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Research paper

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

Farnaz Monajjemzadeh [a,d], Davoud Hassanzadeh [a,e,*], Hadi Valizadeh [a,d], Mohammad R. Siahi-Shadbad [a,d], Javid Shahbazi Mojarrad [b,f], Thomas A. Robertson [c], Michael S. Roberts [c]

[a] Department of Drug and Food Control, Tabriz University of Medical Sciences, Tabriz, Iran
[b] Department of Medicinal Chemistry, Tabriz University of Medical Sciences, Tabriz, Iran
[c] School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, Australia
[d] Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[e] Research Center for Pharmaceutical Nanotechnology, Tabriz University of Medical Sciences, Tabriz, Iran
[f] Biotechnology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

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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-

* Corresponding author. Department of Drug and Food Analysis, Tabriz University of Medical Sciences, Tabriz, Iran. Tel.: +98 411 339 2614; fax: +98 411 334 4798.
E-mail addresses: hasanzadehd@tbzmed.ac.ir, Pharmatest_d@yahoo.com (D. Hassanzadeh).

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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.

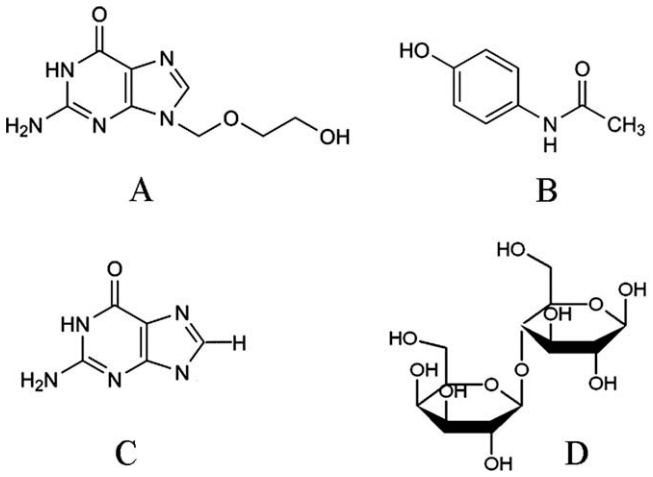

Fig. 1. Structures of (A) ACV, (B) Acetaminophen, (C) Guanine, and (D) Lactose.

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)

was added to each experimental sample (100 μL). The analytical method was validated with respect to parameters such as linearity, intermediate precision, accuracy and selectivity [25,26].

3.1.4. LC–MS/MS

The LC system consisted of a SIL-10AD VP auto injector, SCL-10A VP system controller, LC-10ADVP liquid chromatograph and a DGU-12A degasser, all from Shimadzu (Kyoto, Japan).

Samples were introduced into the mass spectrometer through a C18 Gemini column (2 × 5 × 200 mm, phenomenex) eluted at a flow rate of 0.5 mL/min, at ambient temperature. Elution was performed, with 99% solvent A (1:999 v/v formic acid:water) and 1% solvent B (1:900:50:50 v/v formic acid:acetonitrile:methanol:water). Mass spectrometric detection was performed with an Applied Biosystems MDS Sciex (Ontario, Canada) API 2000 triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) interface in the positive ion mode. The tandem mass spectrometer was operated at unit resolution in the multiple reaction monitoring (MRM) mode, monitoring the transition of the protonated molecular ions to the product ions. Q1 was used from 150 and 600 amu in a mass-resolving mode to select the parent ion. The ion source temperature was maintained at 350 °C. The ionspray voltage was set at 5500 V. The curtain gas (CUR) (nitrogen) was set at 15 and the collision gas (CAD) at 7. The collision energy (CE), declustering potential (DP), focusing potential (FP) and entrance potential (EP) were set at 25, 75, 200 and 8 V, respectively. The system was used in the MRM mode following selection of precursor ions, dissociating them with a collision gas and finally detecting the fragment ions produced by dissociation. Use of this mode results in high selectivity and sensitivity suitable for analysis and detection of specific molecules. Two ion pairs (a = 226.4|135.1 and b = 550.3|194.2) were used in the MRM mode. Data acquisition and processing were accomplished using the Applied Biosystems Analyst version 1.4.1 software.

3.1.5. TLC

Diluent solution was a mixture of methanol and water (2:3 v/v). Developing solvent prepared as a mixture of ethyl acetate and methanol (1:3 v/v) containing 0.25% v/v glacial acetic acid. Standard solution prepared by dissolving lactose in diluent solution. At least 20 units of each brand tablet were weighed and the average tablet weight was calculated. Assuming that the whole excipient content of the mean tablet weight is lactose, the equivalent of 25 mg lactose in powdered tablet was transferred to a 25-mL volumetric flasks and diluted to yield Test solution. Separately, 2 μL each of Standard solution and Test solutions were applied to a thin-layer chromatographic plate (20∗20, Silica gel-60 F254, 0.25 mm thickness) (Merck, Germany). The spots were dried and developed in a paper-lined chromatographic chamber equilibrated with developing solvent for about 1 h prior to use. The chromatogram was developed until the solvent front had moved about three-quarters of the length of the plate. The plate was removed from the chamber, dried in a current of warm air, sprayed evenly with staining solution and heated at 130 °C for 10 min. The staining solution contained thymol (0.5 g) in a mixture of alcohol (95 mL) and sulfuric acid (5 mL). As shown in Fig. 7, the presence of lactose was confirmed because the principal spot obtained from each Test solution corresponds in appearance and R_f value to that obtained from Standard solution.

3.2. Formulation methods

3.2.1. Preparation of ACV–lactose adduct mixture

ACV (0.5 g) and lactose monohydrate (3.3 g) were dissolved in 50 mL of United States Pharmacopoeia (USP) borate buffer (0.1 M, pH = 9.2) with the aid of stirring and ultrasound [26]. The ionic strength of the solution was adjusted to 25 mM with sodium chloride. Triethylamine was added in an equimolar ratio with ACV to aid solubility. The clear solution was then refluxed at 60 °C in a water bath (Contherm Scientific Ltd., New Zealand) for 12 h and dried overnight at the same temperature in an open Pyrex™ beaker using an oven. The dried mixture is referred to as the adduct mixture. Adduct mixtures were dissolved in mobile phase to get a 200 μg/mL concentration with respect to the ACV, and this solution was subjected to reversed-phase chromatography and LC–MS/MS. The presence of brown color was also measured at 490 nm. Different samples of ACV (solid state, solutions with pH = 9.2), aqueous mixture of ACV and lactose (pH = 9.2) and commercial tablets were heated in order to yield degradation products. All solid and liquid samples were heated in a 90 °C oven or in a 60 °C water bath for 24 and 72 h, respectively.

3.2.2. Screening test and commercial tablets

For screening tests, an isothermal stress testing (IST) procedure derived from Serajuddin et al. [27] and Sims et al. [9] with minor modifications was employed to monitor the probable solid-state interaction of drug with excipient in an accelerated manner. Briefly, drug and excipients (Table 1) were weighed directly in 4 mL glass vials (n = 2) and mixed in a 1:1 molar ratio on a vortex mixer for 2 min. All samples were kept for 3 days at room temperature in desiccators containing silica gel. After drying, whenever needed, 1% w/w magnesium stearate and/or 20% v/w water were added to the mixture. The total weight of drug: excipient blend in a vial was kept at 200 mg. Each vial was tightly capped and stored at 95 °C in a hot-air oven. Controls samples were also prepared (Table 1). These samples were examined after 24 h of storage under the above-mentioned conditions using HPLC. Solid samples were dissolved in the mobile phase to yield appropriate concentrations and centrifuged. In each case, the supernatant was filtered through a 0.45-μm nylon membrane filter and then injected onto the HPLC system.

The presence of lactose in commercial tablets was initially examined according to the British Pharmacopoeia (BP) by heating a mixture of lactose (equivalent to 0.25 mg) with added ammonia (5 mL) and water (5 mL). Development of a red color confirms the presence of lactose in the formulations [17]. As some tablets were already colored, a TLC method was used to check the color test results. Twenty commercial tablets of three different brands were finely powdered and assayed according to the United States Pharmacopoeia (USP) and were then kept at 95 °C for 24 h with or without water (Table 1).

3.2.3. Solid-state kinetic study

Lactose and ACV were mixed (1:1 molar ratio) thoroughly with a mortar and pestle, and 200 mg of the mixture was added to at least 10 glass vials (4 mL). The vials were dried for 3 days in silica gel chambers and then capped and placed in an equilibrated oven at 95 °C. Samples of the solids were removed at 2, 6, 24, 48, 72 and 240 h and assayed. Pure ACV was also heated under the same conditions as control samples.

4. Results and discussion

4.1. Analytical methods

4.1.1. DSC

Selected DSC scans of drug, excipient and drug–excipient mixtures are shown in Fig. 2. The thermal behavior of pure drug, respective excipient and the combination of drug and excipient is compared in the DSC thermograms. The peak temperature and

Table 1
Composition and assay results of screening samples.

Samples	Composition					Assay		
	Acyclovir	Mg stearate	Lactose anhydrous	Lactose monohydrate	Water	Acyclovir (%)	Unknown-1 (%)	OD
1[a]	+[c]	–[d]	+	–	–	102.5	0.00	0
2	+	–	+	–	+	88.8	1.08	0
3	+	+	+	–	–	95.6	0.00	0
4	+	+	+	–	+	95.4	0.59	0
5	+	–	–	+	–	94.2	0.00	0
6	+	–	–	+	+	83.8	1.23	0
7	+	+	–	+	–	90.7	0.00	0
8	+	+	–	+	+	83.6	0.87	0
9	+	–	–	–	–	108.2	0.00	0
10	+	–	–	–	+	65.4	0.00	0
11[b]	Brand-1	–	–	–	–	90.21	0.00	0
12	Brand-1	–	–	–	+	86.31	0.00	0
13	Brand-2	–	–	–	–	103.58	0.00	0
14	Brand-2	–	–	–	+	84.67	0.00	0
15	Brand-3	–	–	–	–	95.78	0.00	0
16	Brand-3	–	–	–	+	89.80	0.07	0
17[a]	–	–	+	–	–	–	0.00	0
18	–	–	+	–	+	–	0.00	0
19	–	–	–	+	–	–	0.00	0
20	–	–	–	+	–	–	0.00	0

[a] Physical mixtures of the contents.
[b] Commercial tablets.
[c] Presence.
[d] Absence.

heat of fusion or enthalpy values for drug, excipient and drug–excipient mixture are summarized in Table 2.

The ACV presented its melting point at (255.27 °C) and heat of fusion of (46.75 J/g). The endothermic peak of anhydrous lactose was at 241.72 °C in the pure sample. The melting endothermic peak of ACV was missing in the ACV–anhydrous lactose mixture, which suggested incompatibility (Fig. 2A). A new endothermic peak also appeared at 227 °C (starting from 205.53 °C and ending at 259.34 °C), which may be due to drug and excipient incompatibility.

The monohydrate lactose showed two peaks: one due to dehydration at (152.7 °C) and the second peak related to the melting point at (218.38 °C) (Fig. 2B). The lactose melting peak (218 °C) was characteristic of a monohydrate α-lactose form [6]. In the DSC thermogram of ACV, in the presence of lactose monohydrate, the ACV melting peak was missing, which can be related to the drug and excipient interaction. It should be noted that the peak at 277.04 °C in the ACV and anhydrous lactose mixture was not observed in this sample. The second peak in the ACV–lactose monohydrate thermogram (Fig. 2B) at 218.58 °C starts nearly at the same point as the second peak of pure lactose monohydrate (218.38 °C), but ends almost differently at the later time (253.57 °C vs. 229.05 °C). This finding indicates that the broad peak that appeared at 218.58 °C in the ACV–lactose monohydrate mixture is not the same as the peak in the pure lactose sample and may conceal another peak (Fig. 2C). According to Table 2, this can be proved as the ΔH value for this peak in the pure sample is 137.68 J/g, but in the mixture increases to 314.62 J/g. Thus, it can also be concluded that there is an incompatibility between ACV and monohydrate lactose.

4.1.2. FTIR

The absorption pattern of ACV, lactose, ACV–lactose blends immediately after mixing, and adducts mixture of drug and excipient is shown in Fig. 3a–d, respectively. The possible interaction between ACV and lactose can be proposed to be a Maillard type reaction, which would lead to imine band formation in the FTIR spectrum. The C=N stretching band appears at 1630–1650 cm^{-1} in the infrared spectra of imine-containing compounds [26,28–30] including the Schiff's base, which would form in a Maillard reaction. The absorption band at about 1723 cm^{-1} in Fig. 3a and c is consistent with the ACV carboxylic acid functional group vibration (Fig. 6b). The only difference seen between the adduct mixture and pure drug is a visible shift of this band to 1688 cm^{-1}, which can be related to intermolecular hydrogen bonding. Shepherd et al. have reported a similar shift in IR spectra of ACV [31]. According to the FTIR results, no interaction between ACV and lactose can be detected. This finding can be explained by common phenomenon of peak overlapping in IR spectroscopy as the absorption region of the expected imine and the carboxylic group overlaps.

4.1.3. HPLC

Although different HPLC methods have been used in ACV identification, only a small number have been performed using an internal standard. Guanine, vanillin and salicylic acid are used in this study [32–34]. The previous methods using these internal standards were not looking for the Maillard reaction product of lactose and ACV, and our results also indicated that the Maillard reaction product peak did not separate using these methods. Acetaminophen was tried as a new internal standard and acceptable results were produced. The ACV and internal standard chromatogram are presented in Fig. 4A.

4.1.4. HPLC method validation

The standard solutions for the linearity test were prepared five times at different concentration levels. Peak area ratios of ACV to internal standard were calculated and plotted versus respective concentrations, and a linear regression analysis was performed. The constructed calibration curve was linear over the concentration range of 0.98–250 μg/mL. The correlation coefficient was found to be more than 0.999 with relative standard deviation (RSD) values ranging from 0.21% to 2.08% within the concentration ranges studied. Repeatability of measurement of the peak area was carried out using seven replicates of the same concentration (200 μg/mL). The RSD was found to be 0.23%.

The intra- and inter-day precision of the method were carried out at four different concentrations (31.25, 62.5, 125 and 250 μg/mL).

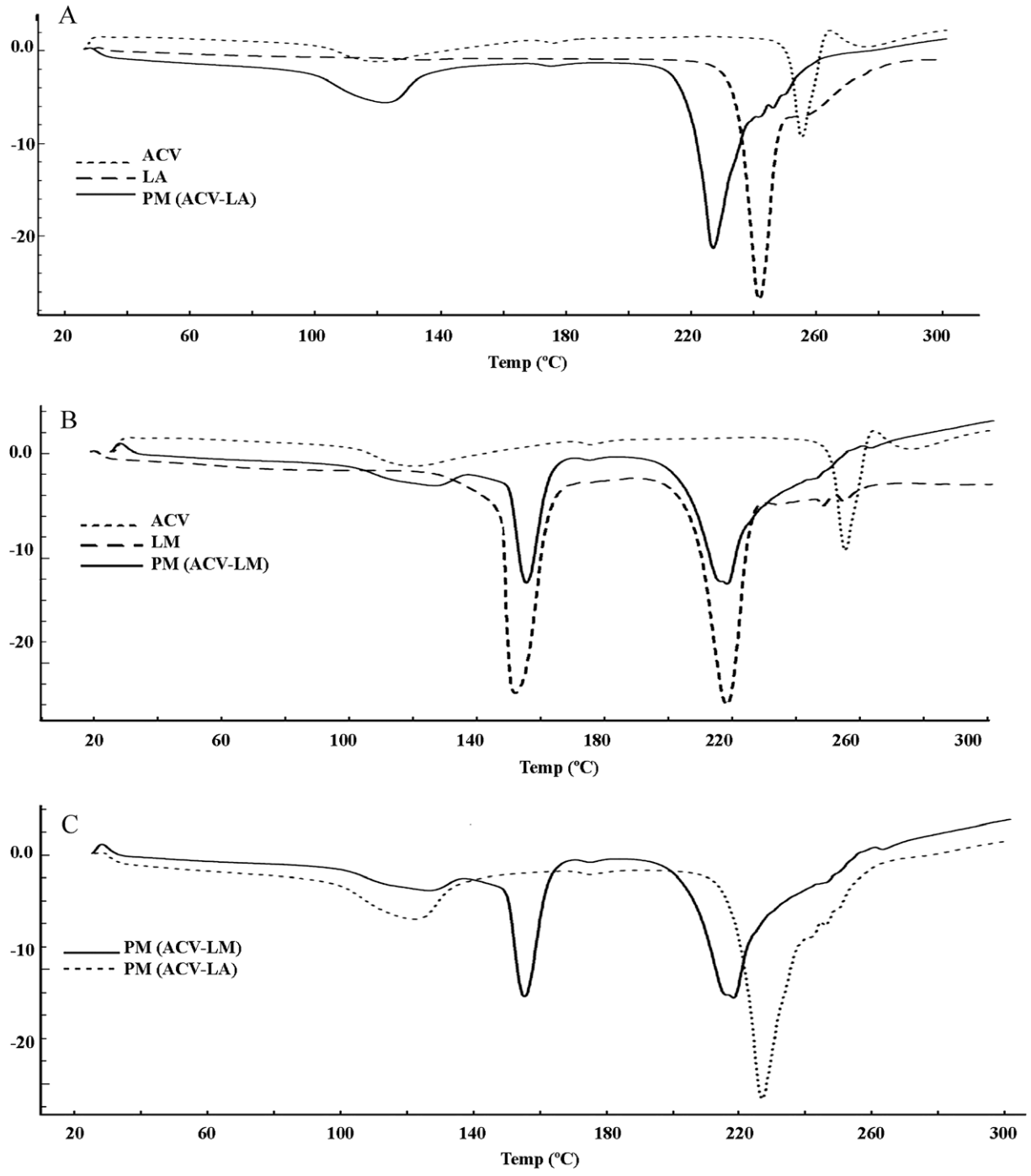

Fig. 2. Selected DSC scans of drug and drug–excipient physical mixture (PM). (A) ACV–Anhydrous lactose with 1:1 molar ratio, (B) ACV–lactose monohydrate with 1:1 molar ratio, (C) ACV–lactose monohydrate lactose and ACV–Anhydrous lactose thermograms comparison.

The low RSD values of within-day and day-to-day variations revealed that the proposed method is precise (Table 3).

The limit of detection (LOD) and limit of quantification (LOQ) were determined based on signal-to-noise ratios using an analytical response of three and ten times the background noise, respectively [26]. The LOD and LOQ were found to be 1.3 and 3.9 μg/mL, respectively. The selectivity of the method was tested using heated samples of ACV with or without lactose. The chromatograms are presented in Fig. 4. Some useful standard chromatographic parameters have been calculated and reported in Table 4.

4.1.5. HPLC analysis of the adduct mixtures

The adduct mixture was dissolved in mobile phase to produce a solution with a nominal ACV concentration of 200 μg/mL. A control sample was prepared using heated ACV without lactose (Fig. 4C). Heated ACV resulted in a peak (labeled as c), which was related to guanine. The chromatogram of the sample spiked with guanine is shown in Fig. 4B. In comparison to control, HPLC analysis of the ACV–lactose adduct mixture revealed one extra peak (labeled as d) and named as Unknown-1 (Fig. 4D and C), which eluted before ACV. Either anhydrous or hydrous lactose samples that were heated alone showed no extra peaks under

Table 2
Peak temperature and enthalpy values for drug, excipient and dug–excipient mixture.

Sample	Peak	Time (min)			Temperature (°C)			ΔH (J/g)
	Name	Start	Peak	End	Start	Peak	End	
ACV[a]	1	3.9	5	6.34	98.91	120.14	141.34	25.70
	2	10.84	11.93	12.33	234.19	255.27	264.02	46.75
	3	12.33	12.92	13.81	264.02	275.69	293.85	20.84
LM[b]	1	5.69	7.08	7.93	126.66	152.07	169.94	135.86
	2	9.24	10.48	10.96	195.05	218.38	229.05	137.68
LA[c]	1	10.25	11.6	11.17	223.37	241.72	251.67	102.62
ACV-LM	1	3.97	5.38	5.88	99.61	126.9	136.72	25.54
	2	5.93	6.9	7.49	137.68	155.62	168.04	117.31
	3	8.89	10.13	11.86	195.08	218.58	253.57	314.62
ACV-LA	1	3.96	5.13	5.72	99.87	122.35	133.97	88.42
	2	9.29	10.57	12.13	205.53	227.04	259.34	376.4

[a] Acyclovir.
[b] Lactose monohydrate.
[c] Anhydrous lactose.

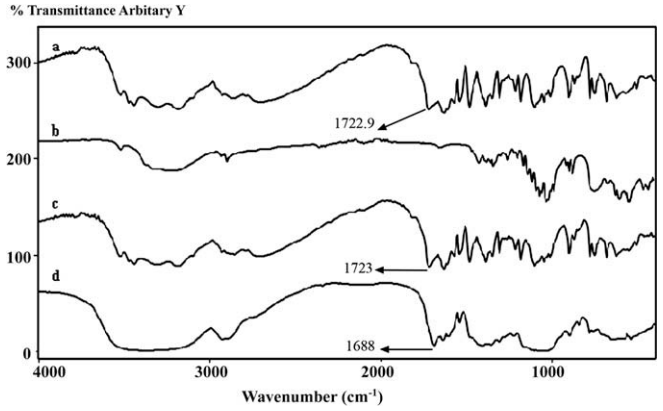

Fig. 3. FTIR spectra of (a) ACV, (b) Lactose, (c) ACV–lactose monohydrate immediately after mixing, (d) ACV–lactose adducts mixture.

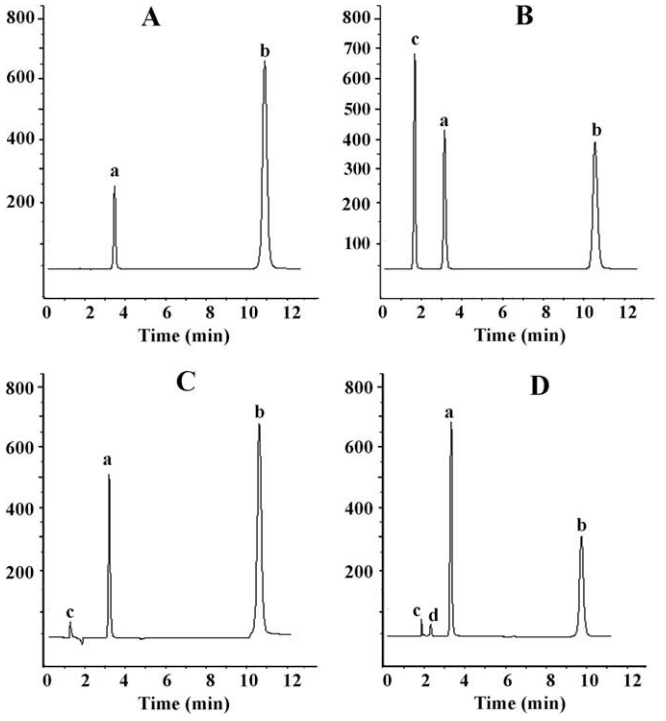

Fig. 4. HPLC chromatogram of (A) ACV and internal standard, (B) sample A spiked with standard guanine, (C) Heated acyclovir in aqueous media, (D) Adducts mixture a = ACV, b = internal standard, c = Guanine, d = Unknown-1.

the same chromatographic conditions when compared to that obtained with the mixture.

4.1.6. Mass spectrometry

An adduct mixture solution was prepared and injected into the LC–MS/MS system. A mass spectrometer compatible (salt-free) method was developed to give similar separation to the HPLC method. Mass spectra (MS2) are presented in Fig. 5A–D. Manually collected HPLC fractions of Unknown-1 were injected into the tandem mass spectrometer system. The full-scan positive ion electrospray product ion mass spectra showed that the precursor ions of ACV and Unknown-1 were the protonated molecules, $[M+H]^+$ of m/z 226.3 and 550.3, respectively (Fig. 5A and C). ACV was also identified as a trimmer shown in Fig. 5B. After collision-induced dissociation, the characteristic ions in the product ion mass spectrum were at m/z 152.2 and 194.2, respectively. Similar molecular [35,36] and daughter ions [36] have been previously reported for ACV LC–MS/MS analysis. Proposed structures for Unknown-1 have been presented in Fig. 6. The nominal molecular mass of Unknown-1 is consistent with the ACV–lactose condensation product formed by the elimination of a single molecule of water from the parent compounds (Fig. 5C). This reaction has been known as the Maillard reaction [37]. The MRM mode was set for detection of ACV (226.4/152.2) and Unknown-1 (550.3/194.2). The MRM chromatogram shown in Fig. 5D indicates the presence of the condensation product (Schiff base) before ACV in LC–MS/MS system, which is in accordance with the HPLC results (Fig. 4D).

Liquid chromatography/mass spectrometry (LC/MS)-based techniques provide unique capabilities for pharmaceutical analysis. LC/MS methods are applicable to a wide range of compounds of pharmaceutical interest and they feature powerful analytical figures of merit (sensitivity, selectivity, speed of analysis and cost effectiveness) [38]. Harmon et al. have detected the lactose–hydrochlorothiazide condensation product (m/z = 622 and 620) using mass spectrometry. Similar approaches have been used by Qiu et al. and Wirth et al. in the analysis of lactose–metoclopramide and lactose–fluoxetine condensation products, respectively [12,13,21].

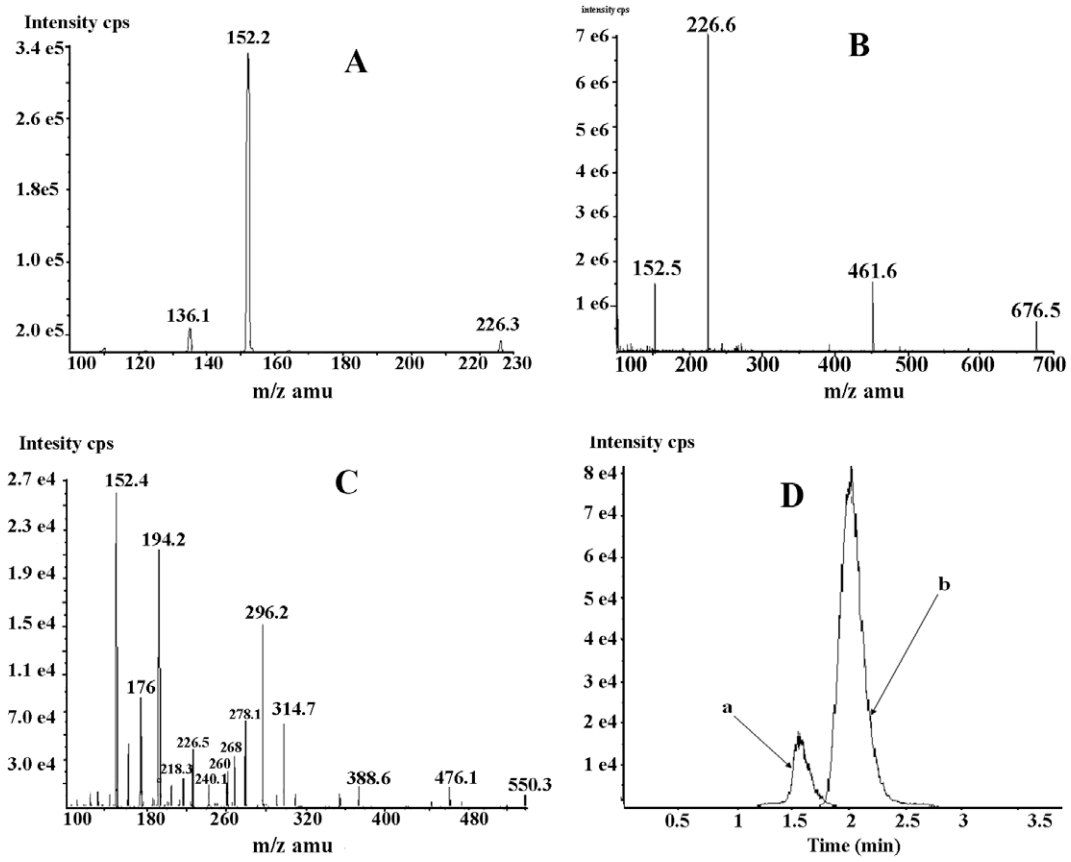

Fig. 5. Positive ion mode electrospray mass spectrum of (A) ACV, (B) ACV trimer, (C) Unknown-1, (D) MRM chromatogram of two pairs; (a) ACV: 226.4/152.2 amu, (b) Unknown-1: 550.3/194.2 amu.

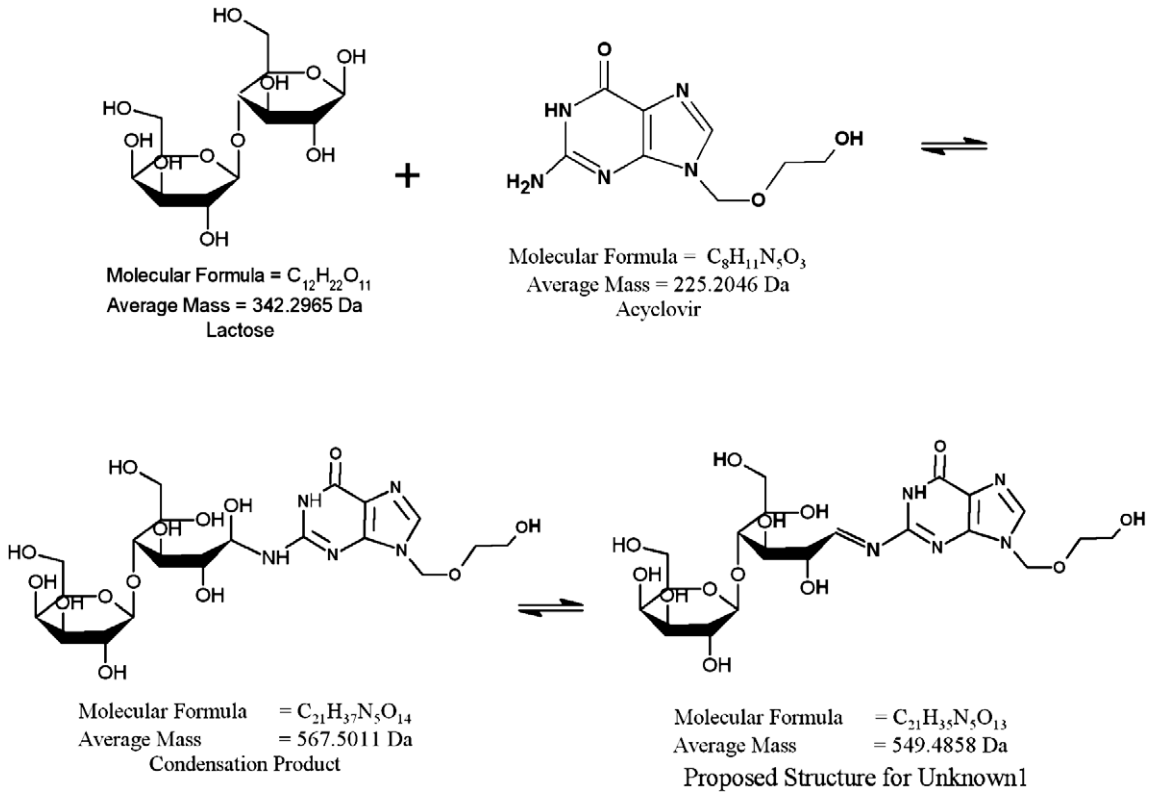

Fig. 6. Proposed Maillard reaction condensation products of ACV and lactose.

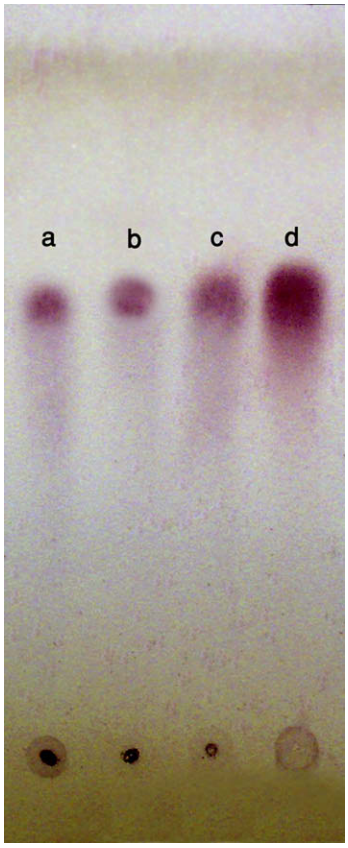

Fig. 7. TLC results of (a) Brand-1, (b) Brand-2, (c) Brand-3, and (d) lactose.

4.2. Formulation methods

4.2.1. Screening tests and commercial tablets

For ease of comparison, the screening test results are shown in Table 1. As expected [14,27], the presence of water promoted the degradation reactions including the Maillard reaction (even samples except 20). An interesting finding was that Unknown-1 was only detected in wet physical mixtures of drug and excipient under the test conditions used here (samples 2, 4, 6 and 8). In addition, no coloring was seen even in samples with detectable amounts of Unknown-1 as a marker for the occurrence of the Maillard reaction. These results may be explained by the fact that the end point of the reaction has not been reached and no melanoidines, which are responsible for brown color, have been produced [8]. This may be due to the storage conditions used in this study. According to Table 1, the reaction has been observed in wet physical mixtures of drug and both excipients (monohydrate and anhydrous lactose) even in the presence of magnesium stearate.

Although the red color test was a positive evidence for the presence of lactose in all commercial tablets, TLC was performed to confirm this finding as some tablets had initial colors that interfered with red color visualization. The method used for TLC is novel and includes an agent to minimize tailing (glacial acetic acid) [39]. TLC results are shown in Fig. 7. It is clear that all the tested brands did have lactose in their formulations. The amount of ACV remaining in heated brands with or without water was analyzed using HPLC. Unknown-1 or ACV–lactose condensation product (Schiff base) was checked with HPLC and LC–MS/MS. It is interesting to report that Brand-1 and Brand-2 although containing lactose as excipient did not show any evidence of the Maillard reaction even under wet conditions. The only detectable reaction product was seen in Brand-3 only under wet conditions. These findings indicate that real formulations, which have different ingredients and/or different ACV:lactose ratios, may not undergo the Maillard reaction even under the accelerated test conditions used in this study.

Table 4
Some standard chromatographic parameters

Peak name	Retention time	Selectivity factor[a]	Resolution[b]
Solvent front	0.2	–	–
Internal standard	10.7	–	–
ACV	3.2	4.409[a]	7.500[a]
Guanine	1.8	12.125 [a]	12.71[a]
Unknown-1	2.2	8.083[a]	14.17[a]
c–d		1.5[b]	1.33[b]
d–a		1.833[c]	1.00[c]
c–a		2.750[d]	1.27[d]

[a] Compared to internal standard.
[b] Peak c compared to peak d.
[c] Peak d compared to peak a.
[d] Peak c compared to peak a.

4.2.2. Kinetic study

Unlike rate laws in homogenous kinetics, which usually depend on reaction order (i.e., first, second, etc.), a rate law for an elementary solid-state reaction could depend on factors such as rate of nuclei formation, interface advance, diffusion, and/or geometrical shape of solid particles. These factors lead to several decomposition models that do not exist in homogenous kinetics and are summarized in Table 5, where α (ranging from 0 to 1) is called the conversion fraction and is a measure of reaction progress as a function of time or temperature, $f(a)$ is the differential reaction model, $g(a)$ is the integral reaction model and (k) is the rate constant. Based on the mechanistic assumptions, models are divided into nucleation, geometrical contraction, diffusion and reaction order (Table 5). The kinetic of many solid-state reactions have been described by nucleation models, specifically the Avarami models. These reactions include crystallization, crystallographic transition, decomposition, adsorption, hydration and desolvation. The rate-limiting step is assumed to be the formation and growth of nuclei, which are finite quantities of product in the reactant lattice. After formation, a nucleus grows and the nucleation rate is different from that of the nuclei growth. Nucleation models (Power and Avrami models) account for both nucleation and nuclei growth rates. Prout–Tompkins model is also classified in this group. In

Table 3
Intermediate precision and accuracy of the HPLC method.

Actual concentration (μg/mL)	Measured concentration (mg/ml), RSD (%)				Accuracy (%)	
	Intra-day		Inter-day		Inter-day	Intra-day
	Measured concentration (mg/mL)	RSD (%)	Measured concentration (mg/mL)	RSD (%)		
31.25	31.8	1.06	32.1	0.1	101.8	102.8
62.5	65.1	0.34	65.8	0.19	104.1	105.2
125	129.1	0.41	129.9	0.24	103.3	103.9
250	247.3	0.53	246.7	0.23	98.9	98.7

Table 5
Solid-state rate expressions for different reaction models. Adopted with permission [15,16].

Model	Differential form[a] $f(\alpha) = 1/k(d\alpha/dt)$	Integral form[a] $g(\alpha) = kt$
Nucleation models		
Power law (P2)	$2\alpha^{(1/2)}$	$\alpha^{(1/2)}$
Power law (P3)	$3\alpha^{(2/3)}$	$\alpha^{(2/3)}$
Power law (P4)	$4\alpha^{(3/4)}$	$\alpha^{(3/4)}$
Avarami–Erofe'ev (A2)	$2(1-\alpha)[-\ln(1-\alpha)]^{1/2}$	$[-\ln(1-\alpha)]^{1/2}$
Avarami–Erofe'ev (A3)	$3(1-\alpha)[-\ln(1-\alpha)]^{2/3}$	$[-\ln(1-\alpha)]^{2/3}$
Avarami–Erofe'ev (A4)	$4(1-\alpha)[-\ln(1-\alpha)]^{3/4}$	$[-\ln(1-\alpha)]^{3/4}$
Prout–Tompkins (B1)	$\alpha(1-\alpha)$	$\ln[\alpha/(1-\alpha)]$
Geometrical contraction models		
Contracting area (R2)	$2(1-\alpha)^{1/2}$	$[1-(1-\alpha)^{1/2}]$
Contracting volume (R3)	$3(1-\alpha)^{2/3}$	$[1-(1-\alpha)^{1/3}]$
Diffusion models		
1-D diffusion (D_1)	$1/2\alpha$	α^2
2-D diffusion (D_2)	$[-\ln(1-\alpha)]^{-1}$	$[(1-\alpha)\ln(1-\alpha)] + \alpha$
3-D diffusion-Jander eqn. (D_3)	$3(1-\alpha)^{2/3}/2(1-(1-\alpha)^{1/3})$	$[1-(1-\alpha)^{1/3}]^2$
Ginstling–Brounshtein (D_4)	$(3/2((1-\alpha)^{-1/3}-1)$	$1-(2\alpha/3)-(1-\alpha)^{2/3}$
Reaction-order models		
Zero (F0/R1)	1	α
First (F1)	$(1-\alpha)$	$-\ln(1-\alpha)$
Second (F2)	$(1-\alpha)^2$	$(1-\alpha)^{-1}-1$
Third (F3)	$(1-\alpha)^3$	$0.5((1-\alpha)^{-2}-1)$

[a] In some references, $f(a)$ and $g(a)$ have opposite designations.

Geometrical contraction models, nucleation is assumed to be instantaneous throughout the surface and the rate-limiting step is the progress of the product layer from the surface of the crystal inward and is different for various crystal morphologies (cubic, cylindrical, spherical, etc.). Geometrical contraction models are divided into two groups; contracting area (for cylindrical solid particles) and contracting volume (for a spherical or cubical solid particle). In diffusion-based models, the rate-limiting step is the diffusion of reactants into reaction sites or products away from reaction sites. Order-based models are the simplest models as they are similar to those used in homogenous kinetics. In these models, the reaction rate is proportional to concentration, amount or fraction remaining of reactants raised to a particular power (integral or fractional), which is the reaction order. Some kinetic analysis methods force data into an order-based model that may not be appropriate [16,17].

Attempts have been made to develop a kinetic model for the loss of the amine in the solid-state Maillard reaction from a pharmaceutical aspect. ACV loss was correlated to different kinetic models and the best fit was accomplished using a diffusion model (D_3). Data are presented in Table 6. Recently, Khawam and Flanagan have reviewed the basics and applications of solid-state kinetics and solid-state kinetic models in two separate articles [16,17]. Solid-state reactions are not usually controlled by mass transfer except for a few reversible reactions or when a large evolution or consumption of heat occurs. Diffusion usually plays a role in the rates of reaction between two reacting solids when reactants are in separate crystal lattices [16,17].

In diffusion-controlled reactions, the rate-limiting step is the diffusion of reactants into reaction sites or products away from reaction sites, and the rate of product formation decreases proportionally with the thickness of the product barrier layer. The model proposed by Qiu et al. describing metoclopramide loss kinetics cannot be used here because the molar ratio of ACV lactose in our study was 1, resulting in Ln 1, to be zero and turning all equation to zero. Full kinetic analysis as a function of the amine, carbohydrate, and reaction conditions is left for future studies.

Table 6
Parameters obtained from the fitting of the reaction rate data (at 95 °C) to various solid-state kinetic models.

Models	Intercept	Slope	RSQ
Nucleation models			
Power law (P2)	0.3495	0.0016	0.5005
Power law (P3)	0.2708	0.0015	0.5926
Power law (P4)	0.2389	0.0015	0.6309
Avarami–Erofe'ev (A2)	0.3739	0.0020	0.5595
Avarami–Erofe'ev (A3)	0.4741	0.0019	0.4312
Avarami–Erofe'ev (A4)	0.5356	0.0018	0.3562
Prout–Tompkins (B1)	−0.3158	−0.0009	0.0433
Geometrical contraction models			
Contracting area (R2)	0.0891	0.0008	0.7557
Contracting volume (R3)	0.0609	0.0005	0.7667
Diffusion models			
1-D diffusion (D_1)	0.0425	0.0006	0.9056
2-D diffusion (D_2)	0.0237	0.0004	0.9219
3-D diffusion-Jander eqn. (D_3)	0.0059	0.0001	0.9374
Ginstling–Brounshtein (D_4)	0.0055	0.0001	0.9274
Reaction order			
Zero-order	0.1656	0.0013	0.7222
First-order	0.1922	0.0018	0.7883
Second-order	0.2260	0.0024	0.8488
Third-order	0.2695	0.0034	0.9000

5. Conclusions

Compatibility studies were completed using different analytical methods. DSC, HPLC and tandem mass spectrometry provided useful information on ACV–lactose compatibility, whereas FTIR was not helpful due to overlapping peaks.

The ability to predict reactions in dosage forms depends on the similarity of the prepared mixture to the formulation. The novel TLC method introduced in this study is a valuable and fast colorimetric test method to identify lactose in commercial solid dosage forms. Low levels of the ACV–lactose condensation product were confirmed by liquid chromatography–tandem mass spectrometry, indicating the formation of a covalent link between ACV and lactose with the loss of a single water molecule. Although all the three tested brands contained lactose in their formulations, only one brand formed Schiff base adduct and only under wet conditions. It can be concluded that the Maillard reaction of acyclovir and lactose in a solid-state formulation is less possible than in the aqueous phase. Thus, there may be some other important factors such as restricted mobility affecting the Maillard reaction in real solid dosage forms. It is also advisable to avoid wet conditions in the formulation process and/or storage of acyclovir solid-state dosage forms containing lactose.

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