Thermodynamic Stability and Crystal Structures for Polymorphs and Solvates of Formoterol Fumarate

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ABSTRACT: Polymorph screening of formoterol fumarate was performed in 12 solvents, followed by evaluations of thermodynamic stability. Three anhydrates, a dihydrate, a diethanolate, a diisopropanolate, and a dibensylalcoholate were found. The crystal structure of three solvated modifications and of the most stable anhydrate was investigated. This indicated that solvation is needed to get a stable and well packed crystal structure. Thermodynamic testing suggests that five crystal modifications are thermodynamically stable, at different conditions, since they are all reversibly related to each other. © 2006 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 95:1144–1161, 2006

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INTRODUCTION

At the beginning of a new drug project there is usually only the neutral form of the active ingredient available. From this moment up until the launch there are a lot of activities going on, all aiming at optimizing the chemical, physical, and biological properties of the active ingredient. Two such activities are the salt screening and the polymorph screening. These activities are presently becoming more and more automated leading to more and more salts and crystal modifications (anhydrates, hydrates, and solvates) being found.[1,2]

However, for polymorph screening it is not the actual number of crystal modifications found which is important, but rather the quality of those actually found. Thus, excessive time spent on finding a large number of crystal modifications may not be cost effective. Instead, one should concentrate on finding those, which work well for formulation, large-scale production, and long-term storage. In the vast majority of cases these crystal modifications are the ones, which are thermodynamically stable. Thus, it is very important to complement the polymorph screening experiments with a number of polymorph evaluation experiments. These will also give information about the kinetic stability.

In the drug product only thermodynamically stable anhydrates and hydrates are of importance, whereas for formulation and large-scale production, knowledge about thermodynamically stable solvates may also be very useful. Kinetically stable crystal modifications may also be important, but one does not need to specially search for these, they will, for obvious reasons, always be found in ordinary polymorph investigations.

Once a polymorph screen has been performed, the thermodynamic stability of the modifications

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found should be evaluated. Unfortunately it is still not possible to definitely determine that certain crystal modifications are actually the most stable ones at certain conditions of pressure, temperature, and chemical environment (i.e., solvent). One can only compare the modifications found and state that some of them are more stable than others. For this reason, fear for the sudden disappearance of a crystal modification has developed.[3] With careful design of the polymorphism experiments this can probably be completely eliminated. The AstraZeneca approach is to perform the following tests, to support the assumption that the most stable crystal modifications have actually been found:

- As a start all anhydrates found should be investigated for enantiotropy or monotropy.[4–6] First the melting point for each anhydrate is determined from DSC measurements. Then one can either perform solubility measurements or competitive slurry experiments in a nonsolvate forming solvent in which the substance has moderate solubility. In competitive slurry experiments two relevant anhydrates (ideally 50:50% w/w) are allowed to compete at temperatures from 0°C up to the highest temperature of interest (below the melting point of the highest melting anhydrate).
- If the anhydrate with the highest melting point competes successfully at low temperatures, the system is monotropic in this temperature range and the highest-melting anhydrate is the thermodynamically most stable one found so far. The long-term stability of this anhydrate should then be tested by slurry experiments for a sufficiently long time (at least 2 months) in a number of nonsolvate forming solvents with varying properties (hydrophilic, hydrophobic, protic, nonprotic, etc.).
- If a number of enantiotropically related anhydrates are found, it is important that the phase transition temperatures are found, and that the phase transformations below and above these are very carefully tested for reversibility. This is because true reversibility is yet another thing which cannot be definitely determined. However, support can be found by the crossing of solubility curves or, better by competitive slurry experiments at more and more refined temperatures. Finally, when the temperature stability range for each anhydrate is known, each enantiotropic anhydrate is long-time slurried, at a temperature in its stability range, as described above. This ascertains that all enantiotropic anhydrates are thermodynamically stable.
- From the above, one anhydrate, which very likely is thermodynamically stable at room temperature has been found. This is then allowed to compete, at room temperature, with any hydrates and solvates found, in slurry experiments in the respective solvent or mixtures thereof. Any hydrate or solvate, which successfully competes against the most stable anhydrate, is also very likely thermodynamically stable at the chosen conditions of temperature, pressure, and solvent. If possible the reversibility of these solvate-ansolvate or hydrate-anhydrate equilibria should also be carefully tested by determining the solubility curves in the corresponding binary or ternary phase diagrams.[4,6,7]
- Summing up, a number of reversible relationships between one (monotropic system) or several (enantiotropic system) anhydrates and a number of hydrates and solvates have now been determined (a schematic example is given in Fig. 1). In such a system, determined under thermodynamic relevant conditions (wet systems studied during long times), it can be asserted, with a very high degree of certainty, that all modifications involved are thermodynamically stable, and all phase transitions involved are truly

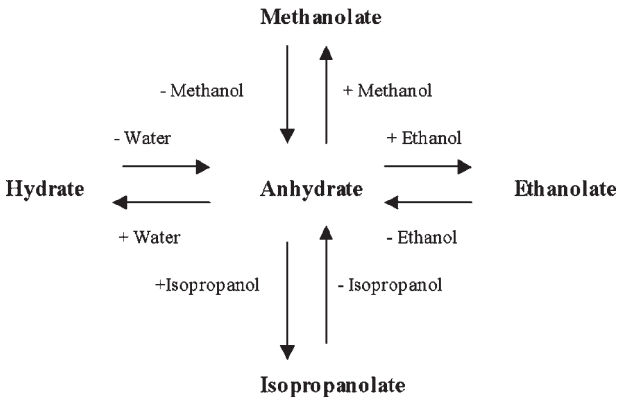


Figure 1. A typical scheme over the reversible equilibria between various crystal modifications of a drug substance.

reversible. The results are conveniently summarized as a number of phase diagrams.
- It should be noted that experiments where the type of false reversibility, which often is obtained by drying/wetting a hydrate in a water sorption instrument or by drying hydrates or solvates with dry gas or in vacuum or heat, must not be involved in this. For safety, reversibility testing should always be performed by long-term slurry experiments in solution.

For formoterol, $C_{19}H_{23}N_2O_4$ a hemifumarate salt was chosen for drug development [(R*,R*)-(±)-*N*-[2-hydroxy-5-[1-hydroxy-2-[[2-(4-methoxyphenyl)-1-methylethyl]amino]ethyl]phenyl]formamide, (E)-2-butendioate (2:1)]. As a simplification in this article, the combination of two formoterol molecules and one fumaric acid molecule will be addressed as "formoterol fumarate." For this salt a polymorph screen was designed. The evaluation of the crystal modifications found concentrated on finding thermodynamically stable ones. Those found have been characterized using standard techniques.

EXPERIMENTAL

Materials

All solvents used were of at least p.a. grade. Water was deionised. The two batches of formoterol fumarate dihydrate used were obtained from AstraZeneca R&D in Lund, Sweden and from AstraZeneca Bulk Production in Södertälje, Sweden. Their purities as determined by HPLC were around 99.8%.

Dry formoterol fumarate was obtained by drying the dihydrate at 85°C for more than 2 h in a stream of dry nitrogen gas or for more than 4 h in a vacuum oven. The dried substance was then stored in a desiccator containing dry silica gel. It should be noted that formoterol is a very potent substance and must not be unintentionally inhaled. All experiments must be performed in a fume hood and cleanliness is of vital importance.

Polymorph Screen Crystallisation Experiments

Depending on solubility, 30–400 mg of formoterol fumarate dihydrate (series 1) or of dry formoterol fumarate (series 2) was added to 1–250 mL of solvent. The solutions or slurries were mixed for a couple of hours and then filtered. The saturated solutions were treated in one of three ways:

- evaporation to dryness at room temperature,
- evaporation at room temperature to crystal formation followed by separation of the crystals by filtration,
- rapid cooling followed by separation of the crystals by filtration.

Twelve solvents with different hydrogen bonding properties and covering a span in dielectricity constant from 2 to 80 were used (see Tab. 1). The two least polar solvents, toluene and cyclohexane, did not dissolve enough substance to make crystallization experiments feasible.

Table 1. The 12 Solvents Used for the Polymorphism Screening Experiments

Solvent	H-Bond Property	Dielectricity Constant at 20°C
Water	Donor–acceptor	80
Acetonitrile	Acceptor	38
Methanol	Donor–acceptor	34
Ethanol	Donor–acceptor	24
Acetone	Acceptor	21
Isopropanol	Donor–acceptor	18
Ethylmethylketone	Acceptor	18
Pentanol	Donor–acceptor	14
Benzyl-alcohol	Donor–acceptor	13
Dimethylamide	Donor–acceptor	6
Toluene	Not H-bond active	2
Cyclohexane	Not H-bond active	2

The H-bond properties indicated do not indicate weak hydrogen bonding involving carbon atoms or π-interactions.[8–10]

Slurry Experiments

As part of the polymorph screening, 50–100 mg of dry formoterol fumarate was shaken for 1–6 days in 4–5 mL of dry methanol, isopropanol, ethanol (95 and 99.5%), and pentanol. This was done in order to produce more solvates.

As a check for thermodynamic stability, 50–100 mg of each of the anhydrates found were shaken for 1–6 days in 4–5 mL of dry ethyl acetate.

Further stability testing of the solvates was made as competitive slurry experiments for 3 days. These were performed using 0.2–0.55 g of dry formoterol fumarate in 5–12 mL of the respective solvent. To this seeds of other crystal modifications were added together with varying amounts of water.

At the end of each slurry experiment a wet sample of the solid phase was extracted and analyzed with XRPD.

Solubility Measurements

Having proven by using slurry experiments, with subsequent XRPD-analysis of the solid phase, that the salt did not disproportionate in water at ambient conditions (room temperature and in contact with atmospheric carbon dioxide), the solubility measurements were performed in water, at room temperature as a function of time during a period of 120 h. Eleven samples containing 6 mg of formoterol fumarate dihydrate were shaken in 2 mL of water. At given moments of time a sample was withdrawn, filtered, and analyzed for the amount of formoterol fumarate with HPLC. The maximum value on the time-concentration curve was used as a measure of apparent solubility. For anhydrates A, B, and C and the amorphous sample a similar procedure was used but using 15 mg of substance.

Single Crystal Growth Experiments

A saturated solution of formoterol fumarate in ethanol at 50°C was prepared by dissolving dry formoterol fumarate in 99.5% ethanol. The solution was not filtered but placed in a vacuum desiccator and allowed to cool. It was then pumped occasionally and after a couple of days, large agglomerates of platelike, anhydrate B, single crystals began to appear. The procedure was repeated twice with the same result. From one such agglomerate a single-crystal of anhydrate B, suitable for X-ray diffraction was selected.

To prepare the diethanolate a slightly different procedure was used. A saturated solution of formoterol fumarate in ethanol at 50°C was prepared by dissolving dry formoterol fumarate in 99.5% ethanol and the solution was filtered through a 0.50 μm filter and then placed in a vacuum desiccator and allowed to cool. It was then pumped occasionally and after 1 day large agglomerates of needle shaped diethanolate single crystals began to appear. The procedure was repeated twice with the same result. From one such agglomerate a single-crystal of the diethanolate, suitable for X-ray diffraction was selected.

The only difference between the two experiments in ethanol described above is the filtering. Obviously, seeds affect crystallization in ethanol. This is further discussed in subpart Kinetic Competition between Anhydrate B and the Diethanolate in Ethanol.

A saturated solution of formoterol fumarate dihydrate in DMSO was prepared at room temperature. The solution was placed in a vacuum desiccator together with a beaker containing water. The desiccator was pumped occasionally and after a couple of days large agglomerates of rhomb shaped single crystals of the dihydrate began to appear. The procedure was repeated twice with the same result. From one such agglomerate a single-crystal of the dihydrate, suitable for X-ray diffraction was selected.

A saturated solution of dry formoterol fumarate in isopropanol was prepared at room temperature. The solution was filtered through a 0.50 μm filter and then placed in a vacuum desiccator. It was pumped occasionally and after 1 day large agglomerates of needle shaped crystals of the diisopropanol solvate began to appear. The procedure was repeated twice with the same result. From one such agglomerate a single-crystal of the diisopropanolate, suitable for X-ray diffraction was selected.

Experiments similar to the ones above were also performed in benzyl alcohol but the solvated crystals obtained were too small for single crystal X-ray diffraction investigations.

Differential Scanning Calorimetry

Using a Perkin-Elmer DSC7, thermograms were recorded between 40 and 200°C in dry nitrogen atmosphere, with a scan speed of 10°C/min. Due to the extreme hygroscopicity of anhydrate A, an extra isothermal period of 10 min was added at 90°C. In this way water taken up during sample

preparation was removed. In all measurements 1–4 mg of sample was used.

Moisture Sorption/Desorption Isotherms

Moisture sorption/desorption isotherms for anhydrates A, B, and C and for the dihydrate were recorded at 25.0°C using a Surface Measurement Systems Dynamic Vapour Sorption instrument. One cycle consisted of an initial drying phase and then analyses were performed in % RH steps of 10%. Equilibrium was considered obtained when the weight change per minute was less than 0.002%. Each cycle was recorded twice. After the second cycle, the samples were analyzed with XRPD.

Single Crystal X-Ray Diffraction

X-ray diffraction intensity data were collected at room temperature or at 200 K using an Enraf-Nonius κ-CCD diffractometer equipped with graphite monochromatised Mo-Kα radiation. The Denzo-SMN and the Maxus Software packages[11,12] were used to determine unit cells, space groups, perform data reductions, and to solve and refine the crystal structures. More information about the structure determinations can be found in Table 2 and in Supplementary Material.

Thermogravimetric Analysis

A Perkin-Elmer TGA7 was used to record the weight loss between 40–110°C. Dry nitrogen gas was used as purge gas and the scan speed was 10°C/min. An isothermal period at 110°C at the end of the experiment was run until a constant weight was observed. In all measurements 3–10 mg of sample was used.

X-Ray Powder Diffraction

XRPD diffractograms were collected using CuKα-radiation, on a Scintag XDS theta/theta diffractometer, equipped with a liquid nitrogen cooled Germanium solid-state detector. An angular range from 2 to 35° in 2θ was covered in steps of 0.03° at a scan speed of 1°/min.

XRPD diffractograms were also collected using CuKα-radiation, on a Philips X'Pert PRO diffractometer, equipped with an X'Celerator detector. An angular range from 2 to 40° in 2θ was covered using various scan rates. With the aid of the very fast X'Celerator detector semi-dynamic experiments could be performed where a wet sample was allowed to dry up while being analyzed multiple times.

RESULTS AND DISCUSSION

Results from the Polymorphism Screen

From the two series of crystallization experiments and the initial slurry experiments, seven crystal modifications were obtained (see Tab. 3 and Fig. 2):

Table 2. Summary of Crystal Data and Experimental Results from the Single Crystal X-Ray Crystallographic Investigations

	Dihydrate	Diethanolate	Diisopropanolate	Anhydrate B
Mw/g/mol	840.91	897.04	925.08	804.9
Crystal system	Triclinic	Monoclinic	Monoclinic	Monoclinic
Space group	P (−1)	P21/n	P21/n	P21/c
a/Å	6.736 (1)	17.507 (1)	17.292 (1)	19.615 (1)
b/Å	10.383 (1)	7.548 (1)	7.549 (1)	5.769 (1)
c/Å	16.571 (1)	18.328 (1)	19.605 (1)	21.070 (1)
α/Å	103.07 (1)	90	90	90
β/Å	98.01 (1)	98.24 (1)	100.85 (1)	111.83 (1)
γ/Å	104.12 (1)	90	90	90
V/Å	1071.4 (1)	2396.9 (4)	2513.4 (4)	2213.3 (4)
Z	2	4	4	4
Dc/mg/m^3	1.303	1.243	1.222	1.208
Radiation/θ-range/Å	Cu/2.80–74.04	Mo/1.12–27.76	Mo/1.06–27.44	Mo/1.04–24.93
Total no. of reflections	4606	7623	6668	4577
No of unique reflections	1637	2095	1489	1225
No. of parameters refined	293	290	299	NA
R/Rw	0.051/0.094	0.060/0.103	0.058/0.124	NA
Packing coefficient	68.0	66.0	65.5	62.6

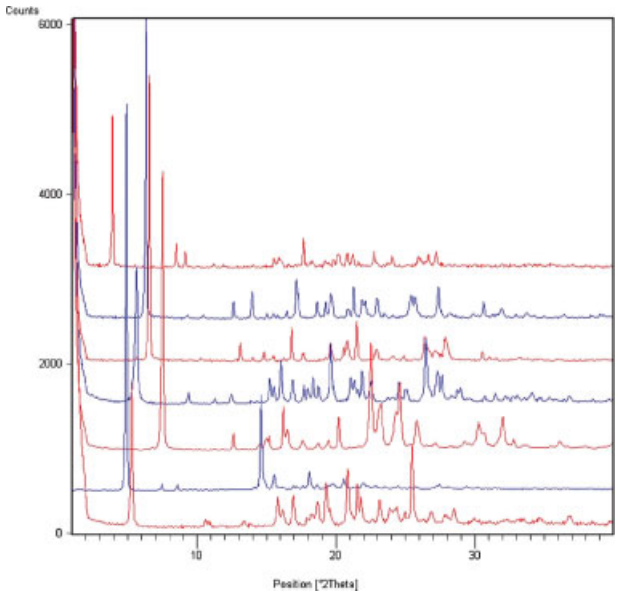


Figure 2. XRPD patterns for the seven crystal modifications obtained from the polymorphism screen. From bottom and up: anhydrate A, anhydrate B, anhydrate C, dihydrate, diethanol solvate, diisopropanol solvate, and dibenzyl alcohol solvate.

- a dihydrate was known already at the start of the polymorph screening and was also obtained in experiments where water was present (the only exception was that in benzyl alcohol below),
- a dibenzyl alcohol solvate was obtained from the series 1 experiment where the dihydrate was dissolved in benzyl alcohol,
- anhydrate A was obtained by drying the dihydrate in a stream of dry nitrogen at 85°C,
- anhydrate B was obtained from the series 2 crystallization experiments where anhydrate A was dissolved in dry acetone and in ethylmethylketone, respectively,
- a partially crystalline diethanol solvate was obtained from slurry experiments with anhydrate A in ethanol,
- a diisopropanol solvate was obtained together with anhydrate C from slurry experiments with anhydrate A in isopropanol,
- anhydrate C was obtained by leaving the diethanol or diisopropanol solvate to dry in a desiccator for a couple of days.

It should be noted that anhydrate B is very sensitive to grinding and pressing. Too much force will create changes in the XRPD diffractogram so that the first peak moves to higher 2θ values, whereas other peaks move in a nonparallel manner. Moisture has a similar influence due to the gradual transformation to the dihydrate. In order to get good diffractograms one must thus use dry conditions and mild sample preparation.

As can be seen from Table 3, the observed and theoretical weight losses match very well for the dihydrate and the dibenzyl alcohol solvate whereas there is less solvent than expected in the diethanol and the diisopropanol solvates. The reason for the misfit is the presence of amorphous material in the diethanol solvate and of anhydrate C in the diisopropanol solvate. Both the presence of amorphous material and of anhydrate C was detected with XRPD.

Thermodynamic Stability of Anhydrates

From the polymorph screening experiments there were indications that three crystal modifications might be more thermodynamically stable than the others. First of all, the dihydrate, which appeared almost every time water was present, was obviously quite stable. So was the dibenzyl alcohol solvate, the only solvate which could be crystallized in the presence of water and which thus

Table 3. The Seven Different Crystal Modifications Encountered Together with Experimental (TGA) and Calculated Weight Losses in Weight %

Modification	Weight Loss TGA/% w/w	Theoretical % Weight Loss/% w/w
A	0	0
B	0	0
C	0	0
Dihydrate	4.4	4.3
Diethanol solvate	8.2	10.3
Diisopropanol solvate	10.7	13.0
Dibenzyl alcohol solvate	21.6	21.2

Table 4. Observed Melting Range (Hot Stage Microscopy), Melting Points and Melting Enthalpies (DSC), and Apparent Water Solubility of Selected Crystal Modifications of Formoterol Fumarate at Room Temperature

Modification	Melting Range/°C	Melting Point/°C	ΔH Melting/kJ/mol	Solubility/mM
Anhydrate A	125–129	127	49	4.7
Anhydrate B	143–148	150	61	3.5
Anhydrate C	120–125	123	52	7.5
Dihydrate	120–127	125	126	1.5
Amorphous	NA	NA	NA	5.3

It should be noted that modification C has in fact a higher solubility than amorphous material. The enthalpy for the dihydrate is for a combined dehydration and melting event.

could resist dihydrate formation. Finally, of the three anhydrates, only anhydrate B had appeared by crystallization. This anhydrate also had the highest melting point of the three.

Solubility data at room temperature (in Tab. 4 apparent solubilities are given) indicates that anhydrate B is more stable than the other two anhydrates at room temperature. DSC data show that anhydrate B has a higher melting point than anhydrates A and C. This shows that anhydrate B is actually more stable than A and C over the whole temperature interval from room temperature up to its melting point at150°C. As a further test of this, anhydrates A, B, and C were each slurried at room temperature in dry ethylacetate. B remained unchanged whereas both A and C were transformed to B. It can thus be concluded that A and C most probably are monotropic anhydrates. However, if three anhydrates are related with three different melting points and if the one with the highest melting point has been proven stable at room temperature, there is also the possibility that they are enantiotropically related, with phase transition temperatures far below room temperature. However, it is not very likely that this is the case since

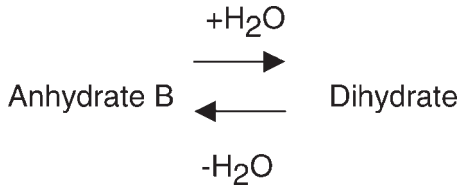
- anhydrates A and C are both obtained by dehydration/solvation (A from the dihydrate and C from the diethanolate or-isopropanolate) and are thus potentially a so called dehydrated hydrate and a desolvated solvate (i.e., have crystal structures, which are related to the corresponding solvates),
- there are quite large differences in solubility between anhydrates A, B, and C.

It should also be noted that anhydrate B cannot be a desolvated solvate since it can be crystallized from saturated solutions of nonsolvate forming solvates such as acetone and ethylacetate.

Thermodynamic Stability of the Dihydrate

If anhydrate B and the dihydrate are thermodynamically stable they should form a phase diagram of the type[13] shown in Figure 3. Two slightly different cases exist, with congruently or incongruently melting hydrates. DSC-data indicate that the dihydrate melts incongruently but this has not been investigated in detail.

If a slurry of anhydrate B in water at 25°C is made, its total composition will be somewhere along the dotted line in area B, with a saturated solution of composition indicated by the arrow. Since this is in the area of thermodynamic stability of the hydrate, this means that the anhydrate after some time will be converted to the hydrate. This is exactly what happens to anhydrate B in water and this proves one direction of the reversibility in Scheme 1.



Scheme 1.

To test the other direction one has to cross the point between areas B and D. Being the point where a slurry of the dihydrate crystals in saturated solution dries up, this borderline cannot be crossed in a slurry experiment. To solve this problem one more solvent component is added to get a ternary system of two solvents and the drug substance (Fig. 4).

In such a system all areas (A, B, C, and D) are "wet" so that slurry experiments can be performed reversibly across two lines in Figure 4:

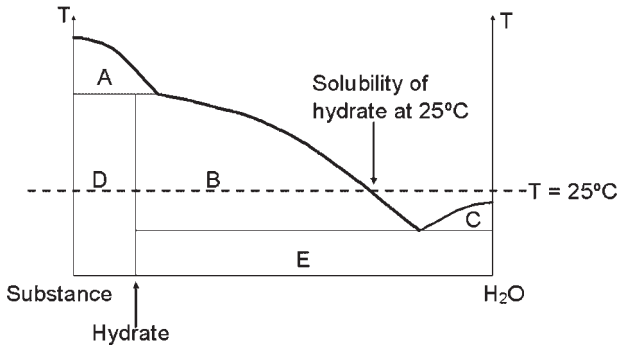


Figure 3. A schematic phase diagram for a drug substance forming thermodynamically stable anhydrate and hydrate. A, anhydrate in equilibrium with saturated solution; B, hydrate in equilibrium with saturated solution; C, ice in equilibrium with saturated solution; D, miscibility gap with anhydrate and hydrate; E, miscibility gap of ice and hydrate. T indicates temperature in °C and the composition axis may be chosen to read weight % or molar %.

- from area B to D and back, which means starting with a saturated solution of the hydrate and adding more nonaqueous solvent,
- from area C to D and back, which means starting with a saturated solution of the anhydrate and adding more water.

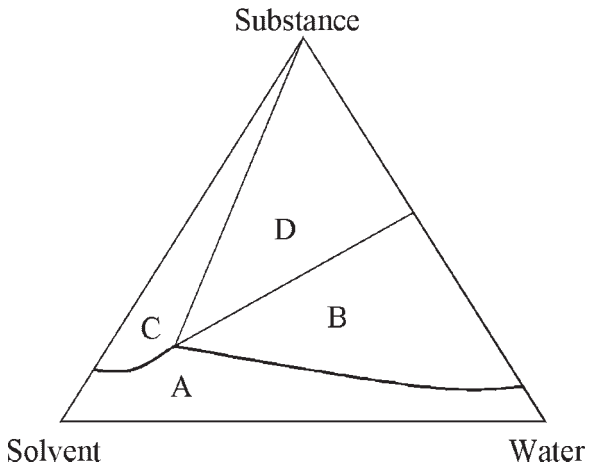


Figure 4. A schematic, ternary phase diagram for a drug substance having a thermodynamically stable anhydrate and a hydrate, in water and an organic solvent. A, nonsaturated solution; B, hydrate in equilibrium with saturated solution; C, anhydrate in equilibrium with saturated solution; D, anhydrate and hydrate in equilibrium with saturated solution of invariable composition (eutectic at crossing of areas A, B, C, and D). The composition axis may be chosen to read weight % or molar %.

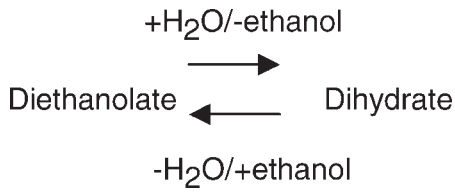
To test the reversibility of the equilibrium between anhydrate B and the dihydrate of formoterol fumarate, the second route was attempted. The competitive slurry experiments were performed in ethanol with 0.6–5.3 weight % water. The saturated solution was prepared using anhydrate A and seeds of anhydrate B and the dihydrate were then added. The results are summarized in Table 5. The experiments show that there is a reversible equilibrium between anhydrate B and the dihydrate according to Scheme 1.

Thermodynamic Stability of the Diethanol Solvate

From the results in Table 5 there was little evidence that the diethanolate was thermodynamically stable. Still this had to be definitely proven so the experiments in Table 5 were repeated using seeds of anhydrate B, the dihydrate, and the ethanlolate. The results are shown in Table 6. From the table it is obvious that a eutectic point is positioned at a water-ethanol composition somewhere between ethanol:water 83.0:5.3% w/w and 85.3:2.7% w/w. Assuming that the formoterol fumarate substance is without influence, the water activity, as calculated with Aspen Properties 12.1 (Aspen Technologies, Inc., Cambridge, MA) in the point mid-way (ethanol: water = 95.5:4.5% w/w) between these two points is approximately 0.10.

Comparing Tables 5 and 6 anhydrate B is substituted for the diethanolate.

The experiments show that there is a reversible equilibrium according to Scheme 2.



Scheme 2.

It is obvious that the equilibrium observed in Scheme 1 is only reversible when seeds of the diethanolate are lacking. In other words it is a quasi-reversible equilibrium in the formoterol fumarate-water-ethanol system. Still it has been observed that the dihydrate, at low water contents, is less stable than anhydrate B, so by using a nonsolvate forming solvent, for example acetone, this equilibrium would be observed as truly

Table 5. Competitive Slurry Experiments in Ethanol Plus Water

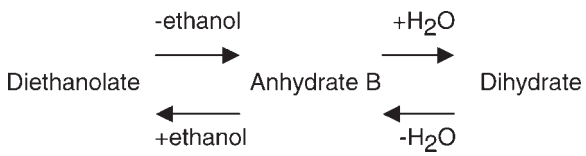
Starting Solid	Seeding Solids	% w/w Ethanol	% w/w Water	% w/w Solid	Resulting Wet Crystal Modification
A	B + dihydrate	87.2	0.6	12.2	B
A	B + dihydrate	86.7	1.1	12.2	B
A	B + dihydrate	85.3	2.7	12.0	B + small amount dihydrate
A	B + dihydrate	83.0	5.3	11.7	dihydrate + small amount B

reversible. The result of this is shown in Table 7. It is obvious that anhydrate B and the dihydrate are in equilibrium in this system and that the experiment with 93.9/0.9/5.2% w/w acetone/water/substance contains a eutectic solution. The clear liquid was separated and analyzed for the amount of solid giving that the composition of the eutectic is approximately 99% w/w acetone, 1% w/w water, and 0.16% w/w formoterol fumarate. The water activity in this point, as calculated with the same assumptions as above, using Aspen Properties 12.1 (Aspen Technologies, Inc.) is approximately 0.05.

Summing up, it has been shown that:

- crystals of anhydrate B can be transformed to crystals of the diethanolate in an ethanol-rich slurry,
- crystals of anhydrate B can be transformed to crystals of the dihydrate in a water-rich slurry,
- crystals of the dihydrate can be transformed to crystals of anhydrate B in a water-poor slurry,
- crystals of the diethanolate can be transformed to crystals of the dihydrate in a water-rich slurry.

Even though it has not been directly shown, it follows from this that the diethanolate must be transformed to anhydrate B in a medium, which contains very little ethanol and water. The results can be summarized in Scheme 3.



Scheme 3.

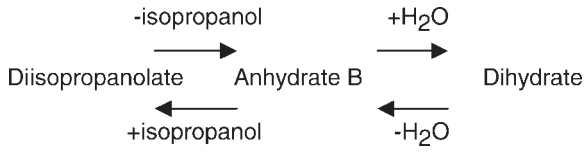
Since the formoterol fumarate-water-ethanol system contains a diethanolate it is not of the type drawn up in Figure 4 but rather that in Figure 5.

It should be noted that, due to the equilibrium between the solvate and hydrate, the reversible equilibrium between the anhydrate and the hydrate or solvate, normally cannot be proven in such a system. However, this is not the only type of phase diagram possible in such a system. If the anhydrate is more dominating a diagram of the type shown in Figure 6, where the hydrate and solvate does not have a common area of existence, is obtained. In such a diagram the reversibility of all three solid phases can be proven by crossing the lines separating areas B and E, E and C, C and F or F and D.

Thermodynamic Stability of the Diisopropanol Solvate

To test if the diisopropanolate was appearing from a kinetic or a thermodynamic route a saturated solution of anhydrate A with isopropanol and water in different amounts was made. Seeds of anhydrate B and the dihydrate were then added. The results are summarized in Table 8.

Obviously at low water contents, the diisopropanolate is thermodynamically more stable than the dihydrate and at high water contents the dihydrate is more thermodynamically stable than the diisopropanolate. The phase diagram is then of the type in Figure 5 or 6 but since two solid phases in equilibrium with a solution has not been observed in any of the experiments it is not possible to say which. Still it has been shown that Scheme 4 (which should be interpreted in the same way as Scheme 3) holds:



Scheme 4.

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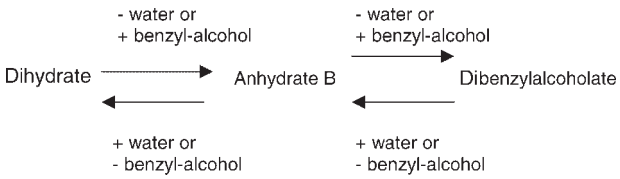
Table 6. Competitive Slurry Experiments in Ethanol Plus Water

Starting Solid	Seeding Solids	% w/w Ethanol	% w/w Water	% w/w Solid	Resulting Wet Crystal Modification
A	B + dihydrate + diethanolate	87.2	0.6	12.2	Diethanolate
A	B + dihydrate + diethanolate	86.7	1.1	12.2	Diethanolate
A	B + dihydrate + diethanolate	85.3	2.7	12.0	Diethanolate + small amount dihydrate
A	B + dihydrate + diethanolate	83.0	5.3	11.7	Dihydrate + small amount diethanolate

Thermodynamic Stability of the Dibenzyl alcohol Solvate

From polymorph screening it was known that the dibenzyl alcohol solvate crystallized from solutions with small amounts of water. If this solvate and the dihydrate are both thermodynamically stable phases (although in different chemical environments), a phase diagram, which is similar to that in Figure 5 or 6 that is, the reversible equilibrium in Scheme 5, should once more be



Scheme 5.

investigated (it should be noted that the addition or subtraction of solvents indicated in this scheme does not relate to solvent molecules added or withdrawn from the crystal structure but rather shows the direction of movements along the water-solvent axis in the phase diagrams in Figure 5 or 6).

However, in Figures 5 and 6, it is presumed that the solvent and water are completely miscible. This is not the case for benzyl alcohol and water. Instead the solubility of benzyl alcohol in water at room temperature is limited to 3.8 weight %[14] and that of water in benzyl alcohol at room temperature to 10.4 weight % (a literature value for this could not be found so it was determined by gradually adding water to pure benzyl alcohol, while stirring, until a second liquid phase separated). This means that, along the benzyl alcohol-water axis of the phase diagram there is a miscibility gap from 3.8 weight % water to 89.6 weight % water. Proceeding into this gap in the substance direction will reveal a number of miscibility gaps. To determine the exact shapes and extensions of these is out of the scope of this investigation, so only crystal modifications stable on the benzyl alcohol side and on the water side of the miscibility gap were tested. The result of this is shown in Table 9.

Obviously at the limits of the miscibility gaps, the dibenzyl alcohol solvate and the dihydrate are transformed into each other so the outer parts of Scheme 5 have in fact been proven. Since the equilibrium between anhydrate B and the dihydrate has already been proven no further tests needs to be made.

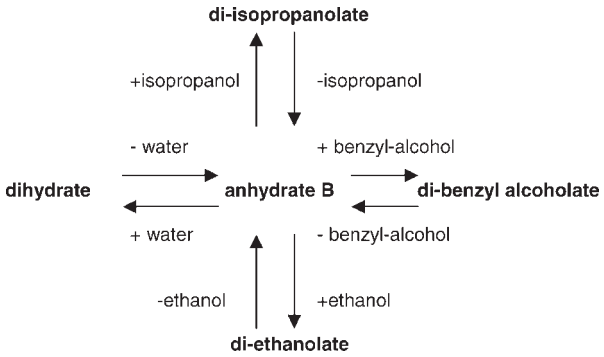
Table 7. Competitive Slurry Experiments in Acetone and Water

Starting Solid	Seeding Solids	% w/w Acetone	% w/w Water	% w/w Solid	Resulting Wet Crystal Modification
A	B + dihydrate	94.8	0.2[a]	5.0	B
A	B + dihydrate	94.2	0.5[a]	5.3	B
A	B + dihydrate	94.3	0.7[a]	5.0	B
A	B + dihydrate	93.6	0.8[a]	5.6	B
A	B + dihydrate	93.9	0.9[a]	5.2	B + dihydrate
A	B + dihydrate	93.7	1.0[a]	5.3	Dihydrate
A	B + dihydrate	97.0	1.0[a]	2.0	Dihydrate
A	B + dihydrate	96.0	2.0[a]	2.0	Dihydrate
A	B + dihydrate	95.0	3.0[a]	2.0	Dihydrate

[a]Maximum 0.2% water in the acetone pa.

Summary of Thermodynamic Relationships

Summing up the results obtained it has been shown that there exist reversible equilibria according to Scheme 6. This scheme states that,



Scheme 6.

for the solvates involved, all solvated phases transform to only one ansolvate phase (anhydrate B) when desolvated in a thermodynamically "correct" way. Then, on readdition of the same solvent, this ansolvate retransforms to the same solvate as before desolvation. This strengthens the belief that these are truly reversible equilibria between five thermodynamically stable phases. It is important to note that Scheme 6 does not sum up all equilibria investigated, that is, it does not say that the dihydrate in water slurry, upon addition ethanol, must pass *via* the anhydrate before it is transformed to the diethanolate.

Other alcoholates could possibly be added to the scheme but this was not investigated further.

It should be noted that anhydrates A and C does not fit into this scheme. No reversibility has been found between these modifications and any of the five above. They are thus all thermodynamically unstable at the temperatures and pressures investigated.

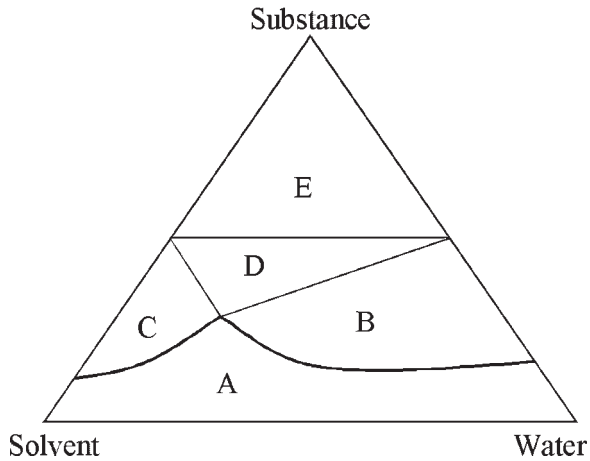


Figure 5. A schematic, ternary phase diagram for a drug substance having thermodynamically stable anhydrate, hydrate, and solvate in water and an organic solvent. A, nonsaturated solution; B, hydrate in equilibrium with saturated solution; C, solvate in equilibrium with saturated solution; D, hydrate and solvate in equilibrium with eutectic saturated solution; and E, dry miscibility gap with solvate, hydrate, and anhydrate.

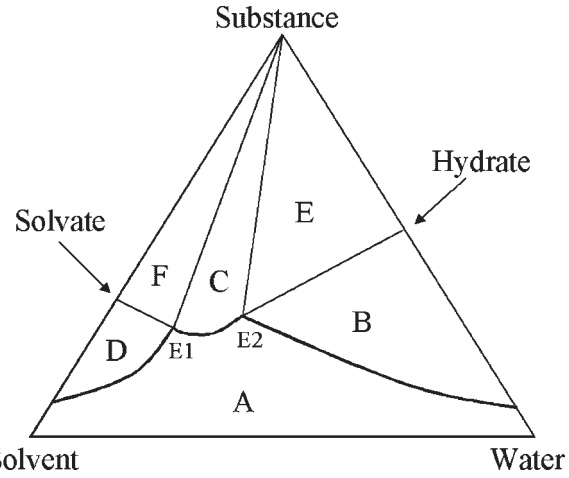


Figure 6. A schematic, ternary phase diagram for a drug substance having thermodynamically stable anhydrate, hydrate and solvate in water and an organic solvent. A, nonsaturated solution; B, hydrate in equilibrium with saturated solution; C, anhydrate in equilibrium with saturated solution; D, solvate in equilibrium with saturated solution; E, anhydrate and hydrate in equilibrium with saturated solution of invariable composition; and F, anhydrate and solvate in equilibrium with saturated solution of invariable composition. Points E_1 and E_2 are eutectic points.

Kinetic Competition between Anhydrate B and the Diethanolate in Ethanol

As noted in subsection Single Crystal Growth Experiments, the crystallizations of the diethanolate and anhydrate B in ethanol were kinetically affected. The whole polymorphism screen and the slurry experiments with only one solid phase were performed in Lund, whereas the single crystal growth experiments and the competitive slurry experiments were performed in Södertälje. In the first two, nonfiltered single crystal growth experiments in ethanol, anhydrate

Table 8. Competitive Slurry Experiments in Isopropanol Plus Water

Starting Solid	Seeding Solids	% w/w Isopropanol	% w/w Water	% w/w Solid	Resulting Wet Crystal Modification
A	Diisopropanolate + B	87.7	—	12.3	Diisopropanolate
A	Diisopropanolate + B + dihydrate	85.3	8.7	6.0	Diisopropanolate
A	Diisopropanolate + B + dihydrate	81.8	12.5	5.7	Dihydrate
A	Diisopropanolate + B + dihydrate	78.5	16.0	5.5	Dihydrate

B was obtained as large single crystals. These crystals were used in the competitive slurry experiments with anhydrate B and the dihydrate in ethanol shown in Table 5 which were also performed in Södertälje. So far the diethanolate had never appeared in the Södertälje laboratory. In a third single crystal growth experiment performed as described in subsection Single Crystal Growth Experiments but including the filtration step, large crystals of the diethanolate appeared. Using these crystals together with crystals of the dihydrate and anhydrate B, the competitive slurry experiments in Table 6 were performed. From this point on, crystallization of anhydrate B single crystals in Södertälje, using the method described in subsection Single Crystal Growth Experiments, became considerably more difficult, even though it could still be done. The diethanolate on the other hand, consistently crystallized from filtered solutions and also frequently from unfiltered solutions.

It is obvious that once the diethanolate had been obtained in the Södertälje lab, its presence affected the subsequent crystallizations, even though intentional seeding was not performed. Being aware of the possibility of disappearing polymorphs[3] cleaning of the lab space was carried out. This significantly increased the possibility of getting anhydrate B (fast crystallizations) or a mixture of anhydrate B and the diethanolate (slower crystallizations) in nonfiltered crystallizations.

It can be concluded that crystallization of anhydrate B in ethanol requires not only the absence of seeds of the diethanolate, but probably also the presence of seeds of itself or maybe of anhydrate A and/or the dihydrate.

The Crystal Structure of the Dihydrate

Crystal and structure data for the four crystal modifications investigated with single crystal X-ray diffraction are given in Table 2. Positional and thermal parameters as well as lists of bond angles and distances are given as Supplementary data. All crystal structures contain racemic mixtures of formoterol molecules with (S,S)- and (R,R)-enantiomer configurations. It should be noted that in all structures the fumarate anion is placed in an inversion center (in the middle of the C=C double bond) so there is only half a fumarate ion in the crystallographic asymmetric unit. The molecular ratio of the formoterol fumarate dihydrate is thus (2:1:2).

The conformation of a molecule in the solid state is a consequence of its intra- and intermolecular interactions.[15] This is clearly exemplified for the formoterol molecule in the dihydrate structure. The molecule consists of two substituted benzene rings separated by a 5-membered chain. In the middle of this chain there is a secondary ammonium ion (see Fig. 7). To be able to approach the secondary ammonium cation to the fumarate anion the formoterol molecule has to bend so as to form a V-shaped conformation. Had it not been for the alcohol group O21–H21, which participates in the intermolecular interaction with the fumarate ion, and also accepts an intramolecular hydrogen bond from N9, the bending might have been even sharper. The formoterol molecule behaves in a similar way in the chiral (R,R)-tartrate structure.[16] The hydroxy-formamide substituted benzene ring, containing two hydrogen bond donors (O25 and N22) and one acceptor (O24), has an intramolecular hydrogen bond N22–H22...O25, which to a large extent affects the orientation of the formamide moiety. The whole of this benzene moiety then rotates, primarily to satisfy intermolecular hydrogen bond demands and secondarily weak hydrogen bonding, π-interactions and van der Waals packing. The rest of the molecule, which lacks hydrogen bond donors and only has a shielded, potential H-bond acceptor O18, will orient so as to optimize packing. The final result is a rather un-strained conformation with planar benzene rings and normal bond distances and bond angles.

The crystal structure of the dihydrate is completely dominated by hydrogen bonds. The

Table 9. Competitive Slurry Experiments in Benzyl-Alcohol Plus Water

Starting Solid	Seeding Solid	% w/w Benzyl-Alcohol	% w/w Water	% w/w Solid	Resulting Wet Crystal Modification
A	B	90.4	—	9.6	Dibenzylalcoholate
A	B and dihydrate	83.2	8.0	8.8	Dibenzylalcoholate
A	B and dihydrate	77.0	14.8	8.2	Dibenzylalcoholate
A	B and dihydrate	1.8	88.5	9.7	Dihydrate

three different molecules in the asymmetric unit (one formoterol, one water, and half a fumarate ion) together have seven hydrogen bond donors and eight hydrogen bond acceptors (of which four are nondonors). All donors are donating hydrogen bonds to four of the acceptors. The only acceptor, which is not involved in hydrogen bonding is O18. This atom is however completely embedded in hydrophobic surroundings in the crystal packing scheme. There are no hydrogen bonds between formoterol molecules but instead all hydrogen bonds stretch directly or indirectly to the fumarate molecule, so that one full fumarate molecule saturates all its "free" electron pairs by accepting 10 hydrogen bonds, 8 from neighboring formoterol molecules and 2 from bridging water molecules. Viewing the crystal structure along the a-axis, as in Figure 8, gives a very clear picture of the crowding around the fumarate molecules. In this way, two-dimensional, hydrogen bonded, hydrophilic sheets are formed parallel to the ab-plane. When these sheets approach each other along the c-axis the nonhydrogen bonding part of the molecules pack to hydrophobic sheets. The bonds within these sheets are mainly of van der Waals type (the distance between ring centroids between pairs of benzene rings is 4.2 Å, too long to be regarded as pi-pi-stacking).

It should be noted that the water molecules, forming two bridges from the formoterol molecule to the fumarate ion, play a very important role in the crystal structure of the dihydrate. By using these water bridges, the main part of the formoterol molecules do not have to come so close to the fumarate ions, leaving it less sterically strained. This means that it will gain conformational freedom, which in turn will give more flexibility to pack its nonhydrogen bonding parts.

The Crystal Structure of the Diethanol and Diisopropanol Solvates

The diethanolate and the diisopropanolate are isostructural. Therefore the description will be restricted to the diethanolate and only refer to the diisopropanolate when necessary.

The conformations of the formoterol molecules in the two solvates (Fig. 9) are very similar. As in the dihydrate the conformation is V-shaped with the two sides of the molecule bending backwards from the central secondary ammonium cation. As in the dihydrate, the conformation of the hydroxyl, foramide-substituted benzene ring is stabilized by an intramolecular hydrogen bond, N22–H22...O25.

The crystal structure, as the dihydrate, is dominated by hydrogen bonds. The fumarate anions are surrounded by formoterol molecules, which donate eight hydrogen bonds to one full fumarate ion. Two intermolecular hydrogen bonds (O21–H21...O25 and O25–H25...O24) connect the formoterol molecules so that hydrogen bonded sheets are formed in the plane defined by the b-axis and the ac-diagonal (see Fig. 10).

Between these sheets the methoxy substituted benzene rings pack to hydrophobic layers. The solvent molecules reside in the hydrophobic layers, but turn their alcohol groups towards the fumarate ions, forming one hydrogen bond. As in the dihydrate, one fumarate ion, altogether accepts 10 hydrogen bonds, which equals its number of "free"

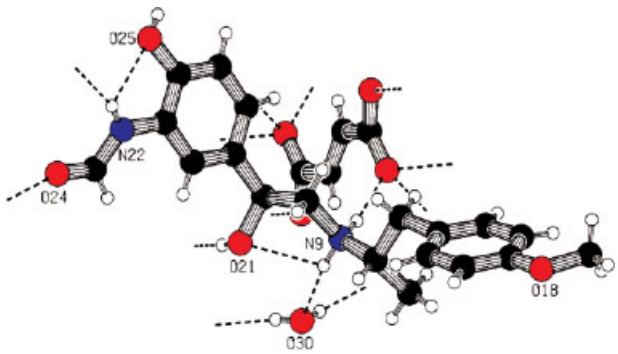


Figure 7. The asymmetric unit in formoterol fumarate dihydrate. Hydrogen bonds are marked as dotted lines.

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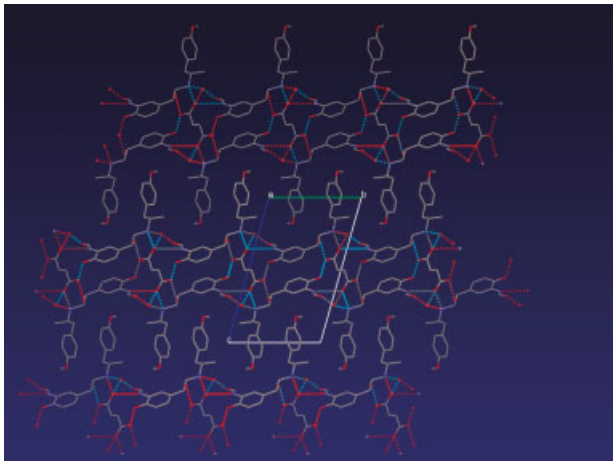

Figure 8. The crystal structure of formoterol fumarate dihydrate viewed down the a-axis. The b-axis is horizontal and the c-axis near vertical.

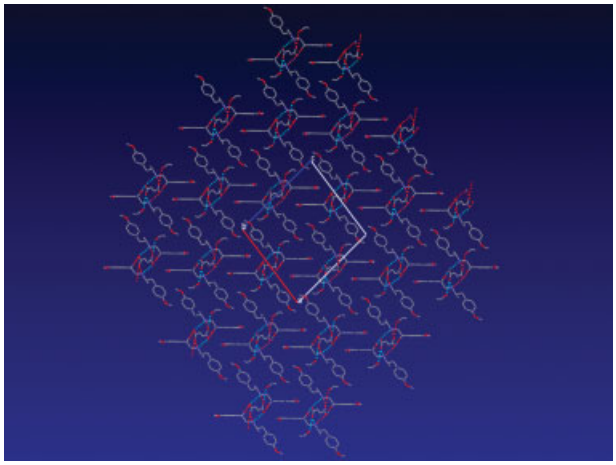

Figure 10. The crystal structure of formoterol fumarate diethanol solvate viewed down the b-axis. The ac-diagonal is horizontal (a-axis red, c-axis blue).

electron pairs. Further proof of the large importance of hydrogen bonds in these structures is that the thermal motion in the formoterol molecule is much larger in the hydrophobic part than in the hydrophilic part (see Fig. 11). This shows clearly that the demand for complete hydrogen bonding greatly supersedes the demand for maximized van der Waals packing.

The Crystal Structure of Anhydrate B

The attempts to fully determine the crystal structure of anhydrate B have not been successful, since the X-ray crystal structure solution cannot be completely resolved. The reason for this is substantial disorder, in the region of the central carbon chain from the secondary amine to the methoxy substituted benzene moiety (see Fig. 12). From solid state NMR investigations it is known that this disorder is dynamic and also that cooling to 223 K does not stop the movement.[17] Only a limited improvement was achieved by collecting X-ray data at 200 K.

Using the best single crystal data to calculate a powder diffraction pattern fits rather well with experimental XRPD data (see Fig. 13). This shows that the structure determination, although not fully refined, is close to the true crystal structure.

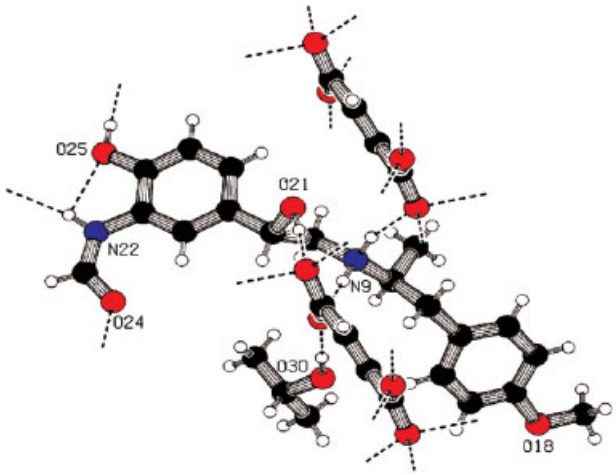

Figure 9. The asymmetric unit of formoterol fumarate diethanol solvate. Hydrogen bonds are indicated as dotted lines.

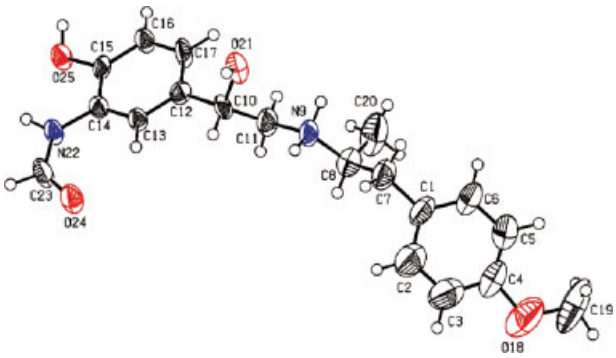

Figure 11. The formoterol molecule in the diethanol solvate showing thermal ellipsoids. Note the large ellipsoids in the molecular part to the right side of the central ammonium ion compared to that on the left side.

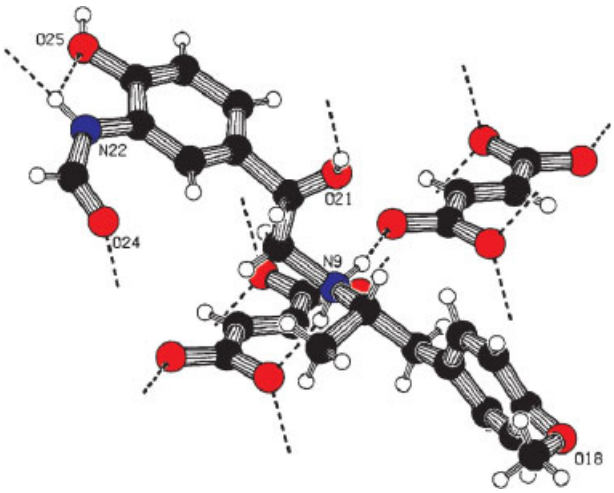

Figure 12. The asymmetric unit in formoterol fumarate anhydrate B. Hydrogen bonds are marked as dotted lines.

On these grounds it is judged to be relevant to describe the crystal structure.

The crystal structure of anhydrate B is surprisingly similar to that of the dihydrate (see Fig. 14). It contains alternating hydrophilic and hydrophobic layers parallel to the ab-plane. In the hydrophilic layer each fumarate ion accepts eight hydrogen bonds from formoterol molecules and between neighboring formoterol molecules there is one hydrogen bond O21–H21...O24.

The Extraordinary Stability of Formoterol Fumarate Dihydrate

The water sorption/desorption experiments with the dihydrate and anhydrates A, B, and C show that, upon water sorption, all anhydrates are gradually transformed to the dihydrate, but at very different rates and at different relative humidities (see Figs. 15 and 16). Quite interestingly the relative stabilities, as based on the solubilities (see subsection Thermodynamic Stability of Anhydrates), have no relation to the sorption behavior, during the first cycle. Anhydrate C, the anhydrate with the highest apparent solubility is surprisingly stable up to 70% RH, after which it adsorbs more than 12% water. Anhydrate A, which is formed upon dehydration of the dihydrate, adsorbs already below 10% RH, whereas anhydrate B adsorbs in a linear fashion up to 70% RH. During the second cycle, the sorption behaviors for the three anhydrates are more similar to that of the dihydrate, showing the gradual transformation to this phase.

The dihydrate, on the other hand, is quite resistant to change and it is actually not possible to dehydrate it using dry nitrogen at room temperature and "normal" experimental times. Dehydration of the dihydrate has to be made at higher temperature. The fact that the dihydrate can be transformed to anhydrate A at high temperature and that anhydrate A can be trans-

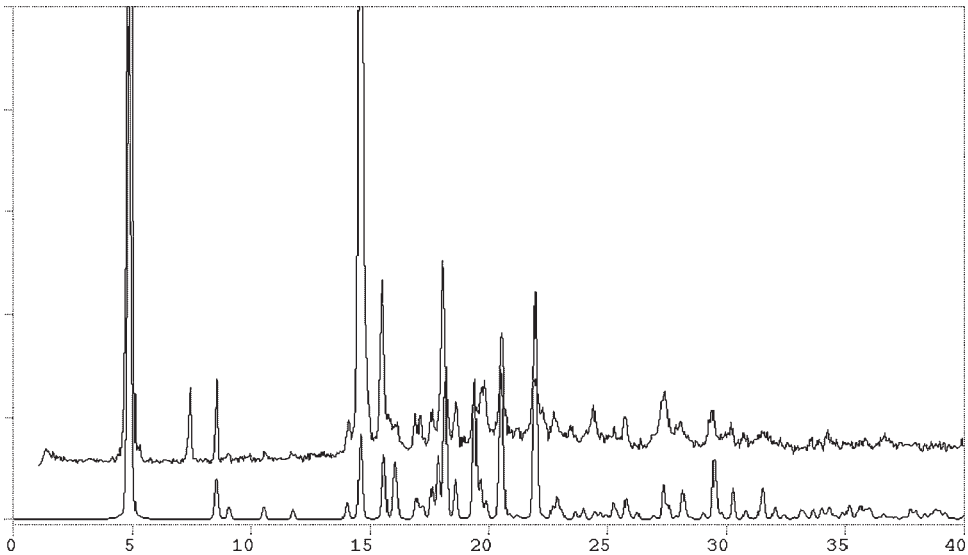

Figure 13. Experimental powder diffractogram for Formoterol Fumarate anhydrate B (top) and diffractograms calculated from single crystal structure data. The peak at 7.5° 2θ in the experimental diffractogram is due to the presence of a small amount of anhydrate C.

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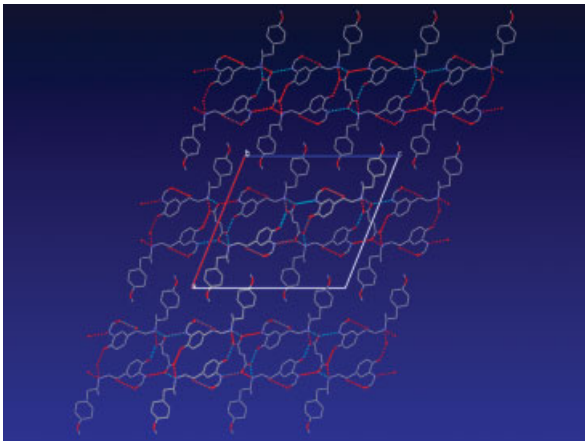
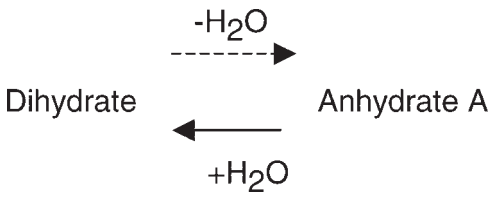


Figure 14. The crystal structure of formoterol fumarate anhydrate B viewed down the a-axis. The b-axis is horizontal and the c-axis near vertical.

formed to the dihydrate when exposed to moisture may give the impression that there exists a reversible equilibrium between these two phases (Scheme 7).



Scheme 7.

In this type of dry experiment anhydrate A appears at completely dry conditions, that is, in a frozen state and would, if thermodynamics had ruled, not appeared at all. So, from a thermodynamic perspective the reversibility is false, which is indicated by the dotted arrow from the dihydrate to anhydrate A. It is important not to rely on this type of dry experiments when evaluating thermodynamic equilibrium. Instead it is recommended to perform such measurements in the presence of a solution phase.

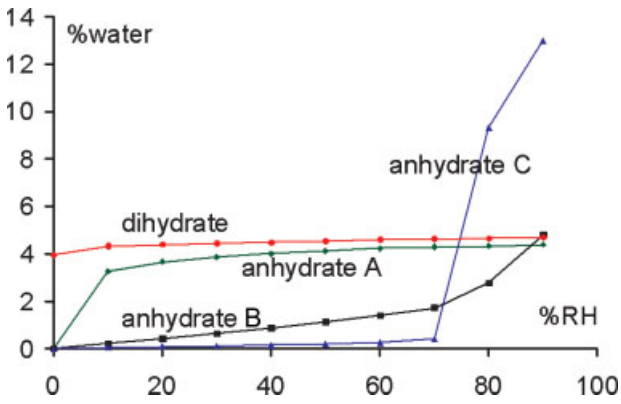


Figure 15. The first sorption cycle for, from the bottom, anhydrate C, anhydrate B, anhydrate A, and the dihydrate.

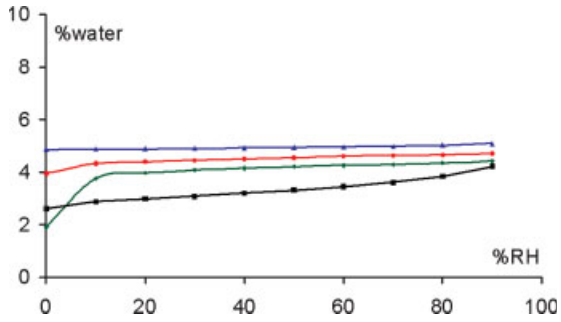


Figure 16. The second sorption cycle for, from the bottom, anhydrate B, anhydrate A, the dihydrate, and anhydrate C.

The observations noted above can be interpreted by means of the crystal structures. The high stability of the dihydrate compared to the anhydrates is directly reflected in its denser and more well packed crystal structure (see densities and packing coefficients[18] calculated from the single crystal structure data, in Table 2). In the dihydrate, the water molecule form bridges between the formoterol and fumarate molecules, which gives the formoterol molecules steric release and more conformational freedom, so that a better packed crystal structure without disorder in the hydrophobic parts can be built. For similar reasons alcohols also form crystal structures, which are more stable than the anhydrates. The crystal structures of the alcoholates are, however less well packed than the dihydrate due to the differences in size and hydrophobicity of the solvent molecules. The water molecules in the dihydrate reside in the hydrophilic sheets whereas the alcohols reside in the hydrophobic sheets pointing their hydrophilic heads towards the hydrophilic parts. Obviously the water molecules fit extremely well whereas the alcohols are not perfectly fitted. This is proven by the fact that the hydrophobic moiety of the formoterol molecule has comparably high temperature parameters even in the diisopropanolate and diethanolate structures, showing that this group moves. Moreover, the dibenzyl alcohol solvate is quite stable, even in moist air, indicating that the benzyl group could be helping the methoxy substituted phenyl group by some sort of pi–pi interaction. Having the crystal

structure of this solvate would of course show more exactly how this is obtained.

The four crystal structures also explain the differences in water sorption behavior between anhydrate A, B, and C. Anhydrate A, which is obtained by dehydration of the dihydrate, most probably has a crystal structure, which is almost identical to that of the dihydrate, but containing more or less open channels through which the water molecules have left and into which the water molecules will penetrate upon rehydration. These channels penetrate the hydrophilic sheets of the crystal structure, which explains the extreme water sorption behavior of anhydrate A. In the crystal structure of anhydrate B, the formoterol and fumarate ions have moved slightly and changed their conformations in order to compensate for the lack of water. This structure is similar to the dihydrate but it lacks the open channels, probably present in anhydrate A. For this reason anhydrate B is less hygroscopic than anhydrate A. Still there is a great deal of relationship between anhydrates A, B, and the dihydrate.

In the crystal structures of the diethanol and diisopropanol solvates, the solvent molecules reside mainly in the hydrophobic parts of the crystal structure. The desolvated structure, anhydrate C, will have channels through which the solvent molecules have left, but since these are in the hydrophobic parts of the structure, they will not be very attractive for water molecules. Also, an intermediate phase seen in the XRPD experiments, indicate that anhydrate C is not just a "residue" of the solvates but rather a crystal structure where the area around the channels has been slightly rebuilt. The channels may have become more or less blocked, which would further decrease the water sorption behavior of anhydrate C.

Another difference between the crystals of the diethanolate and diisopropanolate and anhydrates A and B and the dihydrate is that the alcoholates form needle shaped crystals whereas the others form plate like crystals. The needle axis coincides with the unit cell b-axis for the dialcoholates whereas the direction perpendicular to the plane of the dihydrate and anhydrate B coincides with the unit cell c-axis (dihydrate) and a-axis (anhydrate B).

CONCLUSIONS

Polymorphism screening followed by thermodynamic evaluations of the crystal modifications found and single crystal investigations show that the formoterol fumarate salt needs solvate molecules to form stable, well-packed crystal structures. The solvent molecule, which fills this purpose best, is water, being of a good size and also having the potential of forming hydrogen bonds between the formoterol and fumarate molecules. For this reason the dihydrate of formoterol fumarate is the superior solid phase, which dominates large areas of the ternary phase diagrams investigated. In view of these findings it is logical that the dihydrate has been chosen as the phase for full-scale production.

SUPPORTING INFORMATION

CIF files containing data about the single crystal X-ray diffraction experiment has been filed as Supporting Information. The data includes coordinates, thermal parameters, distances, angles, etc.

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REFERENCES

1. Almarsson Ö, Hickey MB, Peterson ML, Morissette SL, Soukasene S, McNulty C, Tawa M, MacPhee JM, Remenar JF. 2003. High-throughput surveys of crystal form diversity of highly polymorphic pharmaceutical compounds. Crystal Growth Des 3: 927–933.
2. Storey RA, Docherty R, Higginson PD. 2003. Integration of high throughput screening methodologies and manual processes for solid form selection. Am Pharm Rev 6:100–105.
3. Dunitz JD, Bernstein J. 1995. Disappearing polymorphs. Acc Chem Res 28:193–200.
4. Ricci JE. 1966. The phase rule and heterogeneous equilibrium. New York: Dover Publications, Inc. pp 39–47.
5. Bernstein J. 2002. Polymorphism in molecular crystals. Oxford: Oxford University Press. pp 34–38.
6. Findlay A, Campbell AN. 1945. The phase rule and its applications, 8th edition. New York: Dover Publications, Inc. pp 32–38.

7. Crocker LS, Ge Z, Abrahim A, Hartman R, Xu J. 2003. An etoricoxib phase diagram: Hemihydrate and anhydrate. Org Proc Res Dev 7:958–961.
8. Jeffrey GA. 1997. An introduction to hydrogen bonding. New York: Oxford University Press. pp 85–97.
9. Hunter CA, Lawson KR, Perkins J, Urch CJ. 2001. Aromatic interactions. J Chem Soc Perkin Trans 2: 651–669.
10. Steiner T. 2002. The hydrogen bond in the solid state. Angew Chem Int Ed 41:48–76.
11. Otwinowski Z, Minor W. 1997. Denzo-SMN program package. In: Carter CW, Jr., Sweet RM, editors. Processing of X-ray diffraction data collected in oscillation mode. Methods in enzymology. New York: Macromolecular Crystallography Academic Press, Inc. 276:302–326.
12. MacKay S, Edwards C, Henderson A, Gilmore C, Stewart N, Shankland K, Donald A. 2000. The maXus program package. A state-of-the art computer program for solving, refining and publishing crystal structures from X-ray diffraction data. Chemistry Department, The University, Glasgow, Scotland, Developed for Mac Science Co., Japan and Nonius, The Netherlands.
13. Purdon FF, Slater VW. 1946. Aqueous solution and the phase diagram. London: Edward Arnold & Co.
14. In Budavari S, O'Neil MJ, Smith A, Heckelmann PE, Kinneary JF, editors. 1996. The merck index an encyclopedia of chemicals, drugs and biologicals, 12th edition. Whitehouse Station: Merck & Co., Inc. 189p.
15. Bernstein J. 2002. Polymorphism in molecular crystals. Oxford: Oxford University Press. pp 151–187.
16. Kurihara H, Fujita S, Mase T. 1997. (–)-Formoterol, a selective beta2-adrenoreceptor agonist. Acta Cryst C53:1887–1889.
17. Apperley DC, Harris RK, Larsson T, Malmström T. 2003. Quantitative nuclear magnetic resonance analysis of solid formoterol fumarate and its dihydrate. J Pharm Sci 92:2496–2503.
18. Kitaigorodski AI. 1973. Molecular crystals and molecules. New York: Academic Press. pp 18–21.