Supplementary Material

Gold-based immunochromatographic strip for the detection of sirolimus in human whole blood

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Experimental section

Materials and instruments

Gelatin was purchased from Sigma-Aldrich (Shanghai, China). Tetramethylbenzidine and horseradish peroxidase (HRP) were purchased from Aladdin (Shanghai, China). All other reagents and chemicals were supplied by the Sinopharm Chemical Reagent Co., Ltd (Beijing, China), and they were of analytical grade.

Results for the indirect enzyme-linked immunosorbent assay (ic-ELISA) were produced using a Multiskan MKS microplate reader (Thermo Labsystems Company, Beijing, China). AB SCIEX QTRAP 5500 system was used to obtain Liquid chromatography-tandem mass (LC-MS/MS) spectra.

Procedure of ic-ELISA

The procedure of ic-ELISA was performed as previously reported[1]. The sirolimus-CMO-BSA was diluted to 0.03 μg/mL in carbonate buffer (CB, 50 mM, pH 9.6), and added to 96-well microplates (100 μL/well). The microplates were stood at 37 ℃ for 2 h. After washing three times with wash buffer (PBS containing 0.05% Tween 20),

the 96-well microplates were added blocking buffer (2% gelatin in 0.05% CB, 200 μL/well) and stood at 37 ℃ for 2 h. After washing three times, 50 μL of sirolimus standard solutions and 50 μL of anti-sirolimus mAb were added to 96-well microplates and incubated at 37 ℃ for 0.5 h. After washing three times, HRP-labeled goat anti-mouse IgG (100 μL/well) was added and 96-well microplates were allowed to stand at 37 ℃ for 0.5 h. After washing three times again, substrate solution was added (100 μL/well) and 96-well microplates were stood at 37 ℃ in the dark for 15 min. Without washing, 2 M sulfuric acid was added to stop the reaction (50 μL/well). The absorbance was measured at 450 nm. OriginPro8.5 software was used to analyze the data we need.

LC-MS/MS analysis

LC-MS/MS analysis of sirolimus was performed as reported previously with some modifications[2]. A C18 column (Agilent Zorbax Eclipse Plus, 2.1×100 mm, 2.7 μm) was used for separation. The mobile phase was water phase (2 mM ammonium acetate and 0.1% fromic acid, A) and methanol phase (2 mM ammonium acetate and 0.1% fromic acid, B). The gradient conditions used in this paper were as follows: 0.0 min, 60 : 40 (A : B); 0.4 min, 60 : 40 (A : B); 0.4 - 2.0 min, 60 : 40 (A : B) - 0 : 100 (A : B); 5.0 min, 0 : 100 (A : B); 5.0 - 5.5 min, 0 : 100 (A : B) - 60 : 40 (A : B); 7.0 min, 60 : 40 (A : B). The flow rate was 0.3 mL/min with the column temperature of 30 ℃. The injection volume of 10 μL.

The mass spectrometry was choosing the positive electrospray ionization mode with an ion source temperature of 550 ℃ and ion spray voltage of 5500 V. The curtain gas,

ion source gas 1, and ion source gas 2 were 37 psi, 60 psi, and 15 psi, respectively. Multiple reaction monitoring (MRM) was selected to monitor the ions, and the parameters were shown in Table S1.

Under these conditions, a series of sirolimus standards in acetonitrile were prepared for LC-MS/MS analysis to establish the calibration curve.

Sample preparation for LC-MS/MS

Human whole blood samples were prepared according to a previously reported method with slight modifications[2]. 20 μL of sirolimus standard solutions were added into 180 μL of blank whole blood sample and after mixing thoroughly, 200 μL of 0.1 M zinc sulfate solution was added to lyse the red cells. Before centrifugation at 10,000 g for 15 min, 200 μL of acetonitrile: water solution (70:30, v/v) was added, and the solution was mixed fully and left to stand at room temperature for 15 min. The supernatant was collected and filtered through a 0.22 μm filter before LC-MS/MS analysis. The final concentrations of sirolimus in human whole blood were 0.5, 1.5, and 3 ng/mL for the recovery studies.

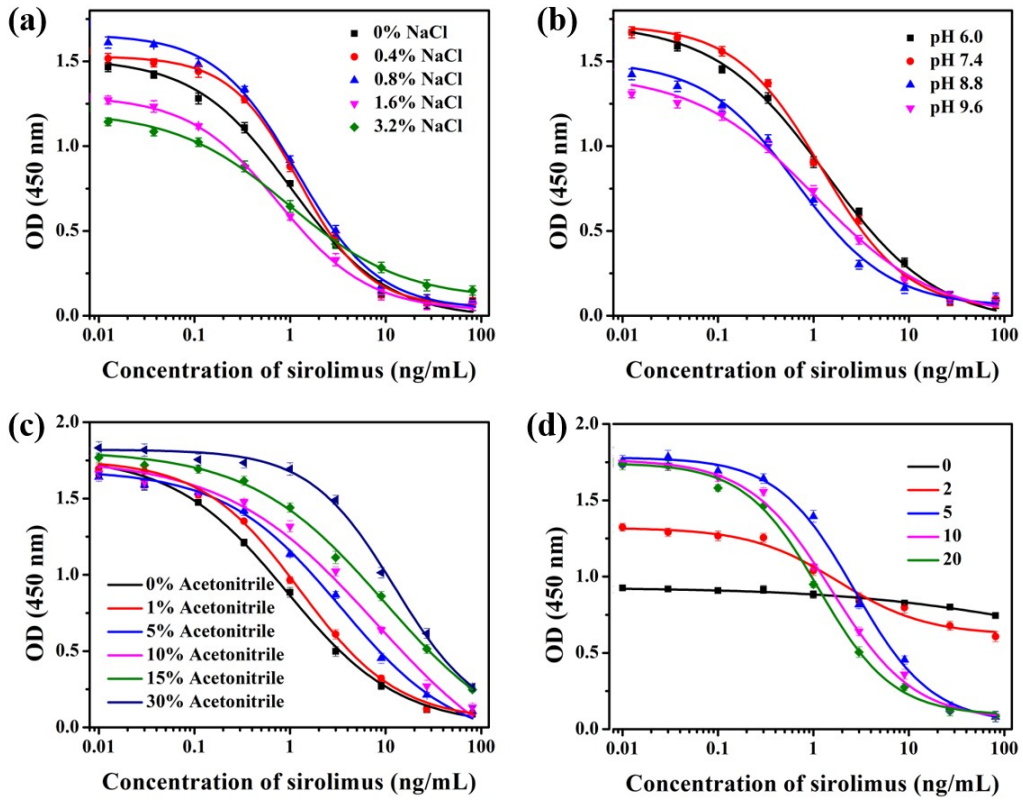

Fig. S1 (a) The optimization of ic-ELISA with different content of NaCl in PBS buffer; (b) the optimization of ic-ELISA with different pH values in PBS buffer; (c) the optimization of ic-ELISA with different content of acetonitrile in PBS buffer; (d) the standard curve for sirolimus detection in human whole blood samples with different dilution ratios.

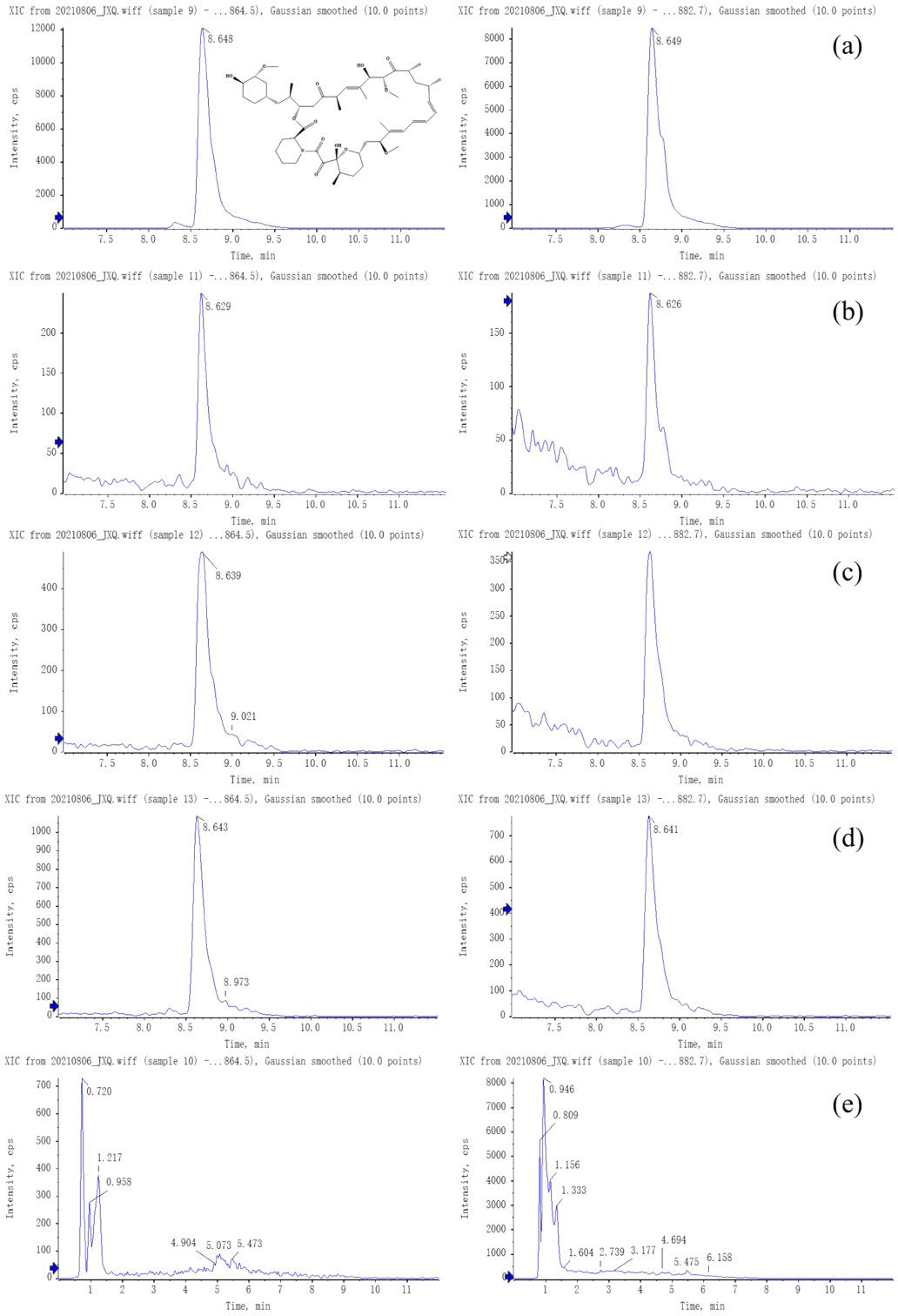

Fig. S2 LC-MS/MS chromatograms of sirolimus: (a) sirolimus standard in acetonitrile (50 ng/mL); (b) 0.5 ng/mL of sirolimus spiked in human whole blood; (c) 1.5 ng/mL of sirolimus spiked in human whole blood; (d) 3 ng/mL of sirolimus spiked in human whole blood samples; (e) 0 ng/mL of sirolimus spiked in human whole blood.

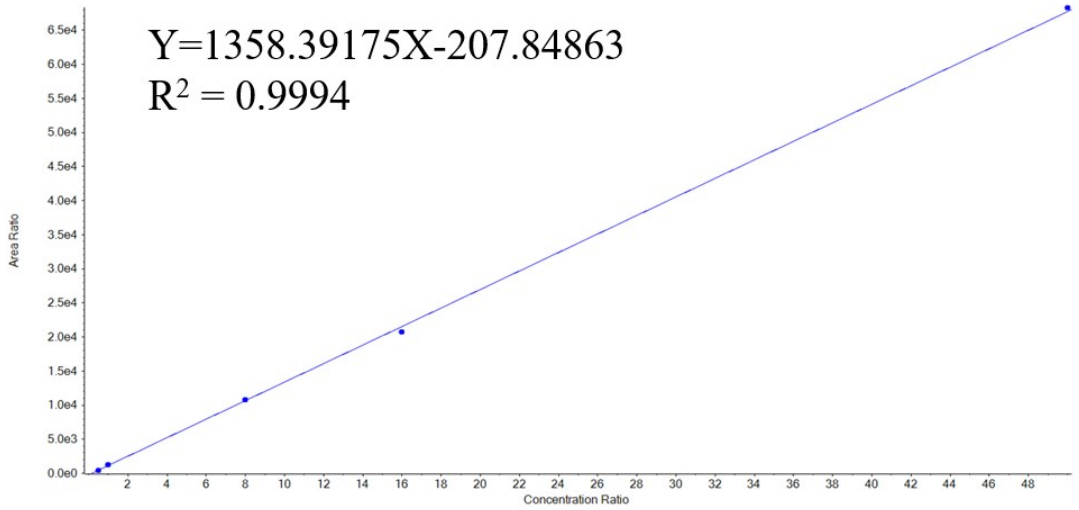

Fig. S3 The calibration curve of sirolimus in acetonitrile by LC-MS/MS.

Table S1. The MS/MS parameters for sirolimus monitoring.

	Parents (m/z)	Daughter (m/z)	Declustering Potential (V)	Entrance Potential (V)	Collision Energy (V)
Sirolimus	931.6	864.5	50	11	25
	931.6	882.3	50	11	18

Table S2. Comparison of different methods for sirolimus detection.

Method	LOD	Sample	Sample pretreatment	Reference
Ic-ELISA	0.19 ng/mL	whole blood	protein precipitation -	this work
Gold-based immunochromatographic strip	20 ng/mL	whole blood	protein precipitation	this work
Chemiluminescence immunoassay (CLIA)	0.25 ng/mL	Plasma	Diluted	3
Chemiluminescent magnetic microparticle immunoassay (CMIA)	1 ng/mL	whole blood	protein precipitation	4
Microparticle enzyme immunoassay (MEIA)	0.68 ng/mL	whole blood	protein precipitation	5
LC-MS/MS	0.5 ng/mL	whole blood	protein precipitation	2
Liquid chromatography–electrospray ionization mass spectrometry (LC-ESI-MS)	1 ng/mL	blood	DBS	6
Coated blade spray-tandem mass spectrometry (CBS-MS/MS)	1 ng/mL	whole blood	protein precipitation	7

Surface-assisted laser desorption/ionization mass spectrometry (SALDI/MS)	14 nM	urine and serum	DLLME procedure and protein precipitation	8
Micellar electrokinetic chromatography (MEKC)	25 ng/mL	serum	solid-phase microex-traction	9

References

1. L. Zeng, X. Wu, L. Liu, L. Xu, H. Kuang and C. Xu, *J. Mater. Chem. B*, 2020, **8**, 1935-1943.

2. Z.-S. Gong, Z.-H. Wu, S.-X. Xu, W.-N. Han, X.-M. Jiang, H. P. Liu, L. Yan, H. Wei and W. Yan, *Clin. Chim. Acta*, 2019, **498**, 21-26.

3. L. Zhang, H. Long, X. Li, K. Xu, M. Meng, Y. Yin and R. Xi, *Anal. Methods*, 2016, **8**, 6298-6304.

4. R. W. Schmid, J. Lotz, R. Schweigert, K. Lackner, G. Aimo, J. Friese, T. Rosiere, D. Dickson, D. Kenney and G. T. Maine, *Clin. Biochem.*, 2009, **42**, 1543-1548.

5. D. Wilson, F. Johnston, D. Holt, M. Moreton, J. Engelmayer, J. M. Gaulier, H. Luthe, P. Marquet, D. Moscato, M. Oellerich, R. Mosso, F. Streit, M. Brunet, C. Fillee, R. Schmid, P. Wallemacq and G. Barnes, *Clin. Biochem.*, 2006, **39**, 378-386.

6. R. N. Rao, P. K. Maurya, M. Ramesh, R. Srinivas and S. B. Agwane, *Biomed. Chromatogr.*, 2010, **24**, 1356-1364.

7. G. A. Gomez-Rios, M. Tascon, N. Reyes-Garces, E. Boyaci, J. J. Poole and J. Pawliszyn, *Anal. Chim. Acta*, 2018, **999**, 69-75.

8. P.-S. Chen, Y.-H. Cheng, S.-Y. Lin and S. Y. Chang, *Anal. Bioanal. Chem.*, 2016,

408, 629-637.

9. I. Oledzka, P. Kowalski, A. Plenis, N. Miekus, N. Grabow, T. Eickner and T. Baczek, *Electrophoresis*, 2018, **39**, 2590-2597.