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Research paper

Compatibility studies of acyclovir and lactose in physical mixtures and commercial tablets

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ABSTRACT

This study documents drug–excipient incompatibility studies of acyclovir in physical mixtures with lactose and in different tablet brands. Differential scanning calorimetry (DSC) was initially used to assess compatibility of mixtures. The Fourier-transform infrared (FTIR) spectrum was also compared with the spectra of pure drug and excipient. Although DSC results indicated incompatibility with lactose, FTIR spectra were mostly unmodified due to overlapping peaks. Samples of isothermally stressed physical mixture were stored at 95 °C for 24 h. The residual drug was monitored using a validated high-performance liquid chromatography (HPLC) assay and data fitting to solid-state kinetic models was performed. The drug loss kinetics followed a diffusion model. The aqueous mixture of drug and excipient was heated in order to prepare an adduct mixture. HPLC analysis revealed one extra peak that was fractionated and subsequently injected into the liquid chromatography–mass spectrometry/mass spectrometry (LC–MS/MS) system. The MRM (Multiple Reaction Monitoring) chromatograms characterized the peak with molecular mass corresponding to an acyclovir–lactose Maillard reaction product. The presence of lactose in commercial tablets was checked using a new TLC method. Overall, the incompatibility of acyclovir with lactose was successfully evaluated using a combination of thermal methods and LC–MS/MS.

1. Introduction

The study of drug–excipient compatibility is an important process in the development of a stable solid dosage form [1]. A new chemical entity or drug substance becomes a drug product after formulation and processing with excipients [2]. Incompatibility between drugs and excipients can alter the stability and bioavailability of drugs, thereby affecting its safety and/or efficacy. Despite the importance of this issue, there is no universally accepted protocol for drug–excipient compatibility testing [1,2]. In recent years, thermal analysis has been used in the development and improvement of pharmaceutical formulations [3,4]. Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) are the most commonly used thermal techniques in drug–excipient compatibility assessments [1,5,6]. Isothermal stress testing (IST) is another method that involves storing the drug–excipient blends with or without moisture at high temperature and determining the drug content [2,7,8]. One of the IST methods adopted by Serajuddin et al. [2] involved the storage of formulated samples with 20% v/w added water at 50 °C for 1–3 weeks. Later, Sims et al. modified their method to a more rapid one by changing the storage temperature and time to 100 °C and 1–3 days, respectively. DSC can be used in combination with IST to evaluate compatibility of drugs with the selected excipients [1,9].

Fourier-transform infrared (FTIR) spectroscopy is another approach used in compatibility tests based on the hypothesis that some functional groups change during drug–excipient interaction [5,10,11].

In the most detailed studies, degradation products can also be identified by mass spectral, NMR, and other relevant analytical techniques [2,11–14]. The identification of degradation products in dosage formulations plays an important role in the drug development process. During the past decade, with the commercialization of mass spectrometers using soft ionization techniques such as electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI), the coupling of high-performance liquid chroma-

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tography (HPLC) and mass spectrometry (MS) has become one of the most powerful techniques for pharmaceutical analysis. The separation by time provided by an HPLC system combined with a mass spectrometer enables a chemist to acquire the structural information of a specific impurity or degrade without a time-consuming isolation process. Liquid chromatography–mass spectrometry (LC–MS) analysis is very sensitive for the detection of low-level unknowns in complex mixtures such as formulations. The great advantage of an LC–MS system is largely based on the fact that soft ionization techniques usually provide molecular weight information for the analytes. In general, protonated, ammoniated and sometimes sodiated molecules are produced in the positive ion mode, while deprotonated molecules are generated in the negative ion mode. Furthermore, these pseudomolecular ions often produce structurally informative fragment ions via collision-induced dissociation (CID) processes. Fragments of fragment ions can also be collected using tandem mass spectrometry [15].

The kinetics of the reaction in the solid state is considerably more complicated than in the case of solution-phase kinetics. First, a solid system is inherently non-homogenous making the reaction dependent on the physical configuration of the system and not only dependent on its composition at any given time. Secondly, molecules in the solid state have significantly more limited molecular mobility than molecules in solution [13]. Solid-state kinetic studies have appeared in the pharmaceutical literature over many years and can be mechanistically classified as nucleation, geometrical contraction, diffusion and reaction order models [16,17].

Lactose (molecular weight, MW = 342.3) is one of the most commonly used pharmaceutical excipients. A survey of the Physician's Desk Reference database shows that there are many pharmaceutical formulations where amino compounds and lactose are both present [13,18]. Recently, the possible reaction of the amine groups of drug entities with the carbonyl groups of common tablet excipients, such as lactose, starch and cellulose, has gained the interest of pharmaceutical scientists [12–14,19–21].

An acyclic nucleoside acyclovir (MW = 225.2) is used in the treatment of varicella infections and prophylaxis of herpes simplex infections. Acyclovir is an amine-containing drug, which makes it a good candidate for the Maillard reaction with a reducing agent like lactose [22]. Tu et al. increased the liver distribution of acyclovir using an acyclovir–dextran conjugate, which was synthesized by the formation of a Schiff base [23]. Later, Desai et al. studied the stability of low concentrations of three guanine-based antivirals (entecavir, lobucavir and acyclovir) in sucrose and maltitol solutions and concluded that the formation of isomeric adducts of the drugs and reducing sugars [24] occurs.

All previous investigations have been conducted in solutions, and the possibility of the acyclovir–lactose reaction has not yet been investigated.

In this report, we focus on the determination of the early-stage Maillard reaction products (ESMRP) between the amine-containing antiviral acyclovir (ACV) and lactose (Fig. 1) in solid-state mixtures and tablet brands. For this purpose, the adduct mixture was analyzed using HPLC, FTIR and LC–MS/MS. Thin layer chromatography (TLC) was also used to confirm the presence of lactose in brand formulations. Finally, acyclovir loss data with or without lactose were fitted to common solid-state kinetic models.

2. Materials and methods

2.1. Materials

Acyclovir (ACV) (2-amino-1,9-dihydro-9-(2-hydroxyethoxymethyl)-6H-purin-6-one) and guanine (2-amino-1,7-dihydro-6H-purin-6-one) (acyclovir related compound) were obtained from Arastoo Pharmaceutical Chemicals Incorp., Tehran, Iran (Fig. 1). Lactose monohydrate (Pharma grade 200 Mesh) and anhydrous lactose were provided from DMV Chemical Co., Netherlands. Acetaminophen was received from Sigma Aldrich. All other chemicals were of HPLC or analytical grade and obtained from Labscan analytical science, Ireland. Commercial tablets of ACV named Brand-1–3 were acquired in Iran and Australia from local pharmacies.

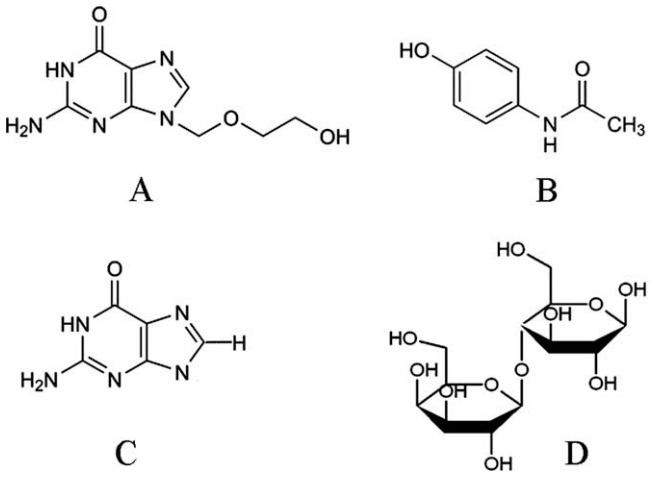

Fig. 1. Structures of (A) ACV, (B) Acetaminophen, (C) Guanine, and (D) Lactose.

3. Methods

3.1. Analytical methods

3.1.1. DSC (differential scanning calorimetry)

A differential scanning calorimeter (DSC-60, Shimadzu, Japan) was used for thermal analysis of drug and mixtures of drug and excipient in a 1:1 w/w ratio. Individual samples (drug and excipients) as well as physical mixtures of drug and excipients were weighed to about 5 mg in the DSC aluminum pan and scanned in the temperature range of 25–300 °C. A heating rate of 20 °C per minute was used, and the thermograms were reviewed for evidence of any interaction. Enthalpy calculations were completed using TA-60 software (version 1.51).

3.1.2. FTIR (Fourier-transform infrared) spectroscopy

FTIR spectra of drug and drug–excipient blends were recorded immediately after mixing and/or heating on an FTIR spectrophotometer (Bomem, MB-100 series, Quebec, Canada) in the range of 400–4000 cm^{-1} using potassium bromide discs. The spectrum was a mean of ten consecutive scans on the same sample. Processing of the FTIR data was performed using GRAMS/32 version 3.04 (Galactic Industries Corporation, Salem, NH).

3.1.3. HPLC

The HPLC system consisted of a SCL-10A XL auto injector, SCL-10A VP system controller, LC-10AT liquid chromatograph and a SPD-M10AVP, UV–Vis, photodiode array (PDA) detector and a FRC-10A fraction collector, all from Shimadzu (Kyoto, Japan). Samples were injected onto a C18 column (100 mm, 4.60 mm, 5 μm; Agilent, USA) maintained at ambient temperature. The two eluting solutions used were A (Deionized water) and B (a mixture of acetonitrile:water:formic acid (95:5:0.1 v/v)). Mobile phase was a mixture of B and A (5:95, v/v). A volume of 1 mL/min was used as the flow rate, and detection was performed at 250 nm. Data were analyzed with Class VP software (version: 6.14 SP1). A solution of Acetaminophen (4 mg/mL in mobile phase) was used as the internal standard (Fig. 1). Internal standard solution (10 μL)