

DIDANOSINE CONCEPT ARTICLE

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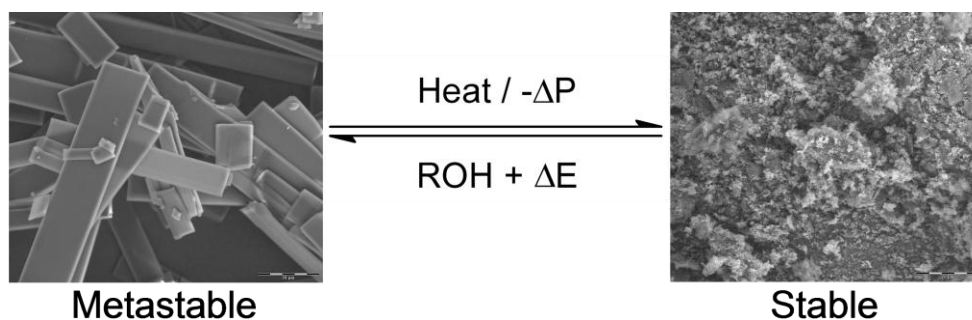
The extent of didanosine's metastability in the solid state

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Graphical Abstract



Abstract

In this work we present an investigation into the metastability of the antiretroviral agent, didanosine, in the solid state. Commercial didanosine was recrystallised from acetonitrile, several alcohols and methanol/water mixtures, in an attempt to prepare different polymorphs of the compound. Despite morphological differences, physicochemical, spectroscopic and powder X-ray diffraction (PXRD) analyses showed no definitive difference between the recrystallisation products and commercial didanosine. Upon drying, the polymorphs lost their crystal morphology and reverted to commercial didanosine. The same phenomenon was observed for the recrystallisation products upon storage for periods longer than one month, even under the recrystallisation medium. Energy, in the form of heating and dissolution, was needed to turn the powder obtained from drying or storage back into the metastable polymorph, suggesting not only that commercial didanosine is the most stable solid-state form. This interconversion between didanosine and its metastable recrystallisation products is an important consideration for pharmaceutical scientists, as it can lead to unpredictable bioavailability and stability of solid didanosine dosage forms.

Keywords: Didanosine, recrystallisation, metastable, interconversion.

1. Introduction

Didanosine (2',3'-Dideoxyinosine, ddI) is a purine analogue and potent nucleoside reverse transcriptase inhibitor. Despite its potency (Mitsuya and Broder, 1986; Perno et al., 1989), low toxicity (Bhalla et al., 1989) and dose dependent adverse effects (Lambert et al., 1990), didanosine is not prescribed as a first-line antiretroviral (ARV) drug to patients suffering from human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS). Factors that contribute to the sparse dispensing of didanosine include its BCS classification as a class 3 drug, with high solubility but low permeation, and the rapidly hydrolyzes of its glycosidic bond (see Fig. 1) in acidic media. Since didanosine requires triphosphorilation of the hydroxyl group on carbon 5' to yield the active metabolite, 2',3'-dideoxyadenosine-5'-triphosphate (Hartman et al., 1990), hydrolysis effectively inactivates the drug. Considering the nature of AIDS and the frequency of dosing, the oral route is the most viable. The bioavailability of didanosine ranges from 41 % $\pm$ 7% (mean $\pm$ s.e.m.) to 25 % $\pm$ 5 % in fasting patients and drops to 17 % $\pm$ 6 % when administered with food (Hartman et al., 1991), and although antacids are included to buffer or outcompete didanosine's hydrolysis, these inclusions lead to severe gastric intolerance, especially when administered to fasting patients.

Several years passed between the first clinical trials and the first published investigations into the solid-state properties of didanosine. Martins et al. (2010) and Bettini et al. (2010) found the same two conformers of didanosine to exist in both the commercial product and their respective recrystallisation products, differing slightly from each other only by rotation on the glycosidic bond axis. The recrystallisation product obtained by Bettini et al. (2010) differed from commercial didanosine only in hydrogen bond strengths, suggesting similar structural conformations and interaction networks but differences in molecular packing, resulting in different unit cell volumes and densities. This polymorph proved to be thermodynamically metastable and was more susceptible to external stresses than commercial didanosine. The recrystallisation product obtained by Martins et al. (2010) exhibited a similar melting point, X-ray diffraction pattern and FT-Raman spectrum as the commercial product, indicating that the two solids were isostructural. Didanosine's chemical structure is most likely the reason for its inability to produce varying amounts of polymorphic or amorphous forms, since such rigid molecules would have only a few naturally "preferred" conformations and molecular coordinates, resulting in fewer energy minima on its energy landscape (Stillinger, 1995).

In this paper we present a thorough investigation into the extent of didanosine's metastability in the solid-state, including thermal, spectroscopic and diffraction studies as well as scanning electron microscopy (SEM) and solubility studies on each sample.

2. Materials and methods

2.1. Materials

Commercial didanosine (HPLC assay > 99 %) was purchased from Sri Sai Nikitha Pharma Pvt. Ltd., Hyderabad, India, as a fine white powder.

2.2 Thermal analysis

To investigate the thermal behaviour of commercial didanosine and its recrystallisation products, differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) were employed. The percentage weight losses determined from TGA were related to theoretic stoichiometric weight loss percentages for 1:1 solvent/drug ratios. Karl Fischer (KF) titrations, carried out on a Metrohm 870 KF Titrino Plus (Metrohm, Switzerland), were used supplementary to TGA to determine the percentage weight loss due to water. DSC experiments were performed on a Shimadzu DSC-60A (Shimadzu, Japan). The DSC cell was purged with nitrogen at 35 mL/min. Indium and tin standards were used to calibrate the temperature and heat of fusion. All samples were accurately weighed (5 – 6 mg) and analysed in aluminium pans with pierced lids to facilitate evolution of volatiles during heating. TGA experiments were performed using a Shimadzu DTG-60 (Shimadzu, Japan). The TGA chamber was purged with nitrogen at 35 mL/min. Indium and tin standards were used to calibrate the temperature. All samples were accurately weighed (7 – 8 mg) and analysed in open aluminium pans. Data from both DSC and TGA experiments were analysed using ta60 software version 2.11.

Hot stage microscopy was employed supplementary to DSC and TGA to visualise volatile evolution. Micrographs were taken on a Nikon Eclipse E400 microscope (Nikon, Japan) equipped with a Nikon DS-Fi1 camera and the images were acquired with NIS-Elements software version 3.22.

2.3. Vibrational analysis

Fourier transform infrared spectroscopy (FTIR) was used to investigate the molecular interactions in each recrystallisation product and compare it with that of commercial didanosine. FTIR analyses were performed using a Shimadzu IRPrestige-21 (Shimadzu, Japan). Peak positions were confirmed using polystyrene film. Spectra were recorded over a range of 500 – 4000 cm⁻¹. All samples were homogeneously dispersed in a ground matrix of KBr. The data was analysed using Shimadzu IRsolution software version 1.40.

2.4. Diffraction analysis

To investigate unit cell dimensions, powder X-ray diffraction (PXRD) analyses were carried out on a PANalytical X'Pert Pro (PANalytical, Netherlands). Measurement conditions were: Anode, Cu; K α_1 ,

65 1.5405 Å; $K\alpha_2$, 1.54443 Å; K-Beta, 1.39225 Å; $K\alpha_1/K\alpha_2$ ratio, 0.5; Generator settings, 40 mA, 45 kV; divergence slit, 0.957°, fixed; step size, 0.017° in 2θ ; scan step times, 19.685 s; temperature, 25 °C. The data was analyzed using X'Pert Data Collector software version 4.0A.

2.5. Scanning electron microscopy

To investigate the morphologies of the crystals prepared in this study, scanning electron microscopy (SEM) was employed. SEM micrographs were taken using a FEI Quanta 200 ESEM. Measurement
70 conditions: Voltage, 10 kV; vacuum, high and low depending on the nature of the individual analysis, coating, Au/Pd to a thickness of 20 nm.

2.6. Karl Fischer titration

The water content of crystals believed to be hydrates were obtained from binary solvent mixtures was
75 determined with a Metrohm 870 KF Titrino Plus. Accurately weighed samples (100 mg) were dissolved in methanol and the water content was titrated using hydranal.

3. Results and discussion

Commercial didanosine was recrystallised from acetonitrile, methanol, ethanol, 1- and 2-propanol and 1-butanol. All the recrystallisation products shared the same macro- and microscopic morphologies,
80 being that of fine mesh networks of elongated blades. The morphologies of the recrystallization products were consistent with that presented by Martins et al. (2010). Thermal analyses of commercial didanosine and its recrystallisation product from acetonitrile revealed no endothermic peaks prior to melting, no weight loss on TGA and no evolution of volatiles on their hot stage micrographs, confirming that these samples were dry. The recrystallisation products obtained from
85 alcohols displayed large endothermic peaks prior to melting, as well as volatile evolution on hot stage micrographs and weight loss on TGA (supplementary information, Fig. S1 to S14). Despite being dry to the touch prior to analysis, volatile evolution and weight loss occurred from the start of the heating runs (Fig. 2). Since the preparation conditions, heating rates and date of analysis for each sample's DSC, TGA and hot stage microscopy study were identical, the only remaining variable that could
90 account for the discrepancies in onset temperature of volatile evolution is the analysis environment. As stated in the methodology section, TGA was performed using open pans, while DSC was performed using closed pans with pierced lids to facilitate volatile evolution. Based on the observations that, in an open environment, volatile evolution and weight loss occurred from the onset of heating while, in an enclosed environment, an abrupt evolution of volatiles can be found at
95 temperatures corresponding to the boiling points of each sample's recrystallisation medium, the results suggest solvent loss from the surfaces of the crystals. The melting points of commercial didanosine and its recrystallisation products differed only by $\pm 1.8^\circ\text{C}$, on average, and all the

recrystallisation products shared similar FTIR interferograms to commercial didanosine. These observations suggest that commercial didanosine shares similar inter- and intramolecular interactions with its recrystallisation products. Apart from a very small peak at position $10.7^{\circ} 2\theta$, the PXRD diffractograms (Fig. 3) indicated that all the recrystallisation products shared similar unit cell dimensions with commercial didanosine. However, this peak is too small to draw any definitive conclusions from, and could simply have arisen from sample preparation or some preferred orientation. All the recrystallisation products were therefore isostructural to commercial didanosine, corresponding to the findings of Martins et al. (2010).

To investigate how the recrystallisation products respond to stresses, it was dried at low temperatures *in vacuo* and at high temperatures in the oven, to cover both low and high stress conditions. The samples were deemed completely dry when no further weight loss could be observed on their individual TGA. Upon reaching full dryness, the recrystallisation products had completely lost their crystal morphologies and reverted to powder (Fig. 4). Thermal, vibrational and PXRD analyses of the powder revealed it to be similar to commercial didanosine. The addition of alcohols to the residual didanosine powder did not reform it into the pre-dried product. Energy, e.g. during the recrystallisation process, was needed for the powder to return to the mesh crystal network. Furthermore, all the recrystallisation products lost their morphologies within one month, even while stored under recrystallisation medium (see Fig. 4). Analyses of the powder hereby obtained showed it too was commercial didanosine.

To broaden the search for didanosine polymorphs, a binary solvent mixture, consisting of methanol/water dilutions was used as recrystallisation medium. All the recrystallisation products obtained from the methanol/water mixtures exhibited macro- and microscopic morphologies similar to the recrystallisation products from pure alcohols described above. Thermal analyses revealed endothermic peaks prior to melting for all the recrystallisation products, as well as large *ab initio* weight losses and volatile evolution. Karl Fischer titration was utilised complimentary to TGA, to better elucidate the contribution of water to the overall weight loss. The large standard deviations on the average weight losses of the recrystallisation products (Table 1) can be directly attributed to the difficulty in reproducible drying of the fine mesh crystal networks without compromising structural integrity. All the recrystallisation products from methanol/water mixtures displayed the same morphology changes upon drying and also upon storage within one month, with the products obtained from lower methanol concentrations displaying the most rapid conversion and those from pure methanol the slowest. However, unlike the recrystallisation products obtained from the alcohols, the products from methanol/water mixtures all displayed different unit cell dimensions, not only relative to commercial didanosine and didanosine recrystallised from 100 % methanol, but also each other (Fig. 5), suggesting that these recrystallisation products are polymorphs of didanosine.

As a classic indicator for stability, solubility studies were performed on the didanosine polymorphs obtained from methanol/water mixtures. As mentioned above, the polymorphs obtained from low methanol concentrations exhibited the most rapid conversion back to commercial didanosine, indicating an inherent instability in the system, while the product obtained from pure methanol took the longest time to revert. These observations were in excellent correlation to the solubility study (Table 2), where polymorphs obtained from lower methanol concentrations displayed higher solubility values than products obtained from higher methanol concentrations. Therefore, although polymorphs of didanosine can be prepared, and advantage of their metastability can be taken to increase the aqueous solubility of didanosine, the rapid conversion of these polymorphs back to commercial didanosine makes it doubtful if this will ever be practically feasible. Both the morphologic and polymorphic form of commercial didanosine is clearly the most stable, and will most often be encountered in practice.

4. Conclusion

Recent studies (Martins et al., 2010; Bettini et al., 2010) have described the difficulty in preparing polymorphs of the antiretroviral (ARV) drug didanosine, and mention was made of its metastability. However, the exact extent of didanosine's inability to form polymorphs and the metastability of its polymorphs are yet to be reported. In this study, we present a thorough investigation into the metastability of polymorphs and recrystallisation products obtained from didanosine. The interconversion between stable and metastable didanosine polymorphs is definitely a point of interest for the pharmaceutical scientist, since it can lead to unpredictable bioavailability and stability of solid dosage forms. Several stages in the formulation or preparation processes can induce stable/metastable polymorph interconversions, e.g. the presence of alcohols or during tableting where pressure (force divided by area) is applied to the system. Giron (2005) emphasized the need for the pharmaceutical scientist to obtain adequate knowledge regarding the thermodynamic properties of the polymorphs used in context of drying, granulation and storage under atmospheric conditions. In the case of didanosine, the importance of such considerations has never been truer. The results obtained from this study suggest that commercial didanosine is the most stable form, not only concerning morphology, but also polymorphism. Future investigations can look for similar behavior in drugs that are structurally similar to didanosine, or that display similar crystal morphology.

Acknowledgements

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Figures and captions

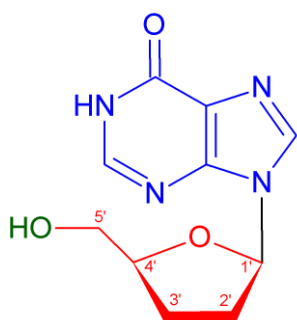


Fig. 1. Chemical structure of didanosine, indicating the hypoxanthine moiety, dideoxyribose ring and glycosidic bond (black).

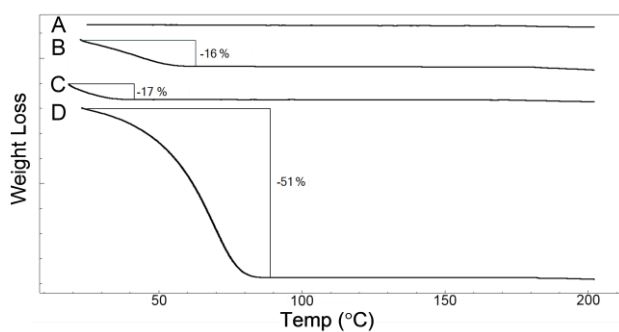


Fig. 2. TGA traces of commercial didanosine (A), and its recrystallisation products from methanol (B), 1-propanol (C) and 1-butanol (D).

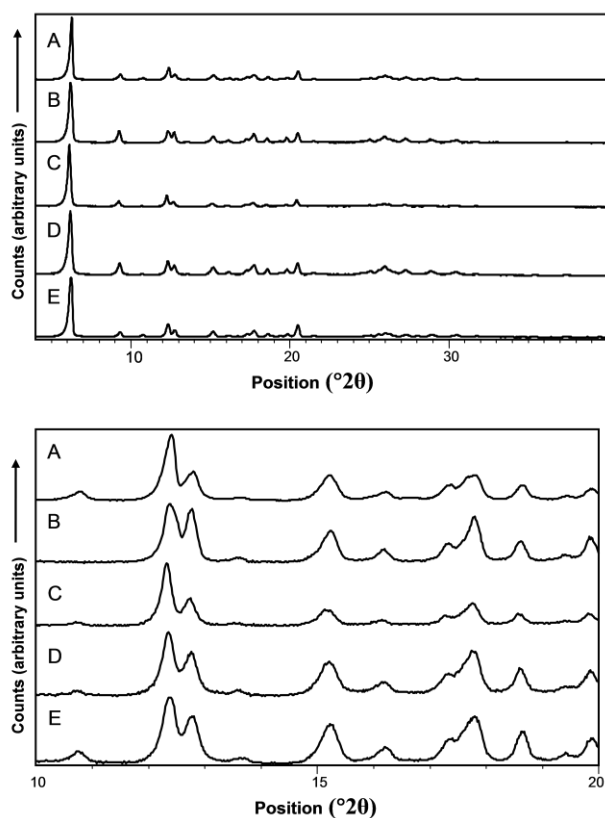


Fig. 3. PXRD diffractograms of commercial didanosine (A), and its recrystallisation products from methanol (B), ethanol (C), 1-propanol (D) and 1-butanol (E), with an enlargement of the diffraction patterns obtained between 10 and 20° 2θ .

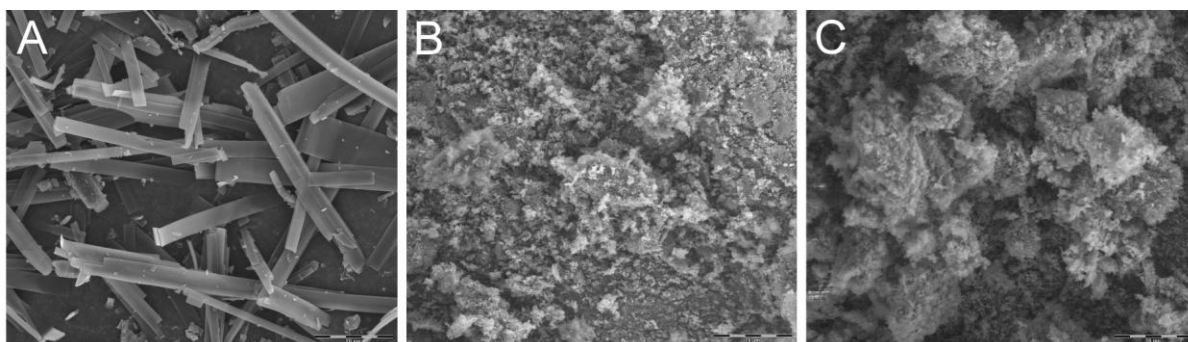


Fig. 4. SEM micrographs of didanosine crystals obtained from methanol after preparation (A), after drying *in vacuo* (B) and after storage for a month in recrystallisation medium (C). The scale bars indicate 20 μm.

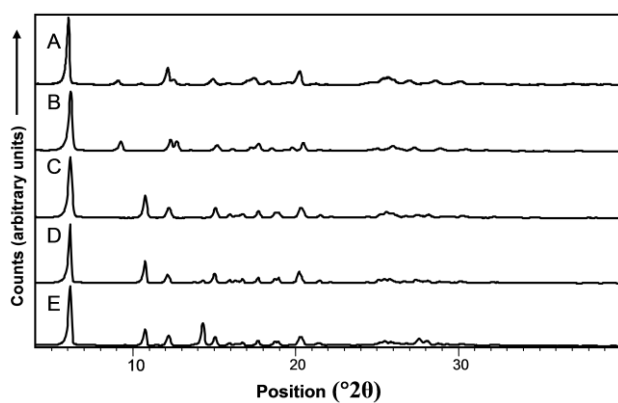


Fig 5. PXRD diffractograms of commercial didanosine (A), and didanosine recrystallised from 100 % methanol (B), 70 % methanol (C), 50 % methanol (D) and 10 % methanol (E).

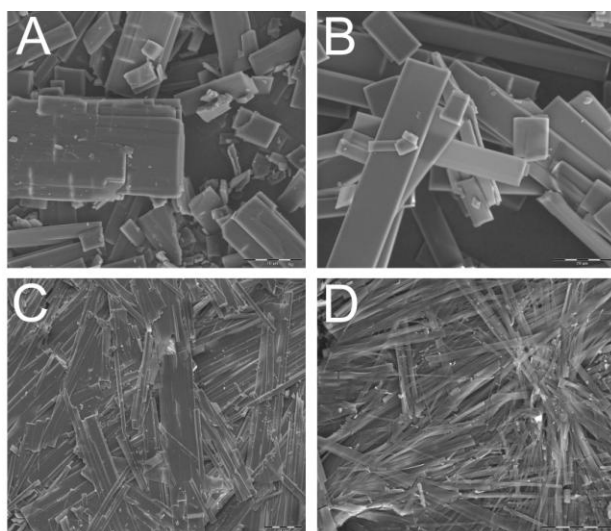


Fig. 6. SEM micrographs of didanosine recrystallised from 70 % methanol (A), 50 % methanol (B), 30 % methanol (C) and 10 % methanol (D). Scale bars indicate 20 μm.

Tables

Table 1

Weight loss (mean $\pm$ S.D.) of didanosine recrystallisation products from methanol/water mixtures

Sample (% methanol)	Weight loss (%)	
	TGA	KF
100	18.4 $\pm$ 2.11	1.4 $\pm$ 0.2
90	33.2 $\pm$ 7.73	12.9 $\pm$ 7.42
80	19.0 $\pm$ 11.79	11.2 $\pm$ 2.72
70	15.4 $\pm$ 0.69	12.2 $\pm$ 7.87
60	28.5 $\pm$ 5.22	18.1 $\pm$ 2.4
50	31.1 $\pm$ 0.86	22.1 $\pm$ 7.55
40	43.7 $\pm$ 10.81	31.6 $\pm$ 11.82
30	36.1 $\pm$ 11.59	27.9 $\pm$ 10.94
20	33.5 $\pm$ 5.77	27.5 $\pm$ 6
10	40.2 $\pm$ 0.88	34.1 $\pm$ 11.6

Table 2

Aqueous solubility (mean $\pm$ S.D.) of commercial didanosine and its recrystallisation products from methanol/water mixtures

Sample	Solubility (mg/ml)
Commercial	30.5 $\pm$ 1.07
100 % methanol	22.5 $\pm$ 3.02
90 % methanol	18.7 $\pm$ 5.15
80 % methanol	29.6 $\pm$ 3.82
70 % methanol	22.9 $\pm$ 3.50
60 % methanol	31.7 $\pm$ 2.03
50 % methanol	27.7 $\pm$ 0.52
40 % methanol	31.9 $\pm$ 0.69
30 % methanol	33.8 $\pm$ 1.79
20 % methanol	43.5 $\pm$ 2.14
10 % methanol	44.3 $\pm$ 2.42

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Supplementary Information

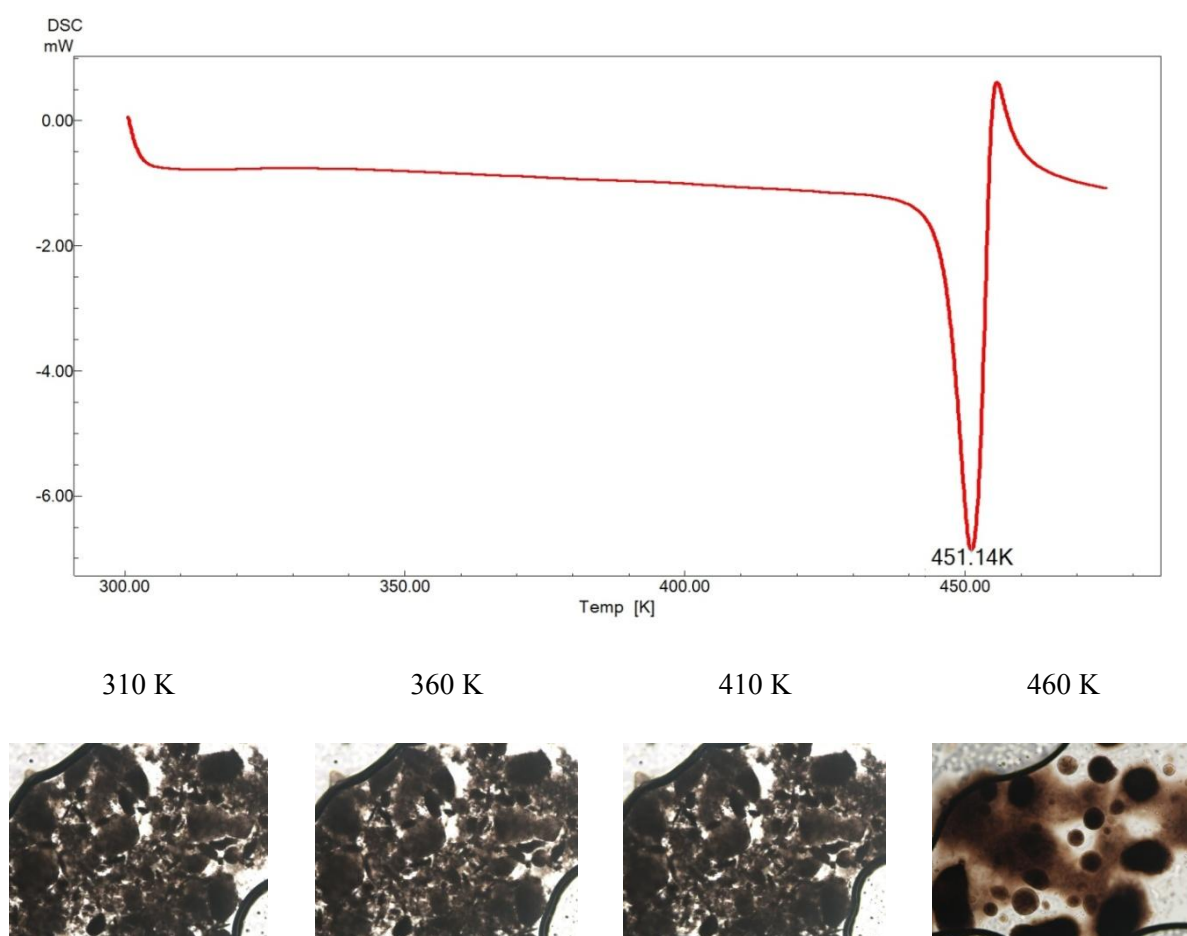


Figure S1: DSC thermogram and hot stage micrographs of didanosine raw material.

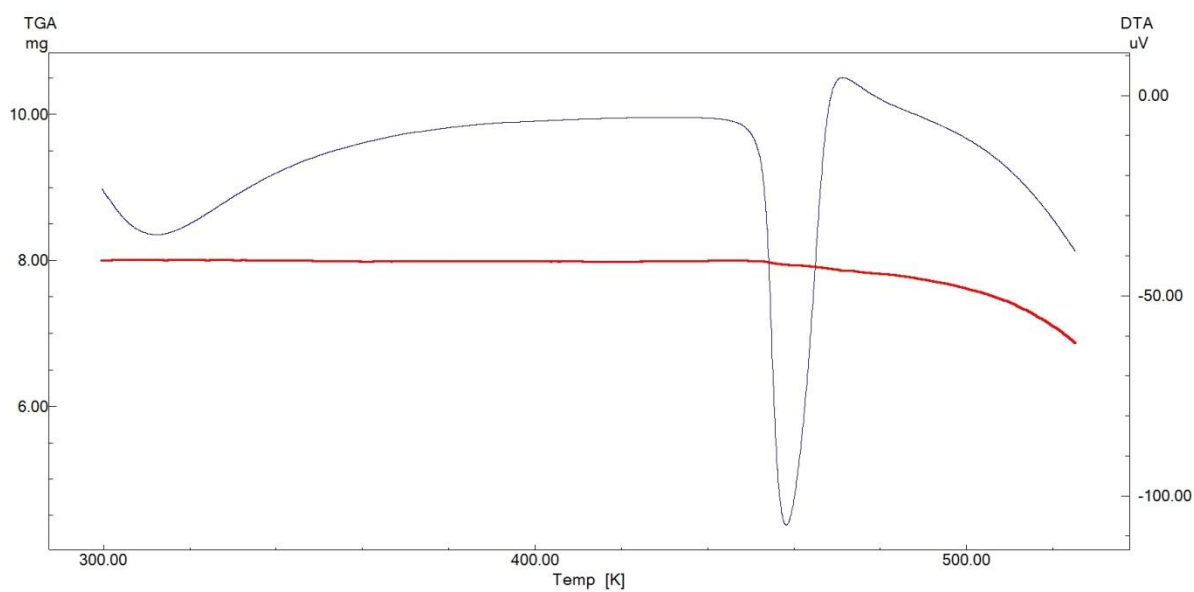


Figure S2: TGA of didanosine raw material.

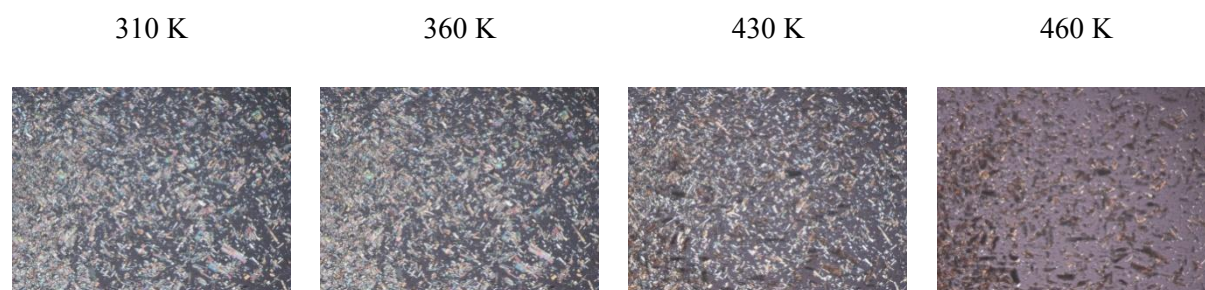
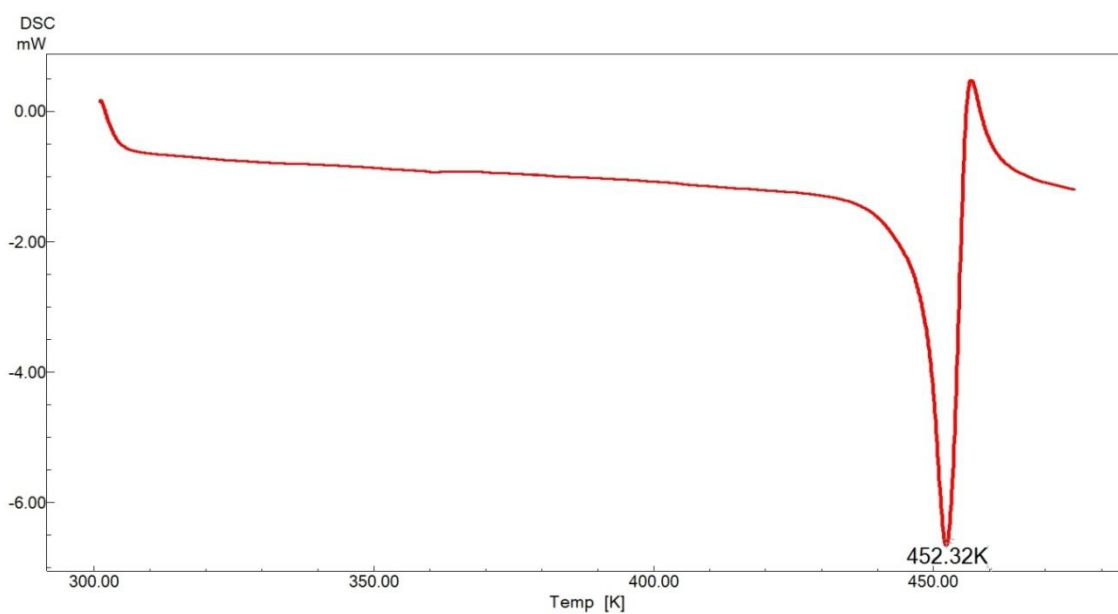


Figure S3: DSC thermogram and hot stage micrographs of didanosine recrystallised from acetonitrile.

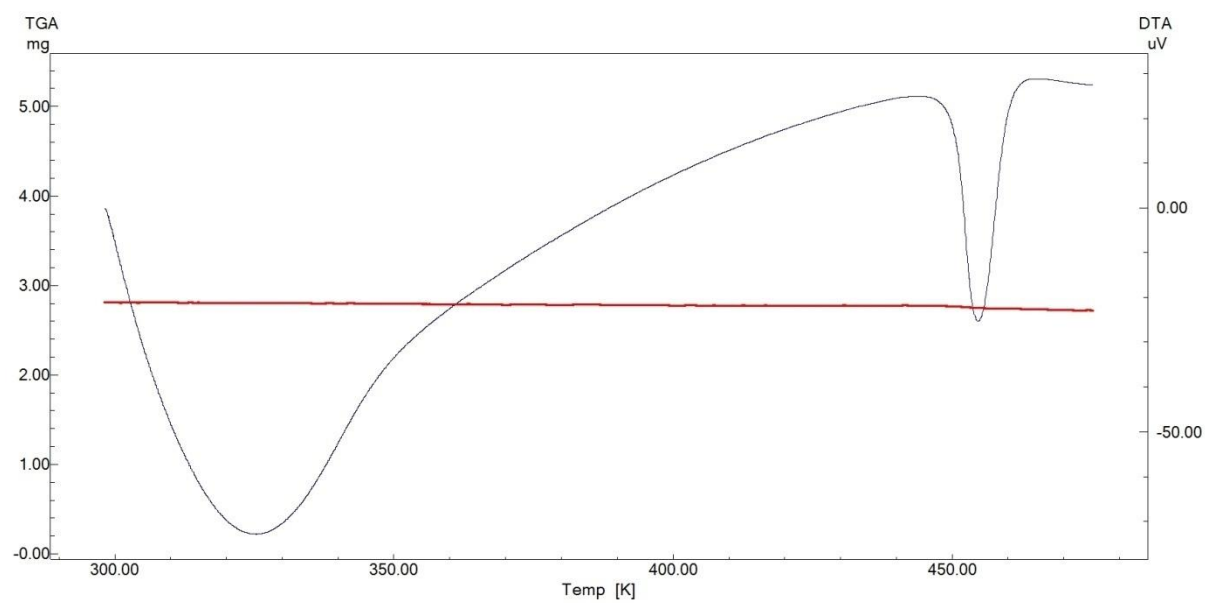


Figure S4: TGA of didanosine crystals obtained from acetonitrile.

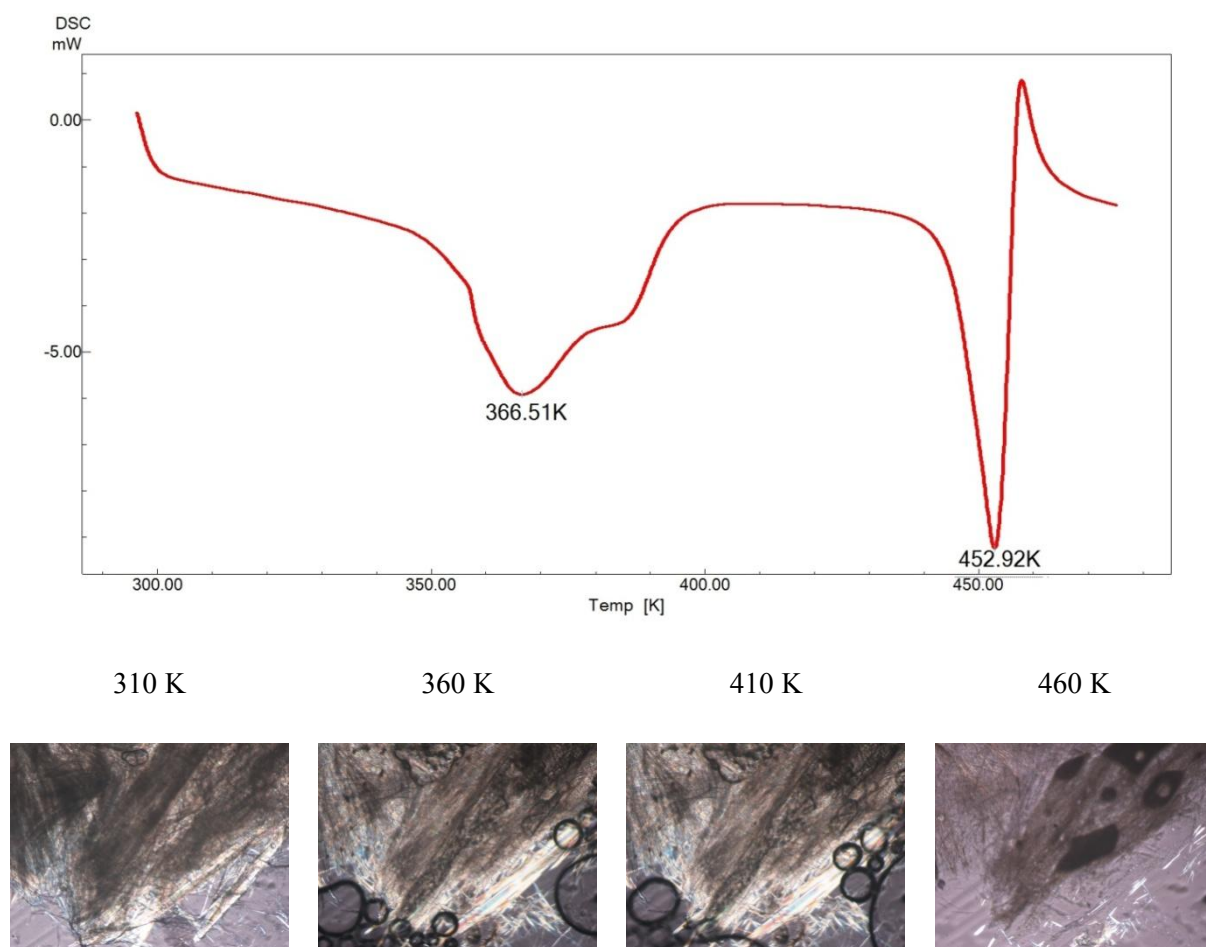


Figure S5: DSC thermogram and hot stage micrographs of didanosine recrystallised from methanol.

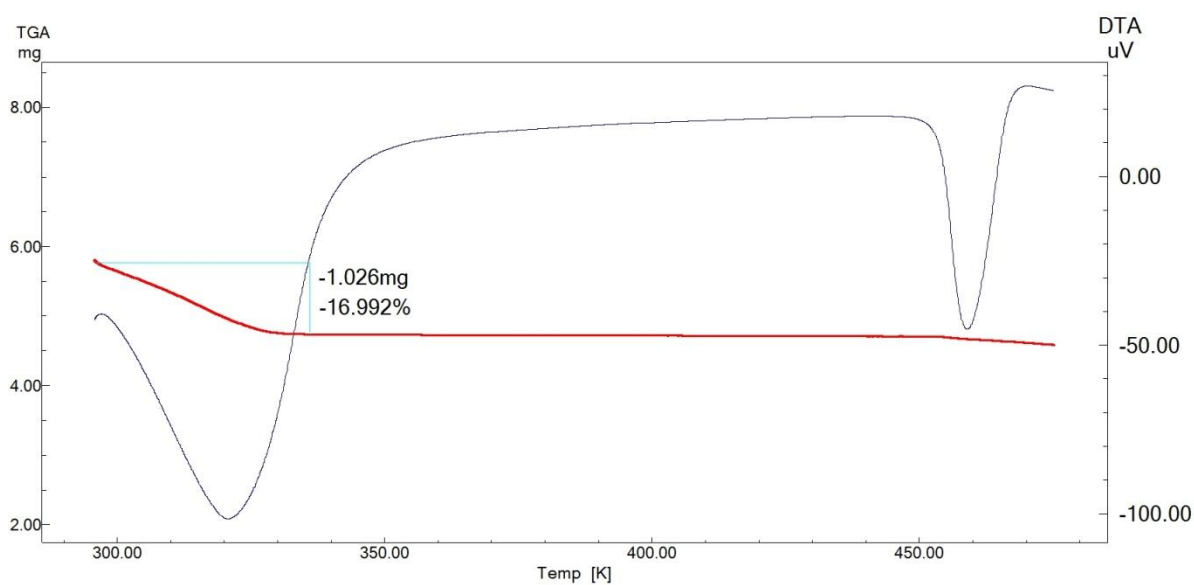


Figure S6: TGA of didanosine crystals obtained from methanol.

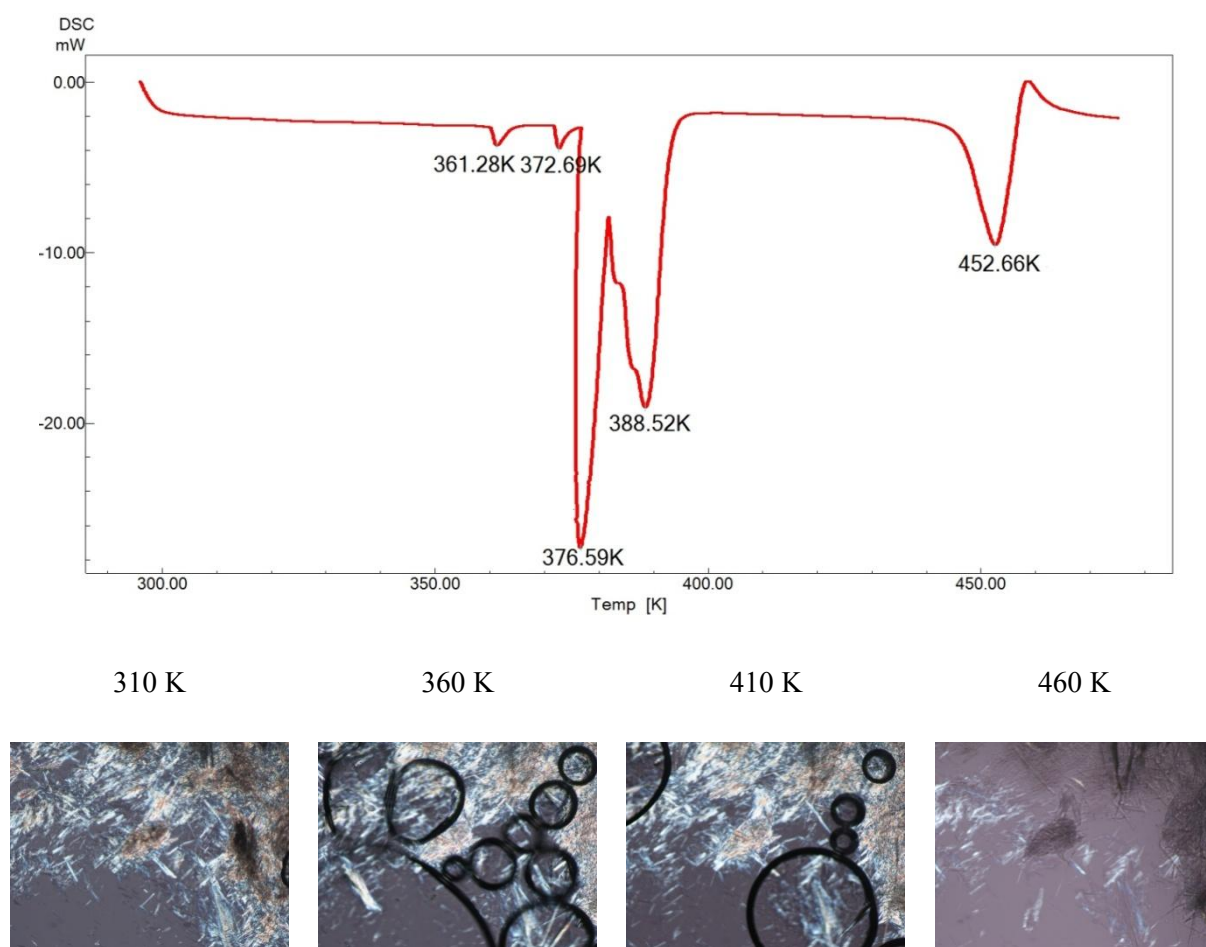


Figure S7: DSC thermogram and hot stage micrographs of didanosine recrystallised from ethanol.

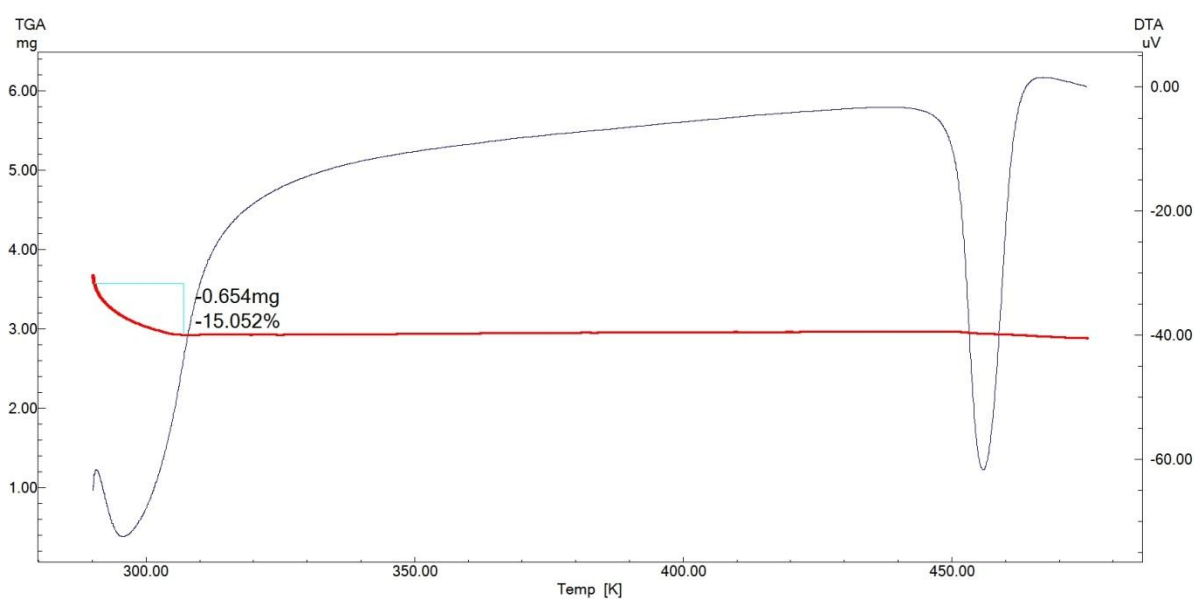


Figure S8: TGA of didanosine crystals from ethanol.

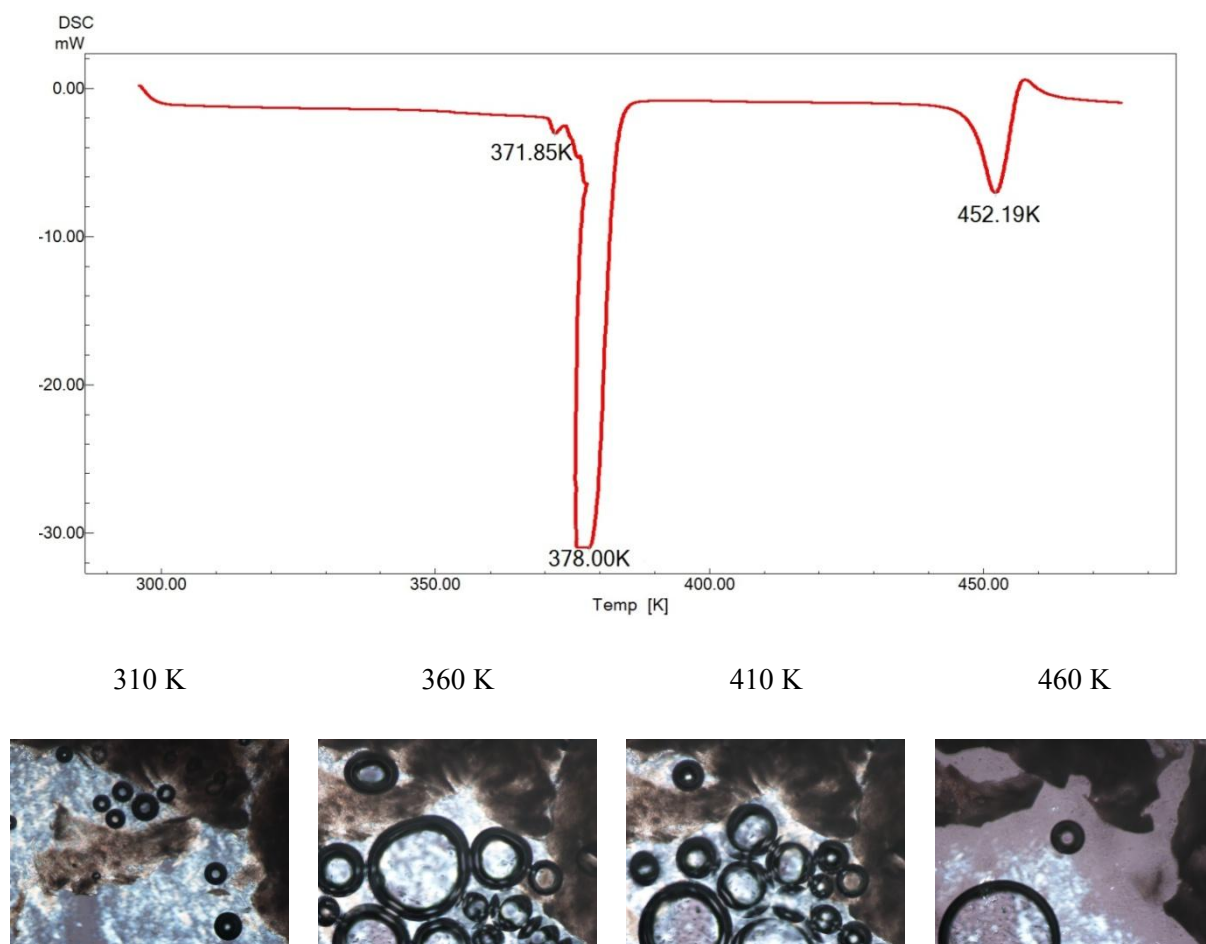


Figure S9: DSC thermogram and hot stage micrographs of didanosine recrystallised from 1-propanol.

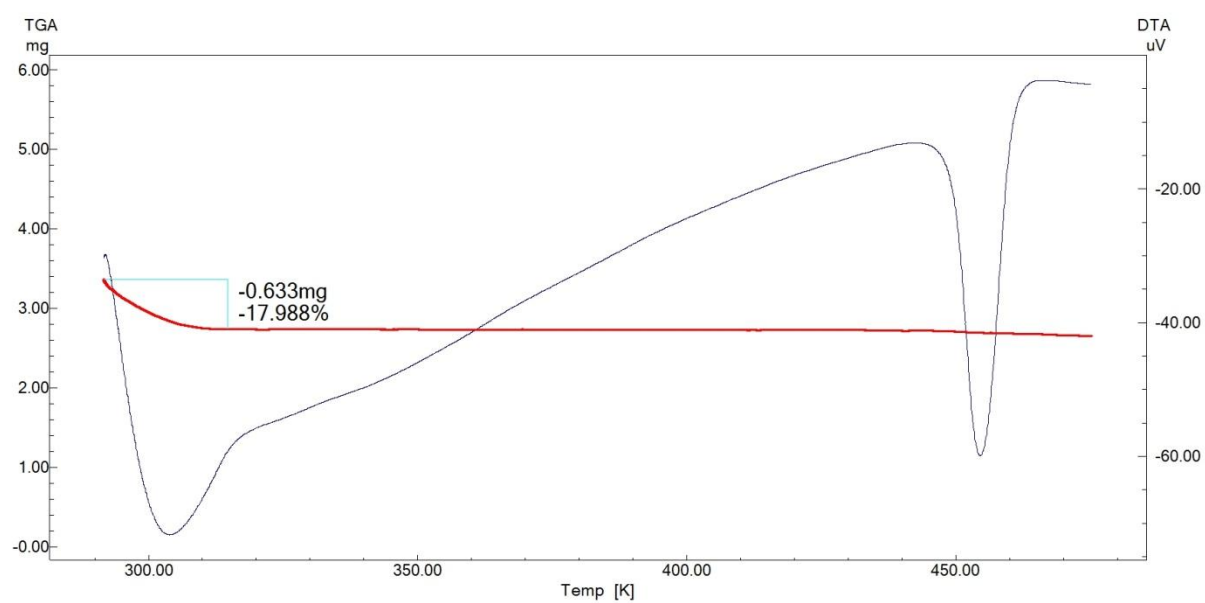


Figure S10: TGA of didanosine crystals from 1-propanol.

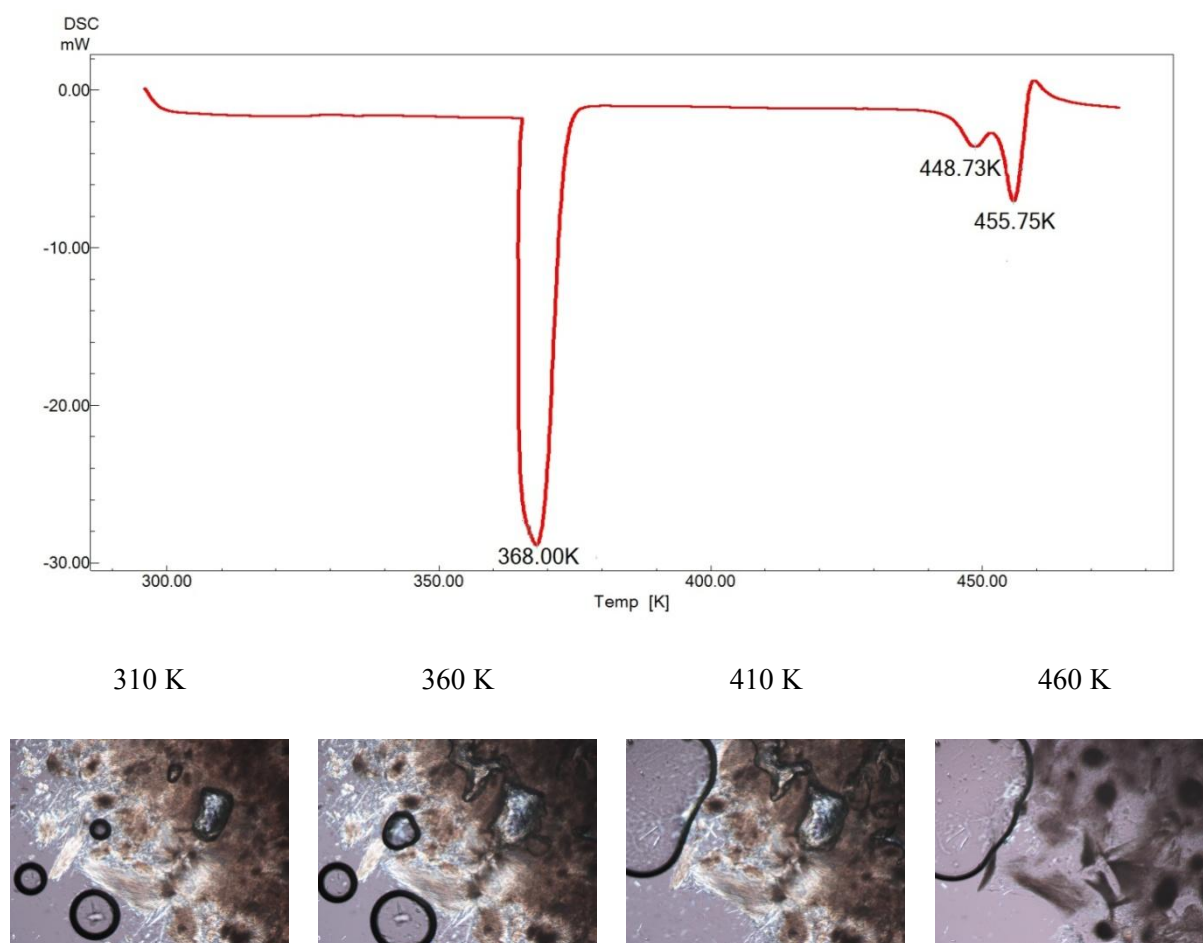


Figure S11: DSC thermogram and hot stage micrographs of didanosine recrystallised from 2-propanol.

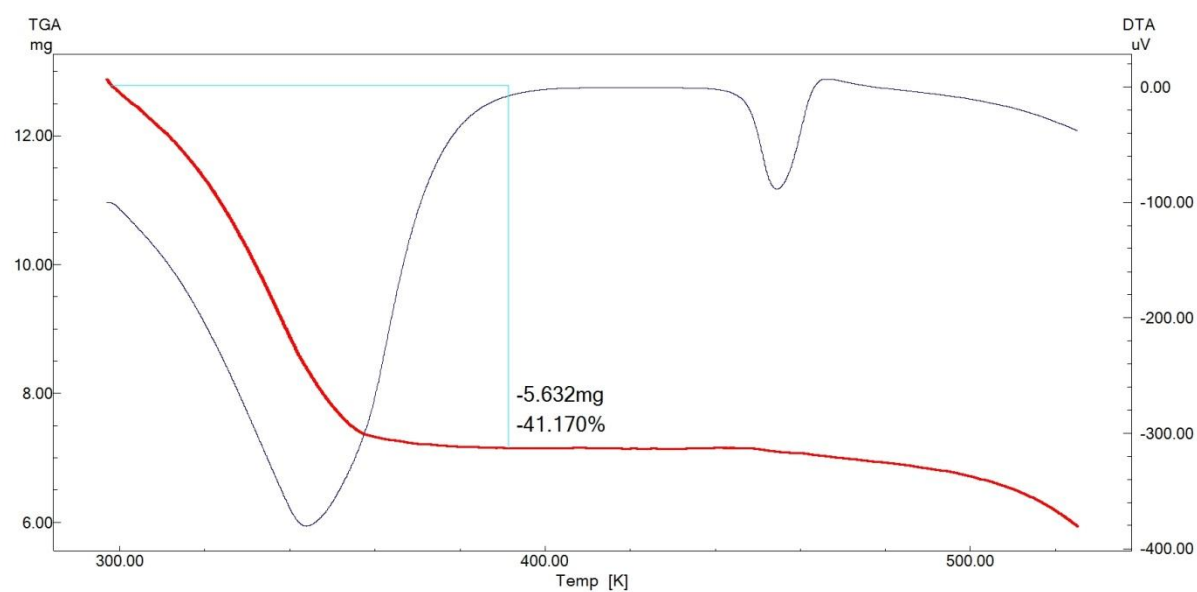


Figure S12: TGA of didanosine recrystallised from 2-propanol.

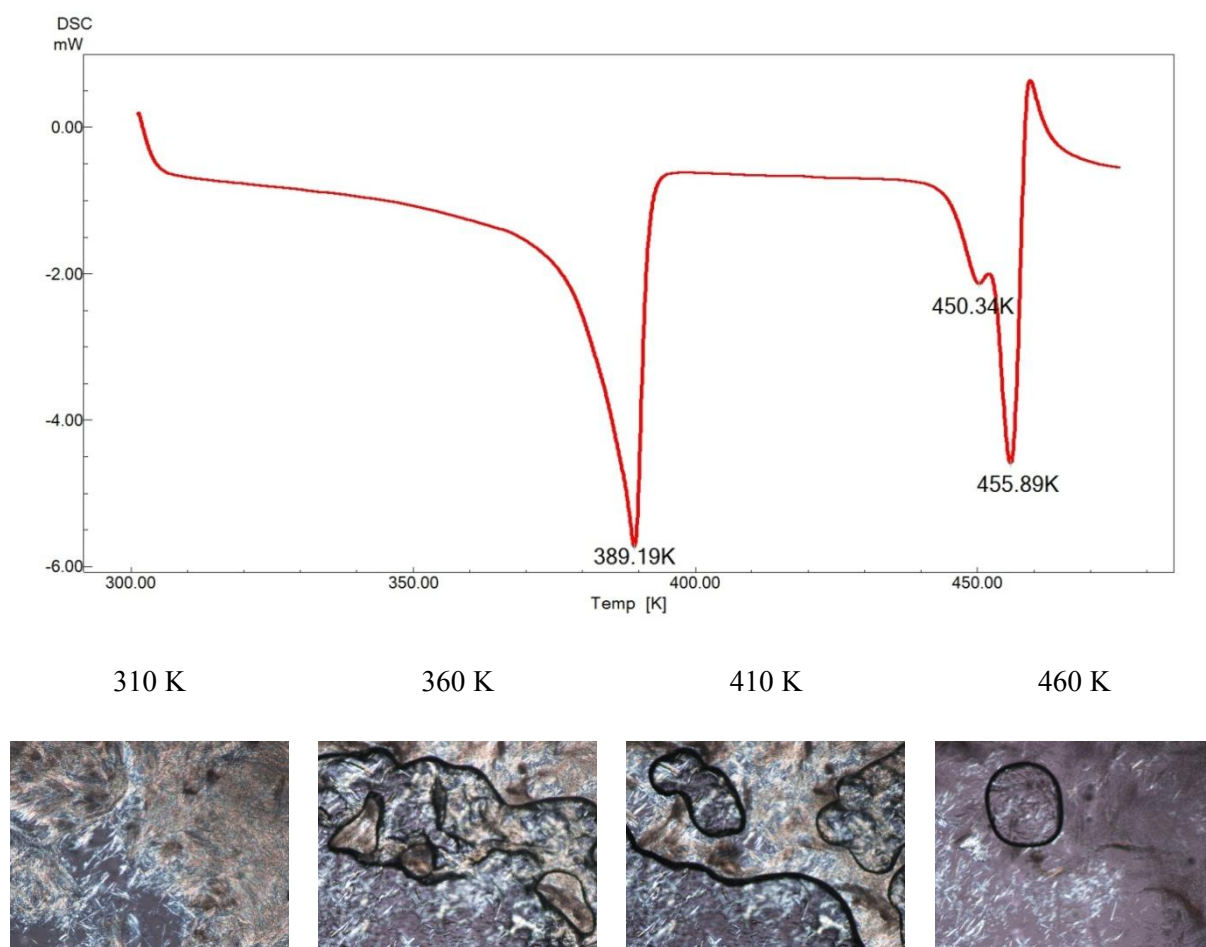


Figure S13: DSC thermogram of didanosine recrystallised from 1-butanol.

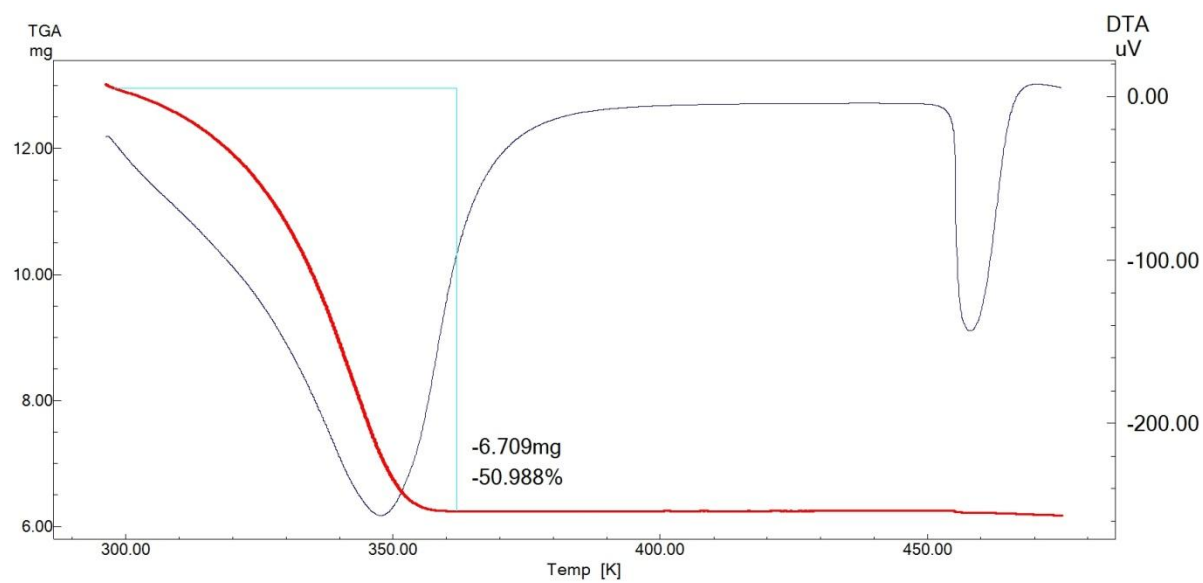


Figure S14: TGA of didanosine crystals from 1-butanol.

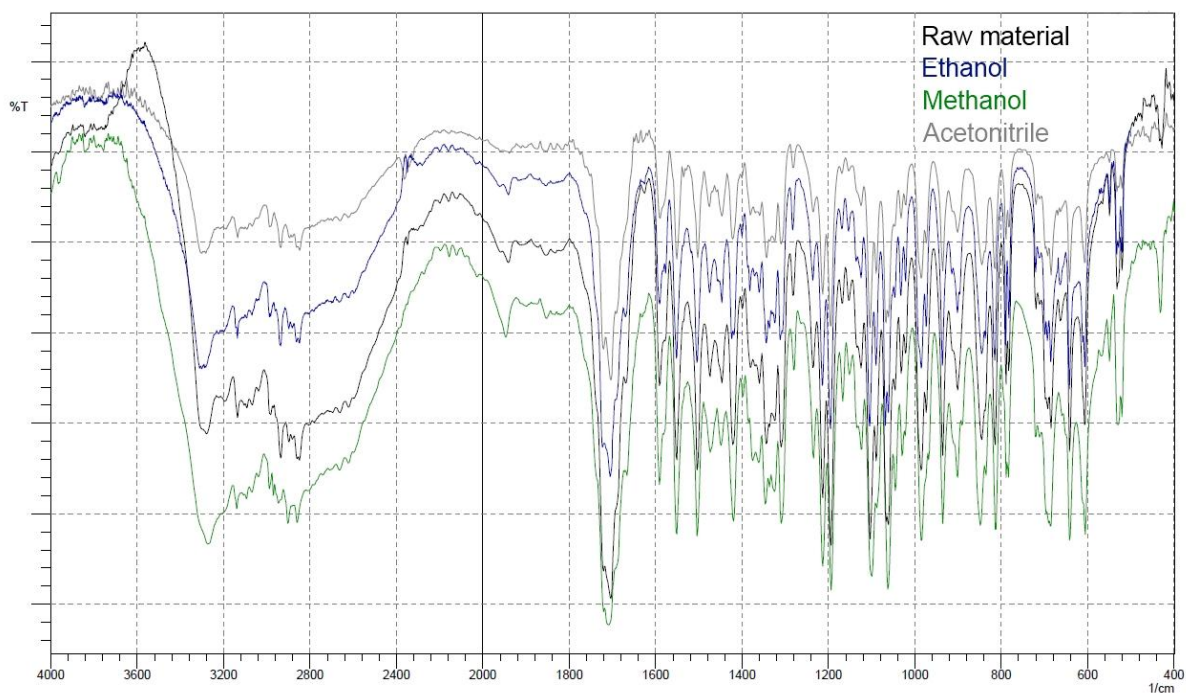


Figure S15: Overlay of the FTIR interferograms of didanosine raw material and its recrystallisation products.

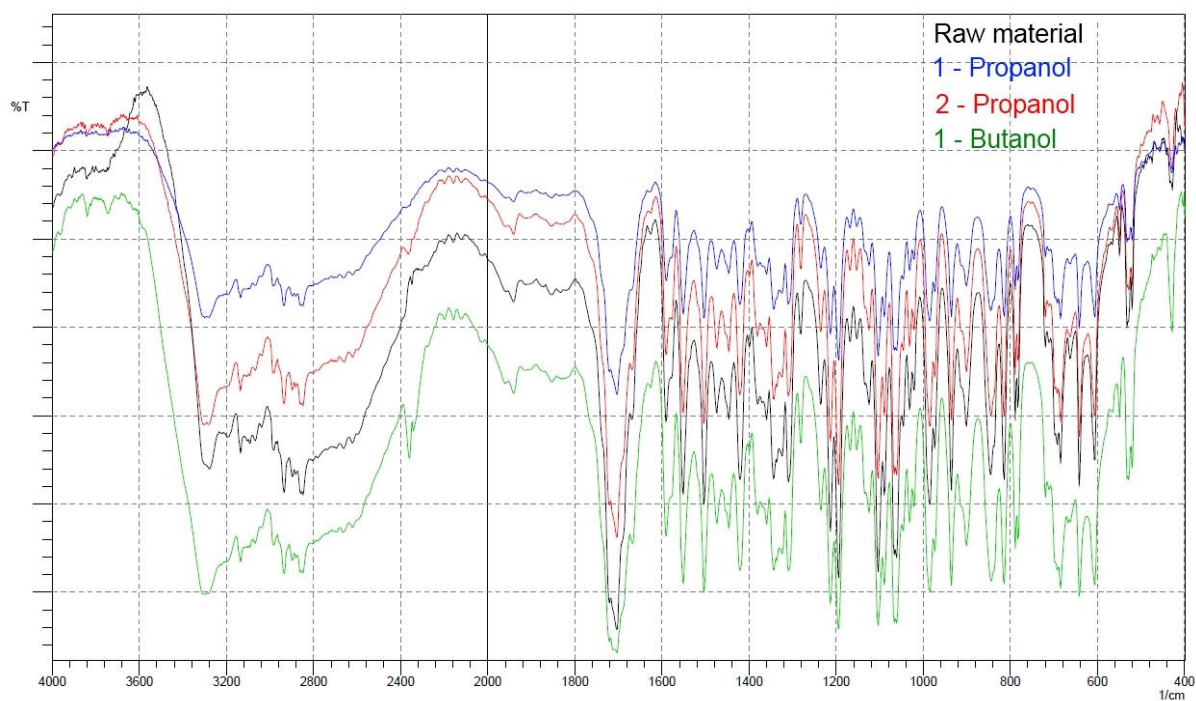


Figure S16: Overlay of the FTIR interferograms of didanosine raw material and its recrystallisation products.

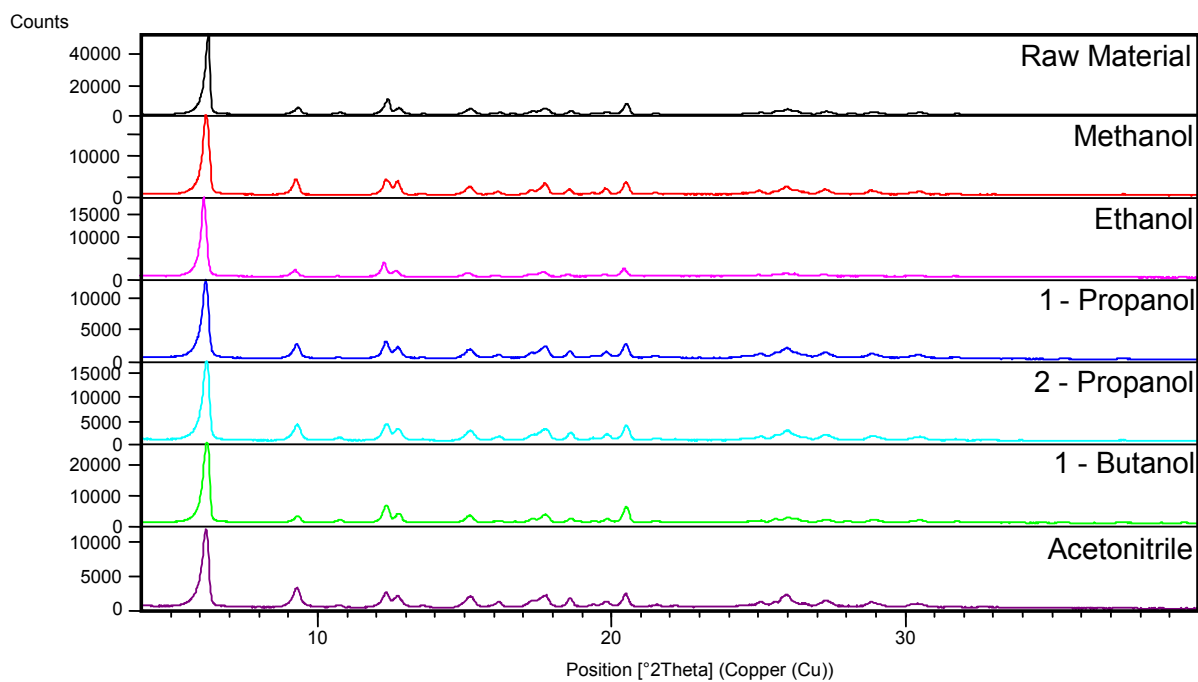


Figure S17: PXRD diffractograms of didanosine raw material and its recrystallisation products.

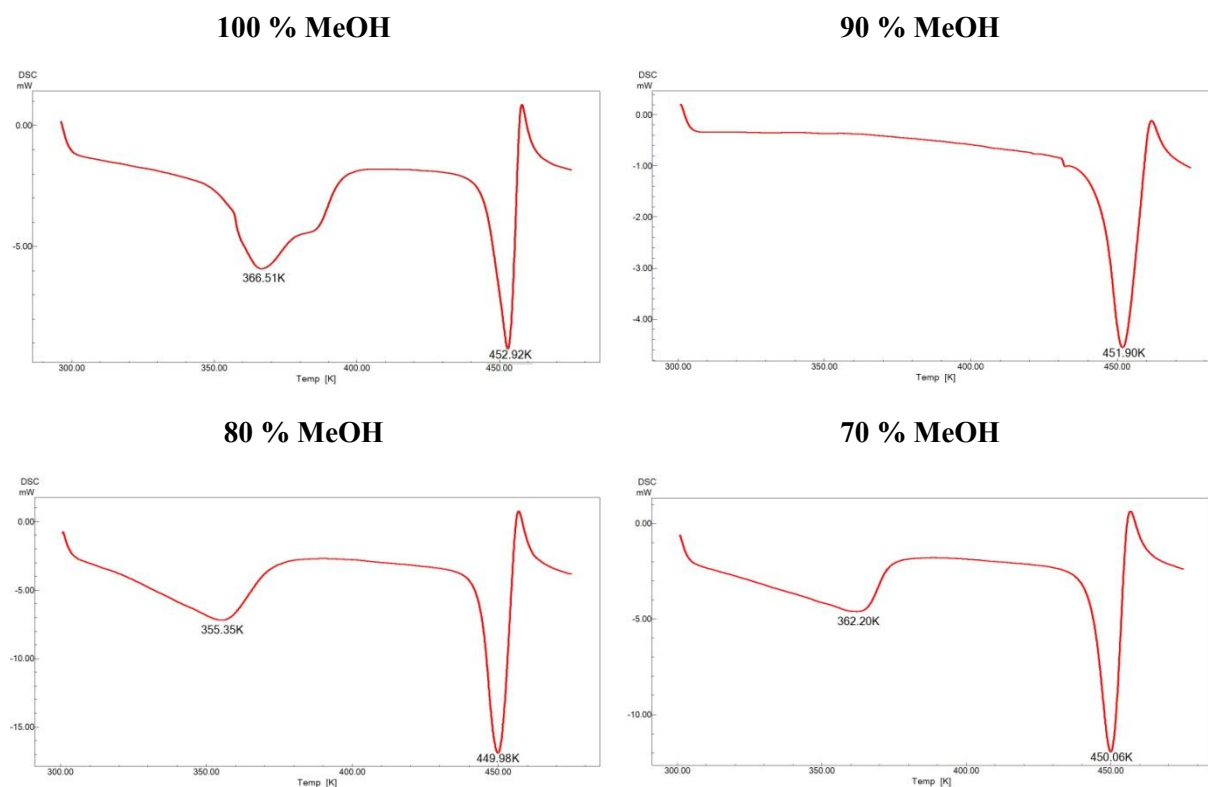


Figure S18: DSC thermograms of the didanosine crystals obtained from methanol and water mixtures.

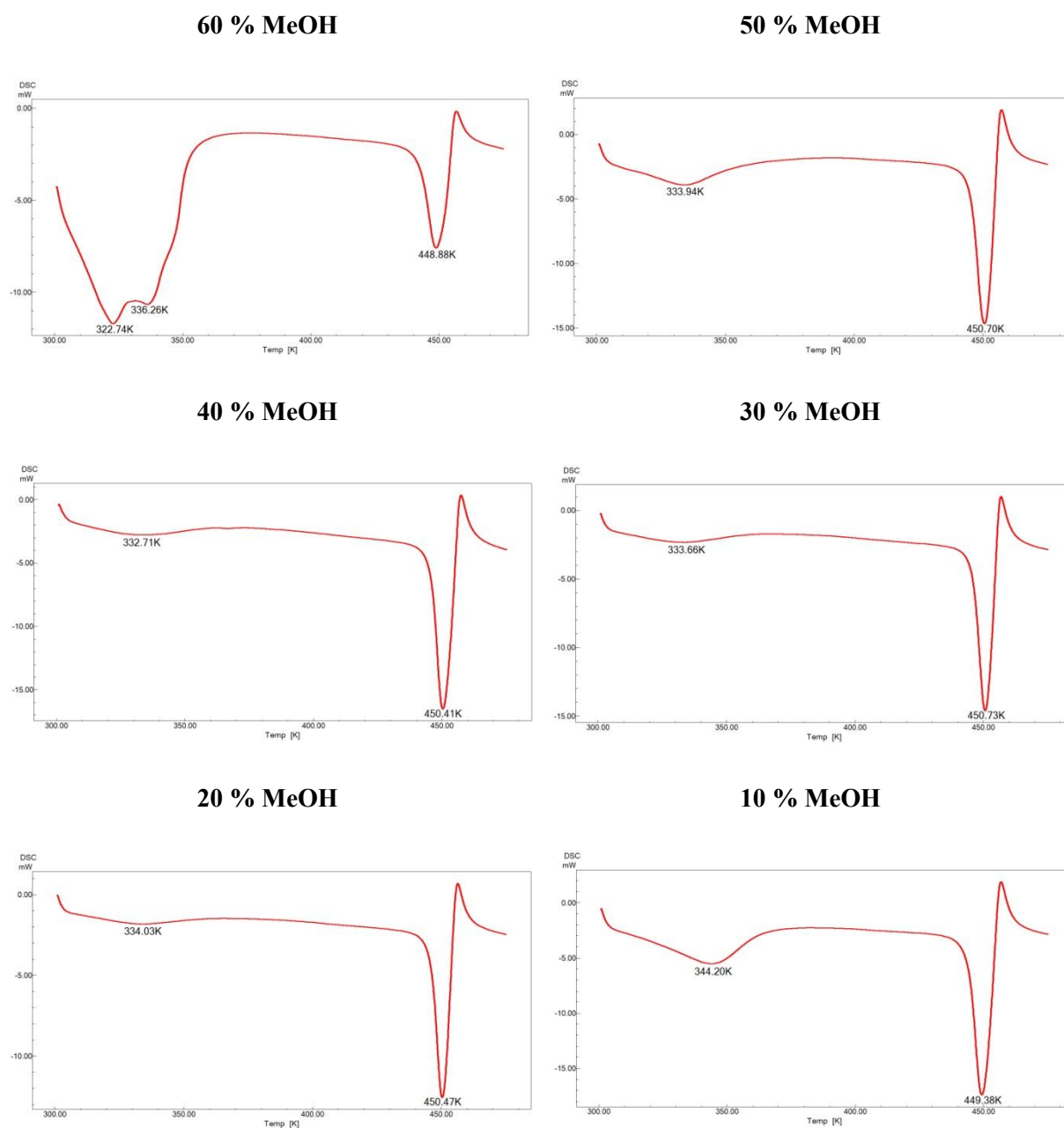


Figure S19: DSC thermograms of didanosine crystals obtained from methanol and water mixtures.

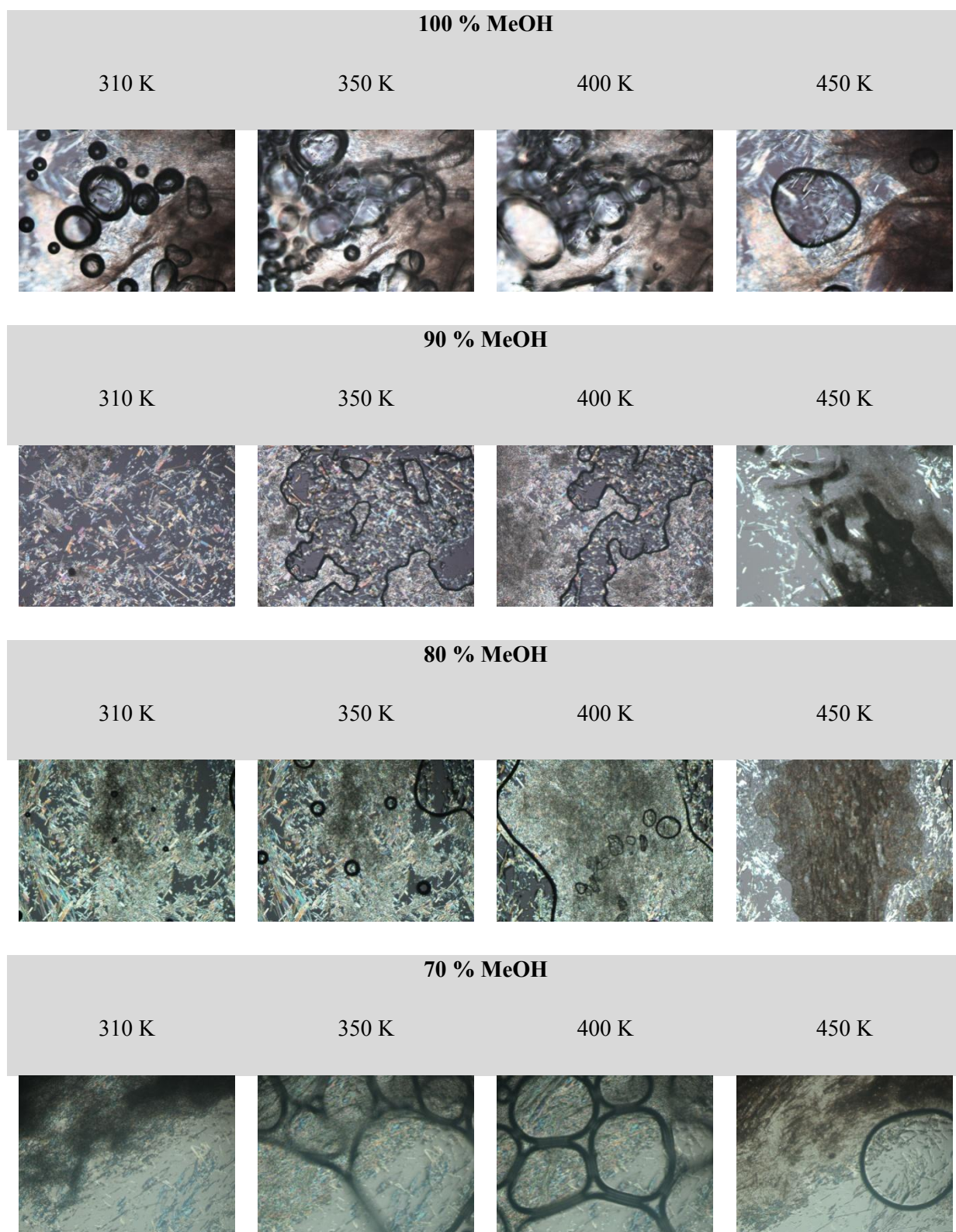


Figure S20: Hot stage micrographs of didanosine crystals from methanol and water mixtures.

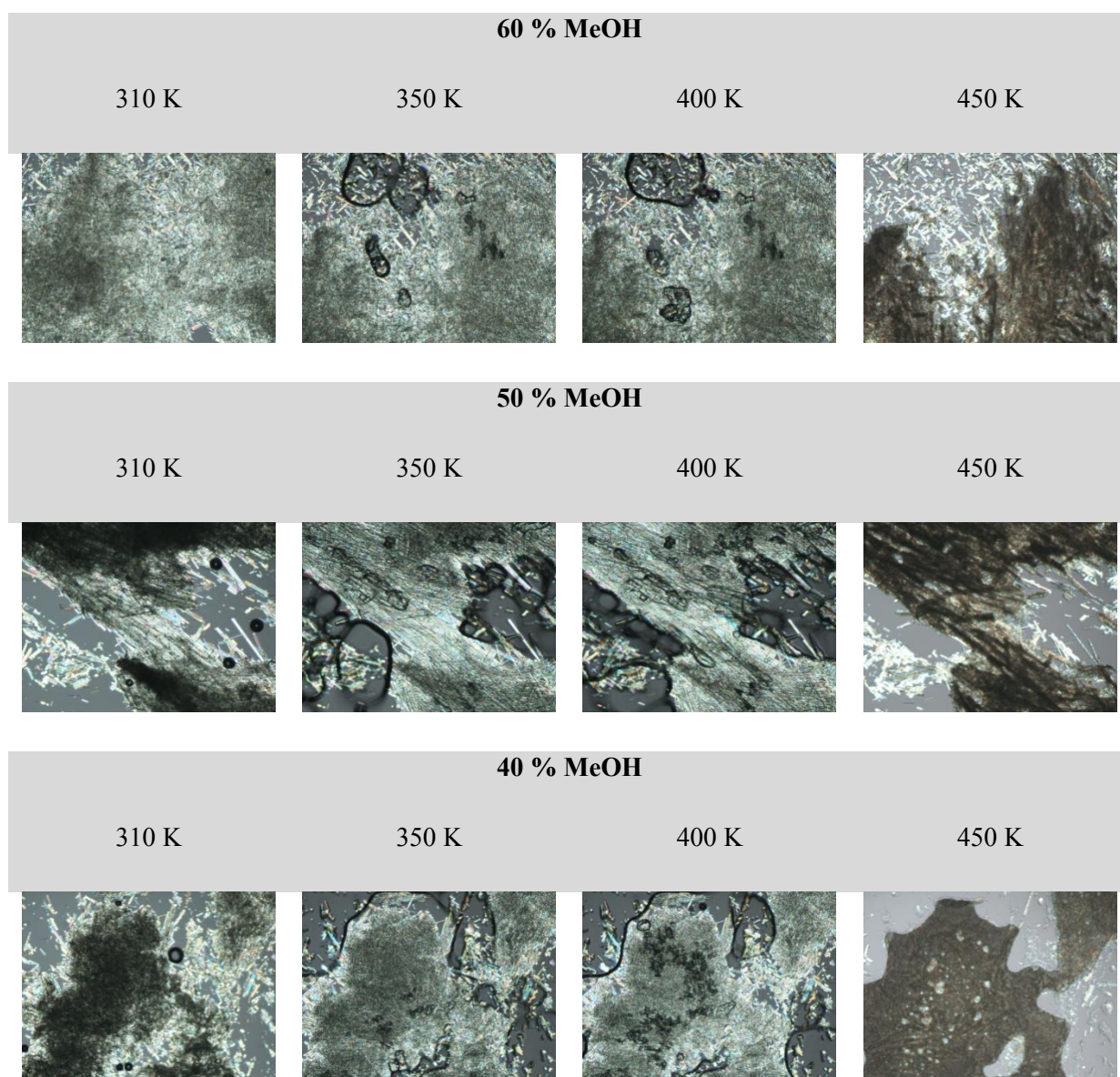


Figure S21: Hot stage micrographs of didanosine crystals from methanol and water mixtures.

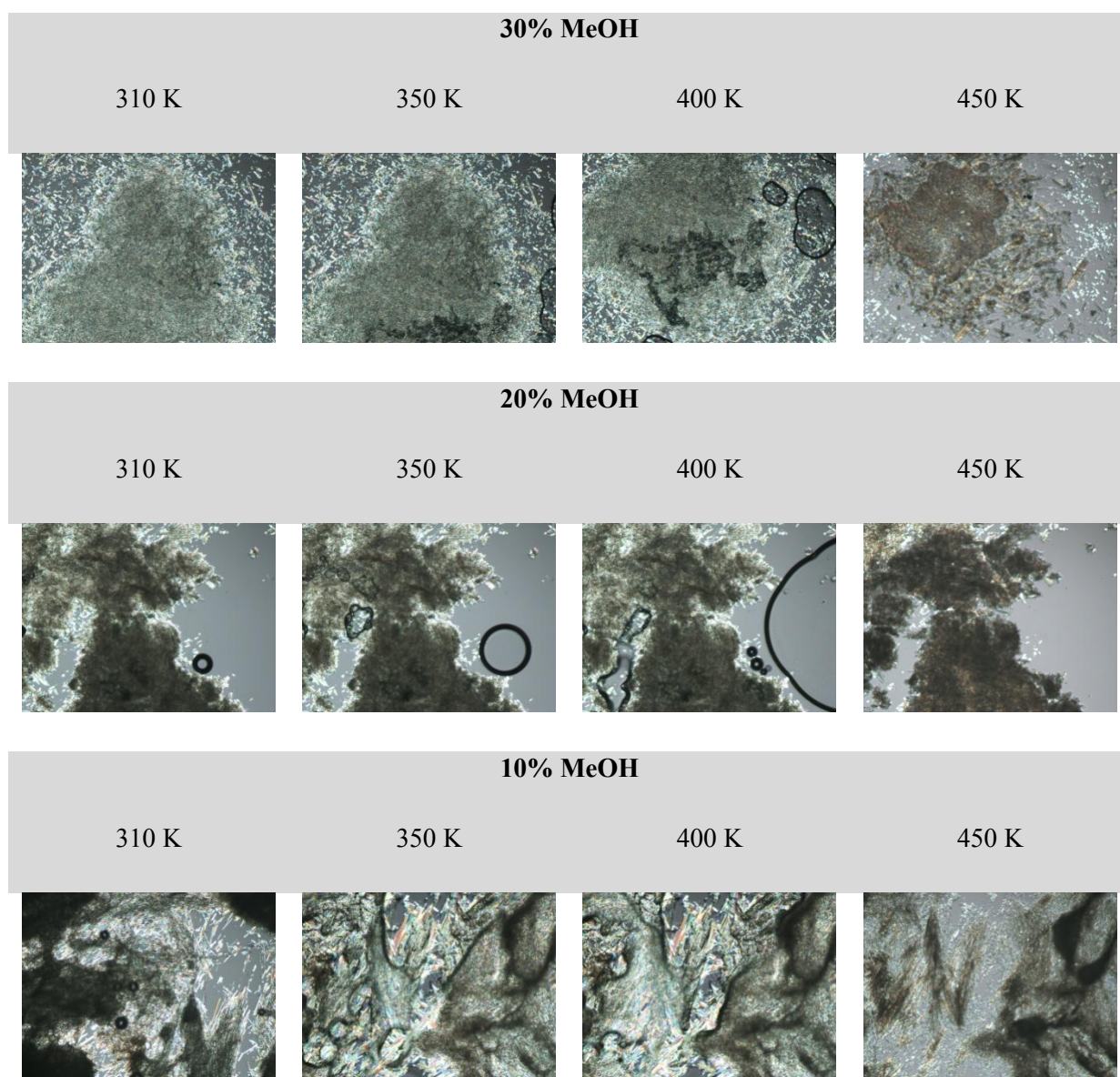
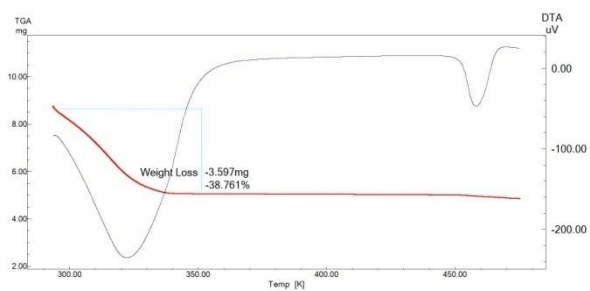
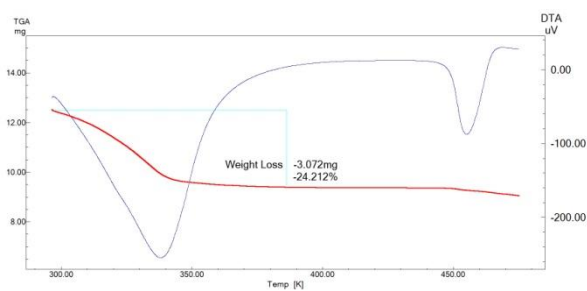


Figure S22: Hot stage micrographs of didanosine crystals from methanol and water mixtures.

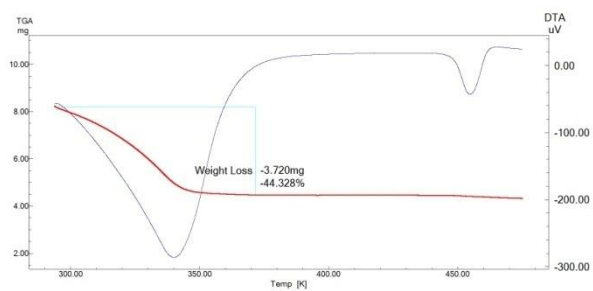
90% MeOH



60% MeOH



30% MeOH



10% MeOH

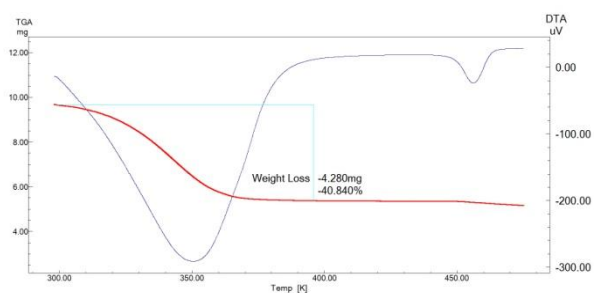
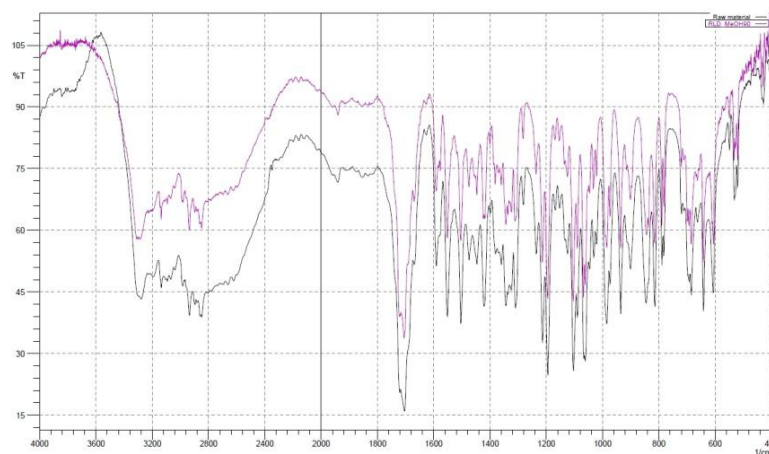
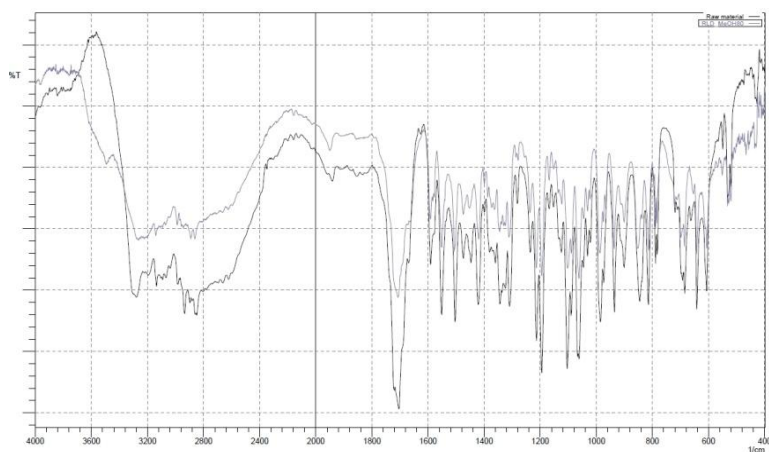


Figure S23: TGA of some of the recrystallisation products from methanol and water mixtures.

90% MeOH



80% MeOH



70% MeOH

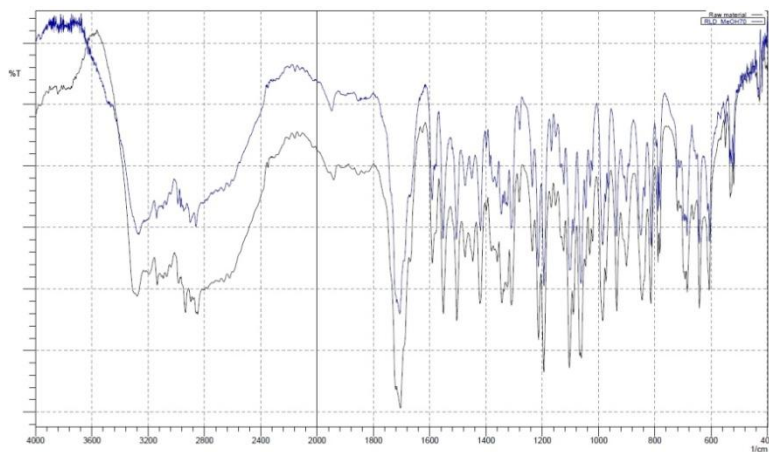
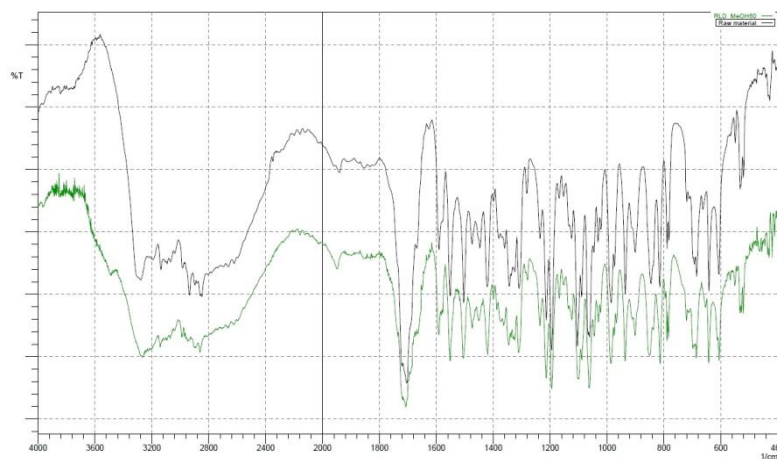
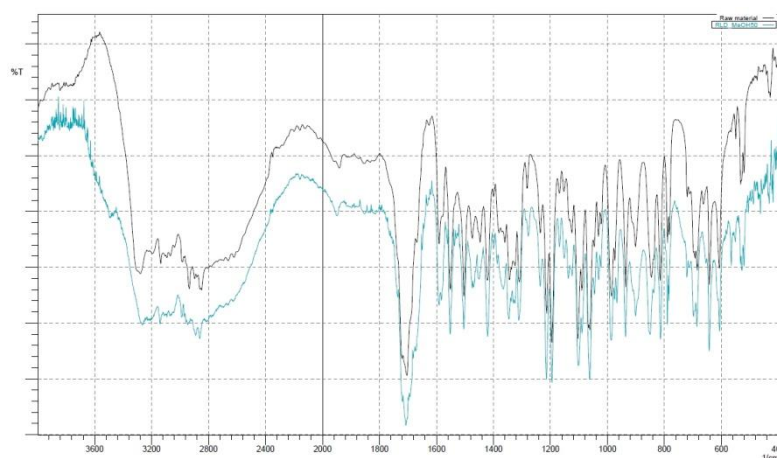


Figure S24: FTIR overlays of didanosine raw material (black interferogram) and its recrystallisation products from methanol and water mixtures.

60% MeOH



50% MeOH



40% MeOH

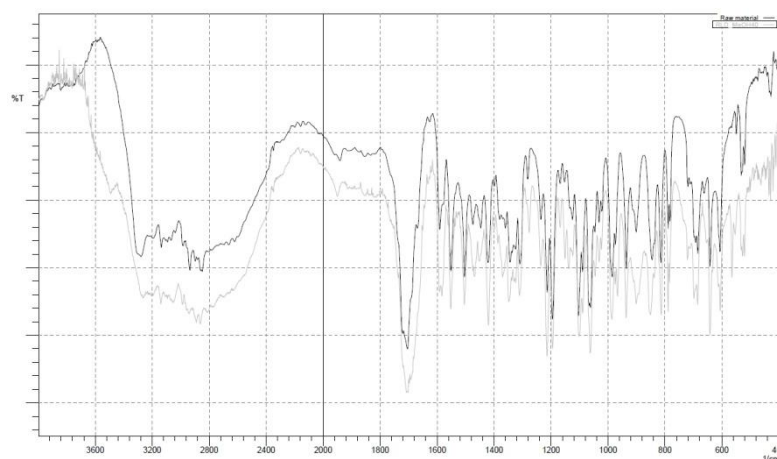
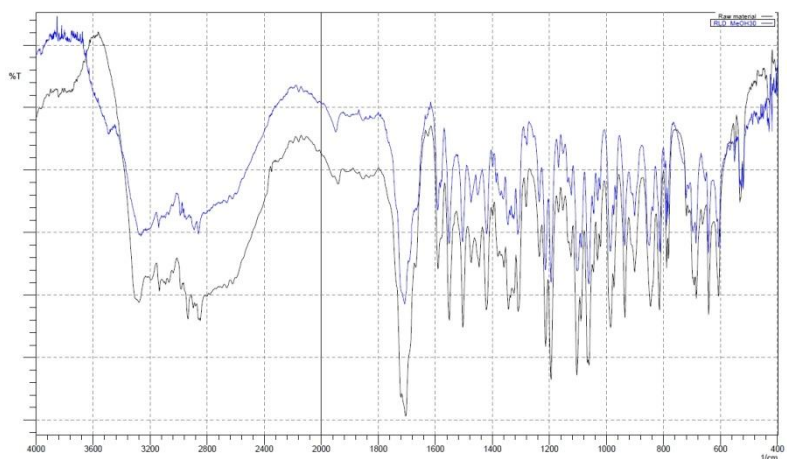
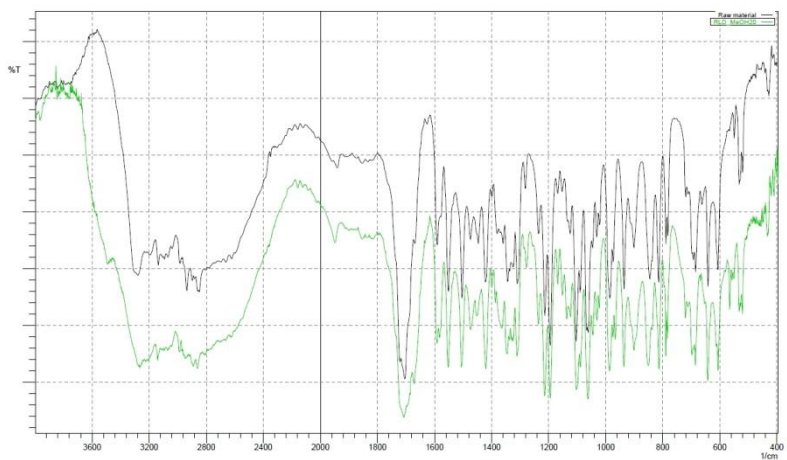


Figure S25: FTIR overlays of didanosine raw material (black interferogram) and its recrystallisation products from methanol and water mixtures.

30% MeOH



20% MeOH



10% MeOH

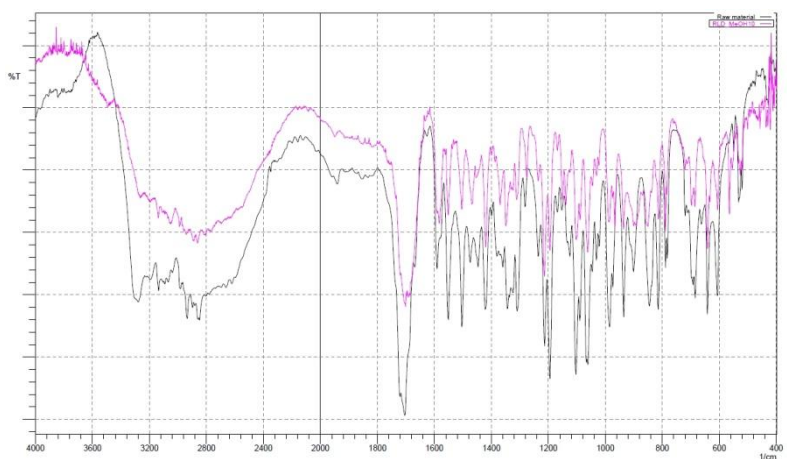


Figure S26: FTIR overlays of didanosine raw material (black interferogram) and its recrystallisation products from methanol and water mixtures.

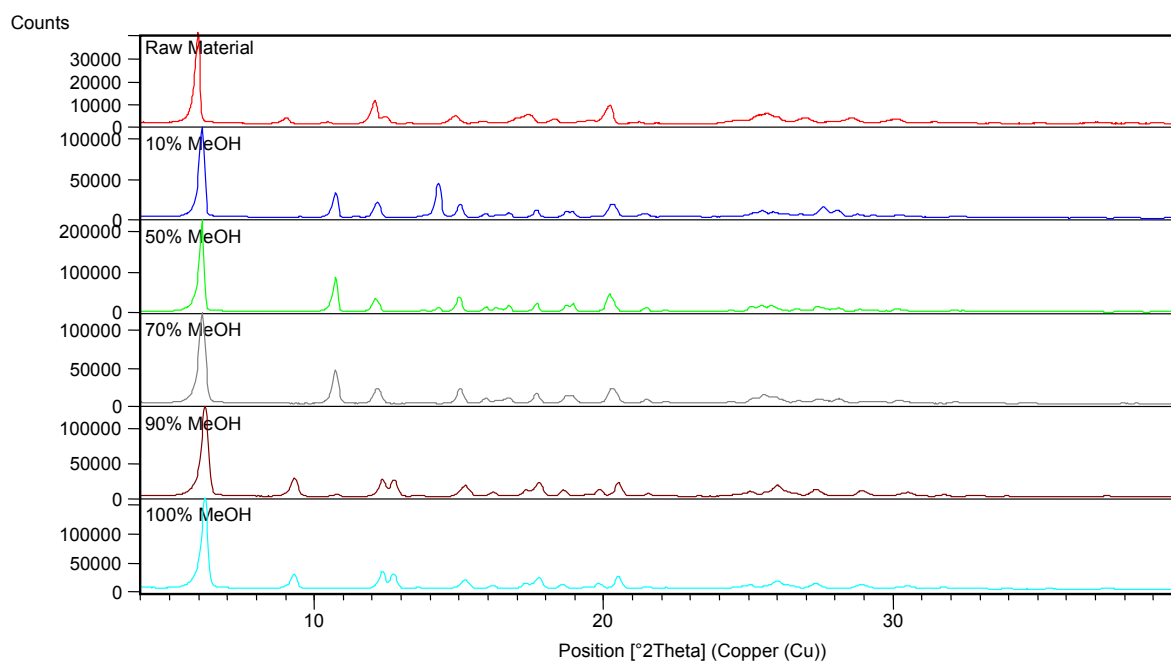
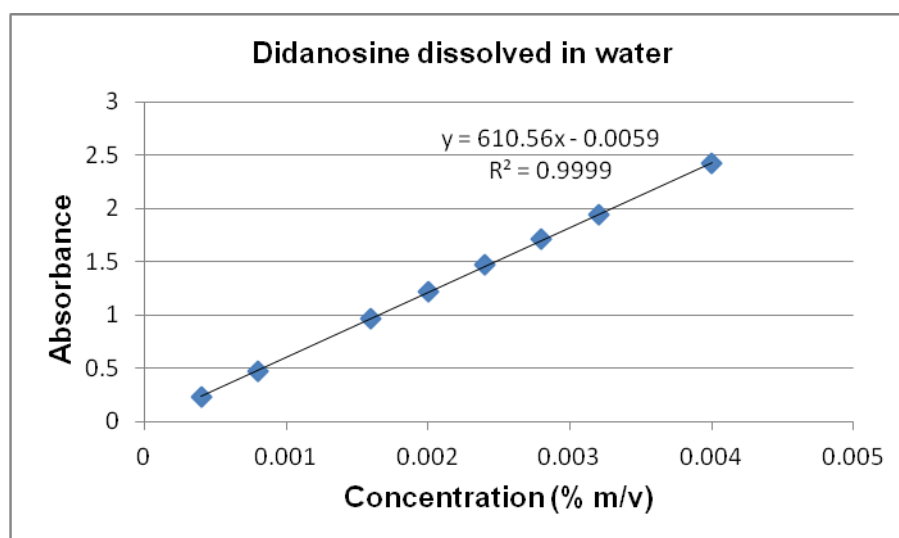
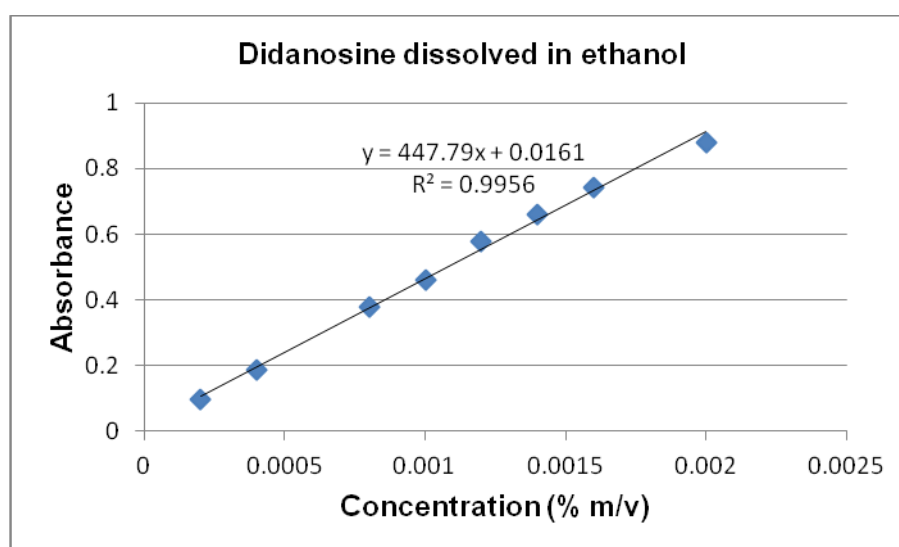


Figure S27: PXRD analysis of the differences in unit cell dimensions between didanosine raw material and its recrystallisation products from methanol/water mixtures.

Water



Ethanol



0.1 N HCl

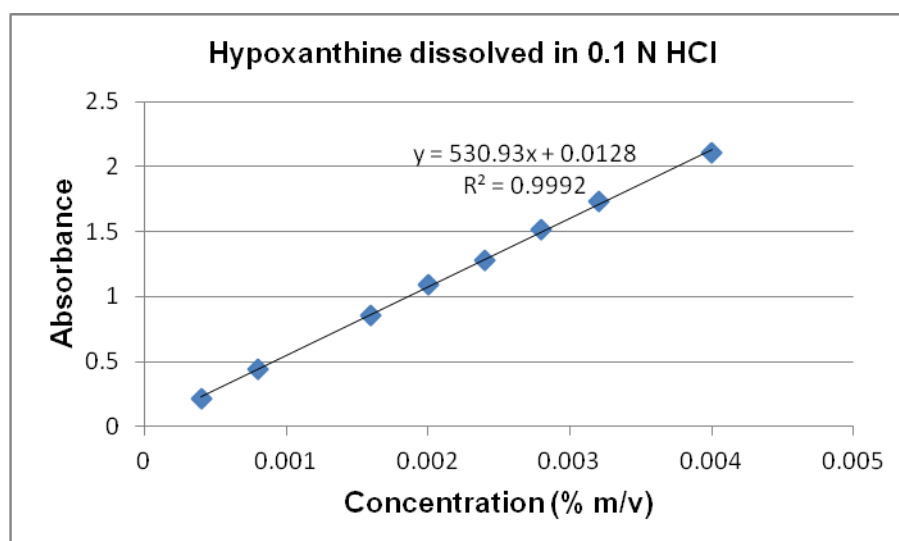


Figure S28: Standard curves for didanosine and hypoxanthine.

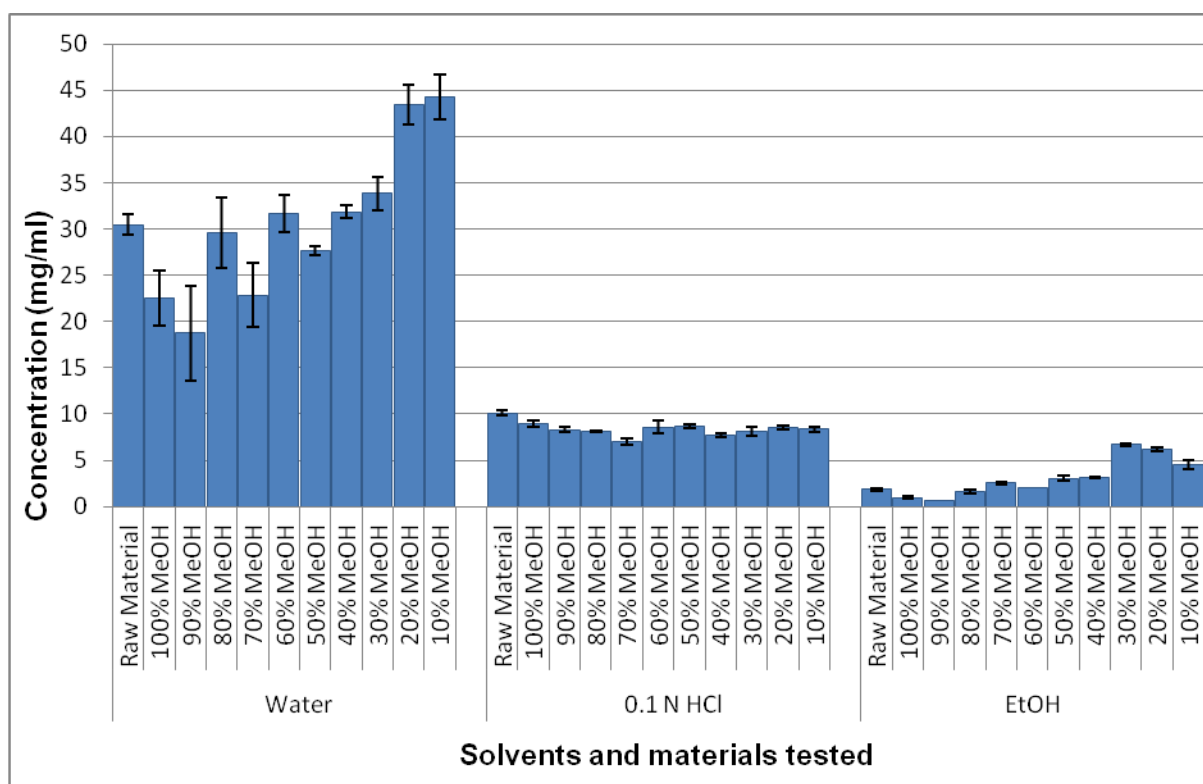
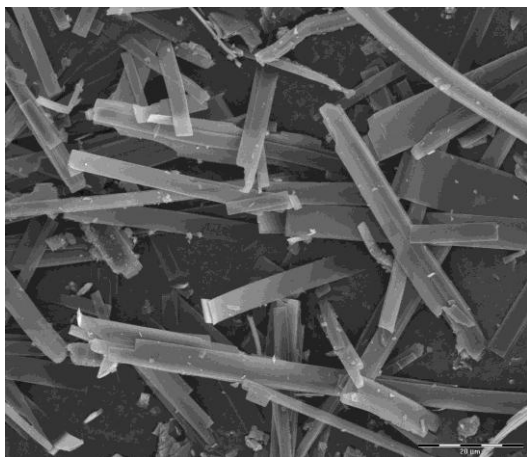
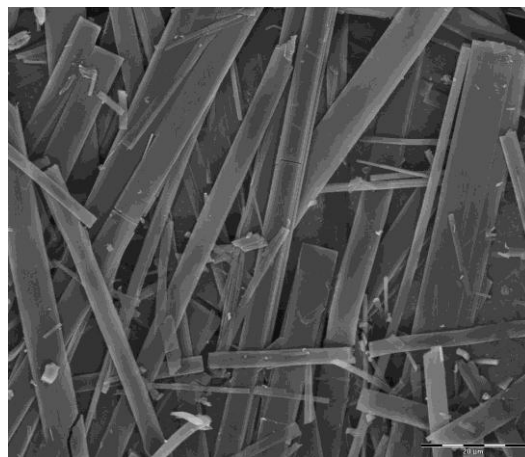


Figure S29: Solubility of didanosine raw material and its recrystallisation products from methanol and water mixtures.

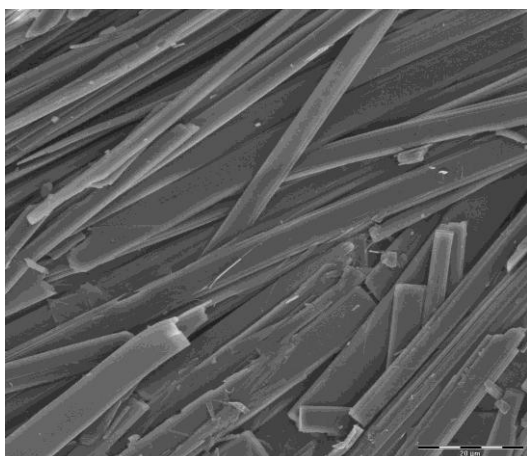
100 % methanol



90 % methanol



80 % methanol



70 % methanol

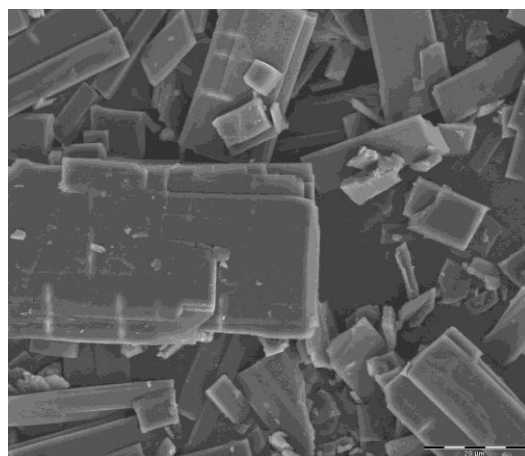
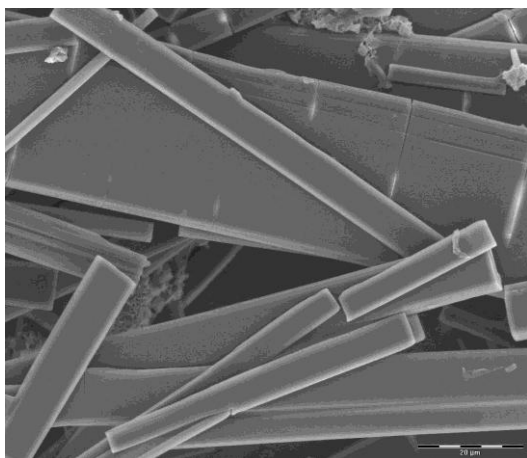
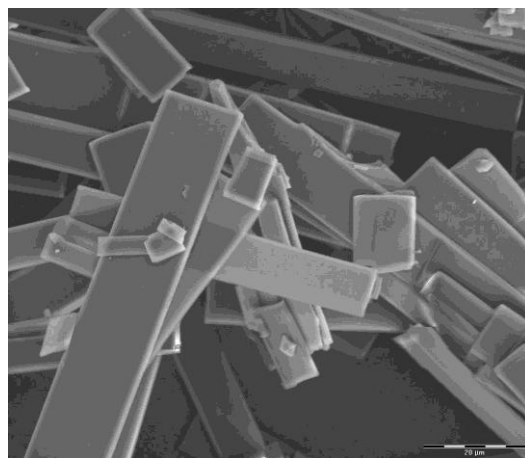


Figure S30: SEM micrographs of didanosine crystals from methanol and water mixtures.

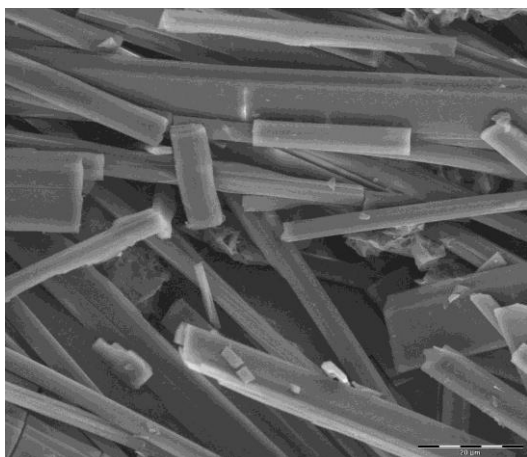
60 % methanol



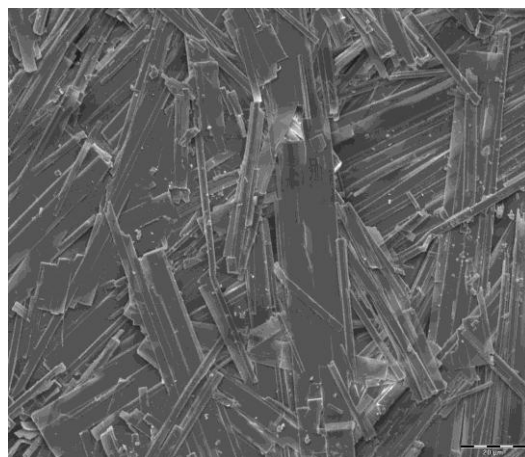
50 % methanol



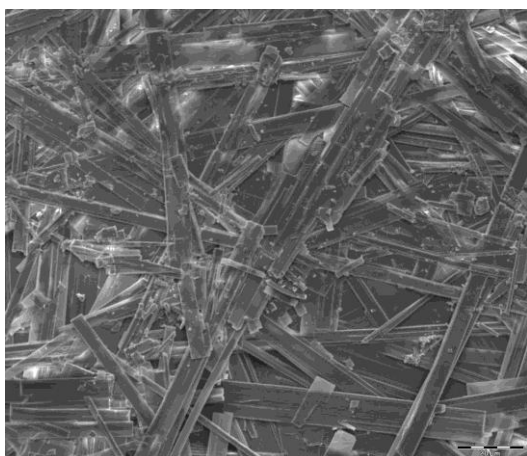
40 % methanol



30 % methanol



20 % methanol



10 % methanol

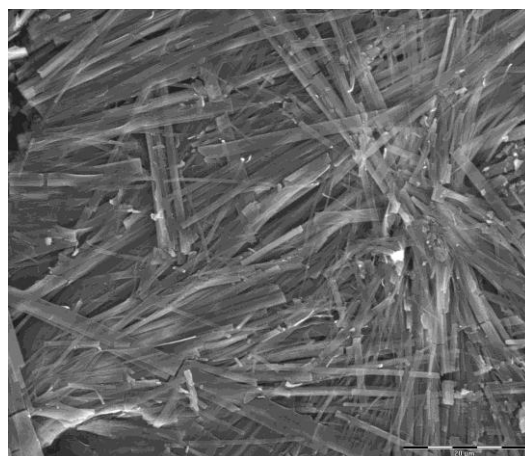


Figure S31: SEM micrographs of didanosine crystals from methanol and water mixtures.