contaminations and chemical compositions and crystalline structures can be examined using SEM. It is a destructive method as it requires sample preparation. Another drawback is its size and cost. Trained persons are required to prepare sample and to operate SEM machine.

Vibrational spectroscopic techniques such as Infrared (IR), Near Infrared (NIR) and Raman Spectroscopy (RS) are proving very beneficial for pharmaceutical quality analysis. It is more accurate, less costly and reliable than the traditional methods. No sample preparation is required for these analyses (Thermo Scientific, 2010). These tests are nondestructive so the sample product can be further packaged or used again for other tests. Near Infrared

Spectroscopy (NIRS) is widely being used in pharmaceutical industry to replace traditional time consuming, destructive, liquid chromatography techniques or wet-testing methods used for the analysis for the medicines. No sample preparation is required for NIRS. According to (Perkinelmer, 2003) NIRS can be used for Micro structure as well as Macro chemical properties of the tablets. Through macro chemical properties analysts can determine the active concentration of the tablet ingredients. Micro structure defines the distribution and size of the components (APIs and excipients) within a tablet. NIRS is recognized for the analysis of raw material, process monitoring and quality control of pharmaceutical products (Gowen, et al., 2008).

In a review prepared by (Roggo, et al., 2007), Analysis of Solid, liquid and biotechnological pharmaceutical products using NIRS along with Chemometrics becomes more powerful. NIR Spectra can provide information about various physical parameters of pharmaceutical product like hardness, compaction force, particle size, dissolution rate etc. These physical parameters can be obtained from tablets as well as powders. (Morisseau & Rhodes, 1997) uses different regression models such as Partial Least Square (PLS) and Multiple Linear Regression (MLR) along with NIRS to find hardness of the tablets. NIR accuracy results highly depend on the drug products and their formulation. Ensuring the correct polymorphic form of a drug is very important as polymorphs of a drug can be helpful for the identification and detection of counterfeits. This confirmation can also be done through NIRS (Roggo, et al., 2007).

In RS, a laser source of visible IR and monochromatic radiation are used for the analysis of the samples. It is a non-destructive method that can be used for the analysis of bulk and final products directly in their packaging. RS can also be used for online monitoring of the drug's quality and requires minimum trained personnel. RS can be helpful for identification of raw material, quantity determination of APIs and screening of polymorphs (Vankeirsbilck, et al., 2002).

2.3 Spectral Image Based Assessment Techniques

Image based quality assessment techniques provide more detailed information about the concentration and distribution of the drug ingredients. These techniques are combination of both vibrational spectroscopic techniques and digital image processing. Image based quality assessment involves two major techniques Hyperspectral Imaging (HSI) and Multispectral Imaging (MSI)

HSI is also known as Chemical Imaging (CI). CI is used to capture spatial as well as spectral information from an object. Initially it was developed for remote sensing but recent research proved that it can be used for nondestructive analysis of pharmaceutical products (Gowen, et al., 2007). HSI is related to MSI, the basic difference between them is the number of bands or the type of measurements.

Hyperspectral sensors are used to collect information of each spatial position as a set of images. Each of the images is based on spectral band range which means that each pixel in the image contains a spectrum of that specific position (Gowen, et al., 2007). CI is three dimensional blocks of data based on one wavelength and two spatial dimensions. CI can be formed by combining either Near-Infrared (NIR) or Raman Spectroscopy (RS) with digital imaging. Both of them can be used for the analysis of pharmaceutical dosage forms. So in general CI can be Near-Infrared Chemical Imaging (NIR-CI) and Raman-CI which can be used for analysis of raw ingredients of drugs, drug development process monitoring and quality control.

NIR-CI is an emerging technology as compared to simple NIR spectroscopic technique in pharmaceutical industry. NIR-CI is used for the prediction of APIs and excipients concentrations from the solid pharmaceutical dosage forms (Ravn, et al., 2008). Another research NIR-CI was used for the detection of counterfeit pharmaceutical tablets where no prior knowledge of the composition of sample is required (Dubois, et al., 2007). NIR-CI is also used to assess content uniformity from the batch of tablets. Content uniformity was evaluated by applying different quantitative algorithms to global hyperspectral image of ten tablets (Cruz & Blanco, 2011). Another recent research demonstrates the use of single point NIRS along with NIR-CI and statistical variance analysis for the detection of counterfeit tablets (Puchert, et al., 2010). NIR-CI is also used for the quantification of coating thickness of the tablets and their chemical structure of the tablet core and coating (Lewis, et al., 2005). High throughput quality analysis is highly required in pharmaceutical industry. NIR-CI can also be applied to perform analysis on multiple sample tablets at a time even if they are packed. This results in fast and nondestructive identification of APIs and excipients (Hamilton & Lodder, 2002).

In pharmaceutical industry Raman-CI can be applied to find particle size estimation, minor component detection and tablet characterizations. For analysis purpose data at each pixel of the sample is compared to a standard spectrum of the sample that has APIs and excipients to its correct level (Gowen, et al., 2008). Sasic applied Raman-CI to capture spectrums from the drugs for the detection of low content API pharmaceutical formulations. Author reported that PCA is more helpful for such kind of detections (Sasic, 2007). Another research conducted by (Doub, et al., 2007) focuses the application of Raman-CI for ingredient specific particle size characterization of

nasal spray formulation. It is suitable for identification of APIs as well as placebo. Similar chemical compositions in drugs can be effectively described using Raman-CI. Cluster analysis is used for the segmentation of images which enable the visualization of distinct regions for the characterization of solid dosage (Bell, et al., 2004).

MSI systems use MSI sensors which can collect spectra from less than 20, generally noncontiguous spectral bands (Shippert, 2003). These bands can detect information in a specific combination from the desired region of the spectrum. Unique combination of spectral information can be achieved by varying number and position of bands within MSI system (Vagni, 2007). Another research uses MSI for the determination of moisture and salicylic acid from a single packaged Asprin tablet. They conclude that MSI offers high speed advantage approximately 30000 times over HPLC. They also concluded that MSI of a field of tablets is almost 1000 times faster than spectrometry of a single tablet (Malik, et al., 2001).

Table 1 describes the comparison between various techniques that can be used for quality assessment of drugs. These techniques are compared against different features. Some of these techniques provide only spectral information about the sample, others provide only spatial information. Multispectral and Hyperspectral imaging techniques provide both spectral and spatial data of the sample. Both of these provide much more detailed information about the sample being studied than any other. Some of the techniques require sample preparation before the analysis which destroys the sample so are destructive. Drugs used in such kind of analysis cannot be used again for any other purpose. Techniques that do not destroy the sample are known as non-destructive techniques and are more appropriate when we do not want to waste the sample. Table 1 also provides comparison of these techniques against the time required for the analysis, their processing complexities and the cost in terms of machinery and man power.

According to the comparison in Table 1, destructive techniques are more complex, time-consuming and costly as they require sample preparation before analysis. XRD and SEM are more suitable for semi-solid drugs which are of crystalline form. NIRS and RS can be used for solids and are nondestructive methods of analysis requiring no sample preparation, but provide only spectral information of the sample.

On the other hand MSI and HSI are also nondestructive methods of analysis but have advantage over other techniques by providing both spectral and spatial data of the sample. This allows application of image processing techniques along with different classification methods for more detailed analysis. MSI is better than HSI as it requires less time for data processing. HSI contains more redundant data requiring more time for processing and is more complex than MSI.

These chemical imaging techniques are also known as surface based techniques. Each measurement captured from the penetration of the rays into the sample material provides information about the surface of the sample. Homogeneity of the data captured from the surface of the tablets represents its correctness. Analysis of the surface area of the tablets can provide information about the correct shape, size, color, hardness and dissolution. Homogeneous nature of the surface can also be used to determine oxidization reaction of the APIs and excipients.

Table 2 provides a quick review and comparison of different medicine analysis techniques. NIR-CI is mostly used for the analysis of solid medicines especially tablets. Analysis of tablets using NIR-CI provides information about content uniformity, composition determination, identification of counterfeits and tablet classification. This is based on both spectral and spatial data of the medicines and mostly require no sample preperation. MSI can also be used for the analysis of solid medicines especially tablets. Analysis through MSI can also be beneficial even the medicines are in packaged form. Contrast enhancement, histograms, binarization, noise reduction and gray scaling are commonly used image analysis techniques. Classification process can be performed mostly using PLS, Support Vector Machine (SVM), Naïve Bayes and KNNs. Principal Component Analysis (PCA) is the most common feature reduction technique. Savitzky-Golay Derivative, Standard Normal Variate (SNV) and Multiplicative Scatter Correction (MSC) are mainly used pre-processing techniques.

3. Proposed Methodology

We have proposed a new methodology for efficient, non-destructive and fast validation of solid medicines using their surface morphology. The complete flow of the proposed methodology is shown in Figure 1. The proposed methodology comprises of six modules: Data Acquisition, Data Pre-processing, Data Analysis, Feature Extraction, Feature Reduction and Classification.

3.1 Data Acquisition

The first stage of our proposed methodology is the acquisition of data. Our proposed methodology requires two different types of data from the sample being analyzed; one is the spectrum from non-visible region of Electromagnetic Spectrum (ES) and the other is from the visible region of the ES. So first stage is further divided into two sub-categories: Spectrum Acquisition and Multi-Channel Image Spectrum Acquisition.

MSI systems is used for acquisition of

wavelengths representing multichannel images of visible spectra known as RGB (Red-Green-Blue) which provides spatial information of the sample and going to Infrared (IR) wavelengths. IR region is classified into Near-Infrared (NIR), Mid-range Infrared (MIR) and Far-Infrared (FIR).

3.2 Data Pre-processing

Data pre-processing is the pre step of data analysis which is used to prepare the acquired data for further analysis. It is further divided into: Reflection Calibration, Spectral Pre-processing, Image Reconstruction and Image Pre-processing.

3.2.1 Reflection Calibration

The raw data captured from the specimen during acquisition phase may also contain dark camera response and background spectral response of the instrument along with its chemical composition. These additions in the data lead towards wrong analysis. To overcome this problem we are applying reflection calibration on both spatial and spectral data. This requires a camera response from the uniform high reflectance standard or white ceramic to capture background and a measurement of dark response by turning off all lights and covering the lens with its cap.

3.2.2 Spectral Pre-processing

Spectral information gathered from the sample provides knowledge about its chemical composition. However, multiple external factors cause systematic variations between spectra. These nonchemical factors include scattering effects due to surface inhomogeneity, specular reflections, random noise, interference from external light sources etc. For this reason after reflection calibration we are using spectral pre-processing techniques. Different pre-processing techniques can be used at this stage to remove such nonchemical biases from the spectral information such as (Cullen, et al., 2012): Smoothing, Normalization, Standard Normal Variate Correction (SNVC), Multiplicative Scatter Correction (MSC), Offset correction and Savitzky-Golay Derivative Conversion.

3.2.3 Image Reconstruction

After using reflection calibration on the RGB spectrum, we will need to combine all three spectrums to get one single color image against these three spectrums. This step is known as image reconstruction.

3.2.4 Image Pre-processing

Image pre-processing techniques are used for enhancing color images prior to computational processing. Some of the pre-processing techniques which can be used to pre-process the images are Noise Removal, Image Smoothing, Grayscale Conversions, Binarization and Normalization.

3.3 Data Analysis

After pre-processing both spectral and spatial data, next step is to perform analysis on this data. Again we will perform analysis on both spectral and spatial data.

3.3.1 Spectral Analysis

Spectral analysis is helpful to determine different components which are present in the sample, their concentration and distribution. This analysis can be performed by evaluating either single wavelength intensity, multi-wavelengths intensities or integrated intensity.

3.3.2 Image Analysis

Image Analysis is required to process images to get useful information like depiction of component composition and distribution of the medicines. Different image processing techniques are available to extract these features such as Edge Detection, Segmentation, Histograms, Image Fusion, Image Morphology, Arithmetic Operators, Convolution and Image Statistics.

Table 1: Comparison of Various Quality Assessment Techniques for Drugs

Technique	Dosage Forms	Applications	Spectral Information	Spatial Information	Sample Preparation	Destructive/ Non-Destructive	Time Consumption	Complexity	Cost	Disadvantage
HPLC	Solids	Raw ingredients and final drug testing	No	No	Yes	Destructive	High	Max	High	May lend to inaccurate compound categorization
Mass Spectrometry	Solids	Detection of low quantities in compounds	No	No	Yes	Destructive	High	Moderate	High	Inability to discriminate between enantiomers, most diastereomers, and salt forms of drugs
RGB Imaging	Solids	Image enhancement	No	yes	No	Non Destructive	Low	Min	Low	Sensitivity depends on detector device
XRD	Semi-solids	Crystallinity measurement, amount of API determination	Yes	No	Little/ No	Semi Destructive	High	Max	High	Cannot examine solutions and non-crystalline drug forms
SEM	Solids, Semi-solids	Determine particle morphology and size distribution	No	Yes	Yes	Semi Destructive	Medium	Moderate	High	Characterize only small area of a tablet
NMR	Liquids, Solid (Limited)	Crystallinity measurement, API and excipients interaction investigation	Yes	No	Little/ No	Semi Destructive	Medium	Moderate	High	More suitable for liquid drugs.
NIRS	Solids	Monitoring final quality of drugs, identification of organic compounds and counterfeit drug, quantitative measurement of API	Yes	No	No	Non Destructive	Low	Min	Low	Low structural selectivity

Table 1: Comparison of Various Quality Assessment Techniques for Drugs (Contd.)

Technique	Dosage Forms	Applications	Spectral Information	Spatial Information	Sample Preparation	Destructive/ Non-Destructive	Time Consumption	Complexity	Cost	Disadvantage
RS	Solids	Crystallinity measurement, determination of multi-components, Analysis of Polymorphic forms	Yes	No	No	Non-Destructive	Low	Min	Medium	Not suitable for Moisture analysis
MSI	Solids, Liquids	Tablet identification / composition determination, surface analysis	Yes	Yes	No	Non-Destructive	Low	Min	Low	--
HSI	Solids, Liquids	Distribution and Identification of counterfeit, contaminated and minor components of drugs, surface analysis	Yes	Yes	No	Non-Destructive	High	Max	High	Require large data storage

Table 2: Comparison of Different Medicines Analysis Techniques

Reference	Technique	Dosage Form	Type of Processing	Sample Preparation	Features		Pre-processing	Feature Reduction	Segmentation/ Image processing techniques	Classification	Software
					Spectral	Spatial					
(Ramirez, et al., 2001)	NIRS	Tablets	Whole tablet uniform content checking	Yes	Yes	No	Mean Centering	No	No	Partial Least Square - I	PLS-IQ
(Laitinen, et al., 2003)	Mono-chromatography	Solids (Powder, Granules)	Particle size characterization	Yes	No	Yes	No	No	Noise Reduction, Binarization, Gray scale Difference Matrix	Partial Least Squares	-
(O'Connell, et al., 2005)	Raman Spectroscopy	Solids	Target substance and excipients discrimination	No	Yes	No	No	PCA	No	Support Vector Machine, k-Nearest Neighbours, Ripper, Naïve Bayes	Unscrambler, MATLAB
(Cruz & Blanco, 2011)	NIR-CI	Tablets	Content uniformity checking	No	Yes	Yes	No	No	Histograms	Multivariate Curve Resolution, Alternating Least Squares	TS Capture, MATLAB
(Malik, et al., 2001)	MSI	Tablets	Multiple tablet simultaneous identification / composition determination	No	Yes	Yes	No	PCA	No	-	-
(Laksmana, et al., 2009)	Confocal Scanning Laser Microscopy	Solids	Coating thickness and pore distribution evaluation	No	No	Yes	No	No	Binarization, Image Contrast Enhancement	Fuzzy c-Means Cluster, Euclidean Distance Method	MATLAB
(Lopes, et al., 2009)	NIR-CI	Tablets	Composition determination	No	Yes	Yes	No	No	No	Classical Least Squares	-
(Lopes & Wolff, 2009)	NIR-CI	Tablets	Tablet classification / sourcing	No	Yes	Yes	Multiplicative Scatter Correction, Standard Normal Variate, Savitzky-Golay Derivative	PCA	Histograms	k-Means Clustering	ISys, MATLAB
(Ravn, et al., 2008)	NIR-CI	Solids	Chemical Image generation	No	Yes	Yes	Savitzky–Golay Derivatives, Standard Normale Variate, Multiplicative Scatter Correction	No	Noise Removal	Partial Least Squares -I, Classical Least Squares	MATLAB, PLS Toolbox
(Puchert, et al., 2010)	NIR-CI	Tablets	Counterfeit tablet identification	Yes	Yes	Yes	No	PCA	No	Partial Least Squares	Unscrambler

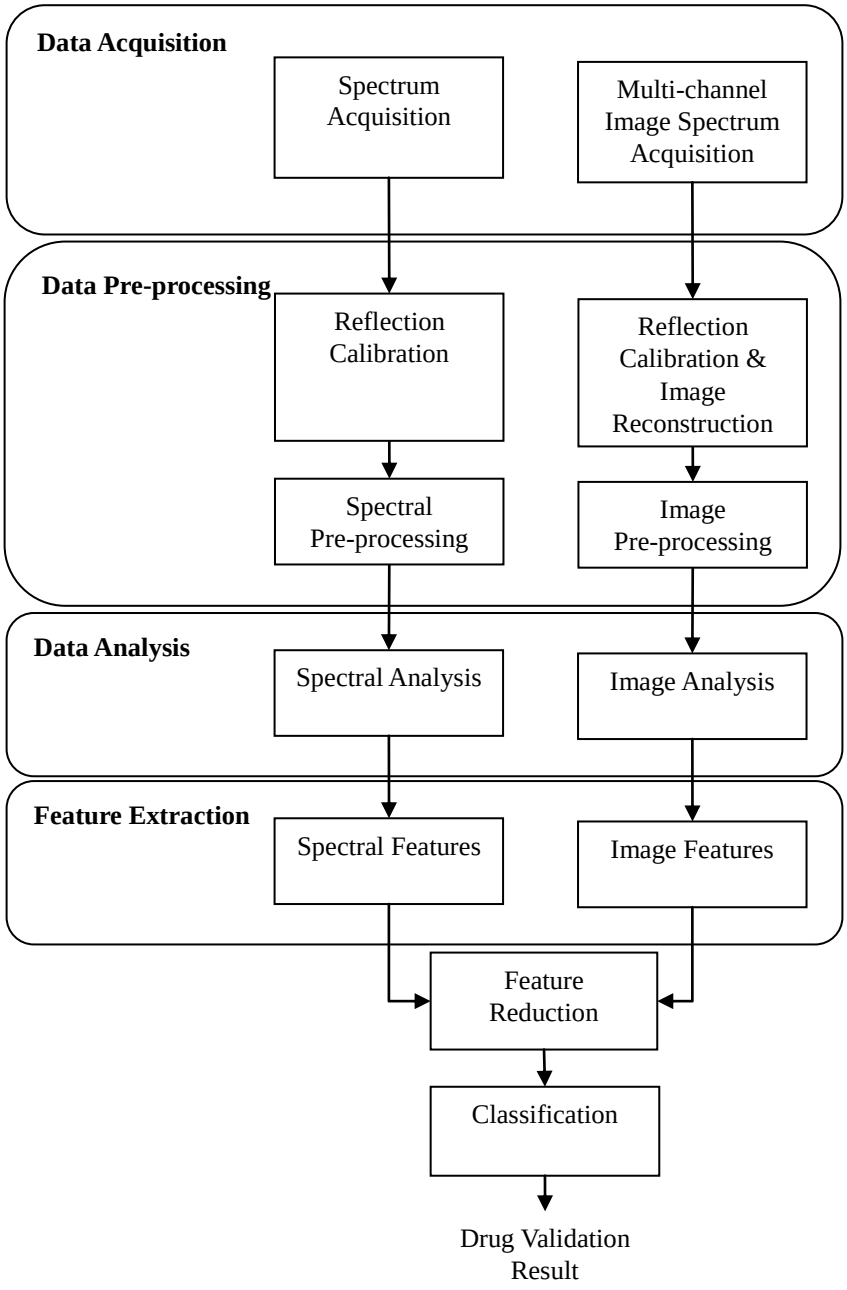

Figure 1: Flow Diagram of the Proposed Methodology

3.4 Feature Extraction

Spectral and Image analysis results in multiple features extracted from the spatial and spectral data of the sample medicine. These features represent the physical structure (like hardness, shape, size smoothness etc.) of the medicine or its chemical compositions. These features will be further used for the classification purpose.

3.5 Feature Reduction

After spectral analysis and feature extraction, it is necessary to reduce the number of available feature, by keeping only those that have maximum variation in their data and discarding all other ones. This can be performed by using multivariate chemometric method PCA (Cullen, et al., 2012). Some other commonly used techniques for feature reduction are kernel PCA, Locally Linear Embedding (LLE), Maximum Variance

Unfolding (MVU), Classical Multidimensional Scaling (CMS) and Canonical Correlation Analysis (CCA).

3.6 Classification

For analysis and comparison, spectrums of samples are compared with reference spectrums from the external library. Different similarity measures can be used like (Van der Meer, 2006) Pearson's Correlation Coefficient, Euclidean Distance and Spectral Angles.

Clustering and Classification is required for the identification of regions having similar spectral characteristics, which provide information about chemical and physical properties of the sample, their concentration and distribution. Clustering can be done by using unsupervised classification techniques, such as K-mean Clustering, Fuzzy Clustering and Support Vector Machines (SVM).

These methods do not require any prior knowledge about the sample being tested and helpful for the extraction of important features. Some other supervised techniques can also be used for classification purposes. These methods require prior knowledge about the data. They use training and testing datasets for classification. Supervised Classification methods are Partial Least Squares (PLS), Linear Discriminant Analysis (LDA), Fishers Discriminant Analysis (FDA) and Artificial Neural Networks (ANN).

The classification phase provides results about the validation of solid drug, i.e. either it is a genuine drug or a substandard drug.

4. Conclusion

The proposed method is helpful for formulating a nondestructive method for quality validation of the solid dosage forms of medicines requiring no sample preparation using MSI, Image processing and different pattern recognition techniques. The research is a contribution to the pharmaceutical industry and is beneficial for quick, cost effective and nondestructive quality assessment of the end products.

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