Electronic Supplementary Information†

Polymorphism in an API ionic liquid: Ethambutol dibenzoate trimorphs

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Materials and Methods

S,S-Ethambutol dihydrochloride (Lot#090M0189V) was purchased from Sigma-Aldrich and used without further purification. All other chemicals were of analytical or chromatographic grade.

Ethambutol base

The free base of ethambutol was prepared as per the method reported by Bhutani et al.[1a] Ethambutol dihydrochloride (2 g) was added to 5N aqueous NaOH (20 ml). The mixture was stirred for 10-15 min and extracted with methylene chloride (25 ml). The organic extract was dried (Na_2SO_4) and left for evaporation at ambient temperature to afford big block crystals of ethambutol in a day. The product crystals are characterized by DSC, NMR and XRD.

Ethambutol dihydrochloride ($C_{10}H_{26}Cl_2N_2O_2$):

^{1}H NMR (DMSO-d_6): δ 0.92 (3H, t, *J* 8), 1.68 (2H, m), 3.06 (1H, m), 3.71 (2H, m), 5.40 (1H, s), 9.29 (2H, d, *J* 80 (N–H coupling)). Protons of $–CH_2$ group (attached to $–NH_2^+$ group) merged with dissolved water peak of DMSO-d_6.

^{13}C NMR (DMSO-d_6): δ 10.24, 20.78, 41.27, 57.99, 60.74.

Ethambutol ($C_{10}H_{24}N_2O_2$):

^{1}H NMR (DMSO-d_6): δ 0.82 (3H, t, *J* 8), 1.31 (2H, m), 2.32 (1H, m), 2.55 (2H, s), 3.27 (2H, m). NHs and OHs exchange in solvent.

^{13}C NMR (DMSO-d_6): δ 10.51, 24.22, 47.31, 60.72, 63.07.

EDB salt crystallization

a. Solution crystallization: An alcoholic (MeOH or EtOH) solution of ethambutol and benzoic acid (in 1:2 molar ratio) was mixed and kept for slow crystallization (i) at room temperature, (ii) in –20 °C refrigerator, and (iii) desiccator separately. For fast crystallization of salts, solutions were subjected to rotavaporization. Crystallization from desiccator resulted in form 1 and that from rotavapor gave form 2. Liquid like products were obtained at other temperatures.

b. Co-melting: Ethambutol and benzoic acid (in 1:2 molar ratio) were taken together in a sublimation tube and heated in an oil bath beyond the melting point of the higher melting compound (ca. 140 °C). The tube was held until a uniform liquid with no trace of solid material was formed and then kept aside for cooling to room temperature. The molten material crystallized to give form 1.

c. Phase transformation

EDB form 3 was obtained from form 1 material subjected to VT-PXRD up to 90 °C, the phase transition temperature in DSC. The product from two VT-PXRD experiments on form 1 was combined and confirmed as form 3 by DSC, ATR-IR and Raman analysis.

Ethambutol dibenzoate ($C_{24}H_{36}N_2O_6$):

^{1}H NMR (DMSO-d_6): δ 0.86 (3H, t, *J* 8), 1.47 (2H, m), 2.65 (1H, m), 2.90 (2H, s), 3.39 (2H, m), 7.39 (2H, d, *J* 8), 7.47 (1H, d, *J* 8), 7.90 (2H, d, *J* 8). NHs and OHs exchange in solvent.

^{13}C NMR (DMSO-d_6): δ 10.52 (2C), 22.65 (2C), 44.22 (2C), 60.51 (2C), 60.96 (2C), 128.43 (4C), 129.59 (4C), 131.64 (2C), 135.07 (2C), 169.31 (2C).

The three polymorphs exhibited identical solution NMR spectra.

Powder X-ray diffraction

PXRD were recorded on Bruker D8 Advance diffractometer using Cu-Kα X-radiation (λ = 1.5406 Å) at 40 kV and 30 mA. Diffraction patterns were collected over 2θ range of 5-50° at scan rate of 1° min^{-1}. VT-PXRD was performed on the same instrument equipped with a variable temperature stage (TTK450 chamber, working temperature range –190 °C to 450 °C). The sample was packed in a Cr–plated copper holder and heated from 30 °C to 90 °C at 5 °C/min. Scans were taken at selected temperatures over 2θ range of 5-42°, using a step size of 0.08° 2θ and time per step of 1 sec. Powder cell 2.4 was used to plot the diffraction patterns.

Spectroscopy

Nicolet 6700 FT-IR spectrometer equipped with a single bounce ATR and an NXR FT-Raman module was used to record IR and Raman spectra. IR spectra were recorded on samples placed on Zn-Se crystal. Raman spectra were recorded on compressed samples contained in a gold-coated sample holder. Solution and solid state NMR spectra were recorded on a Bruker Avance spectrometer at 400 MHz. The identity and stoichiometry of the components of the salts were established through solution ^{1}H NMR integration and ^{13}C NMR spectra. SS-NMR spectra were recorded on a Bruker 4 mm double resonance CP-MAS probe in zirconia rotors at 5.0 kHz with a cross-polarization contact time of 2.5 ms and a recycle delay of 8 s. ^{15}N CP-MAS spectra were recorded at 40 MHz and referenced to glycine N atom, and then the chemical shifts were recalibrated to nitromethane N ($\delta_{\mathrm{glycine}} = -347.6$ ppm).

Thermal analysis

DSC was performed on a Mettler Toledo DSC 1 module and calibrated with indium (T_m = 156.60 °C; ΔH_f = 28.45 J g^{-1}) and zinc (T_m = 419.50 °C; ΔH_f = 107.50 J g^{-1}) as per the manufacturer's specifications. Samples of 5-10 mg were placed in crimped but vented aluminium pans and were purged by a stream of nitrogen flowing at 50 mL min^{-1}. The temperature range used for solid samples in DSC is 30–150 °C at 5 °C min^{-1}. For liquid samples, a heat-cool-reheat DSC cycle through liquid nitrogen cooling in the temperature range –100 to +150 °C at 5 °C min^{-1} was employed.

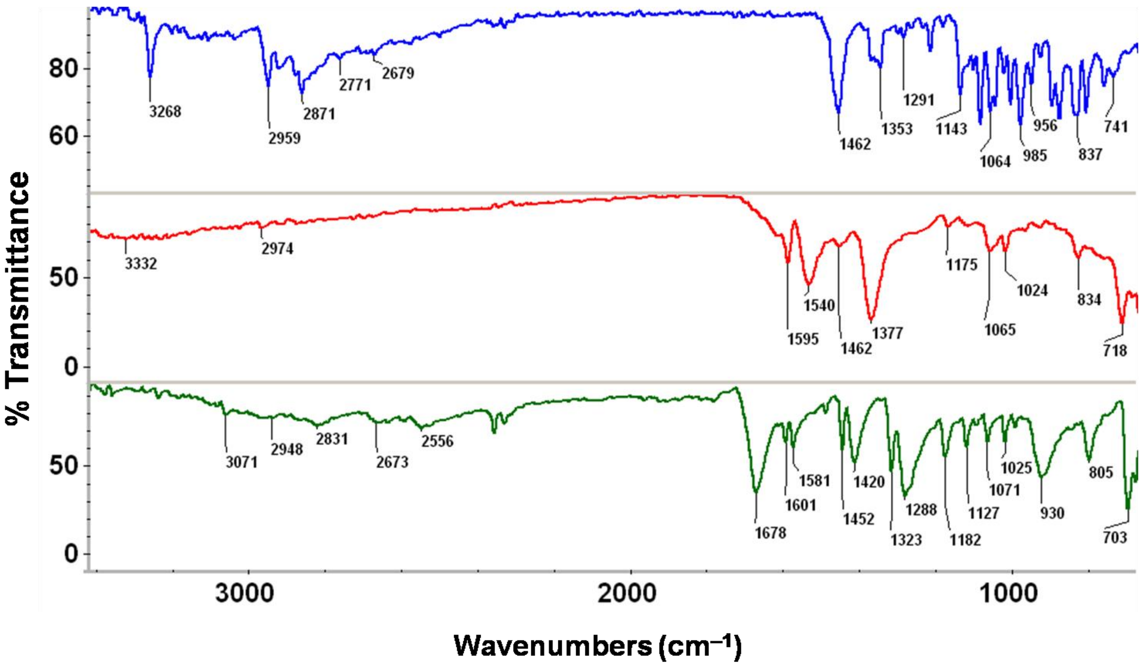

Figure S1 Ethambutol dibenzoate liquid product (red) exhibits distinct ATR FT-IR spectrum compared to its parent compounds ethambutol (blue) and benzoic acid (green) with respect to C=O, N–H, O–H and C–H bands.

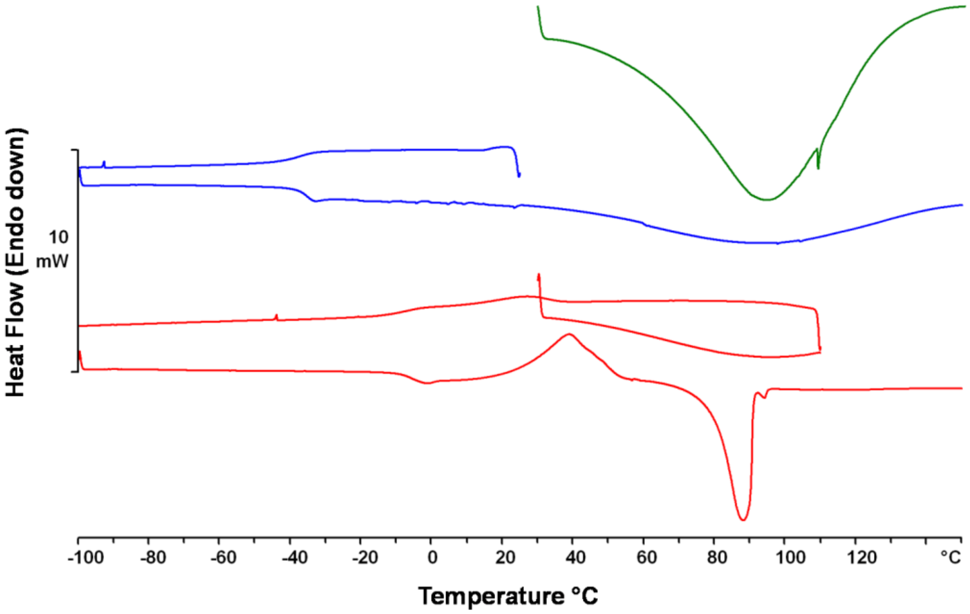

Figure S2 DSC of ethambutol dibenzoate hygroscopic liquid product. (a) Direct heating of the sample (green) in the temperature range 30-150 °C shows a broad endotherm around 100 °C corresponding to dehydration. (b) Cool-heat DSC (blue) also shows a broad endotherm at about the same temperature due to water vapor evolution. (c) Heat-cool-reheat DSC (red) reveals the thermal transitions of the typical solid phase obtained after dehydration of the liquid sample (explained in main text).

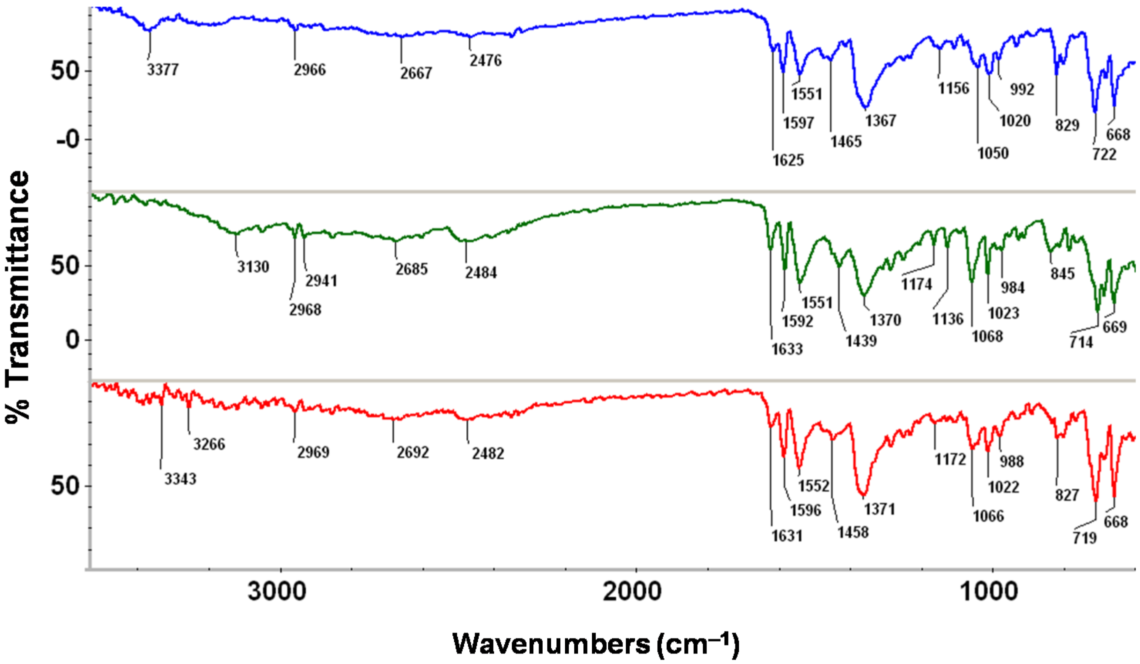

Figure S3 ATR-IR spectra of ethambutol dibenzoate forms 1 (blue), 2 (green) and 3 (red) show differences in their C=O, N–H, O–H and C–H bands.

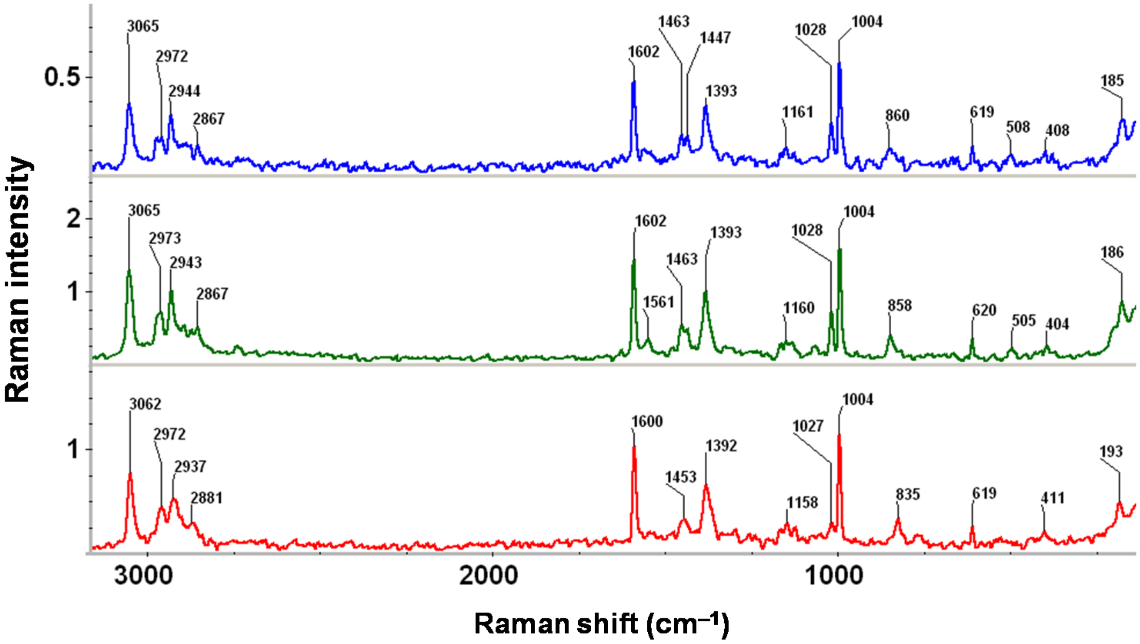

Figure S4 Raman spectra of ethambutol dibenzoate form 1 (blue), form 2 (green) and form 3 (red) show differences in their C–H and C–C vibrations.

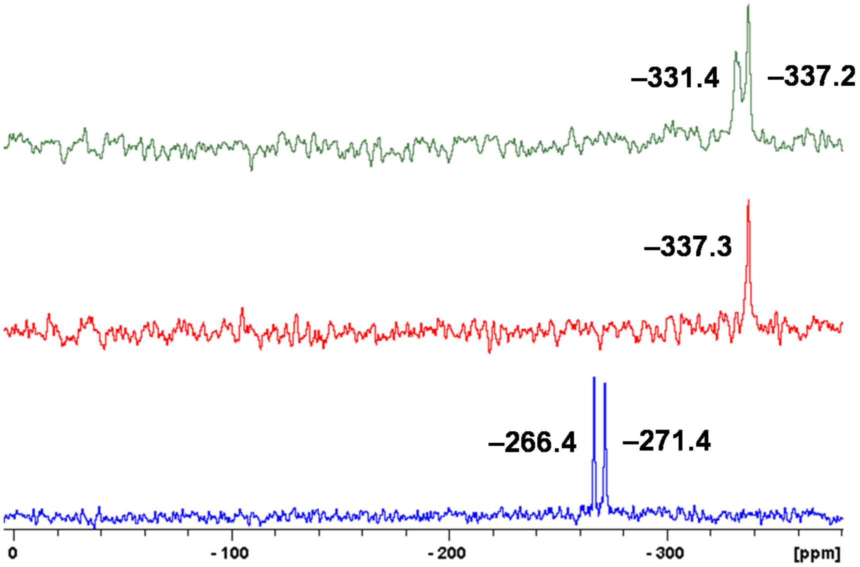

Figure S5 ^{15}N ssNMR spectra of ethambutol dibenzoate forms 1 (red) and 2 (green) and ethambutol (blue). Since proton is accepted by base (from acid proton to 2° amine of ethambutol to result in protonated amine NH_2^+ in the salt), characteristic upfield shift takes place in the salt due to an increase in electron density on the nitrogen. As such, the ethambutol dibenzoate polymorphs exhibit upfield shift of about 60 ppm with respect to ethambutol. The singlet of form 1 and the doublet of form 2 demonstrate that their crystalline environments are different implying them to be polymorphs.

Table S1 X-ray powder diffraction lines of ethambutol dibenzoate polymorphs.

Form 1			Form 2			Form 3		
Angle 2θ (°)	d spacing (Å)	Relative Intensity (%)	Angle 2θ (°)	d spacing (Å)	Relative Intensity (%)	Angle 2θ (°)	d spacing (Å)	Relative Intensity (%)
7.4	11.9	83.3	8.5	10.4	20.1	6.2	14.1	15.6
12.0	7.4	6.2	10.2	8.6	51.8	7.7	11.5	61.6
13.7	6.4	16.7	12.7	6.9	16.7	8.6	10.3	23.1
14.9	5.9	11.1	13.8	6.4	6.7	10.4	8.5	25.9
15.3	5.8	14.8	15.7	5.6	6.9	12.8	6.9	10.2
18.1	4.9	14.2	16.4	5.4	9.7	13.3	6.6	6.3
19.0	4.7	39.8	17.1	5.2	10.6	14.0	6.3	29.4
19.6	4.5	22	18.8	4.7	17	15.2	5.8	8.7
21.1	4.2	100	19.4	4.6	27.7	15.6	5.7	10.4
21.6	4.1	27.1	20.8	4.3	5.3	15.9	5.5	27.3
23.5	3.8	26.9	21.8	4.1	5.6	16.6	5.3	30.4
24.9	3.6	69.9	22.7	3.9	84.4	17.3	5.1	21.9
27.2	3.3	12.1	23.0	3.9	100	17.8	5.0	7.2
27.7	3.2	8.1	23.5	3.8	12.6	18.6	4.8	24.3
28.8	3.1	9.1	24.2	3.7	22.4	19.0	4.7	40.9
29.8	3.0	13.7	25.5	3.5	16	19.4	4.6	24.3
30.8	2.9	10.6	25.8	3.4	18.3	19.8	4.5	80.6
31.9	2.8	12.6	28.7	3.1	8.5	20.6	4.3	7.7
32.8	2.7	20	28.9	3.1	8.3	21.0	4.2	7.3
33.6	2.7	13	30.0	3.0	7	21.5	4.1	42.1
34.1	2.6	16.8	31.3	2.8	5.6	21.9	4.0	32.2
36.2	2.5	9.7	32.2	2.8	6.4	23.0	3.9	100
37.5	2.4	13.3	33.7	2.7	15.9	23.7	3.7	48.3
38.9	2.3	7.9	36.2	2.5	18.3	24.4	3.6	33.7
			37.0	2.4	5.1	25.4	3.5	32.2
			38.5	2.3	5.9	25.6	3.5	32.2
			39.2	2.3	9.3	26.0	3.4	11.6
						27.0	3.3	9.1

						27.8	3.2	9.4
						28.5	3.1	9.3
						29.0	3.1	11.7
						30.1	3.0	9.4
						31.5	2.8	7.7
						32.4	2.8	12
						33.8	2.6	17.5
						34.4	2.6	8.2
						35.1	2.5	6.2
						35.8	2.5	8.3
						36.3	2.5	23.3
						37.2	2.4	7.7
						38.3	2.3	8.7
						39.3	2.3	10.2