Supporting Information

Sub-ppt Mass Spectrometric Detection of Therapeutic Drugs in Complex Biological Matrices Using Polystyrene-Microsphere-Coated Paper Spray

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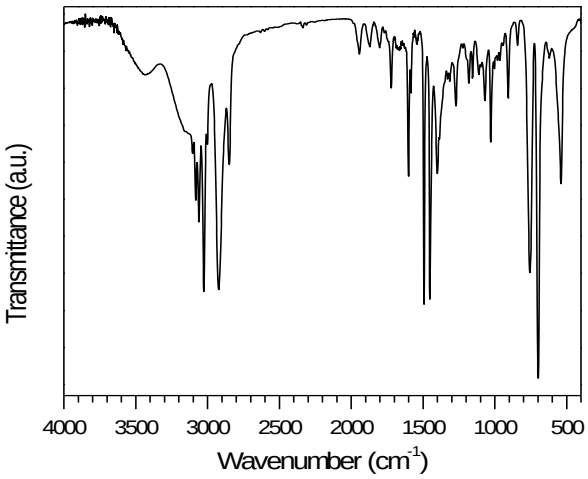

Figure S1. FT-IR spectra of the PS microspheres from the reaction of 25 mL styrene, 1.0 g BPO and 1.0 g PVP in the presence of 80 mL ethanol.

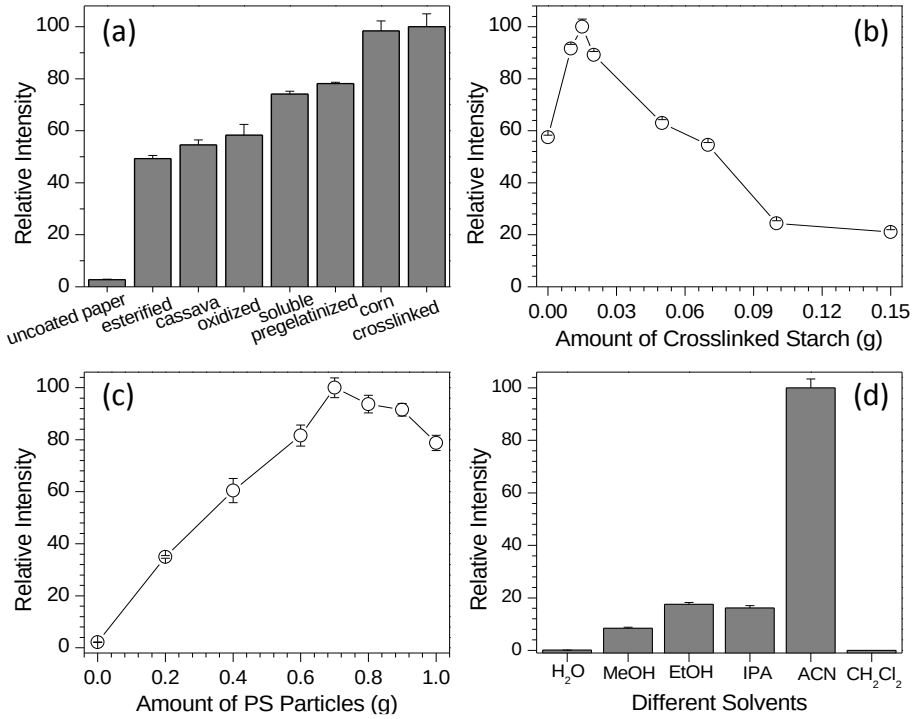

Figure S2. **(a)** Effect of the starch type on the performance of the as-prepared PS coated paper substrates (starch amount: 0.05 g and PS coating amount: 0.6 g); **(b)** effect of the amount of crosslinked starch on the paper performance (PS coating amount: 0.6 g); **(c)** effect of the coating amount of PS particles on the resulting paper performance (amount of crosslinked starch: 0.015 g); **(d)** Effect of spray solvent on the analysis performance of the resulting paper, in which H_2O means water, MeOH means methanol, EtOH means ethanol, IPA means isopropanol, ACN means acetonitrile and CH_2Cl_2 means dichloromethane.

Note: The solution volume for coating was 100 mL aqueous solution, and the evaluation was based on the analysis of 100 ng mL^{-1} amitriptyline [$(M + H)^+$, m/z 278, product ion, m/z 84] in whole blood sample using paper spray mass spectrometry (spray solvent: acetonitrile; applied voltage: 3.5 kV).

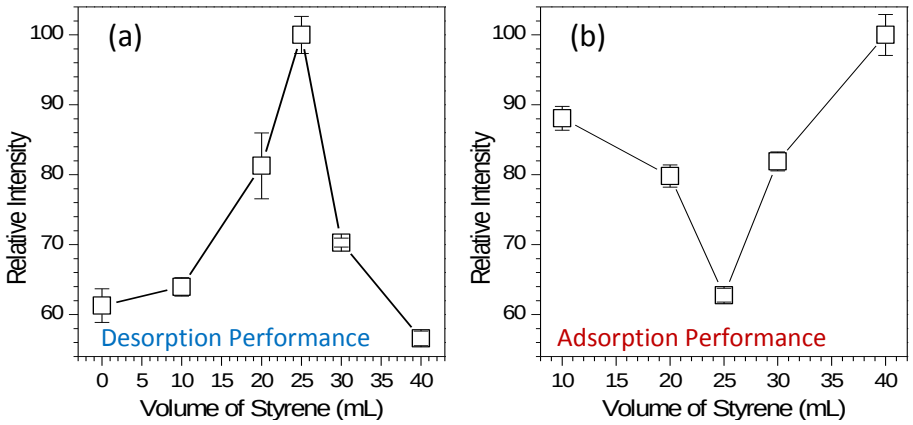

Figure S3. **(a)** Desorption behavior of methylene blue from PS coated paper substrates and **(b)** adsorption behavior of methylene blue onto PS particles in methanol solution, in which PS was prepared in the presence of various volumes of styrene (10 – 40 mL) by fixing the amounts of BPO (1.0 g) and PVP (1.0 g) in 80 mL ethanol.

Note: In **(a)**, "0" mL of styrene means uncoated filter paper; In **(b)**, the adsorption performance of these PS particles was carried out by mixing 0.7 g PS particles and 10 mL of methanol solution containing 1.0 µg mL^{-1} methylene blue followed by stirring for 30 min. Afterwards, the upper clear solution was collected, separated and analyzed with paper spray using grade 1 chromatography paper as substrate. The quantitative analysis was performed with paper spray, and the desorption and adsorption performance of various PS particles was evaluated by the normalized peak intensity of the characteristic fragment ion (m/z 268) from protonated methylene blue (m/z 284); spray solvent or sample, 25 µL methanol or adsorption solution; applied voltage, 3.5 kV]

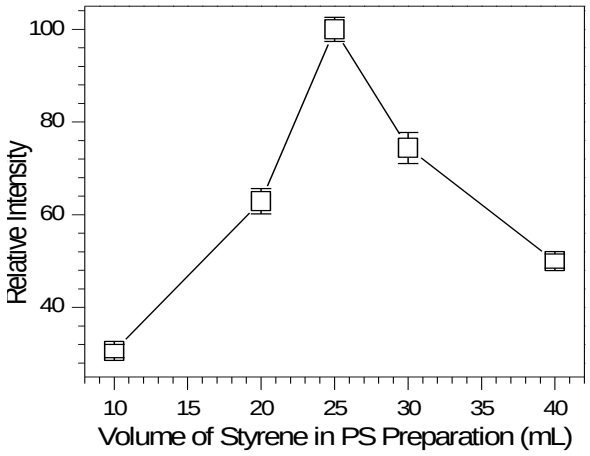

Figure S4. Effect of the volume of styrene on the performance of the resulting PS particles in analysis of verapamil in dried blood spot, in which PS particles were prepared in the presence of various volumes of styrene (10 – 40 mL) by fixing the amounts of BPO (1.0 g) and PVP (1.0 g) in 80 mL ethanol solution and the obtained PS particles were coated onto filter paper via vacuum filtration approach for paper spray analysis.

Note: The quantitative analysis was performed with paper spray mass spectrometry, and the performance of various PS particles was evaluated by the peak intensity of the characteristic fragment ion m/z 303 from protonated verapamil (m/z 455) spiked in dried blood spot; blood volume, 2 μL; spray solvent, 25 μL acetonitrile; applied voltage, 3.5 kV).

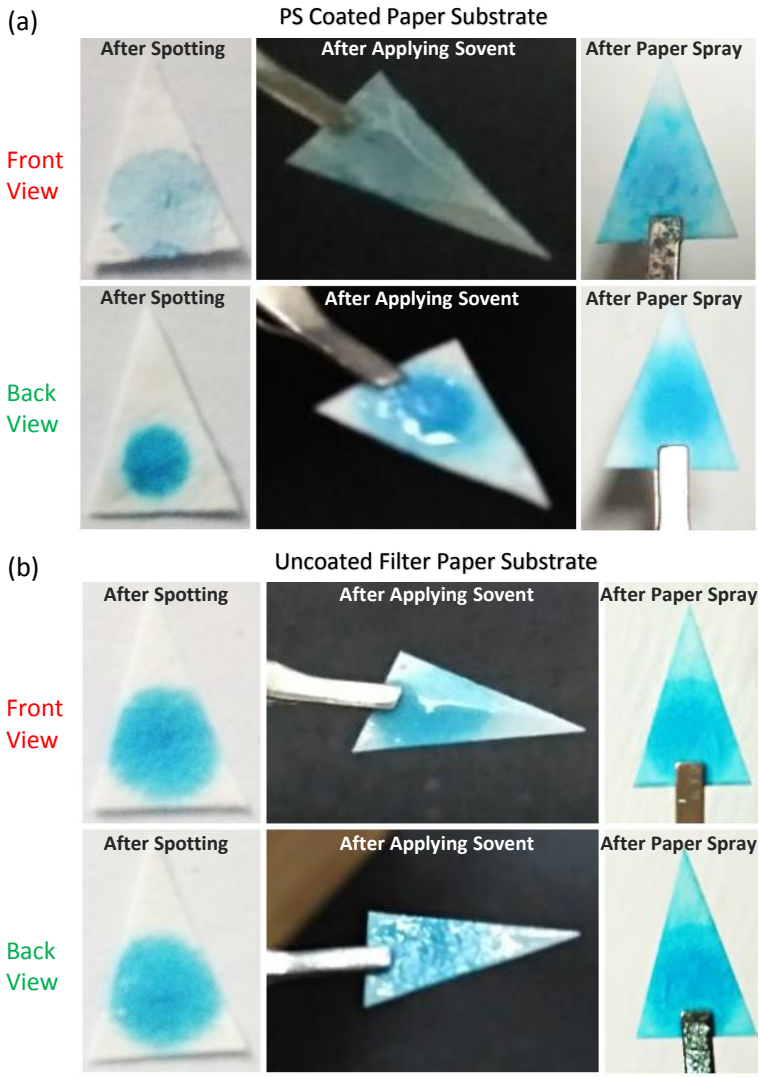

Figure S5. The front and back views of **(a)** PS-coated paper substrate and **(b)** uncoated filter paper substrate after spotting 2 µL of 1 mg mL^{-1} methylene blue, after applying 25 µL methanol and after paper spray (applied voltage, 3.5 kV) as indicated in the figures.

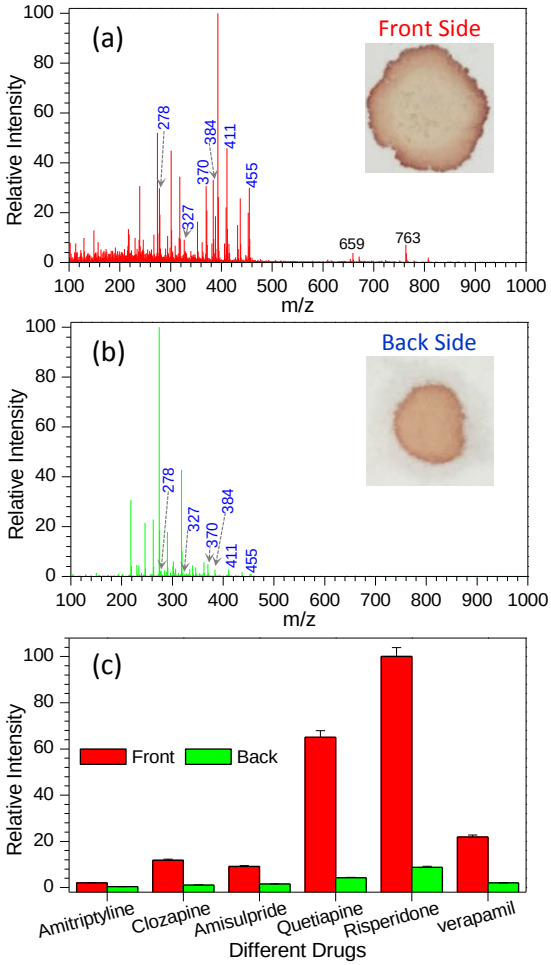

Figure S6. Mass spectra of the extracts from **(a)** the front side coated PS particles and **(b)** the back side paper substrate bearing blood spot [*Note*: ①The experiments were carried out by first spotting 2 μL blood sample containing 50 μg mL^{-1} amitriptyline (m/z 278), clozapine (m/z 327), amisulpride (m/z 370), quetiapine (m/z 384), risperidone (m/z 411), and verapamil (m/z 455) on a PS coated paper substrate. After drying in air, the front side coated PS particles and the back side paper substrate bearing blood sample were scratched off, respectively. Then the collected products were extracted with 1:1 methanol/water followed by analysis using nano-electrospray mass spectrometry (applied voltage: 1.2 kV; tip size of capillary for nano-electrospray: 10 μm). ② The inserts in the top-right corners of **(a)** and **(b)** are the photograph images of the **(a)** front and **(b)** back sides of PS coated paper with blood spots]; **(c)** Comparison of the analysis performance of paper spray by varying the spotting directions with SRM mode, in which "Front" means spotting blood samples on the front side (namely PS coated side) first followed by adding solvent on the front side for spray, "Back" means spotting blood samples on the back side (namely the opposite side of coated PS) first followed by adding solvent on the front side for spray (sample volume: 2 μL blood sample containing 100 ng mL^{-1} studied drugs; solvent: 25 μL acetonitrile; voltage: 3.5 kV).

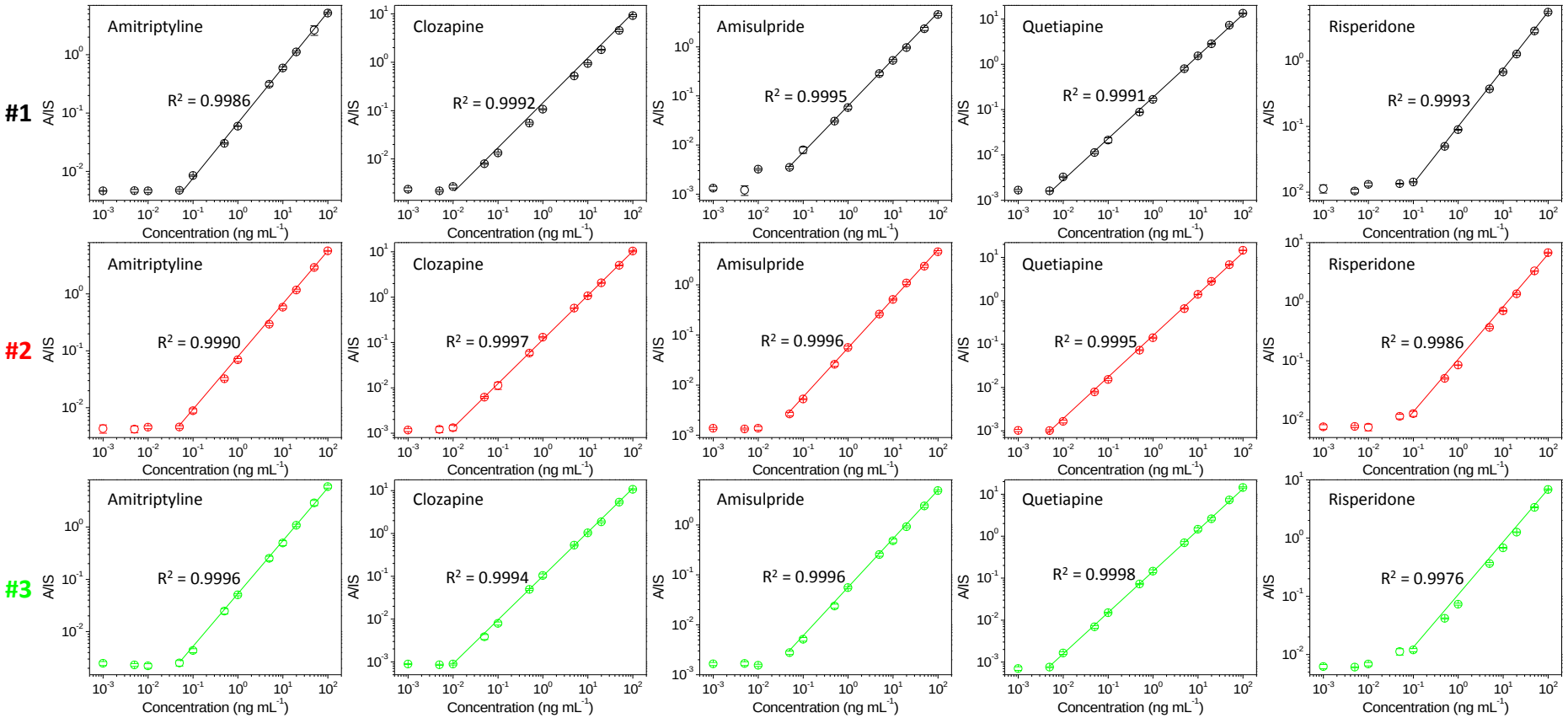

Figure S7. Comparison of quantitative analysis of whole blood spiked with amitriptyline, clozapine, amisulpride, quetiapine and risperidone (0.001 – 100 ng mL^{-1}) and its isotopomers (20 ng mL^{-1}) using PS coated paper in different days such as **Day #1**, **Day #2** and **Day #3**.
Note: Spray solvent: 25 µL of acetonitrile; Applied voltage: 3.5 kV; Bars represent the standard deviation of analysis for four replicates.

Table S1. Selected reaction monitoring (SRM) conditions

Analyte	Parent Ion m/z	Fragment Ion *m/z*	Collision Energy (V)	Tube Lens (V)
Amitriptyline	278, $[M + H]^+$	84	20	75
D_3-Amitriptyline	281, $[M + H]^+$	87	25	76
Clozapine	327, $[M + H]^+$	270	22	87
D_8-Clozapine	335, $[M + H]^+$	275	25	70
Amisulpride	370, $[M + H]^+$	112	26	99
D_5-Amisulpride	375, $[M + H]^+$	117	22	76
Quetiapine	384, $[M + H]^+$	253	23	101
D_8-Quetiapine	392, $[M + H]^+$	258	20	87
Risperidone	411, $[M + H]^+$	191	27	80
D_4-Risperidone	415, $[M + H]^+$	195	27	87

Table S2. Comparison of estimated lower limit of quantitation (LLOQs) of analytes in whole blood using PS coated paper substrates for paper spray mass spectrometry from different days.

Analyte	Matrix	LLOQs (ng mL^{-1})		
		Day #1	Day #2	Day #3
Amitriptyline	Blood	0.051	0.055	0.057
Clozapine	Blood	0.029	0.023	0.018
Amisulpride	Blood	0.038	0.047	0.042
Quetiapine	Blood	0.005	0.005	0.004
Risperidone	Blood	0.065	0.088	0.078