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Article

Chemical Interactions Study of Antiretroviral Drugs Efavirenz and Lamivudine Concerning the Development of Stable Fixed-Dose Combination Formulations for AIDS Treatment

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Lamivudina e efavirenz estão entre os fármacos mais utilizados em todo o mundo para o tratamento da síndrome da imunodeficiência adquirida (AIDS). Foram utilizadas as técnicas de ressonância magnética nuclear de estado sólido (ssNMR), espectroscopia no infravermelho por transformada de Fourier (FTIR), calorimetria exploratória diferencial (DSC), e análise termo-óptica (TOA) para estudar possíveis interações entre esses fármacos, visando o desenvolvimento de uma combinação em dose fixa dos mesmos. DSC e TOA evidenciaram deslocamentos significantes das temperaturas de fusão dos fármacos na mistura, o que sugere uma interação entre eles. Apesar de os resultados de DSC e TOA indicarem incompatibilidade entre as drogas, os espectros de FTIR não apresentaram modificações devido à sobreposição de bandas. As análises de ssNMR mostraram mudanças significativas nos valores de deslocamento químico da mistura quando comparados aos dos fármacos puros, especialmente nos sinais referentes a átomos de carbono deficientes em elétrons. Essses resultados confirmam as interações sugeridas pelo DSC e TOA, devido provavelmente a interações ácido-base entre átomos eletronegativos e deficientes em elétrons dos fármacos.

Lamivudine and efavirenz are among the most worldwide used drugs for acquired immune deficiency syndrome (AIDS) treatment. Solid state nuclear magnetic resonance (ssNMR), Fourier-transformed infrared spectroscopy (FTIR), differential scanning calorimetry (DSC) and thermo-optical analysis (TOA) were used to study possible interactions between these drugs, aiming the development of a fixed-dose drug combination. DSC and TOA have evidenced significant shifts on the melting points of both drugs in the mixture, which may be due to interaction between them. Although DSC and TOA results indicated incompatibility between the drugs, FTIR spectra were mostly unmodified due to overlapping peaks. The ssNMR analyses showed significant changes in chemical shifts values of the mixture when compared with spectra of pure drugs, especially in the signals relating to the deficient electron carbon atoms of both drugs. These results confirm the interactions suggested by DSC and TOA, which is probably due to acid-base interactions between electronegative and deficient electron atoms of both lamivudine and efavirenz.

Keywords: lamivudine, efavirenz, new formulation, incompatibility, solid state

Introduction

Acquired immune deficiency syndrome (AIDS) is a degenerative disease of the immune system caused by the human immunodeficiency virus (HIV), a lentivirus belonging to the family of the Retroviridae.[1,2] Lamivudine and efavirenz (Figure 1) are among the most worldwide used antiretrovirals (ARVs) for the AIDS treatment. Lamivudine (3TC), $C_8H_{11}N_3O_3S$ [cis-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)pyrimidin-2(1H)-one] is a nucleoside reverse transcriptase inhibitor which was approved by the United States Food and Drug Administration (US FDA) in 1995. This drug is in clinical use for HIV infected and hepatitis B-positive patients.[3,4] Efavirenz (EFZ), $C_{14}H_9ClF_3NO_2$ [8-chloro-5-(2-cyclopropylethynyl)-5-(trifluoromethyl)-4-oxa-2-azabicyclo[4.4.0]deca-7,9,11-trien-3-one] is a

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non-nucleoside reverse transcriptase inhibitor (NNRTI). EFZ was approved by the US FDA in 1998. NNRTIs are highly potent antiretroviral agents that can be combined with nucleosides without added toxicity and they should always be used in combination with other antiretrovirals to insure suppression of viral replication.[5] EFZ and 3TC were included in the 15th model list of World Health Organization (WHO) of essential medicines[6] and generally are used together as part of the first-line ARV regimen.[7,8] Currently, they are administrated in separate tablets. The WHO Committee recommends the use of fixed-dose combinations of ARVs (e.g., two or more active pharmacological products in the same capsule, tablet or solution),[6] but such a system could present serious interactions or incompatibilities between their components, which may be extensively investigated before the development of a stable formulation containing EFZ and 3TC.

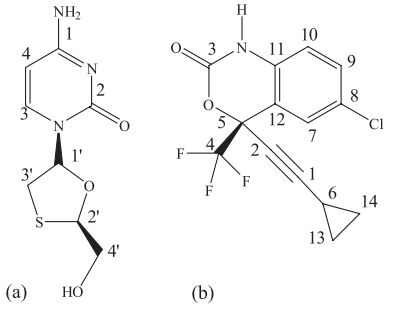

Figure 1. Structures of (a) lamivudine and (b) efavirenz.

The study of drug-excipient compatibility is an important process in the development of a stable solid dosage form.[9] 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-drug and drug-excipient compatibility testing.[9,10] In recent years, thermal analysis has been used in the development and improvement of pharmaceutical formulations.[11-15] Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) are the most commonly used thermal techniques in drug-excipient and drug-drug compatibility assessments. Fourier-transform infrared (FTIR) spectroscopy is another approach used in compatibility tests based on the hypothesis that some functional groups change during drug-excipient and drug-drug interactions.[16-18] Other techniques, in spite of their great utility in compatibility studies, are still under used in pharmaceutical tests, like solid state nuclear magnetic resonance (ssNMR) and a special technique of thermal analysis called thermo-optical analysis (TOA). Therefore, solid state nuclear magnetic resonance, Fourier-transformed infrared spectroscopy, differential scanning calorimetry and thermo-optical analysis of 3TC, EFZ and their mixture were performed to study possible interactions between these drugs, in order to contribute to the future development of a new formulation which may be another alternative for the treatment of patients with AIDS.

Experimental

Materials

Samples of lamivudine (3TC, $C_8H_{11}N_3O_3S$, pharmaceutical grade) and efavirenz (EFZ, $C_{14}H_9ClF_3NO_2$, pharmaceutical grade) were obtained from Hangzhou Coben Pharmaceutical (China) and Nortec (Brazil), respectively. Each drug was individually weighed and the physical mixture was prepared at a 1:1 wt/wt ratio.

Methods

DSC and TG/DTG

DSC curves were obtained in a DSC-60 cell (Shimadzu, Japan) using aluminum pans containing about 1 mg of samples, under dynamic nitrogen atmosphere (50 mL min^{-1}) and heating rate of 10 °C min^{-1} in the temperature range from 25 to 450 °C. The DSC cell was calibrated with indium (mp 156.6 °C; ΔH_{fus} = 28.54 J g^{-1}) and lead (mp 327.5 °C).

TG/DTG curves were obtained in a thermobalance model DTG 60 (Shimadzu, Japan) in the temperature range from 25 to 700 °C, using alumina pans with about 3 mg of samples, under dynamic nitrogen atmosphere (50 mL min^{-1}) and heating rate of 10 °C min^{-1}.

TOA

Thermo-optical analysis is a technique in which a sample is observed with a microscope during a controlled temperature program. TOA images were obtained at different time intervals during heating using a hot stage FP82 (Mettler, Switzerland), with a heating rate of 2 °C min^{-1} and a DM 4000B microscope (Leica, Germany), coupled with a Leica digital camera model DFC 280. The images were captured at 100× magnification.

FTIR spectroscopy

The FTIR absorption spectra of 3TC, EFZ and the physical mixture were recorded on a spectrophotometer Spectrum 1000 (Perkin Elmer, United States) at room temperature in the range 4000-400 cm^{-1} employing an attenuated total reflectance (ATR) accessory. The samples were pressed into a zinc selenide crystal, and the spectra were obtained from 32 scans with a resolution of 4 cm^{-1}.

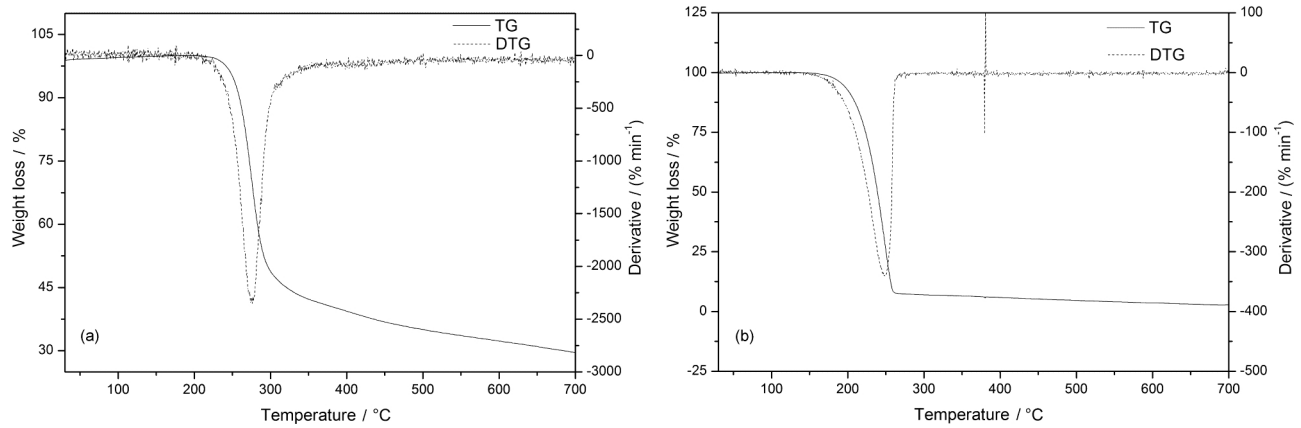

Figure 2. TG and DTG curves of (a) lamivudine and (b) efavirenz.

ssNMR

Proton-decoupled ^{13}C solid-state NMR spectra were acquired on a Bruker Avance DRX 400 NMR spectrometer (Bruker, Germany) using a commercial double-resonance 4 mm MAS probe. A cross polarization pulse sequence[19] was applied with the following typical acquisition parameters: 90° pulse width 4 μs, spin lock time 5000 μs, recycle delay 4.0 s, 3072 data points, 3072 acquisitions, spectral width 28.3 kHz and spinning speed of 4 kHz. Adamantane was used as reference (high field signal at 29.5 ppm).

X-ray diffraction (XRD)

Powder X-ray diffraction (XRD) data were collected in a XRD-6000 diffractometer (Shimadzu, Japan) under 40 kV, 30mA, using Cu Kα (λ = 1.54056 Å) coupled with a graphite monochromator, scanned over an angular range of 10-80° (2θ) with a step size of 0.01° (2θ) and a time constant of 5 s step^{-1}. The sample holder was submitted to a spinning of 60 cycles *per* min to reduce any eventual preferred orientation and minimize rugosity effects. The samples were submitted to a Rietveld analysis to precisely determine the lattice parameters. Rietveld analysis of the powder X-ray diffraction experiment refined 1370 reflections peaks for 3TC and 997 reflections peaks for EFZ in the range of 4 to 80° 2θ.

Results and Discussion

Characterization of lamivudine and efavirenz

DSC and TG/DTG

TG curve of 3TC shows thermal stability until 232 °C. The TG/DTG curves (Figure 2a) indicate that the thermal decomposition process of 3TC occurs in one stage from 232 up to 315 °C with a weight loss of 54%. After this, there is a weight loss of 15% due to drug carbonization process.

DSC curve of 3TC (Figure 3) shows a sharp endothermic peak that corresponds to melting of the drug (T_{onset} = 173 °C, ΔH_{fus} = 355 J g^{-1}). After melting, an exothermic event is observed at about 260 °C, characteristic of 3TC decomposition process.

TG curve of EFZ shows thermal stability until 170 °C. The TG/DTG curves (Figure 2b) indicate that the thermal decomposition process of EFZ occurs in one stage from 170 up to 267 °C with a weight loss of 91.8%. After that, there is a weight loss of 5% due to carbonization process of the drug.

DSC curve of EFZ (Figure 3) shows a sharp endothermic peak that corresponds to drug melting (T_{onset} = 136 °C, ΔH_{fus} = 124 J g^{-1}). After melting, an exothermic event is observed at about 260 °C, characteristic of EFZ decomposition process.

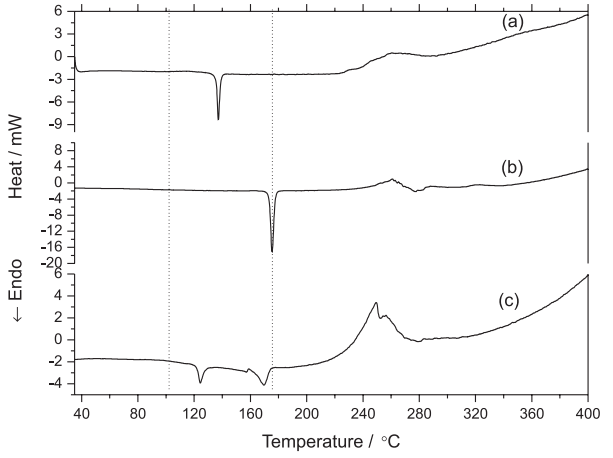

Figure 3. DSC curves of (a) efavirenz, (b) lamivudine and (c) mixture.

TOA

TOA analysis showed that 3TC melts in the temperature range from 175 up to 176 °C, and EFZ melts from 134 up to 138 °C (Figure 4), which is in agreement with the melting points observed in DSC experiments.

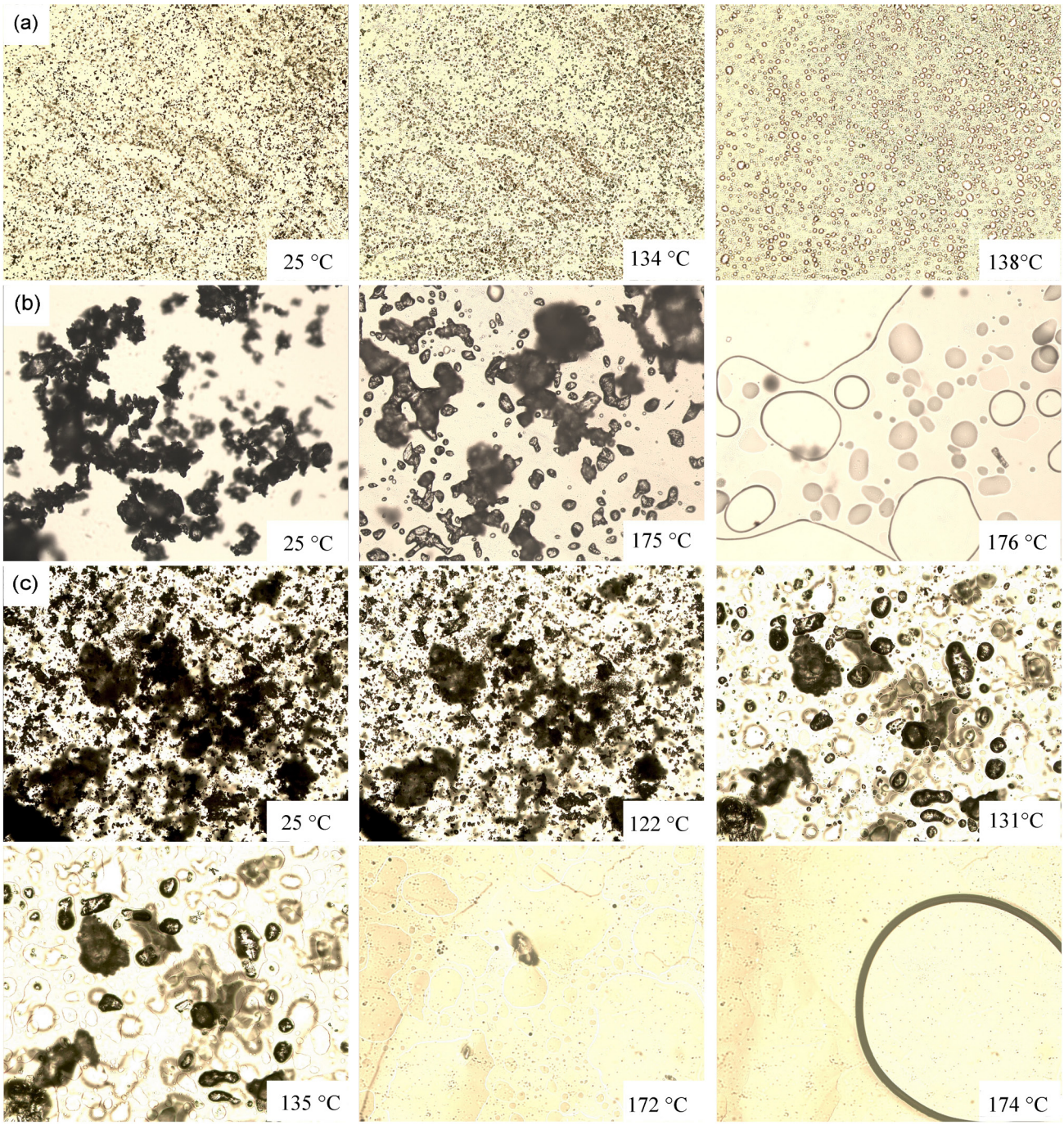

Figure 4. TOA images at 100× magnification of (a) efavirenz, (b) lamivudine and (c) mixture. Three images in different temperatures were shown for each pure drug and six for the mixture.

FTIR spectroscopy

The assignments of the spectra peaks were made in accordance to the literature.[20] The FTIR absorption patterns of 3TC, EFZ and mixture are shown in Figure 5. The scale was changed for clarity. 3TC exhibits main characteristic bands of carbonyl (C=O–NR_2) stretching at 1632 cm^{-1}. This band overlaps the band due to N–H bending at 1607 cm^{-1}. The band due to stretching vibration of the imine group (R_2–C=NR) is observed at 1648 cm^{-1}. Broad bands due to the stretching vibration of –NH_2 and –OH group are observed at 3300-3500 cm^{-1} (Figure 5a). EFZ exhibits a characteristic band due to C=O stretching vibration at 1743 cm^{-1} and a band due to –NH– stretching vibration at 3310 cm^{-1}. An intense band due to the stretching vibration of the –C–F bonds is observed at 1183 cm^{-1}. The band due to stretching vibration of the

triple bond is observed at 2242 cm^{-1}. Stretching vibrations of the C=C bonds in the aromatic ring occur at 1600 and 1494 cm^{-1} (Figure 5b).

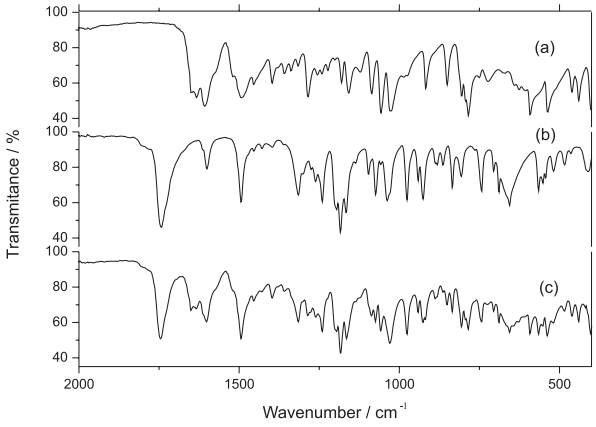

Figure 5. FTIR spectra of (a) lamivudine, (b) efavirenz and (c) mixture.

ssNMR

The ^{13}C NMR spectra of 3TC and EFZ are presented in Figure 6 (panels (a) and (b), respectively) and the respective chemical shifts are summarized in Table 1. Eight signals are observed in the ^{13}C NMR spectrum of 3TC. The carbamide carbon resonates at 157.0 ppm, while the amino-substituted imino carbon resonates at 167.0 ppm. The other sp^2 carbons (C-3 and C-4) resonate at 141.1 and 96.0 ppm. The other signals are attributed to sp^3 carbons bounded to one or two electronegative atoms and are observed between 38 and 92 ppm. Six signals are observed between 106 and 148 ppm in the ^{13}C NMR spectrum of EFZ, which correspond to the resonances of the aromatic carbons. The carbonyl carbon resonates at 154.3 ppm, which is in agreement with the cross conjugation of the carbamate functional group. The shielded resonances observed between –0.5 and 8 ppm are in agreement with the highly tensioned three-carbon-atom ring. Resonances of substituted *sp* carbons are observed at 74.8 and 89.0 ppm, whereas the C–O signal is observed at 79.6 ppm. Finally, the unshielded trifluoromethyl carbon resonates at 114.6 ppm.

XRD

X-ray diffraction measurements show no evidence of any other contaminants, phases or clear existence of different polymorphs for this produced batch. 3TC semi-hydrate crystalizes under monoclinic $P2_1$ space group (Figure 7a). The asymmetric unit cell has four molecules of lamivudine and two of water. Fitted lattice parameters obtained by Rietveld analysis are: a = 11.59 ± 0.01 Å, b = 11.24 ± 0.01 Å and c = 16.05 ± 0.01 Å with $\alpha = \gamma = 90.000$ and $\beta = 94.172 \pm 0.005°$. EFZ crystalizes under orthorhombic $P2_12_12_1$ space group (Figure 7b). Fitted lattice parameters obtained by Rietveld analysis are a = 8.018 ± 0.004 Å, b = 13.417 ± 0.007 Å and c = 24.75 ± 0.01 Å, with $\alpha = \beta = \gamma = 90.000$.

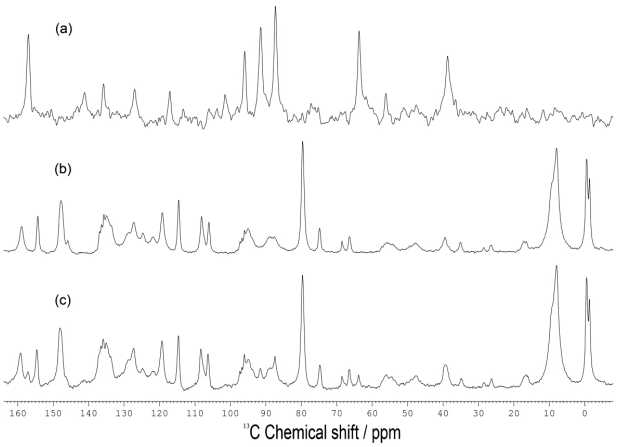

Figure 6. ssNMR spectra of (a) lamivudine, (b) efavirenz and (c) mixture.

Table 1. Comparison between ^{13}C chemical shifts observed for lamivudine and efavirenz in pure state and in the mixture

Carbon number	Efavirenz	Efavirenz in mixture	Lamivudine	Lamivudine in mixture
	δ / ppm			
1	89.0	89.0	167.0	167.4[a]
2	74.8	74.8	157.0	157.1[a]
3	154.3	154.6[a]	141.1	141.1
4	114.60	114.7[a]	96.0	96.0
5	79.6	79.6	–	–
6	–0.5/–1.3	–0.5/–1.3	–	–
7	135.70	135.9[a]	–	–
8	124.7	124.7	–	–
9	128.8	128.8	–	–
10	106.1	106.3[a]	–	–
11	147.8	148.1[a]	–	–
12	121.9	121.6[a]	–	–
13	7.9	7.9	–	–
14	7.9	7.9	–	–
1’	–	–	91.4	91.5[a]
2’	–	–	87.3	87.4[a]
3’	–	–	38.7	39.3[a]
4’	–	–	63.7	63.8[a]

[a]Change in δ value of the drug in the mixture when compared with pure drug.

Compatibility study

DSC

DSC curve of the physical mixture (Figure 3 curve c) has evidenced significant shifts of the melting points of both drugs, if compared with their analysis in pure state. The melting point of 3TC shifted from 173 to 162 °C, and the melting point of EFZ shifted from 136 to 122 °C. These

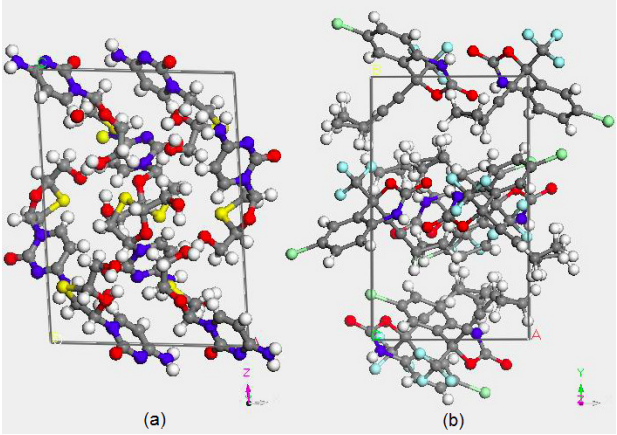

Figure 7. Crystal structure of (a) lamivudine and (b) efavirenz as obtained by Rietveld refinement of the pure samples.

shifts suggest the presence of drug-drug interaction, which is also evidenced by changes in the values of the heat of fusion of the drugs. In a 1:1 wt/wt ratio mixture, the ΔH values of the melting peaks must be half of the pure drug. The ΔH value of 3TC melting in the mixture was 97 J g^{-1}, less than one third of the pure drug (355 J g^{-1}), although the ΔH value of the EFZ melting in the mixture (57 J g^{-1}) was approximately half of the pure EFZ (124 J g^{-1}).

TOA

The TOA analysis of the mixture (Figure 4c) shows that the melting of EFZ starts at about 120 °C, presenting a shift of about 16 °C relating to the pure drug. The melting of 3TC starts at about 122 °C. This large shift of the beginning of 3TC melting compared with pure drug is indicative of strong interaction with EFZ. TOA also shows that complete melting of 3TC occurs at 174 °C, before that complete melting of pure drug, which was at 176 °C. In order to evaluate if this interaction occurs only after EFZ melting, with 3TC dissolution in the EFZ melted, or is due to an incompatibility in solid state, FTIR and ssNMR analysis were performed.

FTIR spectroscopy

There is no difference between the mixture and pure drugs spectra (Figure 5). According to the FTIR results, no interaction between 3TC and EFZ can be detected, probably due to its low resolution and peak overlapping. Monajjemzadeh *et al*. have reported a similar phenomenon in IR spectra of acyclovir, lactose and their mixture.[21]

ssNMR

The ssNMR mixture spectrum (Figure 6c) showed significant changes in chemical shift values when compared with spectra of pure drugs (Table 1). Although the changes in ppm are seemingly small, they clearly indicate alterations of the chemical environment felt by the two compounds (Figure 8). The changes in chemical shifts are more pronounced for the nuclei resonating at higher frequencies for both 3TC and EFZ. These results confirm that the interaction between EFZ and 3TC observed by DSC and TOA occurs in the solid state and involves especially electronegative and deficient electron atoms, probably due to weak acid-base interactions, since the changes in δ values are rather small.

These incompatibilities evidenced in DSC, TOA and ssNMR analyses are an important contribution for the development of a fixed-dose combination, since for the manufacture of a medicament stable, safe and effective it is clearly important to isolate the two drugs in two distinct layers in a multi-layer tablet, each of them containing excipients compatible with the drug. The development of this new formulation is another step in the treatment of AIDS, as patients can take fewer tablets per day, which can increase the treatment adherence.

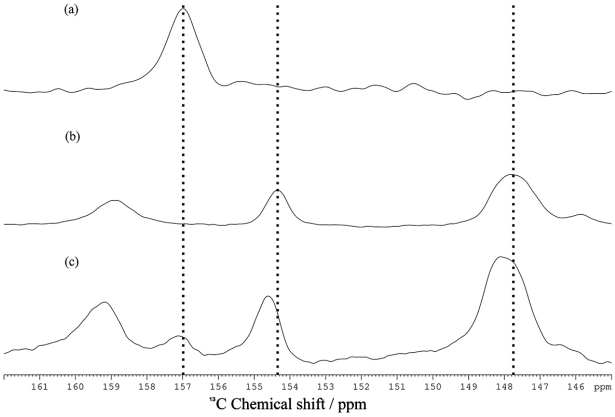

Figure 8. Spectra region amplification of (a) lamivudine, (b) efavirenz and (c) mixture, showing the changes in chemical shifts when drugs are mixed.

Conclusions

Thermoanalytical results have evidenced an incompatibility between lamivudine and efavirenz, which was confirmed by ssNMR analyses. NMR spectroscopy showed that the interactions occur mostly between electronegative and deficient electron atoms of both drugs, probably due to an acid-base interaction. This study shows the great utility of ssNMR, DSC and TOA analysis in pharmaceutical studies of drug-drug and drug-excipient compatibility, which allows the development of new stable formulations.

An alternative for the development of a stable pharmaceutical formulation containing both lamivudine and efavirenz in a fixed-dose combination, in spite of their incompatibility, is the manufacture of a multi-layer tablet, each one containing a unique drug.

Acknowledgment

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