Mechanistic and kinetic insight into spontaneous cocrystallisation with a volatile organic compound

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Supplementary information

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Experimental

Materials

Benzoic acid and isoniazid were procured from commercial suppliers. 5 g of isoniazid and 5 g of benzoic acid were milled separately in the Retsch MM301 ball mill (15 mL stainless steel vials with one 1 cm stainless steel ball in each) for 15 minutes to reduce the particle size. The milled samples were annealed by keeping at ambient conditions for 2 weeks and sieved to obtain the fraction of 75 to 150 µm.

Mechanochemical cocrystallisation

0.2743 g of isoniazid and 0.2442 g of benzoic acid (1:1 molar ratio) were mildly gently blended together in a mortar with the pestle before co-milling to reduce the effect of composition inhomogeneity. Cocrystallisation was performed by milling the mixture of the starting compounds in a Retsch MM301 ball mill. Isoniazid and benzoic acid were milled in 5 mL stainless steel grinding jars with two 8 mm and three 6 mm stainless steel balls in each milling jar. Two different milling ball sizes were chosen after initial experiments, since they offered better data reproducibility compared to equally sized milling balls. Samples were ground at ambient conditions (45-55% relative humidity (RH) and 20-22 °C) for various time periods with a frequency of 20, 25 or 30 Hz. The average composition of the reaction mixture after milling was determined by removing the powder from the grinding jar and quickly mixing it in a mortar to homogenize the composition. The milling product was analysed by powder X-ray diffraction (PXRD) immediately after the experiment.

Cocrystallisation kinetics in pre-milled isoniazid and benzoic acid 1:1 mixtures

The cocrystallisation kinetics in pre-milled isoniazid and benzoic acid 1:1 mixture (0.2743 g of isoniazid and 0.2442 g of benzoic acid) was investigated using *in situ* powder X-ray diffraction experiments at elevated temperature and relative humidity. The pre-milled mixtures were loaded into the humidity chamber and subsequent diffraction patterns were recorded until no further changes in the diffraction patterns were observed. Several sets of experiments were performed to evaluate the effect of relative humidity, temperature and the initial milling frequency. The effect of the temperature was studied by performing experiments at 35, 40, 45, 50 and 55 °C temperature and 60% RH for samples that were pre-milled for 5 minutes at 20 Hz. The influence of the relative humidity was evaluated by acquiring the X-ray diffraction data at 60, 70 and 80 % RH and 40 °C for samples that were pre-milled for 5 minutes at 20 and 25 Hz. The influence of the pre-milling frequency was considered by performing the milling for 5 minutes with a frequency of 20, 25, 30 Hz and

recording the kinetic data of cocrystallisation at 60% RH and 40 °C.

Spontaneous cocrystallisation kinetics in isoniazid and benzoic acid 1:1 physical mixtures

Physical mixtures (1:1) of isoniazid (0.0686 g) and benzoic acid (0.0611 g) were stored in desiccators with various relative humidity (38, 58, 75, 85 and 97 % RH) at 30 °C. Diffraction patterns were periodically recorded over a period of 27 days. The humidity in the desiccators was maintained using saturated salt solutions (NaI for 38% RH; NaBr for 58% RH; NaCl for 75 % RH; KCl for 85% RH and K_2SO_4 for 97% RH).[1]

Cocrystallisation in pre-milled 1:1 mixtures of isoniazid and benzoic acid at elevated temperature and dry air

Pre-milled (20 Hz, 5 min) isoniazid and benzoic acid 1:1 mixtures were stored in desiccators containing P_2O_5 to ensure dry air. These desiccators were maintained at 30, 50 and 70 °C temperature. The composition of the samples was monitored using PXRD analysis.

In-situ PXRD at controlled temperature and humidity

The cocrystallisation experiments of isoniazid and benzoic acid were performed using a Bruker AXS D8 Discover powder diffractometer (Bruker AXS GmbH, Germany) equipped with an MRI humidity chamber. Copper K_α radiation (λ=1.5418 Å) was used in the experiments. Data were collected using a flat sample (depth of 0.8 mm) holder in the Bragg–Brentano geometry.

The X-ray diffraction measurements were performed at various temperatures (35, 40, 45, 50 and 55 °C) and various RH (60, 70, 80%). Diffraction patterns were recorded with 0.02° step size and a scan speed of 0.1 s per step in the 2θ range from 3 to 35°.

Rietveld analysis

The quantitative Rietveld analysis of the X-ray diffraction data were performed using the Bruker Topas 4.3 software[2] with the fundamental parameters (FP) approach. The crystal structures of isoniazid[3] (CSD refcode INICAC01), benzoic acid[4] (CSD refcode BENZAC01) and the isoniazid – benzoic acid cocrystal[5] (CSD refcode SETRIU, 10.5517/ccyw9jm) were obtained from the Cambridge Structural Database (CSD)[6] and used in the calculations.

A 3rd order Chebyschev polynomial was used to describe the background of the powder patterns. It was assumed that the sample is crystalline and does not contain an amorphous phase, as significant background changes were not observed during the experiments. The unit cell parameters were refined and the results were corrected for absorption and sample displacement. The average composition of the sample over the time period necessary to record the pattern was obtained and ascribed to the midpoint of the pattern recording time.

Cocrystallisation in 1:1 unmilled and pre-milled mixtures of isoniazid and benzoic acid under conditions of Magic Angle Spinning (MAS) using solid-state NMR

Physical mixtures (1:1) and pre-milled mixtures (5 min, 20 Hz) of isoniazid (0.2743 g) and benzoic acid (0.2442 g) were prepared according to the procedure described in the previous sections immediately before solid-state NMR analysis. The materials were packed in zirconia rotors and placed in the NMR probe immediately after the sample preparation to avoid any impact of environmental changes. ^{1}H-^{13}C CP (cross polarisation) MAS NMR spectra were acquired at 25 °C using spinning rates of 5 or 10 kHz. Due to the long pulse delay (180 s) of the reacting compounds, spectra of sufficient quality could only be acquired over a period of 6 hours after 128 scans. Five or three experiments for each spinning rate were carried out over a period of 30 hours in the case of pre-milled materials and 18 hours for physical mixtures, as no changes after this time were observed (Supplementary Information, Figs. 4 and 5). The resulting solid-state NMR spectra show the average pictures of the structural and dynamical processes taking place during cocrystallisation of isoniazid and benzoic acid in the six-hour time intervals.

Solid-state NMR analysis

Solid-state NMR spectra were acquired using a Bruker AVANCE III spectrometer equipped with a 4 mm triple resonance probe at 400.23 MHz for ^{1}H and 100.64 MHz for ^{13}C. The ^{1}H-^{13}C cross-polarization/magic angle spinning (CP/MAS) NMR spectra were acquired using a RAMP CP pulse sequence. The MAS rates were 5 or 10.0 kHz; ^{1}H $\pi/2$ pulse length and pulse delay were optimized to 3.20 μs and 180.0 s, respectively. The contact time during CP was set to 2.0 ms and a SPINAL64 decoupling was applied during acquisition. The Hartmann-Hahn conditions were set with hexamethylbenzene (HMB). 512 scans were acquired for pure crystalline compounds and 128 during cocrystallisation experiments. The ^{13}C chemical shifts were recorded with respect to tetramethylsilane (TMS).

Analysis of the cocrystallisation kinetics under MAS conditions

The spectra were de-convoluted using a Gaussian-shape fitting function using Fityk 0.9.8.[7] and integrated peak areas were compared for both MAS rates. Comparison of the cocrystallisation kinetics of isoniazid and benzoic acid at MAS rates of 5 and 10 kHz was based on the intensity of the peak at 172.3 ppm, which is attributed to the carbonyl group of benzoic acid.

DVS analysis

The water vapour sorption–desorption isotherms of isoniazid and benzoic acid 1:1 physical mixture were measured with a TA Q5000 SA instrument at 30, 40 and 50 °C. The

relative humidity was increased with a step size of 5% from 5% to 95% RH and decreased to 5% RH again. The dwell time at each RH was 1 h. The sample was dried at 60 °C for 1 hour prior to analysis. Sorption–desorption isotherms of pure isoniazid and pure benzoic acid were also recorded. Two experiments were performed at each temperature and the average values of both experiments were calculated.

Scanning electron microscopy (SEM)

The particle size and morphology of isoniazid, benzoic acid and the cocrystal were analysed by scanning electron microscopy (SEM) using a JEOL JSM-5910 LV SEM Scanning Electron Microscope with an accelerating voltage of 20 kV. The working distance was 9 mm. The samples were sputtered with gold in an argon atmosphere at room temperature before examination.

Hot stage microscopy (HSM)

Formation of the isoniazid–benzoic acid cocrystal upon heating (at 90 °C) was followed using a Leica DM LS2 optical microscope, equipped with the Mettler Toledo FP 82 HT hot stage and FP 90 temperature controller. The microscope was also used to observe the sublimation of smaller benzoic acid crystals at ambient conditions (45–55% RH and 20–22 °C) for 20 hours.

AFM analysis

The AFM height images (25 μm × 25 μm) and force measurements were recorded in QI mode (Quantitative Imaging mode[8]) at room temperature in air at a resolution of 256×256 pixels using the NanoWizard 3 (JPK Instruments AG) AFM system. The maximum force determined by the vertical deflection of the cantilever (i.e., setpoint) was set to 0.7–1 nN, and the scan rates were automatically controlled by the Z length (1 μm), extension time (5 ms) and retraction time (5 ms). Soft cantilevers (NanoWorld Arrow-Cont) were used and the typical force constant of the cantilever was 0.2 N m^{-1}. The force constant for each of the cantilevers was calibrated using the thermal noise method.[9] For the *in situ* AFM humidity studies an enclosure was constructed and the humidity inside the enclosure was followed by a Fischer Scientific Humidity/Temperature Pen. For each change in humidity the sample was allowed to equilibrate for at least 48 hours.

FTIR analysis

The FTIR spectra were recorded using a Perkin Elmer Spectrum BX instrument in the range 550–4000 cm^{-1} with a resolution of 2 cm^{-1}. Each spectrum was generated by co-addition of 64 interferograms.

Detailed discussion of FTIR and solid-state NMR spectra

The differences between the structures of the isoniazid–benzoic acid cocrystal and its components are highlighted by FTIR and solid state NMR spectra, which demonstrate the changes in hydrogen bonding. The N─H stretching vibrations of isoniazid in the FTIR spectrum at 1334 cm^{-1} are shifted to 1343 cm^{-1} in the cocrystal and the N─NH$_2$ vibrations at 1142 cm^{-1} are not observable for the cocrystal. In the crystal structure of isoniazid the carboxyhydrazide oxygen atom does not participate in hydrogen bonding while in the cocrystal it forms two hydrogen bonds with a terminal hydrazide N─H. Consequently the IR absorption bands arising from the C=O stretching vibrations are shifted from 1667 cm^{-1} for isoniazid to 1658 cm^{-1} for the cocrystal. The corresponding ^{13}C peak of isoniazid carboxyhydrazide (C7) in the solid-state NMR spectra is shifted from 164.7 ppm[10] to 166.4 ppm in the cocrystal.

The carboxyl group of benzoic acid forms an O─H···N hydrogen bond with the pyridine ring of isoniazid in the cocrystal, causing a shift from 1635 cm^{-1} to 1622 cm^{-1} in the frequency of the asymmetric stretching of the isoniazid aromatic C⁛N bonds. The formation of the O─H···N hydrogen bond also shifts the C=O stretching vibration IR band of benzoic acid from 1680 cm^{-1} to 1692 cm^{-1}. The IR absorption band of the C─O stretching at 1289 cm^{-1}, characteristic of benzoic acid, has been shifted to 1278 cm^{-1}. The O─H bending vibrations at 1423 and 934 cm^{-1} of benzoic acid are not observable in the FTIR spectra of the cocrystal. In the NMR spectra, these structural changes result in a substantial upfield shift of the benzoic acid carboxyl (C13) peak at 172.3 ppm to 168.3 ppm. The isoniazid aromatic carbon (C6) peak shifts from 151.3 to 153.3 ppm and the isoniazid aromatic (C2) peak shifts from 149.4 to 148.9 ppm.
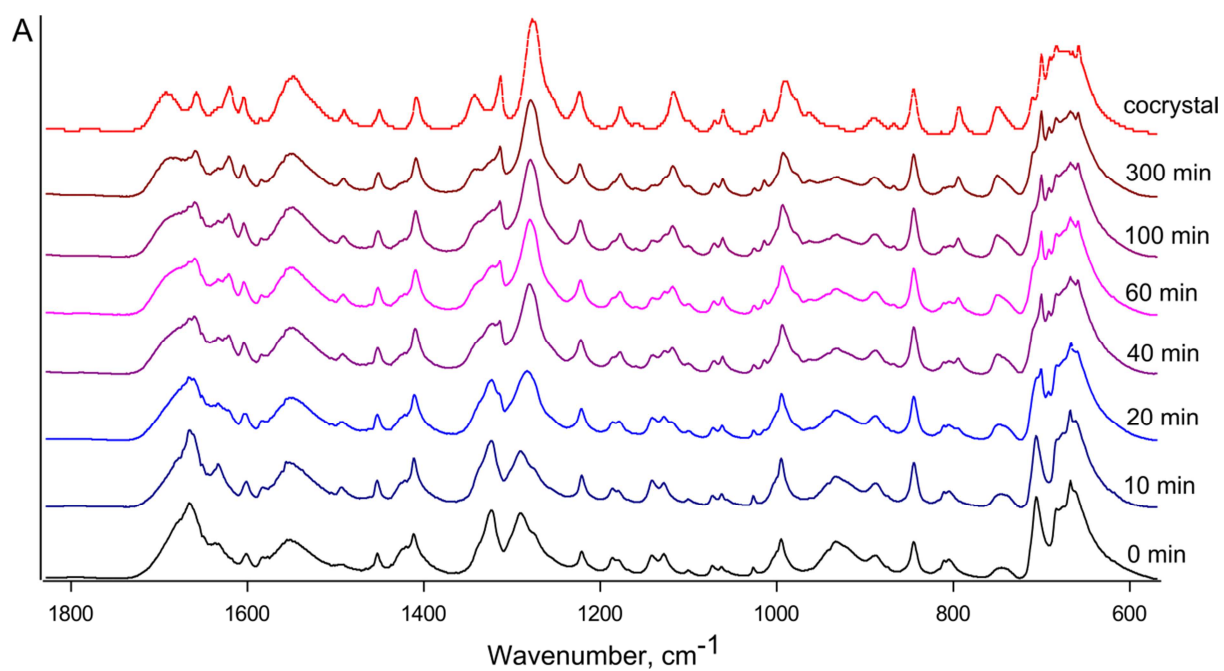
Fig. S1 FTIR spectra of an isoniazid–benzoic acid physical mixture stored at 40 °C and 80% RH
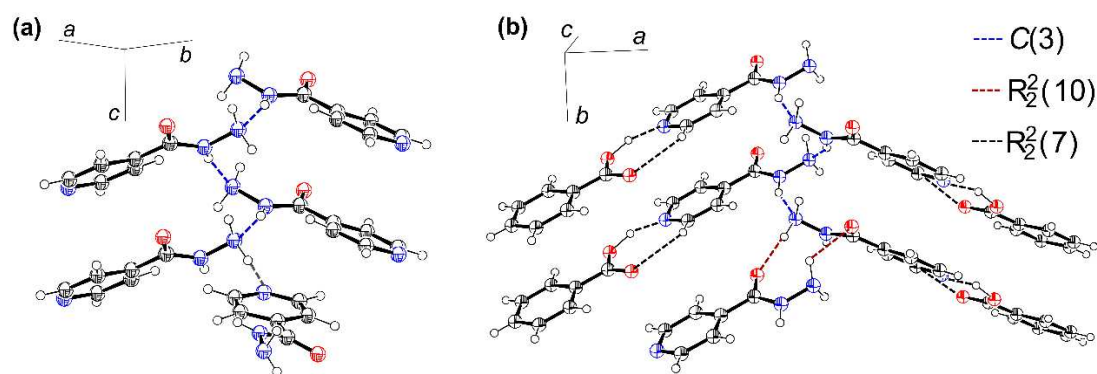

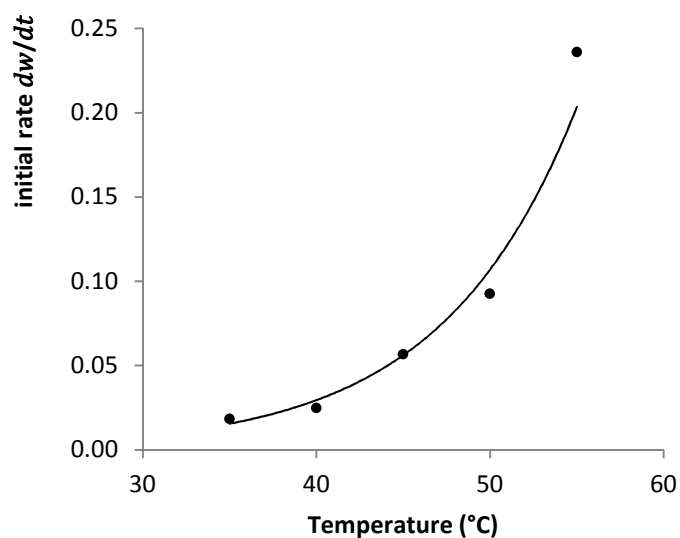**Fig. S2** Hydrogen bonding scheme of (a) isoniazid and (b) isoniazid–benzoic acid cocrystal

Fig. S3 Initial rates $(\dfrac{dw}{dt})$ of isoniazid cocrystallisation with benzoic acid depending on temperature (60% RH, samples pre-milled for 5 min at 20 Hz).
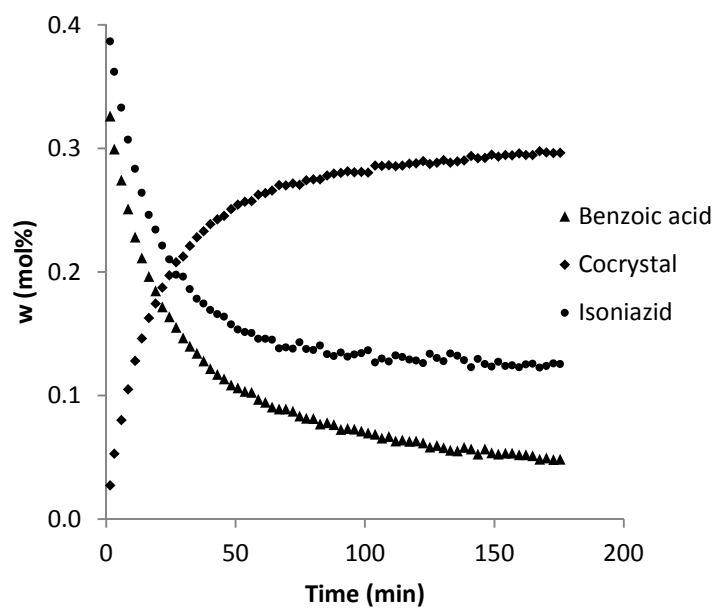

Fig. S4 Content of isoniazid, benzoic acid and their cocrystal in the sample during the cocrystallization at 40 °C and 60% RH (20 Hz, 5 min pre-milled)
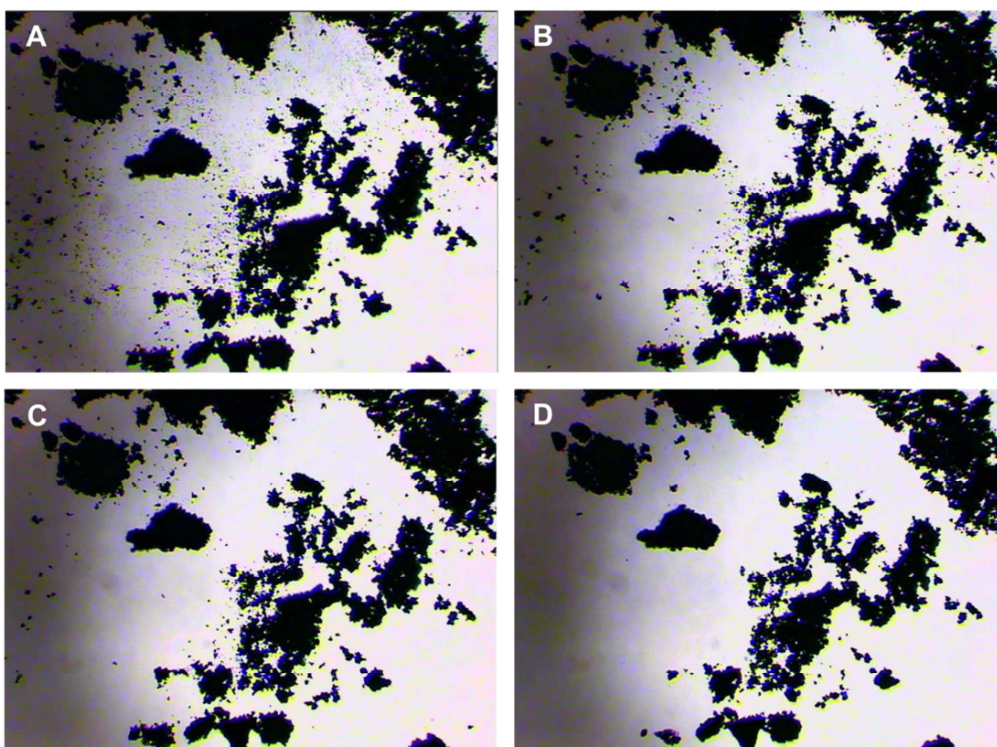
Fig. S5 Vaporization of benzoic acid from a milled sample (30 Hz, 5 min). A- sample after milling, B – after 2 h, C – after 4 h, D – after 20 h at 22 °C
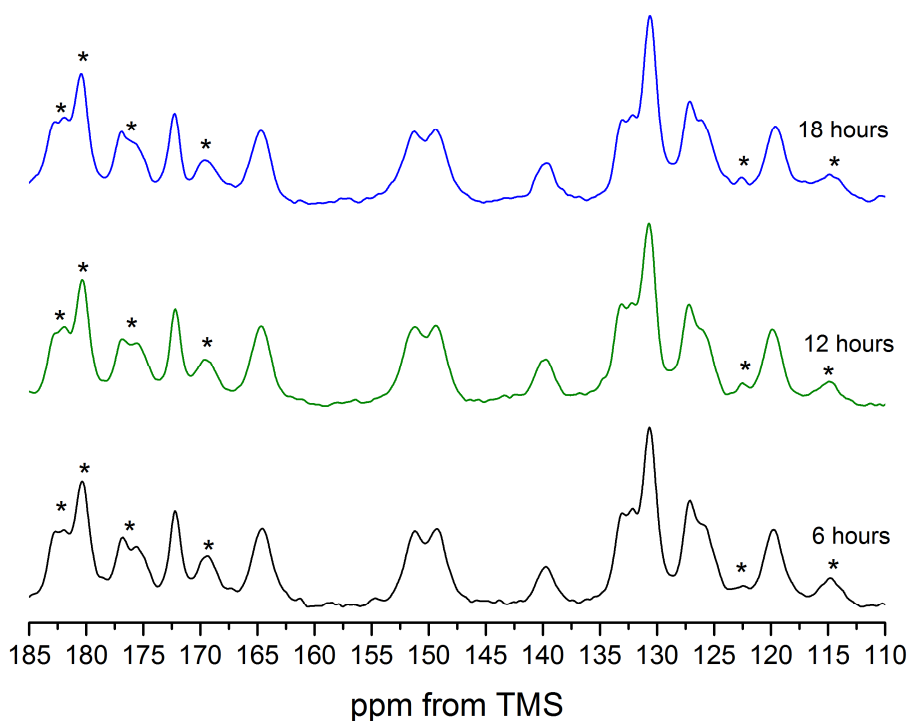

Fig. S6 ^{1}H-^{13}C CP MAS solid state NMR spectra of isoniazid – benzoic acid physical mixture over a time of 18 hours acquired at an MAS rate of 5 kHz (spinning sidebands are labelled with asterisks)
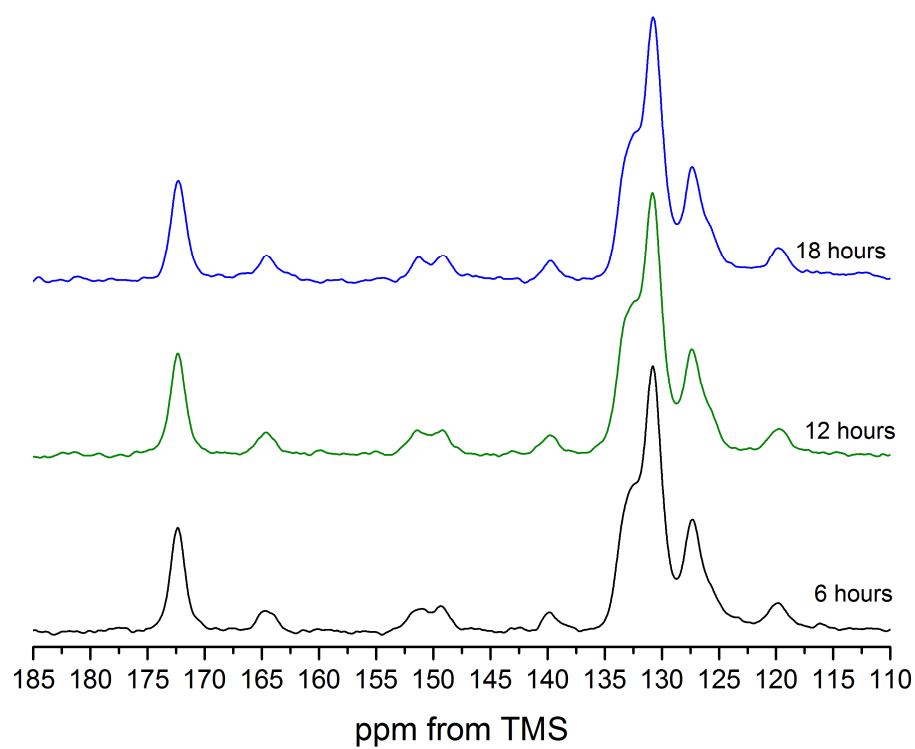
Fig. S7 ^{1}H-^{13}C CP/MAS NMR spectra of isoniazid – benzoic acid physical mixture over a time of 18 hours acquired at an MAS rate of 10 kHz

Table S1 Intensities of the deconvoluted peaks of benzoic acid, isoniazid, cocrystal and sum of aromatic peaks during cocrystallisation under MAS conditions at 5 kHz spinning speed over 30 hours experimental time

| Area of the peaks at 5 kHz MAS rate						
Compound	Peak (ppm)	Time of experiment (hours)				
		6	12	18	24	30
Benzoic acid	172.3	0.683	0.637	0.581	0.522	0.524
	139.9	0.231	0.217	0.193	0.145	0.195
Isoniazid	119.8	0.473	0.407	0.326	0.326	0.269
	149.4	0.334	0.293	0.242	0.256	0.193
	151.3	0.354	0.316	0.307	0.280	0.229
	164.7	0.508	0.448	0.331	0.358	0.393
Cocrystal	117.1	0.095	0.138	0.174	0.199	0.189
	122.8	0.255	0.329	0.299	0.289	0.248
	137.2	0.091	0.092	0.183	0.150	0.193
	148.9	0.094	0.084	0.119	0.110	0.170
	153.3	0.085	0.118	0.122	0.123	0.196
	168.3	0.099	0.186	0.228	0.196	0.247
	166.4	0.095	0.133	0.202	0.181	0.168
Aromatic peaks	127.3	1.454	1.508	1.407	1.583	1.211
	130.8	1.453	1.935	1.481	1.627	1.296
	132.4	1.549	0.907	1.412	1.048	1.568
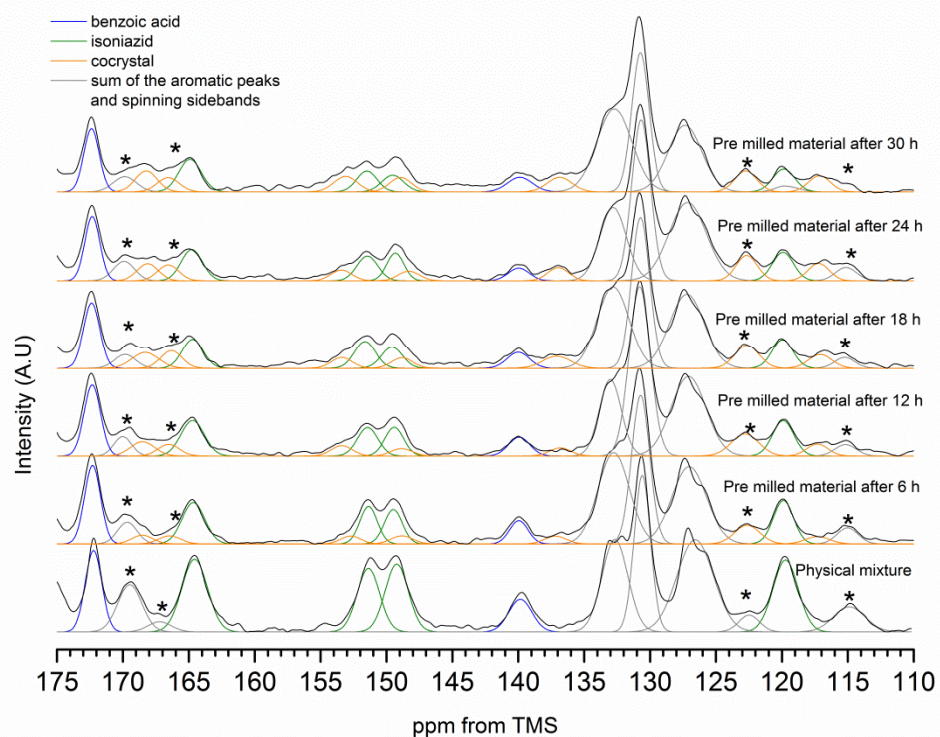
Fig. S8 ^{1}H-^{13}C CP/MAS NMR spectra and deconvoluted spectra of pre-milled (5 min, 20Hz) isoniazid – benzoic acid mixture over a time of 30 hours acquired at an MAS rate 5 kHz compared to physical mixture (spinning sidebands are labelled with asterisks)

Table S2 Intensities of the deconvoluted peaks of benzoic acid, isoniazid, cocrystal and sum of aromatic peaks during cocrystallisation under MAS conditions at 10 kHz spinning speed over 30 hours experimental time

| Peak areas at 10 kHz MAS rate						
Compound	**Peak (ppm)**	**Time of experiment (hours)**				
		6	**12**	**18**	**24**	**30**
Benzoic acid	172.3	0.500	0.414	0.370	0.330	0.323
	139.9	0.198	0.186	0.146	0.125	0.131
Isoniazid	119.8	0.264	0.229	0.193	0.214	0.181
	149.4	0.286	0.228	0.229	0.162	0.162
	151.3	0.317	0.223	0.201	0.209	0.193
	164.7	0.248	0.223	0.222	0.162	0.155
Cocrystal	117.1	0.188	0.140	0.188	0.175	0.178
	122.8	0.187	0.196	0.251	0.224	0.221
	137.2	0.123	0.192	0.202	0.184	0.210
	148.9	0.092	0.133	0.167	0.199	0.169
	153.3	0.083	0.153	0.180	0.143	0.193
	168.3	0.131	0.232	0.249	0.249	0.274
	166.4	0.117	0.103	0.120	0.168	0.167
Aromatic peaks	127.3	1.530	1.316	1.399	1.461	1.366
	130.8	1.791	1.746	1.803	1.803	1.644
	132.4	0.773	0.809	0.713	0.572	0.786
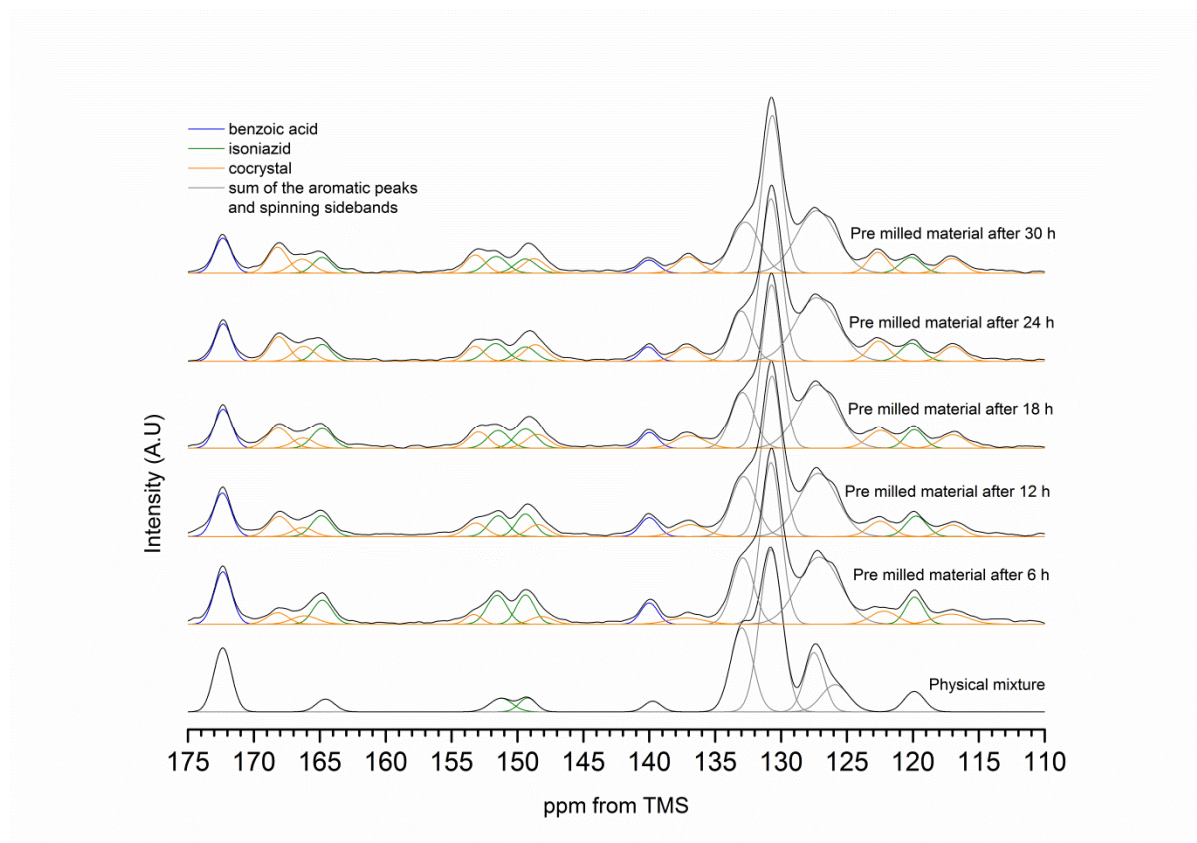
Fig. S9 ^{1}H-^{13}C CP/MAS NMR spectra and deconvoluted spectra of pre-milled (5 min, 20Hz) isoniazid – benzoic acid mixture over a time of 30 hours acquired at an MAS rate of 10 kHz compared to physical mixture

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