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Original Article

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Evaluation of Ampicillin- Lactose Incompatibility Reactions at Solid-State Using Physicochemical Methods

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Abstract

***Background & Aims*:** One of the significant steps in the preformulation of pharmaceutical dosage forms is to examine active pharmaceutical ingredient (API) compatibility with the excipients applied with it. Drug-excipient incompatibility affects the stability, effectiveness, safety, and quality of the final product. Therefore, it is vital, for fruitful drug production, to sort out excipients compatible with the active pharmaceutical ingredient. The aim of this study was to evaluate the compatibility of ampicillin with lactose through differential scanning calorimetry (DSC) and Fourier-transform infrared spectroscopy (FTIR) physicochemical methods.

Materials & Methods: To formulate powder samples, 300 mg ampicillin and lactose powder was discharged into a vial in a ratio of 1: 1, and 20% v/w water was added and then vortexed. Also, samples of pure drug and pure excipient were prepared by the same method. The direct compression method was applied to formulate the tablet samples. The samples were stored at 60° C in the oven and possible incompatibilities were examined for four consecutive weeks by observing the DSC thermograms and FTIR spectra.

Results: According to the results, it is recommended to eschew the formulation of ampicillin with lactose for possible incompatibilities.

Conclusion: Based on the obtained results and the changes in the main peaks of absorption of the drug-excipient mixture in the fourth week compared to the first day, there is the possibility of incompatibility in the mixture of ampicillin and lactose. Also, the observation of discoloration in the powder and tablets in the fourth week compared to the first day indicates the occurrence of a kind of incompatibility in the drug-excipient mixture.

***Keywords*:** Incompatibility, Ampicillin, Lactose, Differential scanning calorimetry, Fourier-transform infrared spectroscopy

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Introduction

Drug-excipient compatibility consideration is an essential phase in the drug preformulation to examine the effects of excipient on the active ingredient. Hence, consideration of the physical and chemical properties of the drug and excipient is significant for an efficacious formulation (1).

Excipients are substances applied, other than as active pharmaceutical ingredient (API), in pharmaceutical formulations measured innocuous in terms of safety and are used to facilitate the manufacturing process, increase stability, and bioavailability (2).

Although measured pharmacologically ineffective, excipients can contribute to the physical or chemical interactions with formulation components, consequently then affecting the efficacy and safety of the formulation (3).

The reaction of a pharmaceutical ingredient containing the amine functional group with reducing sugars such as lactose is the example of direct reactions of API and excipient. These drugs, when mixed with reducing sugars, engender products that cause discoloration (Millard reaction). This brown discoloration is the non-enzymatic reaction and can occur during the storage of the final product. Previous studies have indicated that the products of the Millard reaction have cytotoxic effects (4).

Numerous analysis methods, such as thermal analysis methods, are applied to explore this type of incompatibility. Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) are two widely applied techniques in thermal analysis methods that are used in drug-excipient compatibility studies (5).

Ever-increasing DSC studies are used for the initial and general exploration of incompatibilities, yet it is required to take advantage of other analytical techniques including XRD (X-ray Diffraction), NMR (Nuclear magnetic resonance spectroscopy), and FTIR (Fourier-transform infrared spectroscopy), etc. to confirm incompatibility (6).

Spectroscopic techniques, such as FTIR, are another method in evaluating drug-excipient interaction, which results in information about the reaction between the drug and excipient and the functional groups involved in the reaction (7). A binary mixture of drug and excipient is typically formulated in 1:1 mass ratio, with or without water, and is stored under stress conditions. By keeping the dual mixture at a high temperature for a specific time, the rate of the possible reaction of the API with the excipient and ultimately the degradation of the drug is accelerated. The stored samples are finally analyzed by the FTIR method and the presence or absence of chemical reaction between components is inspected (8).

Ampicillin ($C_{16}H_{19}N_3O_4S$) is an antibiotic grouped among penicillins (aminopenicillin) influencing a wide range of bacterial infections. It is used to treat urinary tract infections, middle ear infections, meningitis, chronic bronchitis, and infections caused by type B staphylococci, gonorrhea, invasive salmonellosis and listeria (9).

Lactose is the most common and widely used type of filler in tablet dosage forms. Lactose is a type of disaccharide consisting of two monosaccharides glucose and galactose. (10).

It is commercially accessible in crystalline and spray-dried forms. The spray-dried form, for its high flowability and good compressibility, is chiefly designed for tablet formulations, but the tendency to change color is higher in spray-dried than crystalline form. Although Millard's reaction is broadly recognized, it is still applied in a wide range of solid pharmaceutical forms in the pharmaceutical industry (11).

In this study, the compatibility of ampicillin (containing type 1 amine) with lactose excipient (a type of reducing sugar) by DSC and FTIR methods was appraised in terms of incompatibility reaction. Besides, the incompatibility of ampicillin with lactose was evaluated in tablets produced by the direct compression method.

Materials & Methods

Ampicillin trihydrate powder ((2S, 5R, 6R) -6 - [[((2R) -Aminophenylacetyl] amino] -3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptane -2-carboxylic acid) was prepared from Dana Pharmaceutical Company (Tabriz - Iran).

Lactose monohydrate and potassium bromide powder were prepared from Merck Company (Darmstadt, Germany).

DSC:

Thermal analysis of the drug-excipient in 1: 1 w/w ratio was performed by a differential scanning

calorimeter. Also, each of the samples on their own was prepared in a weight of 5 mg in an aluminum pan. A heating rate of 10 ° c / min from 25 to 300 ° C was carried out. The test result was obtained through pyris software.

FTIR:

Ampicillin and lactose were mixed in 1:1 mass ratios, and according to Serajuddin et al. 20 % (v/w) water was added to each sample (12). FTIR spectra were recorded immediately after mixing and also after being stored in an oven at predetermined time intervals, using the potassium bromide disc preparation method (Spectrum Two, Perkin Elmer, USA).

Tablet preparation:

Ampicillin tablets with lactose were also produced by direct compression method after an equal mixture of powders in a 1:1 mass ratio. The tablets were placed in an oven at 60 ° C for four weeks. The powder was prepared from the inside of the tablets and examined by the FTIR method at determined times.

Result

DSC:

The DSC method can be applied for an appropriate evaluation of the compatibility between the drug and excipient. This method also provides valuable information about the physicochemical properties of the material, such as various polymorphic forms and stability (13).

DSC thermograms of drug, excipient, and the drug-excipient mixture are shown in Figure 1.

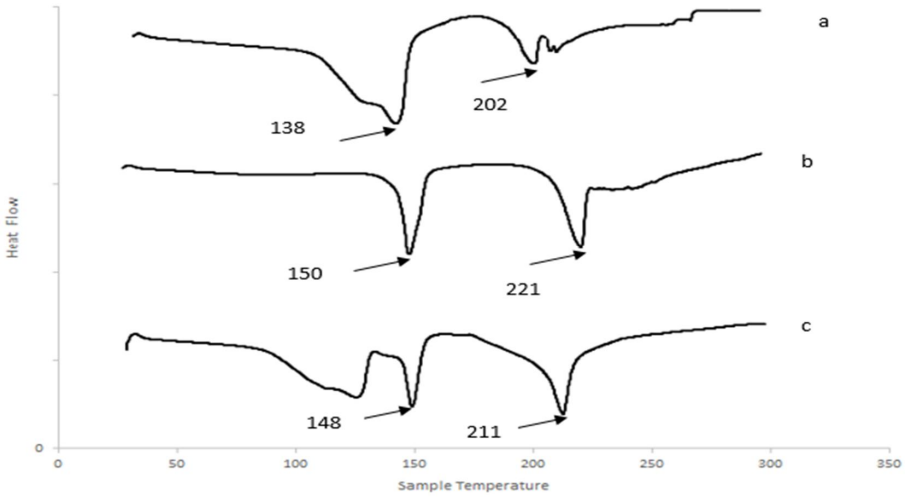

Fig. 1. DSC thermograms of ampicillin (a), lactose (b), and ampicillin-lactose (c) powder samples

According to the thermograms obtained in Figures 1a and 1b, the endothermic peaks of ampicillin at 138 and 202 °C are related to water loss and melting of ampicillin. Also, Endothermic peaks of lactose appeared at 150 and 221 ° C which concerns water loss and lactose melting. Regarding the drug-excipient mixture thermogram (1c), the endothermic peak, concerning drug melting, shifted to a higher temperature which can be due to the drug-excipient incompatibility.

In conclusion, the findings of the DSC indicate the existence of possible incompatibilities between ampicillin and lactose.

FTIR:

FTIR spectroscopy is another technique that can easily, quickly, and accurately examine possible incompatibilities between the drug and the excipient. Spectrum resulting from FTIR is explored concerning the change in position, the intensity of peaks, and

appearance and disappearance of absorption bands. The appearance of a new peak in the spectra can be considered as the interference between components.

Figures 2 and 3 indicate a comparative spectrum of the drug-excipient mixture in powder and tablet form, kept at 60 ° C for four consecutive weeks.

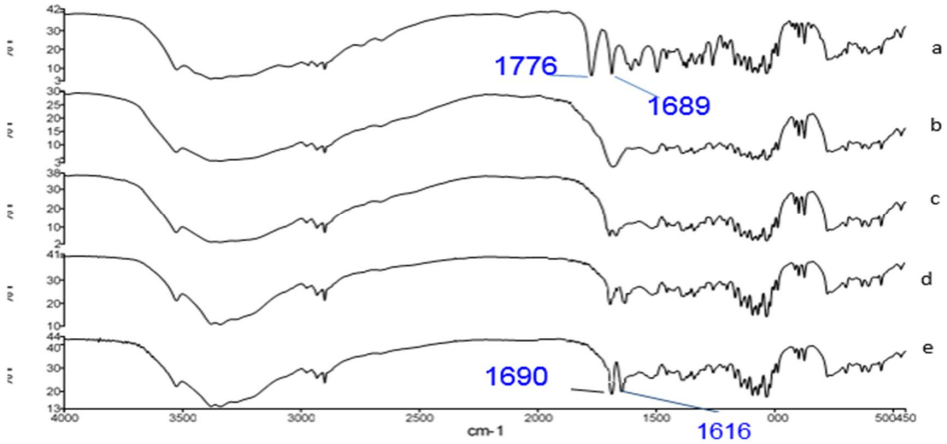

Fig. 2. Comparison of FTIR spectra of ampicillin-lactose powder sample at 60 ° C on the first day (a), first week (b), second week (c), third week (d) and fourth week (e)

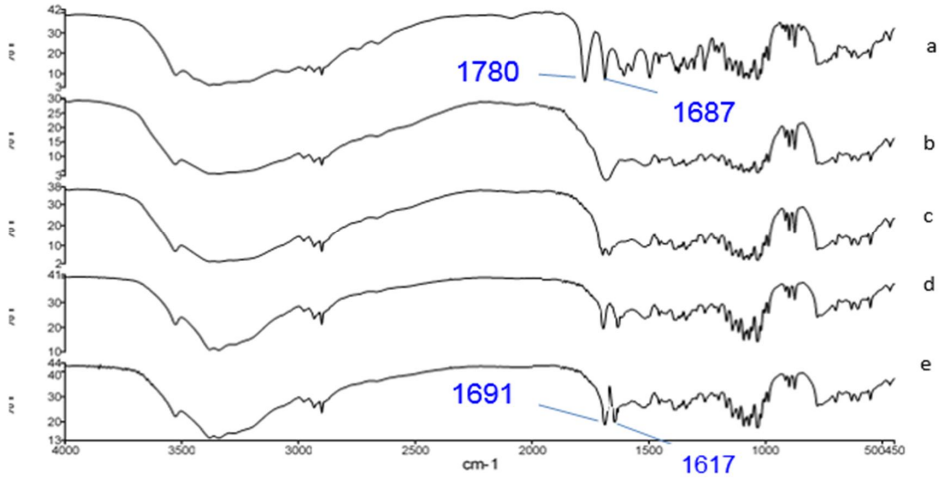

Fig. 3. Comparison of FTIR spectra of ampicillin-lactose tablets at 60 ° C on the first day (a), first week (b), second week (c), third week (d) and fourth week (e)

According to Figures 2 and 3, in the C = O band, which concerns the carboxylic group or beta-lactam, the peak is formed in both powder and tablet forms in the range of 1800-1700. Besides, the peak regarding the C=O band, which is related to the amide group, is observed in 1689 (in the powder sample) and 1687 (in the tablet sample). According to the figures in the samples of powder and tablets of the mixture, kept at 60 degrees, from the first week onwards, peaks of 1776 and 1780 have been eliminated. This peak is associated with

the beta-lactam ring of the ampicillin structure, the removal of which may indicate the halt in the beta-lactam ring following the reaction of hydroxyl group of lactose with the amino part of ampicillin.

Furthermore, in the range of 1500 to 1700 in the samples of ampicillin-lactose powder and tablets, from the first week onwards, a new peak was formed in the range of 1616. Given that 1616 and 1690 peaks in the powder sample and 1617 and 1691 peaks in the tablet sample are formed in the fourth week, these peaks probably indicate the formation of C = N and C = O bands, respectively (14, 15), which can be a reason for the formation of Schiff base. It is followed by the formation of a ketone structure in the amorphous rearrangement, which collectively justifies the Millard reaction.

Changes in appearance:

The changes in the appearance and color of the powder and tablet samples during their storage under stress conditions are shown in Figures 4 and 5, respectively.

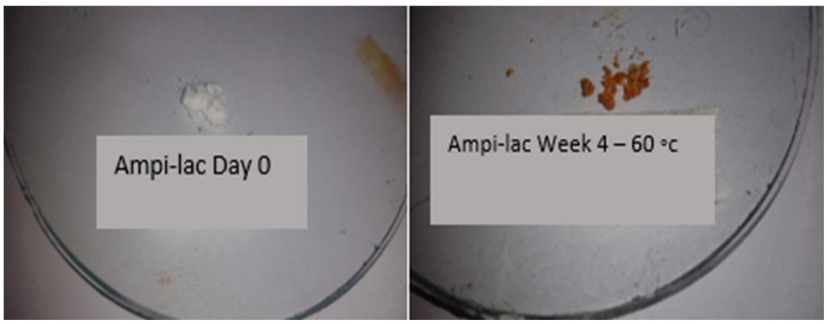

Fig. 4. Color changes of ampicillin-lactose powder from the first day to the fourth week at 60 ° C

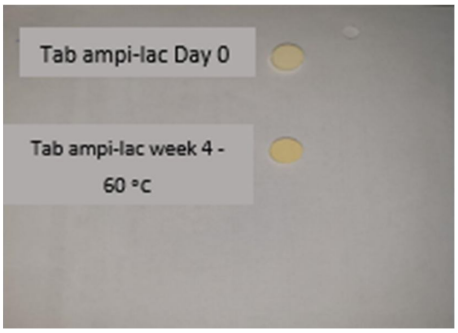

Fig. 5. The color changes of ampicillin-lactose tablets from the first day to the fourth week at 60 ° C

Conclusion

Although the Millard reaction between amines and reducing agents is widely known, lactose is still used as an excipient in the pharmaceutical industry due to its good compressibility and cost-effectiveness.

In this study, the compatibility between ampicillin and lactose excipient in both powder and tablet forms was examined through DSC and FTIR methods.

In DSC thermal studies, the observation of a change in the melting endotherm of the drug is considered as interference. According to the results obtained from theampicillin-lactose thermogram, the melting peaks of the drug and the excipient changes occur possibly due to the incompatibility of the drug and the excipient.

It is essential, in order to study the FTIR spectra, to initially analyze the spectrum of the drug and excipient

before being exposed to stress and then compare it with the spectra obtained under stress.

Based on the obtained results and the changes in the main peaks of absorption of the drug-excipient mixture in the fourth week compared to the first day, there is the possibility of incompatibility in the mixture of ampicillin and lactose.

Also, the observation of discoloration in the powder and tablets in the fourth week compared to the first day indicates the occurrence of a kind of incompatibility in the drug-excipient mixture.

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Conflict of interest

The authors declare no financial or other conflict of interest.

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Ethical Statement

The above-mentioned sampling protocols were approved by the Medical Ethics Committee of Urmia University of Medical Sciences, Urmia, Iran (ethical code: IR.UMSU.REC.1397.270).

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