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Fourier transform infrared spectroscopy supported by multivariate statistics in compatibility study of atenolol with excipients

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ABSTRACT

The objective of this study was to use Fourier transform infrared spectroscopy (FTIR) and multivariate statistics to investigate compatibility/incompatibility of atenolol as a representative of active pharmaceutical ingredients and excipients, such as β-cyclodextrin, methylcellulose, starch and chitosan, when used in solid dosage formulations. Two-component physical mixtures consisting of atenolol and selected excipients were studied by FTIR spectroscopy and two methods of multivariate statistical analysis – principal component analysis (PCA) and cluster analysis (CA), which were used as a supplementary tool for interpretation of the FTIR spectra. Taking into account variability explained by the first two principal components, the results of PCA were visualized in the form of a bi-dimensional scatterplot. A lack of interaction was confirmed by two distinct clusters created by both atenolol and a particular excipient with their mixtures. In the case of CA, lack of interaction between both ingredients was also indicated by two large clusters at a level of 33 or 66% of the maximum distance. The results of the investigations show that with the exception of β-cyclodextrin, the remaining excipients are compatible with atenolol. These findings were confirmed by complementary methods, such as differential scanning calorimetry, thermogravimetry and X-ray powder diffraction.

1. Introduction

Infrared spectroscopy (IR) is without doubt one of the most universal analytical methods available to contemporary scientists. One of the benefits of this method is that virtually any sample can be studied in any state [1]. IR spectroscopy has been extensively used in both qualitative and quantitative studies to examine the physical characteristics of solid pharmaceutical materials [1,2]. It provides information on polymorphism, crystallinity, active pharmaceutical ingredient-excipient compatibility, mixing and particle sizing and can also give physical and chemical data that might be used to monitor pharmaceutical manufacturing procedures [2].

One of the crucial problems during any drug discovery programme is the compatibility/incompatibility of active pharmaceutical ingredients (API) with excipients [3]. The term "incompatibility" refers to interactions of API with excipients or other active substances that lead to change in chemical, physical and therapeutic properties of a pharmaceutical dosage form. API interactions that occur during formulation or storage of a dosage form can be categorized as physical or chemical interactions [4,5]. In fact, potential physical and chemical interactions between APIs and excipients can affect the chemical nature, stability and bioavailability of APIs and, in consequence, their therapeutic efficacy and safety [6].

There is no universally accepted protocol to evaluate incompatibility of an API with excipients [3]. Studies are constantly being conducted into expanding the range of efficient methods to confirm the compatibility of API with excipients, and scholars are constantly finding new, more effective protocols for the detection of incompatibilities [7]. IR spectroscopy is one method that is not only focused on solid state behaviour of APIs and their formulations but that is also employed as a compatibility screening tool, since the vibrational changes serve as a probe of potential intermolecular interactions among the components. The method can also be employed to detect pharmaceutical interactions that result in desalting, hydrate formation, dehydration, polymorphic changes or transformation of crystalline to amorphous forms and *vice versa* during processing [4]. The presence of overlapping bands in the spectra may, however, hinder the analysis. Hence, IR

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spectroscopy alone cannot be used to predict the compatibility/incompatibility in a pharmaceutical mixture, since overlapping bands in the spectra of physical mixtures render the detection of changes in the vibrational bands of particular ingredients problematic. Furthermore, different functional groups in the molecule of an ingredient or extraneous molecules can absorb within the same or similar specific range of frequency, so IR spectra can give somewhat equivocal information about the potential incompatibility of API with the excipient. Consequently, to gain information that can be indirectly acquired from the IR spectra, this spectroscopic method should be used in combination with methods of multivariate statistics, such as principal component (PCA) and cluster (CA) analysis. The aim of this study was therefore to demonstrate the usefulness of two unsupervised pattern recognition methods, PCA and CA, as a tool to support the FTIR spectral analysis.

The role of multivariate statistical analysis is well known in the medical [8], pharmaceutical [9,10], food [11,12] and other sciences [13] for solving the complex relations between objects and variables in the multivariate databases. Additionally, the usefulness of PCA and CA for the detection of incompatibilities based on the spectra collected using dispersive IR spectrometer has yet to be confirmed [14]. It is nevertheless interesting to verify to what extent this multivariate approach will be useful to compatibility study using a data set acquired from the FTIR spectra. This is a crucial issue, because, regardless of the fact that FTIR spectrometer has a significant advantage over dispersive spectrometry, *e.g.* it simultaneously collects high spectral resolution data over a wide spectral range, FTIR spectra do not provide direct information about compatibility between ingredients of pharmaceutical mixtures [15]. Accordingly, the use of supplementary multivariate methods becomes a necessity. The results obtained in this work were confirmed with the aid of complementary tools, such as thermal (differential scanning calorimetry, DSC, and thermogravimetry, TG) and X-ray powder diffraction (XRPD) methods.

A screening of the literature data shows that some statistical methods have already been used for the detection of incompatibilities of ingredients in pharmaceutical mixtures. For example, the Pearson's correlation coefficients between the experimental and theoretical FTIR spectra of API and a particular excipient and its mixtures have been calculated [7,16,17]. The deviation from unity implies the probability of incompatibility in the ingredients. Thus, the Pearson's correlation coefficient can be considered as a supplementary tool in the interpretation of FTIR spectra during any examination of compatibility/incompatibility examination. Furthermore, the PCA was also used to improve the interpretation of the FTIR spectra of samples before and after heating in a DSC apparatus [18]. An optimal PCA model was built in which those samples displaying significant changes in their FTIR spectra after heating (and therefore suggesting potential incompatibilities) were grouped above the threshold line. Taking all the above into consideration, a direct and visual interpretation of the PCA scatterplot and CA dendrogram is presented in this paper as a simple and reliable tool for detection of incompatibilities from the FTIR spectra.

2. Materials and methods

2.1. Materials

Atenolol, $C_{14}H_{22}N_2O_3$, 2-{4-[2-hydroxy-3-(propan-2-ylamino)-propoxy]-phenyl}-acetamide, m.p. 153–160 °C, was a gift sample from Polpharma (Starogard Gdanski, Poland). β-Cyclodextrin, $C_{42}H_{70}O_{35}$, HPLC purity ≥99%, m.p. ~290–300 °C with decomposition, was purchased from Fluka (Siegen, Germany). Methylcellulose, purity ≥ 92.5%, was received from Shin-Etsu Chemical Co. (Tokyo, Japan). Starch, $(C_6H_{10}O_5)_n$, where n is 300–1000, pure for analysis, was obtained from POCh (Gliwice, Poland). Chitosan, 2-amino-2-deoxy-(1->4)-β-D-glucopyranan, loss on drying ≤10%, was supplied by Fluka (Siegen, Germany). Atenolol and excipients were used as obtained without further purification.

2.2. Sample preparation

Two-component physical mixtures of the ingredients were prepared in a porcelain mortar by mixing appropriate quantities of atenolol and an excipient. Atenolol to excipient mass ratios were: 9:1; 7:3; 1:1; 3:7; and 1:9.

2.3. Instrumentation

FTIR spectra of atenolol, excipients and their two-component physical mixtures were collected using a Nicolet 380 FTIR spectrometer (Thermo Fischer Scientific, Madison, USA) with a DTGS KBr detector. The spectra were recorded in triplicate using potassium bromide pellets over a range of 4000–400 cm^{-1} with spectral resolution of 0.482 cm^{-1}. Each pellet was prepared from a 1-mg sample and 100 mg of spectroscopy-grade KBr (Merck, Darmstadt, Germany) using a hydraulic press (Specac, Orpington, UK). Before each measurement, background spectra was taken with an average of 16 scans. The potassium bromide pellet was used as a background. All spectra were collected, baselines corrected if necessary, and interpreted using an OMNIC software.

DSC scans were recorded on a Mettler Toledo 822e heat-flux calorimeter (Schwerzenbach, Switzerland). Samples (atenolol, excipients and their mixtures) of approximately 5 mg were weighed into flat-bottomed aluminium pans and scans were collected at a rate of 10 °C/min over the temperature range of 20–300 °C. Nitrogen at a flow rate of 70 mL/min was used as a purging gas. The DSC scans were collected and interpreted using a STAR^e software.

TG traces were obtained using a MOM OD-103 derivatograph (Budapest, Hungary). Samples of approximately 200 mg were placed in four flat-bottomed platinum pans and heated at a rate of 5 °C/min over the range of 25–700 °C in air. As a reference material, alumina was used.

XRPD diffraction patterns were recorded on a Bruker D2 Phaser diffractometer (Karlsruhe, Germany). A CuKα tube (k = 0.154060 nm), current of 10 mA and voltage of 30 kV were used. The samples were analysed under an exposure time of 0.10 s using a step size of 0.02° over the diffraction angle range 7–55° (2θ). The diffraction patterns were plotted and interpreted using a Diffrac.suite software.

2.4. Multivariate statistics

PCA and CA calculations were performed using Statistica 10 software (StatSoft Inc., Krakow, Poland). The starting point for multivariate statistical calculations was a matrix of data acquired from the FTIR spectra of the samples under study. The dimension of the matrix was 7 × 2906, where 7 is the number of rows (atenolol, excipient and their two-component physical mixtures) and 2906 is the number of columns (absorbance values collected at a 0.482 cm^{-1} spectral resolution from the FTIR spectra of API, excipient and their mixtures). All data acquired from the raw FTIR spectra were standardized through centring transformation.

PCA is used to reduce the dimensionality of the complex multivariate data [13,19]. A new set of non-correlated variables is derived, labelled principal components (PCs), and representing a certain quantity of features of the multivariate data set. All are calculated as columns in two new matrices. A new matrix P reveals the main relations among the samples and classifies these samples