Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

Thin film molecularly imprinted polymer (TF-MIP), a selective and single-use extraction device for high-throughput analysis of biological samples

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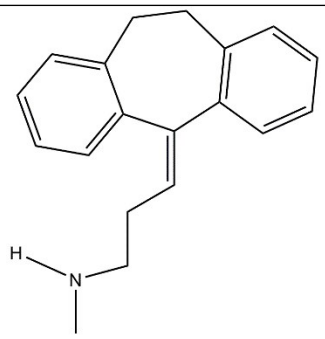
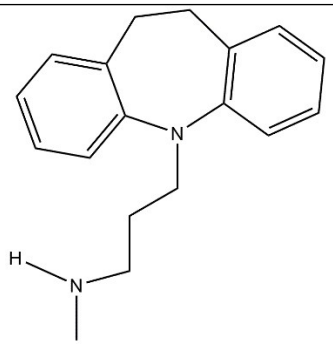
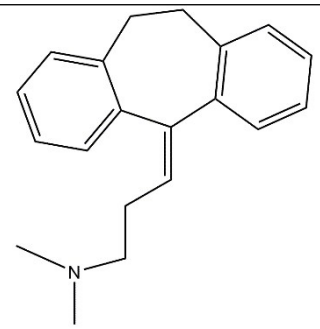
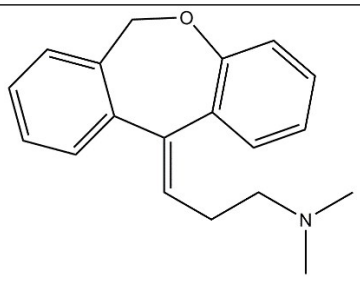
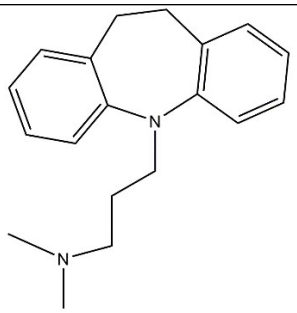
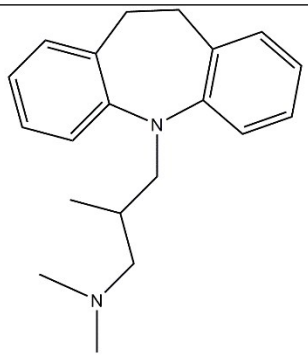
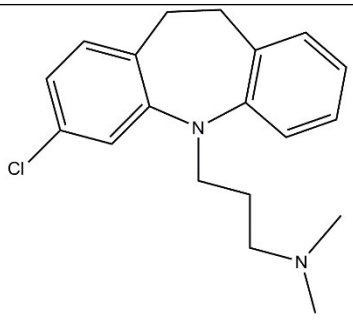
1- Physical chemical properties of TCAs

Table S1. Target drugs and physical chemical properties

Compound	Structure	pKa	logP*
Nortriptyline		10.1	4.51
Desipramine		10.4	4.90
Amitriptyline		9.4	4.92
Doxepin		8.96	4.29

Imipramine		9.4	4.80
Trimipramine		9.42	4.2
Clomipramine		8.98	5.19

* logP: log Ko/w, (octanol−water partition coefficient)

2- Mass spectrometry parameters of TCAs

Table S2. Summary of tandem mass spectrometry parameters of TCAs using LC-MS/MS

TCAs	Precursor ion (m/z)	Cone voltage (V)	Product ion 1 (m/z)	Collision energy (eV)	Product ion 2 (m/z)	Collision energy (eV)
Nortriptyline	264.3	25	233.2	18	105.0	24
Desipramine	267.1	25	208.1	24	72.1	18
Amitriptyline	278.1	35	117.1	28	91.0	26
Doxepin	280.1	35	235.1	28	107.0	28
Imipramine	281.1	25	85.9	20	58.1	35
Imipramine-D3	284.2	30	208.2	30	89.1	15
Trimipramine	295.1	35	192.8	56	100.0	24
Clomipramine	315.1	35	85.9	24	58.0	42

3- Preparation of the pseudo template:

In a 500 mL round bottom flask,15.00 g desipramine hydrochloride (1 eq, 0.05 mol) was dissolved in 200 mL THF and 85 mL 2 M NaOH with stirring under nitrogen. Benzyl Chloroformate (7.4 mL,1.2 eq, 0.056 mol) was then added dropwise over ten minutes. The reaction was stirred overnight at room temperature under nitrogen. The biphasic mixture was transferred to a separatory funnel and allowed to separate. The organic phase was collected, and the aqueous phase was washed three times with 50 mL of ethyl acetate. The pooled organic phases were dried with anhydrous magnesium sulfate and evaporated to dryness under reduced pressure. The crude mixture was then purified by flash chromatography using a SiliaSep bare silica, 25 μm (550 mesh), 90 Å cartridge. An isocratic elution consisting of 70:30 hexanes: ethyl acetate was used to elute the pure compound (Rf = 0.6). The collected fractions were monitored for purity using thin layer chromatography (SiliaPlate 200 μm, 3 x 6 cm, with F254 UV). The pure product, benzyl (3-(10,11-dihydro-5H-dibenzo[b,f]azepin-5-yl)propyl)(methyl)carbamate (CBZ-desipramine) was obtained as a clear colorless oil (19.43g, 97%). The product was characterized by high resolution MS and 1H NMR. MS (ESI) m/z called. for $[C_26H_{28}N_2O_2]^+$: 400.21508 [M+H]+; found: 400.21524; $\delta = 0.41\ ppm$. 1H NMR (500 MHz, Chloroform-d) δ 7.33 (tt, J = 11.1, 3.7 Hz, 3H), 7.32 – 7.24 (m, 2H), 7.09 (dd, J = 16.5, 7.9 Hz, 6H), 6.99 (d, J = 8.2 Hz, 1H), 6.90 (td, J = 7.3, 1.3 Hz, 2H), 5.09 (s, 1H), 4.99 (s, 1H), 3.72 (dt, J = 20.8, 5.8 Hz, 2H), 3.35 – 3.27 (m, 2H), 3.15 (s, 1H), 3.11 (d, J = 5.3 Hz, 2H), 2.78 (d, J = 12.1 Hz, 3H), 1.79 (dt, J = 14.5, 7.1 Hz, 2H).

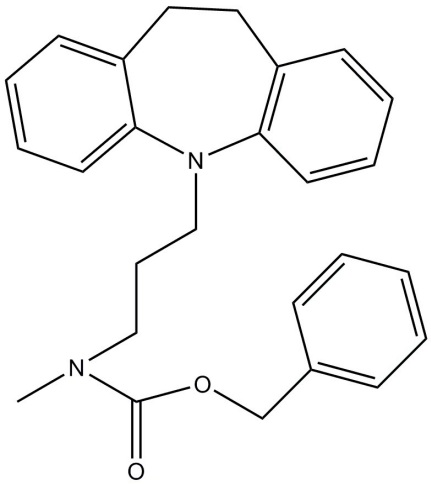


Figure S1. Chemical structure of the synthesized template

4- MIP formula development

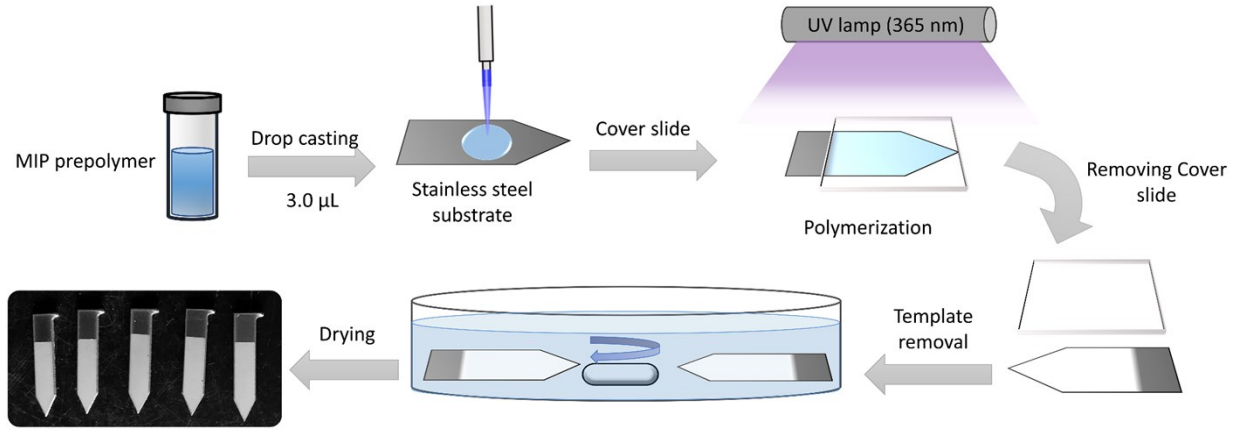


Figure S2. Fabrication of thin film MIP using drop casting technique

Table S3. Details of prepolymer solutions used for porogen dilution study

Porogen volume	1000 µL	1200 µL	1300 µL
Monomer mass and volume	69.4 (68 µL)		
Crosslinker mass and volume	950.3 mg (905 µL)		
Total volume	1973 µL	2173 µL	2273 µL
Monomer + crosslinker mass /volume of prepolymerization solution (mg/µL)	0.52	0.47	0.45
Mass deposited on substrate (mg)	2.08	1.88	1.80

Table S4. Effect of porogen volume on imprinting and repeatability of extraction of TCAs

TCAs	Porogen volume					
	1000 µL		1200 µL		1300 µL	
	No pH adj	pH adj	No pH adj	pH adj	No pH adj	pH adj
	IF (±SD)					
Nortriptyline	0.93 (0.35)	1.99 (0.24)	3.05 (1.32)	2.12 (0.61)	1.48 (0.51)	1.65 (0.49)
Desipramine	0.95 (0.33)	1.89 (0.27)	3.51 (1.58)	2.21 (0.63)	1.51 (0.55)	1.67 (0.48)
Amitriptyline	0.99(0.34)	3.00 (0.42)	3.43 (1.63)	1.87 (0.86)	1.64 (0.61)	1.62 (0.49)
Doxepin	1.00 (0.33)	3.51 (0.65)	4.36 (2.24)	1.94 (0.89)	1.63 (0.67)	1.66 (0.48)
Imipramine	0.96 (0.33)	3.17 (0.48)	3.84 (1.78)	1.93 (0.85)	1.58 (0.57)	1.64 (0.46)
Trimipramine	0.96 (0.32)	2.76 (0.32)	3.78 (1.80)	1.73 (0.82)	1.52 (0.54)	1.60 (0.56)
Clomipramine	0.89 (0.34)	2.23 (0.19)	2.25 (0.97)	1.58 (0.84)	1.38 (0.46)	1.59 (0.59)

Table S5. Effect of porogen volume on recovery and repeatability of extraction of TCAs using MIPs and NIPs

TCAs	Porogen volume											
	1000 µL				1200 µL				1300 µL			
	No pH adj		pH adj		No pH adj		pH adj		No pH adj		pH adj	
Recovery%(RSD%)	MIP	NIP	MIP	NIP	MIP	NIP	MIP	NIP	MIP	NIP	MIP	NIP
Nortriptyline	24.2(29.0)	26.0(24.7)	11.1(6.6)	5.6(10.4)	13.9(26.4)	4.6(34.6)	3.6(27.5)	1.7	5.6(18.1)	3.8(23.4)	3.6(25.8)	2.2(14.8)
Desipramine	23.3(27.2)	24.5(22.2)	10.4(8.1)	5.5(11.4)	13.3(26.4)	3.8(36.6)	3.3(26.6)	1.5	4.7(16.4)	3.1(14.8)	3.3(24.7)	1.9(14.8)
Amitriptyline	25.9(27.9)	26.3(21.1)	10.9(10.8)	3.6(8.8)	14.5(29.1)	4.2(37.6)	3.5(21.4)	1.9	5.8(18.2)	3.5(24.1)	3.3(21.1)	2.1(21.8)
Doxepin	27.0(26.9)	27.1(19.6)	8.7(15.7)	2.5(9.9)	14.3(31.2)	3.3(40.8)	2.3(20.4)	1.2	4.5(19.7)	2.8(23.3)	2.1(20.3)	1.3(20.2)
Imipramine	22.2(27.4)	23.1(21.1)	8.5(12.1)	2.7(9.0)	12.1(29.2)	3.1(36.0)	2.6(20.0)	1.3	4.1(18.1)	2.6(21.5)	2.4(19.9)	1.5(19.8)
Trimipramine	25.8(26.2)	26.9(21.3)	10.9(10.0)	3.9(6.1)	14.1(31.4)	3.7(35.7)	4.0(24.2)	2.3	4.9(18.8)	3.2(21.1)	3.9(24.6)	2.4(24.7)
Clomipramine	23.8(29.4)	26.6(24.0)	13.0(7.7)	5.8(3.3)	15.5(27.3)	6.9(33.0)	5.3(27.1)	3.3	8.1(17.0)	5.9(22.7)	5.4(27.0)	3.4(25.7)

5- MIP formula repeatability:

Fabrication of thin film MIPs by drop-casting method only requires a few microliters of pre-polymer solution. The pre-polymer mixture is stable and can be stored to prepare MIPs. However, we assessed the variability of MIPs formulae prepared on different by using 2 different batches of template. Three thin films from each batch were used for extraction of TCAs (Figure S3). The results depict that two batches of thin film MIPs are similar. A further investigation of the data was conducted by performing a t-test. The results (Table S6) are obtained at 95% of confidence level and show that there is no difference between two batches.

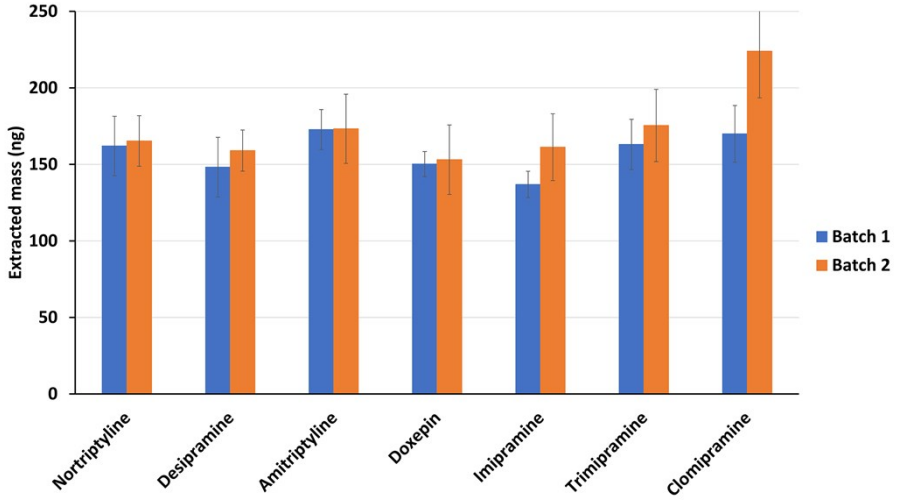


Figure S3. Inter-batch reproducibility of 2 sets of thin film MIPs. All extractions from 20 mL of 50 ng mL^{-1} of TCAs with 1% TEA at pH ~11.

Table S6. T-test at a 95% confidence level for inter-batch reproducibility of thin film MIPs (T_{crit} = 2.776).

TCAs	T-Value	P-Value
Nortriptyline	-0.22	0.835
Desipramine	-0.22	0.835
Amitriptyline	-0.03	0.976
Doxepin	-0.21	0.848
Imipramine	-1.8	0.147
Trimipramine	-0.75	0.497
Clomipramine	-2.62	0.059

6- Characterization of thin films:

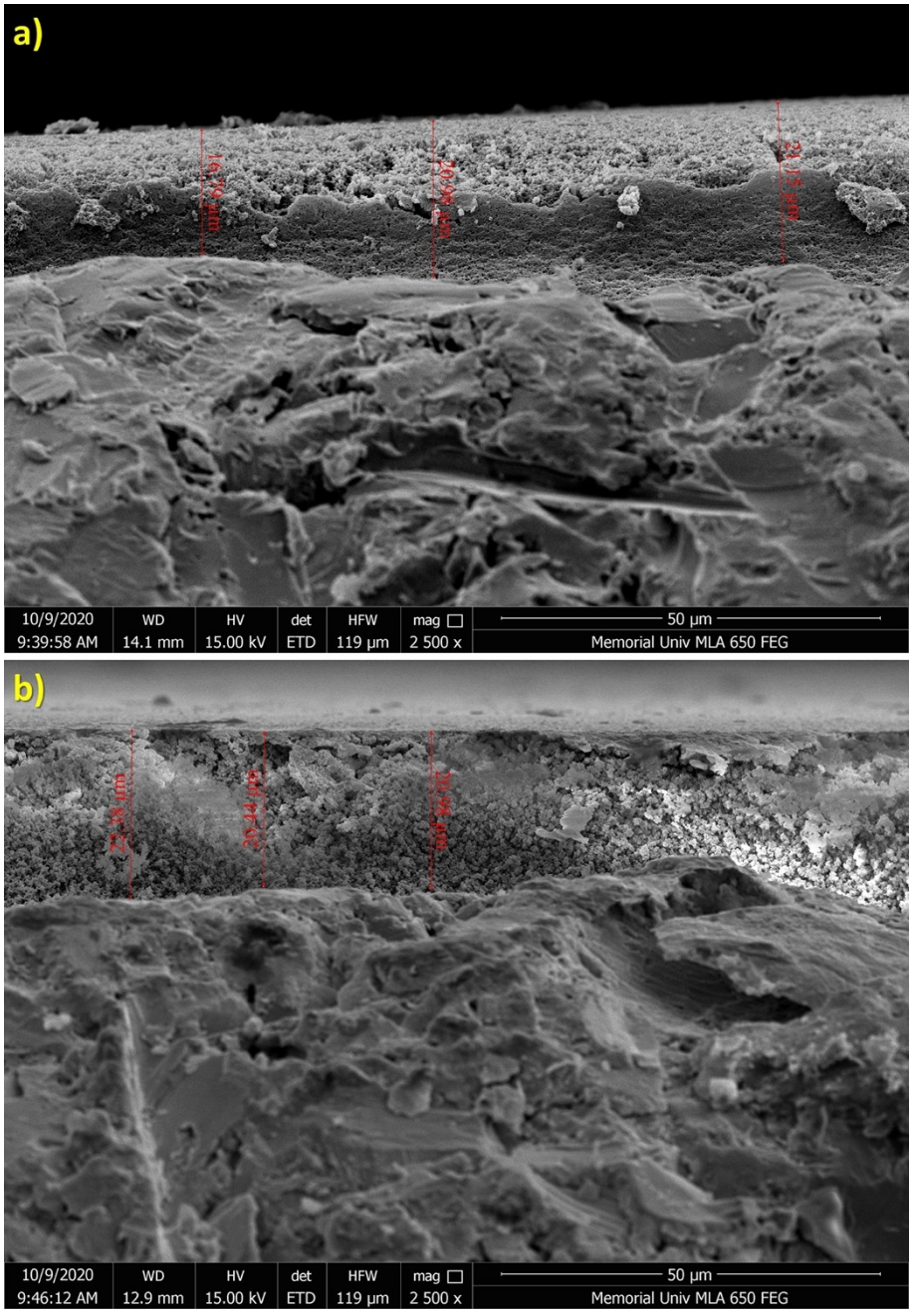


Figure S4. Side view of exemplary a) thin film MIP, and b) thin film NIP prepared on stainless steel substrates obtained at 2500x magnification.

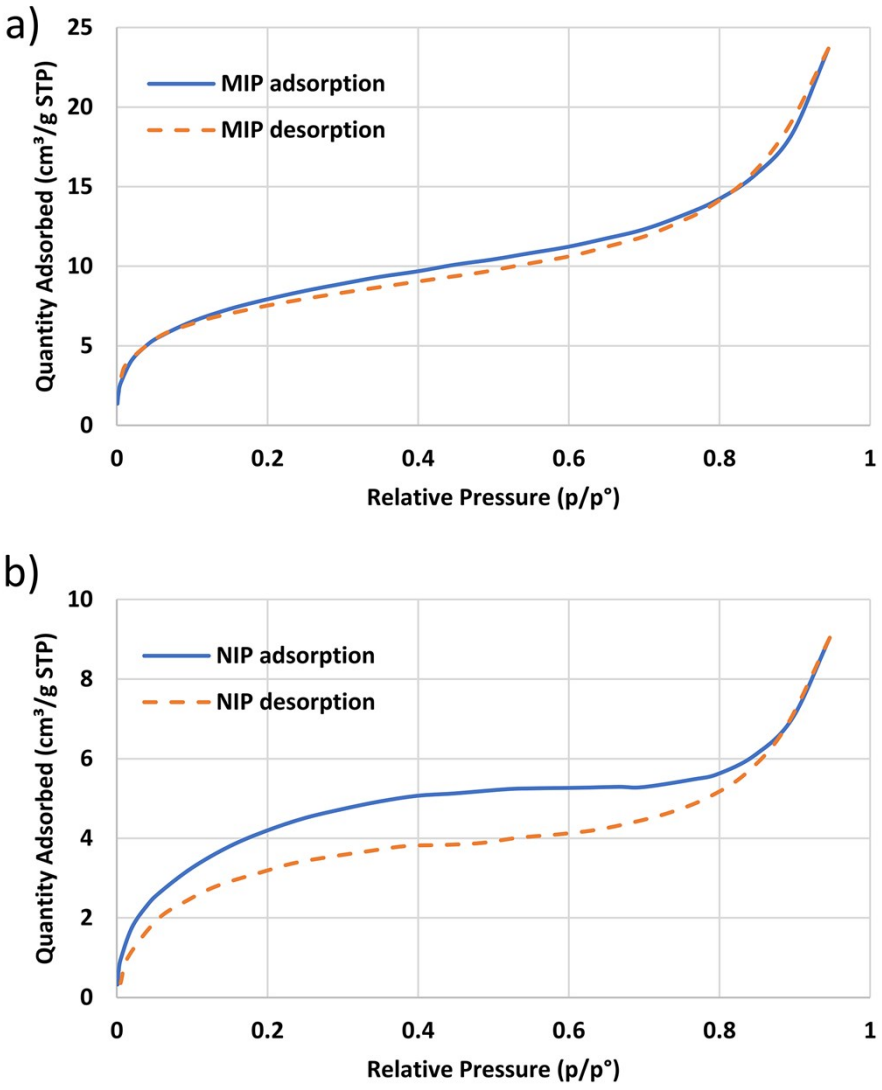


Figure S5. BET isotherm for a) optimized thin film MIP and b) its corresponding NIP

7- Protein binding assessment:

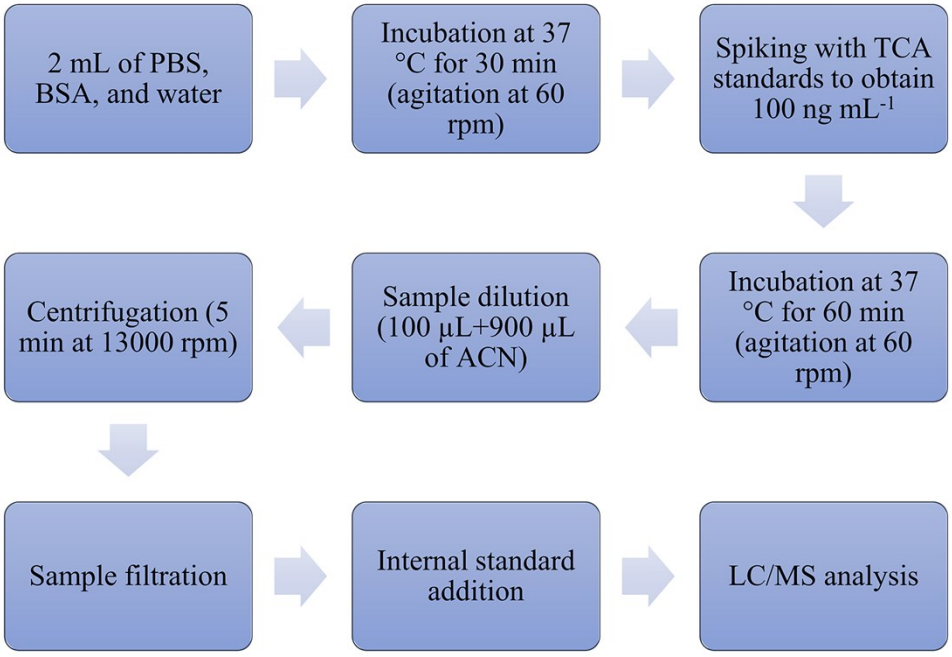


Figure S6. The summary process for assessment of protein binding of TCAs

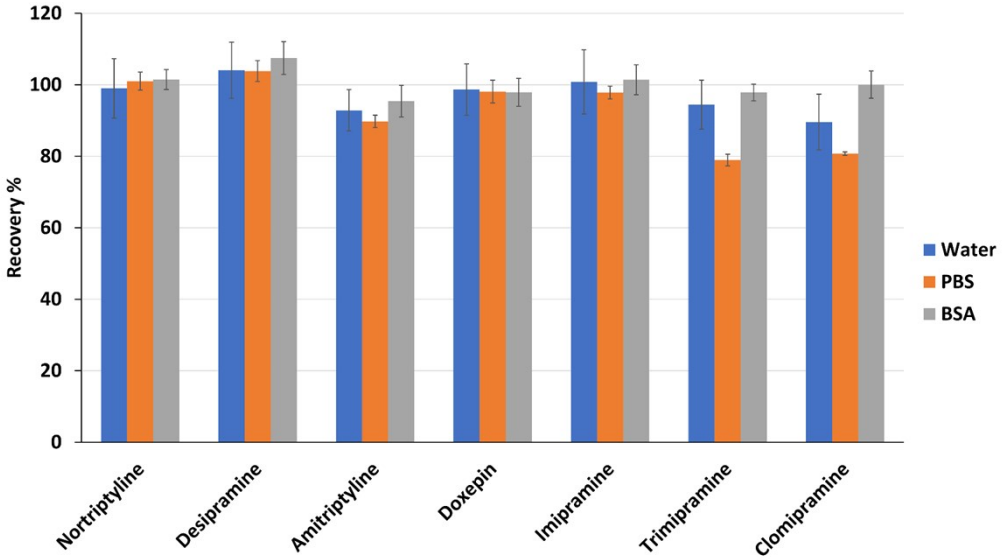


Figure S7. Effect of PBS and BSA on recoveries of TCAs following 1 h incubation at 37 ºC at neutral pH (TCA are protonated under these conditions).

8- Method development

Washing:

Thin film MIPs allow for sample clean-up after extraction. In the previous studies, this step was performed by washing the extraction devices after exposure to sample solution with ultrapure water. This washing step reduces the co-adsorption of matrix components and interfering substances such as salts. In this step, three different washing solutions including ultra pure water, 0.1% aqueous FA, and1% aqueous TEA were tested, and the results were compared with performing the extraction without any washing step (Figure S8). Performing desorption without rinsing the thin films has resulted in low efficiency due to co-extracted matrix components and ionization suppression.

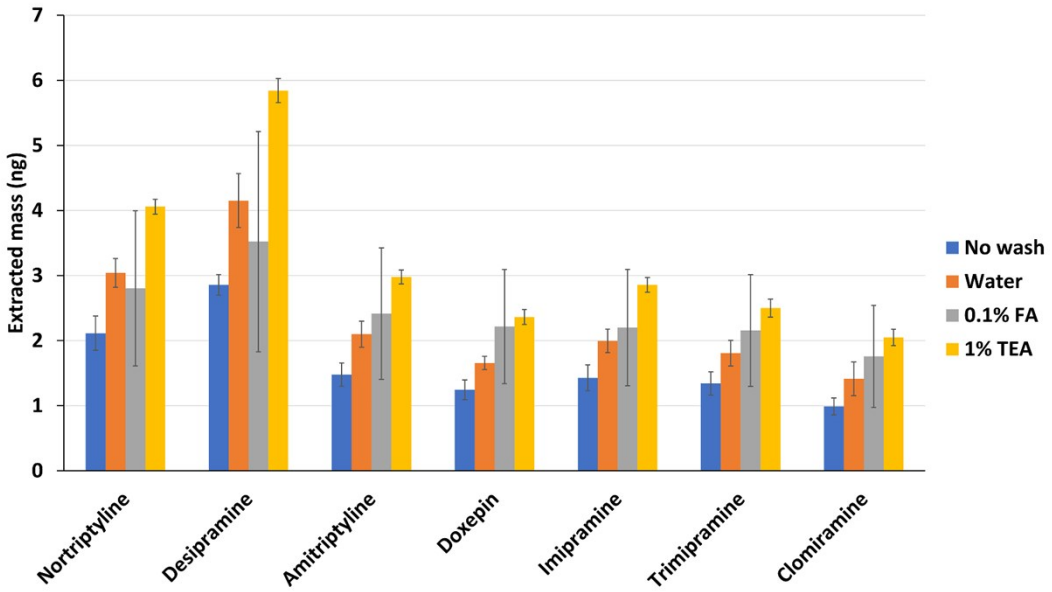


Figure S8. Wash optimization. Wash: 8 s immersion in 20 mL DI water, 0.1 % aqueous FA or 1% aqueous TEA. Sample extraction: 700 µL of 100 ng mL^{-1} of TCAs in BSA with 1% TEA, extraction for 20 min at 1000 rpm, Desorption conditions: 700 µL MeOH, 20 min at 1000 rpm.

Although water has been recommended in the literature for rinsing step after direct immersion extraction, it can cause ionization of adsorbed TCAs on the thin film MIPs and facilitate these drugs desorption into the washing solution. This idea was further demonstrated by the results of

using of water with 0.1% FA as a washing solution leading to high %RSD values. These results can be explained by the basic nature of TCAs. These drugs are ionized in the neutral and acidic pH conditions which lead to wash them off the thin film MIPs. The highest efficiency and repeatability were obtained using immersion of thin films in 1% TEA in water as washing solution. Basic condition of 1% TEA can maintain the adsorbed analytes on the film and rinse co-extracted components.

Desorption:

The composition of desorption solvent was optimized to ensure a reliable and efficient elution of analytes from thin film. TCAs are hydrophobic analytes needing organic solvent (ACN or MeOH) to be desorbed. Additionally, due to the type of the interactions between the TCAs and the MIP sorbent which is mostly hydrogen binding, it was expected that adding FA and water can disturb these interactions and help to a better desorption of the drugs from the thin film MIP. Combination of solvents with different polarity is also effective in releasing the drugs which are trapped in the MIP's specific cavities. Figure S9 demonstrates the desorbed mass (ng) of the drugs using different desorption solvents.

As can be seen in Figure S9, desorption was improved by adding water (1:1 ratio) and FA (0.1%) to the pure solvent. Therefore, mixture of ACN/water (1:1) with 0.1%FA, which is also compatible with LC mobile phase, was used as the desorption solvent. Other factor which can be assessed to improve the desorption efficiency is the desorption time profile to find the equilibrium of analytes between thin film and desorption solvent. Figure S10 depicts that increasing the desorption time can improve the desorption efficiency. Based on these results, 20 minutes was selected as the optimum time for desorption.

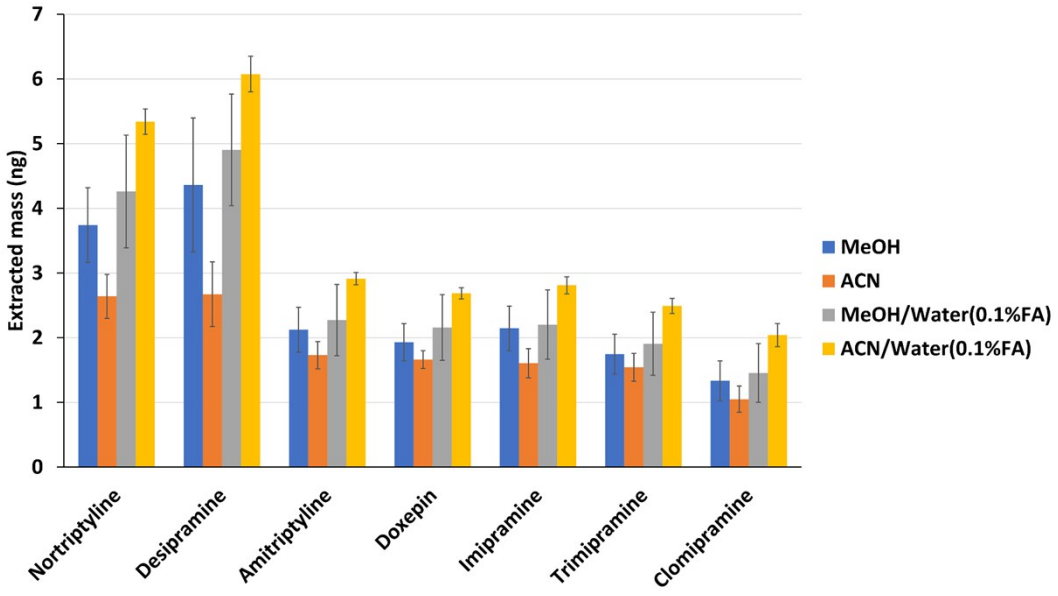


Figure S9. Comparison of the extracted mass of TCAs by using different solvents for desorption. Sample extraction: 700 µL of 100 ng mL^{-1} of TCAs in BSA with 1% TEA, extraction for 20 min at 1000 rpm, washing: 8 sec immersion in water with 1%TEA, Desorption conditions: 700 µL, 20 min at 1000 rpm.

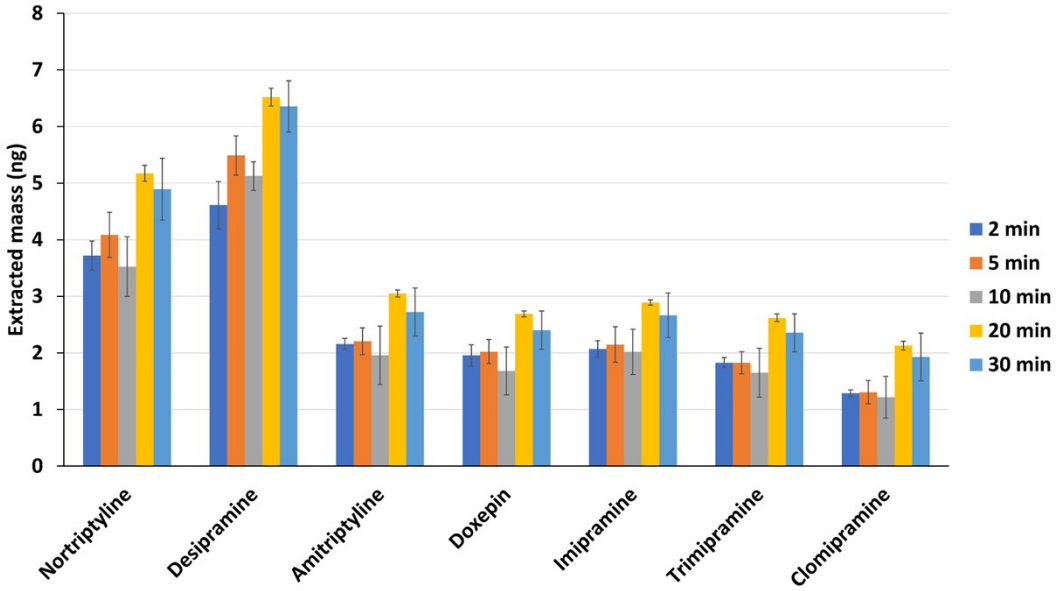


Figure S10. Desorption time profile. Sample extraction: 700 µL of 100 ng mL-1 of TCAs in BSA with 1% TEA, extraction for 20 min at 1000 rpm, washing: 8 sec immersion in water with 1%TEA, Desorption conditions: 700 µL of ACN/water (1:1) with 0.1% FA at 500 rpm.

Extraction:

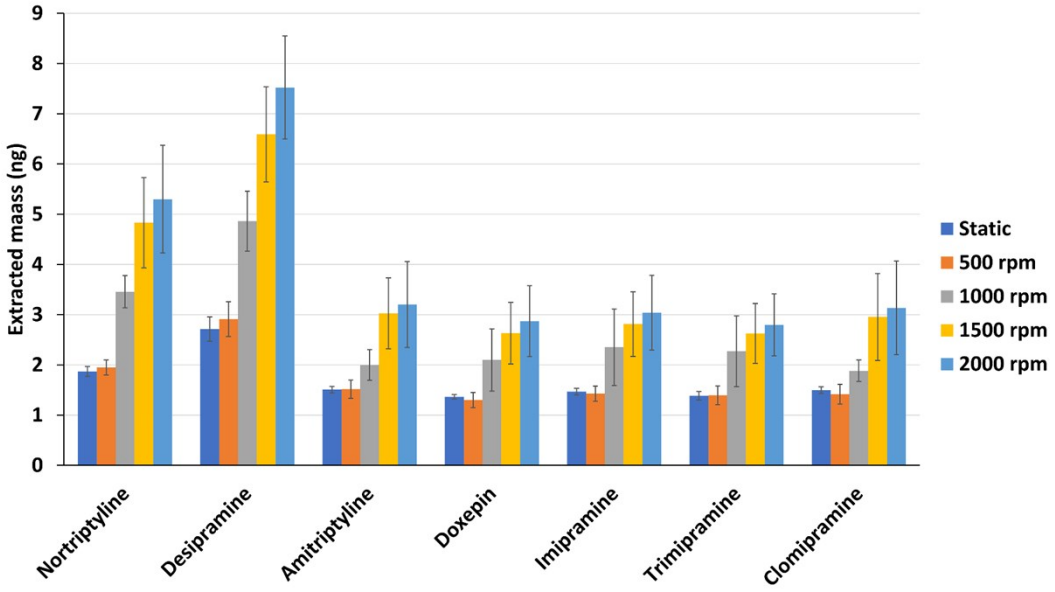


Figure S11. The effect of agitation on the recovered TCAs. Sample extraction: 700 µL of 100 ng mL^{-1} of TCAs in BSA with 1% TEA, extraction for 20 min at various agitation, washing: 8 sec immersion in water with 1%TEA, Desorption conditions: 700 µL of ACN/water (1:1) with 0.1%FA, 20 min at 500 rpm.

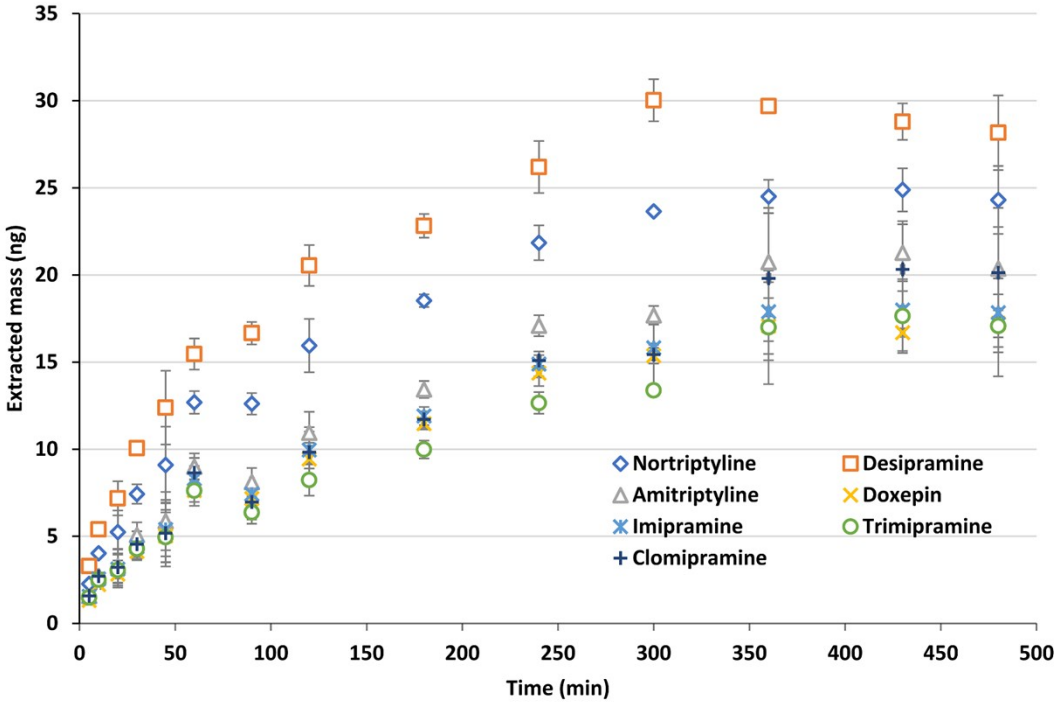


Figure S12. Extraction time profile of MIP. Sample extraction: 700 µL of 100 ng mL^{-1} of TCAs in BSA with 1% TEA at 1500 rpm, washing: 8 sec immersion in water with 1%TEA, Desorption conditions: 700 µL of ACN/water (1:1) with 0.1%FA, 20 min at 500 rpm

9- Calibration curve of TCAs in BSA

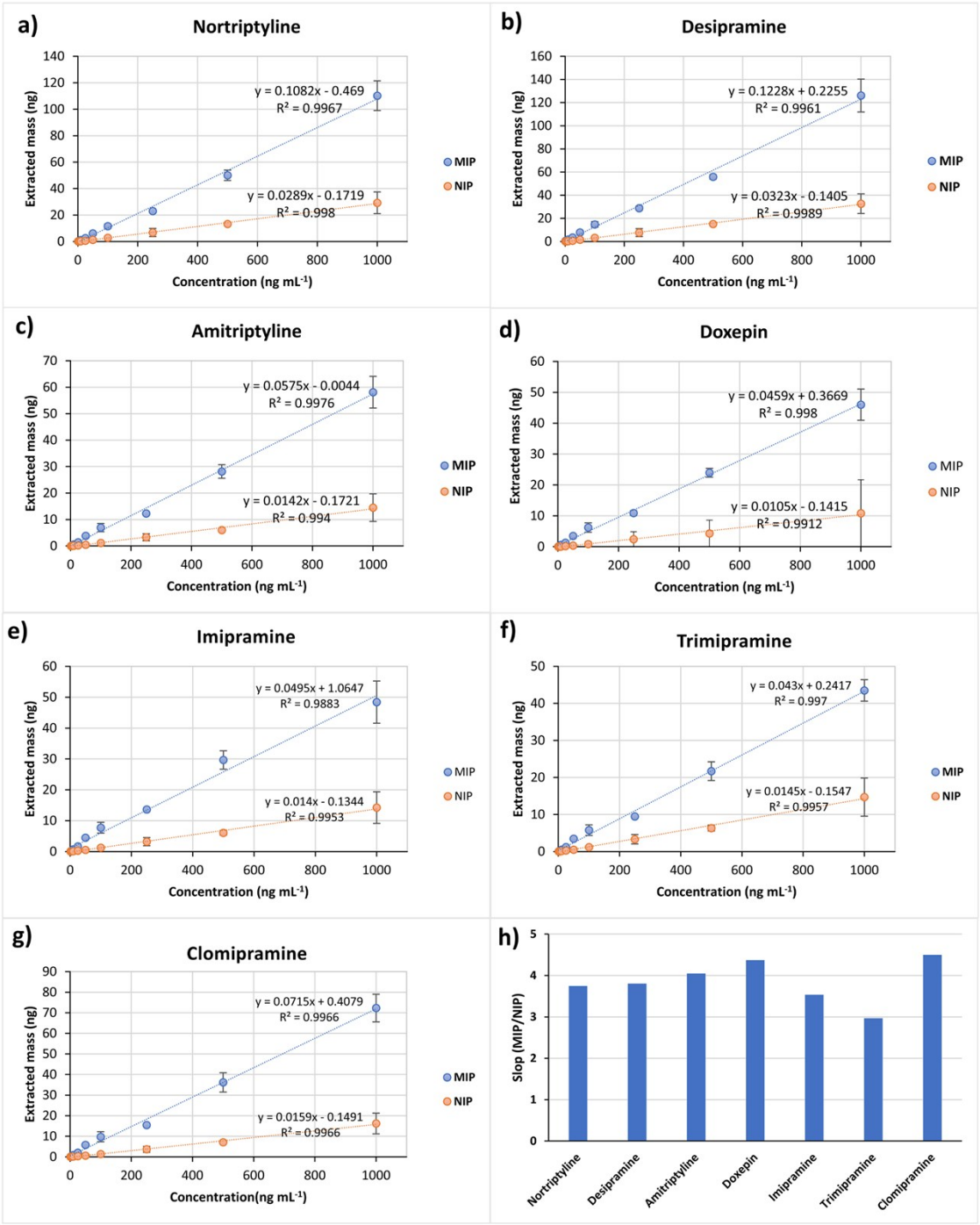


Figure S13. Calibration curves obtained for extraction of a) nortriptyline, b) desipramine, c) amitriptyline, d) doxepin, e) imipramine, f) trimipramine, and g) clomipramine; h) the ratio of slopes of calibration curve for TCAs using thin film MIPs versus NIPs for extraction from BSA.

10- Calibration curve of TCAs in plasma without internal standard

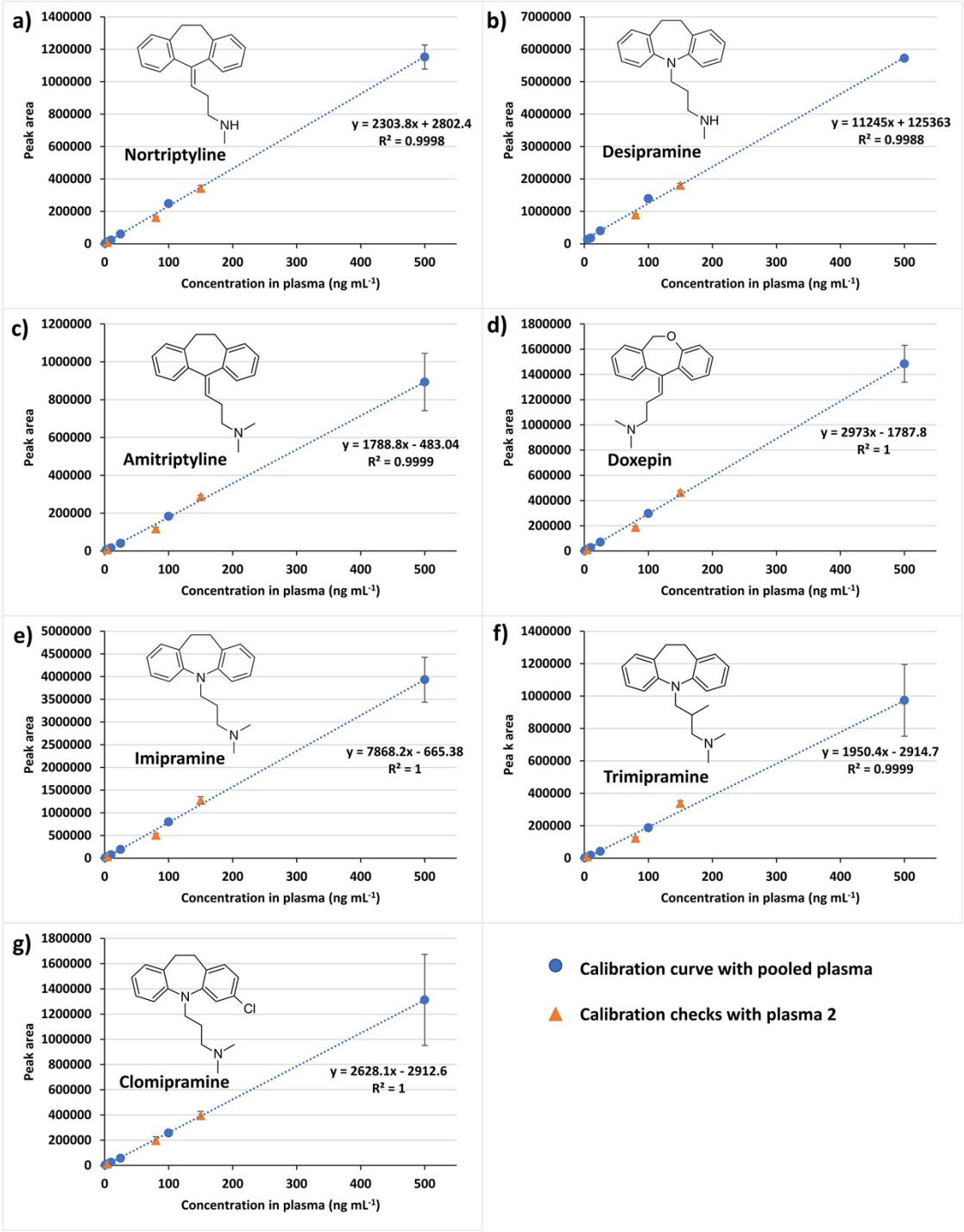


Figure S14. Calibration curves obtained for extraction of a) nortriptyline, b) desipramine, c) amitriptyline, d) doxepin, e) imipramine, f) trimipramine, and g) clomipramine using thin film MIPs from plasma solutions without internal standard correction.