



Is it possible to find presence of lactose in pharmaceuticals? – Preliminary studies by ATR-FTIR spectroscopy and chemometrics



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ABSTRACT

Lactose and saccharose have the same molecular formula; however, the arrangement of their atoms is different. A major difference between lactose and saccharose with regard to digestion and processing is that it is not uncommon for individuals to be lactose intolerant (around two thirds of the population has a limited ability to digest lactose after infancy), but it is rather unlikely to be saccharose intolerant.

The pharmaceutical industry uses lactose and saccharose as inactive ingredients of drugs to help form tablets because of their excellent compressibility properties. Some patients with severe lactose intolerance may experience symptoms of many allergic reactions after taking medicine that contains this substance. People who are specifically “allergic” to lactose (not just lactose intolerant) should not use tablets containing this ingredient.

Fourier Transform Infrared (FTIR) spectroscopy has a unique chemical fingerprinting capability and plays a significant important role in the identification and characterization of analyzed samples and hence has been widely used in pharmaceutical science. However, a typical FTIR spectrum collected from tablets contains a myriad of valuable information hidden in a family of tiny peaks. Powerful multivariate spectral data processing can transform FTIR spectroscopy into an ideal tool for high volume, rapid screening and characterization of even minor tablet components.

In this paper a method for distinction between FTIR spectra collected for tablets with or without lactose is presented. The results seem to indicate that the success of identifying one component in FTIR spectra collected for pharmaceutical composition (that is tablet) is largely dependent on the choice of the chemometric technique applied.

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1. Introduction

Being disaccharides (carbohydrates) – lactose (“milk” sugar) and saccharose (“table” sugar) have the same molecular formula, however the arrangement of their atoms is different. Saccharose is made up of glucose and fructose while lactose is composed of glucose and galactose. Glucose, fructose and galactose have the same chemical formula but different chemical structures, i.e. they are isomers. Carbohydrates are one of the classes of macronutrient compounds which are required in relatively large quantities as they provide cells with energy hence they are indispensable for accurate operation of human body [1].

It is not uncommon for individuals to be lactose intolerant – around two thirds of the population has a limited ability to digest lactose after infancy, whereas being saccharose intolerant is rather unlikely. Lactose intolerance results from an inability to break into its constituent

components as a result of insufficient lactase production, which is the enzyme digesting lactose. Saccharose intolerance is caused by sucrase deficiency [2].

Consumption of lactose containing products by persons who are lactose intolerant leads to transportation of undigested product to the lower intestine which may cause many allergic reactions as well as bloating or swelling of the abdomen, pain, diarrhea, nausea, production of gas [3].

Having excellent compressibility properties, lactose and saccharose are used in the pharmaceutical industry, as inactive ingredients of the drugs to help form tablets. Lactose is also used to form a diluent powder for dry-powder inhalations. The properties of lactose that contribute to its popularity as an excipient are: cost, availability, bland taste, low hygroscopicity, compatibility with active ingredients and other excipients, excellent physical and chemical stability and water solubility. Most medications do not contain enough lactose to cause lactose intolerance having no more than 12.5 to 25 mg of this substance, what is tiny on anyone's scale [4]. One glass of milk (250 ml) contains 12,000 mg of lactose.

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However, there are four classes of people who are specifically “allergic” to lactose (not just lactose intolerant) [1–9]:

1. Those who are extremely sensitive to lactose – this group includes people at the very far end of the sensitivity curve, those who became intolerant through disease or surgery (i.e. Secondary Lactose Intolerance, SLI). People in this group often have absolutely no lactase in their systems.
2. Senior citizens and others who take many pills every day. These people often have the least lactase and the most delicate intestines.
3. People with milk allergies (including the worst, life-threatening allergies).
4. Those who object to animal-derived products, such as the millions of vegans, Orthodox Jews or others with religious or ethical reasons to avoid any pills containing lactose, even if they do not get specific lactose intolerance symptoms.

People hypersensitive to various products avoid them intentionally so that not to expose their body to unwanted allergic reactions. Pharmacists perform various roles to ensure prime health outcomes for their patients. Since pharmacists know about the mode of action of a particular drug, its ingredients, and its metabolism and physiological effects on the human body in great details, they play an important role in optimization of a drug treatment for an individual; while dispensing prescription drug they have a duty to ask patient about any known allergies.

Pharmacists' knowledge relies on patient information leaflets (package insert) containing specific data about tablet including its composition (API – active pharmaceutical ingredient and all excipients). As lactose can be potential source of allergic symptoms, its presence should be also clearly marked.

Spectroscopic methods have proved to be very efficient since they allow for samples characterization by direct measurement of substances in the solid state. They involve straightforward operational steps, and deliver reliable, fast results. FTIR spectroscopy possessing a unique chemical fingerprinting capability plays an important role in providing data on identifying and characterizing analyzed samples and hence has been widely used in pharmaceutical science [10–15].

FTIR spectroscopy can be considered as a detection technique with many desirable features, such as being non-destructive and requiring no/minimal sample preparation while offering results with high chemical uniqueness. FTIR is capable of determining several sample components in a single measurement without needing physical separation. Infrared absorption causes changes in the vibrational and rotational state of molecules; the absorption frequency depends on the molecular vibrational frequency whereas absorption intensity depends on effective energy transfer to the molecule, which in turn depends on any change in the dipole moment occurring due to molecular vibration. Thus a molecule only absorbs infrared light if absorption changes the dipole moment. FTIR spectroscopy measures the vibrations of covalent chemical bonds, creating a ‘spectral fingerprint’. This result can be used to identify and quantify all ingredients present in a sample [16,17].

Typical FTIR spectrum collected from tablets contains a myriad of valuable information hidden in a family of tiny peaks. FTIR characterization is complex mainly because of the high degree of overlap of infrared absorption bands. Only powerful multivariate data analysis can transform FTIR spectroscopy to an ideal tool for high volume, rapid screening and characterization of minor components in tablets. The development of statistical procedures for the fast identification and detection of all pill ingredients is an essential stage in data processing. Multivariate analysis is a powerful tool when dealing with multi-component systems and offers a valuable methodology for extracting significant differences from complicated datasets [18,19].

Further developments in FTIR interferometer architecture suggest that simpler and more compact implementations of these types of sample analysis systems will be available in the near future. New spectrometers as well as new accessories for sampling will significantly contribute to extend the application fields of the infrared techniques. Very

soon FTIR spectrometers will be made as compact, relatively inexpensive and user-independent devices, making the technology available for wider group of users. Due to future advances in instrumentation and data processing techniques, infrared techniques will be invaluable tools for people – helping them, for example, to control the quality and content of consuming pills, easily discriminate not only original and illegal products but also drugs containing lactose.

In this paper a method for distinction between FTIR spectra collected for tablets with or without lactose is presented. Experiments were performed on Furazidin which is a medicine used to treat acute and chronic urinary tract infections and in long-term prevention of the recurrence of these pathological states [20]. The tablets are produced by various companies which use lactose or saccharose as a one of the excipients. The choice of this pharmaceutical was imposed by the fact that plenty of patients taking this drug complained on symptoms related to lactose intolerance even if they selected lactose-free pills (according to patients' leaflet information).

Tablets were measured mainly through placing them in direct contact with the crystal surface to collect spectroscopic data – in an attenuated total reflectance (ATR) mode. As success of identification of minor substance in FTIR spectrum collected for a mixture is largely dependent on the chemometric techniques applied for optimal data evaluation, data analysis was made by employing hierarchical cluster analysis (HCA) and principal component analysis (PCA) to ensure objective interpretation of the results [21]. Described procedure proves that FTIR coupled with chemometric methods is a prevailing technique which can be used when specific analytical questions have to be answered in a non-destructive manner. Discussed in this paper work can be valuable for people allergic to lactose, as it shows the power of method for finding dangerous from their perspective substance in the tablets what can highly assist in leading the daily lives without unpleasant symptoms.

2. Experimental

Furazidin available in Poland is produced by three pharmaceutical companies: Adamet (tablets brand names: Furaginum and uroFuraginum), TEVA Pharmaceuticals (Furaginum and neoFuraginum) and US Pharmacia (FuragiActive); each pill contains 50 mg of Furazidin as API. As is stated in the patients' leaflet information, lactose is present in TEVA and US Pharmacia products. Tablets for analysis were purchased from pharmacies in various cities within Poland in order to assure collection of pills from different batches. Pure grade lactose and saccharose were purchased from Sigma Aldrich company.

Initially, samples for experiments were prepared as thin transparent pellets (diameter 13 mm, thickness <1 mm) being a mixture of small amount (<1 mg) of analyzed substance and KBr (circa 100 mg). The mixtures were first ground into a fine powder, then placed in a standard evacuable pellet press and compressed with a clamping force of 80 kN in a hydraulic press. Taking into account the fact that testing pharmaceuticals should be very fast and at the same time highly reliable – another FTIR sampling method – ATR mode (where there is no requirement for samples to be added in KBr pellets or to undergo any pretreatment) was employed in this study. In our experiments we worked with MIRacle device (produced by PIKE) – a universal sampling accessory which is designed for both single- or multi-reflection ATR and specular reflectance measurements. Single-reflection ATR ZnSe crystal (1.8 mm diameter, cut-off wavenumber at 520 cm⁻¹) was used during experiments. The ATR crystal material, which is also called an internal reflective element, was chosen according to the sample physical properties such as pH and hardness, the spectral region of interest, and the refractive indices of both sample and crystal. The ATR method requires the sample to be in intimate contact with the sampling area. Coupled to MIRacle digital readout a high-pressure clamp enabled reproducible sampling by applying the same pressure to all samples. In order to exclude any possible contamination occurring from one sample to another, special

care was paid to cleaning the face of the crystal and then performing a background scan prior to a new sample spectrum collection.

In total 150 tablets without external coatings were examined (each group mentioned earlier was represented by 30 tablets), every tablet was sampled fourfold. FTIR spectra were collected using a liquid nitrogen-cooled MCT (mercury cadmium telluride) detector consisting of 137 co-added scans which were measured at a spectral resolution of 4 cm^{-1} in the wavenumber range $4000\text{--}520\text{ cm}^{-1}$. The scanner velocity was set to 20 kHz. Interferograms were Fourier transformed with a Blackman-Harris 3-term apodization and a zero filling factor of 4. Spectral data were acquired with OPUS 6.0 software [22] (provided by the spectrometer manufacturer). For ATR mode a single channel spectrum of the ZnSe crystal was taken as a background; for transmission mode background was collected with no sample in the path of IR radiation. All FTIR spectra were divided by the background spectrum to provide a ratio of the transmittance (T) output. All spectra were presented in absorbance units ($A = -\log T$) as a function of wavenumber for transmission experiments and in ATR units for ATR mode experiments.

FTIR analyses of samples were conducted at the Singapore Synchrotron Light Source (SSLS) at the Infrared Spectro/Microscopy beamline (ISMI) [23] equipped with Bruker IFS 66v/S FTIR spectrometer coupled to a Hyperion 2000 (Bruker Optik, Ettlingen, Germany) IR microscope. An internal Globar source can be used as a source of radiation, as well as IR synchrotron radiation (SR). It is known that synchrotron based source offers a versatile infrared source that covers a spectral range of $10,000$ to 10 cm^{-1} . It delivers a considerable brightness advantage over a conventional thermal IR source, which enables measurements with high resolution and studies of extremely small samples which provides a new dimension in both qualitative and quantitative analyses. Furthermore, synchrotron IR light is not destructive since it does not break any bonds or change inherent chemical formulae.

In case of tablets analyzed in ATR mode using MIRacle, SR does not offer any additional advantages – FTIR spectra collected by using either SR or Globar source have similar signal-to-noise ratio (aperture size was set to 3 mm), therefore in our experiments all measurements were performed with the laboratory source.

As highly complex FTIR spectra collected for pharmaceuticals are difficult to accurately ascribe, in spite of up-to-date computer-searchable databases of spectra being currently available, HCA and PCA as most popular multivariate techniques were employed for spectra evaluation to ensure objective interpretation of the results.

HCA and PCA are examples of unsupervised classification, where spectra are classified into groups without a prior knowledge and no assumptions made as to the distribution of variables [24–26].

HCA is a method for dividing a group of objects into classes such that similar objects are placed in the same class. The HCA method searches for objects which are close in variable space. Several methods for searching clusters exists, one of them considers each object as a size one cluster (agglomerative approach) and compares the distances between clusters. Two points which are closest to each other are then joined, forming a new cluster. This procedure is repeated until all points are grouped together. There are various ways of computing the distance between two objects (spectra in our case) – the most popular are Euclidean and Mahalanobis distances. Two clusters containing more than one member are combined using linkage criterium. Some commonly used criteria are single-, complete- or mean-linkage clustering. In case of clustering spectral datasets Ward's algorithm is often used that decrease the variance for clusters being merged. The results of such hierarchical clustering are usually presented as dendrograms.

PCA simultaneously considers correlation among many variables, allowing extraction and visualization of a large body of information. The dataset, composed by samples (rows) and variables (columns) is decomposed in two new sets of data, which are named scores and loadings. Loading plots reveal the covariance among variables and enable the identification of the most relevant variables for variance explanation in the retained components. Score plots reveal any patterns in the data, including clusters, trends and outliers. Each point represents one spectrum (in new coordinates named principal components (PCs)); those points which are closely located denote similar spectra, i.e., samples with similar characteristics, while distant points suggest different types of spectra. Scores and loadings contain information about samples and variables respectively, and by analyzing both types of information together the dataset is visualized in a smaller dimension.

For the evaluation of spectral data an open source R programming environment for statistical analysis, visualization and modelling was used. R is highly extensible through the use of user-submitted packages for specific functions or specific areas of study [27]. In our case hyperSpec, ggplot2 and reshape libraries were also loaded to analyze and visualize the results [28,29].

3. Results and discussions

FTIR spectroscopy detects functional groups and identifies chemical bonds in a molecule by recording an infrared absorption spectrum. All FTIR spectra comprise different peaks as a function of wavenumber [cm^{-1}], producing a sample profile as a distinctive, molecular fingerprint which can be used to screen and scan samples for many different components. As was mentioned in the introduction lactose and saccharose have

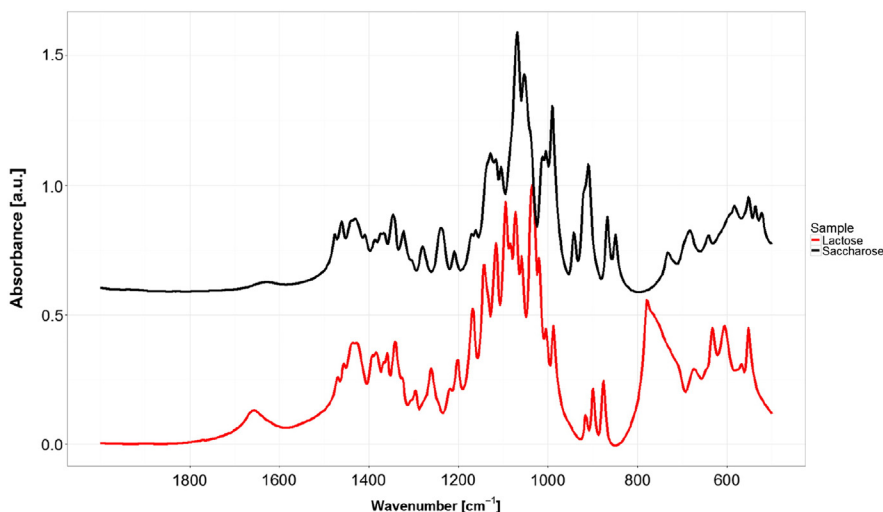


Fig. 1. Comparison of FTIR spectra collected in transmission mode for lactose and saccharose. Samples were mixed with KBr and prepared as thin pellets.

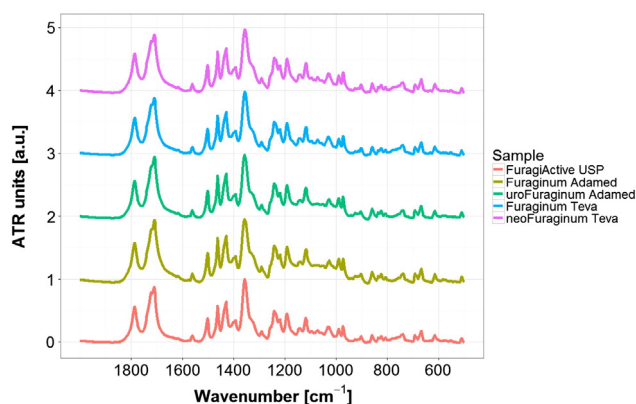


Fig. 2. The mean FTIR spectra for Furazidin tablets produced by Adamet (Furaginum Adamet and uroFuraginum Adamet), TEVA Pharmaceuticals (Furaginum Teva and neoFuraginum Teva) and US Pharmacia (FuragiActive) collected in ATR mode. Subsequent mean spectra are Y-axis shifted by one ATR unit for the clarity of presentation.

the same molecular formula however the arrangement of their atoms is different. As is depicted in Fig. 1 FTIR spectra (only fingerprint region is presented $2000\text{--}520\text{ cm}^{-1}$) for both substances differ significantly, based on this fact some lines characteristic only for lactose will be employed in further analysis of Furazidin tablets.

As with almost all available pharmaceuticals, Furazidin tablets are too thick to allow high quality transmission analysis as the IR beam needs to pass through the sample. That is why samples were prepared as thin pellets – Furazidin was mixed with KBr to undergo experiments in transmission mode. However, using the MIRacle device expedited the procedure – the total ATR analysis time for a thick tablet was <1 min. The only prerequisite is to place the tablet on the ATR crystal and apply pressure.

ATR spectra collected for Furazidin tablets are similar to FTIR spectra collected in transmission mode, however their careful comparison shows that the intensities of the spectral features in ATR spectrum exhibit lower absorbance than those in an absorbance spectrum, especially at high wavenumbers. This is why all ATR spectra were processed with the ATR correction available in OPUS software [22]. The intensity of the ATR spectrum is related to the penetration depth of the evanescent wave in the sample, which depends on the refractive indices of the crystal and the sample and ranges from 0.5 up to $5\text{ }\mu\text{m}$. The relatively

thin penetration depth of the IR beam is the main benefit of ATR sampling.

Fig. 2 presents the mean FTIR spectra collected for five analyzed groups of Furazidin tablets produced by Adamet (Furaginum and uroFuraginum), TEVA Pharmaceuticals (Furaginum and neoFuraginum) and US Pharmacia (FuragiActive). Since all measured pills contain the same API and have very similar excipients (excluding presence of lactose or saccharose), hence alike spectral images have been noticed.

A large number of principal absorption peaks within the fingerprint region of FTIR spectra collected for Furazidin significantly masks the tiny peaks belonging to lactose in TEVA and US Pharmacia products. As can be seen in Fig. 2 the mean spectra typical for five analyzed groups provide little basis for differentiating them on a molecular level.

The main goal in our analysis was finding the answer to the question: whether it is possible to distinguish tablets containing lactose based on tablets complex FTIR spectra. To fulfill this task, statistical procedures HCA and PCA were employed in analysis. Since theoretical and experimental evidence has shown that variable selection can improve multivariate models [24], in this study we mainly focused on the fingerprint region ranging from 2000 to 520 cm^{-1} . In this spectral region primary differences in various compounds are highlighted and characteristic groups show absorption. By selecting this region additionally, we eliminated spectral regions that do not contain information which is directly correlated with the property of interest (e.g. noise or non-linearities), interferences and potential variables that generate a lower signal to noise ratio (indicative of low sensitivity).

Prior to multivariate analysis approach, FTIR spectra were additionally preprocessed. The baseline of each spectrum was corrected using a polynomial fitting function (`spc.fit.poly.below`) from the `hyperSpec` package which iteratively calculates supporting points for the baseline polynomials. After that, normalization of each spectrum was done in order to correct multiplicative scaling effects and gave the same weight to each sample to contribute significantly in the variance. In our case a vector normalization, also called 2-norm, was used. Each mean-centered spectrum was divided by the square root of the sum of the mean-centered intensities squared, so that a spectrum was obtained in which the sum of all intensity values squared is equal to one.

The preprocessed spectra were subjected to HCA using the Euclidean distance and Ward's method for aggregation to evaluate the similarity among FTIR spectra collected for Furazidin, lactose and saccharose samples.

Fig. 3 shows the dendrogram obtained by HCA with color marked group memberships showing evident separation into three major

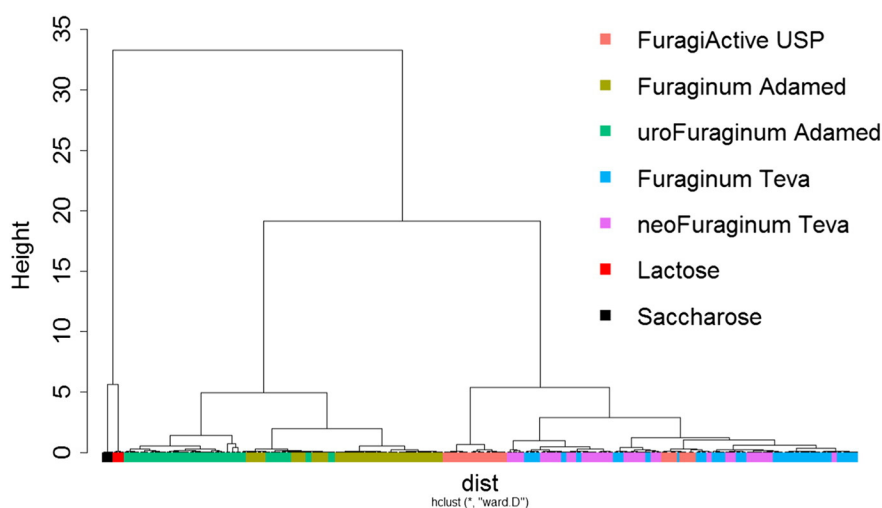


Fig. 3. HCA on FTIR spectra of Furazidin, lactose and saccharose samples. The vertical axis represents the measure of similarity between FTIR spectra taken into analysis. Leaves (single spectrum entries) were color-coded according to key legend presented on the right side. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

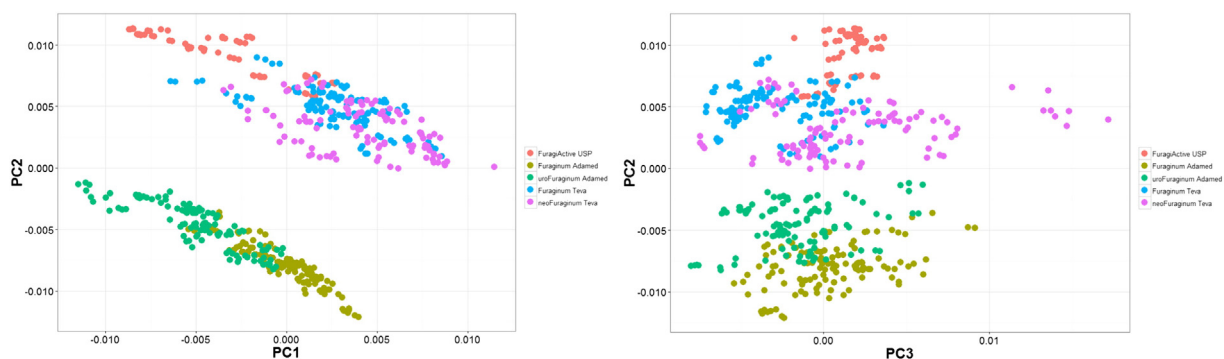


Fig. 4. PC1 vs PC2 (a) and PC2 vs PC3 (b) obtained as a result of PCA done on first derivatives of FTIR spectra collected for Furazidin tablets produced by Adamet, TEVA and US Pharmacia.

groups (at height level equals 15) corresponding to 1. lactose and saccharose, 2. Adamet and 3. TEVA and US Pharmacia products.

FTIR spectrum of commercial tablets contains combined information about mixture of API and excipients. Two separate clusters for Adamet and Teva - US Pharmacia products may point for differences among tablets excipients for these manufacturers, as the API used by these companies is the same. Indeed, Adamet tablets contain saccharose whereas TEVA - US Pharmacia products have lactose.

Additionally, as it was mentioned earlier, both Adamet and TEVA produce two types of tablets: Furaginum and uroFuraginum (Adamet) and Furaginum and neoFuraginum (TEVA). However, as can be seen in Fig. 3, FTIR spectra collected for these tablets are not grouped in single clusters, it may indicate that even if the tablets have different brand names – the same producer use the same constituents for the tablet preparation.

As is presented in Fig. 2 the mean FTIR spectra collected for all Furazidin samples produced by three companies are very comparable; so this is why HCA method “sees” their similarity and link them first in separate clusters but at the height 20 they are treated as one single group. According to HCA, FTIR spectra collected for lactose and saccharose are more similar to each other in terms of Euclidean distance than to any spectrum measured for Furazidin.

So, our initial question has not been addressed adequately yet: HCA correctly divided analyzed FTIR spectra according to the differences in the excipients, but based on this analysis it is impossible to conclude which group contains lactose.

Additional investigation was carried out using PCA. As was mentioned earlier PCA is a data-reduction technique, useful for obtaining a limited number of variables containing the most relevant information for the proper distinction of the spectra. PCA was performed on first derivatives of FTIR spectra to emphasize even the tiny spectral differences between the spectra. The screeplot was performed in order to check the number of significant principal components. The order of the PCs denotes their importance to the dataset. PC1 describes the highest amount of variation, PC2 the second highest, and so on. Examination of the cumulative variance plot revealed that three first PCs explained 99.6% of system variance that is why only three PCs were retained for further analysis.

As shown in Fig. 4 a and b, based on scatter plots PC1 vs PC2 and PC2 vs PC3 all FTIR spectra were divided into two big groups; one group contains spectra collected for Adamet products and the latter one spectra measured for tablets from TEVA and US Pharmacia. In our study, PC2 is the main component which differentiates clearly these two groups. Projections on PC1 and PC3 axis give little or no discrimination among products from three companies.

Apart from differentiation and classification, the main potential of PCA is the possibility to collect information regarding the basis for discrimination among analyzed spectra from the loadings corresponding to each PC. A strong representation of the spectral variability can be seen. Moreover, these loadings can be compared to the original FTIR spectra. PCA can thus provide information on molecular composition or the underlying biochemical differences of the datasets. As was

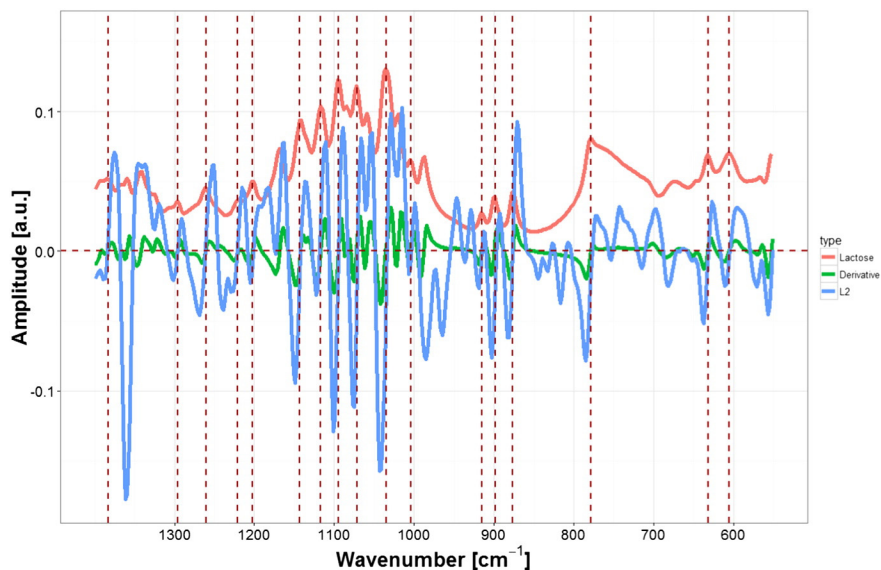


Fig. 5. Plot of the loading for PC2 (L2) compared with the mean FTIR spectrum collected for lactose (Lactose) and its first derivative (Derivative). Vertical dash lines point the wavenumbers for which L2 curve and first derivative of the mean FTIR spectrum collected for lactose is equal 0 and change the sign from – to + what indicates the peaks in FTIR spectrum for lactose.

Table 1

Bands characteristic for lactose found in loading 2 (ν -stretching, δ -deformation, as-antisymmetric) [30–32].

Wavenumber [cm^{-1}]	Bands assignment
1383	$\delta\text{OCH} + \delta\text{COH} + \delta\text{CCH}$
1296	$\delta\text{CH} + \delta\text{OH}$ in plane
1260	δOH in plane; δCCO
1221	$\delta\text{CH} + \delta\text{OH}$ in plane
1202	$\delta\text{CH} + \delta\text{OH}$ in plane
1142	$\nu\text{CO} + \nu\text{CC}$
1117	νCO
1095	νCO
1071	νCO
1035	νCO
915	$\nu\text{CO} + \nu\text{CCH} + \nu_{\text{as}}$
898	δCH
877	δOCC
778	$\delta\text{CCO} + \delta\text{CCH}$
632	νCH_2
605	δCCO

mentioned earlier all Furazidin products are mainly discriminated by PC2 that is why the second loading (L2) has been analyzed. To understand the information contained in L2 a simple evaluation presented in Fig. 5 was made – the loading of PC2 is compared to first derivative of the mean FTIR spectrum collected for lactose (as PCA was done on first derivatives of FTIR spectra collected for Furazidin samples). It is known that relative extrema occur where first derivative ($f'(x)$) changes sign, maxima for $f(x)$ are observed when first derivative changes sign from $+$ ($f'(x) > 0$) to $-$ ($f'(x) < 0$). As we follow convention used in FTIR spectroscopy in presenting the wavenumbers (X-axis) in reverse order (from 2000 to 520 cm^{-1}), maxima corresponding to the peaks within FTIR spectrum for lactose will be found for $f'(x) = 0$ when the sign of the first derivative is changed from $-$ to $+$. For better visualization of described procedure additionally in Fig. 5 the mean FTIR spectrum for lactose is presented. Vertical dash lines point the wavenumbers for which L2 curve and first derivative of the mean FTIR spectrum collected for lactose are equal 0 and change the sign from $-$ to $+$ what indicates the peaks in the original FTIR spectrum for lactose.

Lactose is present in TEVA and US Pharmacia products thus hint about its characteristic lines is expected in features existing in L2. As can be seen in Fig. 5 curve for L2 is quite complex as is not derived only from the individual component. However, most of the lines characteristic for lactose can be recognized in this spectrum based on described

earlier procedure (especially within the region $1400\text{--}520 \text{ cm}^{-1}$); Table 1 presents positions and assignments of these lines [30–32]. As Adamet products contain saccharose is very likely that in L2 hint about some lines typical for saccharose can be found, but identifying them was not a goal of our work.

Based on obtained results we decided to perform once again PCA analysis but this time additionally first derivatives of FTIR spectra for lactose and saccharose were included into investigated dataset.

As can be seen in Fig. 6 value of PC1 is exactly the same for lactose and saccharose what can explain why this component does not differentiate among Adamet, TEVA and US Pharmacia products. Lactose and saccharose are separated by PC2. It is clearly visible that distances between lactose and TEVA and US Pharmacia products (Furaginum, neoFuraginum and FuragiActive) in PC2 projection are smaller than distances between Adamet products (Furaginum and uroFuraginum) and lactose. In these coordinates saccharose “lies” closer to Adamet products.

This analysis proved the fact that indeed lactose is present in TEVA and US Pharmacia products whereas saccharose can be found in Adamet tablets.

4. Conclusions

ATR is a popular sampling technique which almost eliminates the need for sample preparation for most solids, liquids, and gels. This contrasts traditional FTIR sampling by transmission measurements where the sample must be diluted with IR transparent salt, pressed into a pellet or into a thin film, prior to analysis to prevent IR bands being totally absorbed. As was shown, using ATR mode for testing tablets ensures very smooth and fast analysis.

Despite the simplicity of conducting experiments, analysis of pharmaceutical products is a rather challenging task, as collected FTIR spectra contain information not only about API but also about all excipients used for tablets production. Simple comparison of FTIR spectra collected for Adamet, US Pharmacia and TEVA products (Fig. 2) has not provided information regarding the real differences among them, and has not answered the question whether it is possible to detect the presence of lactose manifested as rather tiny peaks within the plenty of bands present in FTIR spectra taken for TEVA and US Pharmacia tablets. Although FTIR spectroscopy is a powerful tool for the examination of chemical compounds with a great level of precision, analysis of the tiny peaks

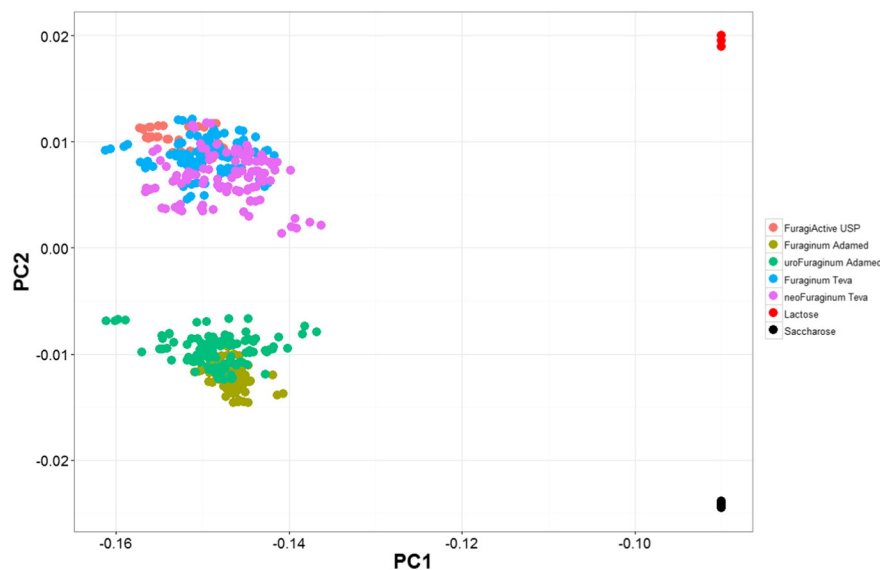


Fig. 6. PC1 vs PC2 obtained as a result of PCA done on first derivatives of FTIR spectra collected for Furazidin tablets produced by Adamet, TEVA and US Pharmacia and first derivatives of FTIR spectra for lactose and saccharose.

hidden in rich spectral signatures requires complex multivariate analytical techniques.

HCA and PCA as unsupervised statistical methods enable (by aggregation and dimension reduction) the graphical display of a whole dataset thus increasing the understanding of the set, indicating the presence or absence of natural groupings among samples. Additionally, PCA generate new variables (PC's) that carry most of the variability of the spectral dataset.

Analysis of the loadings obtained for PCA provided information about the source of variability within a dataset, as derived from variations in the molecular components which contribute to each spectra. In other words, the loading of PC as identified by the PCA method sheds further light on the spectral basis for the differentiation of analyzed groups. As was presented in this work PC2 simply points for differences between TEVA, US Pharmacia and Adamet products but detailed analysis of L2 curve reveals some specific features for molecular characterization of lactose (Table 1). Additionally, PCA repeated on dataset that includes also first derivatives of FTIR spectra of pure lactose and saccharose shows that lactose is present in TEVA and US Pharmacia products.

Our study presented in this paper seems to prove the fact that FTIR spectroscopy combined with proper statistical analysis – in our case PCA – could be used as unique tool for differentiation the medications containing the same API but different carbohydrates (lactose or saccharose) used to form the tablets.

Further development of FTIR portable spectrometers may provide in very near future easy access to these devices enabling in this way instant analysis of tablets. For this purpose, however adequate preprocessing and statistical procedures, as described in this paper, will be required.

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Appendix A. Supplementary data.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.saa.2016.08.003>.

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