Electronic Supplementary Information

The selective response of templated polymer for the cationic drug pentamidine: implications from molecular simulations and experimental data

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Polymers

Briefly, 4-methoxybenzamidine (the template molecule, **T**), the appropriate functional monomer and ethylene glycol dimethacrylate, EGDMA (the cross-linker) were dissolved in methanol (the porogen) in thick-walled glass tubes. The molar ratio of the template to the functional monomer and the cross-linker was equal to 1:4:20. At the end, the polymerization initiator, 2,2'-azobisisobutyronitrile, was added. The homogeneous solutions were purged with nitrogen for approximately five minutes and then the glass tubes were sealed. Subsequently, the polymerization was carried out under a nitrogen atmosphere for 24 hours at 64 ^{o}C. The bulk rigid polymers were ground in a mortar with a pestle and wet-sieved into particles less than 45 μm in diameter. Fine particles were separated by repeatedly decanting them in acetone. **T** was removed from the polymer through continuous extraction in a Soxhlet apparatus (for between 24 and 36 hours, 80 mL, methanol), followed by extensive washing with a methanol – formic acid system (99.5:0.5 v/v). The particles were then dried in a vacuum at room temperature.

Description of the analytical procedures for pentamidine, A1

MISPE of **A1** from standard solution

The solid phase extraction was carried out on a Macherey-Nagel manifold. Empty polypropylene 1 mL SPE columns, secured by glass-fibre frits, were filled with 25 mg of MIP**1** particles. The following steps of the SPE protocol were applied to each column: conditioning (1 mL, methanol – water, 85:15 v/v), loading (2.5 mL, methanol – water, 85:15 v/v standard solution, concentration 1 μmol L^{-1} equal to 34 μg L^{-1}), washing (0.5 mL, methanol – water, 85:15 v/v) and eluting (0.5 mL, methanol – formic acid, 99.5:0.5 v/v). The flow rate of each SPE step was 1 mL min^{-1}. The conditioning, loading, washing and eluting fractions were collected, evaporated to dryness and dissolved in 500 μL of ultra-pure water. An aliquot of 20 μL was used to determine the amount of **A1** through HPLC analysis. The

amount of **A1** that was bound to MIP**1** during the loading step was calculated by subtracting the unbound amount from the initial amount of **A1** in the standard solution. Triplicate cartridges were used for the SPE.

Characterization of analytical performance

The analytical scheme for the analysis of **A1** in spiked human urine was characterized by the determination of linearity, LOD, LOQ and recovery. The linearity of the calibration lines for **A1** was analysed within the range of 0.5 – 10 μmol L^{-1} for human urine spiked with standard solutions of **A1** at five different concentrations of each compound (0.5, 1, 2, 5 and 10 μmol L^{-1}). The peak area ratios of **A1** were plotted against the corresponding concentrations and the calibration lines were constructed by means of the least-squares method. The LOD and LOQ values for **A1** were determined as the signal-to-noise (S/N) ratio of 3 and 10, respectively.

In all of the experiments, the sample of human urine was monitored prior to analysis to exclude the presence of **A1** before spiking. The total recoveries of the analytical scheme were calculated by comparing them to the amount of **A1** present in the human urine before the SPE loading step. Thus, so-called "free" **A1** (not adsorbed to proteins) was determined in the spiked human urine before the loading of the sample.

Analysis of A1 in spiked human urine

The solid phase extraction protocol was used to separate **A1** from the spiked human urine. The human urine sample was collected from a healthy volunteer (aged 47, male), who had not been the subject of any drugs treatment for one week prior to the collection. The sample was analysed within six hours after the collection without being frozen. The sample was diluted with ultra-pure water (5:95 v/v) and was analysed using HPLC.

Empty polypropylene 1 mL SPE columns, secured by glass-fibre frits, were filled with 25 mg of MIP**1** particles or the non-imprinted commercial sorbent MCX Oasis®. Then, SPE was carried out according to the optimized procedure with the loading step consisting of 0.5

mL of human urine sample diluted with ultra-pure water (1:99 v/v) and spiked with the standard solution at a concentration of 0.5 μmol L^{-1} (170 μg L^{-1}), followed by washing (0.25 mL) and eluting (0.5 mL). The eluates were collected and the **A1** analysis was carried out using HPLC-UV. The total recoveries were calculated. Triplicate cartridges of each sorbent were used in the SPE.

Table S1. MISPE protocol for the separation of pentamidine, **A1** on MIP**1** (n = 3).

Step of extraction	Amount of **A1** (μg ± S.D.)
Conditioning (1 mL, methanol – water, 85:15 v/v)	Found
	< L.Q.[a]
Loading (2.5 mL, methanol – water, 85:15 v/v standard	Bound
solution of 1 μmol L^{-1} equal to 340 μg L^{-1})	0.57 ± 0.09
Washing (0.5 mL, methanol – water, 85:15 v/v)	Found
	0.031 ± 0.009
Eluting (0.5 mL, formic acid – methanol, 0.5:99.5 v/v)	Found
	0.53 ± 0.04

[a] below limit of quantification

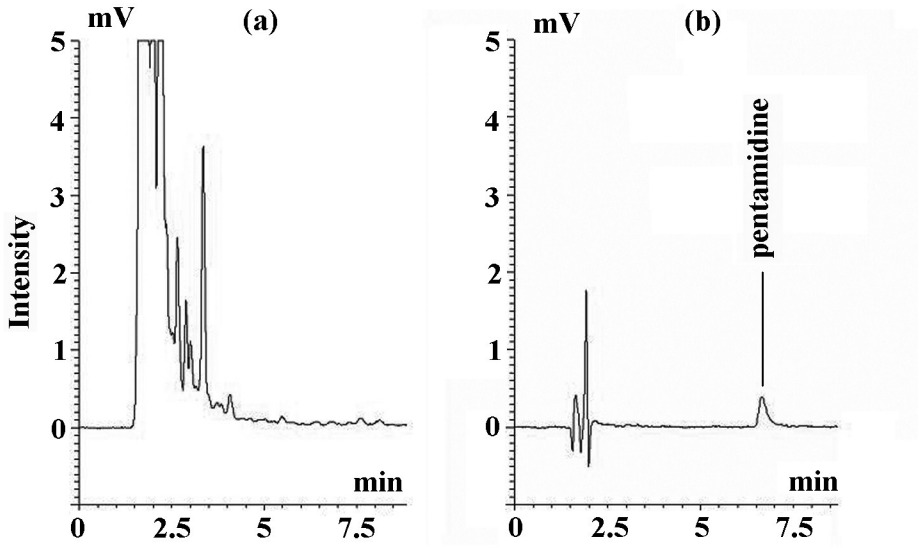

Fig. S1. Chromatograms of neat human urine (a) and the elution fraction after the MISPE of **A1** from spiked human urine (b).