Shimadzu (Kyoto, Japan) chromatograph equipped with an Shimadzu LC 8A pump, SCL-6B control unit, SIL-6B auto injector, and an SPD-7A UV detector. Analytes were separated on a 5 μm LiChrospher 60 RP-select B (250 × 4 mm; Merck, Darmstadt) column. The liquid phase was a mixture of acetonitrile/phosphate buffer (30/70, vols; 0.05 M, pH 4.6) at a flow rate of 1 ml/min. UV detection was at 210 nm to accommodate the low UV absorption of fentanyl. Samples (50 μl) of undiluted infusion solution were injected, and each analysis was replicated 5 times. The content of the test solution was determined with a series of concentrations of Fentanyl (Sigma Chemie) as reference standard in the range of 0.5–5 μg/ml, according to the European Pharmacopoeia. The interday and intraday precision (coefficient of variation) was <10%. The stability-indicating capability of the assay was determined by subjecting a sample to extremes of heat (60 °C, 2 h) and pH (2 and 12). There were no interfering peaks in either chromatograms.

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Variable temperature X-Ray Powder Diffractometry of spironolactone polymorphs

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Spironolactone is a diuretic steroidal aldosterone agonist known to show variable and incomplete oral bioavailability because of poor water solubility and dissolution rate [1]. This might be due to variations in the crystal form because the crystal properties of spironolactone are complex; it can adopt polymorphic, non-stoichiometrically solvated or amorphous glass forms from the same solvents, and can undergo solvent mediated and other solid-state transformations [1–9]. This study reports the usefulness of variable temperature X-ray powder diffractometry (VTXRPD) as a fast method to characterize and measure the transformation between two spironolactone polymorphs, and mixtures thereof, found among raw material samples randomly obtained from pharmaceutical bulk suppliers.

Salole and Al-Sarraj [2] and El-Dalsh et al. [3] published the first extensive reports describing and characterizing spironolactone polymorphs. Their research identified four polymorphic forms – three metastable, and one stable form – and several pseudopolymorphs. Agafonov et al. [1] prepared two non-solvated forms (I and II) and four solvated crystalline forms and described the morphology, symmetry, and the crystallographic parameters of five of these forms. Their results showed that under general crystallization and preparation procedures spironolactone most probably crystallize either as the metastable form I and form II the thermodynamically stable form. Form I was formed by fast crystallization from various organic solvents. It most probably represents a desolvated form since structural evidence indicated that in this form the solvent molecules incompletely occupies the four allowed positions in the unit cell with space group $P2_12_12_1$. These two forms are also classified as monotropic polymorphs, because the two forms do not transform one into another by heating [1]. The most recent report on spironolactone polymorphism identified three true polymorphs similar to those described in the other reports but this study rules out the formation of stable pseudopolymorphs [9]. All these researchers concluded that the facility with which spironolactone crystallize in different crystal forms is due to a flexible lattice allowing the molecule to adopt subtly distorted conformations [5, 6, 8].

In this study, the physicochemical properties of five randomly obtained samples of spironolactone were determined. The median particle sizes by volume of all the samples were identical and small, ≤ 6 μm. There were no

significant differences in the dissolution of the powders in three dissolution media (0.1 N HCl + 0.1% SLS; 0.1 N HCl; H_2O). However, the dissolution rates in the three media decreased in the order 0.1 N HCl + 0.1% SLS > 0.1 N HCl > H_2O. In H_2O and 0.1 N HCl only 17% and 16% respectively dissolved after 60 min for both the stable and the metastable forms. DSC analysis showed that samples 1 and 2 exhibited a single melting endotherm at 204 °C and samples 3, 4 and 5 at 205–206 °C. No additional crystal transformations, other than the melting process, were observed. These results were not in line with the reported melting points of the crystal forms and at first glance suggested that the samples contained the same crystal form. However, small differences in the DRIFTS spectra of the two groups of powders suggest the presence of some impurities (residual solvents) or polymorphic mixtures.

According to XRPD data (Figs. 1 and 2) the five samples represented two distinctive groups of spironolactone powders. Based on the X-ray diffraction data for the different crystal forms of spironolactone reported by Aganofov et al. [1], samples 3, 4 and 5 were the same as the thermodynamically stable form obtained from acetone, i.e. form II. Fig. 1 shows the XRPD patterns of sample 3 with an increase in temperature. These samples did not show any change in crystal form upon heating up to 195 °C and represents pure samples of form II, characterized by a singlet at 9.2 °2θ, a doublet at 11.6 and 12.2 °2θ, and a triplet at 16.1, 16.8 and 17.3 °2θ in the XRPD pattern. The XRPD patterns of samples 1 and 2 were different from that of the thermodynamically stable form II. Careful analysis of the XRPD patterns (Fig. 2) of these powders showed that the samples were mixtures of form I and II. Both the main peaks mentioned above for form II and those characteristic for form I (13.2, 14.6, 15.2, and 17.6 °2θ) were present in the XRPD patterns of these

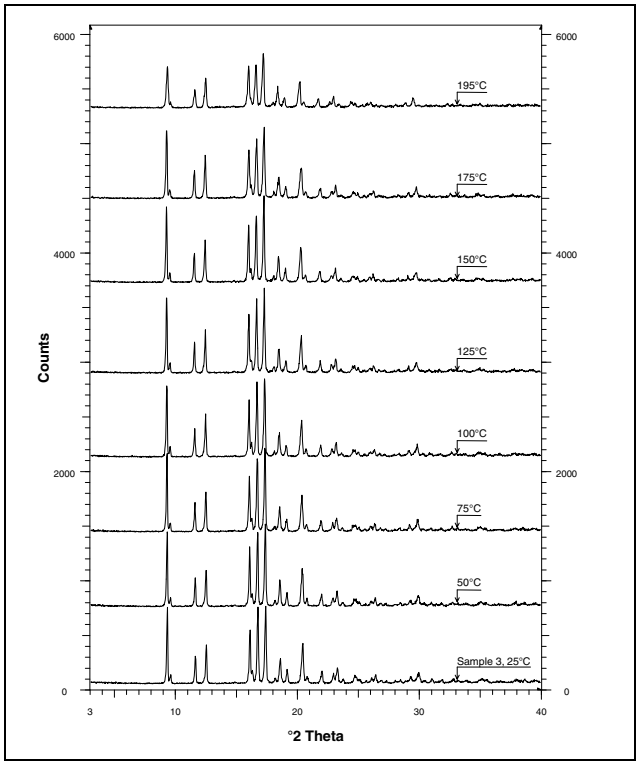

Fig. 1: Variable temperature XRPD patterns of spironolactone sample 3, representing the thermodynamically stable crystal form II as described by Agafonov et al. [1]

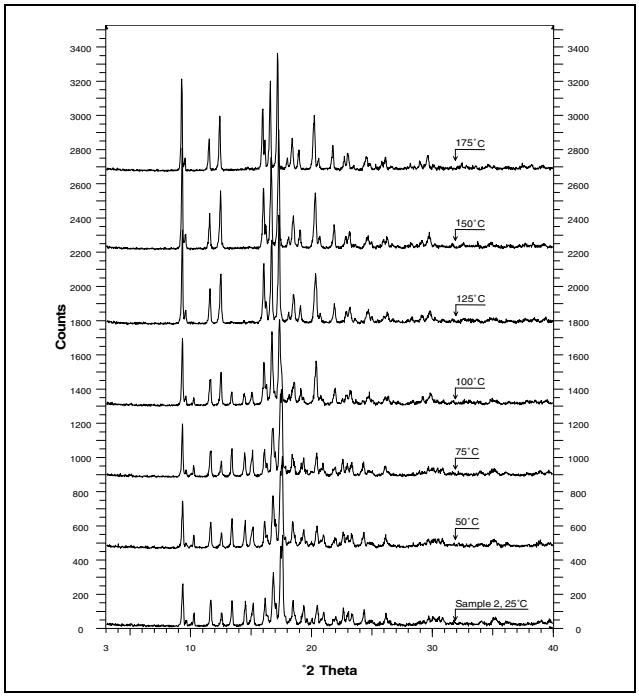

Fig. 2: Variable temperature XRPD patterns of spironolactone sample 2, characterizing the phase changes upon heating of a powder containing a mixture of form I and II

samples. Further analysis of the XRPD patterns showed that these powders contained between 20–50% of form I. Previously another sample from the same supplier of sample 2 spontaneously transformed to form II when stored at room temperature. Upon heating (Fig. 2) the mixture is also complete transformed to form II. The change was gradual in the temperature range from 25–75 °C. As the temperature increased above 100 °C samples 1 and 2 were quickly transformed into form II. This polymorphic change is evident from the disappearance of the peaks at 13.2–15.2 °2θ. The XRPD pattern at 175 °C also matches that of form II shown in Fig. 1. This result is contradictory to previous reports that form I and II are monotropic crystal forms that are not converted into each other upon heating.

This comparative raw material characterization study confirmed that spironolactone exists in different crystal forms, predominantly the thermodynamically stable form II and mixtures of this form and a metastable form I. Out of five samples tested, three were form II and two a mixture of form I and II. Mixtures of the intermediate metastable form and the stable form of spironolactone had comparable melting points and DSC analysis could therefore not be used to determine the polymorphic purity of the samples. IR analysis and dissolution testing were also not able to distinguish between the crystal forms. VTXRPD proved to be very useful in establishing the polymorphic purity of the samples [10]. It also conclusively showed that form I, the metastable form, transformed to form II the thermodynamically stable form. This change was more rapid at higher temperatures.

Experimental

Spironolactone powders were randomly obtained from five suppliers. These samples were characterized by X-ray powder diffractometry (XRPD), Diffuse Reflectance Infrared Spectroscopy (DRIFTS), Differential Scanning Calorimetry (DSC), Thermogravimetric Analysis (TGA), powder dissolution, and variable temperature X-ray powder diffractometry

(VTXRPD). DRIFTS spectra were recorded on a Nicolet Nexus 470-FT-IR spectrometer (Thermo Nicolet, USA) over a range of 600–4000 cm^{-1}. Powdered samples were mixed with KBr prior to the measurement. X-ray powder diffraction profiles were obtained with a Bruker D8 Advance diffractometer (Bruker, Germany). The measurement conditions were: target: Cu; voltage: 40 kV; current: 30 mA; divergence slit: 2 mm; anti scatter slit: 0.6 mm; detector slit: 0.2 mm; monochromator; scanning speed: 2°/min (step size: 0.025°; step time: 1.0 s). The effect of an increase in temperature (variable temperature X-ray powder diffractometry, VTXRPD) on the XRPD pattern was investigated with an Anton Paar TTK 450 low-temperature camera, attached to the Bruker D8 Advance diffractometer. A heating rate of 10 °C/min was used during all the measurements. DSC thermograms were recorded with a Shimadzu DSC-50 instrument (Shimadzu, Japan). The measurement conditions were: sample weight: ≈2 mg; sample holder: aluminum crimp cell; gas flow: nitrogen at 40 ml/min; heating rate: 10 °C/min. Mean volume particle size distributions in suspension were measured with a Galai-Cis-1 particle size analyzer (Israel). Powder dissolution was measured using Method 2, paddle, of the USP 24. The paddle was rotated at 75 rpm and samples were taken at 7.5, 15, 30, 45 and 60 min intervals. The powder sample, 50 mg, was rinsed from the glass weighing boat into a 10 ml test tube with exactly 2 ml of the dissolution solution. Glass beads, 25 mg, with a mean size of 0.1 mm, were added to the suspension and the mixture was agitated for 120 s using a vortex mixer. The contents of each test tube was transferred into the dissolution medium, 1000 ml (0.1 N HCl + 0.1% SLS; 0.1 N HCl; H_2O) and the dissolution rate was measured. The concentration of dissolved powder was calculated from the UV absorbance at 242 nm. Results are the mean of six experiments.

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Use of 1,4-dioxan for preparation of bupivacaine loaded PLGA microspheres with an o/w emulsion extraction process

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The oil-water emulsion extraction process is one of the most popular methods for preparation of microspheres. In this method the organic solvent used for dissolving PLGA is extracted with water what results in polymer precipitation. Benzyl alcohol is the most frequently used solvent in this procedure, however acetone, ethyl-methyl ketone, ethyl formate, ethyl acetate and dimethyl sulphoxide (DMSO) were used in some studies [1–5]. The aim of this study was to prepare microspheres with 1,4-dioxan, to our knowledge not used before for such purpose. According to the pharmaceutical classification, dioxan is a class 2 solvent (methylene chloride, widely used pharmaceutical solvent belongs to the same class) [6], what means that it may be used in technological processes, but its residue in the product must strictly be controlled (LD_{50} after oral delivery is 2 g/kg) [7]. The advantage of this solvent is its good miscibility with water and most organic solvents and a high freezing point (11.8 °C), what enables removing the residues during the freeze-drying step in a process of preparation of microspheres.

Microspheres prepared with dioxan (formulation D) were compared with those obtained with benzyl alcohol (formulation BA) and DMSO (formulation DMSO). Bupivacaine was encapsulated in the microspheres in order to study the relationship between the type of solvent and encapsulation and drug release rate from the PLGA matrix.

Solubility of bupivacaine in dioxan and benzyl alcohol was very good (at least 400 mg/ml) while in DMSO the drug was less soluble (50 mg/ml). PLGA dissolves easily in dioxan as well as in the two other solvents. The microspheres were prepared by a standard procedure [1].

The microscopic observation revealed that microspheres prepared with benzyl alcohol were spherical, sizing in range 1–20 μm (80% in the range 1–5 μm) (Table). In contrast to the formulation BA, formulations D and DMSO were porous and larger in size (80% of the particle in the range 1–15 μm). Particles obtained with dioxan were spherical but a significant portion of the particles prepared with DMSO was irregular in shape.

When the ratio of bupivacaine to PLGA was 10/90 all solvents enabled producing microspheres, which were similar in size and shape to the drug-free particles. However, when the amount of the drug was elevated to 25%, microspheres were only produced if dioxan was used as a solvent. Production of spherical microparticles with other solvents was not possible due to fast and uncontrolled pre-