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Thermal and X-ray powder diffraction structural characterization of two benfluorex hydrochloride polymorphs

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ABSTRACT

Two polymorphic forms of benfluorex hydrochloride, phases **I** and **II**, were isolated as monophasic polycrystalline samples, and structurally characterized using *ab initio* X-ray powder diffraction methods and a global optimization strategy (simulated annealing). Form **I** crystallizes in monoclinic system, space group $P2_1/n$, with $Z=4$, $a=21.0719(10)$ Å, $b=7.0563(4)$ Å, $c=14.8684(7)$ Å, $\beta=116.998(3)^\circ$, $V=1969.8(2)$ Å^3, while Form **II** crystallizes in the orthorhombic space group Pbca, with $Z=8$, $a=33.8031(2)$ Å, $b=15.1451(8)$ Å, $c=7.6138(6)$ Å, $V=3897.9(4)$ Å^3. Crystals of Form **I** and Form **II** of benfluorex hydrochloride are based upon an ionic packing of protonated benfluorex molecules at the most basic site, the N1 atoms, and chloride anions. Form **I** shows the presence of μ-Cl ions, generating centrosymmetric dimers with a N_2Cl_2 moiety, while Form **II** contains antiparallel chains of C–H···O hydrogen-bonded molecules running along *c* axis. DSC and thermodiffractometric measurements showed that heating progressively Form **II** from ambient temperature to 160 °C causes a phase transition to the thermodynamically stable Form **I**, immediately followed by the sample melting, near 165 °C. Recrystallization directly to Form **I** is observed when the melt is cooled back to ambient temperature, with a significant hysteresis (this event being centered near 130 °C).

1. Introduction

In the last years, many leading pharmaceutical companies have begun to strictly control the crystal chemistry of active pharmaceutical ingredients (API's) during their preparation and development stages. In several cases, the presence, or the discovery, of new crystal phases (polymorphs and solvates), initially interpreted as annoying side effect, was positively used to tailor new synthetic processes and more efficient formulation of drugs and, in some cases, to widen the patent panorama with evident economical impact. Actually, polymorph formation and interconversion can occur during manufacturing, altering the sought, well-defined, physicochemical properties and, in many cases, the bioavailability of these compounds [1]. Thus, not surprisingly, the relationship between crystal structure of an API and its solid-state properties is one of the main challenges in polymorphism studies [2], as structural data of a drug can help to predict, among other features, the API behaviour during manufacturing. A well-known example is paracetamol, which in its orthorhombic form possesses sliding planes allowing a better compression and avoiding the tablets break with respect to the monoclinic form [3]. Accordingly, the scientific literature on the polymorphic behaviour of generic drugs is rich and steadily increasing [4], but a complete structural information of these pharmaceutical compounds is often lacking. Despite the bevy of information that a full structure determination by X-ray diffraction methods can provide, often the absence of single crystals of the metastable phases (transforming, upon manipulation, recrystallization from solutions or from the melt or by thermal treatment, into the most stable one) prevents this kind of study.

Recently, our collaboration with some pharmaceutical companies on the structural characterization of their polymorphic API's has led to the complete structural analysis of several polymorphic drugs (acitretrin, linezolid, sibutamine, azelastine, bupropione), to mention a few [5–9]. The crystal chemistry of those has been mostly unravelled by powder diffraction methods, in combination with thermal analyses, thermodiffractometry and, when necessary, solid-state ^{13}C CP-MAS NMR measurements.

Benfluorex 2-{[1-methyl-2-[(3-(trifluoromethyl)phenyl)ethyl] amino]ethyl} benzoate, in its racemic hydrochloride salt, is a known hypolipidaemic compound with possible glucose lowering effects. It has been shown to improve glucose tolerance in obese diabetic individuals by increasing sensitivity to insulin [10]. Reports on its physicochemical and structural properties are scarce: the synthe-

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sis of the compound in the solid state is reported in an old patent, in which this material was characterized using uniquely its melting point value [11]. IR characterization was later reported in the European Pharmacopoeia [12], where the formation of an additional metastable form is briefly mentioned. Since the existence of more than one crystal species of benfluorex hydrochloride has not been certified by solubility, spectroscopic or diffraction analysis, we decided to investigate its crystal chemistry by starting with the isolation of monophasic samples of benfluorex hydrochloride polymorphs. With this goal in mind, we succeeded in obtaining pure batches of two distinct forms, **I** and **II**, which will be the subject of the present contribution. These species being recovered only as polycrystalline materials, forced us to employ powder diffraction methods, thermodiffractometry and DSC analyses to interpret their structure and stability ranges, which are collectively discussed in the following.

2. Experimental

2.1. Material

Samples of benfluorex hydrochloride Form **I** and Form **II** were prepared according to the following procedures.

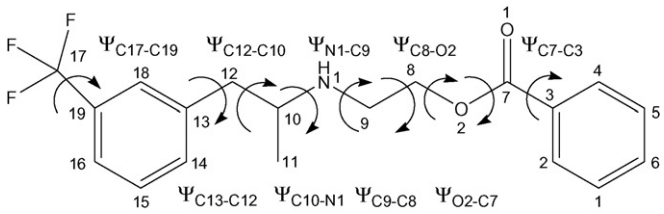
*Form **I***: Benfluorex hydrochloride was suspended in isopropanol and heated under reflux at 82 °C to achieve the complete dissolution. The clear solution was then cooled down to 15 °C (benfluorex hydrochloride Form **I** crystallizes at *ca.* 60 °C), and hold at this temperature for 2 h. After centrifugation, the precipitate was filtered and washed with isopropanol. It was then dried overnight under vacuum at 70 °C.

*Form **II***: Benfluorex hydrochloride was suspended in water, and heated to 90 °C to reach its complete dissolution. The clear solution was then cooled down to 15 °C (benfluorex hydrochloride Form **II** crystallizes at *ca.* 80 °C), and hold at this temperature for 2 h. After centrifugation the precipitate was filtered and washed with water. It was then dried overnight under vacuum at 70 °C.

2.2. Methods

2.2.1. Thermal analysis

Thermal analyses were performed on a DSC 8500 Perkin Elmer apparatus at scanning rate of 10 °C min^{-1}, typically in the 50–200 °C temperature range (heating, as well as upon cooling: *vide infra*).



Scheme 1.

Each sample, accurately weighted, was heated in an open aluminium pan. The sensor and the samples were maintained under a nitrogen purge during the whole experiment. The transition temperatures were determined by the onset method, *i.e.* taking the temperature value measured at the crossing point between the baseline and the tangent segment taken at the lower inflection point of the endothermic peak.

2.2.2. X-ray powder diffraction analysis

Strictly monophasic samples of benfluorex hydrochloride were gently ground in an agate mortar, and then deposited in the hollow of an aluminum sample holder, 0.2 mm deep, equipped with a quartz monocrystal zero background plate (supplied by *The Gem Dugout*, State College, PA). Diffraction data were collected in the 5–105° 2θ range, sampling at 0.02°, on a θ:θ vertical scan Bruker AXS D8 Advance diffractometer, equipped with a linear Lynxeye position sensitive detector, set at 300 mm from the sample (Ni-filtered Cu K$\alpha_{1,2}$ radiation). Standard peaks search method, followed by indexing by TOPAS-R [13], allowed the determination of approximate cell parameters for Forms **I** and **II**. The structures solution was initiated by employing semi rigid molecular fragments [flexible about nine torsion angles; see Scheme 1] built by molecular mechanics optimization [14], and a freely floating Cl^- anion. Simulated annealing, using the default parameters set in TOPAS-R and 1,000,000 iterations, allowed the location and orientation of the used fragments, later refined by the Rietveld method. All computations were performed using TOPAS-R, accounting for the full α_1–α_2 doublet. No antibump or distance restraints were introduced in the final refinement cycles, apart from the rigid-body description of the molecular fragment cited above. The fundamental parameters approach in describing the peak shapes was employed, the background contributions were modeled by a polynomial fit (fourth order Chebyshev model), and preferred orientation correction

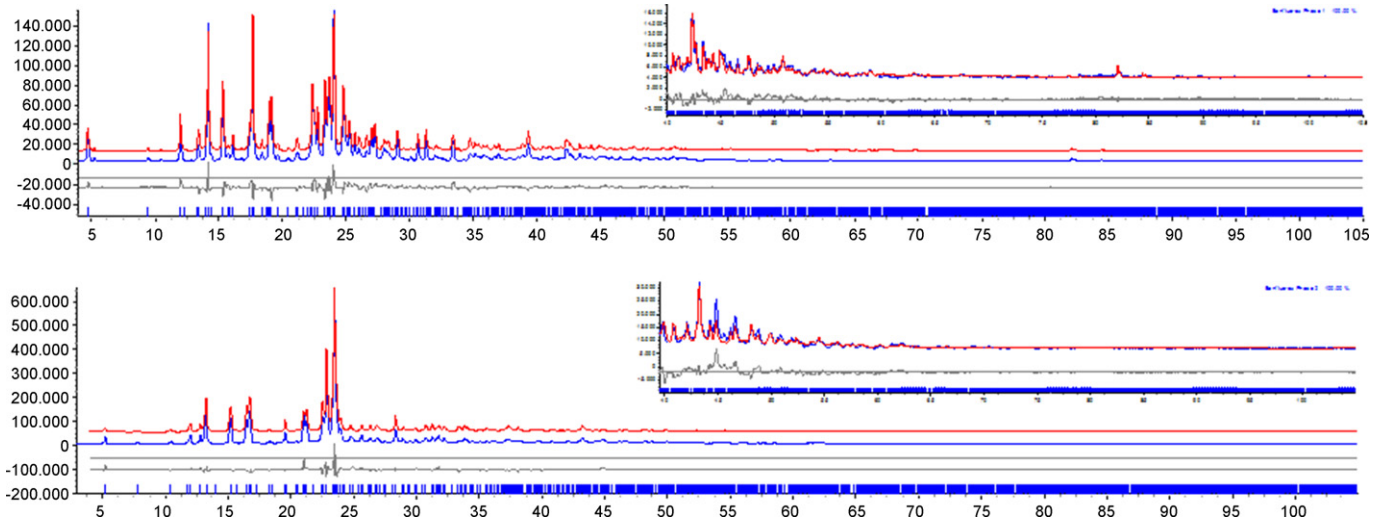


Fig. 1. Final Rietveld refinement plots for Benfluorex hydrochloride Form **I** and Form **II** (up to bottom), with peak markers and difference plot ($y_{obs} - y_{calc}$) at the bottom. Blue: observed data (y_{obs}); red: calculated data (y_{calc}, shifted along the y coordinate for sake of graphical comparison). The inserts show the high angle region ($2\theta > 40°$).

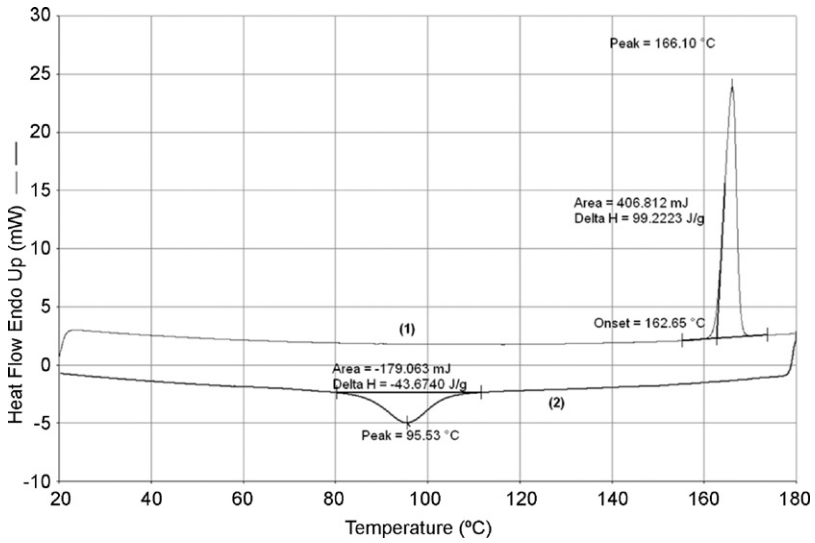


Fig. 2. DSC thermograms for Benfluorex hydrochloride Form **I**, under: (1) progressive heating from 50 °C to 180 °C (melting observed at 163 °C – onset); (2) progressive cooling down to r.t. (crystallization to Form **I** – XRPD evidence – being observed near 100 °C).

for [1 0 0] pole was described by the March-Dollase formulation for both phases [final magnitude Form **I**, $r = 0.7055(2)$; Form **II**, $r = 0.943(3)$]. Fig. 1 shows the final Rietveld refinement plots.

Crystal data for Form **I**: $C_{19}H_{21}F_3NO_2Cl$, fw 387.83 g mol^{-1}, 293 K, monoclinic, $P2_1/n$, $a = 21.0719(10)$ Å, $b = 7.0563(4)$ Å, $c = 14.8684(7)$ Å, $\beta = 116.998(3)^\circ$, $V = 1969.8(2)$ Å^3, $Z = 4$, ρ_{calc}, 1.307 g cm^{-3}, μ (Cu Kα) = 20.8 mm^{-1}, $R_{wp} = 0.105$, $R_p = 0.080$, $R_{Bragg} = 0.062$, 2θ range (5–105)°.

Crystal data for Form **II**: $C_{19}H_{21}F_3NO_2Cl$, fw 387.83 g mol^{-1}, 293 K, orthorhombic, Pbca, $a = 33.8031(2)$ Å, $b = 15.1451(8)$ Å, $c = 7.6138(6)$ Å, $V = 3897.9(4)$ Å^3, $Z = 8$, ρ_{calc}, 1.323 g cm^{-3}, μ (Cu Kα) = 21.1 mm^{-1}, $R_{wp} = 0.113$, $R_p = 0.080$, $R_{Bragg} = 0.058$, 2θ range (5–105)°.

Fractional atomic coordinates have been deposited as CIF files within the Cambridge Crystallographic Database as publications No. 757077 and 757078.

2.2.3. Thermodiffractometry

Phase transition of benfluorex hydrochloride was followed directly into the diffractometer chamber using a custom made sample holder which allows raising the temperature, in air, up to 600 °C. Samples of both phases were heated up to 180 °C in 10 °C temperature steps and at each temperature XRPD spectra were recorded to monitor the transition process. At the end, the samples were cooled back to ambient temperature.

3. Results and discussion

3.1. Thermal properties

The DSC trace recorded for benfluorex hydrochloride Form **I** (see Fig. 2) shows a sharp endotermic peak at T_{onset} of *ca.* 163 °C, corresponding to its melting point. The transition from the solid state to the liquid state involves an enthalpy change of about 38 kJ mol^{-1}. After the melting event, this compound decomposes at *ca.* 230 °C, as determined by TG analysis (not shown here). If, immediately after melting, the liquid of Form **I** is cooled down to room temperature, recrystallization near 100 °C is observed.

At variance, the DSC trace of benfluorex hydrochloride Form **II** (Fig. 3) shows a sharp endotermic peak at a significantly lower

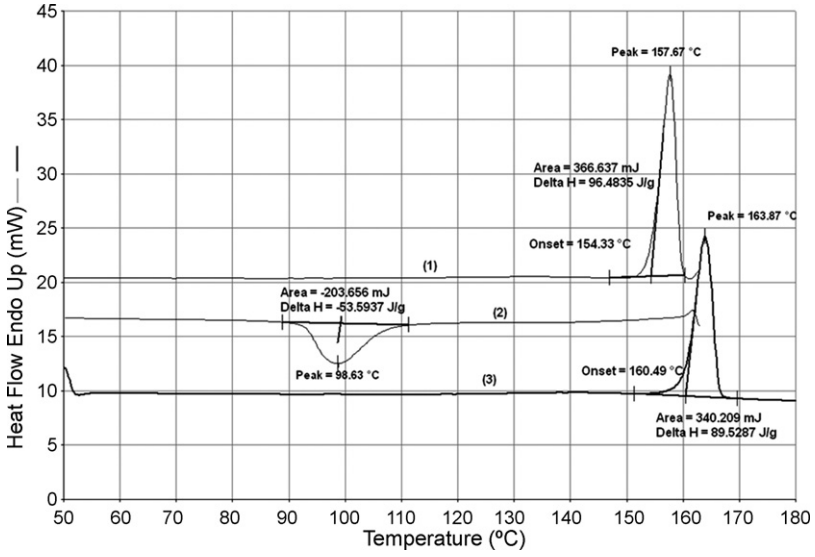


Fig. 3. DSC thermograms for Benfluorex hydrochloride Form **II**, under: (1) progressive heating from 50 to 163 °C (melting observed at 154 °C – onset); (2) progressive cooling down to r.t. (crystallization to Form **I** – XRPD evidence – being observed near 100 °C) and (3) reheating up to 180 °C (melting observed at *ca.* 160 °C).

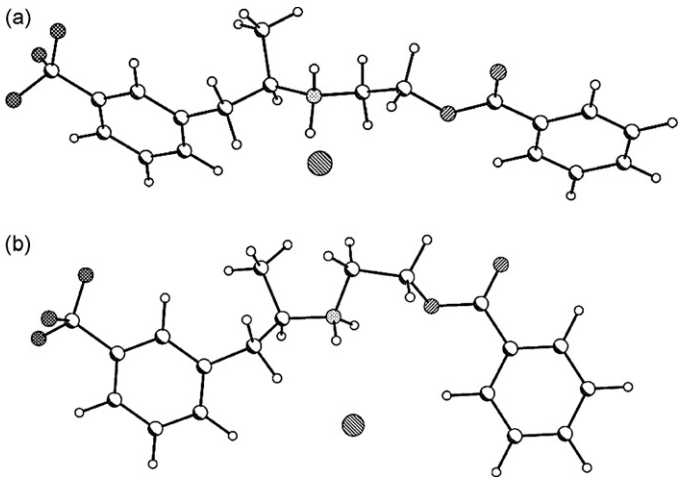


Fig. 4. Schematic drawing of the molecular conformations of Benfluorex hydrochloride Form **I** (a) and Form **II** (b).

temperature, with T_{onset} of *ca.* 154 °C and, upon cooling, recrystallization to Form **I**. The enthalpy change is only marginally lower (about 37 kJ mol^{-1}), as expected for the less thermodynamically stable phase. Subsequent heating showed a new melting process occurring at about 161 °C, suggesting formation of Form **I**, as also confirmed by our XRPD results, and demonstrating that the thermodynamically stable phase is indeed Form **I**, which possesses a higher melting point and a slightly higher melting enthalpy.

*3.2. Crystal and molecular structures of benfluorex hydrochloride in the two polymorphs, Form **I** and Form **II***

Crystals of Form **I** and Form **II** of benfluorex hydrochloride are based upon an ionic packing of protonated benfluorex cations and chloride anions. In both phases, protonation occurs at the most basic site, the N1 atoms, which, therefore, become tetrahedrally bound to two carbons and two hydrogen atoms. While this structural feature could not be derived directly from our powder diffraction experiment, the analysis of packing contacts suggests N1 as the positively charged site. Differentiation of the two polymorphs is driven by the two different conformations adopted by the saturated chain linking the two extremes of the benfluorex cation, two aromatic rings (a trifluoromethylphenyl and a benzoate group)

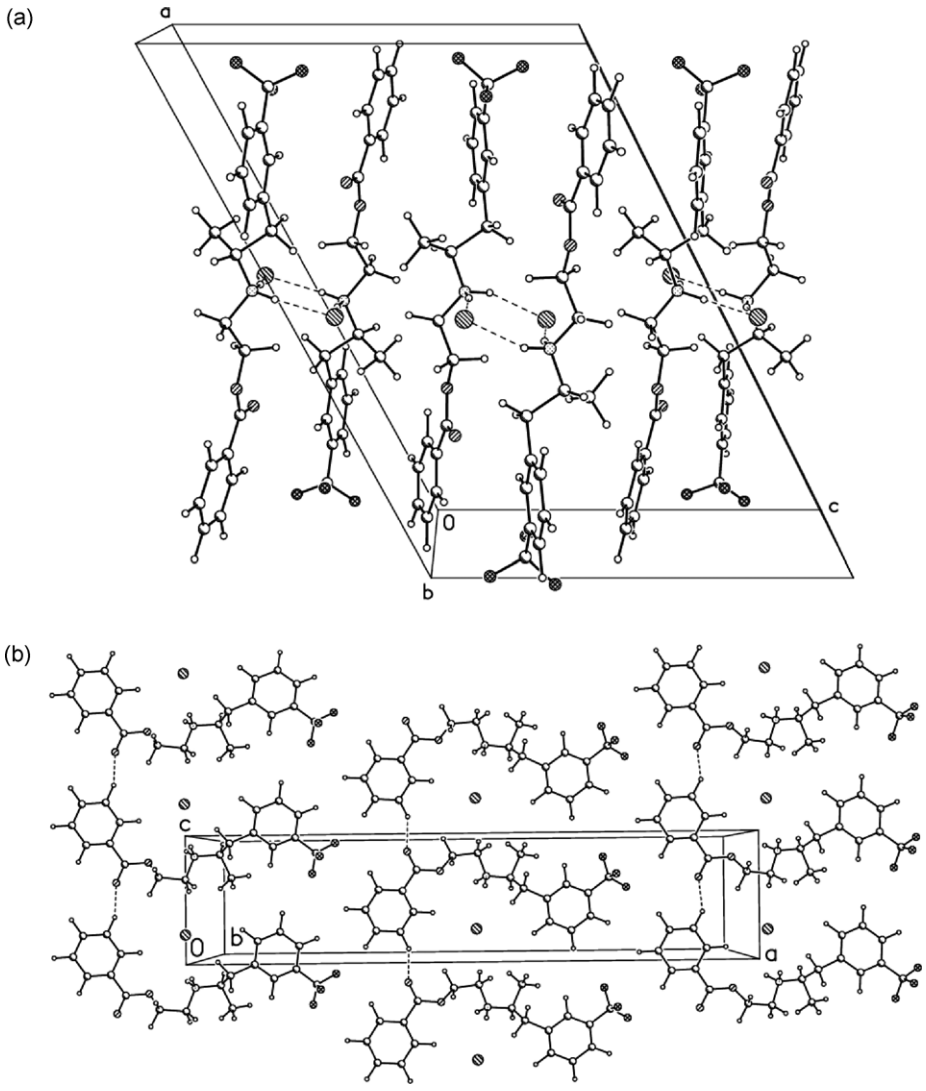


Fig. 5. Schematic drawing of the crystal packing of Benfluorex hydrochloride polymorphs. (a) Form **I**, viewed down [0 1 0] and (b) Form **II**, viewed down [0 1 0]. Intermolecular hydrogen bonds are highlighted with fragmented lines.

Table 1
Selection of the relevant conformational parameters of the S enantiomer of benfluorex hydrochloride polymorphs Forms **I** and **II** and of the fenfluramine hydrochloride (CCDC code: **BABCUC** [15] and **BUHCIQ** [16]).

Torsion angle		Form **I** (°)	Form **II** (°)	**BABCUC** (°)	**BUHCIQ** (°)
C18–C13–C12–C10	$\Psi_{C13-C12}$	114(1)	88(1)	114	126
C13–C12–C10–N1	$\Psi_{C12-C10}$	159(1)	162(1)	176	−178
C12–C10–N1–C9	Ψ_{C10-N1}	−162(1)	103(1)	−64	−58
C10–N1–C9–C8	Ψ_{N1-C9}	−163(1)	−176(1)	−179	−172
N1–C9–C8–O2	Ψ_{C9-C8}	−145(1)	−43(1)	–	–
C9–C8–O2–C7	Ψ_{C8-O2}	−159(1)	−158(1)	–	–
C8–O2–C7–C3	Ψ_{O2-C7}	173(1)	−128(1)	–	–
O2–C7–C3–C4	Ψ_{C7-C3}	−179(1)	−178(1)	–	–

connected by a flexible heteroatomic saturated aliphatic chain. A view of the two molecular conformations derived by our structural analysis are depicted in Fig. 4(a) and (b) where significantly different torsions in the spacer are shown (for the atomic labeling see Scheme 1). The conformational flexibility of the chain is compared with that of a precursor of benfluorex hydrochloride, the fenfluramine hydrochloride [15,16], which exists in two crystalline phases (one racemic, CSD Code: BABCUC, and the other enantiopure: CSD Code BUHCIQ). Table 1 reports the torsional angle values, obtained in the final refinement, for Form **I** and **II**, together with those of fenfluramine. In Form **I**, the saturated chain is nearly stretched (with a distance between the two ipso carbons of 9.70 Å), while it forms a partial arch in Form **II**, lowering this value down to 8.37 Å, as a consequence of a loss of the all-trans conformation present in Form **I**. In the two fenfluramine crystal phases, the torsional angles are very similar, and witness a substantial stability of the observed conformation. However, as in benfluorex hydrochloride Form **II**, also here the *all-trans* conformation is avoided, and arching of the aliphatic residue is evident.

Chloride ions in the crystals of the two polymorphs extensively interact with the most acidic sites of the organic fragments, through evident hydrogen bond contacts of NH···Cl type (3.02 and 3.27 Å in Form **I**, 3.13 Å and a much longer one of 3.46 Å in Form **II**). The intermolecular contacts appear rather different: Form **I**, through the presence of μ-Cl ions, contains centrosymmetric dimers with a N_2Cl_2 moiety (see Fig. 5(a)), while Form **II** contains well defined ionic couples, the chloride anions falling in the pocket generated by the molecular arch described above (as shown in Figs. 4(b) and 5(b)).

The crystal packing of the two forms, evidences that, in both forms, molecules maintain their principal inertial axes closely packed in a parallel fashion. However, in Form **I** (Fig. 5(a)), molecules pack in a head-to-tail fashion forming centrosymmetric dimers connected via hydrogen bonds between two ammonium H atoms and two chloride ions. The experimentally observed preferred orientation poles [1 0 0] find a coherent structural explanation in the structural model presented above. In Form **II** (Fig. 5(b)) the molecules interact *via* C1–H···O1 short contacts [C1–H···O1 3.087 Å, H···O1 2.194 Å, C1–H···O1 135.54°] forming hydrogen-bonded molecular chains running in antiparallel directions along *c* axis.

3.3. Thermodiffractometry

As shown in Fig. 6, which contains two complete 2D thermodiffractograms, we observed the *direct* transformation of benfluorex hydrochloride Form **II** into Form **I** in the diffractometer chamber (by progressively heating Form **II** up to 170 °C, in 10 °C steps and maintaining isothermal conditions form about 10 min at each step, *i.e.* the time necessary for data collection) without observing melting of Form **II**, probably because of thermal gradients in the sample – open to the air – and to the much longer timescale of our XRPD measurements, than in DSC.

As evidenced by thermal analysis, this phase transition is clearly irreversible, leading to the formation of the stable polymorph, Form **I**. After the phase transition, the sample was further heated to about 180 °C, inducing evident melting, and cooled back to ambient temperature within minutes: crystallization to benfluorex hydrochloride Form **I** was observed with large preferred orientation effects, with no detectable traces of Form **II**. In a separate thermodiffractometric experiment, Form **I** was heated up to the melting temperature and cooled back to ambient temperature different times. In agreement with the DSC data, no transitions to Form **II** or to other new phases were observed. The sample melts slightly below 180 °C, giving a liquid, which, upon cooling, solidifies to an amorphous material, which crystallizes back to Form **I** as the temperature decreases (see Fig. 6(b)).

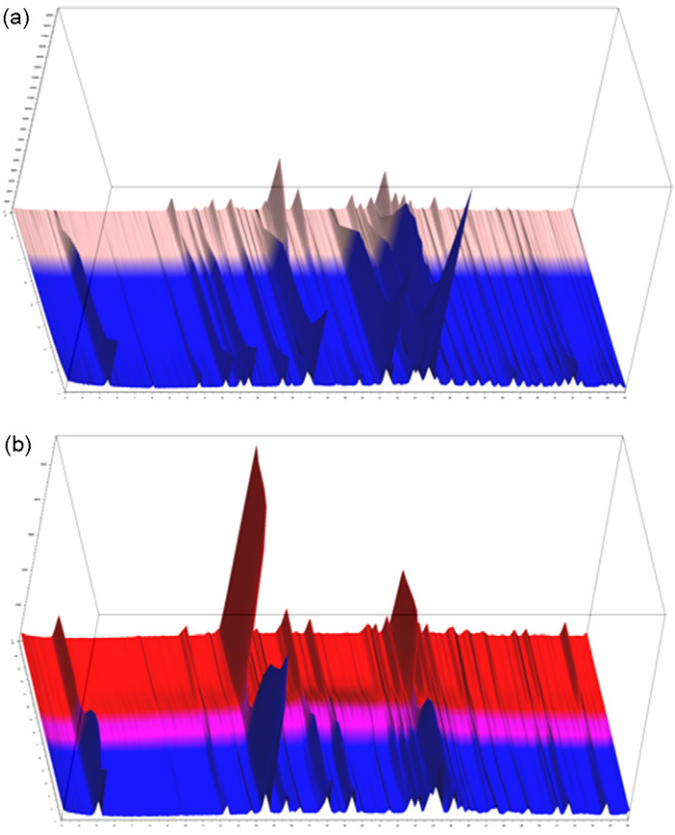


Fig. 6. (a) Thermodiffractometric plot for Benfluorex hydrochloride Form **II** transforming into Form **I** (bottom to top). The lowest portion of the graph refers to the heating steps from 30 to 160 °C while the upper portion represents different patterns measured isothermally at 170 °C. (b) Thermodiffractometric plot for Benfluorex hydrochloride Form **I**. The lowest portion of the graph refers to the heating steps from 60 to 170 °C. The middle portion shows the melt observed at *ca.* 180 °C, while the upper section was measured upon cooling from 140 to 70 °C.

4. Conclusions

The crystal structure of benfluorex hydrochloride, Form **I** and Form **II**, were determined uniquely from laboratory X-ray powder diffraction data using as starting model the semi rigid molecular fragments built by molecular mechanics optimization**,** adding structural flexibility with the aid of nine freed torsional angles. The molecular geometry and crystal packing were fully determined in order to clarify the differences in the solid state. Crystals of Form **I** and Form **II** of benfluorex hydrochloride are based upon an ionic packing of benfluorex cations protonated at the most basic site, the N1 atoms, and chloride anions. Form **I** shows intermolecular contacts through the presence of μ-Cl ions, generating centrosymmetric dimers with a N_2Cl_2 moiety, while Form **II** packs in antiparallel chains of molecules linked by C–H···O interactions.

For Form **II** three subsequent thermal events were monitored by DSC and thermodiffractometry: melting, recrystallization to Form **I**, and final melting of this new form. Our experimental data coherently demonstrated that Form **I** must be considered the thermodynamic stable phase.

Once again, the power of modern laboratory powder diffraction data and the viability of this method in retrieving structural details when single crystals are not available are confirmed. While the overall picture of the derived structural model is somewhat blurred (compared to conventional single-crystal diffraction analysis), the XRPD determination of geometrical parameters which would otherwise remain inaccessible, is a valid alternative [17].

Acknowledgement

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