Supporting Information

Diversity of crystal structures and physicochemical properties of ciprofloxacin and norfloxacin salts with fumaric acid

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Preparation of solid forms via liquid-assisted grinding (LAG)

The capability of ciprofloxacin and norfloxacin to form various crystalline products with fumaric acid was initially observed by analysing the results of mechanochemical treatment of the components in the presence of different solvents, namely, water (H_2O), acetonitrile (ACN), methanol (MeOH), ethanol (EtOH) and water/organic mixtures. Identification of the solid forms obtained by liquid-assisted grinding (LAG) and their phase purity was carried out by PXRD and DSC methods. The water content and composition of the hydrated salts were derived from thermogravimetric analysis (TG). The results of mechanochemical experiments are shown in Table S1.

The PXRD, DSC and TG investigations indicate that the liquid-assisted grinding of **NFX** and fumaric acid in the 1:1 molar ratio leads to formation of two distinct products with the same drug/acid ratio but a different number of water molecules in the crystal lattice, which was calculated from the sample mass loss in the TG experiments (Figure S1 and S2). As Table S1 shows, grinding the components with ACN, EtOH or MeOH or their mixtures with 5% water resulted in the formation of norfloxacin fumarate monohydrate (1:1:1) (Figure S3a). An increase in the mixture water content up to 50% or usage of pure water produces an alternative crystalline form of NFX fumarate which contains 2 molecules of water per 1 molecule of the salt (Figure S3a). Considering the existence of the [**CIP**+**Fum**+**H_2O**] (1:0.5:1.5) salt, where fumaric acid acts as a diprotic acid, NFX and fumaric acid were ground in the 1:0.5 molar ratio, which resulted in the isolation of the third solid form of the salt, later identified as norfloxacin hemifumarate monohydrate (1:0.5:1). The TG analysis of this form revealed the sample mass loss of 4.65% over the temperature range of 25–160°C, which corresponds to 1 water molecule per 1.5 molecule of the salt (Figure S4). It was found that the water content in a mixed solvent had no effect on the final product of the mechanochemical synthesis, in contrast to LAG experiments with 1:1 drug to acid ratio (Table 1).

In the case of ciprofloxacin, PXRD analysis has shown that grinding of the components in the presence of neat ACN, EtOH and MeOH or 5% water/organic mixtures leads to the formation of a product which differs from the known forms of salts in powder patterns, i.e. fumarate monohydrate and hemifumarate sesquihydrate (Figure S3b). In addition, the DSC curve of the new material has only one endotherm, corresponding to the melting process of the salt (227.8 ºC), while there is no evidence of a dehydration process (Figure S5). Therefore, this form is considered to be an anhydrous salt of ciprofloxacin fumarate with 1:1 molar ratio, later referred to as [**CIP+Fum**] (1:1). Adding water or 50% water/organic mixtures to the reactants (CIP and fumaric acid) turns the mechanochemical reaction to the formation of either [**CIP**+**Fum**+**H_2O**] (1:1:1) or [**CIP**+**Fum**+**H_2O**] (1:0.5:1.5), depending on the stoichiometry of the components (Table S1, Figure S3b). Since the crystal structures of these salts are known, the ground products were identified by comparing the experimental and calculated PXRD patterns (Figure S6).

In order to evaluate the relative stability of the solid forms of ciprofloxacin and norfloxacin fumarates, the corresponding salts underwent mechanochemical treatment in the presence of different solvents (Table S2).

The PXRD analysis of the ground solids has shown that the [**NFX+Fum+H_2O**] (1:1:1) form converts to fumarate dihydrate (1:1:2), when water or water/organic mixtures are added to the grinding jar. However, the reverse reaction does not occur as the [**NFX+Fum+H_2O**] (1:1:2) is found to be stable under grinding with ACN, EtOH and MeOH. Partial transformation of [**NFX+Fum+H_2O**] (1:1:2) to [**NFX+Fum+H_2O**] (1:1:1) is observed only under the neat grinding conditions. Anhydrous ciprofloxacin fumarate and its monohydrate behave in the same way, with the latter form appearing to be thermodynamically more stable compared to [**CIP+Fum**] (1:1). The [**NFX+Fum+H_2O**] (1:0.5:1) salt seems to be stable in all the tested solvent systems.

At the next step, all the new phases were characterised by the single crystal X-ray diffraction. Therefore, the bulk phase purity of the samples obtained in the LAG experiments was confirmed

by comparing the experimental PXRD with the calculated lines from the X-ray crystal structures (Figures S7, S8**).**

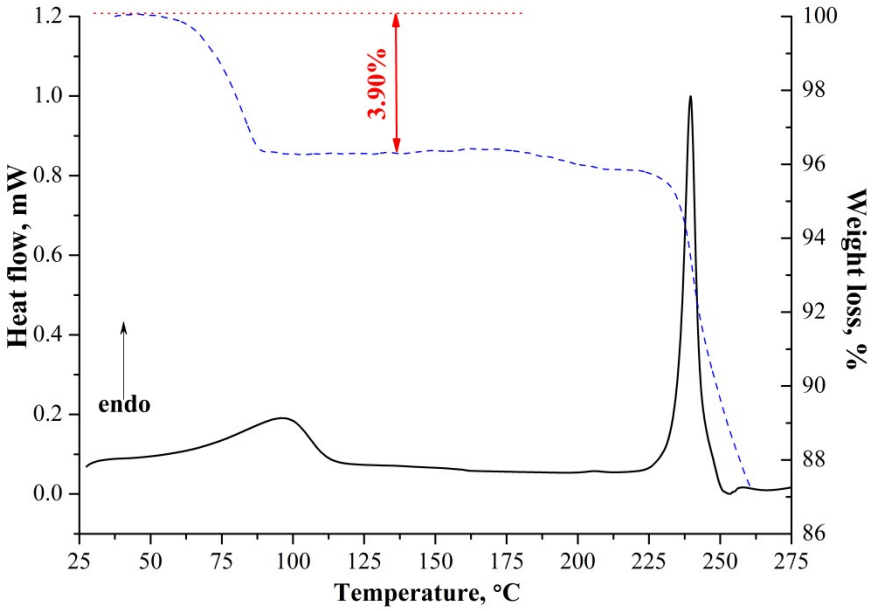


Figure S1. DSC thermogram and TG analysis of [**NFX+Fum+H_2O**] (1:1:1)

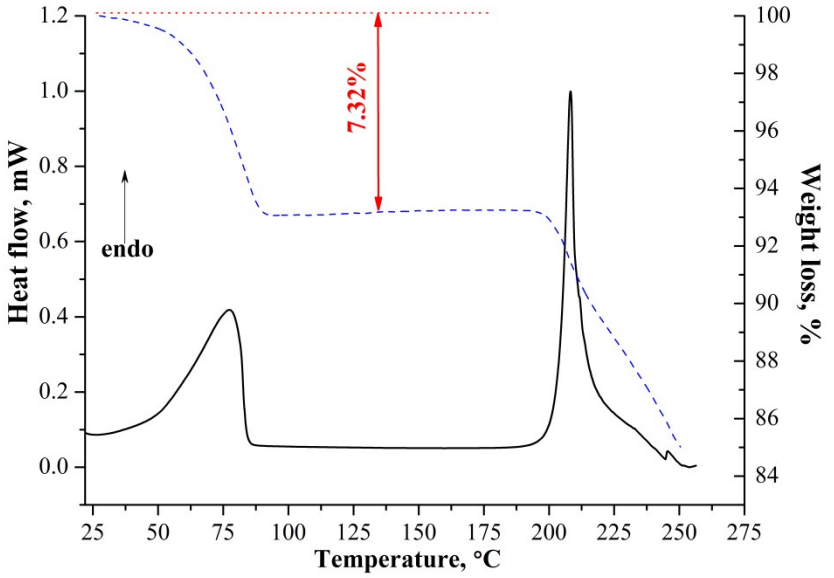
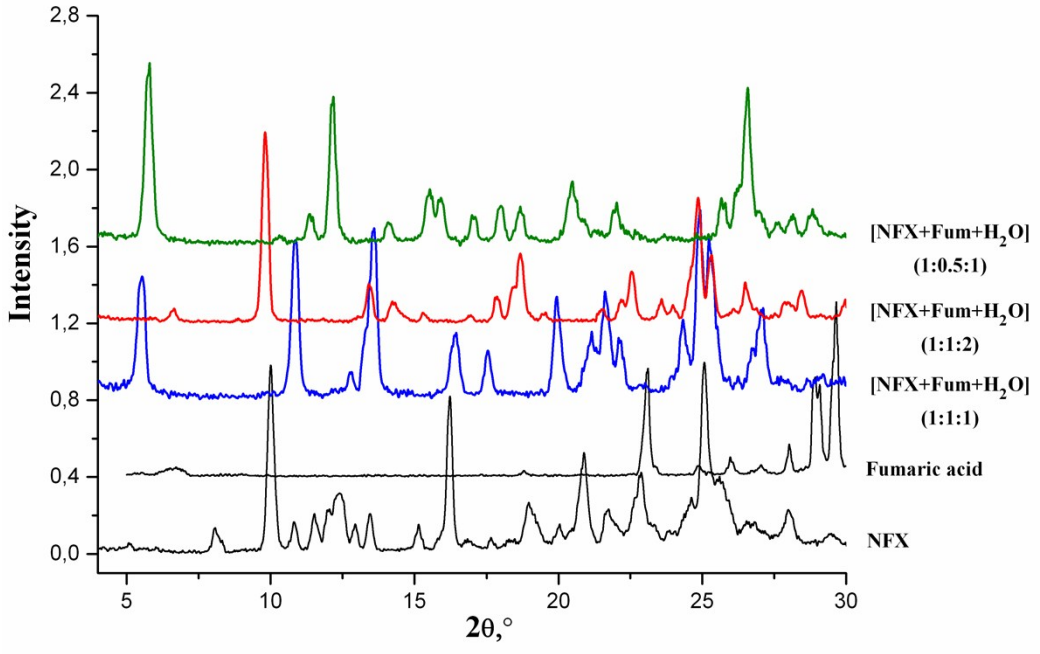
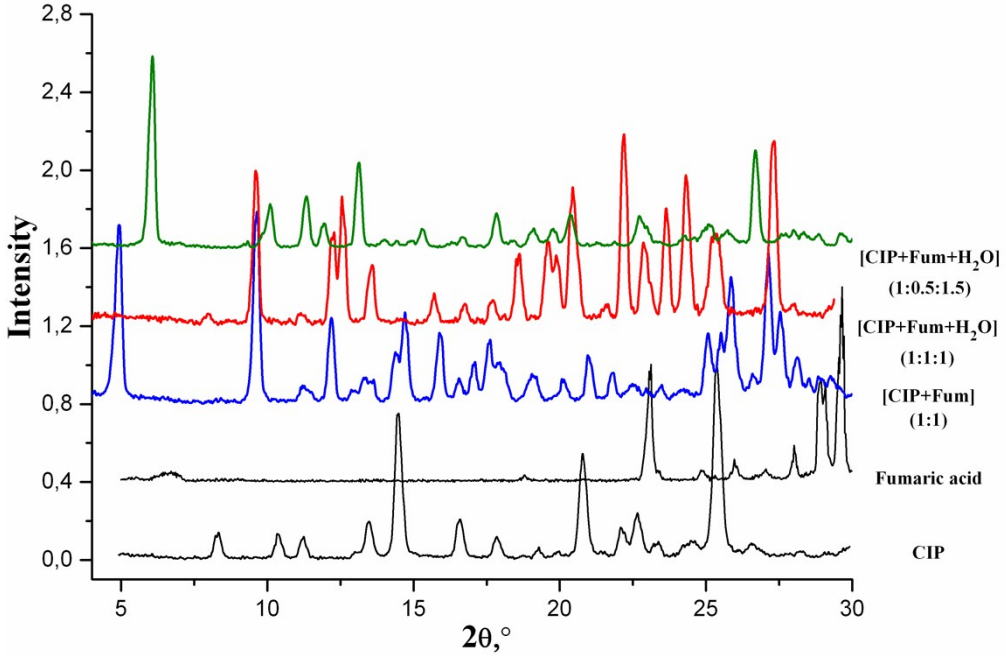


Figure S2. DSC thermogram and TG analysis of [**NFX+Fum+H_2O**] (1:1:2)



(**a**)



(**b**)

Figure S3. Experimental PXRD patterns of fluoroquinolones, fumaric acid and different solid forms of (a) norfloxacin fumarate and (b) ciprofloxacin fumarate obtained by liquid-assisted grinding

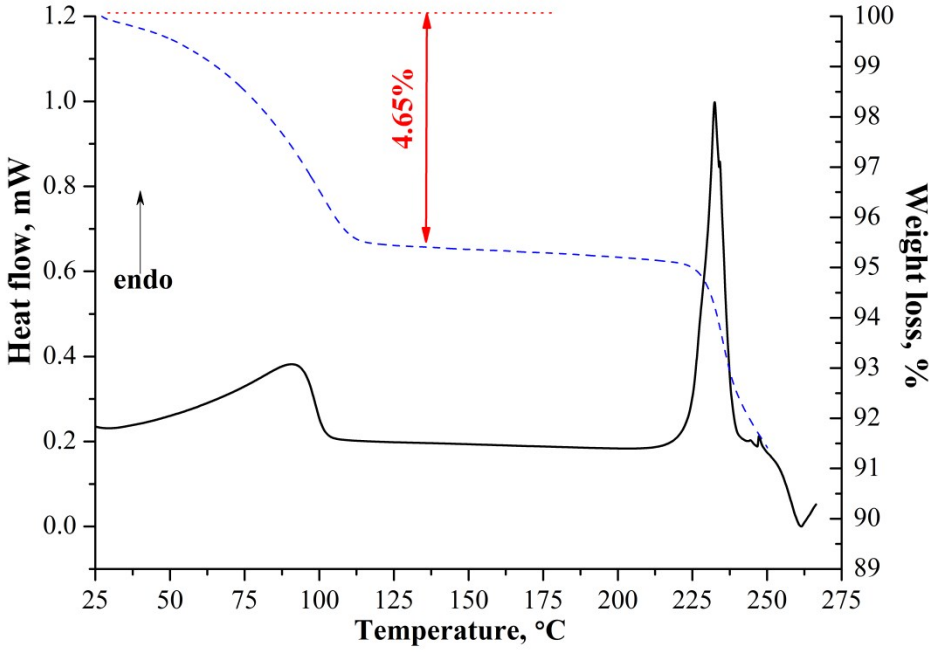


Figure S4. DSC thermogram and TG analysis of [**NFX+Fum+H_2O**] (1:0.5:1)

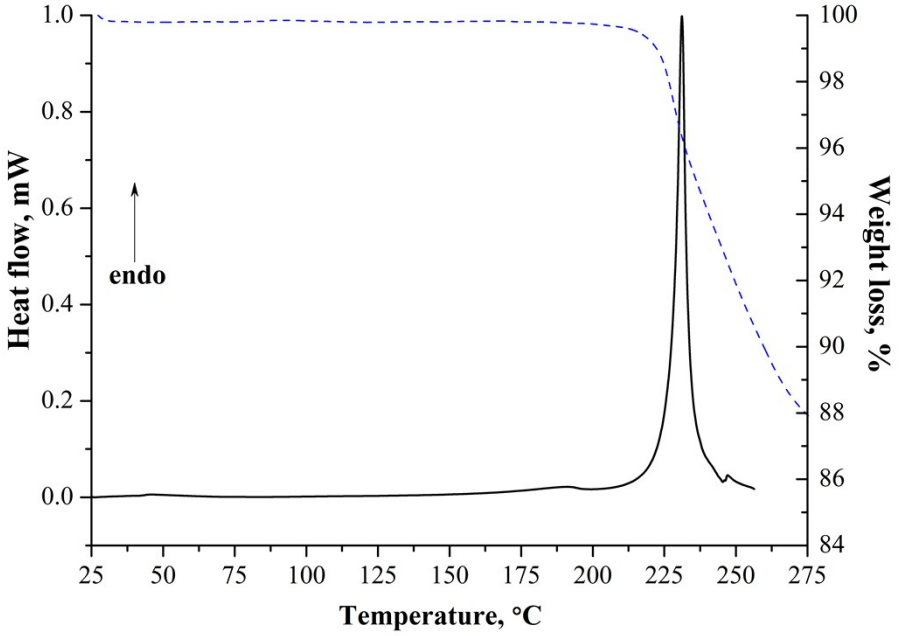


Figure S5. DSC thermogram and TG analysis of [**CIP+Fum**] (1:1)

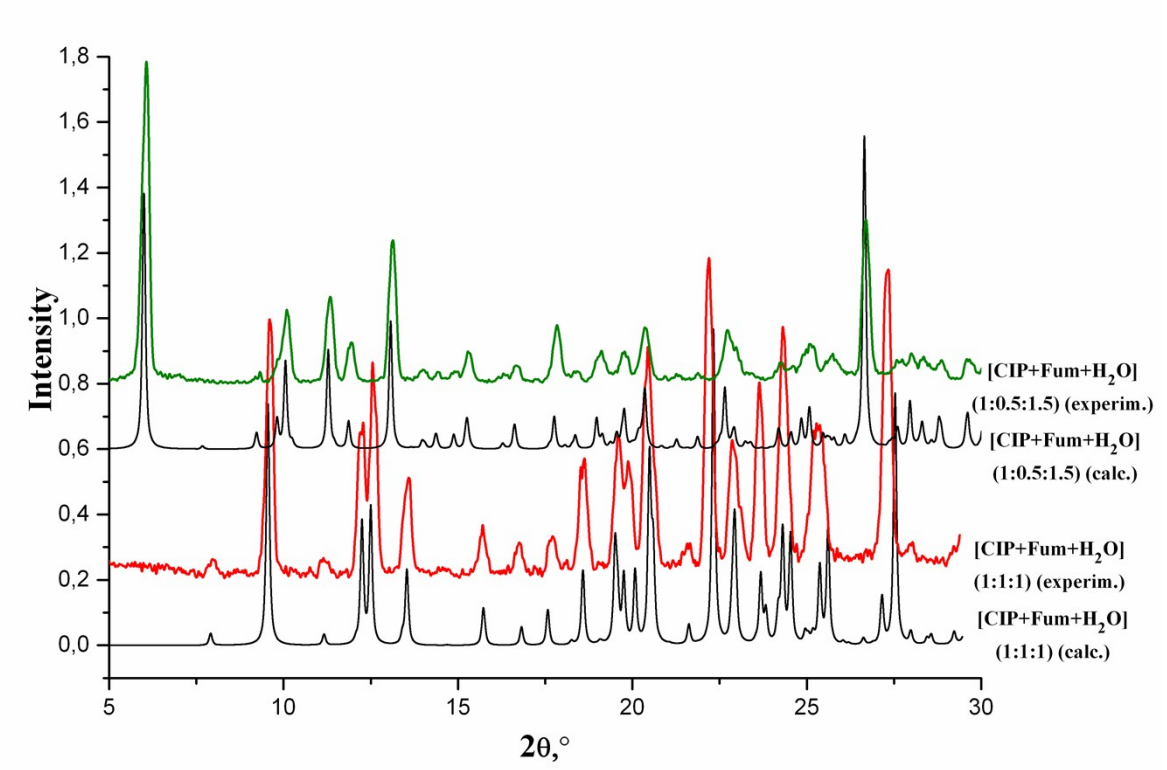


Figure S6. Experimental and calculated PXRD patterns of [**CIP**+**Fum**+**H_2O**] (1:1:1) and [**CIP**+**Fum**+**H_2O**] (1:0.5:1.5)

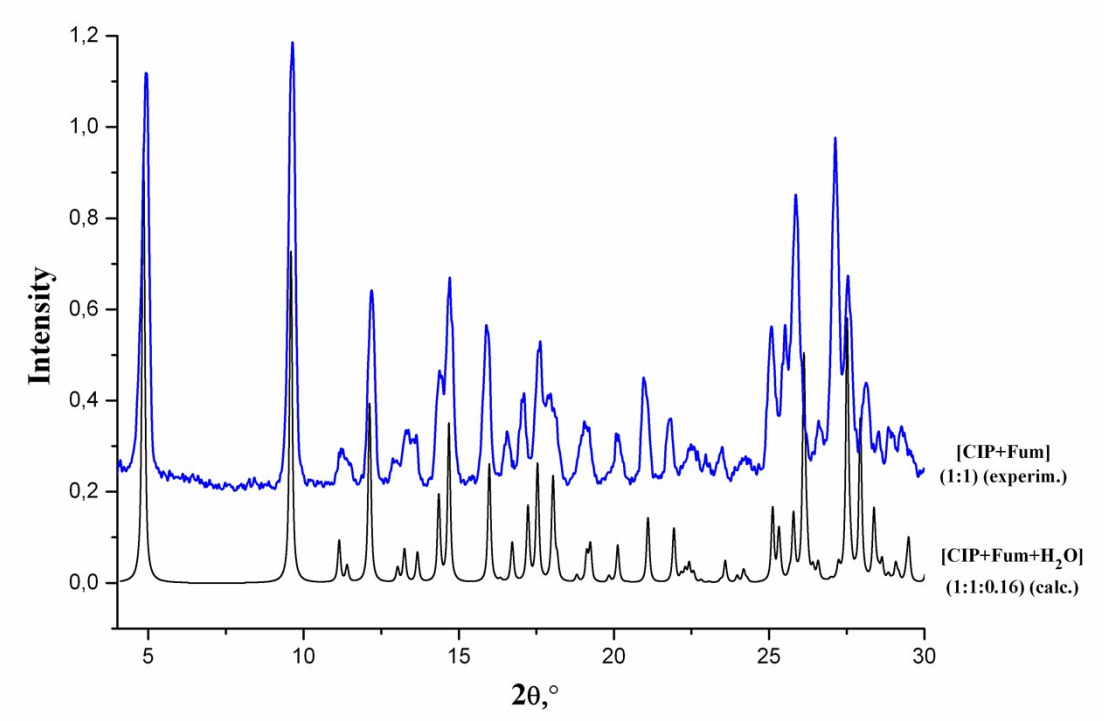


Figure S7. Experimental and calculated PXRD patterns of [**CIP**+**Fum**] (1:1)

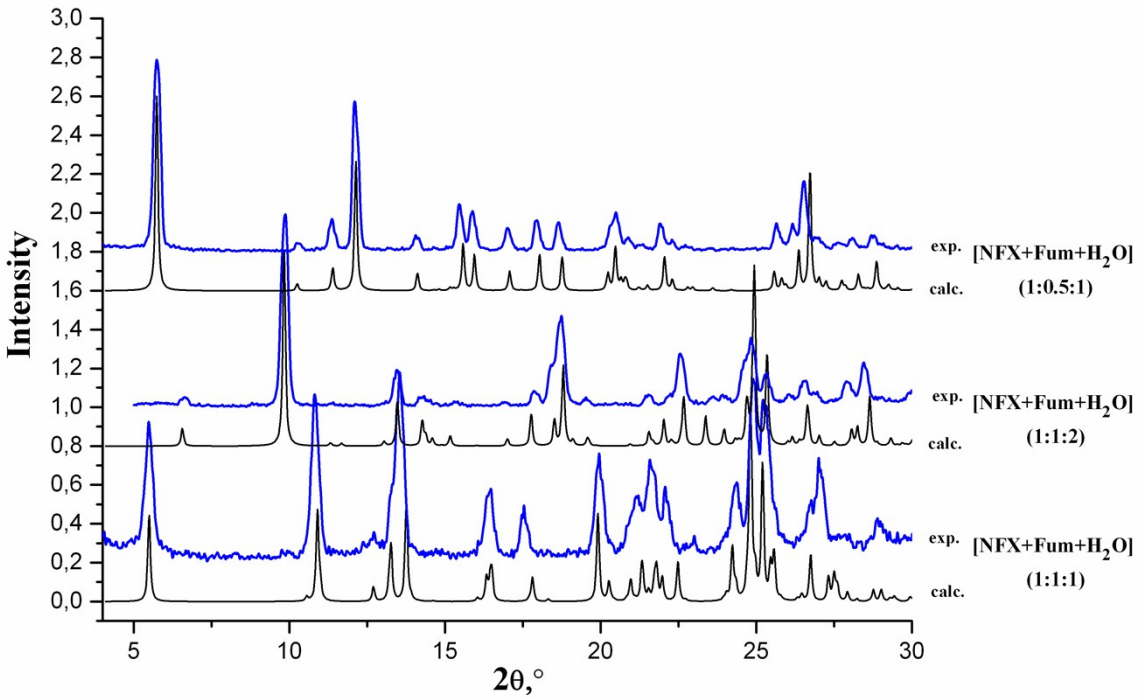


Figure S8. Experimental and calculated PXRD patterns of [**NFX+Fum+H_2O**] (1:1:1), [**NFX+Fum+H_2O**] (1:1:2) and [**NFX+Fum+H_2O**] (1:0.5:1)

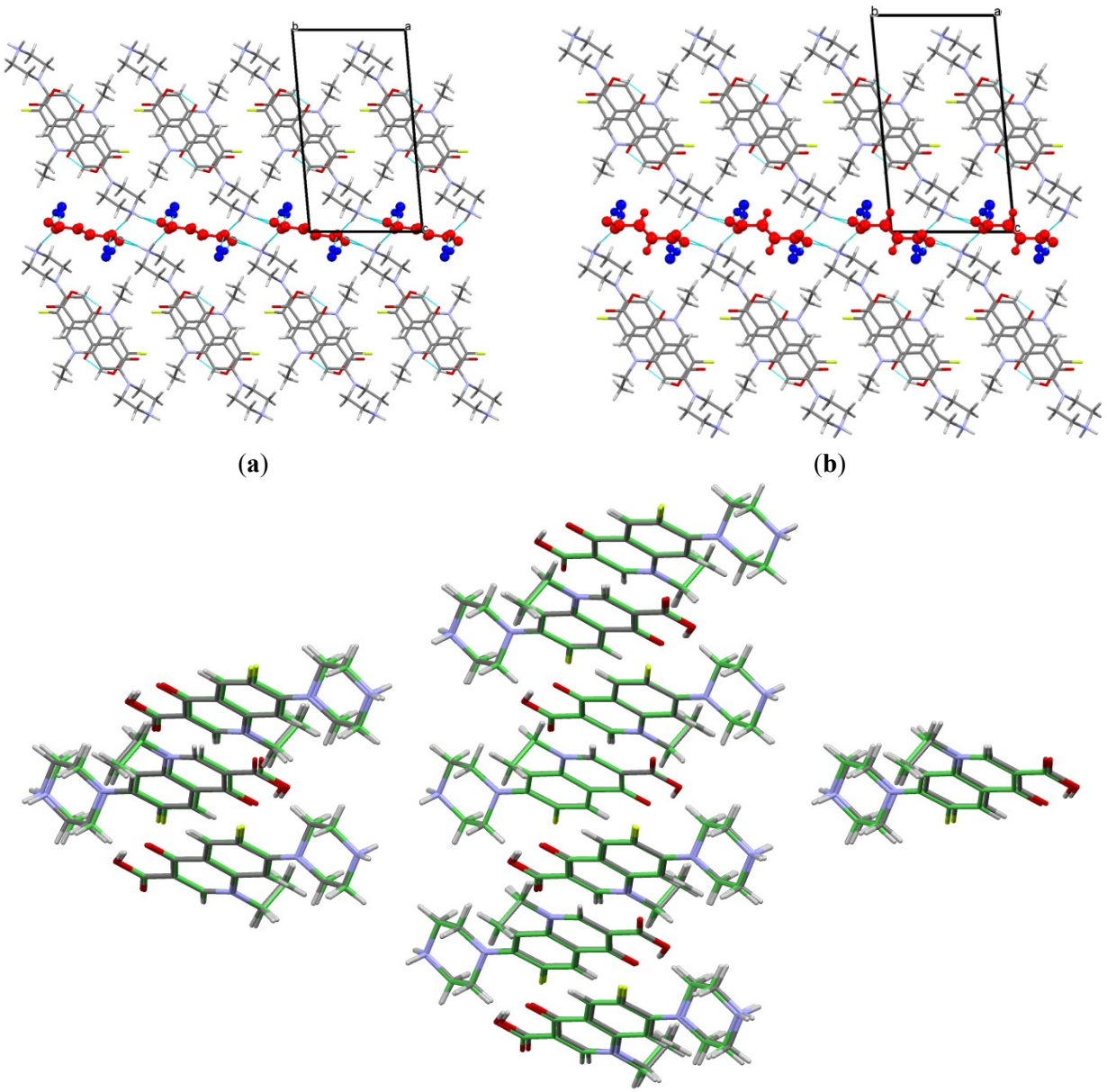


Figure S9. Molecular packing arrangements of the (a) [**NFX+Fum+H_2O**] (1:0.5:1) and (b) [**NFX+Succinic+H_2O**] (1:0.5:1) salts. The counterions are colored red, the water molecules are colored blue. (c) Overlay of the [**NFX+Fum+H_2O**] (1:0.5:1) (gray) and [**NFX+Succinic+H_2O**] (1:0.5:1) (green) crystal structures performed in the Crystal Packing Similarity module[1S] implemented in Mercury (n=20, $rmsd_n$ = 0.114). The counterions and water molecules are not considered

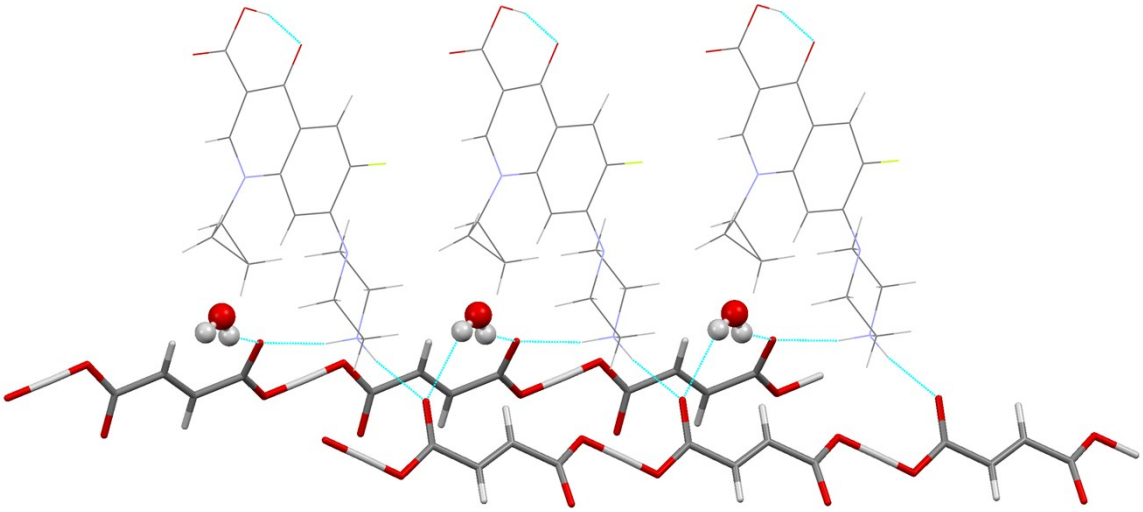
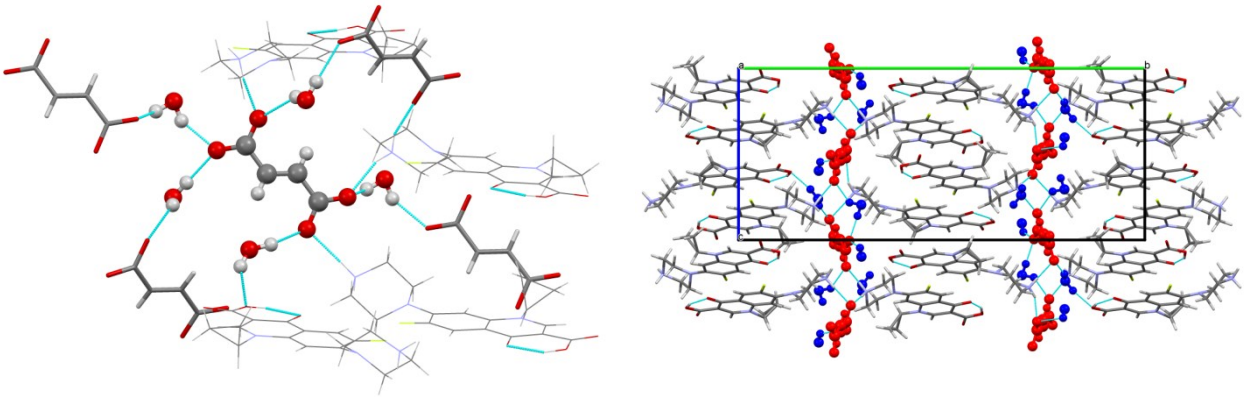


Figure S10. Part of crystal lattice with positions of water molecules with site occupancy 0.16 displayed in the [**CIP+Fum+H_2O**] (1:1:0.16) single crystal



(**a**) (**b**)

Figure S11. Illustration of (a) hydrogen bonds and (b) molecular packing projection in the [**CIP+Fum+H_2O**] (1:0.5:1.5) salt. The fumarate ions are colored red, the water molecules are colored blue

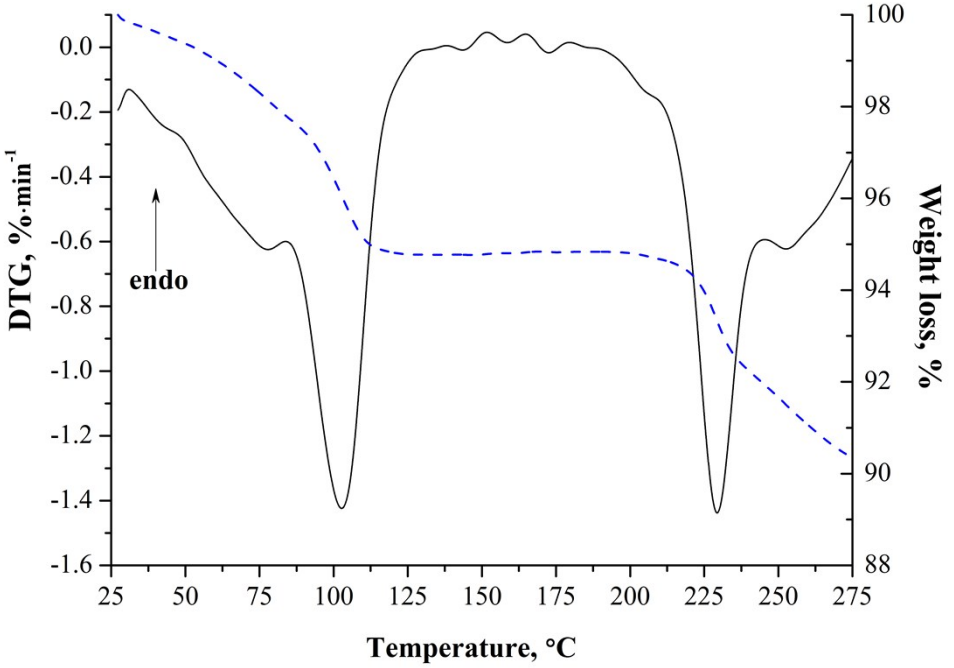


Figure S12. TG and DTG curves of [**CIP+Fum+H_2O**] (1:0.5:1.5)

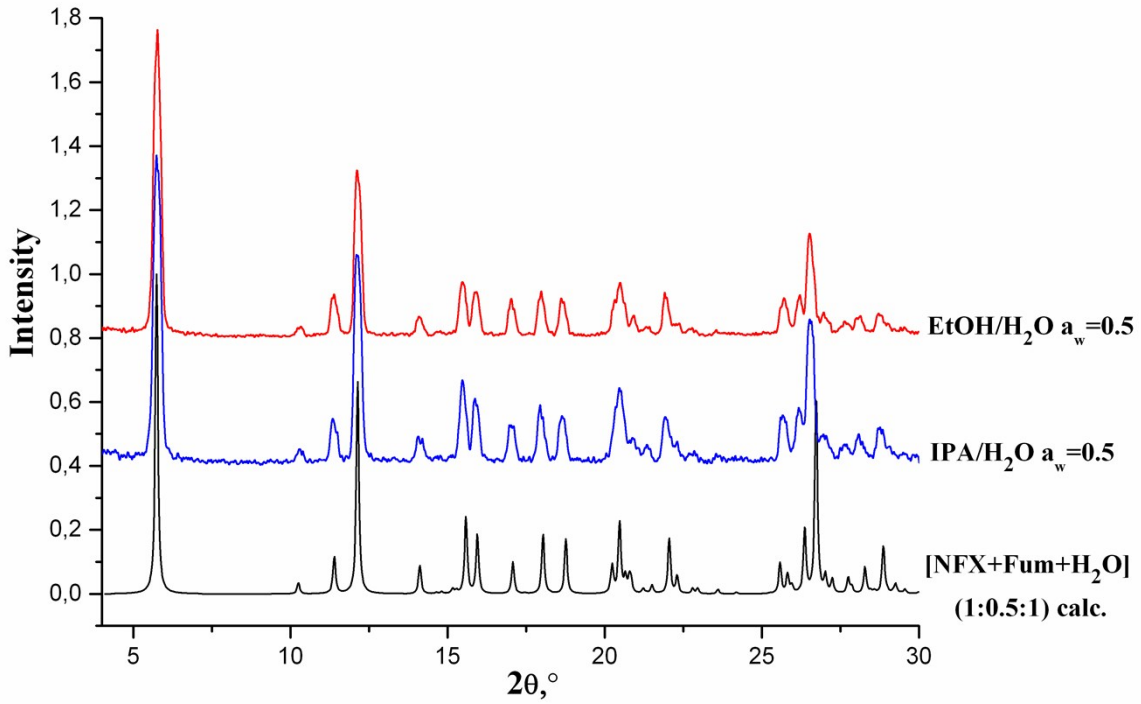


Figure S13. Experimental PXRD patterns of residual materials after slurry of [**NFX+Fum+H_2O**] (1:1:1) in EtOH/H_2O and IPA/H_2O mixtures with a_w=0.5 at 25 °C

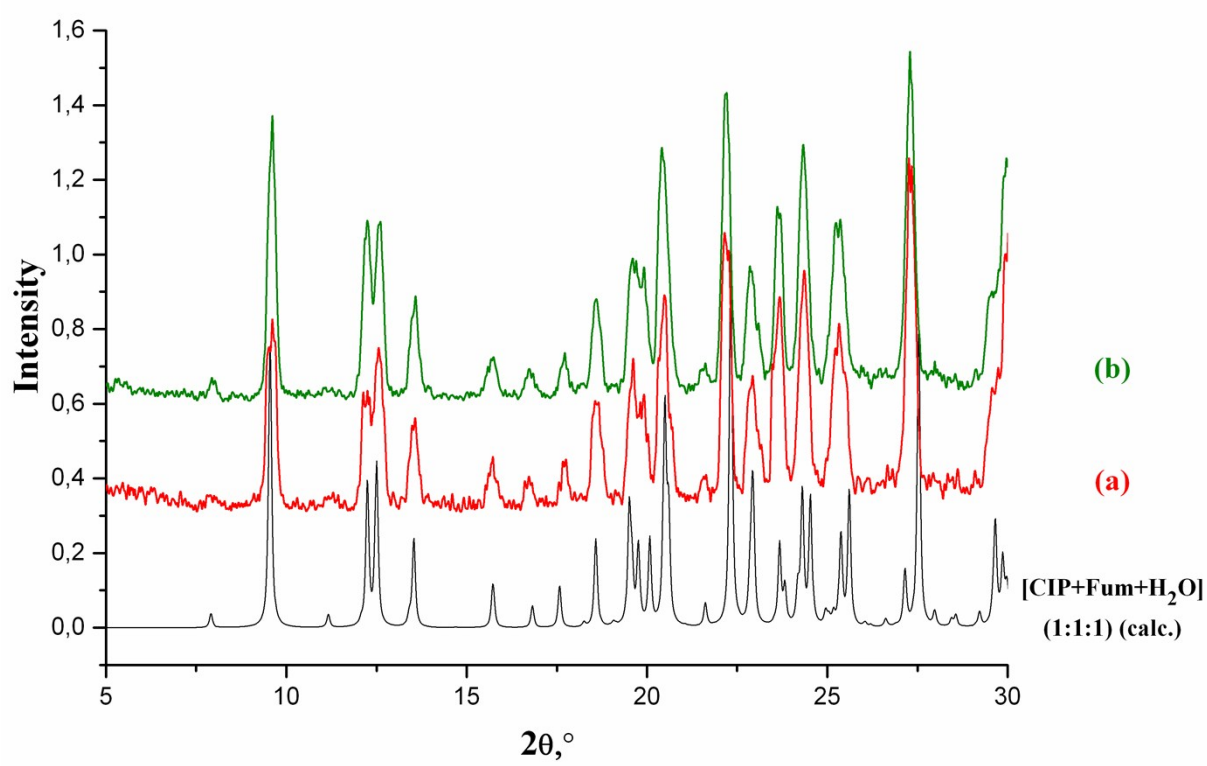


Figure S14. Experimental PXRD patterns of residual materials after dissolution of [**CIP+Fum**] (1:1) in buffer solutions with (a) pH 1.2 and (b) pH 6.8

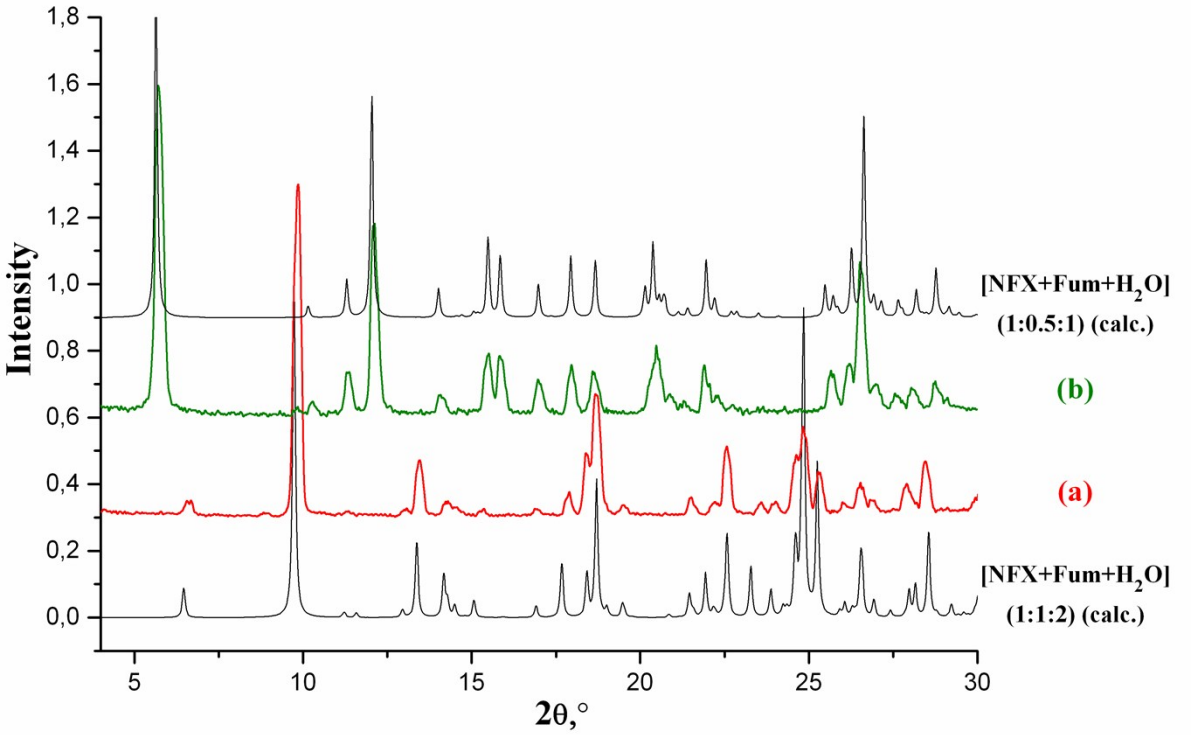


Figure S15. Experimental PXRD patterns of residual materials after dissolution of [**NFX+Fum+H_2O**] (1:1:1) in buffer solutions with (a) pH 1.2 and (b) pH 6.8

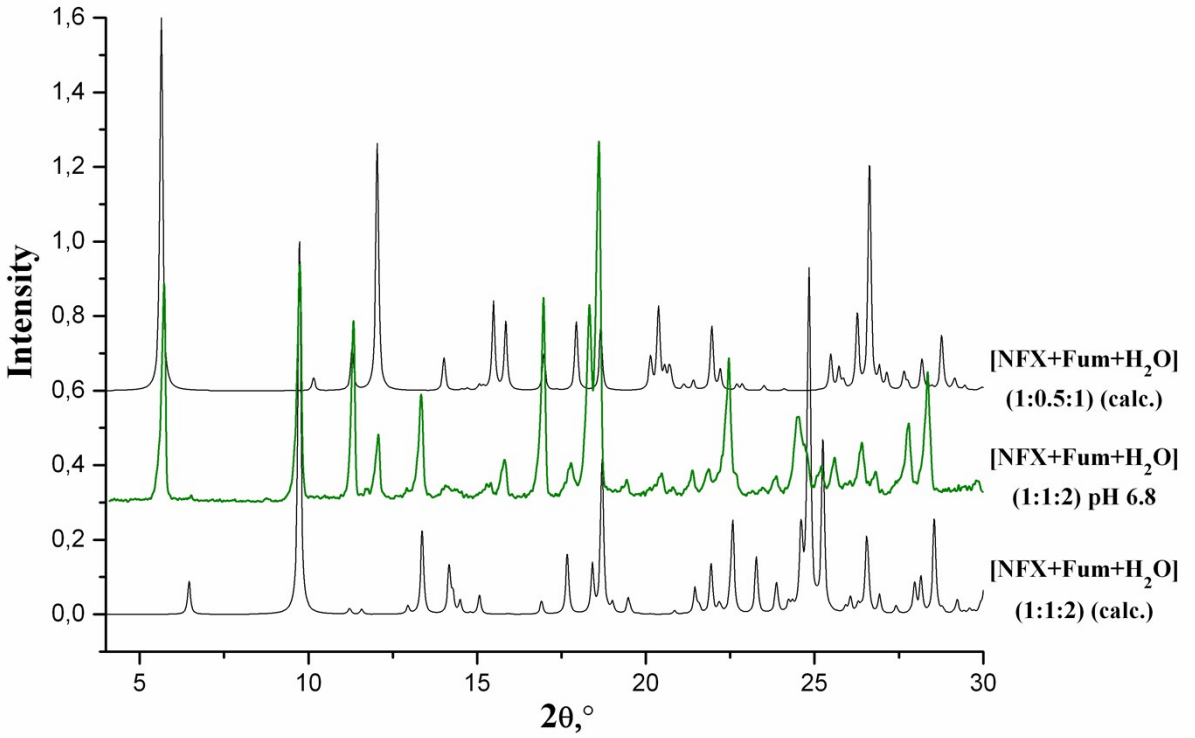


Figure S16. PXRD analysis of residual materials after solubility of [**NFX+Fum+H_2O**] (1:1:2) in the pH 6.8 solution

Table S1. Results of liquid-assisted grinding (LAG) experiments for the physical mixture of components with different solvents

Solvent	**NFX + Fumaric acid**		**CIP + Fumaric acid**	
	1:1	**1:0.5**	**1:1**	**1:0.5**
H_2O	Fumarate dihydrate (1:1:2)	Hemifumarate monohydrate (1:0.5:1)	Fumarate monohydrate (1:1:1)	Hemifumarate sesquihydrate (1:0.5:1.5)
ACN	Fumarate monohydrate (1:1:1)		Fumarate (1:1)	
EtOH	Fumarate monohydrate (1:1:1)		Fumarate (1:1)	
MeOH	Fumarate monohydrate (1:1:1) + NFX		Fumarate (1:1)	
MeOH/H_2O (95:5 v:v)		Hemifumarate monohydrate (1:0.5:1)	Fumarate (1:1)	
ACN/H_2O (95:5 v:v)	Fumarate monohydrate (1:1:1)	Hemifumarate monohydrate (1:0.5:1)	Fumarate (1:1)	
EtOH/H_2O (95:5 v:v)	Fumarate monohydrate (1:1:1)	Hemifumarate monohydrate (1:0.5:1)	Fumarate (1:1)	
ACN/H_2O (50:50 v:v)	Fumarate dihydrate (1:1:2)	Hemifumarate monohydrate (1:0.5:1)	Fumarate monohydrate (1:1:1)	Hemifumarate sesquihydrate (1:0.5:1.5)
EtOH/H_2O (50:50 v:v)	Fumarate dihydrate (1:1:2)	Hemifumarate monohydrate (1:0.5:1)	Fumarate monohydrate (1:1:1)	Hemifumarate sesquihydrate (1:0.5:1.5)
MeOH/H_2O (50:50 v:v)	Fumarate dihydrate (1:1:2)	Hemifumarate monohydrate (1:0.5:1)	Fumarate monohydrate (1:1:1)	Hemifumarate sesquihydrate (1:0.5:1.5)

Table S2. Results of liquid-assisted grinding (LAG) experiments for NFX and CIP fumarates with different solvents

Solvent	**Initial form** [**NFX+Fum+H_2O**] (1:1:1)	[**NFX+Fum+H_2O**] (1:0.5:1)	[**NFX+Fum+H_2O**] (1:1:2)	[**CIP+Fum**] (1:1)	[**CIP+Fum+H_2O**] (1:1:1)
			Results		
H_2O	Fumarate dihydrate (1:1:2)	Hemifumarate monohydrate (1:0.5:1)		Fumarate monohydrate (1:1:1)	
ACN		Hemifumarate monohydrate (1:0.5:1)	Fumarate dihydrate (1:1:2)		Fumarate monohydrate (1:1:1)
EtOH		Hemifumarate monohydrate (1:0.5:1)	Fumarate dihydrate (1:1:2)		Fumarate monohydrate (1:1:1)
MeOH		Hemifumarate monohydrate (1:0.5:1)	Fumarate dihydrate (1:1:2)		Fumarate monohydrate (1:1:1)
ACN/H_2O (50:50)	Fumarate dihydrate (1:1:2)	Hemifumarate monohydrate (1:0.5:1)		Fumarate monohydrate (1:1:1)	
EtOH/H_2O (50:50)	Fumarate dihydrate (1:1:2)	Hemifumarate monohydrate (1:0.5:1)		Fumarate monohydrate (1:1:1)	
MeOH/H_2O (50:50)	Fumarate dihydrate (1:1:2)	Hemifumarate monohydrate (1:0.5:1)		Fumarate monohydrate (1:1:1)	
Neat grinding	Fumarate dihydrate (1:1:1)		Fumarate dihydrate (1:1:2) + Fumarate monohydrate (1:1:1)	Amorphous	Fumarate monohydrate (1:1:1)

Table S3. Intrinsic dissolution rates of CIP, NFX and their salts with fumaric acid in pH 1.2 media at 37°C.

	Intrinsic dissolution rate, $mg \cdot min^{-1} \cdot cm^{-2}$
CIP[a]	6.6 ± 0.1
[**CIP+Fum**] (1:1)	7.64 ± 0.05
[**CIP+Fum+H_2O**] (1:1:1)[a]	3.16 ± 0.07
[**CIP+Fum+H_2O**] (1:0.5:1.5)	11.8 ± 0.1
NFX	11.6 ± 0.3
[**NFX+Fum+H_2O**] (1:1:1)	10.7 ± 0.1
[**NFX+Fum+H_2O**] (1:1:2)	7.60 ± 0.05
[**NFX+Fum+H_2O**] (1:0.5:1)	14.7 ± 0.2

[a]data taken from ref. 2S.

References

1S. J. A. Chisholm, S. Motherwell, *J. Appl. Crystallogr.,* 2005, **38**, 228.

2S. A. O. Surov, A. N. Manin, A. P. Voronin, K. V. Drozd, A. A. Simagina, A. V.Churakov, G. L. Perlovich, *Eur. J. Pharm. Sci.,* 2015, **77**, 112.