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# **Stoichiometric Molecularly Imprinted Polymers for the Recognition of Anti-Cancer Pro-drug Tegafur**

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**Highlights**

- Molecularly Imprinted Polymers for anti-cancer pro-drug tegafur were prepared.
- Stoichiometric imprinting and a custom-made functional monomer were utilised.
- High selectivity for tegafur vs. other analytes was demonstrated.
- Clean extracts and recoveries of up to 96% from aqueous samples were achieved.

## Abstract

Molecularly Imprinted Polymers (MIPs) targeting tegafur, an anti-cancer 5-fluorouracil pro-drug, have been prepared by stoichiometric imprinting using 2,6-*bis*(acrylamido)pyridine (BAAPy) as the functional monomer. Solution association between tegafur and BAAPy was studied by  $^1\text{H}$  NMR titration, which confirmed the formation of 1:1 complexes with an affinity constant of  $574 \pm 15 \text{ M}^{-1}$  in  $\text{CDCl}_3$ . Evaluation of the synthesised materials by HPLC and equilibrium rebinding experiments revealed high selectivity of the imprinted polymer for the pro-drug versus 5-fluorouracil and other competing analytes, with maximum imprinting factors of 25.3 and a binding capacity of  $45.1 \mu\text{mol g}^{-1}$ . The synthesised imprinted polymer was employed in solid-phase extraction of the pro-drug using an optimised protocol that included a simple wash with the porogen used in the preparation of the material. Tegafur recoveries of up to 96% were achieved from aqueous samples and 92% from urine samples spiked with the template and three competing analytes. The results demonstrate the potential of the prepared polymers in the pre-concentration of tegafur from biological samples, which could be an invaluable tool in the monitoring of patient compliance and drug uptake and excretion.

**Keywords:** tegafur, 5-fluorouracil, bioanalysis, molecularly imprinted polymers, solid-phase extraction.

## 1. Introduction

Since its introduction in 1957, 5-fluorouracil (5-FU) remains an essential part of the treatment of a wide range of solid tumours, particularly those found in the gastrointestinal tract, breast, head and neck. However, as with all anti-cancer medications, 5-FU is quite toxic, due to phosphorylation in the digestive tract, with myelotoxicity and gastrointestinal disorders being its major side-effects [1,2]. Furthermore, the activity of 5-FU is restricted by rapid degradation of up to 85% of the drug within the first minutes of entry to the blood stream, thus leaving a very small amount for anti-cancer action. The above limitations, as well as the need for patients to regularly visit the hospital for intra-venous administration of the drug, with the associated increased risks of thrombosis or infection, have driven research into the development of orally administered pro-drugs of 5-FU, usually in the form of substituted fluoropyrimidines. These include floxuridine (5-fluoro-2'-deoxyuridine), doxifluridine (5-fluoro-5'-deoxyuridine) and tegafur or ftorafur (1-(2-tetrahydrofuryl)-5-fluorouracil), the latter being one of the most studied orally administered 5-FU pro-drugs. Tegafur is often co-formulated with uracil or other active ingredients that enhance its bioavailability and reduce its toxicity [3-5]. It is metabolised to 5-FU in the body by cytochrome P450 2A6 and its terminal elimination half-life is 11 hours [6]. Despite its several benefits over 5-FU, orally administered tegafur needs to be taken twice or thrice daily and up to 20% of the pro-drug is excreted in urine un-metabolised. Thus, monitoring patient compliance, as well as drug uptake and excretion, is of vital importance, and tools facilitating analysis of the drug in complex biological samples are essential.

Here, we wish to report, for the first time, on the design, development and application of a synthetic affinity separation phase exhibiting high selectivity for tegafur and capable of extracting the pro-drug from aqueous and biological samples. Our strategy was based on the technique of molecular imprinting, according to which a three-dimensional synthetic polymer network is formed in the presence of a target substance, thus embedding binding pockets complementary to the latter in terms of size, shape and functional group orientation. The resulting materials are capable of reversibly binding the so-called template or closely related substances from complex mixtures, rendering them robust, reusable and

inexpensive alternatives to affinity phases based on natural receptors, such as enzymes and antibodies [7-9].

The design of imprinted polymers for tegafur presented here was based on our prior experience in the development of recognition elements for uracil derivatives, which contain a hydrogen bond acceptor-donor-acceptor (A-D-A) imide moiety [10,11]. Our previous work, as well as that of others' [12-15], has shown that polymerisable functional monomers with complementary donor-acceptor-donor (D-A-D) arrays result in highly selective imprinted polymers, capable of recognising the template substance even in the presence of strongly competing analytes. Thus, of the previously reported custom-made functional monomers, including polymerisable adenine, purine and pyrimidine derivatives, 2,6-*bis*(acrylamido)pyridine (BAAPy) has been shown to be the most successful receptor and was used in the extraction of riboflavin from highly complex samples, such as beer and milk [16,17]. More recently, BAAPy was used in the development of molecularly imprinted polymers for the recognition of pyrimidine nucleosides [18], and in the preparation of composite beads for the enantiomeric resolution of the metastatic breast cancer drug aminoglutethimide by RAFT polymerisation [19]. Lastly, composite poloxamer nanomaterials containing *R*-thalidomide molecularly imprinted polymers based on BAAPy were recently used in enantioselective controlled release and targeted drug delivery [20]. The above examples highlight the versatility of the molecular imprinting technique, as well as the potential of BAAPy, and by extension other custom-made functional monomers, in the preparation of affinity separation phases with high selectivity for their respective targets, even when applied in highly competitive media.

## 2. Experimental

### 2.1. Materials and methods

Tegafur was purchased from Tokyo Chemical Industry UK Ltd. (Oxford, UK). 2,6-*bis*(acrylamido)pyridine (BAAPy) [21] and 9-isobutyladenine [22] were synthesised as described previously. All other tested analytes, shown in Figure 1, ethyleneglycol dimethacrylate (EDMA), HPLC grade solvents and deuterated solvents were purchased from Sigma Aldrich (Gillingham, UK). 2,2'-Azobis(2,4-dimethylvaleronitrile) (ABDV) was purchased from Wako Chemicals GmbH and was used

as received. Polymerisation inhibitors were removed from EDMA by filtration through a basic alumina column.  $^1\text{H}$  NMR spectra were collected on a Bruker ECX 400 MHz NMR spectrometer. An Agilent 1100 HPLC instrument equipped with photodiode array detector and a Phenomenex Kinetex<sup>TM</sup> C<sub>18</sub> column (5 $\mu\text{m}$ , 150 mm  $\times$  4.6 mm i.d.) was used for all chromatographic separations.

## 2.2. $^1\text{H}$ NMR titration experiments

Monomer–template complexation was studied prior to polymer synthesis using  $^1\text{H}$  NMR titrations in order to establish the type and strength of interactions present in the pre-polymerisation solution. Thus, to a solution of BAAPy (0.001 mol L<sup>-1</sup>) in CDCl<sub>3</sub> were added increasing amounts of tegafur, until at least a 10-fold excess was reached. The complexation-induced shifts (CIS) of the BAAPy amide protons were followed and a titration curve was constructed. The stoichiometry of the monomer-template complex was confirmed using Job's method of continuous variation. Hence, equimolar solutions (0.01 mol L<sup>-1</sup>) of BAAPy and tegafur were mixed in different ratios and a plot of  $\Delta\delta$  against the molar fraction of monomer multiplied by the CIS ( $X_i \times \Delta\delta$ ) was constructed [23,24].

## 2.3. Preparation of imprinted polymers

The imprinted (P<sub>TGF</sub>) and corresponding non-imprinted polymer (NIP) used in this study were prepared by thermally initiated free-radical polymerisation. Briefly, tegafur (0.202 g, 1 mmol) and BAAPy (0.219 g, 1 mmol) were transferred into to a glass vial and mixed with 5.6 mL of chloroform. Upon complete dissolution, 3.8 mL (20 mmol) of EDMA were added followed by 0.04 g of ABDV. The pre-polymerisation solutions were ultra-sonicated for 5 min, purged with N<sub>2</sub> and then hermetically sealed. The vials were placed in a water-bath thermostated at 40°C for 24 h. The resulting rigid monoliths were smashed and washed with methanol in a Soxhlet apparatus in order to remove the template and any unreacted monomers. The coarse polymer particles were ground using a mortar and pestle, wet-sieved with acetone and the 25-38  $\mu\text{m}$  fraction was collected, dried and stored at room temperature for further experiments. The corresponding non-imprinted polymer was prepared in the same manner, but without addition of the template in the pre-polymerisation mixture.



#### 2.4. Chromatographic evaluation

Chromatographic evaluation of the prepared polymers was performed using LC columns (50 mm × 4.6 mm i.d.) manually packed with the 25-38  $\mu\text{m}$  fraction of each material. The columns were then connected to a HPLC instrument and equilibrated with 1% acetic acid in acetonitrile until a stable baseline was obtained. Analyses were performed by injecting 5  $\mu\text{L}$  of 1  $\text{mmol L}^{-1}$  solution of each analyte in acetonitrile and recording its elution profile at a flow-rate of 1  $\text{mL min}^{-1}$  and a wavelength set accordingly for each tested analyte. Retention factors ( $k=(t_R-t_o)/t_o$ ), where  $t_R$  is the retention time of each analyte (average of three injections) and  $t_o$  the retention time of the void volume marker (acetone), as well as imprinting factors ( $IF=k_{MIP}/k_{NIP}$ ), were calculated.

#### 2.5. Equilibrium rebinding experiments

The polymers' affinity and capacity for tegafur and 5-FU were estimated using equilibrium rebinding experiments performed in acetonitrile. Thus, 10 mg of each polymer were transferred in 2 mL glass vials and equilibrated with 1 mL of analyte solution of increasing concentration (0-5  $\text{mmol L}^{-1}$ ) for 24 hours. All samples were prepared in triplicate. The supernatants were then analysed by HPLC using a mixture of water/acetonitrile 80:20 as the mobile phase. The flow-rate was 1  $\text{mL min}^{-1}$  and the detection wavelength was set at 270 nm. The amount of analyte bound to each polymer was calculated by subtracting the amount determined after the experiment from the starting amount of the drug. The averaged results ( $n=3$ ) were plotted as concentration of free analyte in solution vs. the amount of analyte bound to the polymer to produce binding isotherms that were fitted by non-linear regression to the appropriate binding model.

#### 2.6. Solid phase extractions (MI-SPE)

50 mg of P<sub>TGF</sub> and NIP particles were dry packed in 3 mL SPE cartridges using 20  $\mu\text{m}$  porous polyethylene frits. The developed SPE protocol consisted of an initial aqueous conditioning step followed by loading of 1 mL of aqueous sample. Initially, a screening study was performed whereby 1 mL of water/acetonitrile mixtures ranging from 0:100 to 100:0 was percolated through the drug loaded cartridges. This was later replaced with an aqueous wash (1 mL), followed by drying of the SPE cartridges on the vacuum manifold by allowing air to flow through for 5 minutes, and a second washing

step with 1 mL of chloroform. Finally, the cartridges were eluted with 1 mL of a 5% acetic acid in methanol solution. SPE analysis of urine samples was performed in spiked samples ( $200 \mu\text{g mL}^{-1}$ ) after filtration and dilution with ultra-pure water. Analysis of SPE fractions was performed by HPLC using the method described above and each extraction cycle was repeated at least three times.

### 3. Results and discussion

The complexation of tegafur with BAAPy was initially studied by  $^1\text{H}$  NMR titration experiments in  $\text{CDCl}_3$  whereby an association constant of  $574 \pm 15 \text{ M}^{-1}$  was calculated, which is in agreement with values measured previously for similar compounds (Figure 2). Furthermore, the stoichiometry of the formed complexes was verified by a Job plot experiment (Figure 2-insert). The corresponding graph shows a maximum at 0.5 equivalents of tegafur, suggesting the formation of 1:1 complexes, as predicted by the potential for formation of three H-bonds between the complementary A-D-A and D-A-D arrays on the template and monomer, respectively (Figure 1).

Imprinted polymers were synthesised by thermally initiated solution polymerisation using chloroform as the porogen and a ratio of tegafur: BAAPy: EDMA of 1:1:20 ( $\text{P}_{\text{TGF}}$ ) and 0:1:20 (NIP). The use of stoichiometric ratios of template and functional monomer is justified by the relatively strong association between the two species that should result in a high number of solution phase complexes, whose polymerisation eventually leads to the formation of the imprinted binding sites. Furthermore, contrary to imprinted polymers prepared using a typical 4-fold or higher excess of functional monomer, the polymers prepared here should exhibit reduced non-specific binding, as the likelihood of randomly distributed functionality outside imprinted binding sites is reduced.

#### 3.1. Polymer evaluation by equilibrium rebinding

Equilibrium rebinding experiments were used as a straightforward method to probe the affinity and calculate the capacity of the prepared polymers. Hence, 10 mg of each of the  $\text{P}_{\text{TGF}}$  and NIP polymer particles were equilibrated with increasing concentrations of tegafur or 5-fluorouracil. The resulting experimental data, together with the corresponding best fit lines obtained by non-linear regression are presented in Figure 3, while the fitting parameters are shown in Table 1. Rebinding results suggest a strong imprinting effect, as demonstrated by the difference in the corresponding affinity constants and

binding capacity of  $P_{TGF}$  and NIP for tegafur, while  $P_{TGF}$  also exhibits strong selectivity for the template vs. 5-FU, a closely structurally related analyte that also possesses an A-D-A hydrogen bond array. The latter observation highlights the importance of analyte size and shape in the molecular recognition event within the imprinted sites: contrary to that which may be expected, 5-FU, despite its similar functionality and smaller molecular size compared to the template, does not bind as strongly to the imprinted sites, while approximately five times less 5-FU was bound by the polymer compared to tegafur. This behaviour can be attributed partially to the potential of the furfuryl functional group to interact, albeit weakly, with monomer units distributed randomly within the polymer matrix as well as the polymer backbone itself. It is also noteworthy that both analytes interact very weakly with the control polymer NIP, underpinning the importance of the templating process as a synergistic effect between using tailored functional monomers and creating binding pockets of the correct size and shape to facilitate binding. The influence of templating is also demonstrated by the binding model that best fitted each experimental isotherm. Thus, the binding of 5-FU on to both  $P_{TGF}$  and NIP was best described using the Langmuir model, assuming a single type of binding sites of moderate to low affinity, which was also the case for tegafur binding to the non-imprinted polymer. However, the same model failed to describe the binding of tegafur on  $P_{TGF}$  and the corresponding results were better fitted used the bi-Langmuir isotherm, assuming two types of binding sites. Over 90% of these sites exhibited an average affinity comparable to that measured on the control polymer and a much lower population of sites showing significantly higher affinity. This behaviour is explained by the moderate template-monomer solution affinity constant that results in a majority of low fidelity binding sites in the final imprinted polymer matrix. However, their near 10-fold higher population compared to the corresponding non-imprinted polymer still results in pronounced selectivity for the template.

### 3.2. Chromatographic evaluation of imprinted polymers

In order to better understand the selectivity profile of the polymers,  $P_{TGF}$  and NIP particles of 25-38 $\mu$ m diameter were packed in LC columns, and a series of competing analytes were sequentially injected and their elution profiles recorded. Similar to our previous findings with BAAPy-based imprints, the polymers studied here exhibited extremely long retention times for the template in 100% acetonitrile

and, thus, 1% acetic acid was added to reduce elution volumes. The results obtained are presented in Figure 4, while detailed retention times are shown in Table 2. Hence, P<sub>TGF</sub> retained tegafur for 10.8 minutes vs. 1.2 minutes for NIP, resulting in an imprinting factor of 25.3, proving the successful imprinting of the pro-drug demonstrated by equilibrium rebinding. In agreement with rebinding experiments, 5-FU was significantly less retained on the imprinted polymer, at just 4.0 minutes, with a corresponding imprinting factor of 8.2, which is approximately three times lower than the template. This strengthens the hypothesis that the furfuryl group of tegafur plays an important role on the recognition and binding processes. Interestingly, none of the other competing analytes was strongly retained on either P<sub>TGF</sub> or NIP, verifying that the hydrogen bonding A-D-A triad was essential for recognition.

### 3.3. Extraction of tegafur from aqueous and urine samples

The performance of the synthesised polymers as solid-phase extraction sorbents was then evaluated, by packing 25-38 $\mu$ m particles in 3 mL SPE cartridges and attempting to extract the drug from aqueous solutions. Loading of aqueous solutions of tegafur on the cartridges resulted in near quantitative binding of the drug on both the imprinted and non-imprinted columns, by non-specific, hydrophobic interactions. We then investigated the influence of water content in the washing, or so-called “molecular recognition”, step, by incrementally increasing the percentage of water in acetonitrile and measuring the final recovery of the drug following elution of the cartridges with MeOH-5% acetic acid (Figure 5). Thus, when the cartridges were washed with pure acetonitrile, tegafur recoveries were 41% and 3% using P<sub>TGF</sub> and NIP respectively, which was the maximum observed difference between the two polymers in this experiment, with a calculated imprinting factor of 11.8. When a mixture of water/acetonitrile 10:90 was used in the washing step, the corresponding recoveries dropped to 11.5% and 1% and the imprinting factor was marginally lower. Subsequently, when the water content was between 20% and 80%, almost no tegafur was recovered from either polymer. However, a dramatic change was observed when a 90:10 water/acetonitrile mixture was used, whereby 66% and 48% of the pro-drug were recovered from P<sub>TGF</sub> and NIP respectively. Lastly, when pure water was used as the washing solvent, recoveries from both polymers were over 93% with virtually no difference in the performance of the two sorbents. These findings reflect the typical behaviour of EDMA-based imprinted polymers, which exhibit their highest

selectivity in low polarity media. Addition of water in the washing stages results in dramatic decrease in binding strength due to disruption of hydrogen bonding; this is followed by non-selective binding in predominantly aqueous environments, driven by hydrophobic interactions.

In view of the reduced recoveries obtained when using water/acetonitrile mixtures in the washing step, it was decided to replace them with chloroform, the solvent in which the polymers were prepared. Using the porogen as a rebinding solvent has been shown to promote specific interactions by re-conditioning the polymer to its original state and, in the case of chloroform, swelling the polymer and promoting hydrogen bonding due to its non-polar nature. Thus, following loading of the aqueous sample of tegafur, the cartridges were washed with water, which did not remove any of the template from either P<sub>TGF</sub> or NIP columns, dried on the manifold by allowing air to pass through the polymer bed for 5 minutes and subsequently washed with 1 mL of chloroform. The cartridges were finally eluted with 1 mL of MeOH-5% acetic acid. Analysis of the collected fractions revealed that chloroform did not remove any of the template from the imprinted polymer cartridge, while over 60% was eluted from its non-imprinted counterpart. Thus, 96% of the drug was recovered from P<sub>TGF</sub> but only 37% was recovered from NIP, proving the important role of the porogen in the recognition process (Figure 6).

Using the optimised solid-phase extraction protocol, we then tested the polymer performance in a more competitive scenario by applying the materials in the extraction of urine samples spiked with a mixture of tegafur, 5-FU, caffeine and theophylline (200 µg mL<sup>-1</sup> each). Remarkably, P<sub>TGF</sub> once again exhibited exceptionally high selectivity for tegafur, with recoveries averaging 92%, comparable with what was achieved in aqueous solutions. Recoveries of competing analytes 5-FU and theophylline were under 12%, while caffeine did not bind to either the imprinted or the non-imprinted polymers (Figure 7). The significant urine sample clean-up, coupled with the speed and simplicity of extraction process, prove that molecular imprinting of tegafur was successful and that P<sub>TGF</sub> can be applied in the selective extraction of the drug from highly competitive samples, including biological fluids.

#### 4. Conclusions

The interaction of previously developed polymerisable receptor 2,6-*bis*(acrylamido)pyridine (BAAPy) with the anti-cancer pro-drug tegafur was studied in solution and the formation of 1:1 complexes with

association constant of  $574 \pm 15 \text{ M}^{-1}$  was verified. Stoichiometric non-covalent molecularly imprinted polymers were then prepared using EDMA as the cross-linking monomer. Equilibrium rebinding experiments exhibited the highly selective nature of the prepared imprinted polymer ( $P_{\text{TGF}}$ ), whose total binding capacity for tegafur was  $45.1 \mu\text{mol g}^{-1}$ , over 10-fold higher than its non-imprinted counterpart (NIP), and over 7-fold higher compared to the binding of 5-FU. Further evaluation of the prepared polymers was performed using them as LC stationary phases, whereby tegafur was by far the most strongly retained analyte on  $P_{\text{TGF}}$ , necessitating the use of an acidic modifier to ensure complete elution of the compound. An imprinting factor of 25.3 was calculated for tegafur, while elution of structurally related 5-FU was completed faster and an imprinting factor of 8.2 was calculated. The prepared materials were used as sorbents in the solid-phase extraction of tegafur from aqueous samples. An initial study on the influence of water percentage in the cartridge washing stage revealed that while  $P_{\text{TGF}}$  exhibited good selectivity in purely organic media and at low water contents, this diminished when highly aqueous washes were employed and non-specific, hydrophobic interactions dominated. Hence, an optimised SPE protocol that included a chloroform washing step was developed, enabling recoveries of the drug up to 96% using  $P_{\text{TGF}}$  compared to 37% using NIP. This extraction protocol was successfully used in the extraction of the drug from urine samples spiked with tegafur and three competing analytes, where tegafur recoveries of up to 92% were achieved, demonstrating the potential of this polymer for use in the extraction of the drug from highly competitive aqueous and biological samples.

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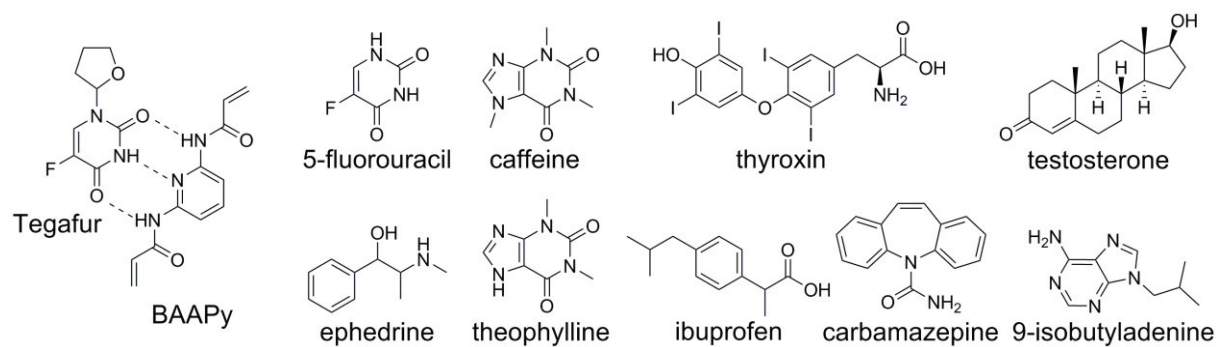


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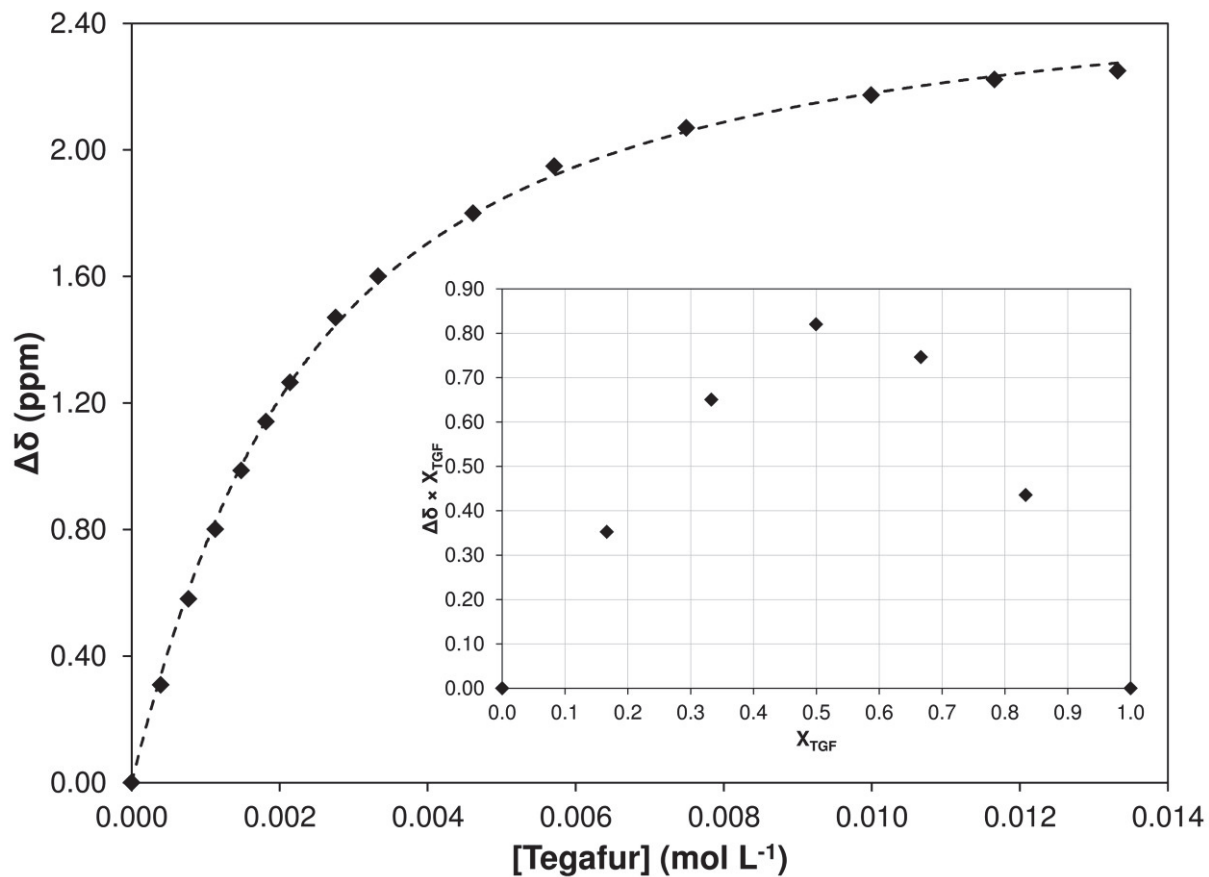


## Figure Captions

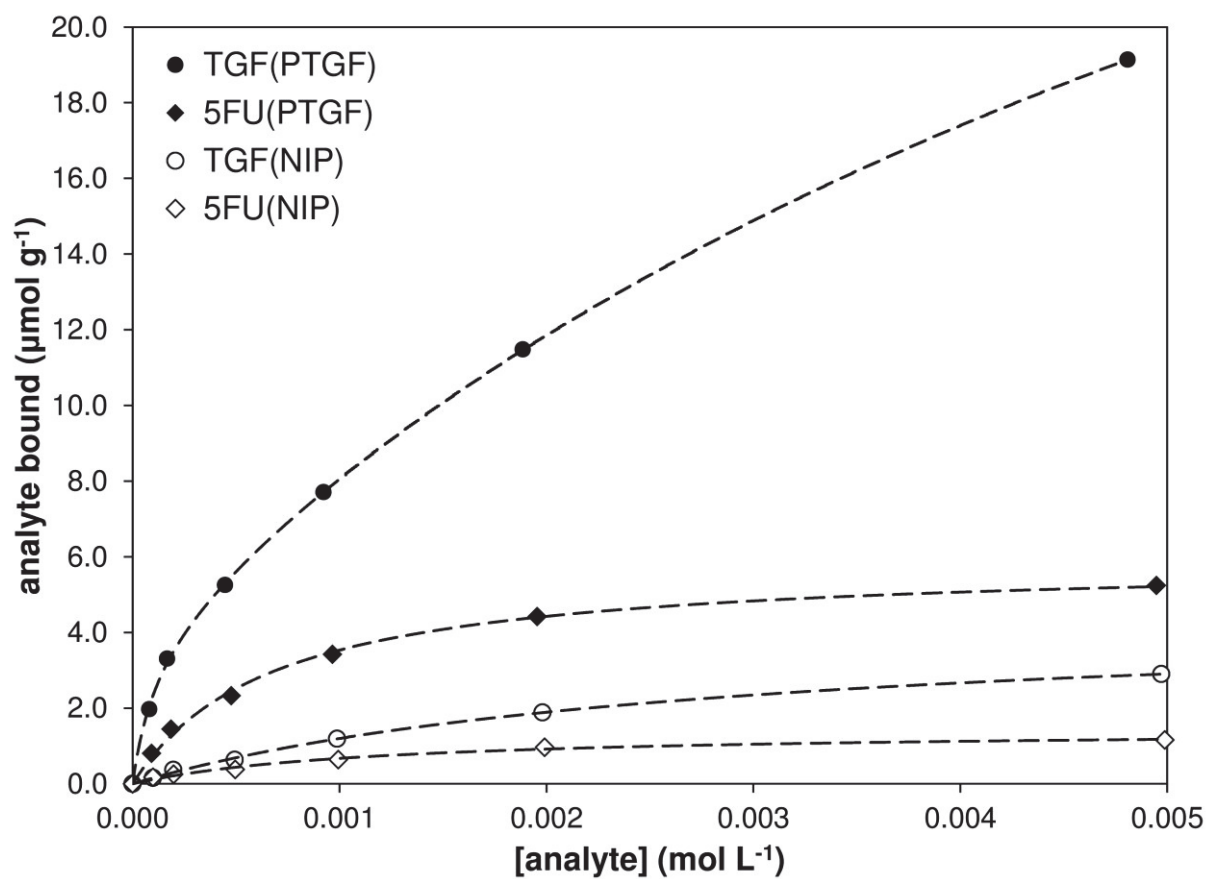
**Figure 1:** Proposed mode of association of tegafur with BAAPy, and chemical structures of all analytes tested in this study.



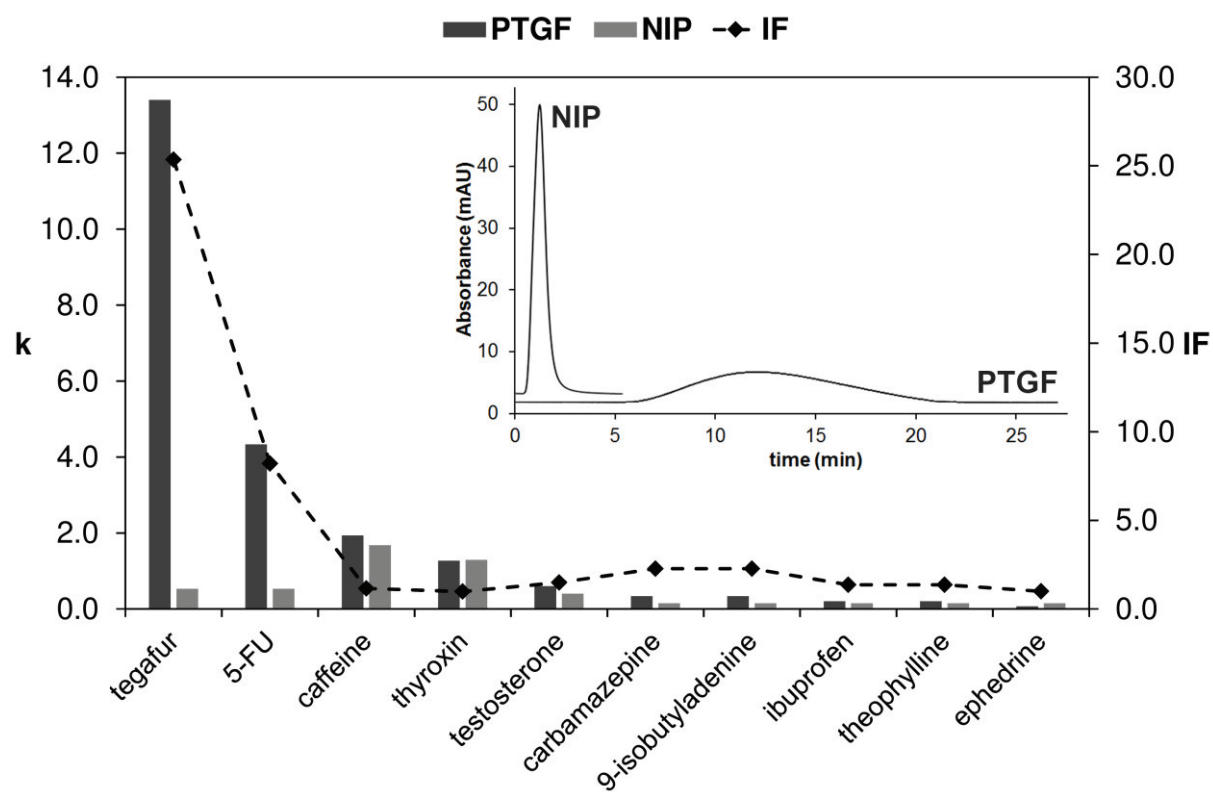
**Figure 2:**  $^1\text{H}$  NMR titration of BAAPy ( $0.001 \text{ mol L}^{-1}$ ) with tegafur in  $\text{CDCl}_3$  and corresponding isotherm fitted to the 1:1 binding model. Insert: Job plot for the association of BAAPy with tegafur suggesting the formation of 1:1 complexes.



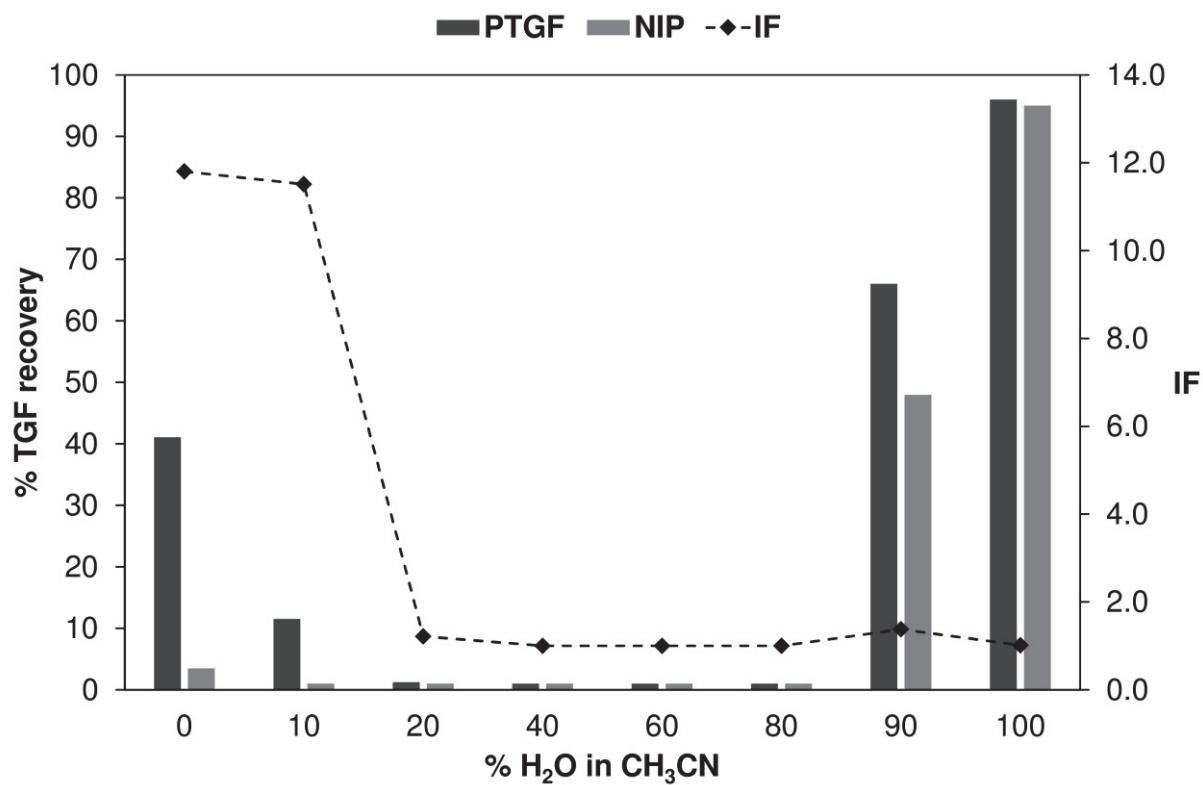
**Figure 3:** Equilibrium rebinding isotherms for the binding of tegafur (circles) and 5-fluorouracil (diamonds) on the prepared imprinted (black symbols) and non-imprinted polymer (open symbols) in acetonitrile. Dashed lines represent the corresponding best fitted isotherms obtained by non-linear regression.



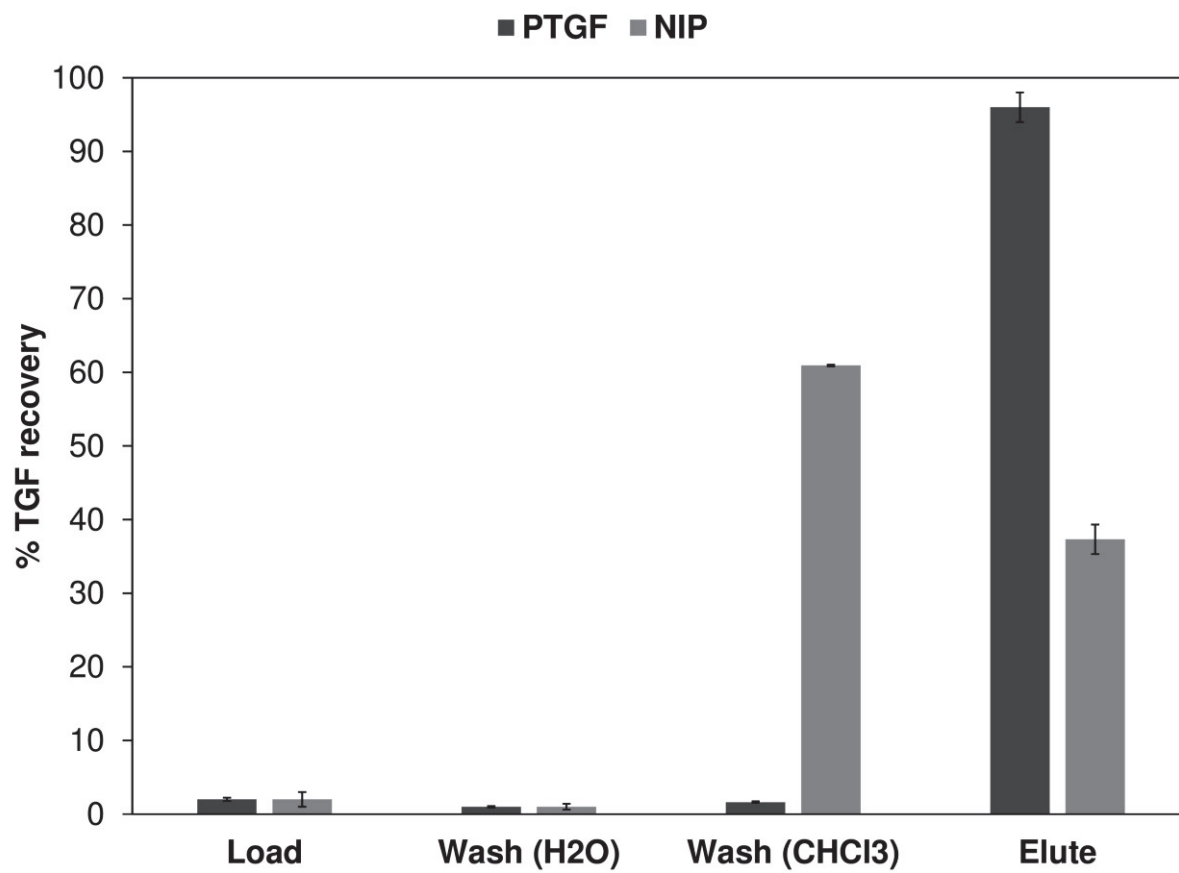
**Figure 4:** Retention factors,  $k$  (bars), and corresponding imprinting factors,  $IF$  (dashed line), for the template and competing analytes, calculated by chromatographic evaluation of the prepared polymers in acetonitrile/acetic acid 99:1. Elution conditions as described in experimental section. Detailed experimental results shown in Table 2. Insert: Characteristic elution profiles of TGF on PTGF and NIP columns.



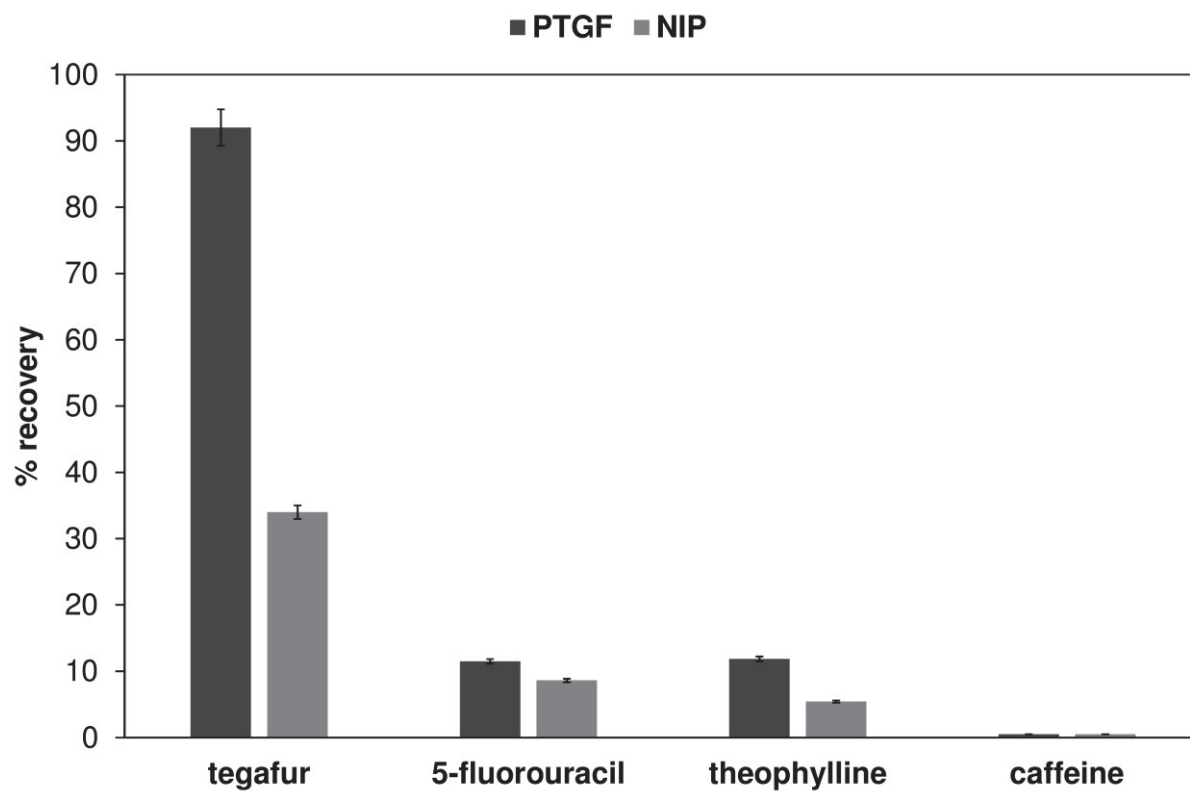
**Figure 5:** Effect of the percentage of water added in the polymer washing step on the recovery (%) of tegafur by solid-phase extraction, and corresponding imprinting factors (dashed line).



**Figure 6:** Recovery (%) of tegafur from aqueous samples using the optimised SPE protocol and the synthesised imprinted and non-imprinted polymers.



**Figure 7:** Recovery (%) of tegafur, 5-fluorouracil, theophylline and caffeine from spiked urine samples using the optimised SPE protocol and the synthesised imprinted and non-imprinted polymers.



**Table 1** Affinity constants ( $K_a$ ) and number of binding sites ( $N$ ) calculated from equilibrium rebinding results presented in Figure 3 by non-linear regression fit to the bi-Langmuir (tegafur on  $P_{TGF}$ ) or Langmuir adsorption model.

analyte	$P_{TGF}$			NIP		
	$K_a$ (L mol <sup>-1</sup> )	$N$ ( $\mu$ mol g <sup>-1</sup> )	$R^2$	$K_a$ (L mol <sup>-1</sup> )	$N$ ( $\mu$ mol g <sup>-1</sup> )	$R^2$
tegafur	$0.1 \times 10^3$	41.2	0.999	$0.4 \times 10^3$	4.5	0.997
	$8.9 \times 10^4$	3.9				
5-fluorouracil	$1.5 \times 10^3$	5.9	0.997	$0.8 \times 10^3$	1.4	0.987



**Table 2** Retention times ( $t_R$ , min, n=3), selectivity factors ( $k$ ) and imprinting factors ( $IF$ ) of tested analytes on imprinted and non-imprinted polymer packed LC columns, in acetonitrile/acetic acid 99:1. Corresponding %RSD < 5%.

analyte	$t_R$		$k$		$IF$
	P <sub>TGF</sub>	NIP	P <sub>TGF</sub>	NIP	
tegafur	10.8	1.2	13.4	0.5	25.3
5-FU	4.0	1.2	4.3	0.5	8.2
caffeine	2.2	2.1	1.9	1.7	1.2
thyroxin	1.7	1.8	1.3	1.3	1.0
testosterone	1.2	1.1	0.6	0.4	1.5
carbamazepine	1.0	0.9	0.3	0.1	2.3
9-isobutyladenine	1.0	0.9	0.3	0.1	2.3
ibuprofen	0.9	0.9	0.2	0.1	1.4
theophylline	0.9	0.9	0.2	0.1	1.4
ephedrine	0.8	0.9	0.1	0.1	1.0