

1 **Supplementary materials**

2
3 **Testing for Ketoprofen binding to HSA coated magnetic nanoparticles under normal conditions and**
4 **after oxidative stress**

5 **Marta Ziegler-Borowska^{1,*}, Kinga Mylkie¹, Pawel Nowak¹, Patryk Rybczynski¹, Adam Sikora²,**
6 **Dorota Chelminiak-Dudkiewicz¹ and Anna Kaczmarek-Kedziera¹**

7 ¹ Faculty of Chemistry, Nicolaus Copernicus University in Torun, Gagarina 7, 87-100 Torun, Poland,
8 martaz@umk.pl

9 ² Faculty of Pharmacy, Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in
10 Torun, dr A. Jurasza 2, 85-089 Bydgoszcz, Poland; mmars@cm.umk.pl

11 * Correspondence: martaz@umk.pl; Tel.: +48-056-611-4916 (M.Z-B.)

12
13 **1. HPLC analysis**

14
15 Based on the data containing the declared value of the analyte concentration and the value of the
16 measured signal, a simple calibration was determined, which was then used to determine the marked
17 values. The data obtained in this way were compared with the declared values in order to verify the
18 calibration function.

19
20 **Table S1.** Results of HPLC analysis for ketoprofen calibration curve

21

Concentration [mg/L]	Peak area	Measured value
1.5625	15713	1.15034
3.125	31926	2.61342
6.25	62480	5.37063
12.5	123109	10.84183
25	272487	24.32180
50	563523	50.58508
100	1132406	101.92146
200	2257901	203.48687
300	3276038	295.36423
400	4467281	402.86279
3.125	32203	2.63841
6.25	63890	5.49786
12.5	127454	11.23392
25	273815	24.44164
50	559139	50.18946
100	1124229	101.18356
200	2237574	201.65255
300	3250830	293.08945
400	4447174	401.04832

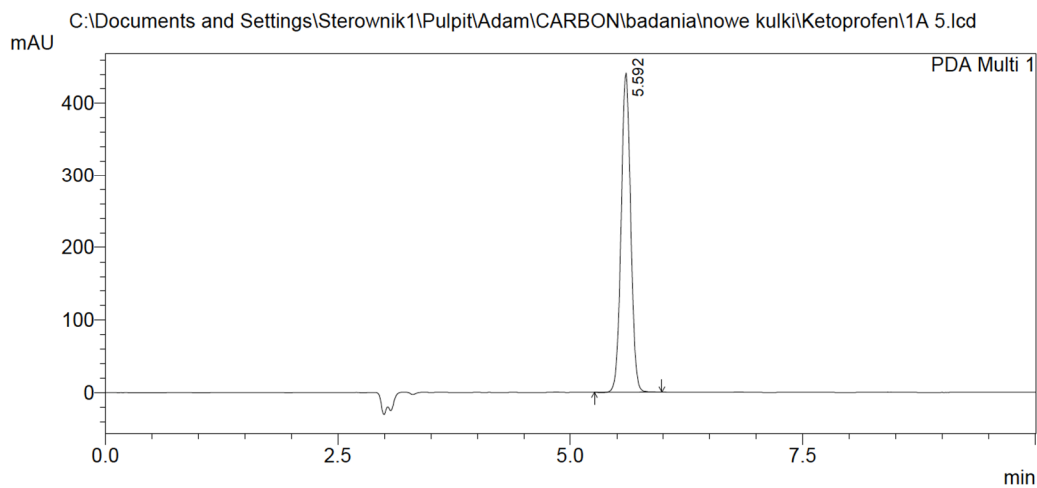
3.125	32713	2.68443
12.5	126853	11.17969
25	280259	25.02315
50	577886	51.88120
100	1155206	103.97891
200	2279431	205.42980
300	3267960	294.63526
400	4453636	401.63146

22
23
24
25

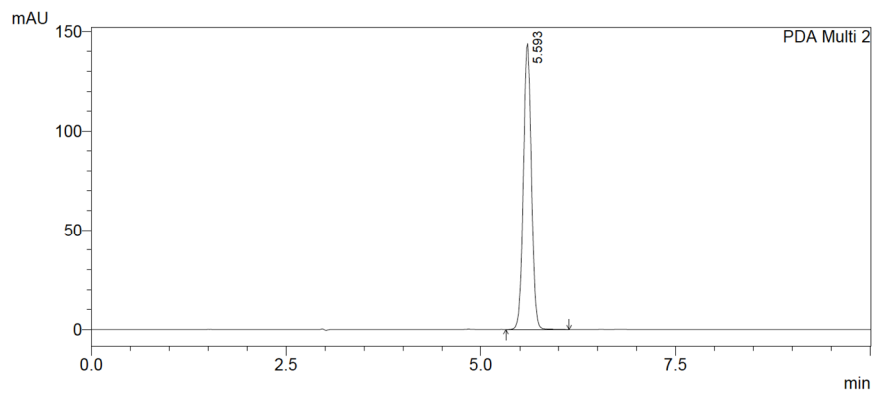
==== Shimadzu LCsolution Analysis Report ====

C:\Documents and Settings\Sterownik1\Pulpit\Adam\CARBON\badania\nowe kulki\Ketoprofen\1A 5.lcd
 Acquired by : Admin
 Sample Name : 1A 5
 Sample ID : 1A 5
 Tray# : 1
 Vail # : 1
 Injection Volume : 5 uL
 Data File Name : 1A 5.lcd
 Method File Name : method.lcm
 Batch File Name : bacz.lcb
 Report File Name : Default.lcr
 Data Acquired : 2018-02-25 12:46:06
 Data Processed : 2018-02-25 12:56:09

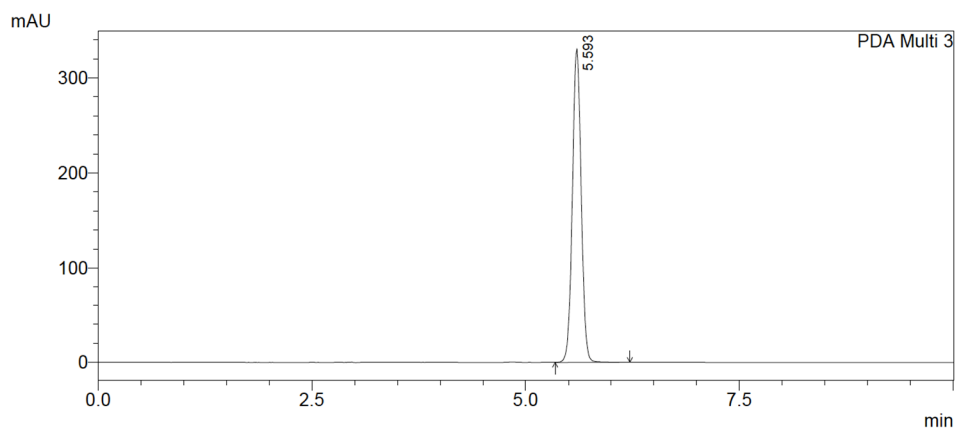
<Chromatogram>



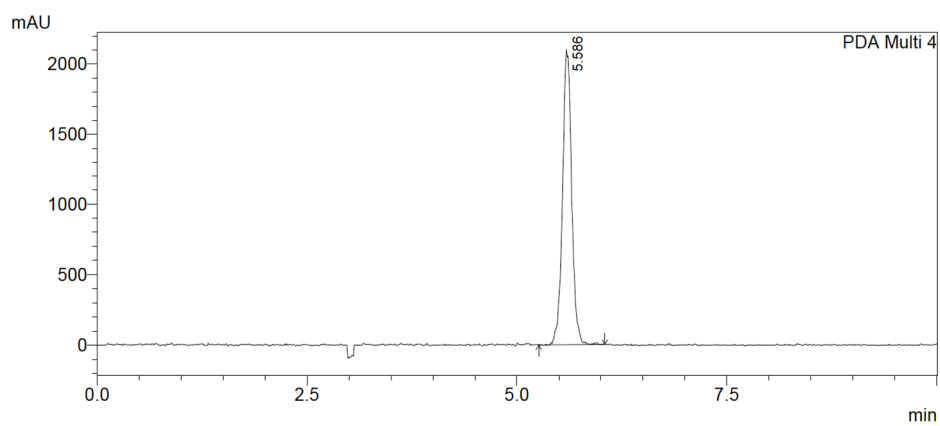
26
27
28
29
30
31



32
33



34



- 1 PDA Multi 1/220nm 4nm
- 2 PDA Multi 2/289nm 4nm
- 3 PDA Multi 3/275nm 4nm
- 4 PDA Multi 4/190nm - 800nm 4nm

35
36
37
38
39
40
41

Figure S1. Ketoprofen chromatograms

2. HSA -ketoprofen interaction results

42 **Table S2.** Results of ketoprofen interaction with HSA without oxidative stress

Nanoparticles	m_0 [mg]	m_s [mg]	bounded ketoprofen		HSA amount [g]	b [mg/g HSA]
			[%]	[mg]		
free HSA	0.02288	0.01024	55.24	0.01264	0.00166	7.60
Fe ₃ O ₄ -CS(Glu)-HSA	0.02288	0.01713	25.12	0.00575	0.00076	7.60
Fe ₃ O ₄ -CS(SqA)-HSA	0.02288	0.01686	26.29	0.00602	0.00079	7.60
Fe ₃ O ₄ -CSEt(NH ₂)-HSA	0.02288	0.01148	49.84	0.01140	0.00150	7.60
Fe ₃ O ₄ -CSEt(NH ₂) ₃ -HSA	0.02288	0.00692	69.77	0.01596	0.00210	7.60
Fe ₃ O ₄ -AS-HSA	0.02288	0.01032	54.91	0.01256	0.00165	7.60

43

44 **Table S3.** Results of HSA -ketoprofen interactions under oxidative stress induced by H₂O₂

Nanoparticles	m_0 [mg]	m_s [mg]	bounded ketoprofen		b [mg/g HSA]
			[mg]	[mg]	
free HSA	0.02288	0.01283		0.01005	6.04
Fe ₃ O ₄ -CS(Glu)-HSA	0.02288	0.01826		0.00462	6.12
Fe ₃ O ₄ -CS(SqA)-HSA	0.02288	0.01807		0.00481	6.08
Fe ₃ O ₄ -CSEt(NH ₂)-HSA	0.02288	0.01373		0.00915	6.10
Fe ₃ O ₄ -AS-HSA	0.02288	0.01284		0.01004	6.08
Fe ₃ O ₄ -CSEt(NH ₂) ₃ -HSA	0.02288	0.01005		0.01283	6.11

45

46

47

48 **Table S4.** Results of HSA -ketoprofen interactions under oxidative stress induced by hydroxyl radical

Nanoparticles	m_0 [mg]	m_s [mg]	bounded ketoprofen		b [mg/g HSA]
			[mg]	[mg]	
free HSA	0.02288	0.01165		0.01123	6.75
Fe ₃ O ₄ -CS(Glu)-HSA	0.02288	0.01761		0.00528	6.98
Fe ₃ O ₄ -CS(SqA)-HSA	0.02288	0.01769		0.00520	6.56
Fe ₃ O ₄ -CSEt(NH ₂)-HSA	0.02288	0.01280		0.01008	6.72
Fe ₃ O ₄ -AS-HSA	0.02288	0.01164		0.01124	6.80
Fe ₃ O ₄ -CSEt(NH ₂) ₃ -HSA	0.02288	0.00864		0.01424	6.78

49

50

51

52

53

54

55

56

57 **Table S5.** Results of HSA -ketoprofen interactions under oxidative stress induced by Chloramine-T

Nanoparticles	m₀ [mg]	m_s [mg]	bounded ketoprofen [mg]	b [mg/g HSA]
free HSA	0.02288	0.01623	0.00665	4.00
Fe ₃ O ₄ -CS(Glu)-HSA	0.02288	0.01980	0.00308	4.07
Fe ₃ O ₄ -CS(SqA)-HSA	0.02288	0.01975	0.00313	3.95
Fe ₃ O ₄ -CSEt(NH ₂)-HSA	0.02288	0.01683	0.00605	4.03
Fe ₃ O ₄ -AS-HSA	0.02288	0.01628	0.00660	3.99
Fe ₃ O ₄ -CSEt(NH ₂) ₃ -HSA	0.02288	0.01446	0.00842	4.01

58

59