Crystal Engineering of Nutraceutical Phytosterols: New Cocrystal Solid-Solution

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Electronic Supplementary Information

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1. Experimental: materials and methods

1.1 Materials

β-sitosterol (SIT CSS) used in this study was used as received from Sigma-Aldrich with purity of 79.3% (batch BCBS0067V). According to its certificate of origin it was purificated/extracted from non-biological natural inorganic/organic source.

Standard sample of β-sitosterol from Ph. Eu. Reference Standard, ref. (Y0001615) (72.5%) has been used to HPLC phytosterols determination and it was purchased from Sigma-Aldrich.

The following coformers have been used and an additional two coformers have been considered after the qualitative solubility experimental results (acetic acid and formic acid): acetic acid, formic acid, propionic acid, malic acid, succinic acid, fumaric acid, citric acid, lactic acid, pyruvic acid (zymonic acid), glutamic acid, glutamine, 3-hydroxybenzoic acid, 4-hydroxybenzoic acid, 2,4-dihydroxybenzoic acid, 3,4-dihydroxybenzoic acid, 2,5-dihydroxybenzoic acid, 2,4-dihydroxybenzoic acid, 3,5-dihydroxybenzoic acid, gallic acid, 2-picolinic acid, nicotinic acid, L-ascorbic acid, orcinol, resorcinol, hydroquinone and phloroglucinol.
The coformers where purchased from Sigma-Aldrich except propionic acid, which was purchased from Reidel-de Haën.

1.2. Solubility qualitative determination of β-sitosterol solid solution (SIT CSS)

The solvents selected to be used in the cocrystal screening are highlighted in black.
SIT CSS was dissolved in 30 solvents in a temperature range of 25-90 ºC. SIT CSS is soluble at 25 ºC in the following solvents: **IPA** (0.6 mL), **butanol** (0.2 mL), **MEK** (0.4 mL), **acetone** (1.6 mL), MiBK (0.4 mL), **cyclohexane** (1.0 mL), **toluene** (0.3 mL), xylene (0.4 mL), **AcOEt** (0.4 mL), diethyl ether (0.3 mL), **THF** (0.2 mL), dimethyl ethylene glycol (0.3 mL), **diisopropyl ether** (0.2 mL), **dioxane** (0.2 mL), **dichloromethane** (0.2 mL), chloroform (0.2 mL), **acetic acid** (1.3 mL), **benzyl alcohol** (0.4 mL) and diethylamine (0.2 mL). At 50 ºC it is soluble in **ethanol** (2.0 mL) and heptane. At 70 ºC it is partially soluble in **methanol** (2.0 mL). At 80 ºC it is soluble in **ACN** (2.0 mL). At 90 ºC it is soluble in DMSO (2.0 mL) and partially soluble in DMF (2.0 mL). It is insoluble in **ethylene glycol**, **formic acid**, water, pentane and NH_3 (32%) in water.

1.3 Solid forms Screening

Screening through net grinding (with liquid coformers) and liquid assisted grinding experiments (LAG) was conducted by grinding. Thus, 20-35 mg of a 1:1 mixture of SIT CSS and each coformer together with one drop (volume estimated 25 μL) of each solvent were grinded using a Retsch MM 2000 grinding mill. The samples were placed in 2 mL volume stainless steel jars, along with two stainless tungsten grinding balls of 3 mm diameter. Grinding was performed for 15-30 minutes, with a frequency of the mill of 30 Hz. Finally, the samples were collected immediately without prior drying for PXRD analysis. The formation of a new solid form was determined by comparing PXRD patterns of starting materials (any known crystal form of both SIT and the respective coformer) and products from cocrystal screening LAG experiments. When evidences of new forms were detected reaction crystallization (RC) experiments were conducted in order to

prepare bigger amounts of material for further characterization by preparing a saturated solution of the most soluble component (SIT CSS or coformer) in a particular solvent in a sealed vial under stirring. A small quantity of the less soluble component was added until it did not dissolve anymore. The suspension was stirred at different times and the resulting solids were filtered and analyzed by PXRD. Screening through solvent mediated transformations (SMT) were conducted by preparing suspensions of SIT and coformer in different molar ratios (40-1200 mg of the final mixture) in selected solvents. The sealed vials were stirred for different times and the resulting solids were filtered and analyzed by PXRD. A summary of the experimental procedures can be found in Table S1.

Table S1. Cocrystal screening of β-Sitosterol CSS

Methodology	Coformers	Nº Experiments	Nº Solids	Positive results[a]	Coformers	Form obtained (according to PXRD)
Solubility Study	-	34	29	2	-	Anhydrous form (LOFFET), monohydrate (TEXQOC) and three solvates: IPA, DMF DMSO, acetic acid and benzyl alcohol (CSS SIT-BzOH-H_2O)
Net grinding at 25 ºC	5	5	5	1 / 2	3 / 2	CSS SIT-ProA-H_2O (I and II) and CSS SIT-ZA-H_2O
Liquid assisted grinding at 25 ºC	22	84	80	1	15	15 new evidences
Reaction Crystallization at 25 ºC	3	84	81	1 / 2	11 / 2	CSS SIT-GA and CSS SIT-3,4-DHBA-H_2O
Solvent mediated transformation at 25 ºC	2	63	32	1/2	5/4	CSS SIT-ProA-H_2O II, CSS SIT-ZA-H_2O and CSS SIT-4-HBA (I and II)
Crystallizations at slow cooling rate	5	50	44	3	2	Hemihydrate (JOPMUP01), CSS SIT-BzOH-H_2O, CSS SIT-ProA-H_2O (I and II), CSS SIT-GA and CSS SIT-ZA-H_2O
Solvent atmosphere	1	1	1	2	1	CSS SIT-ProA-ACN
Preparation of the solid forms: scale up batches	5	100	98	2	5	CSS SIT-BzOH-H_2O CSS SIT-ProA-H_2O (I and II), CSS SIT-ZA-H_2O CSS SIT-GA CSS SIT-4-HBA (I and II) CSS SIT-3,4-DHBA-H_2O

[a] (1) positive: SIT + coformer + new peaks observed in PXRD, (2) positive: cocrystal, (3) single crystal

1.4 Single X-ray crystallographic analysis

Single crystal X-ray diffraction (SCXRD) intensity data of the different crystal forms of β-sitosterol were collected using a D8 Venture system equipped with a multilayer monochromator and a Mo microfocus (λ = 0.71073 Å). Frames were integrated with the Bruker SAINT software package using a SAINT algorithm. Data were corrected for absorption effects using the multi-scan method (SADABS).[1] The structure was solved and refined using the Bruker SHELXTL Software Package, a computer program for automatic solution of crystal structures and refined by full-matrix least-squares method with ShelXle Version 4.8.0, a Qt graphical user interface for SHELXL computer program.[2]

1.5 Powder X-ray Diffraction Analysis

Powder X-ray diffraction (PXRD) patterns were obtained on a PANalytical X'Pert PRO MPD diffractometer in transmission configuration using Cu Kα1+2 radiation (λ = 1.5406 Å) with a focusing elliptic mirror and a PIXcel detector working at a maximum detector's active length of 3.347º. Configuration of convergent beam with a focalizing mirror and a transmission geometry with flat sample sandwiched between low absorbing films measuring from 2 to 40º in 2θ, with a step size of 0.026º and a total measuring time of 30 minutes to 2 hours at room temperature (298 K).

1.6 Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry analysis were carried out by means of a Mettler-Toledo DSC-822e calorimeter. Experimental conditions: aluminium crucibles of 40 μL volume, atmosphere of dry nitrogen with 50 mL/min flow rate, heating rate of 10 ºC/min. The calorimeter was calibrated with indium of 99.99% purity (mp: 156.4 °C; ΔH: 28.95 J/g).

1.7 Thermogravimetric Analysis (TGA)

Thermogravimetric analyses were performed on a Mettler-Toledo TGA-851e thermobalance. Experimental conditions: alumina crucibles of 70 μL volume, atmosphere of dry nitrogen with 50 mL/min flow rate, heating rate of 10 ºC/min.

1.8 Nuclear Magnetic Resonance (NMR)

Proton nuclear magnetic resonance (1H-NMR) spectra has been recorded on a Varian Mercury 400 (400 MHz). Chemical shifts for proton are reported in parts per million (ppm) downfield from tetramethylsilane and are referenced to residual proton in the NMR solvents (dmso-d6: δ 2.50; chloroform-d: δ 7.26). Experimental conditions: delay: 1; pulse: 45°; scans: 32.

2. Crystal data and structure refinement

2.1 Benzyl alcohol solvate hydrate (CSS SIT-BzOH-H_2O) (4:1:1) (CCDC: 2000520)

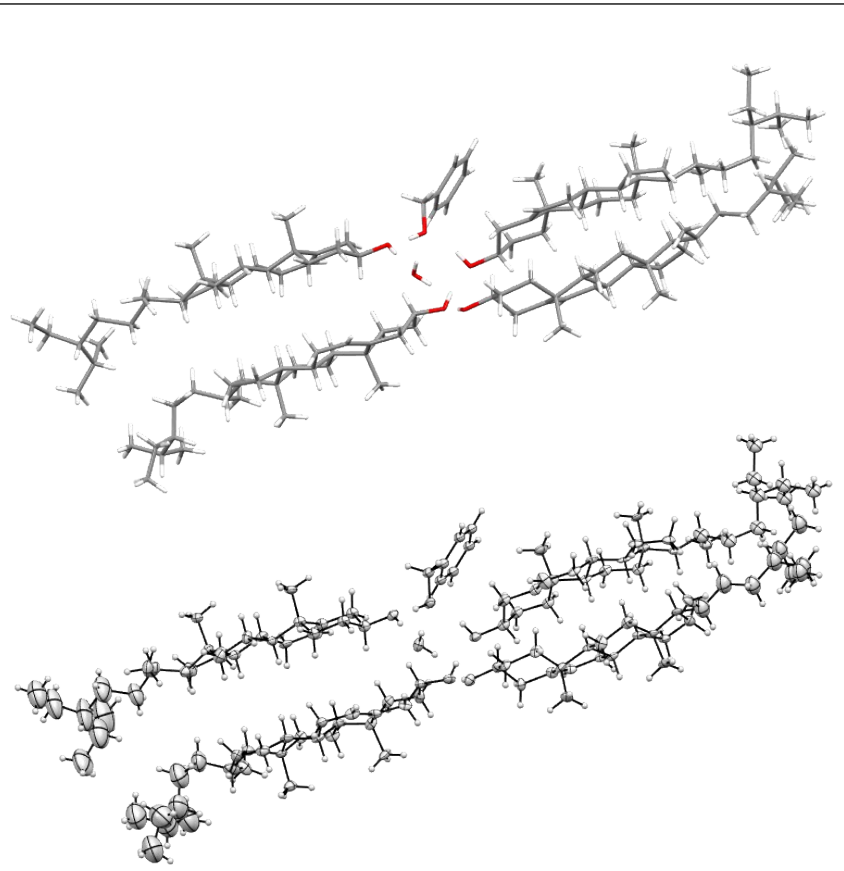
Structure	CSS SIT-BzOH-H_2O	Asymmetric Unit representation
Empirical formula	$C_{123}H_{210}O_6$	
Formula Weight	1784.90	
Temperature (K)	100(2)	
Wavelength (Å)	0.71073	
Crystal system	Triclinic	
space group	P 1	
a, b, c (Å)	7.597(3), 9.730(4), 37.763(14)	
α, β, γ (°)	84.846(9), 86.089(8), 88.219(9)	
Volume (Å^3)	2772.8(19)	
Z, Density (calc.) (Mg/m^3)	1, 1.069	
Absorption coefficient (mm^{-1})	0.063	
F(000)	996	
Crystal size (mm^3)	0.2 x 0.1 x 0.1	
θ range for data collection (°)	2.171 to 26.879	
Limiting indices	-9<=h<=9, -12<=k<=12, -46<=l<=46	
Reflections collected / unique	43250/ 20328 [R(int)= 0.0741]	
Completeness to theta = 25.242°	98.4	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.7453 and 0.5118	
Refinement method	Full-matrix least-squares on F^2	
Data/restrains/parameters	20328 / 34 / 992	
Goodness-of - fit on F^2	0.947	
Final R indices [I > 2σ(I)]	R1 = 0.0461, wR2 = 0.0903	
R indices (all data)	R1 = 0.0729, wR2 = 0.0964	
Largest diff. peak and hole (e.Å^{-3})	0.613 and -0.548	

Table S2. Hydrogen bonds for CSS SIT-BzOH-H_2O [Å and °]

Donor --- H....Acceptor	[ARU]	d(D– H)	d(H...A)	d(D...A)	<(D - H...A)
O1A ---H1AO ...O1C	[ ]	0.84	1.86	2.700(3)	175
O1 ---H1O ...O1D	[ ]	0.84	1.89	2.682(3)	158
O1B ---H1BO ...O1	[ ]	0.84	1.85	2.670(3)	165
O1C ---H1CO ...O1B	[ ]	0.84	1.96	2.774(3)	162
O1D ---H1DO ...O1W	[ ]	0.84	1.85	2.678(3)	166
O1W ---H1WA ...O1A	[ ]	0.85(3)	2.07(3)	2.894(3)	162(3)
O1W ---H1WB ...O1B	[1+x,y,z]	0.83(3)	2.07(3)	2.863(3)	160(3)

2.2 Propionic acid cocrystal hydrate (CSS SIT-ProA-H_2O I) (2:1:1) (CCDC: 2000521)

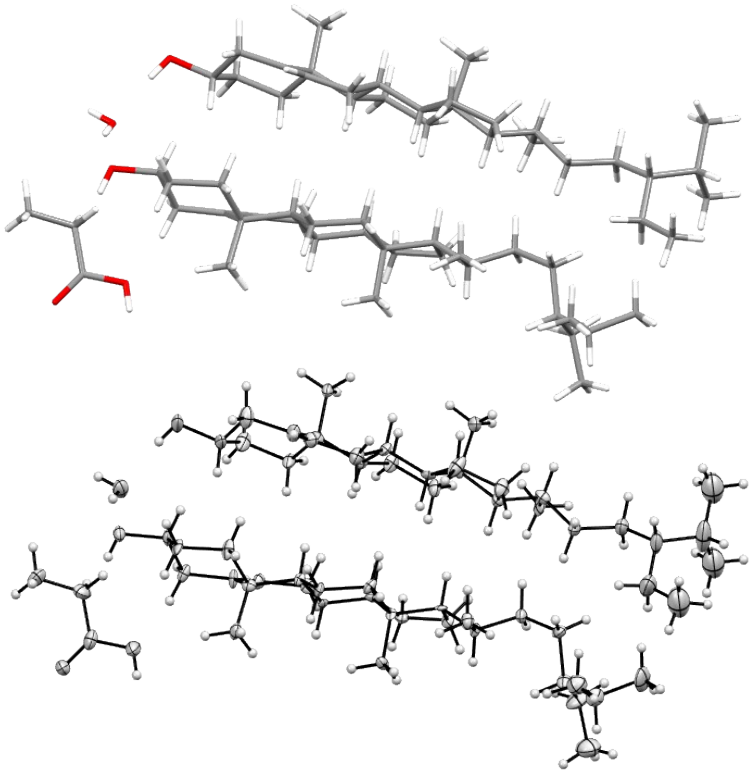
Structure	CSS SIT-ProA-H_2O I	Asymmetric Unit representation
Empirical formula	$C_{61}H_{108}O_5$	
Formula Weight	921.47	
Temperature (K)	293(2)	
Wavelength (Å)	0.71073	
Crystal system	Monoclinic	
space group	P 21	
a, b, c (Å)	9.439(2), 7.5391(16), 39.635(8)	
α, β, γ (°)	90, 95.216(6), 90	
Volume ($Å^3$)	2808.8(10)	
Z, Density (calc.) (Mg/m^3)	2, 1.090	
Absorption coefficient (mm^{-1})	0.066	
F(000)	1028	
Crystal size (mm^3)	0.353 x 0.071 x 0.032	
θ range for data collection (°)	2.064 to 27.656	
Limiting indices	-11<=h<=11, -9<=k<=9, -49<=l<=49	
Reflections collected / unique	53621/ 12975 [R(int)= 0.4570]	
Completeness to theta = 25.242°	99.9	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.7456 and 0.6443	
Refinement method	Full-matrix least-squares on F^2	
Data/restrains/parameters	12975 / 9 / 599	
Goodness-of - fit on F^2	0.743	
Final R indices [I > 2σ(I)]	R1 = 0.0446, wR2 = 0.0582	
R indices (all data)	R1 = 0.3086, wR2 = 0.0920	
Largest diff. peak and hole ($e.Å^{-3}$)	0.339 and -0.328	

Table S3. Hydrogen bonds for CSS SIT-ProA-H_2O I [Å and °]

Donor --- H....Acceptor	[ARU]	d(D – H)	d(H...A)	d(D...A)	<(D - H...A)
O1A---H1AA...O1W	[1-x,1/2+y,-z]	0.80(5)	1.94(5)	2.738(5)	174(5)
O1---H1O...O3W	[x,y,z]	0.82	1.85	2.653(6)	164
O3W---H3WD...O1A	[x,-1+y,z]	0.82(4)	2.04(4)	2.805(6)	156(5)
O3W---H3WE...O1A	[-x,-1/2+y,-z]	0.84(4)	1.93(4)	2.750(6)	166(4)
O2W---H20...O1	[1+x,y,z]	0.82	1.81	2.630(5)	172

2.3 Propionic acid cocrystal hydrate (CSS SIT-ProA-H_2O II) (4:1:1) (CCDC: 2000523)

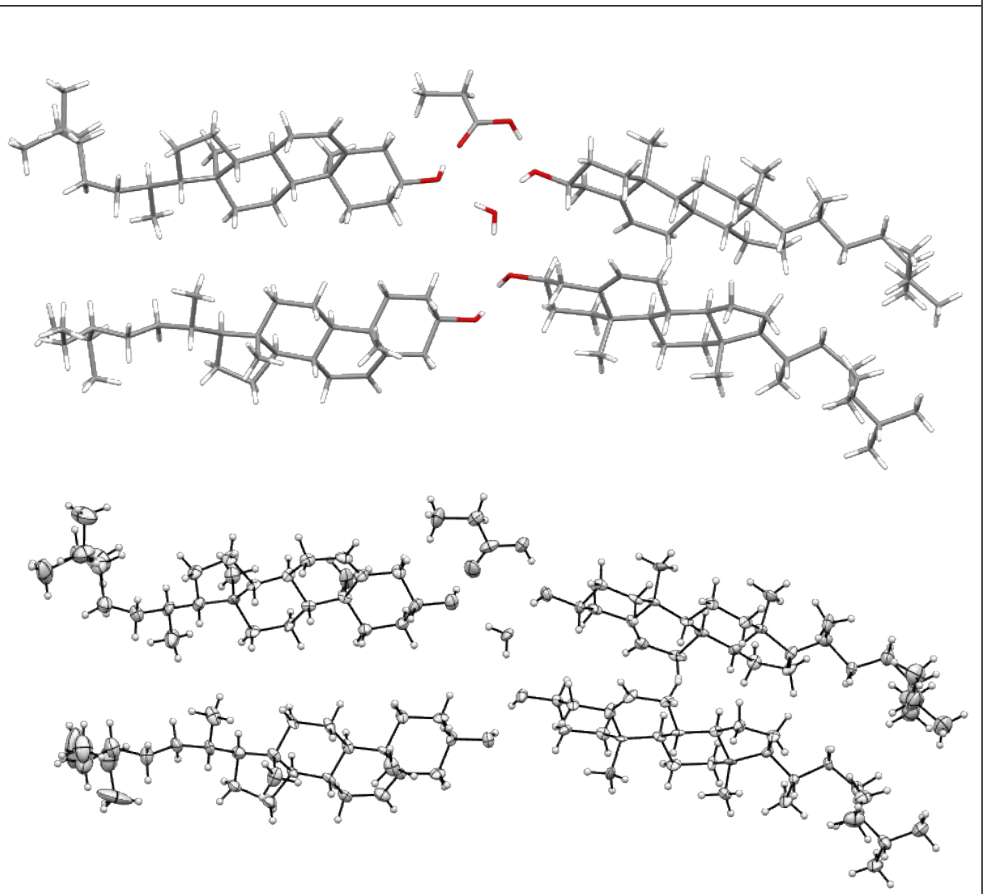
Structure	CSS SIT-ProA-H_2O II	Asymmetric Unit representation
Empirical formula	$C_{119}H_{208}O_7$	
Formula Weight	1750.84	
Temperature (K)	100(2)	
Wavelength (Å)	0.71073	
Crystal system	Monoclinic	
space group	P 21	
a, b, c (Å)	26.373(3), 7.4971(7), 27.183(3)	
α, β, γ (°)	90, 92.569(6), 90	
Volume ($Å^3$)	5369.2(10)	
Z, Density (calc.) (Mg/m^3)	2, 1.083	
Absorption coefficient (mm^{-1})	0.064	
F(000)	1956	
Crystal size (mm^3)	0.317 x 0.087 x 0.032	
θ range for data collection (°)	2.202 to 26.431	
Limiting indices	-32<=h<=32, -9<=k<=9, -33<=l<=33	
Reflections collected / unique	84425/ 21902 [R(int)= 0.5074]	
Completeness to theta = 25.242°	99.8	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.7454 and 0.6268	
Refinement method	Full-matrix least-squares on F^2	
Data/restrains/parameters	21902 / 4 / 1166	
Goodness-of - fit on F^2	0.950	
Final R indices [I > 2σ(I)]	R1 = 0.1012, wR2 = 0.1599	
R indices (all data)	R1 = 0.3448, wR2 = 0.2356	
Largest diff. peak and hole ($e.Å^{-3}$)	0.394 and -0.327	

Table S4. Hydrogen bonds for CSS SIT-ProA-H_2O II [Å and °]

Donor --- H....Acceptor	[ARU]	d(D – H)	d(H...A)	d(D...A)	<(D - H...A)
O1A---H1AO...O3W	[x,y,z]	0.84	1.97	2.793(10)	165
O1C---H1C...O3W	[x,1+y,z]	0.84	2.26	2.870(10)	129
O1W---H1W...O1A	[x,y,z]	0.84	2.37	2.665(10)	101
O1B---H1BO...O1C	[x,y,z]	0.84	1.93	2.741(9)	162
O1D---H1DO...O2W	[x,y,z]	0.84	1.86	2.693(11)	170
O3W---H3OA...O1B	[x,y,z]	0.80(7)	2.09(6)	2.806(9)	149(8)
O3W---H3OB...O1D	[x,y,z]	0.81(5)	1.91(5)	2.691(8)	162(7)

2.4 Propionic acid cocrystal ACN solvate (CSS SIT-ProA-ACN) (4:1:1) (CCDC: 2000525)

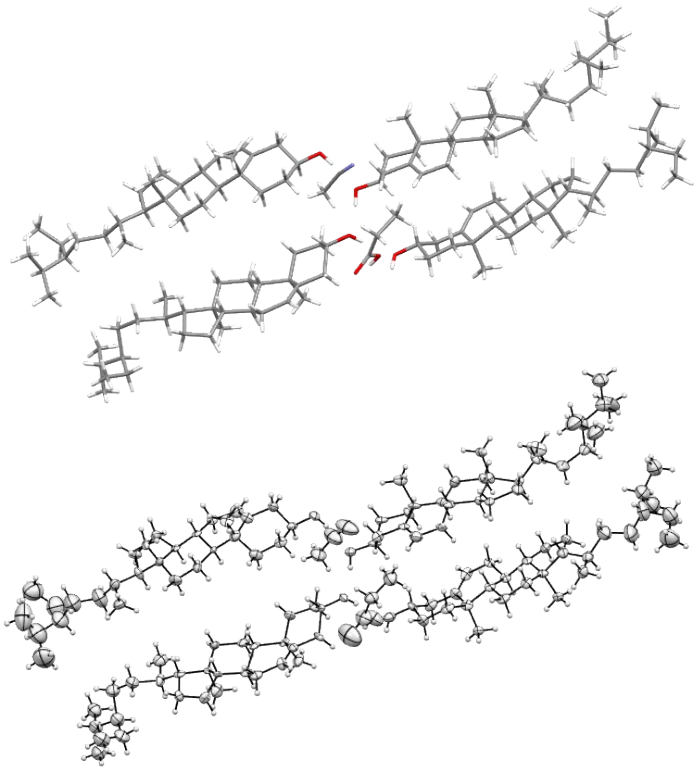
Structure	CSS SIT-ProA-ACN	Asymmetric Unit representation
Empirical formula	$C_{121}H_{209}NO_6$	
Formula Weight	1773.88	
Temperature (K)	100(2)	
Wavelength (Å)	0.71073	
Crystal system	Triclinic	
space group	P 1	
a, b, c (Å)	7.581(2), 9.702(3), 36.973(9)	
α, β, γ (°)	82.962(5), 86.112(5), 89.740(5)	
Volume ($Å^3$)	2692.7(13)	
Z, Density (calc.) (Mg/m^3)	1, 1.094	
Absorption coefficient (mm^{-1})	0.064	
F(000)	990	
Crystal size (mm^3)	0.278 x 0.160 x 0.096	
θ range for data collection (°)	2.225 to 26.458	
Limiting indices	-9<=h<=9, -12<=k<=12, -46<=l<=46	
Reflections collected / unique	32223/ 16659 [R(int)= 01519]	
Completeness to theta = 25.242°	91.9	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.7454 and 0.6155	
Refinement method	Full-matrix least-squares on F^2	
Data/restrains/parameters	16659 / 61 / 1145	
Goodness-of - fit on F^2	1.314	
Final R indices [I > 2σ(I)]	R1 = 0.1056, wR2 = 0.1872	
R indices (all data)	R1 = 0.1676, wR2 = 0.2302	
Largest diff. peak and hole ($e.Å^{-3}$)	0.637 and -0.588	

Table S5. Hydrogen bonds for CSS SIT-ProA-ACN [Å and °]

Donor --- H....Acceptor	[ARU]	d(D – H)	d(H...A)	d(D...A)	<(D - H...A)
O1A---H1AO...O1C	[x,y,z]	0.82	1.98	2.777(9)	162
O2---H2O...O1B	[x,1+y,z]	0.82	1.87	2.565(12)	143
O1B---H1BO...O1D	[x,y,z]	0.82	2.01	2.758(10)	151
O1C---H1OC...N1W	[x,1+y,z]	0.82	2.56	2.879(16)	105
O1D---H1DO...O1A	[x,y,z]	0.82	2.23	2.853(10)	133

2.5 Zymonic acid cocrystal hydrate (CSS SIT-ZA-H_2O) (2:1:1) (CCDC: 2000526)

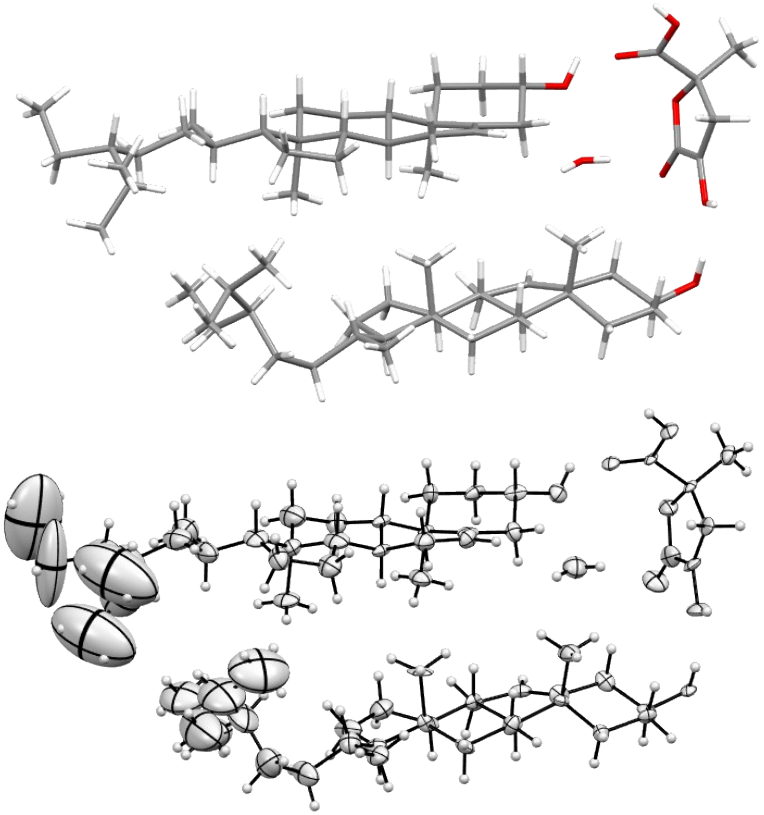
Structure	CSS SIT-ZA-H_2O	Asymmetric Unit representation
Empirical formula	C64 H109 O8	
Formula Weight	1006.51	
Temperature (K)	100(2)	
Wavelength (Å)	0.71073	
Crystal system	Monoclinic	
space group	C 2	
a, b, c (Å)	77.42(2), 7.6086(18), 9.924(3)	
α, β, γ (°)	90, 90.948(7), 90	
Volume ($Å^3$)	5845(3)	
Z, Density (calc.) (Mg/m^3)	4, 1.144	
Absorption coefficient (mm^{-1})	0.073	
F(000)	2228	
Crystal size (mm^3)	0.2 x 0.1 x 0.1	
θ range for data collection (°)	2.127 to 22.238	
Limiting indices	-82<=h<=82, -8<=k<=8, -10<=l<=10	
Reflections collected / unique	38307/ 7367 [R(int)=0.3422]	
Completeness to theta = 25.242°	99.6	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.7447 and 0.4684	
Refinement method	Full-matrix least-squares on F^2	
Data/restrains/parameters	7367 / 41 / 638	
Goodness-of - fit on F^2	0.882	
Final R indices [I > 2σ(I)]	R1 = 0.0602, wR2 = 0.0934	
R indices (all data)	R1 = 0.1580, wR2 = 0.1155	
Largest diff. peak and hole ($e.Å^{-3}$)	0.268 and -0.227	

Table S6. Hydrogen bonds for CSS SIT-ZA-H_2O [Å and °]

Donor --- H....Acceptor	[ARU]	d(D – H)	d(H...A)	d(D...A)	<(D - H...A)
O1A---H1OA...O1	[x,y,z]	0.82	2.43	2.768(7)	105
O2---H2...O1B	[x,y,1+z]	0.82	1.75	2.558(7)	169
O4---H4...O1W	[x,1+y,z]	0.82	1.69	2.514(9)	177
O1B---H1OB...O4	[x,y,z]	0.82	2.08	2.888(7)	168
O1W---H1WA... ?		0.80(3)			
O1W---H1WB...O5	[x,y,z]	0.80(5)	1.89(5)	2.672(9)	164(9)

2.6 Gallic acid cocrystal (CSS SIT-GA) (4:1) (CCDC: 2000527)

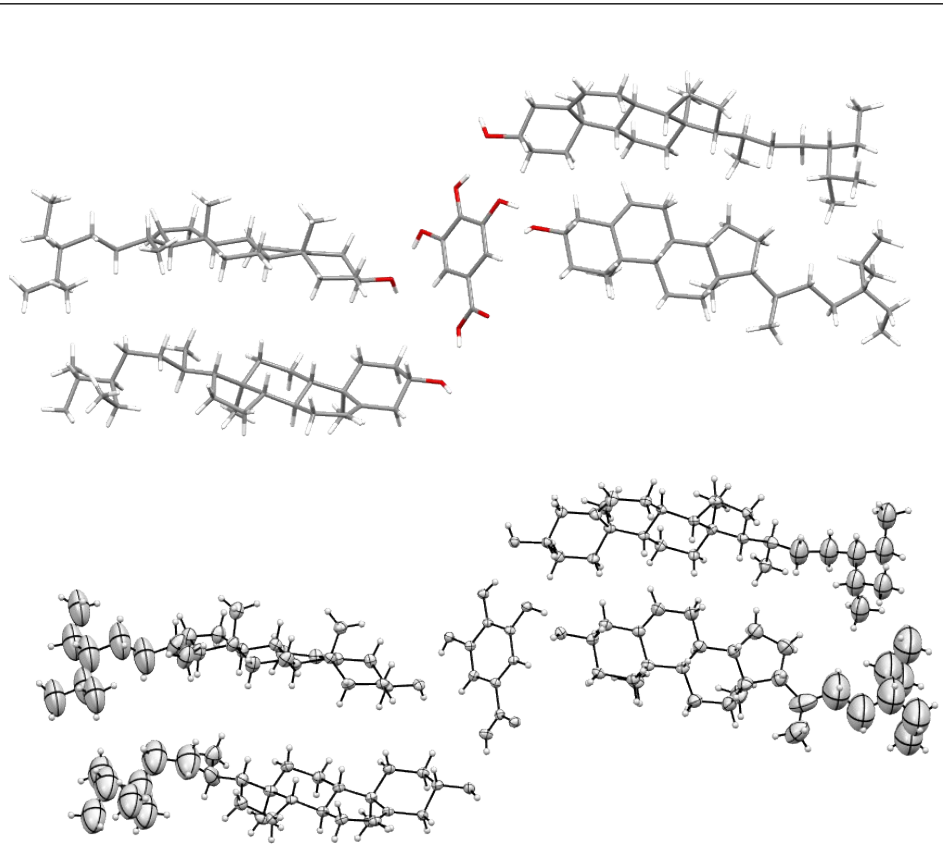
Structure	CSS SIT-GA	Asymmetric Unit representation
Empirical formula	$C_{123}H_{206}O_9$	
Formula Weight	1828.87	
Temperature (K)	100(2)	
Wavelength (Å)	0.71073	
Crystal system	Monoclinic	
space group	P 21	
a, b, c (Å)	10.7439(5), 13.6989(6), 38.1228(19)	
α, β, γ (°)	90, 93.002(2), 90	
Volume (Å^3)	5603.2(5)	
Z, Density (calc.) (Mg/m^3)	2, 1.084	
Absorption coefficient (mm^{-1})	0.066	
F(000)	2032	
Crystal size (mm^3)	0.2 x 0.1 x 0.1	
θ range for data collection (°)	2.140 to 27.136	
Limiting indices	-13<=h<=13, -17<=k<=17, -48<=l<=48	
Reflections collected / unique	99282/ 24652 [R(int) = 0.0712]	
Completeness to theta = 25.242°	99.8	
Absorption correction	Empirical	
Max. and min. transmission	0.5 and 0.5	
Refinement method	Full-matrix least-squares on F^2	
Data/restrains/parameters	24652 / 66 / 1042	
Goodness-of - fit on F^2	1.053	
Final R indices [I > 2σ(I)]	R1 = 0.0897, wR2 = 0.2458	
R indices (all data)	R1 = 0.1117, wR2 = 0.2775	
Largest diff. peak and hole (e.Å^{-3})	1.939 and -0.1669	

Table S7. Hydrogen bonds for CSS SIT-GA [Å and °]

Donor --- H....Acceptor	[ARU]	d(D – H)	d(H...A)	d(D...A)	<(D - H...A)
O1A---H1AO...O3	[1-x,-1/2+y,1-z]	0.84	2.49	2.928(4)	113
O1A---H1AO...O4	[1-x,-1/2+y,1-z]	0.84	1.94	2.779(4)	174
O2---H2...O1C	[ ]	0.84	1.79	2.536(4)	147
O3---H3A...O1D	[ ]	0.84	1.79	2.613(5)	167
O4---H4O...O1B	[ ]	0.84	1.95	2.694(5)	147
O4---H4O...O5	[ ]	0.84	2.30	2.738(4)	113
O5---H5O...O1A	[ ]	0.84	2.05	2.688(4)	132
O1B---H1BO...O1	[1-x,1/2+y,1-z]	0.84	2.08	2.907(4)	170
O1C---H1CO...O3	[2-x,-1/2+y,1-z]	0.84	2.04	2.846(4)	161
O1D---H1DO...O1	[2-x,1/2+y,1-z]	0.84	1.87	2.670(4)	160

3. Synthesis of the different crystal forms

Details of synthesis and characterization of each form can be found at Table S2. β-sitosterol (SIT) used in this study was of reagent grade and used as received from Sigma-Aldrich with purity of 79.3% (according to Gas Chromatography Area %). Stoichiometry has been assessed based on NMR and TGA measurements when crystal structure is not available.

3.1 Benzyl alcohol solvate hydrate (CSS SIT-BzOH-H_2O) (4:1:1). It was obtained by slow evaporation in benzyl alcohol at 25 ºC, (82 days). Its PXRD diagram has been indexed.

3.2 Propionic acid cocrystal hydrate (CSS SIT-ProA-H_2O I) (2:1:1). It was obtained by antisolvent precipitation (water) through CSS SIT propionic acid solution at 25 ºC. Its PXRD diagram has been indexed.

3.3 Propionic acid cocrystal hydrate (CSS SIT-ProA-H_2O II) (4:1:1). **)**. It was obtained by solvent mediated transformation in CSS SIT-propionic acid in acetone at 25 ºC, (1 day). Its PXRD diagram has been indexed.

3.4 Propionic acid cocrystal ACN solvate (CSS SIT-ProA-ACN) (4:1:1). It was obtained by slow precipitation in ACN atmosphere at 25 ºC using CSS SIT-propionic acid acetone solution as raw material, (3 days).

3.5 Zymonic acid cocrystal hydrate (CSS SIT-ZA-H_2O) (2:1:1). It was obtained by slow evaporation in diethyl ether at 25 ºC, (25 days). Its PXRD diagram has been indexed.

3.6 Gallic acid cocrystal (CSS SIT-GA) (4:1). It was obtained by solvent mediated transformation in AcOEt, (m.p 194 ºC). Its PXRD diagram has been indexed.

3.7 4-Hydroxybenzoic acid cocrystal (CSS SIT-4-HBA I) (1:1). It was obtained by solvent mediated transformation in AcOEt, (m.p. 145 ºC). Its PXRD diagram has been indexed.

3.8 4-Hydroxybenzoic acid cocrystal (CSS SIT-4-HBA-H_2O II) (2:2:1). It was obtained by reaction crystallization in AcOEt, (m.p. 163 ºC). Its PXRD diagram has been indexed.

3.9 3,4-Dihydroxybenzoic acid cocrystal hydrate (CSS SIT-3,4-DHBA-H_2O) (1:2:1). It was obtained by reaction crystallization in AcOEt (m.p 157 ºC). Its PXRD diagram has been indexed.

Table S8. Synthesis of the different crystal forms

Crystal form CSS	SIT CSS (mg)	Coformer	Coformer (amount)	Solvents (mL)	T (ºC)	Cocrystal screening	Time (day)	Crystal form	molar ratio[a]
SIT-BzOH-H_2O	20	-	-	BzOH (0.4)	25	Slow evaporation	82	solvate	(4:1:1)
SIT-ProA-H_2O I	20		0.3 mL	Water (0.6)	25	Antisolvent precipitation	seconds	cc	(2:1:1)
SIT-ProA-H_2O II	50		0.5 mL	Acetone (0.5)	25	SMT	1	cc	(4:1:1)
SIT-ProA-ACN	50		0.25mL	Acetone (0.5)	25	ACN atmosphere	3	cc	(4:1:1)
SIT-ZA-H_2O	20	Pyruvic acid	0.1 mL	Et_2O (0.2)	25	Slow evaporation	25	cc	(2:1:1)
SIT-GA	625	Gallic acid	98 mg	AcOEt (5.0)	25	SMT	1	cc	(4:1)
SIT-4-HBA I	100	4-HBA	37 mg	AcOEt (0.3)	25	SMT	1	cc	(1:1)
SIT-4-HBA-H_2O II	2500	4-HBA	4000 mg	AcOEt (15.0)	25	RC, coformer saturated	1	cc	(2:2:1)
SIT-3,4-DHBA-H_2O	45	3,4-DHBA	50 mg	AcOEt (0.1)	25	RC, coformer saturated	1	cc	(1:2:1)

[a] Molar ratio according to SCXRD, ^{1}H-NMR and TGA analysis

4.- Analytical methodologies: determination of phytosterols content (%)

4.1 High-performance liquid chromatography (HPLC)

The tested samples were: β-sitosterol from Ph. Eu., CSS SIT-ProA-H_2O I, CSS SIT-ProA-H_2O II, CSS SIT-ZA-H_2O, CSS SIT-GA, CSS SIT-4-HBA I, CSS SIT-4-HBA-H_2O II and CSS SIT-3,4-DHBA-H_2O.

Sample preparation: 10 mg of the compound were dissolved in 10 mL of THF. Each sample was prepared three times.

Standard sample: β-sitosterol from Ph. Eu. Reference Standard, ref. (Y0001615) (72.5%, $C_{29}H_{50}O$).

Calibration curve: a stock solution of approximately 1500 mg/L β-sitosterol (10 mg/ 5 mL) in THF was prepared. Three more standards between 0.4 mg/L and 1100 mg/L by dilution in THF was prepared. Every standard was injected twice.

Equipment:

- Chromatograph: Waters Alliance 2695.
- Detector: Waters PDA 2996.
- Balance: Mettler Toledo AT261.
- Software: Empower, Waters.

Analytical conditions:

- Column: YMC-Pack Pro C18, 5 µm, 12 nm, 50 x 4.6 mm.
- Mobile phase: Methanol – Acetonitrile 20:80 (v/v).
- Flow rate: 1.0 mL/min
- Injection: 10 µL
- Detection (UV): 210 nm

4.2 High-performance liquid chromatography – high resolution mass (HPLC-HRMS)

The tested sample was CSS SIT-GA.

Equipment:

- Chromatograph: Accela (Thermo Fisher Scientific).
- Detector: Accela (PDA) + LTQ-Orbitrap Velos (HRMS).
- Software: Xcalibur (Thermo Fisher Scientific).

Analytical conditions:

- Column: YMC-Pack Pro C18, 5 µm, 12 nm, 50 x 4.6 mm.
- Mobile phase: Methanol – Acetonitrile 20:80 (v/v).
- Flow rate: 1.0 mL/min
- Injection: 10 µL
- Detection (UV): 210 nm
- Ion source (MS): APCI
- Polarity (MS): Positive

4.3 Gas chromatography-mass spectrometry (GS-MS)

Samples tested were β-sitosterol raw material (BCB50067V) and CSS SIT-GA.

Sample preparation: 1.01 mg of the compound were dissolved in 1 mL of DCM/Methanol (1:1).

Tested and standard samples derivatization: 100 µL of both solutions were totally dried. Then, 150 µL of N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) were added and the resulting solution was heated up to 150 ºC for 1 hour. The solution was cooled down at room temperature and it was totally dried under N_2 flow. Both solids were suspended in 1 mL of hexane. Finally, 1 µL of each solution were injected.

Equipment: Thermo Scientific trace CG Ultra connected to Thermo Scientific ITQ 900.

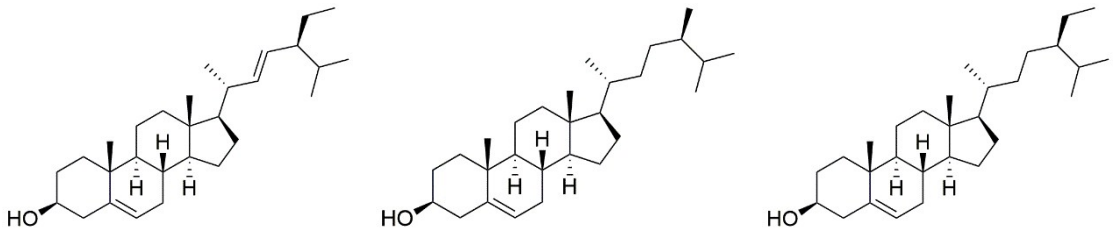
Analytical conditions:

- Column: Teknokroma Sapiens X5-MS 30 m x 0.25 mm d.i. x 0.25 um d.f.
- Injector:
    - Injector split/splitless
    - Injector mode: splitless
    - Splitless time: 1 min.
    - Injector temperature: 300 ºC
    - Gas: He (1 mL/min)
- Oven method:
    - Initial temperature: 40 ºC, Isothermic: 1 min
    - Step 1: Heating from 40 ºC to 180 ºC at a rate of 15 ºC/min.
    - Step 2: Heating from 180 ºC to 320 ºC at a rate of 6 ºC/min., Isothermic: 25 min.
- Detector:
    - Ion source temperature: 200 ºC
    - Interface temperature: 320 ºC
    - Solvent delay: 5 min.
    - Mass range: 50 to 900 uma.

4.4 Results:

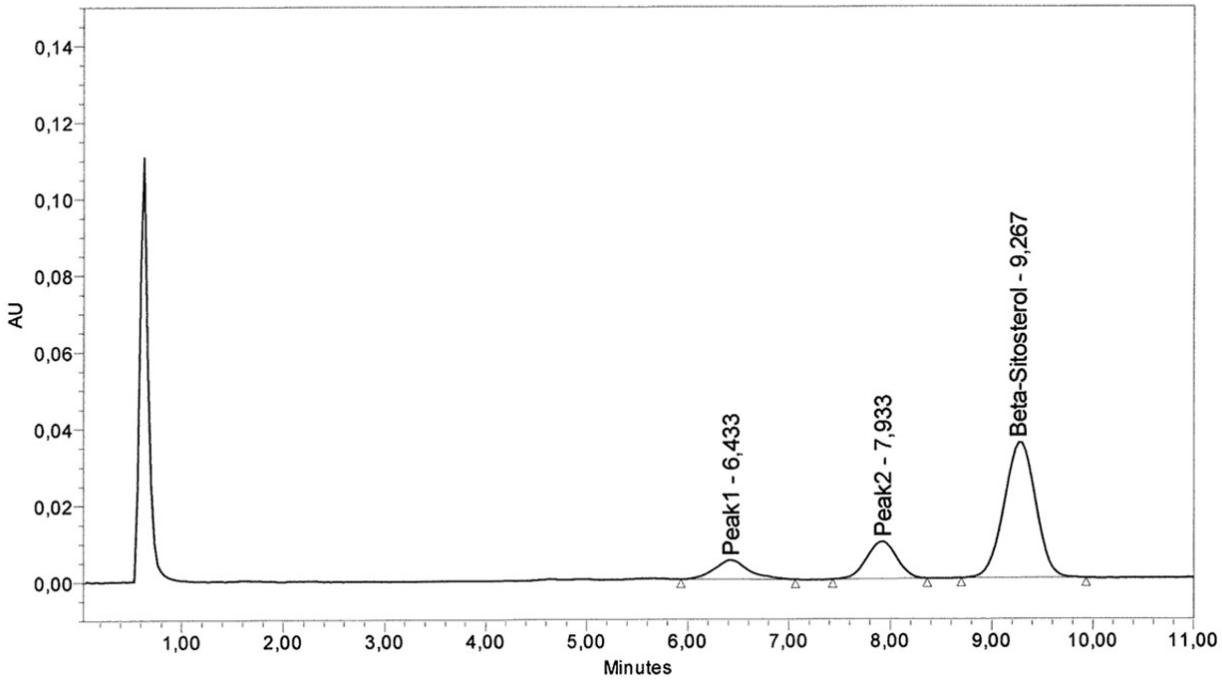
4.4.1 Results obtained by the HPLC analysis:

Three main peaks have been observed during the HPLC analysis of the standard β-sitosterol Ph. Eu (Figure S1) at 6.443, 7.933 and 9.267 min. They were assigned to Stigmasterol, Campesterol and β-Sitosterol respectively according to HRMS analysis of the CSS SIT-GA (Figures S2 to S5) having the following structures:



Stigmasterol	Campesterol	β-Sitosterol
$C_{29}H_{48}O$ M.W.: 412.70	$C_{28}H_{48}O$ M.W.: 400.69	$C_{29}H_{50}O$ M.W.: 414.72

Figure S1: Chromatogram of the HPLC analysis of β-Sitosterol standard. Run time (minutes) of the identified phytosterols: Stigmasterol (~ 6,4), Campesterol (~ 7,9) and β-Sitosterol (~ 9,3)



The molecular weight (without 1 molecule of water) of each compound were the following:

- Stigmasterol (peak 1): MS (ES) (+): 395.37 $[C_{29}H_{47}]^+$
- Campesterol (peak 2): MS (ES) (+): 383.37 $[C_{28}H_{47}]^+$
- β-Sitosterol (peak 3): MS (ES) (+): 397.38 $[C_{29}H_{49}]^+$

Figure S2: Chromatogram of the HPLC analysis of CCS SIT-GA

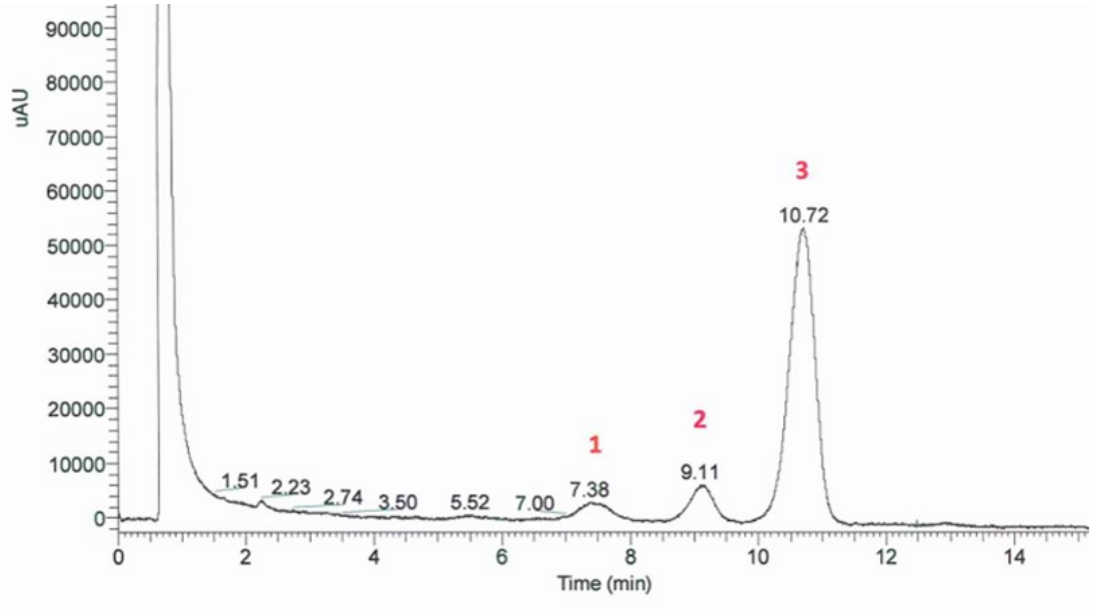


Figure S3: FTMS of peak 1 (Stigmasterol): MS (ES) (+): 395.37 $[C_{29}H_{47}]^+$

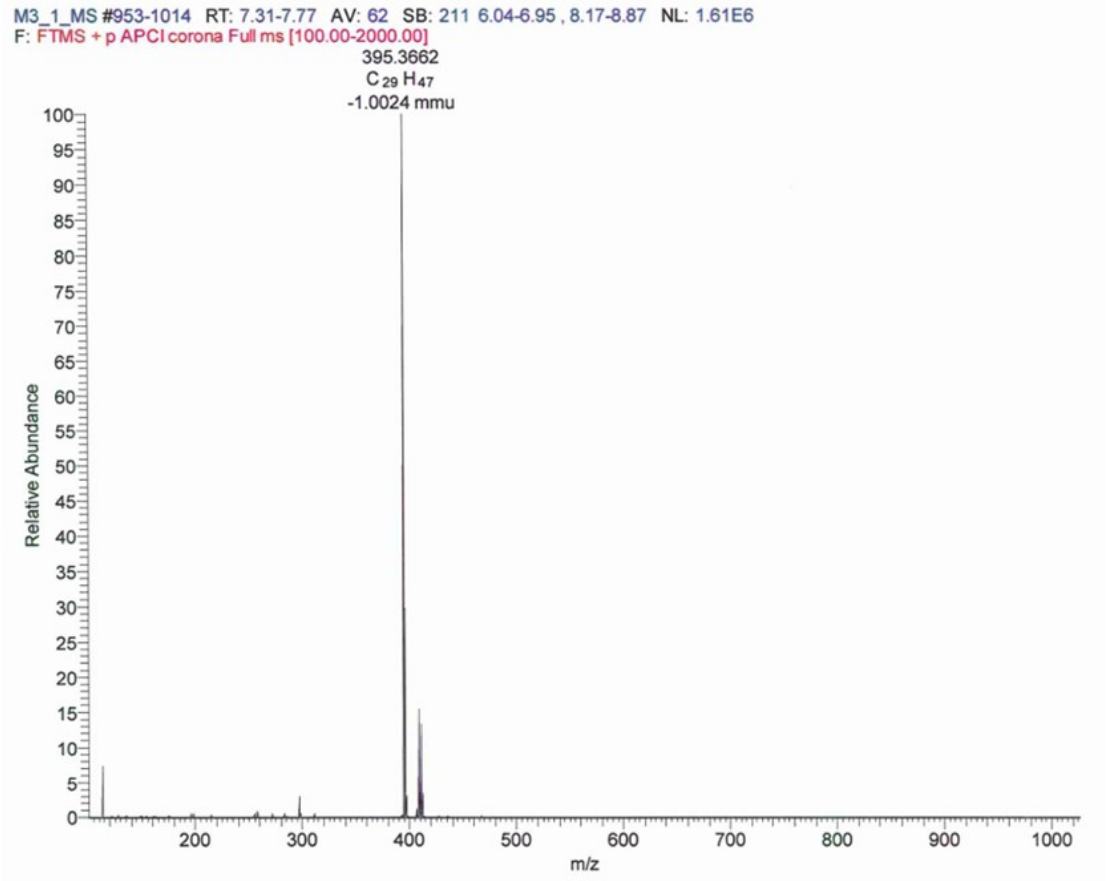


Figure S4: FTMS of peak 2 (Campesterol): MS (ES) (+): 383.37 $[C_{28}H_{47}]^+$

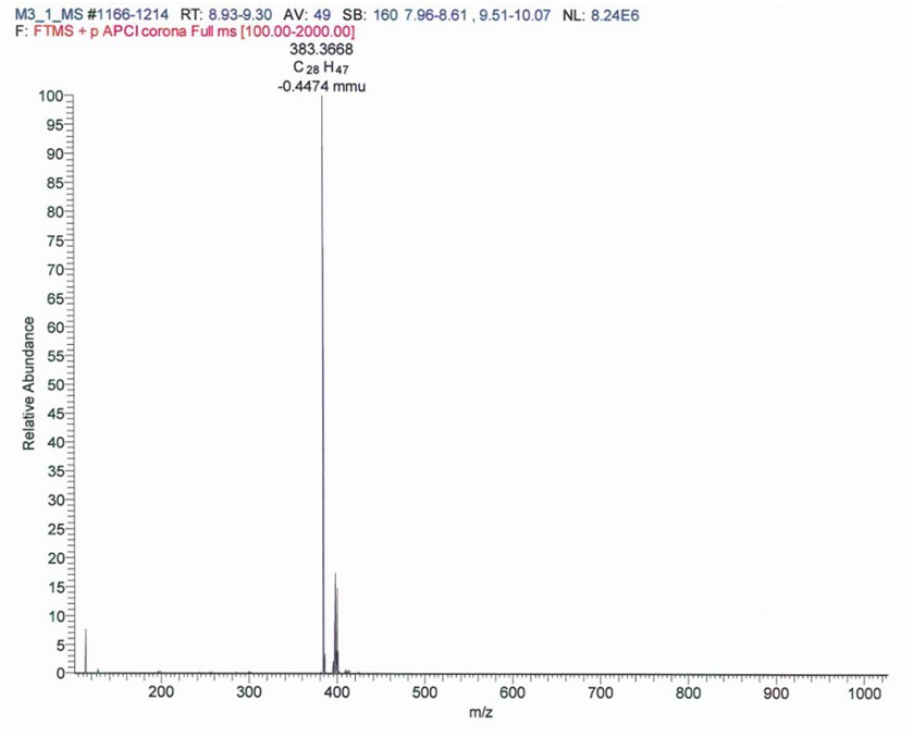
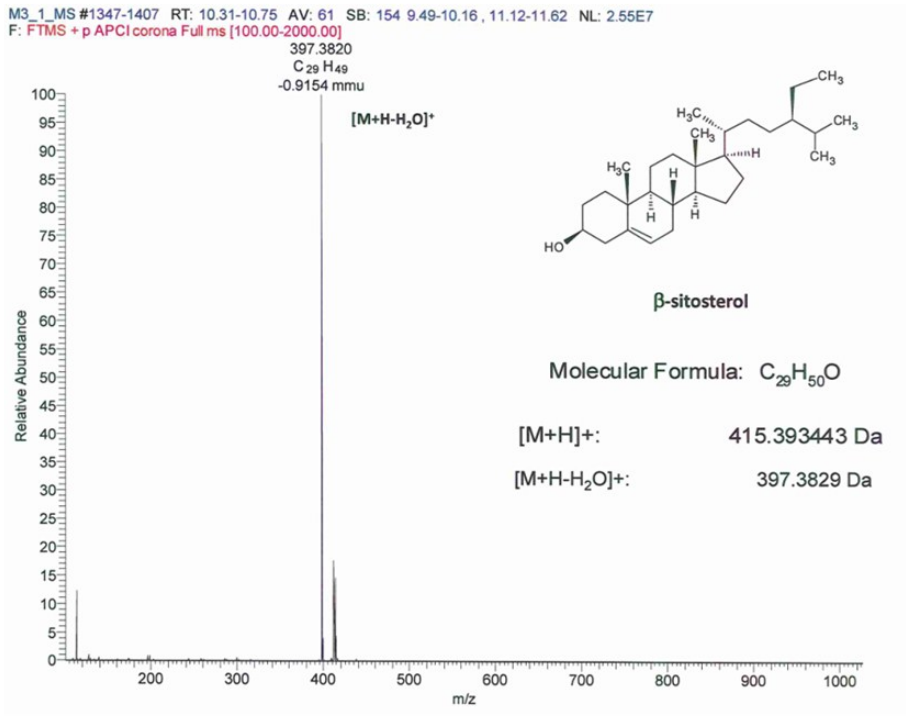


Figure S5: FTMS of peak 3 (β-Sitosterol): MS (ES) (+): 397.38 $[C_{29}H_{49}]$+

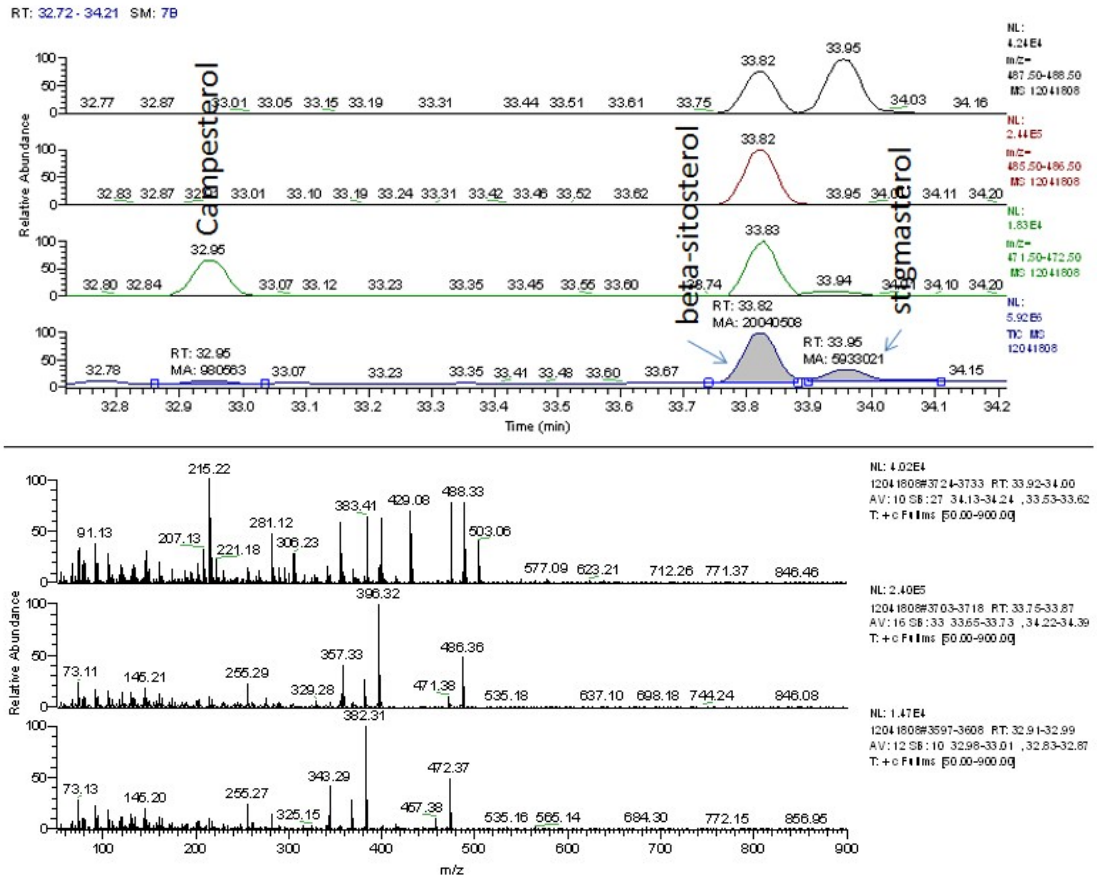


The same peaks have been observed in all the CSS SIT samples as defined above. The results are summarized in the Table 1 of the paper.

4.4.2 Results obtained by the GS-MS analysis:

The main peaks have been observed during GS-MS analysis of both trimethylsilyl (TMS) derivate of two samples above mentioned, which confirm with high precision the identity of the three phytosterols present in all the multicomponent forms of CSS of β-Sitosterol, (Figure S6).

Figure S6: Chromatogram of the GS-MS analysis of CSS SIT-GA (black, red and green) and β-Sitosterol Sigma-Aldrich (BCB50067V) (blue)



The molecular weights of the observed peaks were the following:

- Stigmasterol - TMS: 486.36
- Campesterol – TMS: 472.37
- β-Sitosterol – TMS: 488.33

5.- Characterization of the solids

Figure S7: Comparative PXRD diffractograms between different commercial batches of β-Sitosterol: batch BCBS00067V (79.3%) (green) and batch Y0001615 (72.5%) (purple) and simulated from the cif's files (LOFFET (black) and TEXQOC (blue))

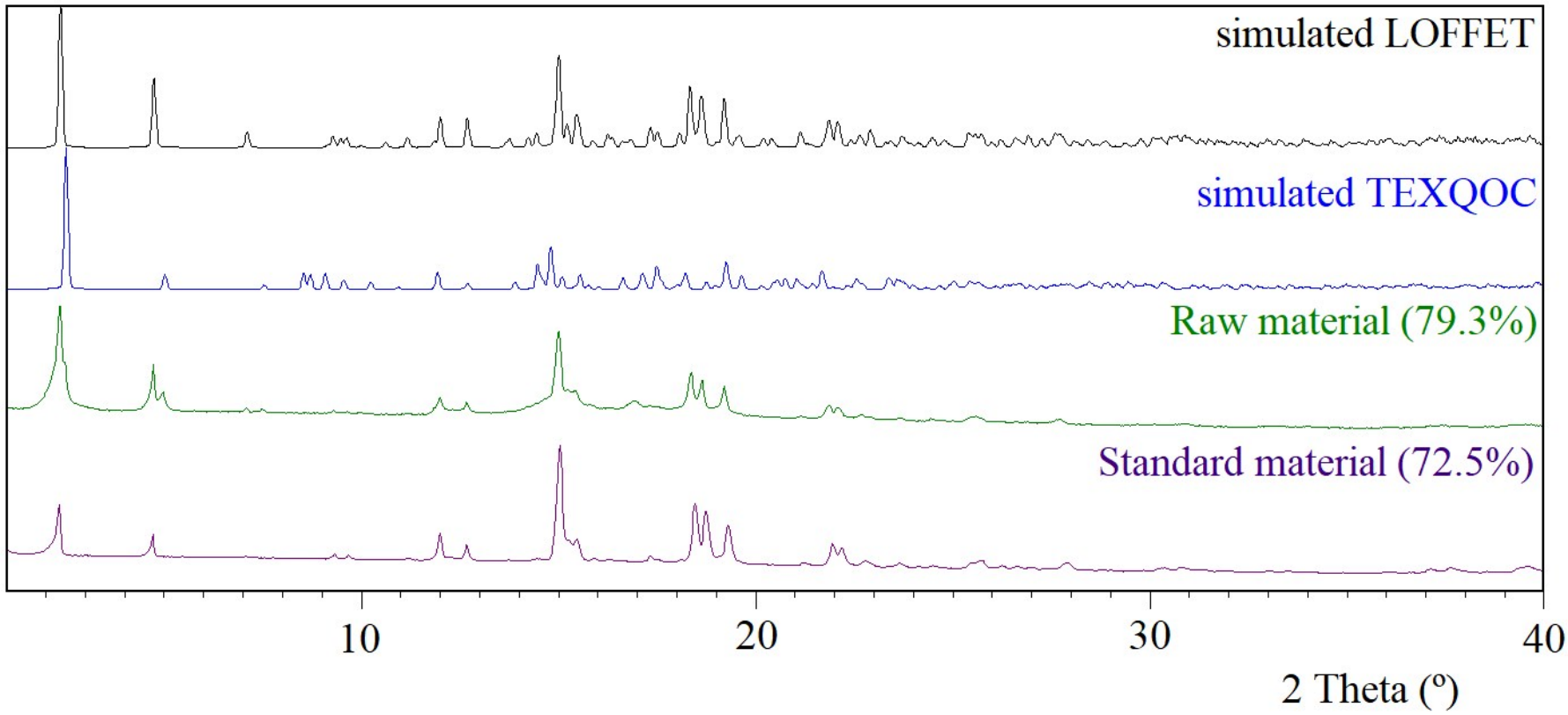


Figure S8: The XRPD of benzyl alcohol cocrystal has been indexed with the following proposed triclinic cell: a=38.21(3) Å, b=9.935(2) Å, c=7.640(2) Å, α = 88.48 (1)º, β= 93.38(3)º, γ= 96.34(4)º, V=2877(2) Å^3 (Figures of Merit: M= 24, F= 94), with number of impurities equal to zero. A *P*1 space group is compatible with the cell and the cell volume is compatible with 4 molecules of CSS β-Sitosterol, 1 molecule of benzyl alcohol and 1 molecule of water. (R_{wp}: 9.62; R_{exp}: 1.62)

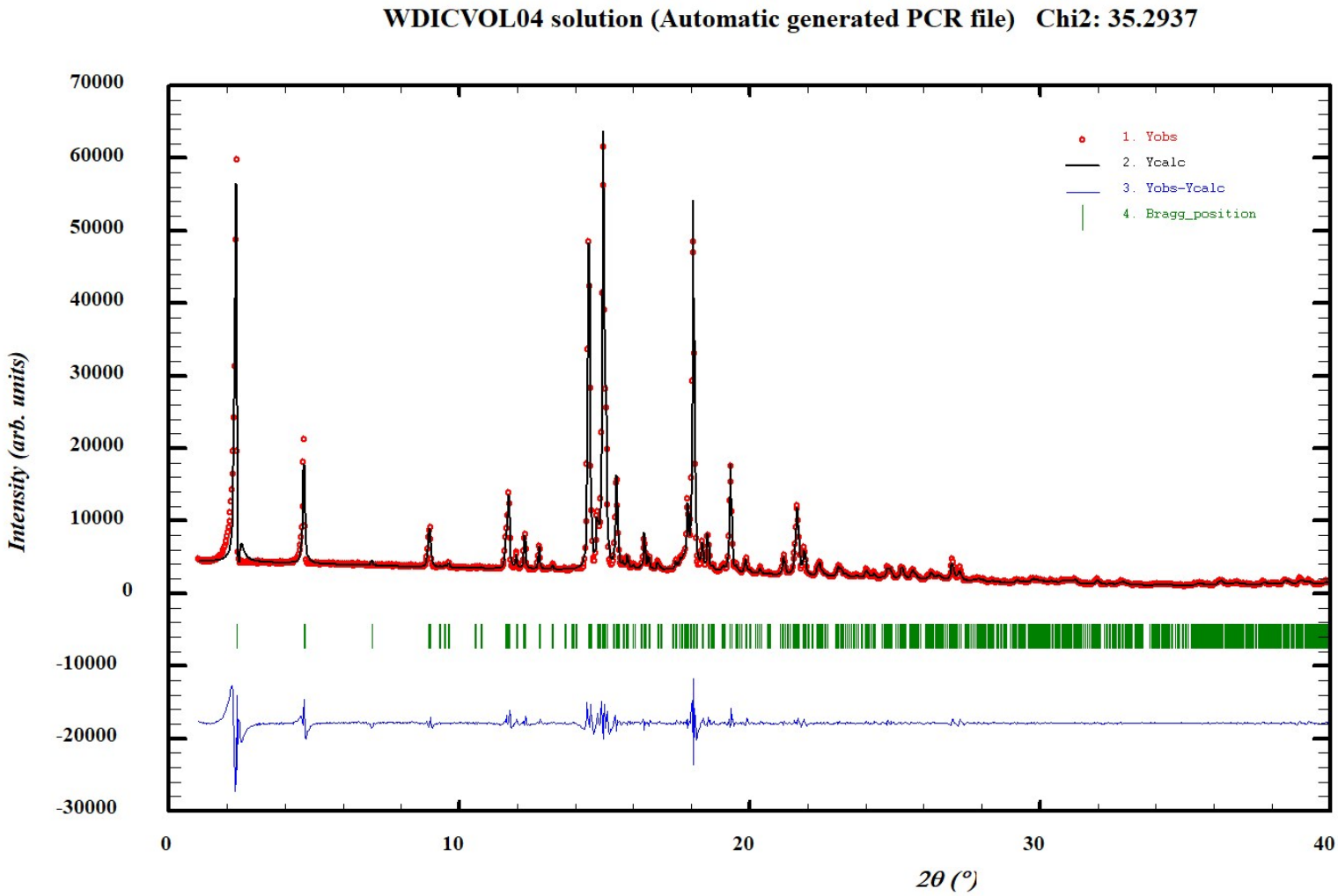


Figure S9: The structure was provisionally determined by single crystal X-ray diffraction at 100K showing a molar ratio 4:1:1 of the cocrystal β-Sitosterol:benzyl alcohol:water, with the following triclinic cell: a=7.597(3) Å, b=9.730(4) Å, c=37.763(14) Å, α= 84.84(6)º, β=86.089(8)º, γ= 88.219(9) º, V=2773(1) Å^3, Z=1 and *P*1 space group. (R_{int} (%)= 7.41; R-Factor (%) = 12.5). Comparative PXRD diffractograms between bulk benzyl alcohol cocrystal and simulated from the cif is shown.

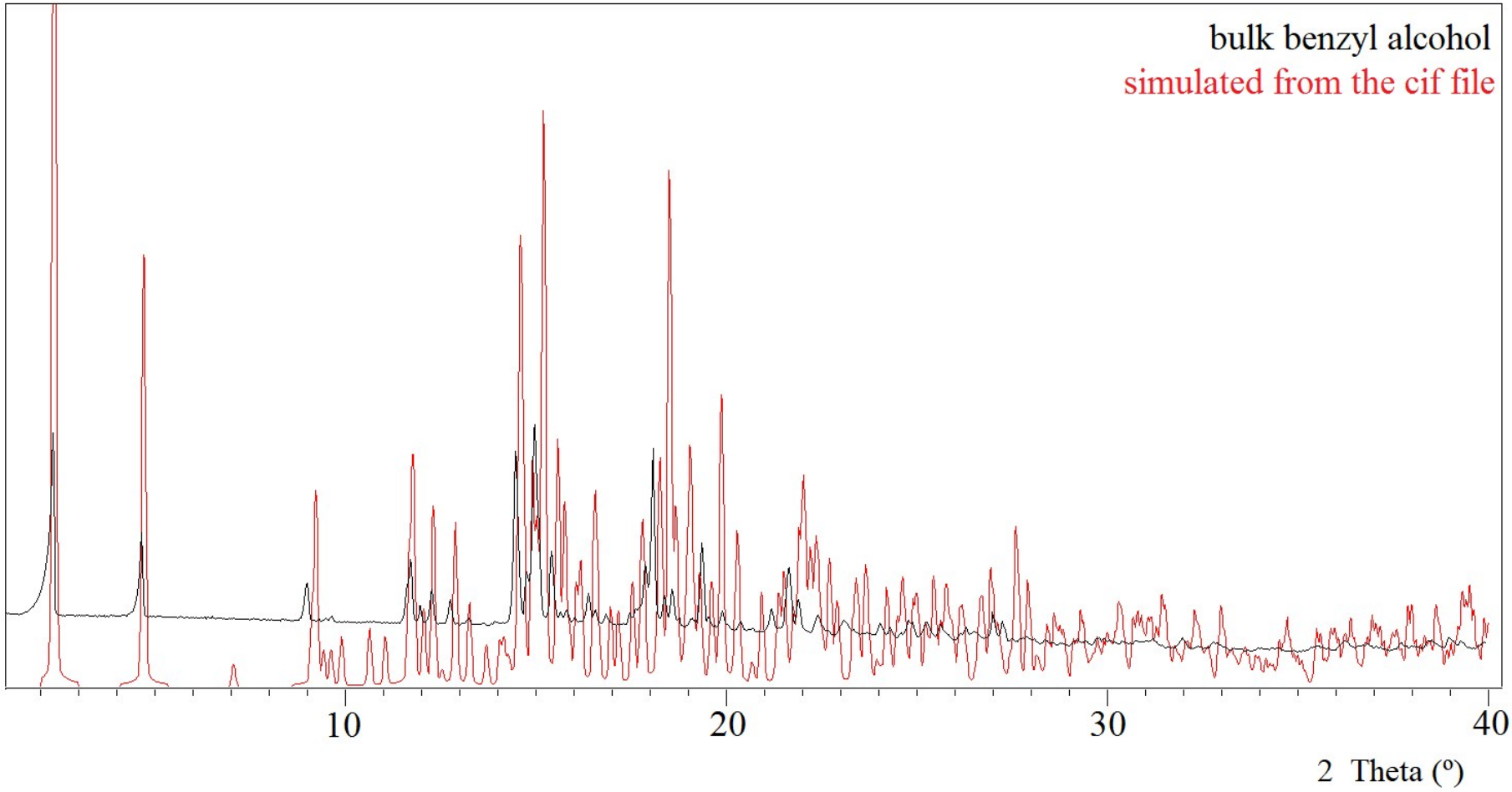


Figure S10: DSC of CSS SIT-ProA-H_2O I

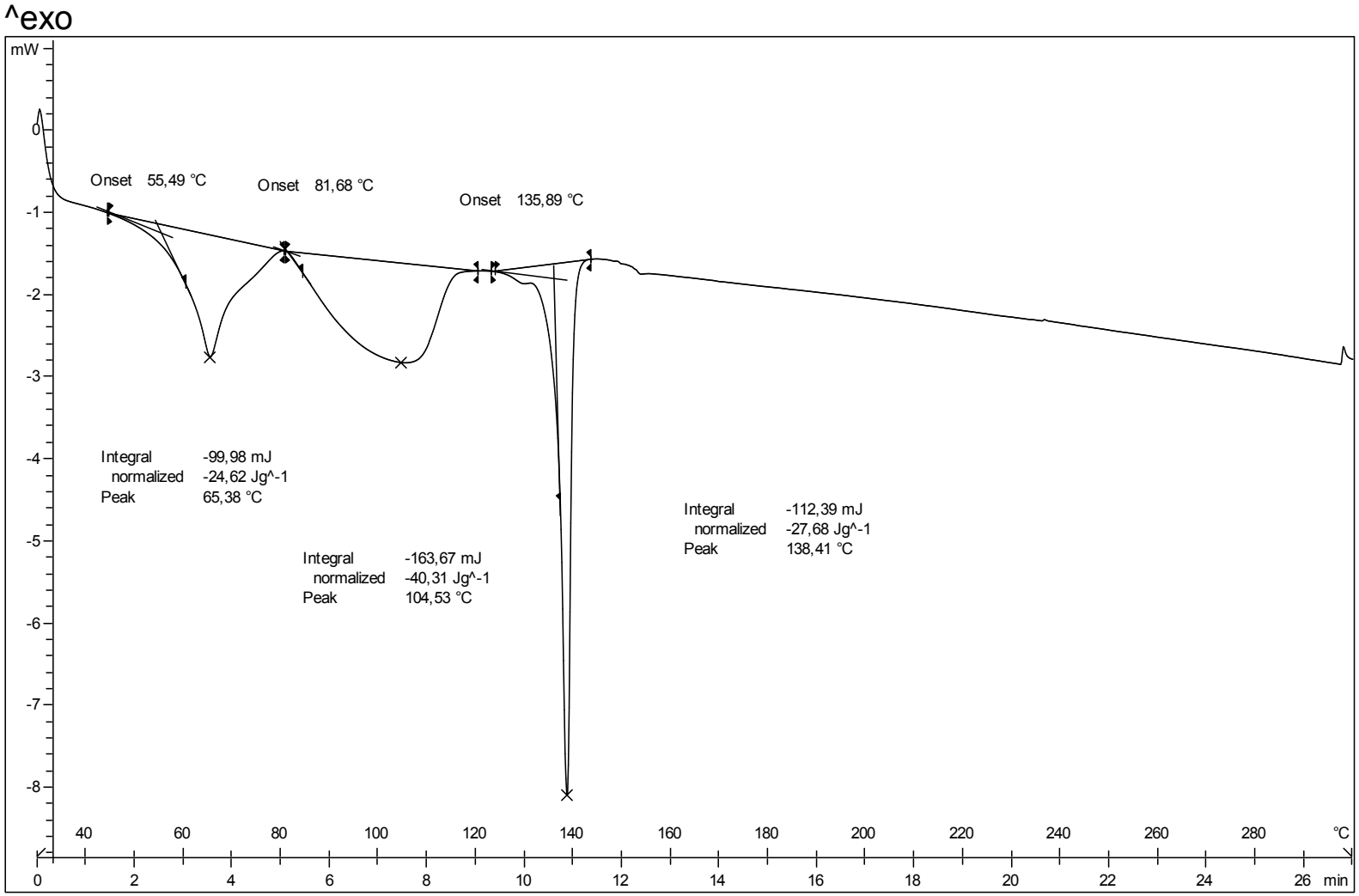


Figure S11: TGA of SIT-ProA-H_2O I: a weight loss of 9.8% is detected from 30 ºC to 155 ºC which could be attributed to 1 molecule of water and 1 molecule of propionic acid per 2 molecules of CSS β-Sitosterol (theoretical weight loss of 10.0%)

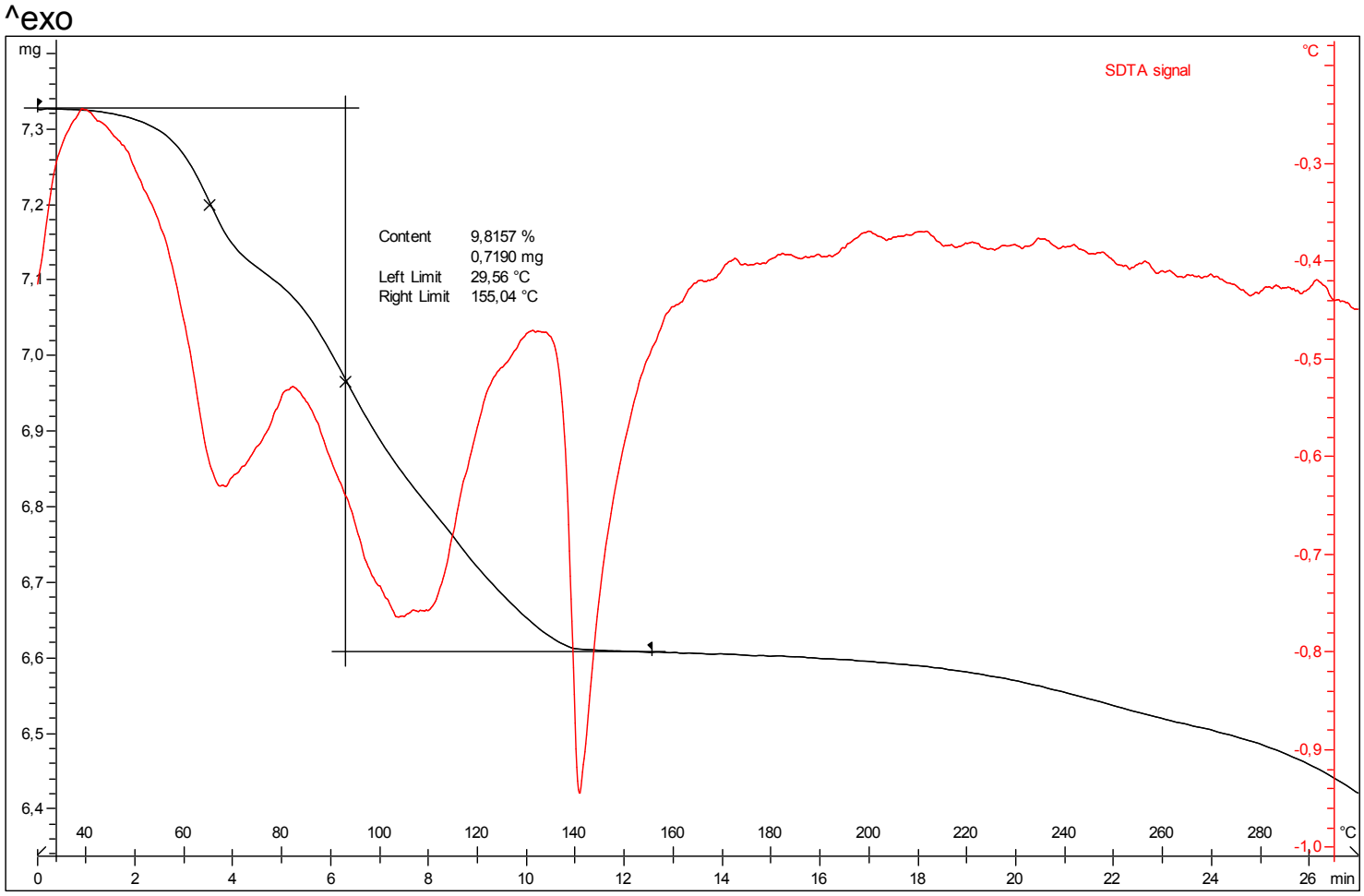


Figure S12: The XRPD of SIT-ProA-H_2O I cocrystal has been indexed with the following proposed monoclinic cell: a=40.0(2) Å, b=7.617(2) Å, c=9.631(1) Å, β= 97.03(3)º, V=2914(2) Å^3 (Figures of Merit: M= 50, F= 140), with number of impurities equal to zero. A $P2_1$ space group is compatible with the cell and the cell volume is compatible with 2 molecules of CSS β-Sitosterol, 1 molecule of propionic acid and 1 molecule of water. (R_{wp}: 6.88; R_{exp}: 1.60)

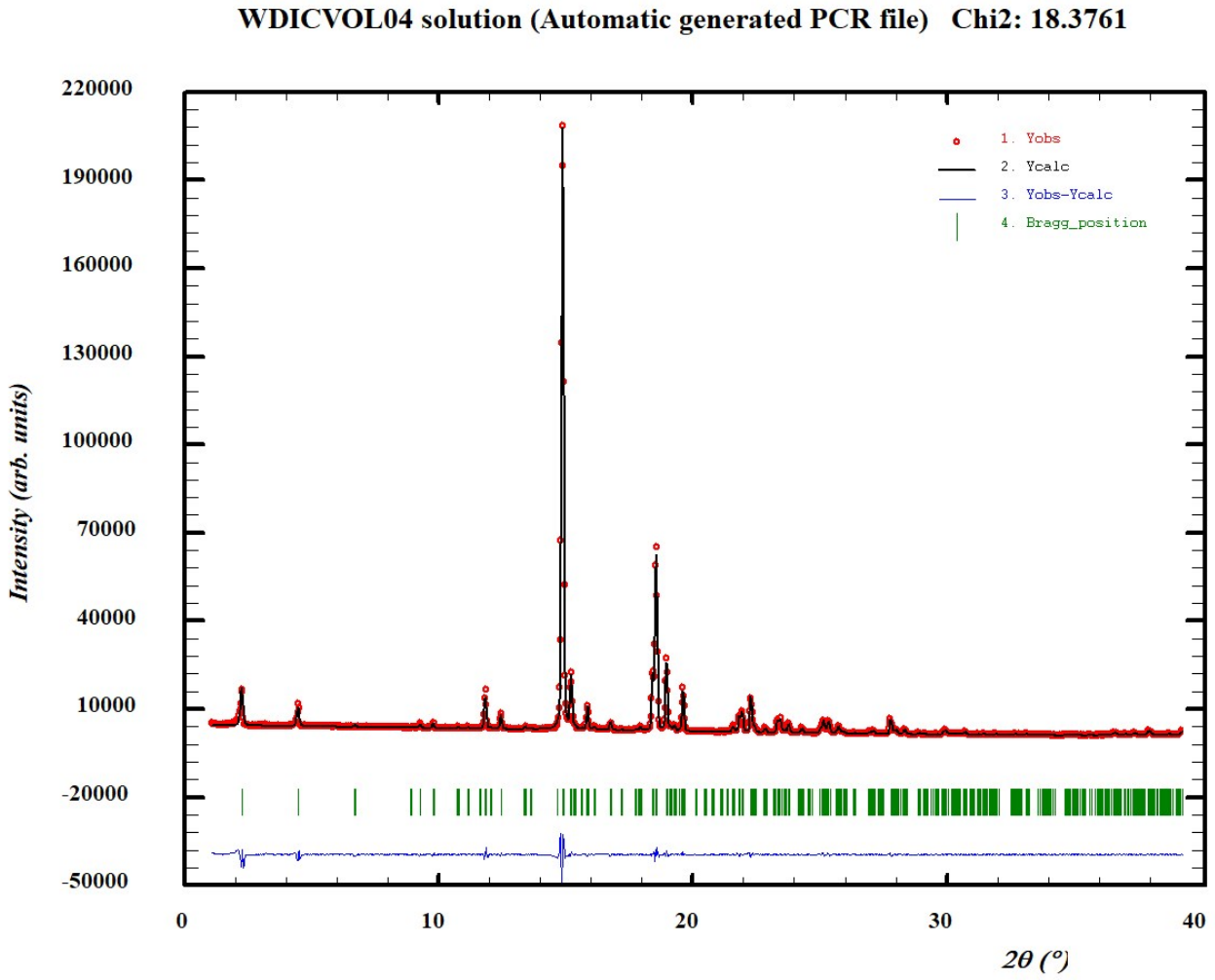


Figure S13: The structure was provisionally determined by single crystal X-ray diffraction at 293K showing a molar ratio 2:1:1 of the cocrystal β-Sitosterol:propionic acid:water, with the following monoclinic cell: a=9.439(2) Å, b=7.5391(16) Å, c=39.635(8) Å, β=95.216(6)º, V=2809(1) Å^3, Z=2, and $P2_1$ space group. (R_{int} (%) = 5.11; R-Factor (%) = 4.5). Comparative PXRD diffractograms between bulk SIT-ProA-H_2O I cocrystal and simulated from the cif is shown.

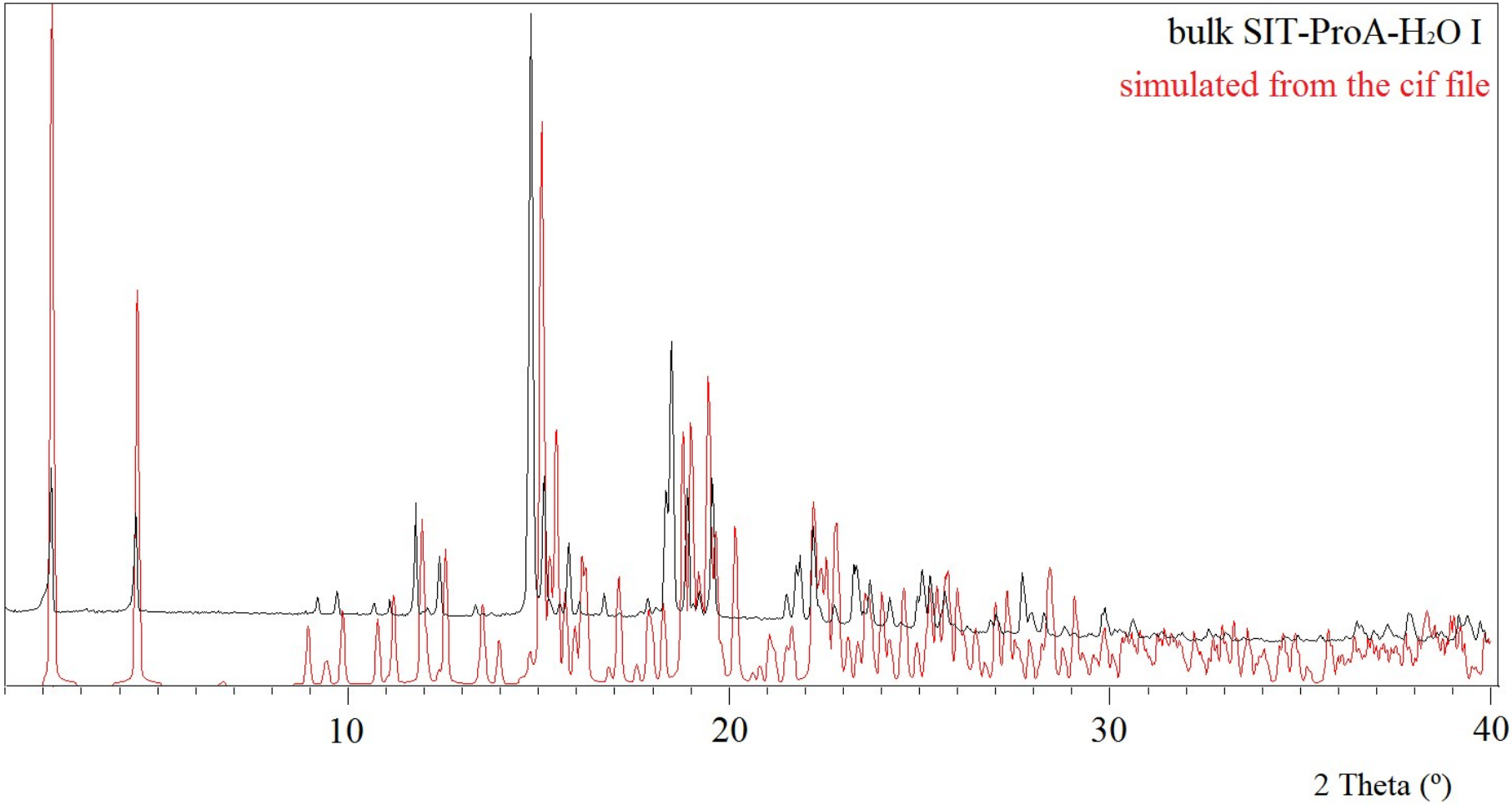


Figure S14: ^{1}H-NMR (chloroform-*d*: delay: 1/pulse: 45°/scans: 32) of SIT-ProA-H_2O I cocrystal

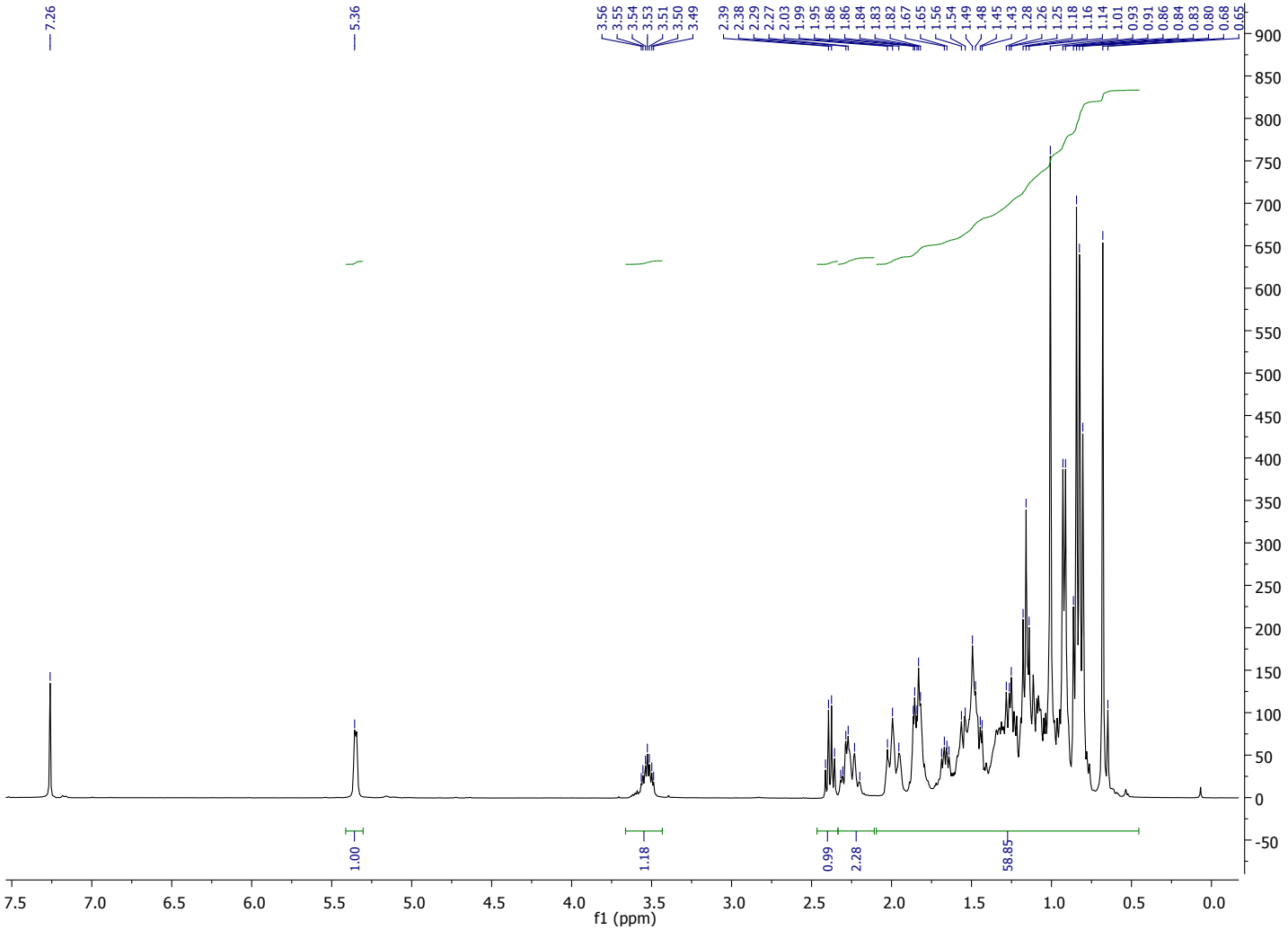


Figure S15: DSC of SIT-ProA-H_2O II

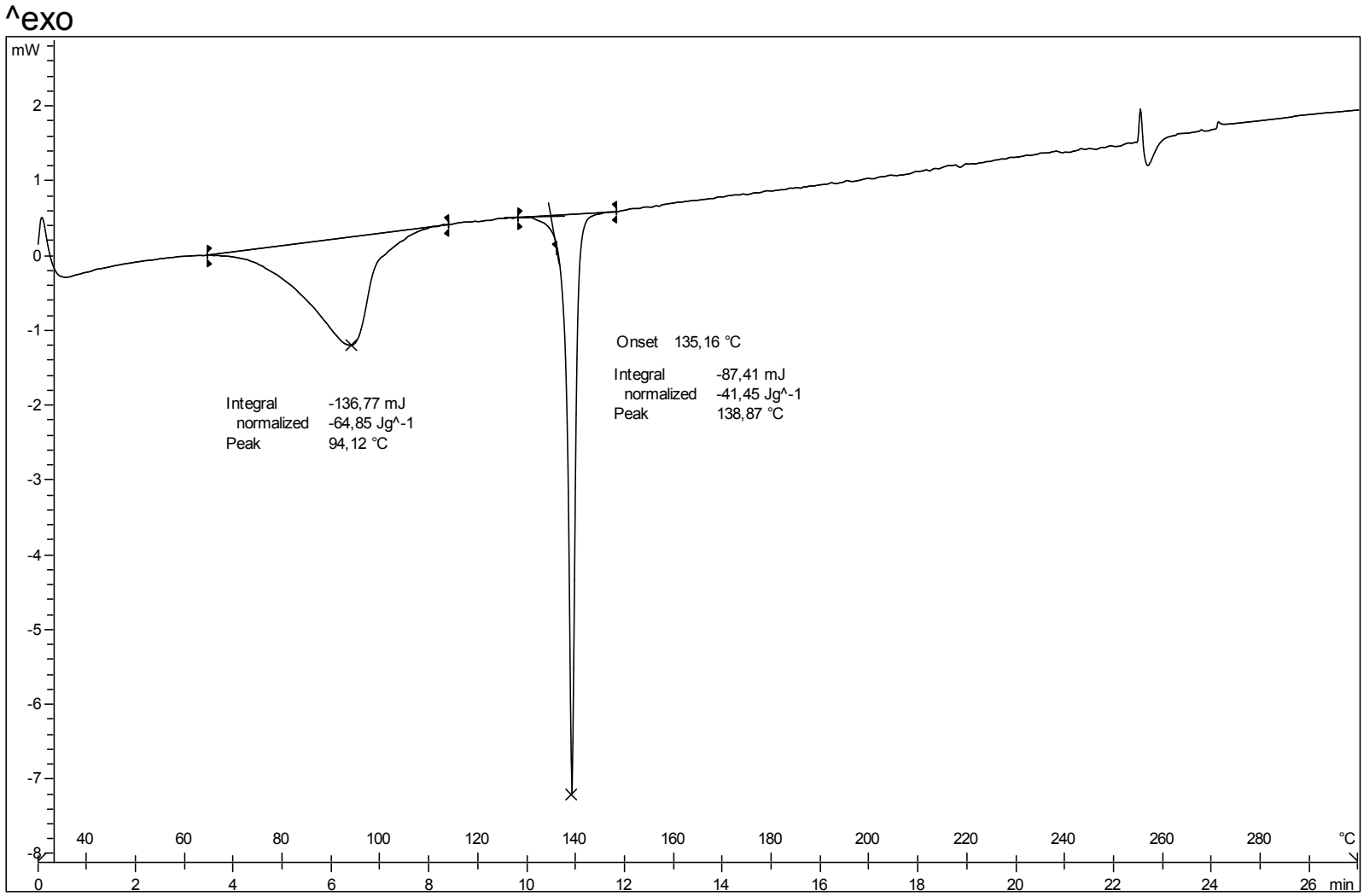


Figure S16: TGA of SIT-ProA-H_2O II: a weight loss of 5.1% is detected from 30 ºC to 154 ºC which could be attributed to 1 molecule of water and 1 molecule of propionic acid per 4 molecules of CSS β-Sitosterol (theoretical weight loss of 5.3%)

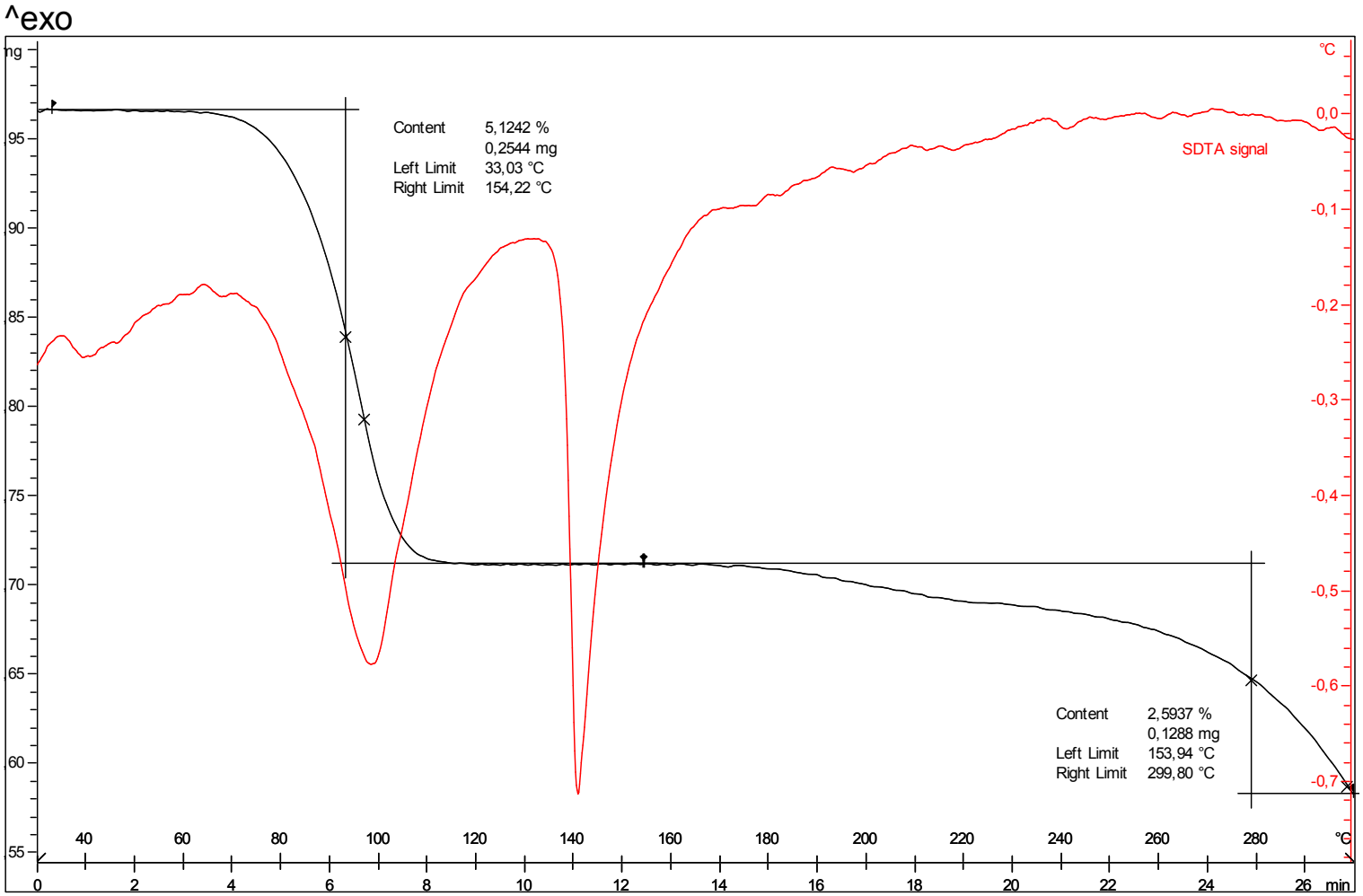


Figure S17: The XRPD of SIT-ProA-H_2O II cocrystal has been indexed with the following proposed monoclinic cell: a=28.16(1) Å, b=7.568(2) Å, c=26.235(9) Å, β= 92.09(2)º, V=5587(3) Å^3 (Figures of Merit: M= 28, F= 102), with number of impurities equal to zero. A $P2_1$ space group is compatible with the cell and the cell volume is compatible with 4 molecules of CSS β-Sitosterol, 1 molecule of propionic acid and 1 molecule of water. (R_{wp}: 11.5; R_{exp}: 1.28)

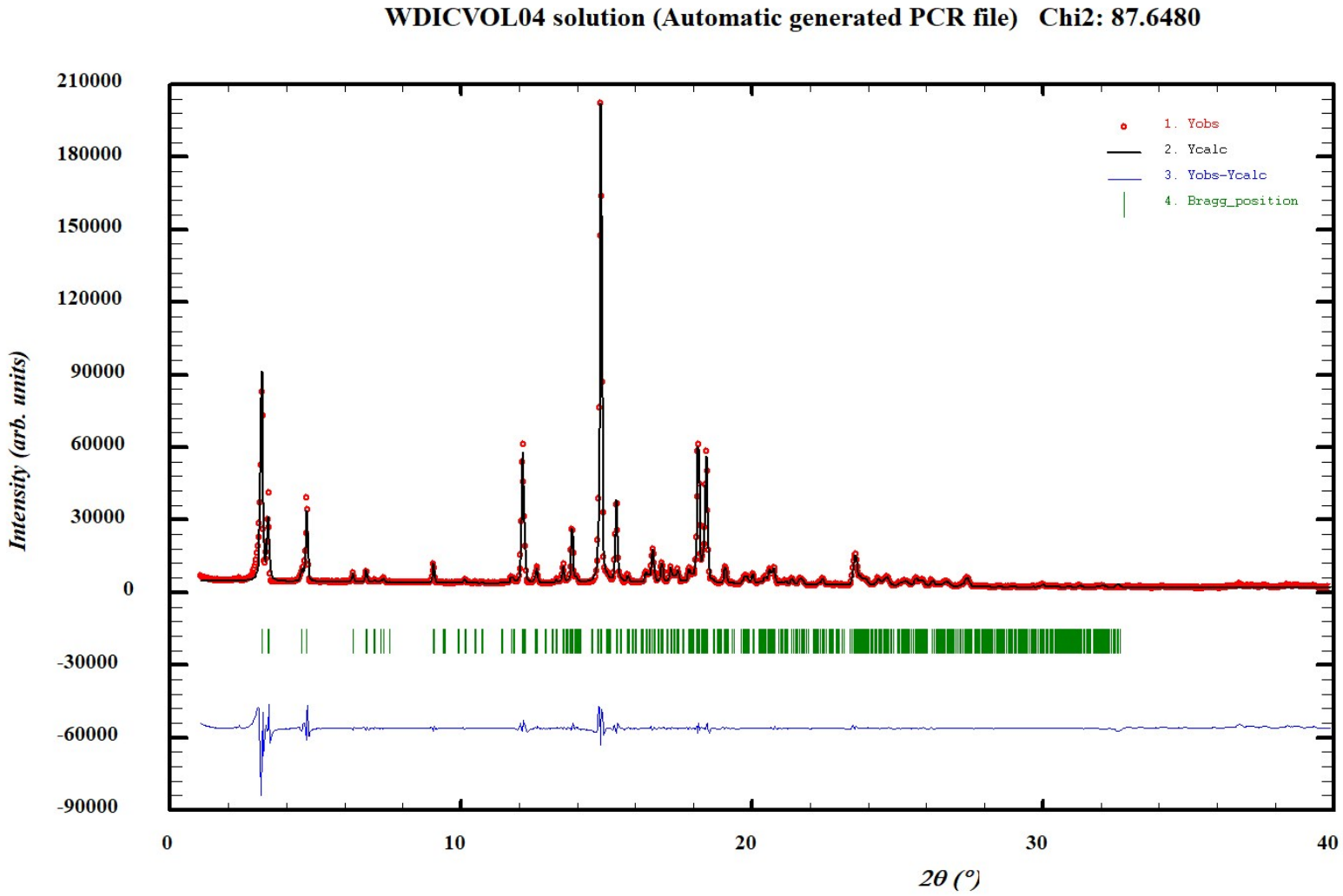


Figure S18: The structure was provisionally determined by single crystal X-ray diffraction at 100K showing a molar ratio 4:1:1 of the cocrystal β-Sitosterol:propionic acid:water, with the following monoclinic cell: a=26.373(3) Å, b=7.4971(7) Å, c=27.183(3) Å, β=92.569(6)º, V=5369(1) Å^3, Z=2, and $P2_1$ space group. (R_{int} (%) = 50.74; R-Factor (%) = 10.1). Comparative PXRD diffractograms between bulk SIT-ProA-H_2O II cocrystal and simulated from the cif is shown.

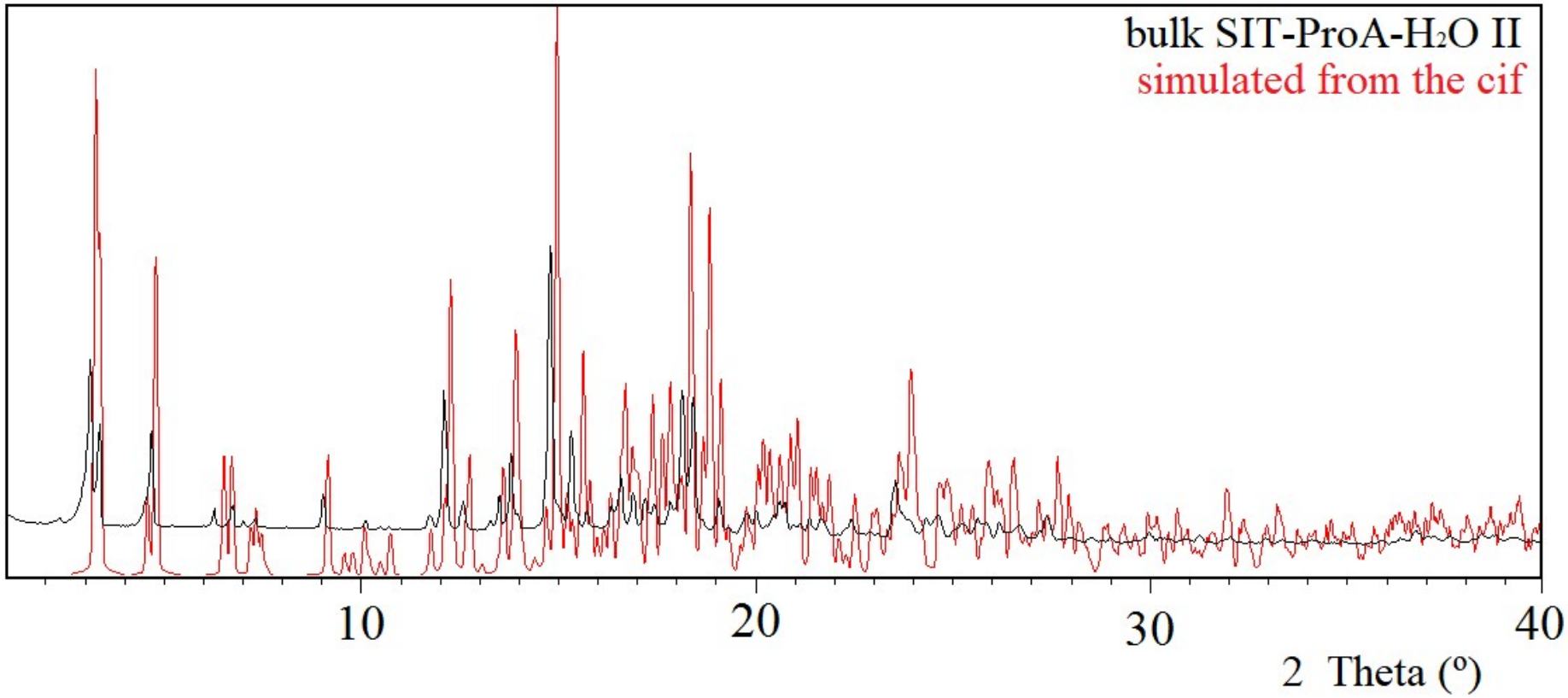


Figure S19: ^{1}H-NMR (chloroform-*d*: delay: 1/pulse: 45°/scans: 32) of SIT-ProA-H_2O II cocrystal

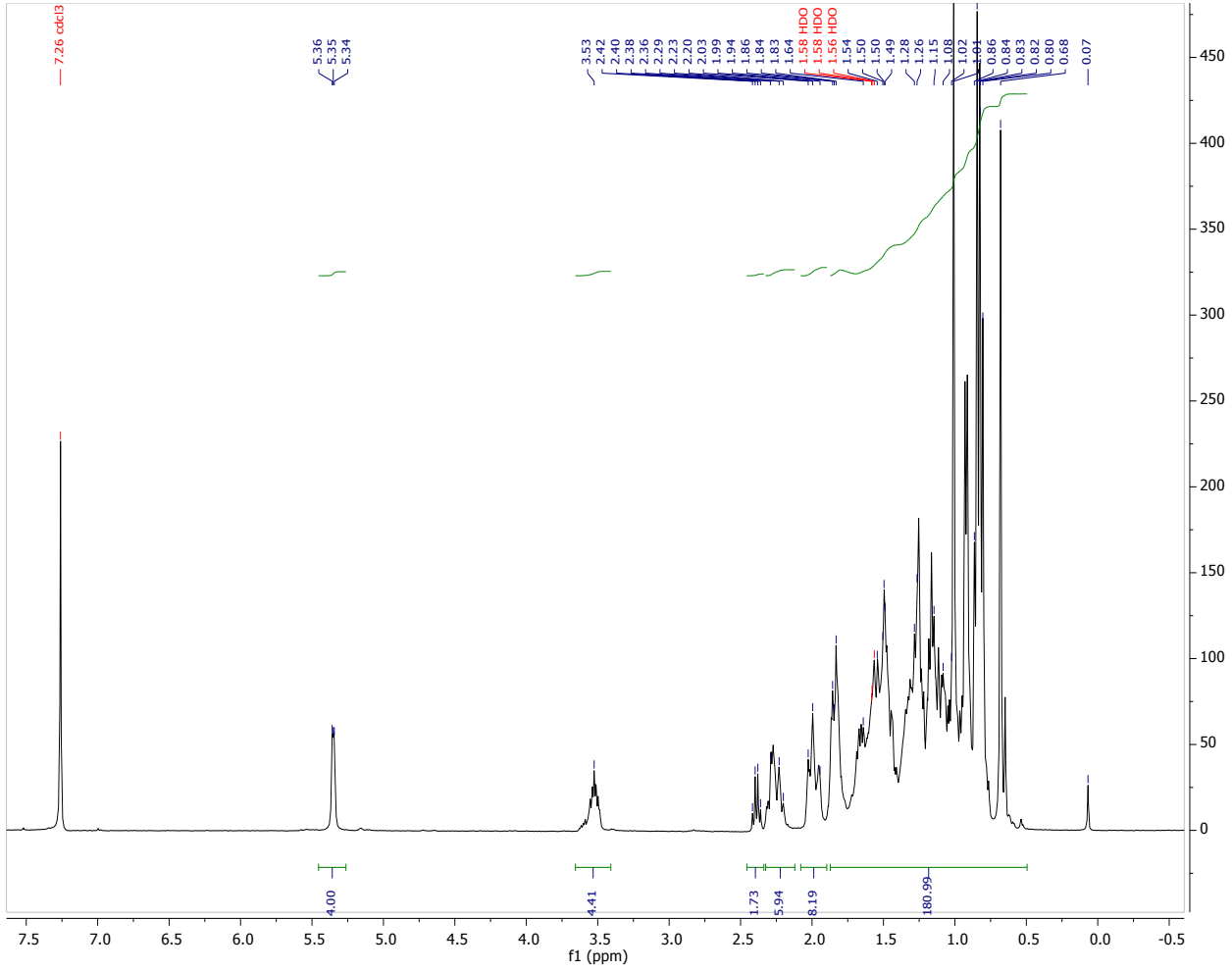


Figure S20: The structure was provisionally determined by single crystal X-ray diffraction at 100K showing a molar ratio 4:1:1 of the cocrystal β-Sitosterol:propionic acid:ACN, with the following triclinic cell: a=36.972(10) Å, b=9.702(3) Å, c=7.581(2) Å, α= 82.96(2), β=86.112(5)º, γ= 89.740(5), V=2693(1) Å^3, Z=1, and *P*1 space group. (R-Factor (%) = 18.6). Simulated PXRD of Form SIT-ProA-ACN is shown

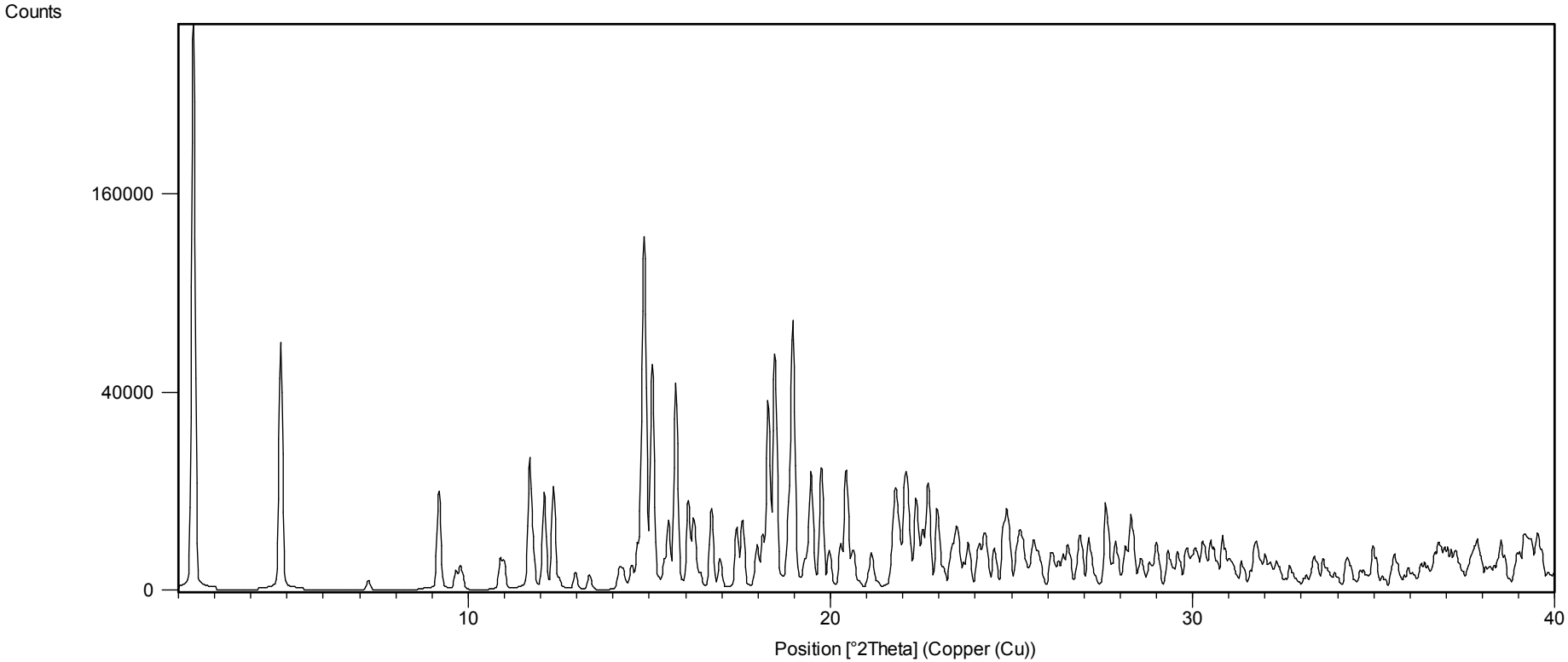


Figure S21: DSC of Form SIT-ZA-H_2O

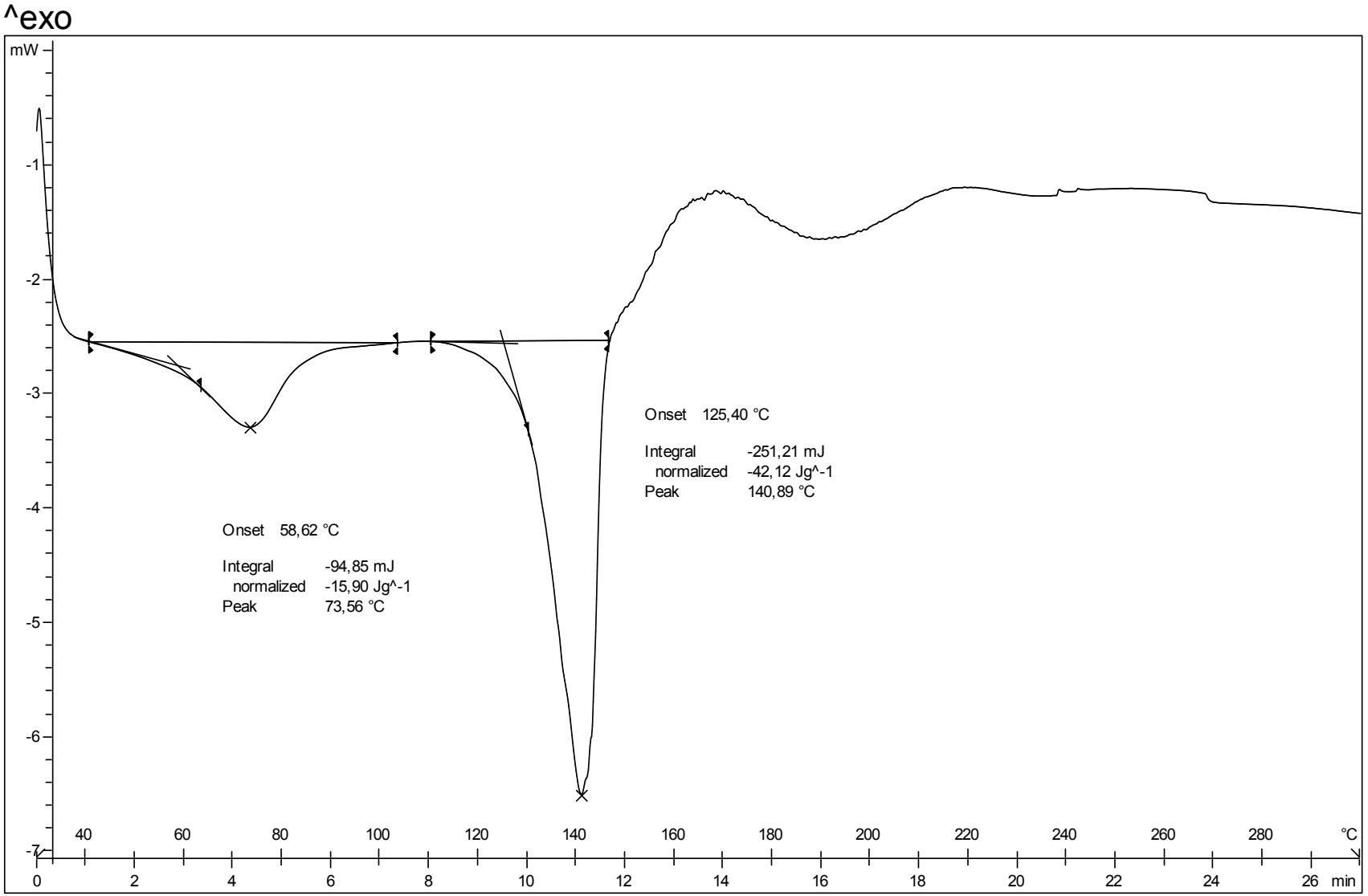


Figure S22: TGA of Form SIT-ZA-H_2O: a weight loss of 2.4 % is detected from 30 ºC to 118 ºC which could be attributed to 1 molecule of water per 1 molecule of zymonic acid and 2 molecules of CSS β-Sitosterol (theoretical weight loss of 1.8%)

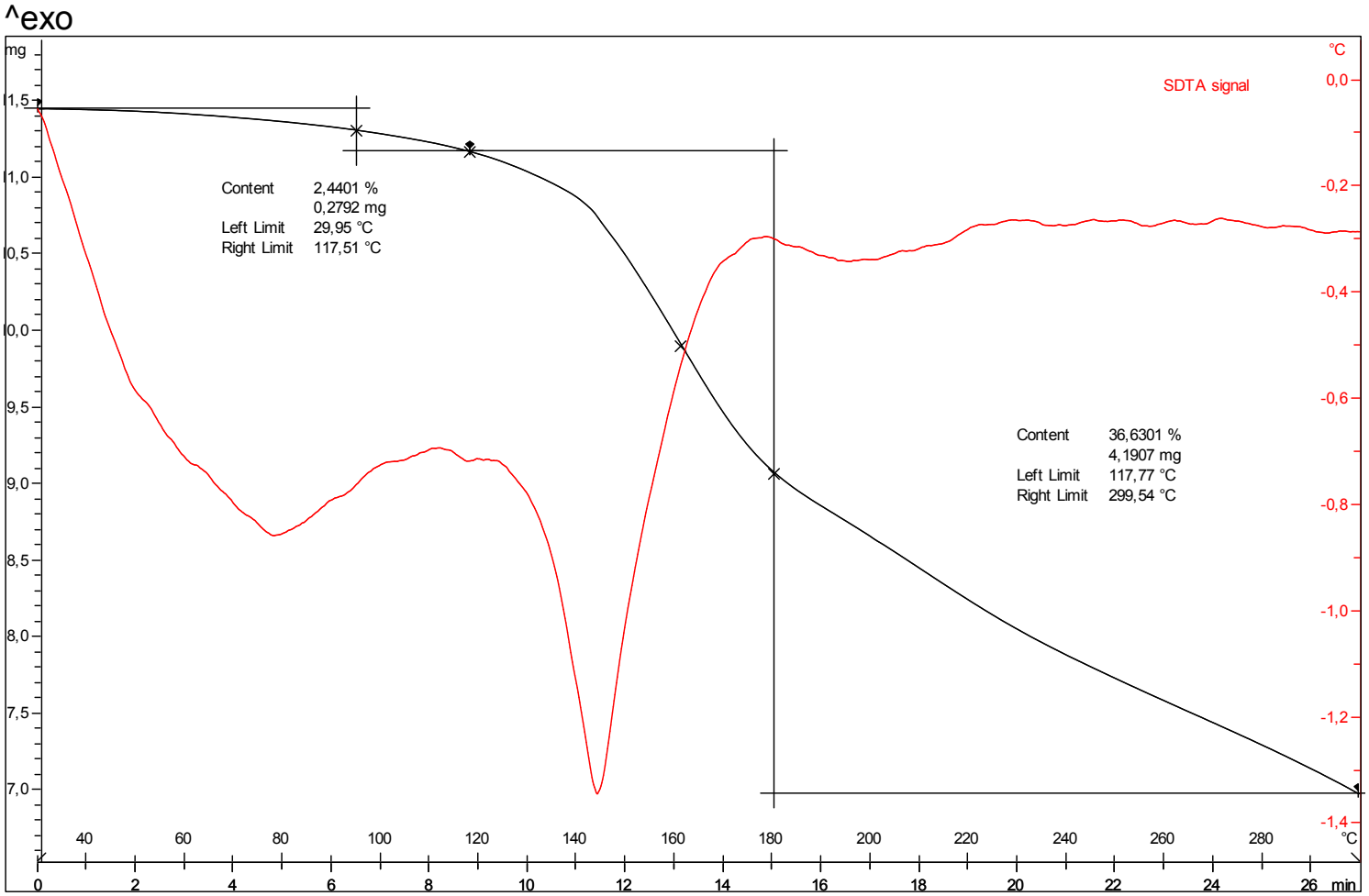


Figure S23: The XRPD of SIT-ZA-H_2O cocrystal has been indexed with the following proposed monoclinic cell: a=39.51(2) Å, b=6.982(3) Å, c=20.126(7) Å, β= 95.18(4)°, V=5528(4) Å^3 (Figures of Merit: M= 10, F= 35), with number of impurities equal to zero. A $P2_1$ space group is compatible with the cell and the cell volume is compatible with 2 molecules of CSS β-Sitosterol, 1 molecule of zymonic acid and 1 molecule of water. (R_{wp}: 10.1; R_{exp}: 2.78)

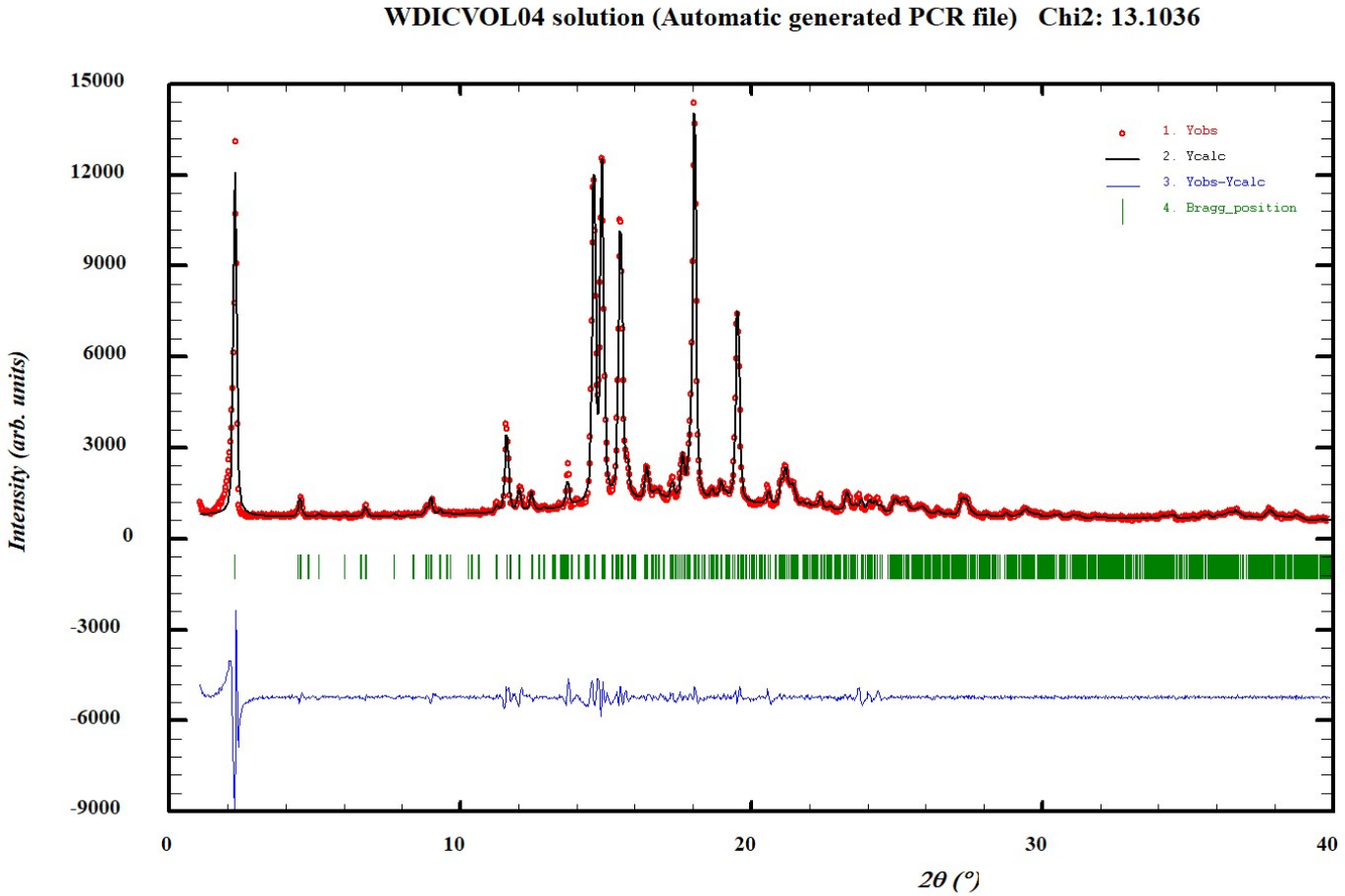


Figure S24: The structure was provisionally determined by single crystal X-ray diffraction at 293K showing a molar ratio 2:1:1 of the cocrystal β-Sitosterol:zymonic acid:water, with the following monoclinic cell: a=77.42(2) Å, b=7.609(2) Å, c=9.924(3) Å, β=90.948(7)º, V=5875(3) Å^3, Z=4, and *C*2 space group. (R-Factor (%) = 15.8). Comparative PXRD diffractograms between bulk SIT-ZA-H_2O cocrystal and simulated from the cif is shown.

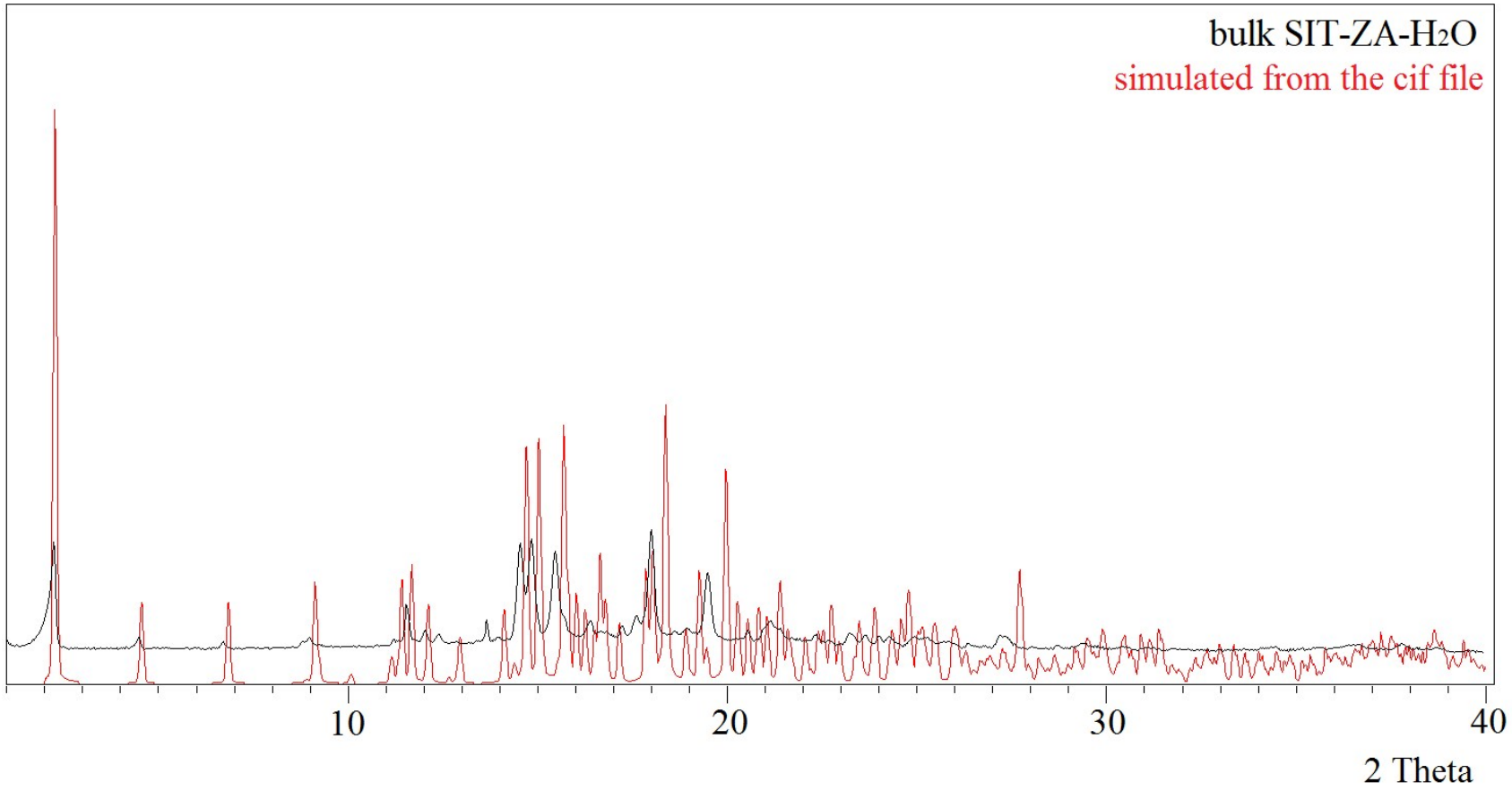


Figure S25: ^{1}H-NMR (chloroform-*d*/delay: 1/pulse: 45°/scans: 32) of Form SIT-ZA-H_2O

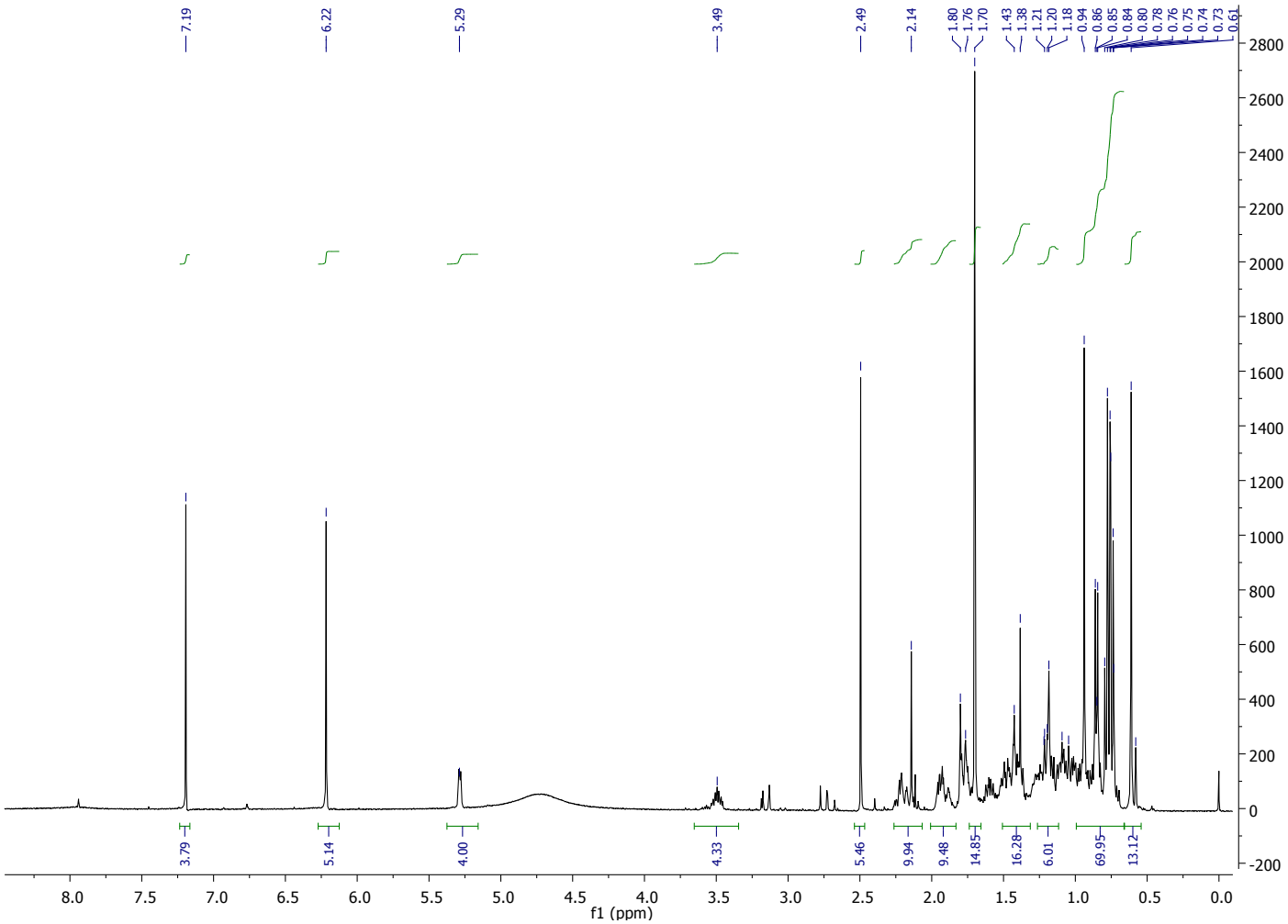


Figure S26: DSC of Form SIT-GA

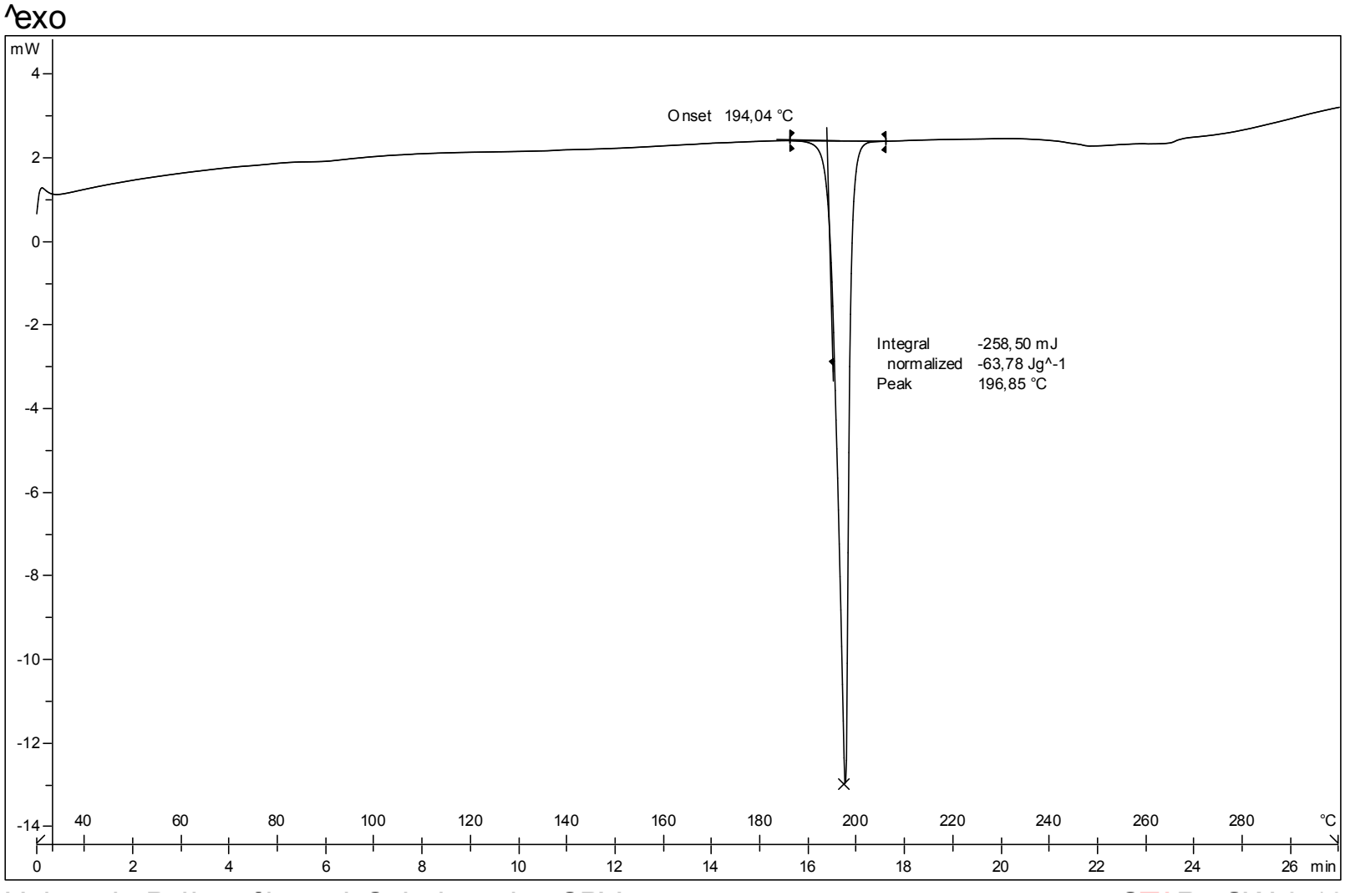


Figure S27: TGA of Form SIT-GA

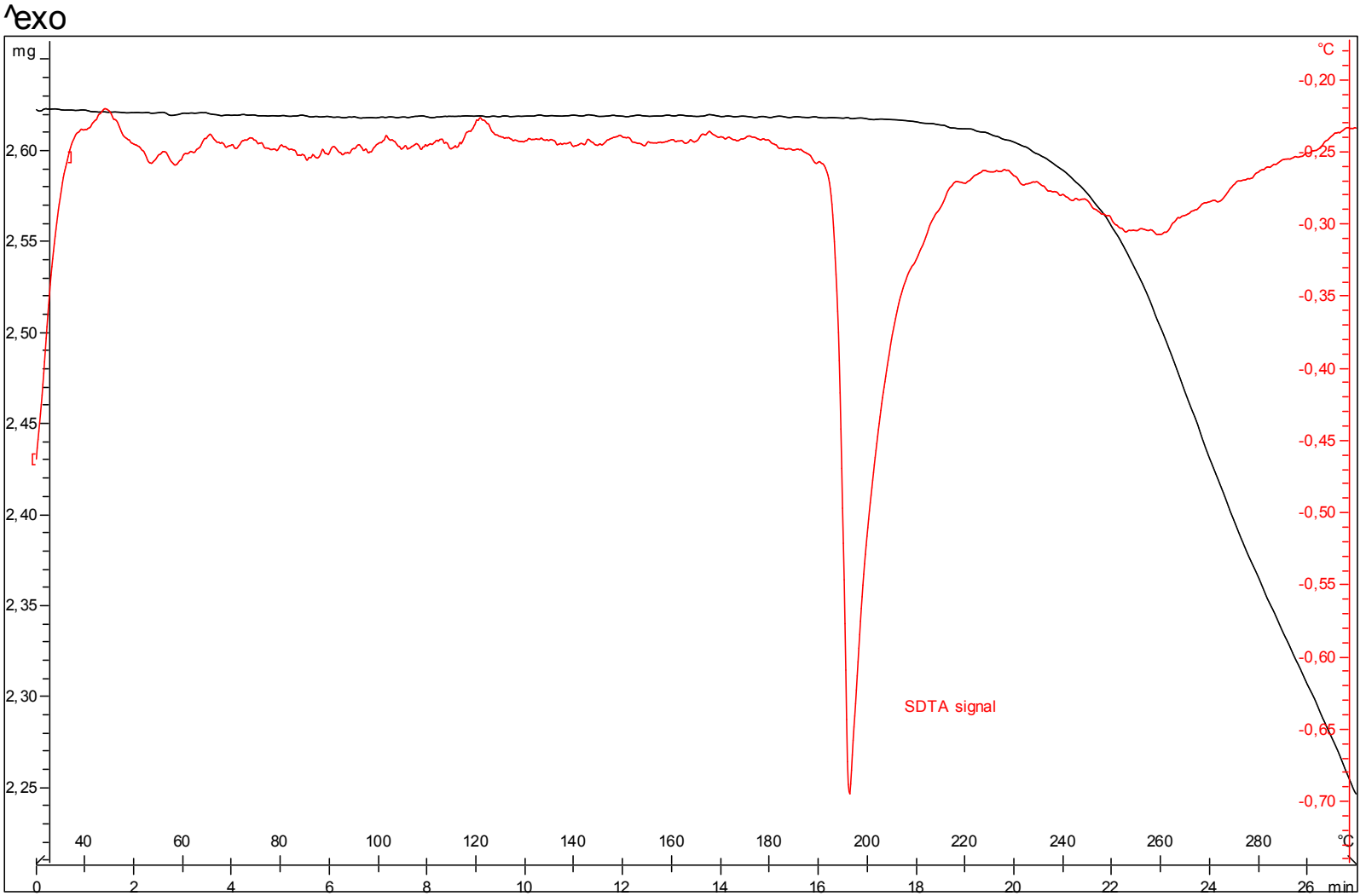


Figure S28: The XRPD of SIT-GA cocrystal has been indexed with the following proposed monoclinic cell: a=38.54(5) Å, b=13.812(4) Å, c=10.882(3) Å, β= 92.33(5)º, V=5788(8) Å ^{3} (Figures of Merit: M= 39, F= 135), with number of impurities equal to zero. A $P2_1$ space group is compatible with the cell and the cell volume is compatible with 4 molecules of CSS β-Sitosterol, 1 molecule of gallic acid and 1 molecule of water. (R_{wp}: 11.1; R_{exp}: 2.05)

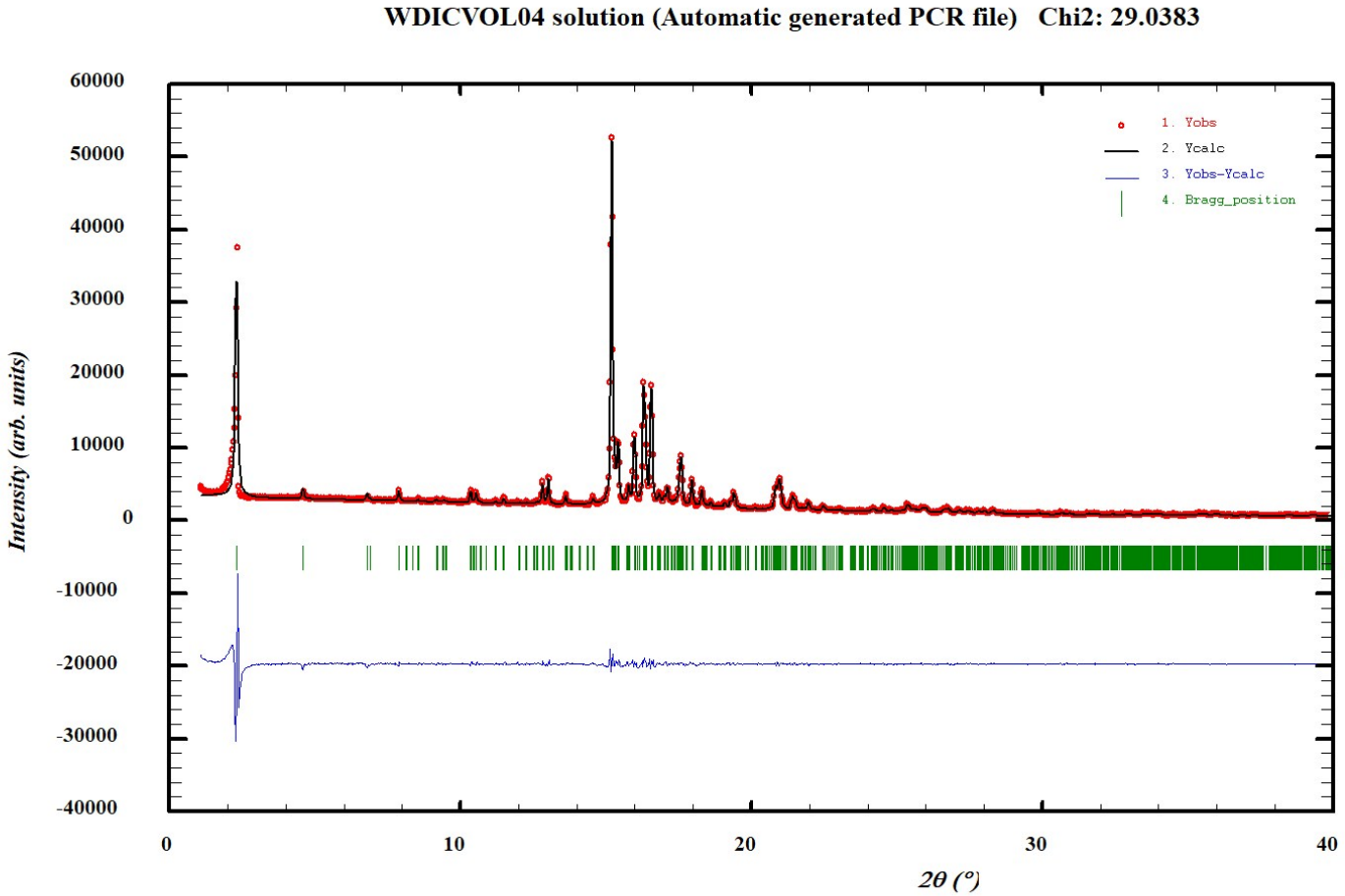


Figure S29: The structure was provisionally determined by single crystal X-ray diffraction at 100K showing a molar ratio 4:1 of the cocrystal β-Sitosterol:gallic acid, with the following monoclinic cell: a=38.123(2) Å, b=13.699(6) Å, c=10.744(5) Å, β=93.002(2)º, V=5603(1) Å^3, Z=2, and $P2_1$ space group. (R-Factor (%) = 13.2). Comparative PXRD diffractograms between bulk SIT-GA cocrystal and simulated from the cif is shown.

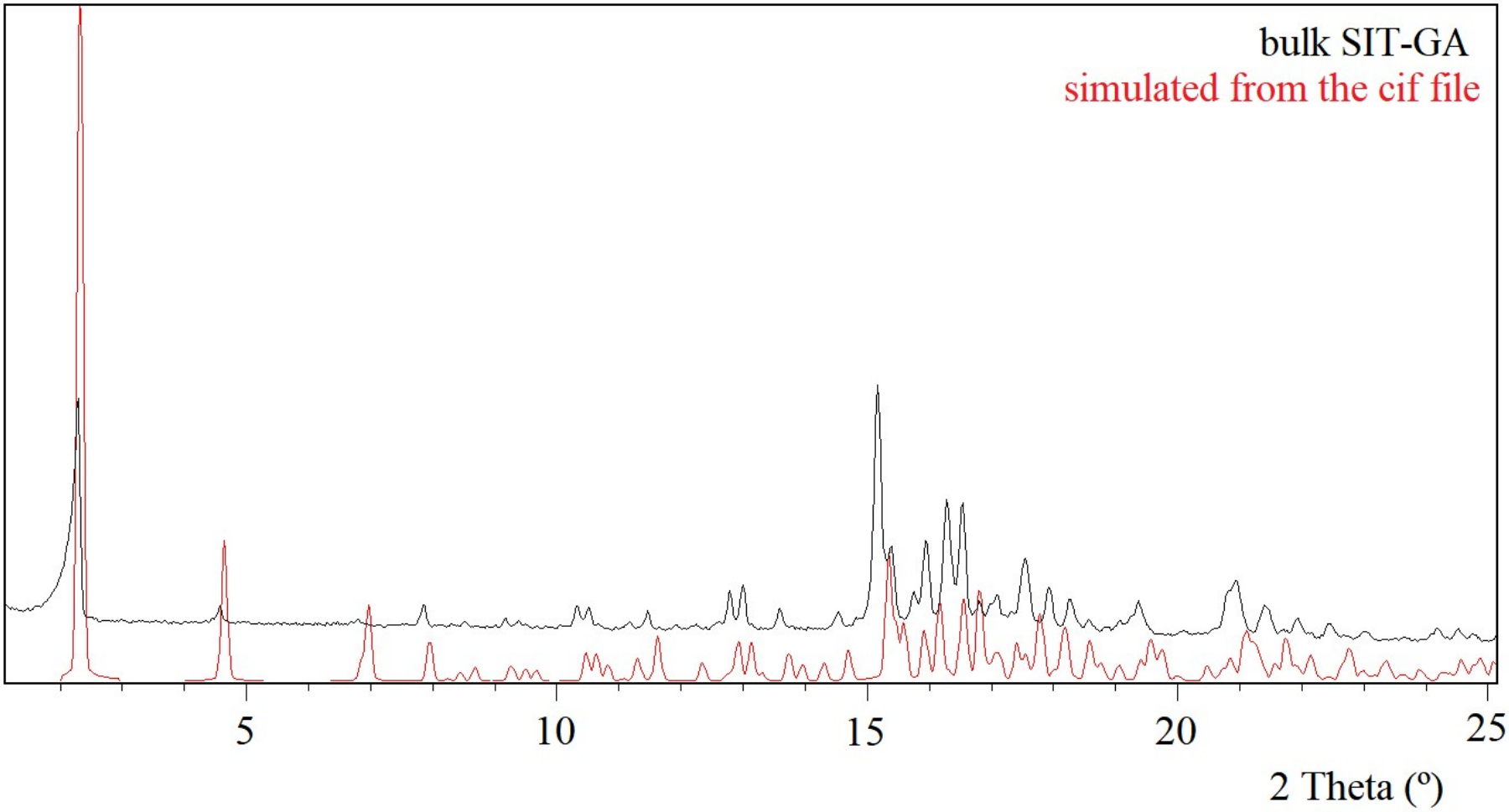


Figure S30: ^{1}H-NMR (chloroform-*d*/delay: 1/pulse: 45°/scans: 32) of Form SIT-GA

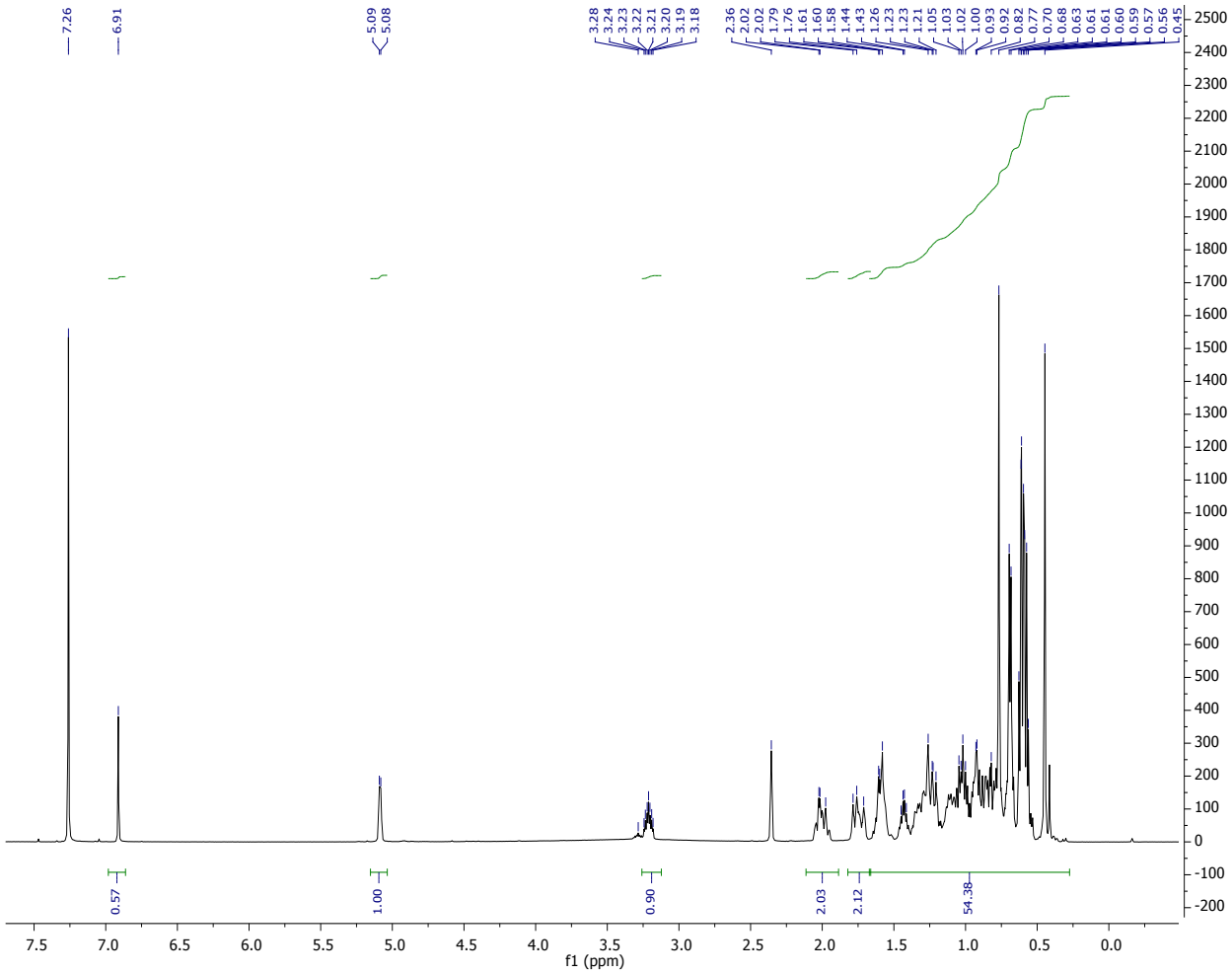


Figure S31: DSC of Form SIT-4-HBA I

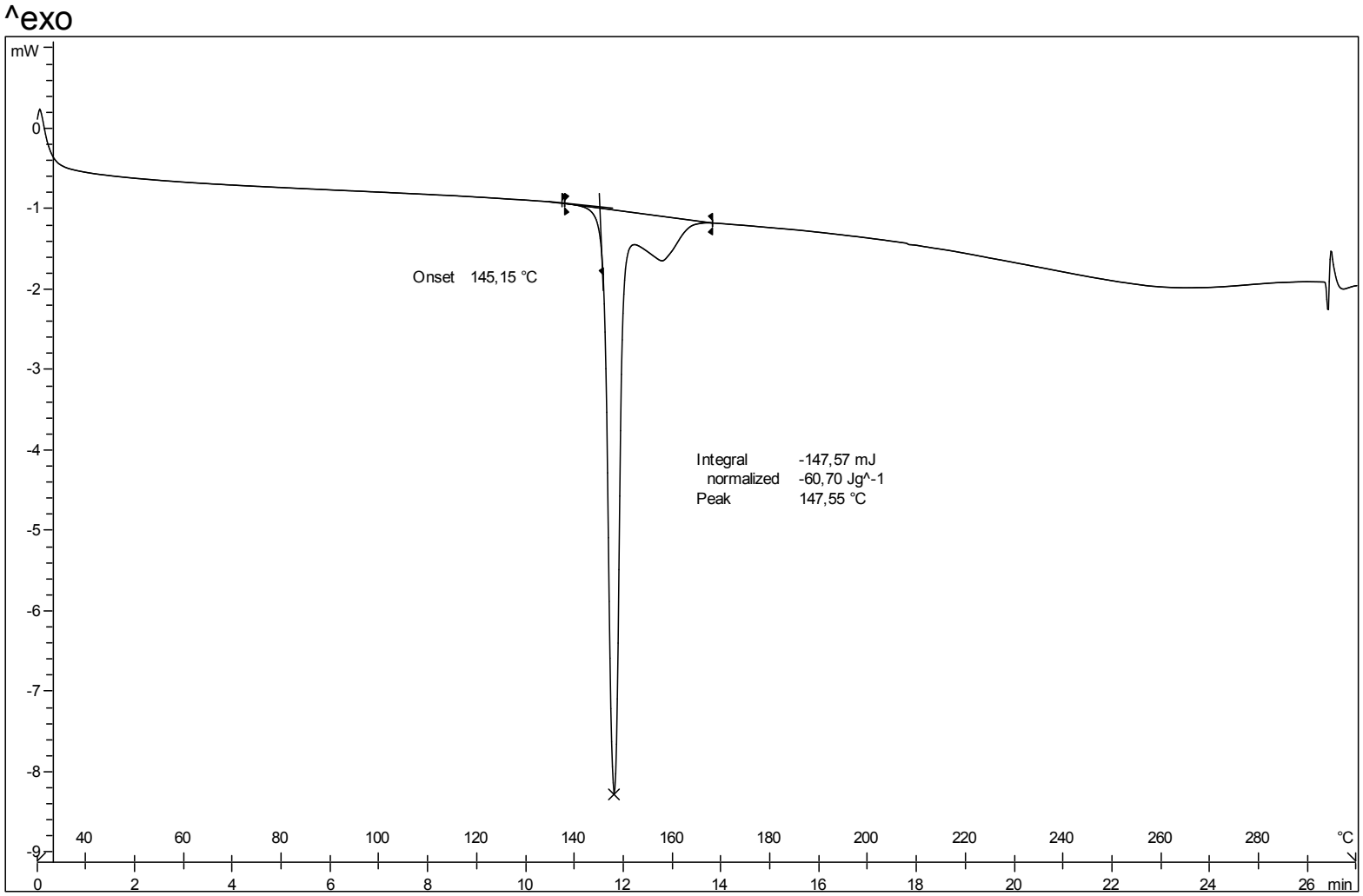


Figure S32: TGA of Form SIT-4-HBA I

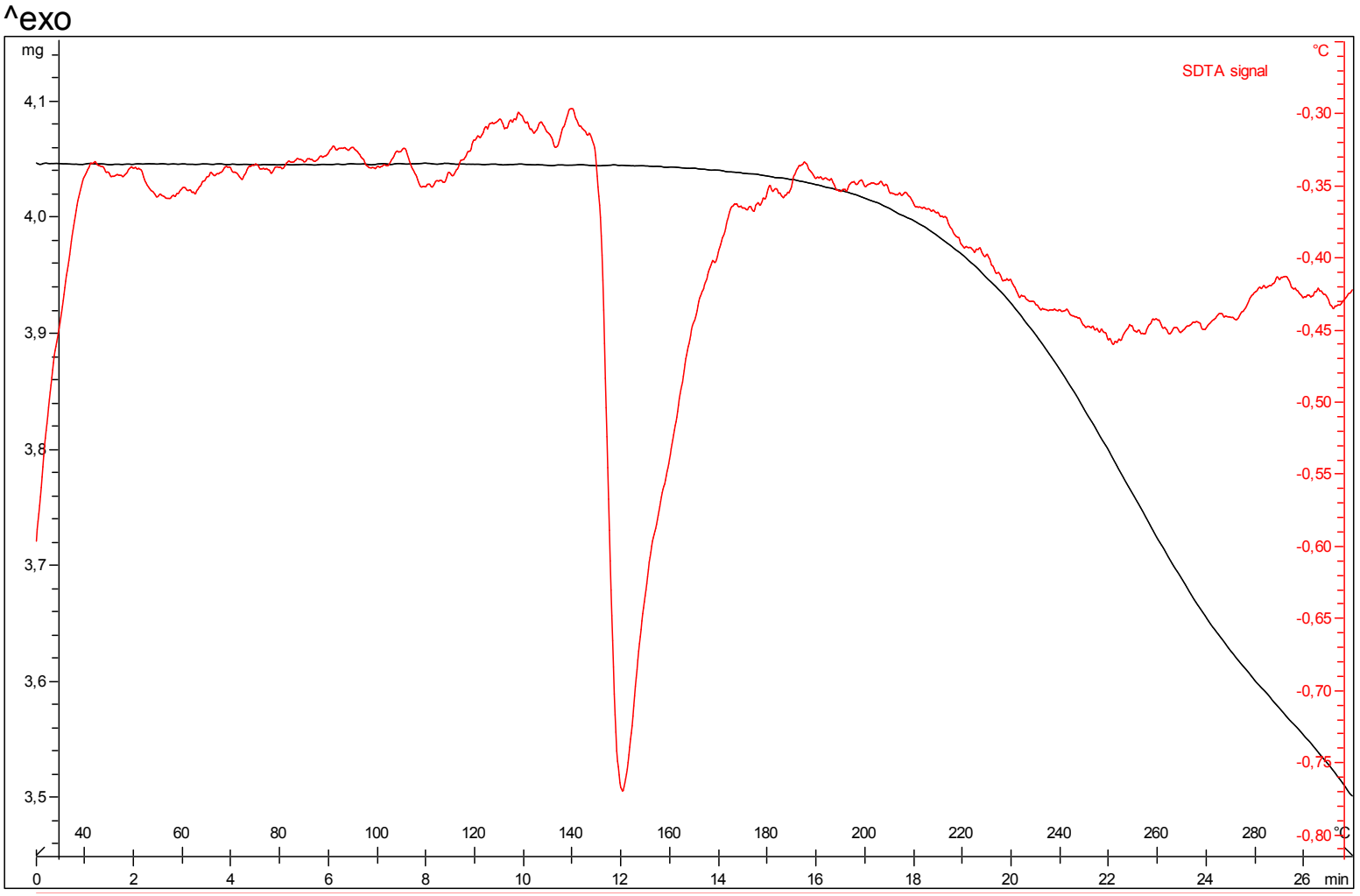


Figure S33: The XRPD of SIT-4-HBA I cocrystal has been indexed with the following proposed monoclinic cell: a=38.16(5) Å, b=14.196(6) Å, c=10.539(4) Å, β= 92.0(1)º, V=5711(8) Å ^{3} (Figures of Merit: M= 18, F= 70), with number of impurities equal to zero. A $P2_1$ space group is compatible with the cell and the cell volume is compatible with 1 molecule of CSS β-Sitosterol and 1 molecule of 4-HBA. (R_{wp}: 21.3; R_{exp}: 1.61)

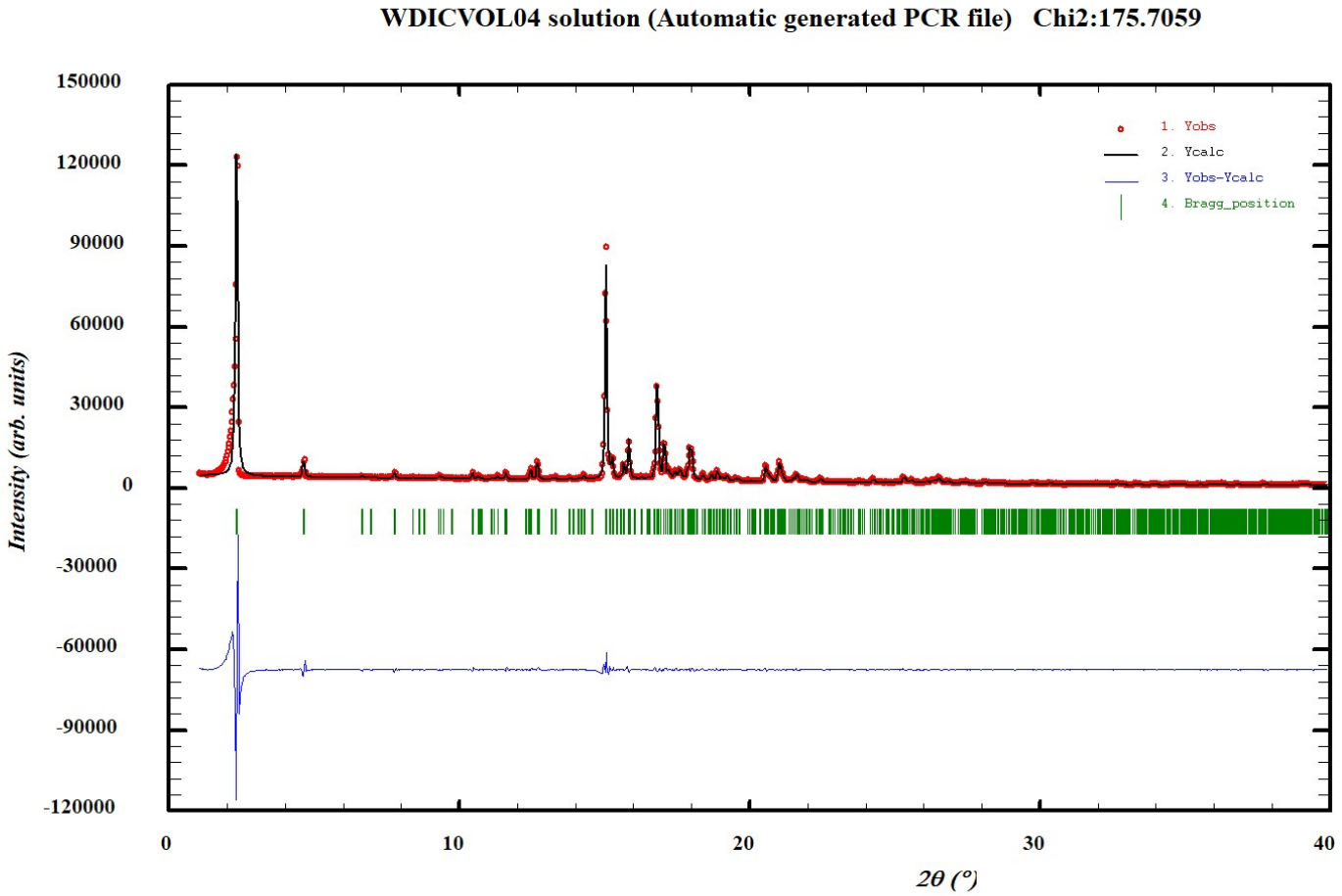


Figure S34: ^{1}H-NMR (dmso-d_6/delay: 1/pulse: 45°/scans: 32) of Form SIT-4-HBA I

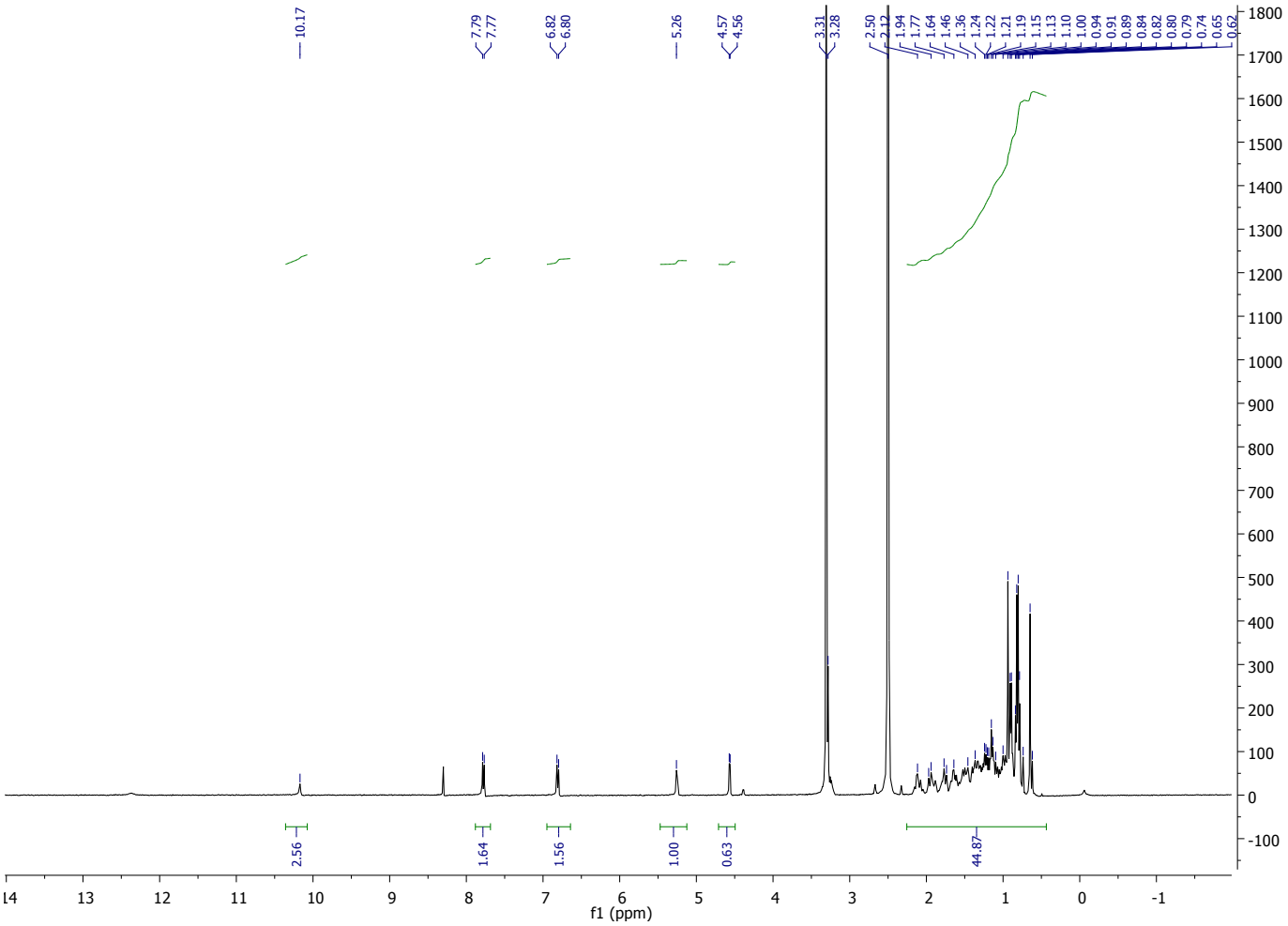


Figure S35: DSC of Form SIT-4-HBA-H_2O II

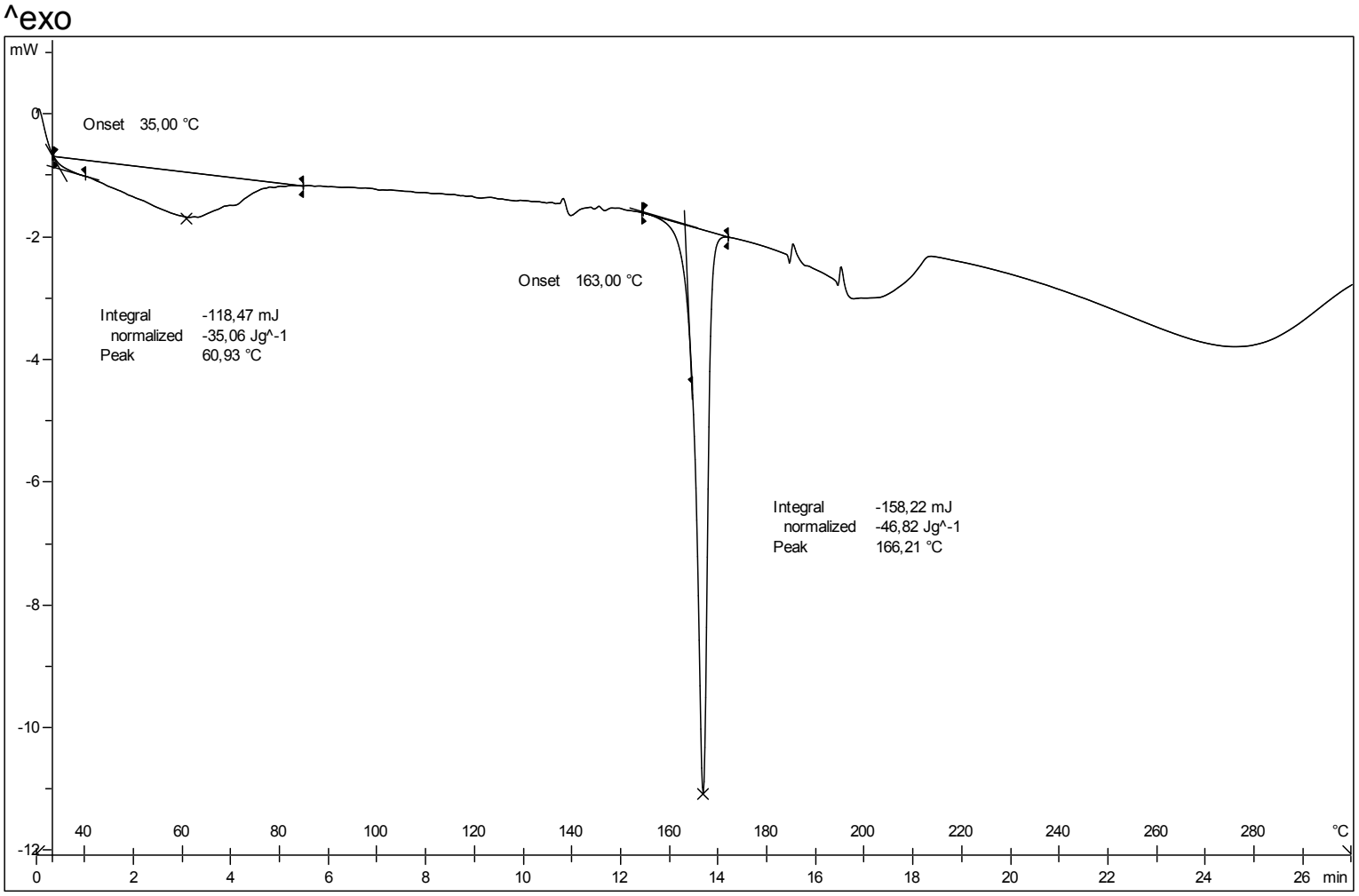


Figure S36: TGA of Form SIT-4-HBA-H_2O II: a weight loss of 1.3 % is detected from 29 ºC to 86 ºC which could be attributed to ½ molecule of water per 1 molecule of 4-HBA and 1 molecule of CSS β-Sitosterol (theoretical weight loss of 1.6%)

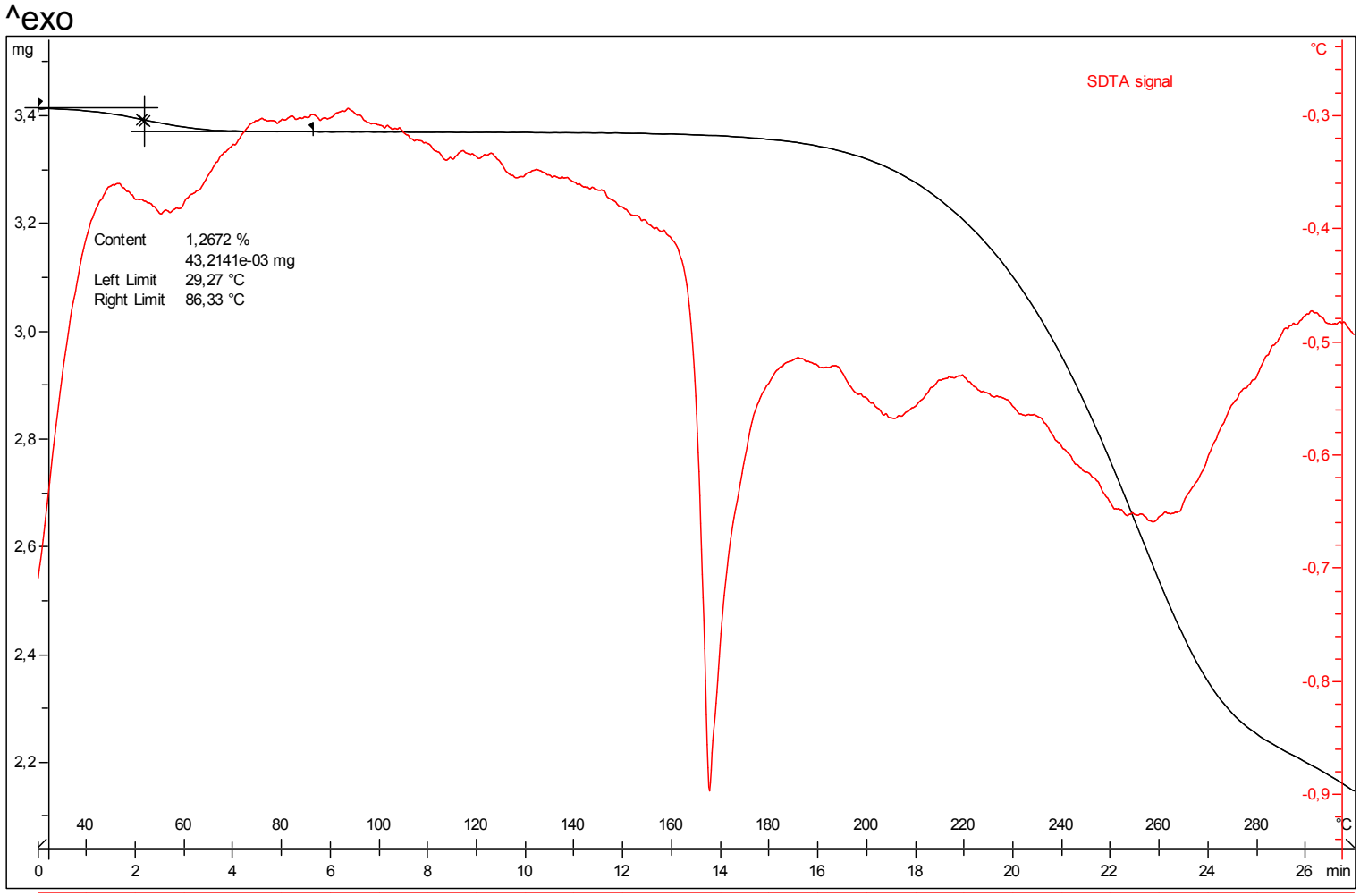


Figure S37: The XRPD of SIT-4-HBA-H_2O II cocrystal has been indexed with the following proposed triclinic cell: a=43.1(1) Å, b=7.05(1) Å, c=8.37(2) Å, α= 107.4(1), β= 108.8(2)°, γ= 89.2(1), V=2290(9) Å ^{3} (Figures of Merit: M= 17, F= 63), with number of impurities equal to zero. A *P*-1 space group is compatible with the cell and the cell volume is compatible with 1 molecule of CSS β-Sitosterol, 1 molecule of 4-HBA and ½ molecule of water (R_{wp}: 21.8; R_{exp}: 1.45)

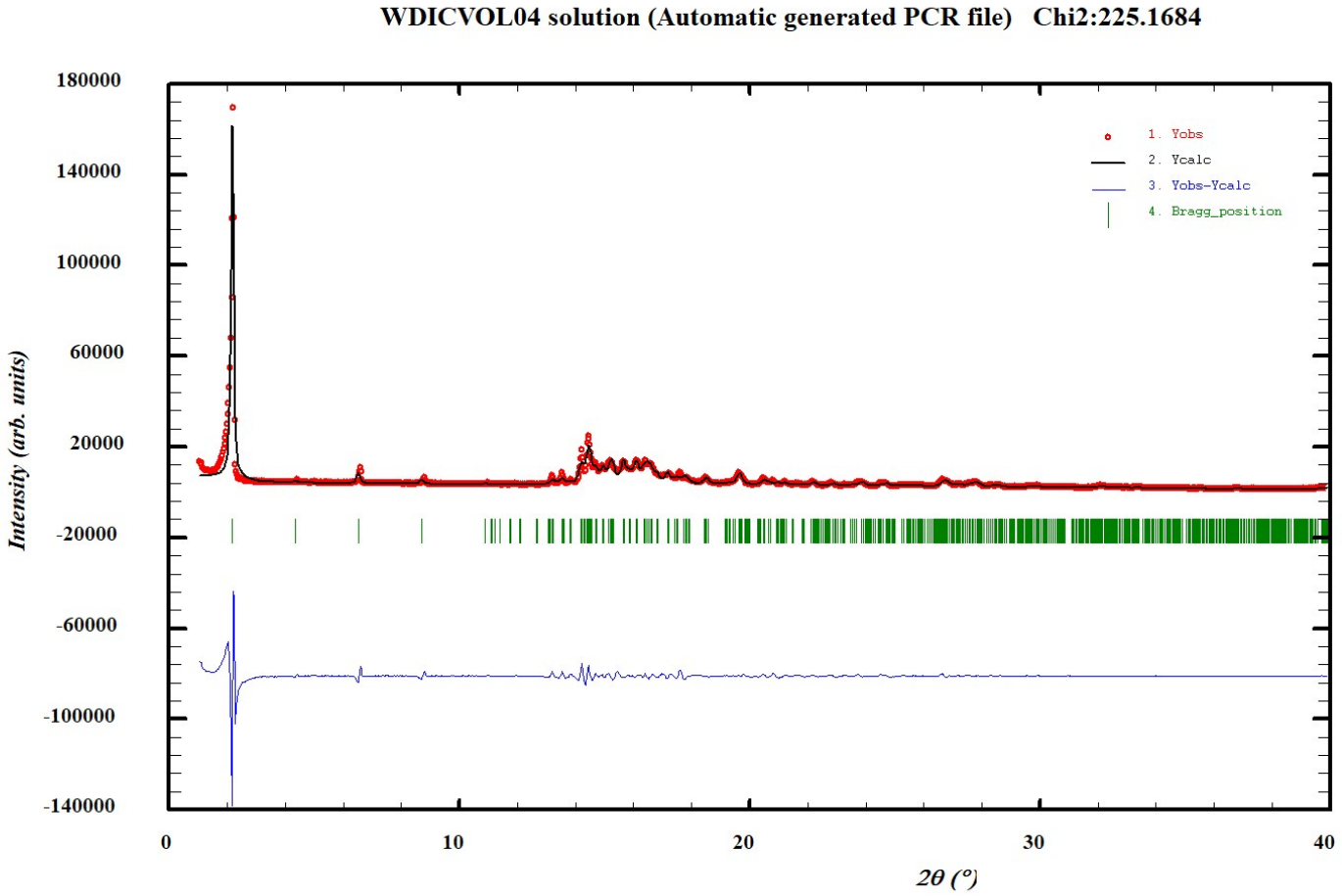


Figure S38: The XRPD of SIT-4-HBA-H_2O II cocrystal has been indexed without the first peak at 2.2 2θ with the following proposed triclinic cell: a=42.87(3) Å, b=7.083(5) Å, c=8.361(5) Å, α= 107.3(1), β= 108.8(1)º, γ= 89.06(3), V=2285(3) Å ^{3} (Figures of Merit: M= 17, F= 63), with number of impurities equal to zero. A *P*-1 space group is compatible with the cell and the cell volume is compatible with 1 molecule of CSS β-Sitosterol, 1 molecule of 4-HBA and ½ molecule of water (R_{wp}: 7.21; R_{exp}: 1.59)

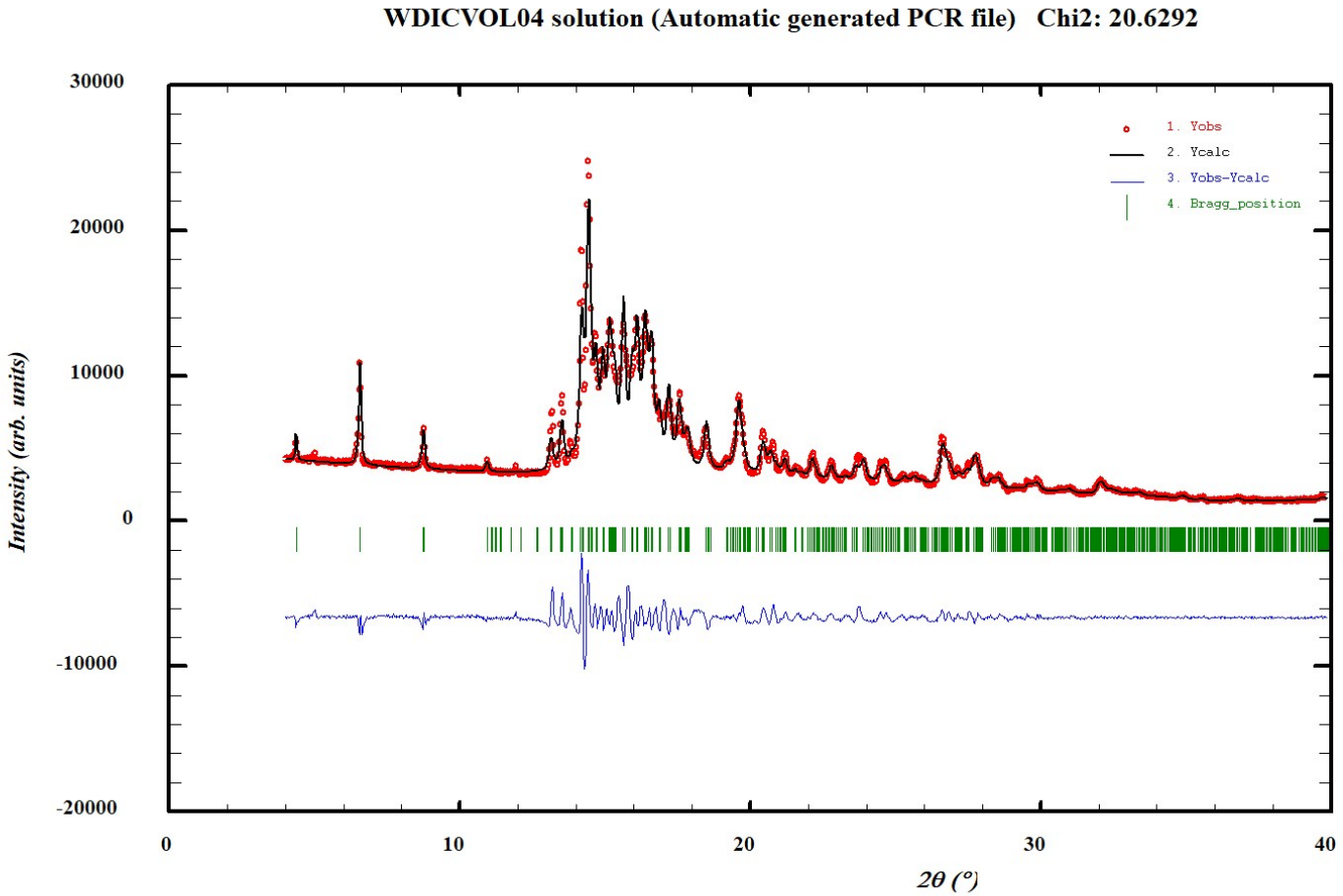


Figure S39: ^{1}H-NMR (dmso-d_6/delay: 1/pulse: 45°/scans: 32) of Form SIT-4-HBA-H_2O II

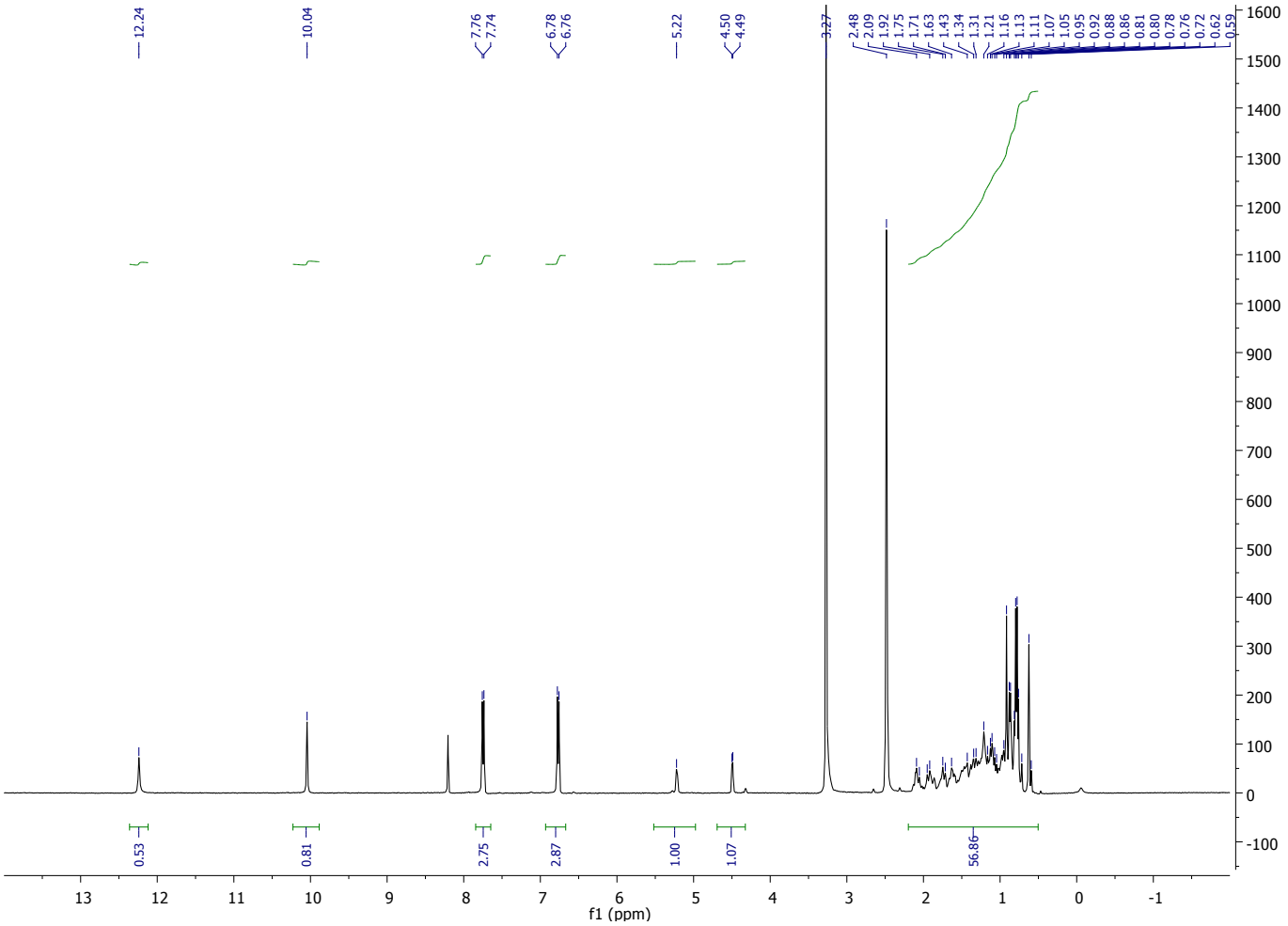


Figure S40: DSC of Form SIT-3,4-DHBA-H_2O

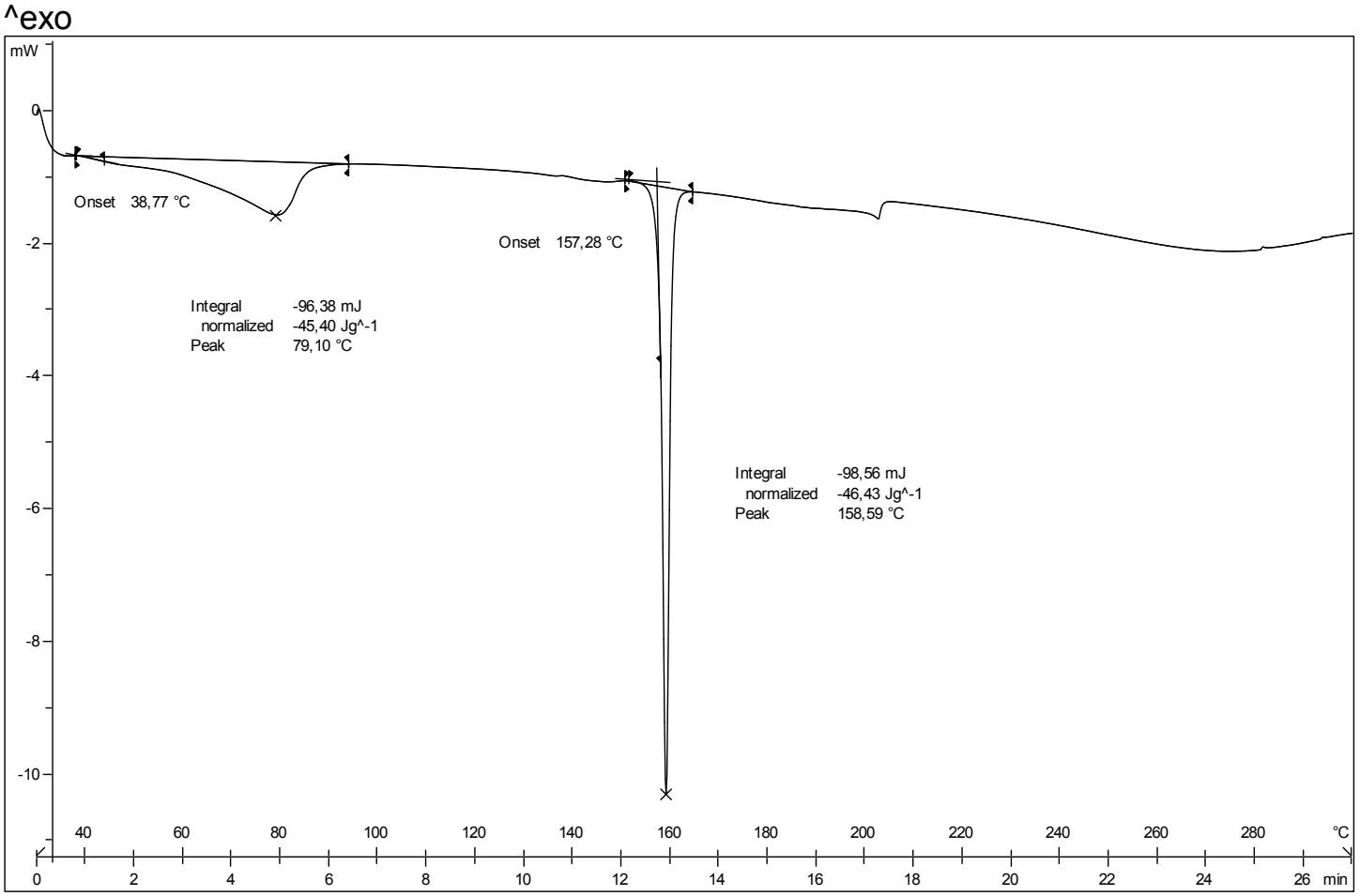


Figure S41: TGA of Form SIT-3,4-DHBA-H_2O: a weight loss of 2.1% is detected from 29 ºC to 116 ºC which could be attributed to 1 molecule of water per 2 molecules of 3,4-DHBA and 1 molecule of CSS β-Sitosterol (theoretical weight loss of 2.4%)

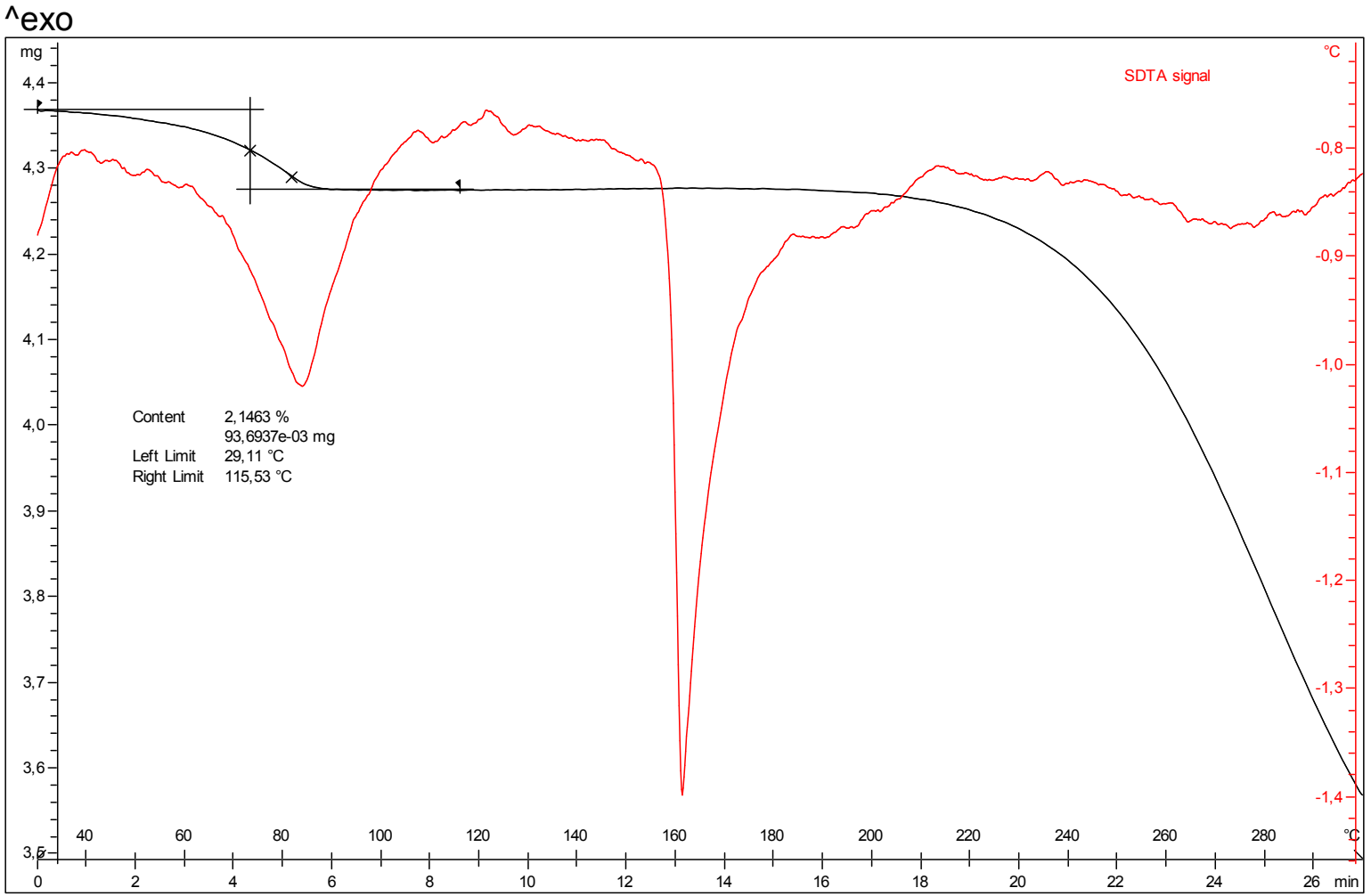


Figure S42: The XRPD of SIT-3,4-DHBA-H_2O cocrystal has been indexed with the following proposed monoclinic cell: a=38.91(4) Å, b=14.017(8) Å, c=10.701(5) Å, β= 92.41(9)º, V=5832(8) Å^3 (Figures of Merit: M= 16, F= 72), with number of impurities equal to zero. A $P2_1$ space group is compatible with the cell and the cell volume is compatible with 1 molecule of CSS β-Sitosterol, 2 molecules of 3,4-DHBA and 1 molecule of water (R_{wp}: 22.6; R_{exp}: 1.46)

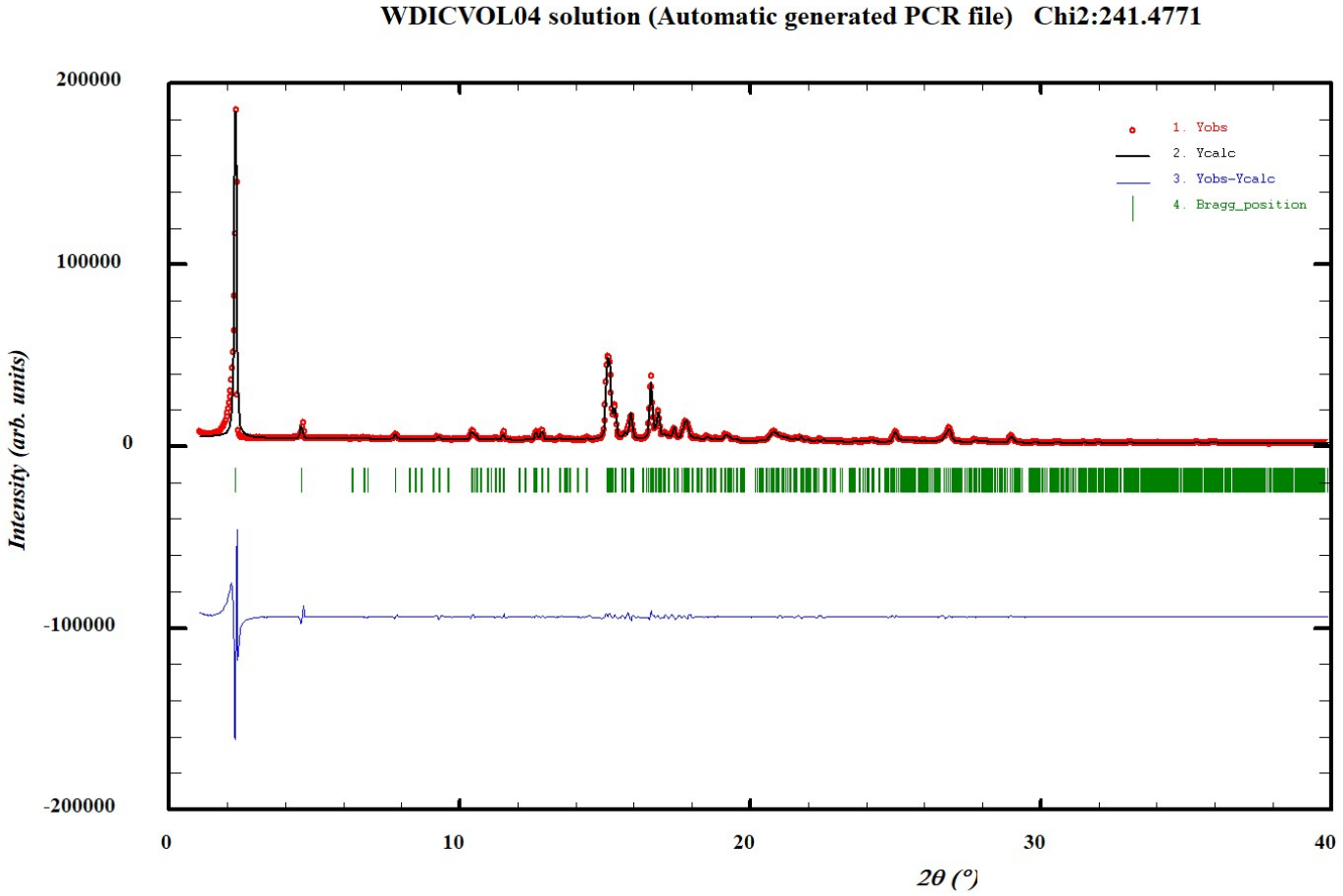
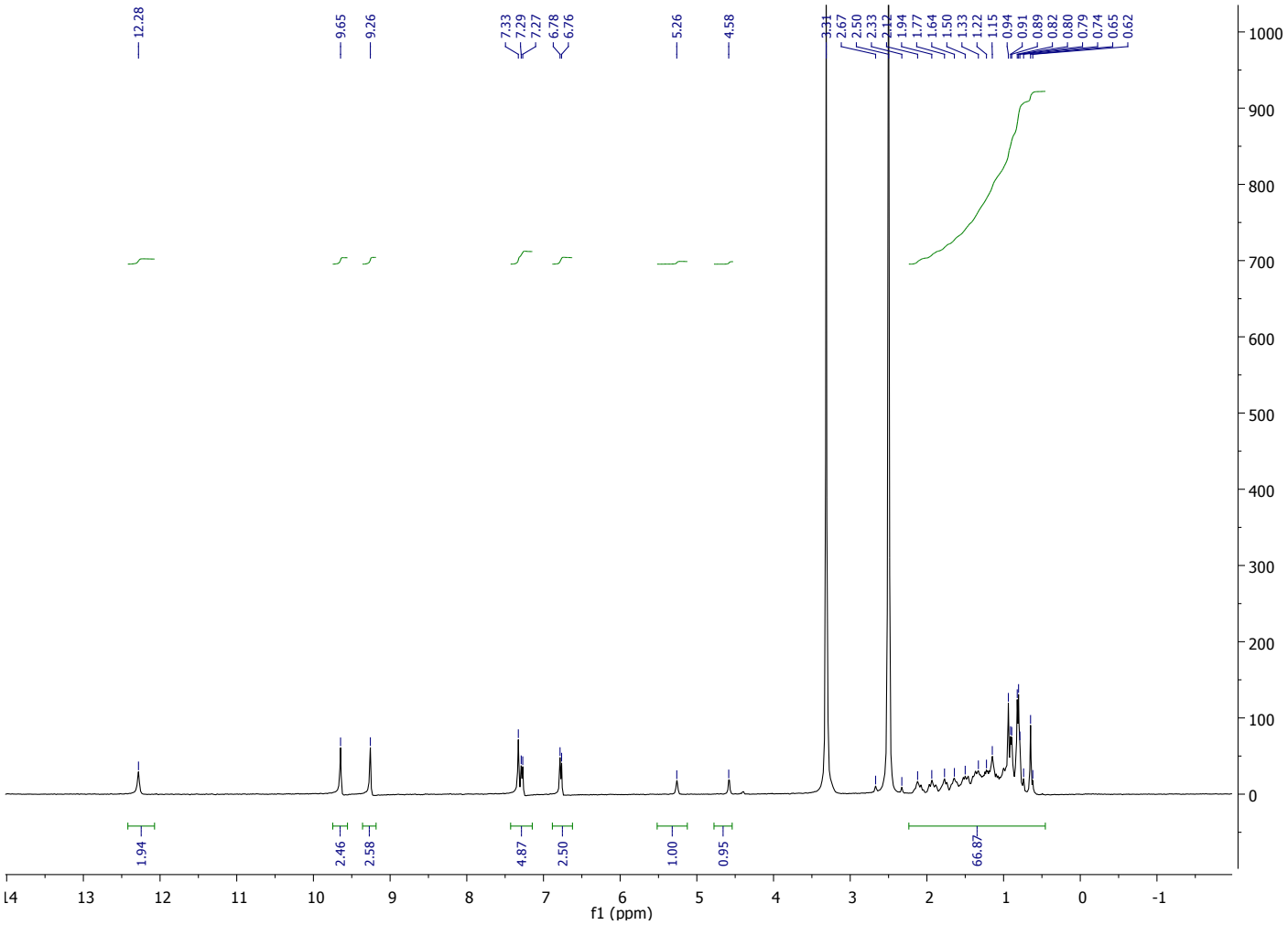


Figure S43: ^{1}H-NMR (dmso-d_6/delay: 1/pulse: 45°/scans: 32) of Form SIT-3,4-DHBA-H_2O



6. References

[1] SADABS Bruker AXS; Madison, Wisconsin, USA, 2004; SAINT, Software Users Guide, Version 6.0; Bruker Analytical X-ray Systems: Madison, WI, 1999. Sheldrick, G. M. SADABS v2.03; Area-Detector Absorption Correction; University of Göttingen: Germany, 1999. Saint, Version 7.60A; Bruker AXS 2008; SADABS, V. 2008-1, 2008.

[2] G. M. A, Sheldrick, *Acta Crystallogr., Sect. A*, 2008, **64**, 112−122.