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Short communication

Solid-state characterization of two polymorphic forms of *R*-albuterol sulfate

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Abstract

R-Albuterol (levalbuterol) is a drug used for asthma therapy and some formulations of it are in solid dosage forms. The aim of this work was to describe and characterize two polymorphic modifications of *R*-albuterol sulfate by means of typical structure-sensitive analytical techniques such as X-ray powder diffraction, FT-IR spectroscopy, visual and microscopic inspection, and DSC. Substantial differences were observed between the solid-state properties of the crystals, confirming the existence of at least two polymorphic forms for *R*-albuterol sulfate: Form I and Form II.

Keywords: *R*-Albuterol; Levalbuterol sulfate; XRPD; IR; Polymorphism; Crystal forms

1. Introduction

(±)-2-*Tert*-butylamino-1-(4-hydroxy-3-hydroxy-methyl) phenyl ethanol, also known as albuterol, is a β_2-adrenoceptor agonist prescribed for the treatment of bronchial asthma [1]. Albuterol is a racemate, and its bronchodilator activity resides in the (*R*)-isomer or levalbuterol [2,3].

In 1997, under the chiral switch strategy [4], levalbuterol hydrochloride and levalbuterol sulfate (RSS, Fig. 1) were approved by the FDA [5] and since 1999 levalbuterol hydrochloride has been marketed as a nebulizer solution. Levalbuterol formulations have been developed in syrup, controlled release tablets, metered dose inhalers, and dry-powder inhalers although some of these are not on the market yet [6].

Polymorphism is defined as the ability of a substance to exist as two or more crystalline phases or forms that have different arrangements and/or conformations of the molecule in the crystal lattice [7]. In consequence, the polymorphic solids have different chemical and physical properties with biopharmaceutical effects in the dissolution rates and/or bioavailability [8–10]. Therefore the physical characterization of the solid state of a drug has become an extremely important area in pharmaceutics and has been the subject of many studies involving different analytical methods [11].

In the context of producing RSS for biological tests, we observed that RSS crystallizes in two different ways, and this work was conducted in order to characterize these two crystal forms by X-ray powder diffraction (XRPD), diffuse reflectance IR Fourier transform (DRIFT) spectroscopy, visual inspection and DSC.

2. Materials and methods

2.1. Materials

RSS was prepared as described in a previous report [12] and the purity of this compound was 99.57% assayed against albuterol sulfate USP standard, with an optical purity of 99.8% by HPLC [13].

All solvents used in this study were HPLC grade (Fisher Scientific, New Jersey, NJ).

2.2. Methods

2.2.1. Optical microscopy

Crystal morphologies of RSS Form I and RSS Form II were observed with an Olympus SZX12 optical microscope (Olympus

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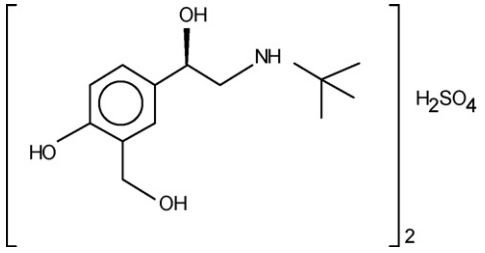

Fig. 1. *R*-Albuterol sulfate (RSS).

Optical Co., Ltd.) equipped with an optical polarizer and linked to a digital camera (Olympus D11, Olympus Optical Co., Ltd.), using Image Processing software.

2.2.2. Powder X-ray diffractometry (PXRD)

The diffraction patterns were collected using a Bruker D8-Advance powder diffractometer, in θ–θ geometry, using Cu Kα radiation and working at 40 kV and 30 mA. The Sol-X® solid-state Si(Li) detector was used. C/Ni Goebel mirrors in the incident beam were used as a monochromator; 1.0 mm divergence and scatter slits and a 0.1 mm receiving slit were used, taking care to avoid introducing preferred orientation of the crystallites.

2.2.3. Infrared spectroscopy

Fourier transform infrared (FTIR) spectra were acquired on a Shimadzu spectrometer (Shimadzu, Kyoto, Japan). Spectra over a range of 4000–500 cm^{-1} with a resolution of 2 cm^{-1} (50 scans) were recorded using KBr pellets. For diffuse reflectance analysis, samples weighing approximately 2 mg were mixed with 200 mg KBr by means of an agate mortar and pestle, and placed in sample cups for fast sampling.

2.2.4. Thermal analysis

DSC thermograms were recorded with a DSC 2920 modulated Differential Scanning Calorimeter (TA Instruments, New Castle, DE). Samples weighing 5–8 mg (Precisa 262SMA-FR Balance) were heated in crimped aluminum pans from 30 to 300 °C at a rate of 10 °C min^{-1} under static air.

2.2.5. HPLC analysis

A Waters 2690 HPLC system with quaternary pump and autosampler, and a Waters 996 photodiode array detector were used. For data acquisition, Millennium 3.20 software was used. All separations were achieved using a 25 mm × 4.6 mm Chirobiotic T column (amphoteric glycopeptide Teicoplanin bonded to a 5 μm silica gel) from ASTEC (Whippany, NJ).

All samples and standard solutions were chromatographed at ambient temperature (22 ± 2 °C) using an acetonitrile/methanol/acetic acid/triethylamine mixture (60:40:0.3:0.2, v/v/v/v) as the mobile phase (flow rate of 1.5 ml min^{-1}), with detection at 276 nm and an injection volume of 10 μl.

2.2.6. Solution-state NMR

NMR spectra were obtained in a Bruker NMR with a Bruker AC 200 console (Bruker, Germany). The spectra were processed with WinNMR software (Bruker, Germany). The samples were prepared dissolving 5 mg of each form in 0.5 ml of DMSO d_6 with 0.03% of tetramethylsilane (TMS, Sigma–Aldrich Chemical Co. Inc., Milwaukee, USA) used as reference for $\delta_H = 0$.

3. Results and discussion

Levalbuterol sulfate is soluble only in water, slightly soluble in methanol and practically insoluble in other polar and apolar organic solvents. It also has poor solubility in ethanol but when it is re-crystallized from this solvent, some products of decomposition are observed. Due to these limitations, water and methanol were the only solvents used for screening polymorphic forms of RSS.

Crystallization of RSS from water always gave the same type of crystals (Form I), while two different types were obtained in the crystallization from methanol.

Crystallization of a saturated solution of RSS in methanol at 15–30 °C, gave a solid that, after being dried at 25 °C, was labeled as Form I. When Form I was dissolved in refluxing methanol, allowing some solid to remain insoluble, crystals with different habits were obtained after the filtration and vacuum drying (25 °C) of that insoluble material. This was named Form II. Both forms were analyzed by HPLC, showing that no by-products or decomposition compounds had been formed during the preparation of the crystals. RSS Form I was also obtained when RSS Form II was vacuum dried at 60 °C for 8 h and again the HPLC analysis showed that the compound had remained unchanged. This transition was also observed when the crystals were heated to 40 °C but this took a longer period of time.

After obtaining the two crystal forms of RSS, images of crystal representing the morphologies of RSS Form I and RSS Form II were recorded. They showed differences in morphology between the two powders. RSS Form I has a crystalline prismatic habit; the crystals reached 3–6 mm in length and formed a twin with an angle of 30° to axis c (Fig. 2a). RSS Form II has a laminate habit and developed colorless pseudohexagonal equidimensional individuals, 0.06 mm in diameter (Fig. 2b). An inspection of the two forms by liquid NMR and DSC (see below) indicated that the crystals did not correspond to solvates or hydrates of RSS and the presence of impurities was also discarded.

The XRPD patterns of RSS Form I and II samples are shown in Figs. 3 and 4, respectively. These show distinct differences in positions and relative intensities. RSS Form I shows characteristic peaks at 10.7; 11.9; and 12.6 ($2\theta \pm 0.2°$ 2θ) that correspond only to this form while RSS Form II shows characteristic peaks at 8.7; 9.6 and 15.2 ($2\theta \pm 0.2°$ 2θ). The comparison between the XRPD powder patterns of RSS Forms I and II indicate that they have different crystal structures, and that therefore they are likely to be two polymorphic forms of RSS.

Beside, the XRPD powder patterns of both forms, were different respect to the XRPD patterns of the commercially available (±) albuterol sulphate [14].

The FTIR spectra of the RSS Form I (Fig. 5) and RSS Form II (Fig. 6) were similar except for the O–H and N–H stretch-

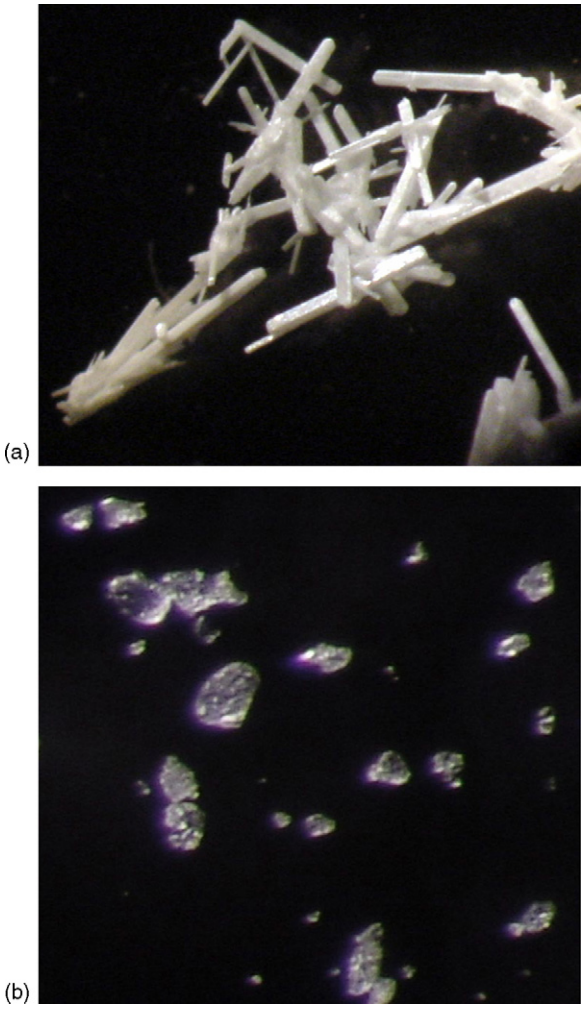

Fig. 2. Images highlighting the different morphologies of (a) RSS Form I (1 cm ≅ 3 mm) and (b) RSS Form II (1 cm ≅ 0.5 mm).

ing frequencies in the region of 3580–2300 cm^{-1}, presumably reflecting differences in hydrogen bonding, and for the absorption at 1600 cm^{-1} that showed a single peak in the spectra of Form I and a split peak in Form II. Most of the absorption peaks in the fingerprint region of RSS Form I were comparable to the

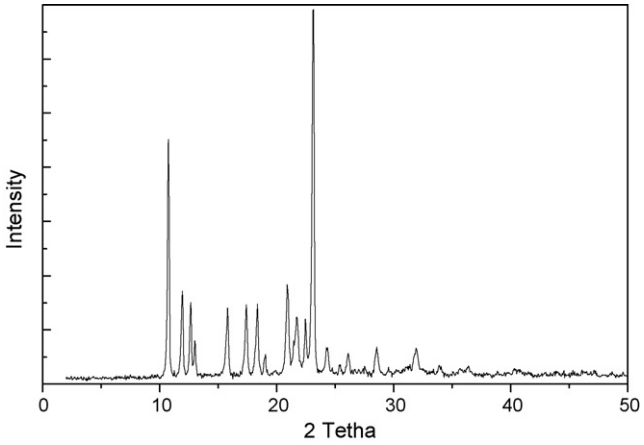

Fig. 3. XRPD Pattern of RSS Form I.

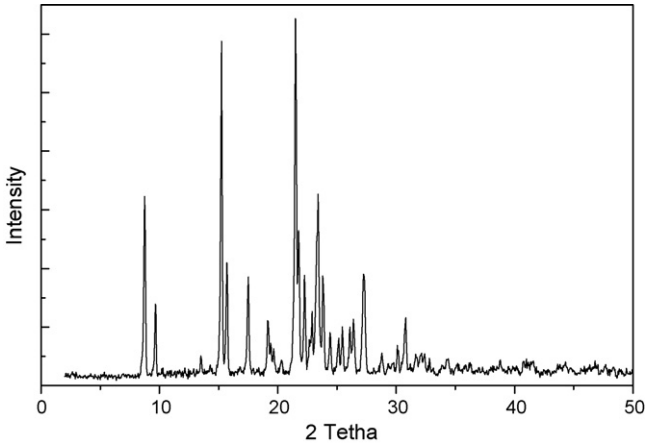

Fig. 4. XRPD Pattern of RSS Form II.

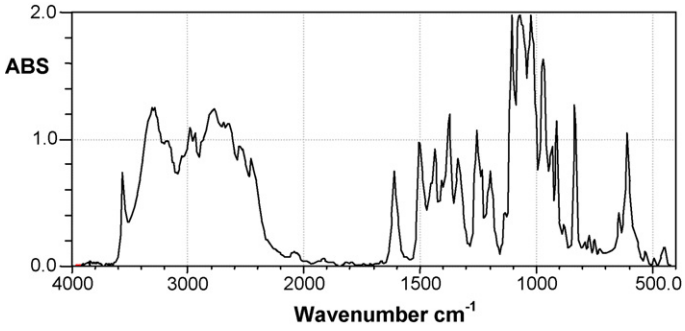

Fig. 5. FT-IR Spectra of RSS Form I.

corresponding peaks in the RSS Form II spectrum. Apart from some similarities between the two spectrums, there were clearly defined differences in the region of 1500–3600 cm^{-1} showing that both compounds have different crystal structures and confirming the differences observed in the XRPD spectra of both forms.

DSC traces of RSS Form I did not show any melting point but a decomposition pathway, characterized by three exothermic peaks at 210, 288 and 328 °C, was observed. RSS Form II showed the same pattern (217, 283 and 328 °C) but the first peak was 7 °C higher in temperature than the corresponding peak of RSS Form I (Table 1). Unfortunately not much information could be obtained from the thermograms because only decomposition processes were detected. However, the small difference

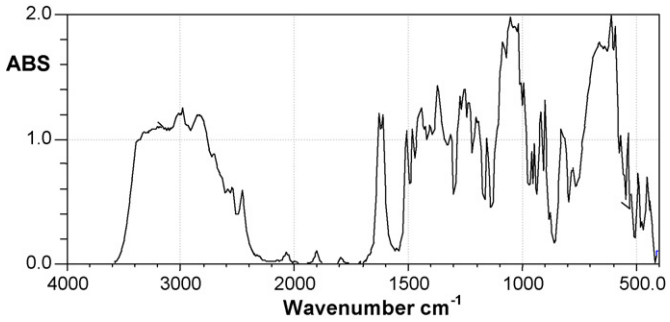

Fig. 6. FT-IR Spectra of RSS Form II.

Table 1
DSC data of RSS Form I and Form II crystals with the corresponding heat required

Thermal events	RSS Form I		RSS Form II	
	Endotherm (°C)	Heat (J/g)	Endotherm (°C)	Heat (J/g)
Decomposition 1	210	14	217	19
Decomposition 2	288	15	283	15
Decomposition 3	328	11	328	9

in the decomposition temperature could be associated with the different crystal morphology of Forms I and II.

No solvate or hydrate were observed in the DSC assay according to the NMR results. The expected transition between the two polymorphic forms was not found either, even though in a bulk experiment the transition from Form II to Form I was seen by heating Form II at 40 °C or higher temperature under vacuum conditions.

4. Conclusions

In summary, two different crystal forms of RSS were studied by XRPD and FT-IR spectroscopy and DSC, leading to the characterization of two polymorphs, Forms I and II, for levalbuterol sulfate. From the results it can be concluded that the most physically stable form of RSS is Form I, with Form II converting to Form I by heating at least 40 °C under vacuum. The X-ray diffractograms as well as the IR-spectra of Form I and II are very different and enable a clear, fast identification of the polymorphs, while the DSC curves did not show significant differences hindering identification of the polymorphs.The significant differences in the polymorphs morphology could impact not only in the dissolution rate and, as a direct consequence, in the bioavailability of the drug, but also in the pharmacotechnology, since compressibility, fluidity, stability of the polymorphs are expected to be different.

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