Electronic Supplementary Material (ESI) for Analytical Methods.

Development of competitive 'pseudo'-ELISA assay for measurement of cocaine and its metabolites using molecularly imprinted polymer nanoparticles

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Supporting Information

1. Glass beads modification

Modification scheme of the surface glass beads with 3-aminopropyltrimethyloxysilane (Figure S1) and immobilisation of the template on the glass beads (Fig. S2) are presented below.

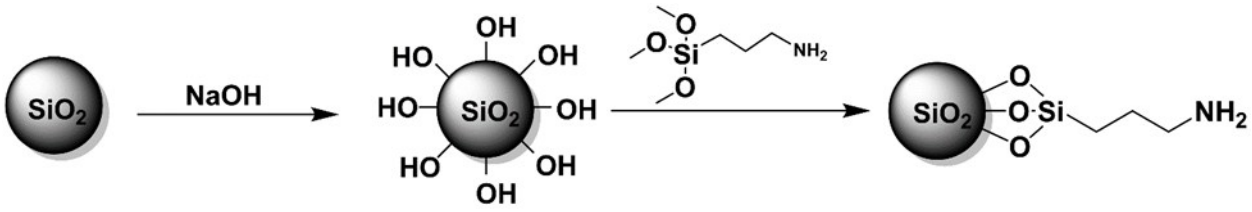

Figure S1. Modification of the glass beads with 3-aminopropyltrimethyloxysilane.

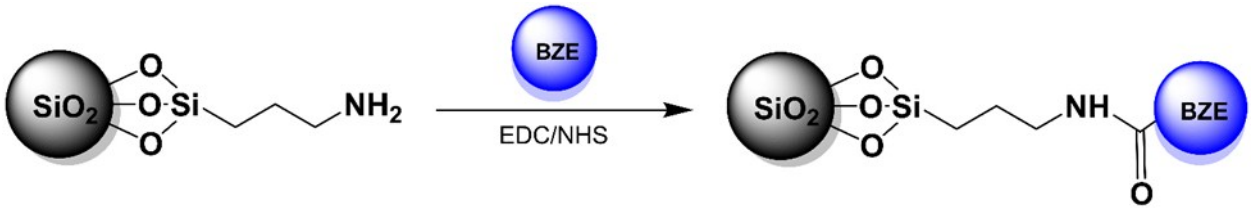

Figure S2. Immobilization of the template on the modified glass beads.

2. NanoMIPs immobilisation in the microplates wells

On the Fig. S3 we show a schematic arrangement of nanoparticles in microplate. Imprinted polymer nanoparticles (40 µL, 0.06 mg mL^{-1}) were dispensed into the wells of a 96-well polystyrene microplate, and left to dry overnight at ambient temperature.

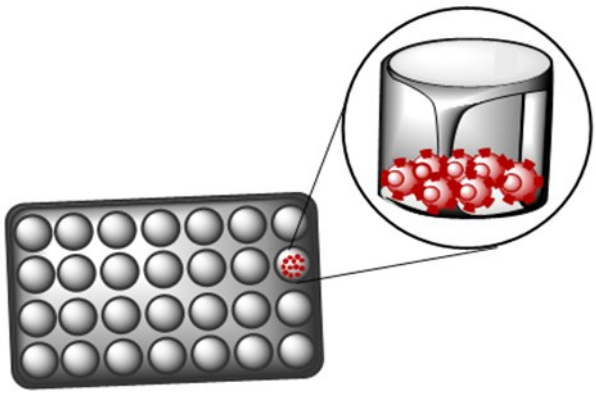

Figure S3. Immobilisation of nanoMIPs on the microplate wells.

3. NanoMIPs size

The determination of size distribution of the nanoMIPs was made using the Dynamic Light Scattering (DLS) technique. The typical size distribution of nanoparticles in aqueous format is shown in Fig. S4.

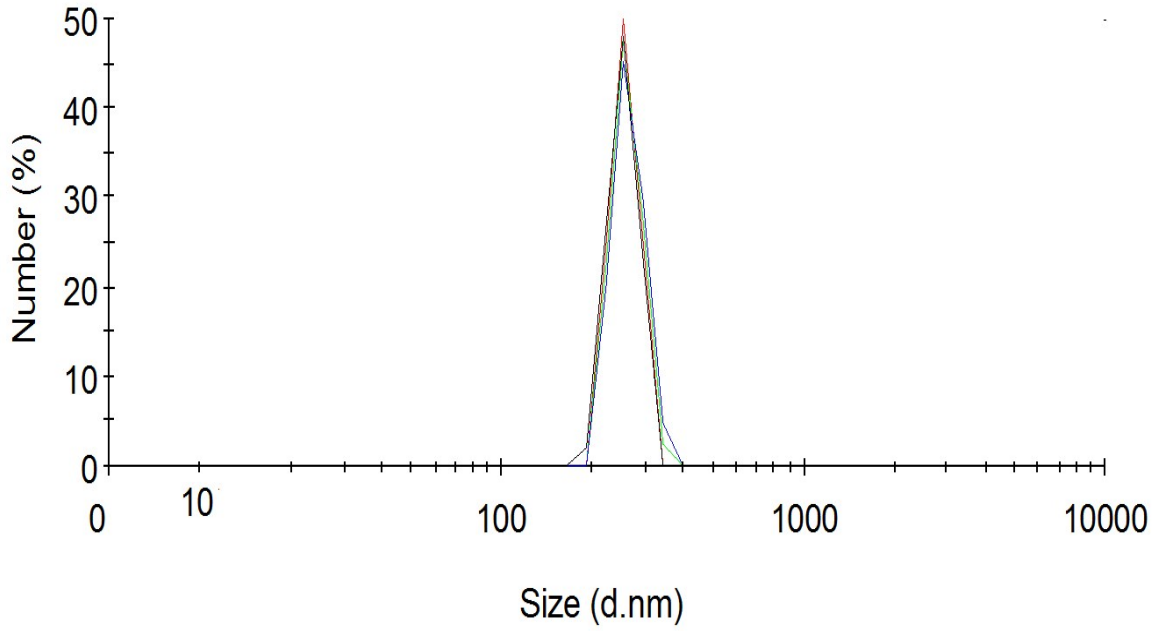

Figure S4. Particles size distribution of nanoMIPs measured by DLS.

4. Optimisation of the blocking conditions

Optimisation of the blocking conditions was performed using response surface methodology for studying the correlation between response and factors. Box-Behnken design (BBD) was employed to evaluate the main interaction and quadratic effects of the protein, the surfactant, and time of incubation of the blocking solution in the ELISA assay. The parameters, specifically, the ranges and constrains for each factor , responses values, design comprising 15 runs and analysis of variance for the response surface quadratic model for optimisation of the blocking solution are presented in Table S1 and Table S2, respectively. Three-dimensional response surface contour plots of the signal between MIPs and wells without MIPs is shown on Figure S6.

Table S1 Factors and responses in Box-Behnken experimental design

Factor	Level		
	Low (-1)	Medium (0)	High (+1)
F_1: BSA concentration (%)	0.1	0.3	0.5
F_2: Tween 20 (%)	0.5	0.75	1
F_3: Incubation time (min)	60	90	120
Responses	**Constraints**		
R_1: Relation of the signal between MIPs and NIPs	Maximize		
R_2: Relation of the signal between MIPs and without MIPs	Maximize		

Blocking solution					
	Factors			Responses	
Exp No	F_1 (%)	F_2 (%)	F_3 (min)	R_1	R_2
1	0.10	0.50	90.00	2.55	9.57
2	0.50	0.50	90.00	2.31	3.99
3	0.10	1.00	90.00	2.45	10.50
4	0.50	1.00	90.00	1.64	3.39
5	0.10	0.75	60.00	2.65	14.76
6	0.50	0.75	60.00	2.40	8.03
7	0.10	0.75	120.00	3.39	12.24
8	0.50	0.75	120.00	1.94	2.04
9	0.30	0.50	60.00	3.13	9.36
10	0.30	1.00	60.00	2.63	6.48
11	0.30	0.50	120.00	2.10	3.76
12	0.30	1.00	120.00	3.13	2.17
13*	0.30	0.75	90.00	1.50	1.64
14*	0.30	0.75	90.00	1.47	1.40
15*	0.30	0.75	90.00	1.26	1.65

*Center points in the design

Table S2 Analysis of variance for the response surface quadratic model for optimization of the blocking solution

Source	Sum of squares	Df	Mean square	*F*-values	P-values
Model	260.14	6	43.36	36.81	0.000
F_1: BSA concentration	109.67	1	109.67	101.31	0.0002
F_2: Tween 20	2.14	1	2.14	1.98	0.2185
F_3: Incubation time	42.41	1	42.41	39.18	0.0015
F_1F_1	76.84	1	76.84	70.99	0.0004
F_1F_2	0.58	1	0.58	0.54	0.4952
F_1F_3	3.01	1	3.01	2.78	0.1563
F_2F_2	2.01		2.01	1.85	0.2315
F_2F_3	0.42	1	0.42	0.38	0.5624

F_3F_3	36.45	1	36.45	33.68	0.0021

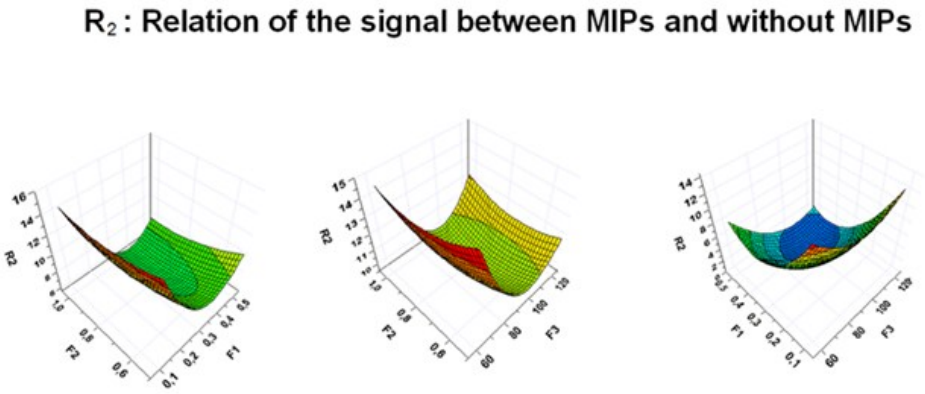

Figure S5. Three-dimensional response surface contour plots of the signal between MIPs and wells without MIPs.

5. Optimisation of the conjugate concentration

To choose the best concentration of the blocking solution blank assay was made. The difference in absorbance between signal from the well with and without nanoMIPs together with absorbance value were analysed (Table S3). The selection of the HRP conjugate dilution 1:100 was based on the higher absorption values for the wells containing MIPs (1.728 ± 0.016 a.u.) that allowed to achieve higher sensitivity of the assay.

Table S3. Absorbance at 450 nm of the dilutions of the HRP-BZE

HRP-BZE			
Dilutions	**MIPs**	**Without MIPs**	**ABS MIPs/ABS without MIPs**
1:100	1.728 ± 0.016	0.880 ± 0.014	1.964
1:200	0.964 ± 0.039	0.475 ± 0.015	2.029
1:400	0.561 ± 0.007	0.246 ± 0.002	2.280
1:800	0.201 ± 0.001	0.116 ± 0.001	1.733

6. Pseudo-ELISA for measurement the cocaine in blood serum using nanoMIPs prepared in water

A sample of human serum was spiked with several concentrations of cocaine and tested using the ELISA. The results are shown in Fig. S7. It shows only a weak response for cocaine in the concentration range between 10^{-12} and 10^{-9} M, which can be explained by high interference from the blood serum matrix. It was decided that nanoMIPs for the measurement

of such small molecules as cocaine in the complex biological samples should be made in organic solvent in order to ensure higher affinity of the interactions.

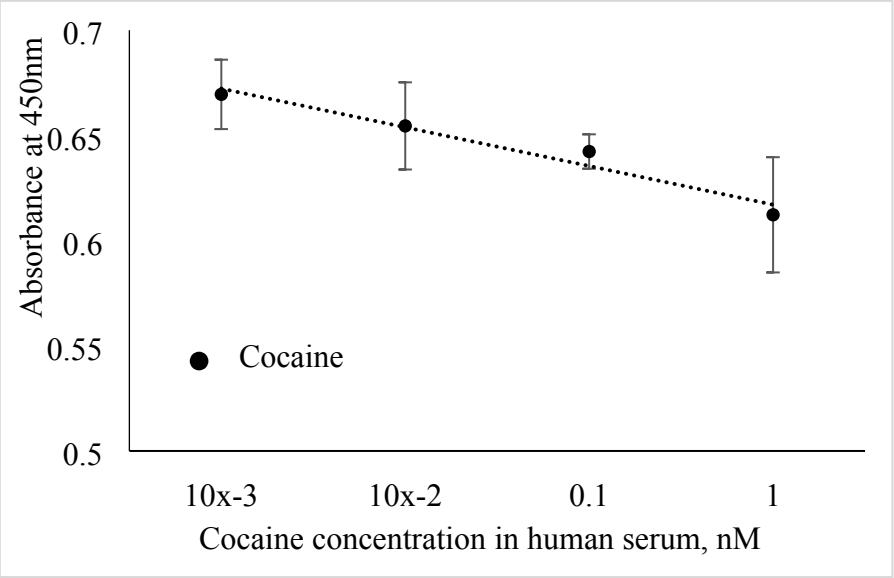

Figure S6. Competitive pseudo-ELISA assay for COC in human serum based on nanoMIPs made in water polymerisation.