Supplementary Materials

Metabolic profiles and fingerprints for the investigation of the influence of nitisinone on the metabolism of the yeast Saccharomyces cerevisiae

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1SM. Optimization of the extraction process

According to the literature, there is still no consensus on which extractor is the most appropriate one for the extraction of catecholamine from a yeast matrix prior to LC analysis. Based on the pre-experiment, 20 mM FA in methanol, 39% AA in water, 20 mM AA in acetonitrile, and 20 mM FA in acetonitrile were selected as extraction solvents, and their extraction efficiencies were evaluated in the presented study. As seen in Figure 1SM, the solution with 20 mM FA in acetonitrile provided the highest extraction efficiency of all analytes among all tested solvents. Therefore, for the following experiments, 8 mM FA in acetonitrile was used, which corresponds to the mobile phase component used for chromatographic analysis.

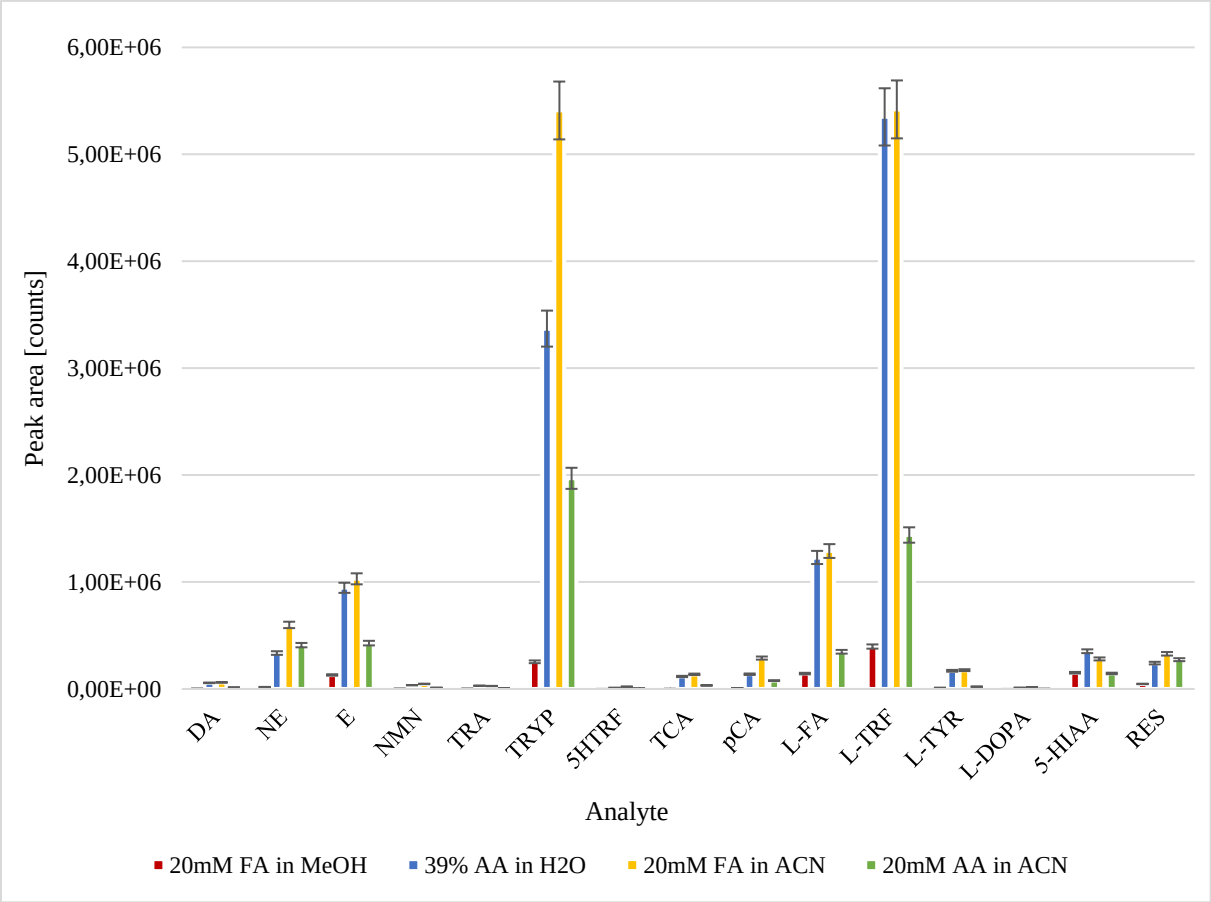

Fig. 1SM. Selection of the extraction solvent. Data are shown as a mean ± CV, n = 3.

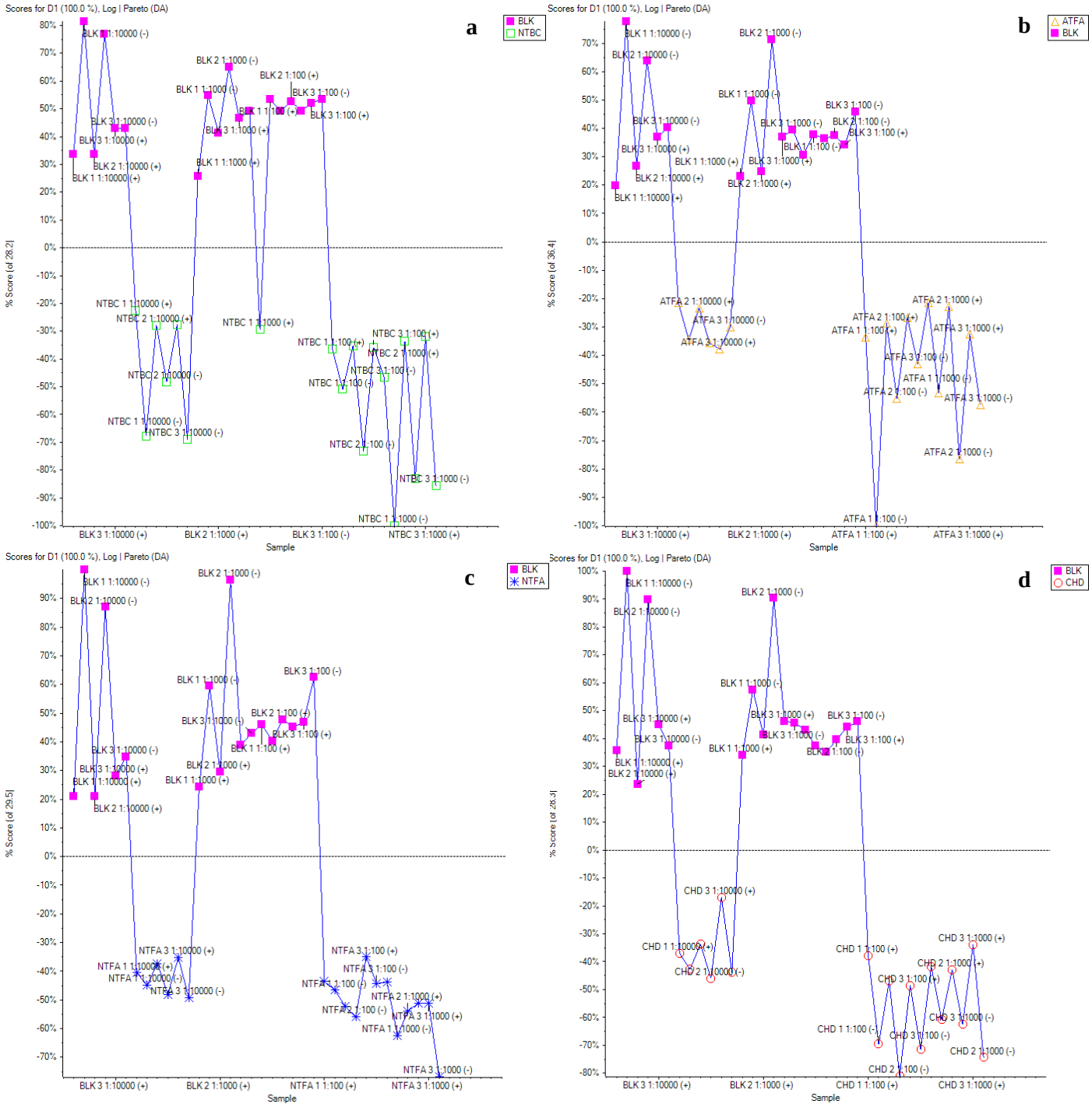

Fig. 2SM. PCA score plots of a data set obtained after 30 minutes of yeast incubation. a) Comparison of data for NTBC and blank. b) Comparison of data for ATFA and blank. c) Comparison of data for NTFA and blank. d) Comparison of data for CHD and blank.

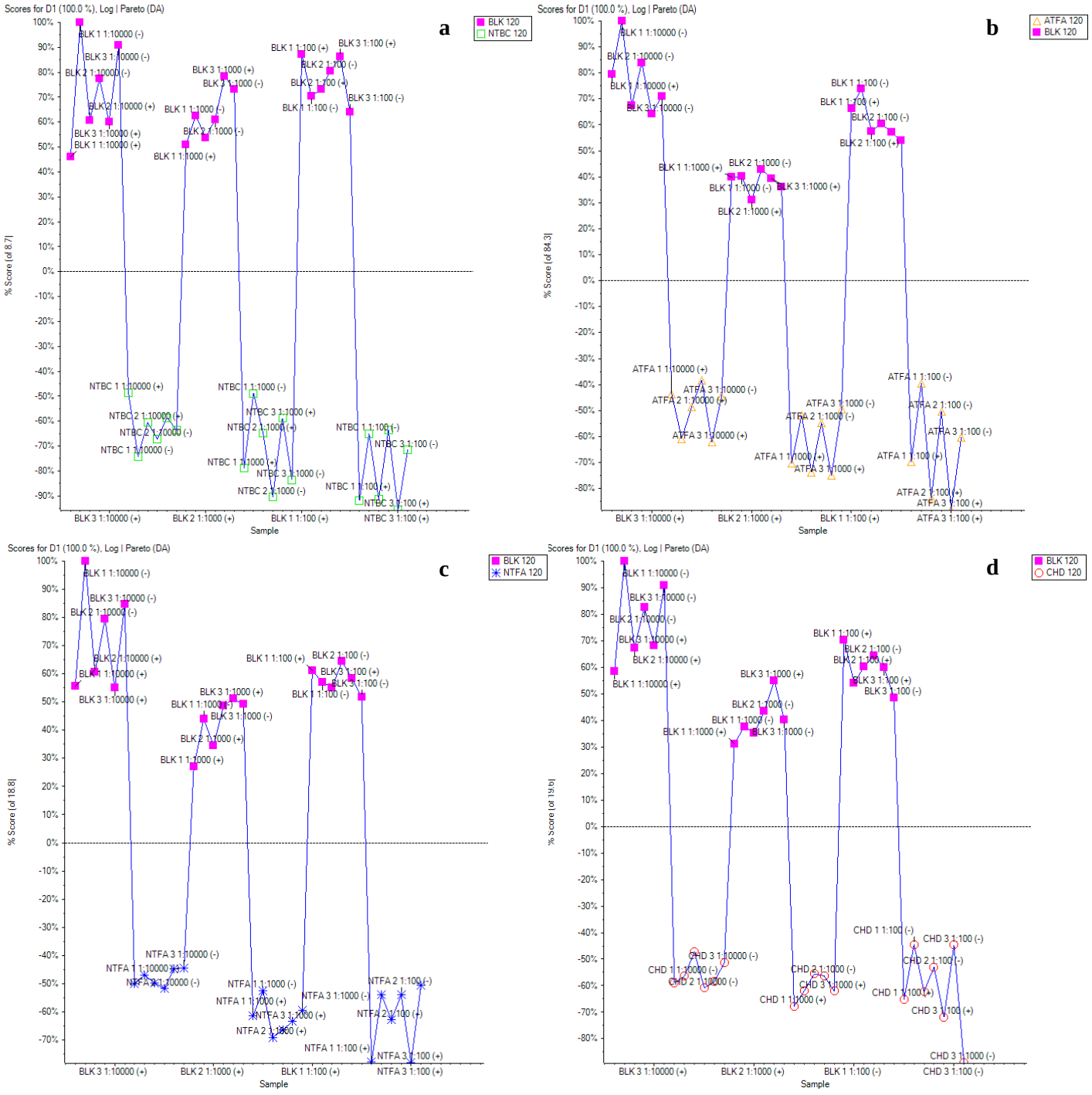

Fig. 3SM. PCA score plots of a data set obtained after 120 minutes of yeast incubation. a) Comparison of data for NTBC and blank. b) Comparison of data for ATFA and blank. c) Comparison of data for NTFA and blank. d) Comparison of data for CHD and blank.

2SM. Visualization of the comparison of blank samples obtained after 30 and 120 minutes of yeast incubation using a PCA score plot.

A comparison was made between the metabolic responses of the blank samples obtained after 30 and 120 minutes of yeast incubation, Fig. 4SM. The graph shows a significant separation of the results for 30 and 120 minutes, regardless of extract dilution and ionization mode. The noticeable change in the matrix extract during the experiment indicates that the model organism exhibited various metabolic processes during incubation. Because of this, the results obtained from the experiments are difficult to interpret, and the validation results are not fully satisfactory.

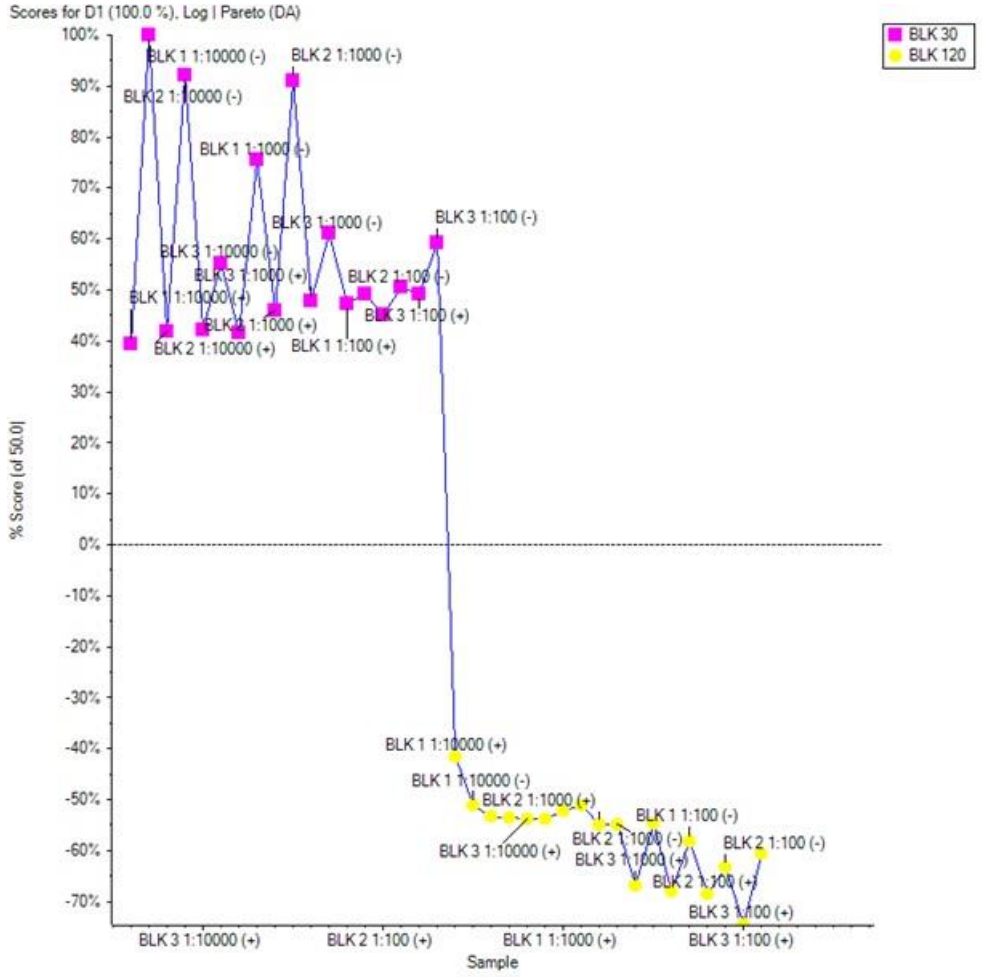

Fig. 4SM. PCA score plot of the blank sample data obtained after 30 and 120 minutes of yeast incubation.

Table 1SM. Time-scheduled gradient elution program.

Type of analysis	Time [min]	Mobile phase composition		Flow rate [mL/min]	Temperature [°C]	Injection volume [µL]
		8 mM FA in ACN [%]	8 mM FA in H_2O [%]			
Determination of NTBC and its metabolites	0.0	50	50	0.8	40	10
	5.0	90	10			
	7.0	50	50			
Determination of L-TYR, L-FA, L-TRF and its metabolites	0.0	2	98	0.8	20	10
	4.5	2	98			
	15.0	100	0			
	15.1	2	98			
	22.0	2	98			
Non-targeted analysis	0.0	10	90	0.5	25	20
	15.0	100	0			
	20.0	10	90			

Table 2SM. Source-dependent parameters for analysis.

Type of analysis	Ion spray voltage [V]	Source temperature [°C]	Nebuliser gas [psi]	Heater gas [psi]	Curtain gas [psi]
Determination of NTBC and its metabolites	-4500	550	40	50	35
Determination of L-TYR, L-FA, L-TRF and its metabolites	4500	450	30	35	35
	-4000	450	30	30	35
Non-targeted analysis	4500	400	25	20	30
	-4500	400	25	20	30

Table 3SM. Optimized MS/MS parameters for the determined compounds.

Analyte	Q1 [a] [m/z]	Q3 [b] [m/z]	DP [c] [V]	EP [d] [V]	CE [e] [V]	CXP [f] [V]
POSITIVE IONISATION						
TRA	138,1	103,2	46	9	29	6
		121,1	31		19	10
tCA	149,0	131,0	56	9	15	10
		103,1	56		29	6
DA	154,0	137,1	51	9	15	10
		91,2	31		19	10
TRYP	161,0	144,0	46	9	17	9
		115,0	46		43	8
pCA	165,0	146,9	56	9	15	10
		118,9	56		27	8
L-FA	166,0	103,0	51	9	37	6
		120,5	51		19	8
NE	170,1	152,0	31	9	11	12
		107,1	31		19	10
5-HT	176,9	160,2	51	9	13	10
		115,1	31		39	8
L-TYR	182,0	136,0	51	9	19	10
		165,0	51		15	12
NMN	184,0	165,9	36	9	11	10
		134,0	36		25	10
E	184,0	166,0	41	9	15	12
		107,0	31		31	10
L-DOPA	197,8	152,0	51	9	19	12
		107,1	51		37	6
L-TRF	205,0	188,0	46	9	15	14
		146,0	46		25	10
5-HTRF	220,9	204,1	51	9	15	10
		162,0	51		27	12
NEGATIVE IONISATION						
NTBC	327.8	281.0	-50	-10	-14	-1
		238.6			-40	-1
ATFA	203.8	159.9	-60	-10	-24	-9
		119.9			-40	-7
NTFA	233.7	190.0	-45	-10	-12	-9
		160.0			-20	-9
CHD	110.8	69.0	-55	-10	-24	-7
		57.0			-30	-5
5-HIAA	189.9	145.9	-60	-10	-16	-9
		143.9			-30	-7
RES	226.8	184.9	-90	-10	-28	-9
		143.0			-36	-9

Table 4SM. Method validation data for NTBC and its metabolites.

Analyte	Range [μg/mL]	[R^2]	LOD[a] [μg/mL]	LOQ[b] [μg/mL]	ME[c] [%]	Recovery [%]		
						Concentration 1 [μg/mL]	Concentration 4 [μg/mL]	Concentration 8 [μg/mL]
NTBC	0.2 – 8.0	0.9850	0.0069	0.0210	75	94	113	85
ATFA	0.2 – 8.0	0.9941	0.0014	0.0042	135	65	43	67
NTFA	0.2 – 8.0	0.9931	0.0025	0.0077	62	52	73	36
CHD	0.2 – 8.0	0.9937	0.0587	0.1779	8	83	35	70

a) LOD – limit of detection. b) LOQ – limit of quantification. c) ME – matrix effect.