SUPPORTING INFORMATION

for

A 3D-Printed Modularized Purification System for Rapid, High-Throughput MALDI-MS Analysis of Small-Volume Biological Samples

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Contents

1. Supporting Experimental Section

Chemicals and materials

The 3D printable photopolymer resin was purchased from Formlabs (Somerville, MA, USA). Ethanol (99.7%) was from Sinopharm Chemical Reagent Co. (Shanghai, China). Ordered mesoporous carbon (CMK-8) and graphene oxide were bought from XFNANO Materials Tech Co. (Nanjing, China). Cetyltrimethylammonium bromide (CTAB) and hexadecyldimethylbenzylammonium chloride (HDBAC) were from Sigma (St. Louis, MO, USA). Perfluorooctane sulfonic acid potassium salt (PFOS) was from J&K Scientific Ltd. (Beijing, China). Pentachlorophenol (PCP) was purchased from Dr. Ehrenstorfer (Augsburg, Germany). 2,2',4,4'-Tetrabromodiphenyl ether (BDE-47) was bought from Accustandard (New Haven, CT, USA). Tetrabromobisphenol A (TBBPA) was purchased from TCI (Tokyo, Japan). The ultrapure water was made by Millipore Milli-Q system (Billerica, MA, USA). HPLC grade methanol was from J. T. Baker (Phillipsburg, NJ, USA). All reagents were of analytical grade unless otherwise noted. PTFE filter membranes (diameter 13 mm, pore size 0.22 μm) were used in sample filtration (Jinteng, China).

3D printing of sample purification modules

The digital models of different modules were designed using SolidWorks 2017 (Dassault Systèmes SE, France) (see Figure 1B). The designed digital models were separately converted to .STL files before 3D printing (see Supporting Information). The 3D fabrication was carried out with a desktop stereolithography printer (Form 2, Formlabs Inc., Somerville, USA) with 50 μm layer thickness using photopolymer resin. Multiple modules can be printed in the same batch. After printing, modules were rinsed with 99.7% ethanol (Sinopharm Chemical Reagent Co., Shanghai, China) to remove unreacted resin.

Sample purification procedures

Prior to MS analysis, sample purification procedures including extraction, filtration, elution, and sample collection were all conducted by using the fabricated MSPS (see Supporting Movies S1-S4). Briefly, prior to sample purification, sorbent

materials (e.g. OMC) were initially dispersed in water with the aid of ultrasonication. Next, the dispersed sorbent materials and sample solution were added to the extraction module using a multi-channel pipette where the target analytes were extracted for half an hour. After extraction, the sample was transferred to the filtration module with a 0.22 μm filter membrane being placed inside. Then, the filtrate was transferred to and collected in the sample collection module which was connected to a vacuum pump to accelerate this process. The collected solution in the sample collection module was directly dropped onto the MALDI plate for the following MALDI-TOF MS analysis via a multi-channel stopcock. The sample collection module also has multiple channels and has been designed to fit the MALDI plate.

In the step of elution, 0.5 mL of eluent (DCM/acetone 1:1 (v/v) or methanol) was added into the filtration module. After eluting for 10 min, the filtrate was collected at the sample collection module for the MS analysis. If a matrix-free MALDI plate was used,[1] the filtrate was directly used for the MALDI-MS analysis. If an additional MALDI matrix was used, it can be added and mixed with the filtrate from the top inlet of the sample collection module before being loaded to the MALDI plate. To recognize the whole system assembling and sample purification process more clearly, we strongly recommend the readers to watch the Supporting Movies S1-S4 in SI.

MALDI-TOF MS analysis

MALDI-TOF MS was conducted on a Bruker Daltonics Autoflex III Smartbean MALDI-TOF mass spectrometer in reflector mode controlled by a FlexControl software. A 355 nm Nd:YAG laser with a frequency of 200 Hz was used. The spectra were recorded by summing 100 laser shots. The laser power was set to 35% and 5% in negative and positive ion mode, respectively. An aliquot (2 μL) of sample solution was dropped onto the MALDI plate followed by air-drying. The data processing was performed by the FlexAnalysis software 3.4.

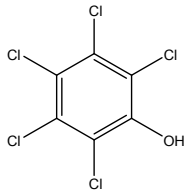
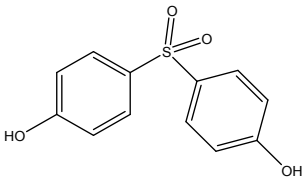
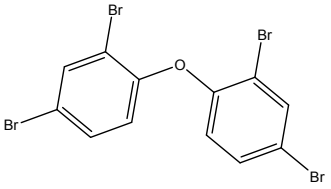
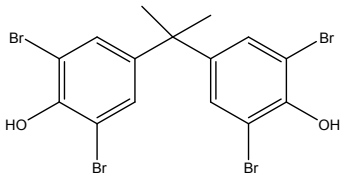
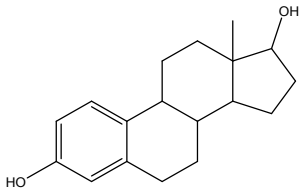
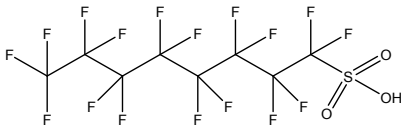
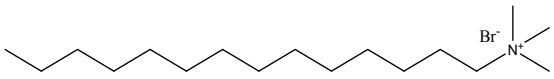
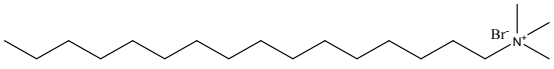
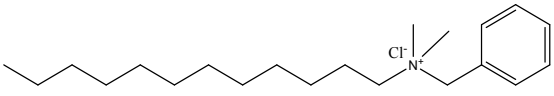
Collection of real samples

Tap water samples were collected from this lab (Beijing, China) and stored in dark at 4 °C. Human serum and whole blood samples were provided by the National Institute of Sports Medicine of China (Beijing, China) and stored at -20 °C. The

whole blood samples were from workers in a perfluorochemical plant located in Wuhan, China[2] and stored at -20 °C. Human lavage fluid samples were collected in the Fifth Hospital in Wuhan (Wuhan, China) and stored at -20 ℃.

2. Supporting Tables

Table S1. Chemical structures of model analytes.

compound	*m/z*	chemical structure
Pentachlorophenol (PCP)	264.2	
Bisphenol S (BPS)	248.3	
2,2',4,4'-Tetrabromodiphenyl ether (BDE-47)	250.1	
Tetrabromobisphenol A (TBBPA)	542.4	
Estradiol (E2)	270.5	
Perfluorooctanesulfonate (PFOS)	498.5	
Tetradecyltrimethylammonium bromide (TTAB)	255.9	
Cetyltrimethylammonium bromide (CTAB)	284.0	
Dodecyldimethylbenzylammonium chloride (DDBAC)	304.0	

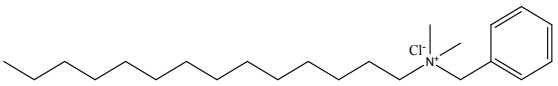
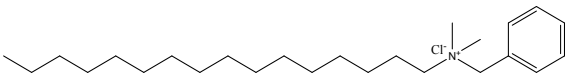
Tetradecyldimethylbenzylammonium chloride hydrate (TDBAC)	332.1	
N-hexadecyl-*N*,*N*-dimethylbenzylammonium chloride (HDBAC)	360.1	

Table S2. Reproducibility and recovery tests of typical analytes in whole blood samples.

Analyte	*m/z*	Recovery	Sample-to-sample RSD (n = 15)[b]	Channel-to-channel RSD (n = 5)[c]
BPS	248.4	84%	20.4%	5.2%
BDE-47	250.2	117%	22.4%	10.1%
PCP	264.2	94%	20.3%	7.7%
PFOS	498.5	106%	19.7%	5.6%
TBBPA	542.4	81%	25.6%	12.7%
TTAB	255.9	80%	24.3%	11.9%
CTAB	284.0	119%	22.2%	3.2%
DDBAC	304.0	114%	21.4%	10.8%
TDBAC	332.1	94%	22.9%	10.9%
HDBAC	360.1	82%	24.9%	4.0%

[b] The sample-to-sample RSDs were measured based on 15 samples in different batches.

[c] The channel-to-channel RSDs were measured based on 5 channels controlling by a stopcock.

3. Supporting Figures

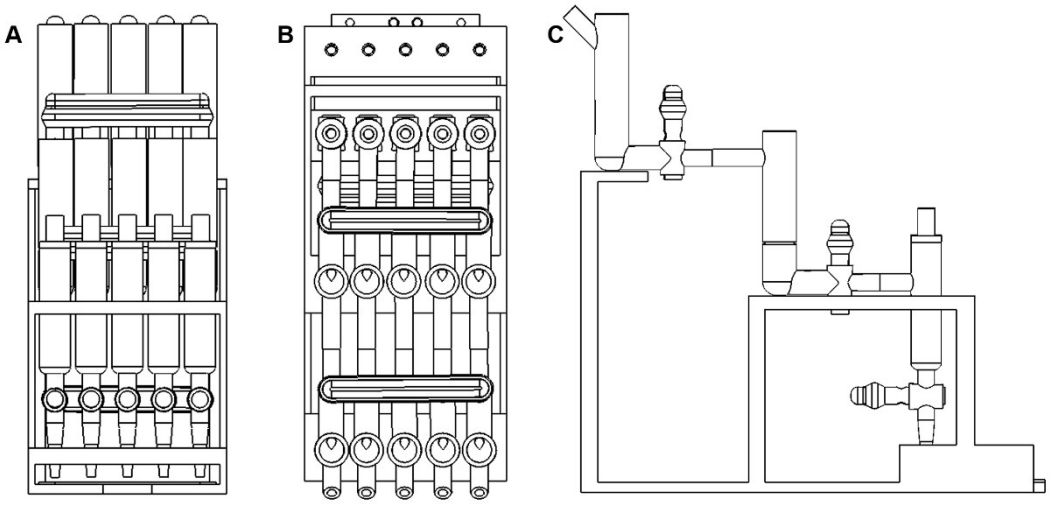


Figure S1. Three views of the assembled five-channel MSPS. (**A**) Front view, (**B**) top view, and (**C**) side view.

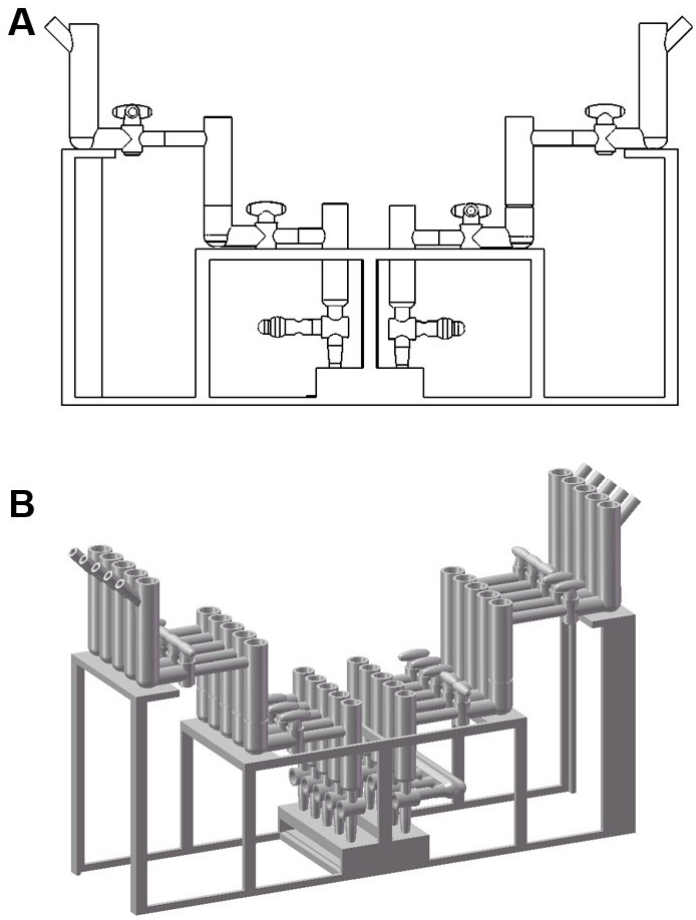


Figure S2. Double-sided MSPS. (**A**) Side view and (**B**) 3D model. The two five-channel systems were used in a head-to-head manner. In this way, the analytical capacity could be doubled and ten samples could be loaded simultaneously to the MALDI plate. Undoubtedly, the throughput can be easily increased by integrating more channels in the system.

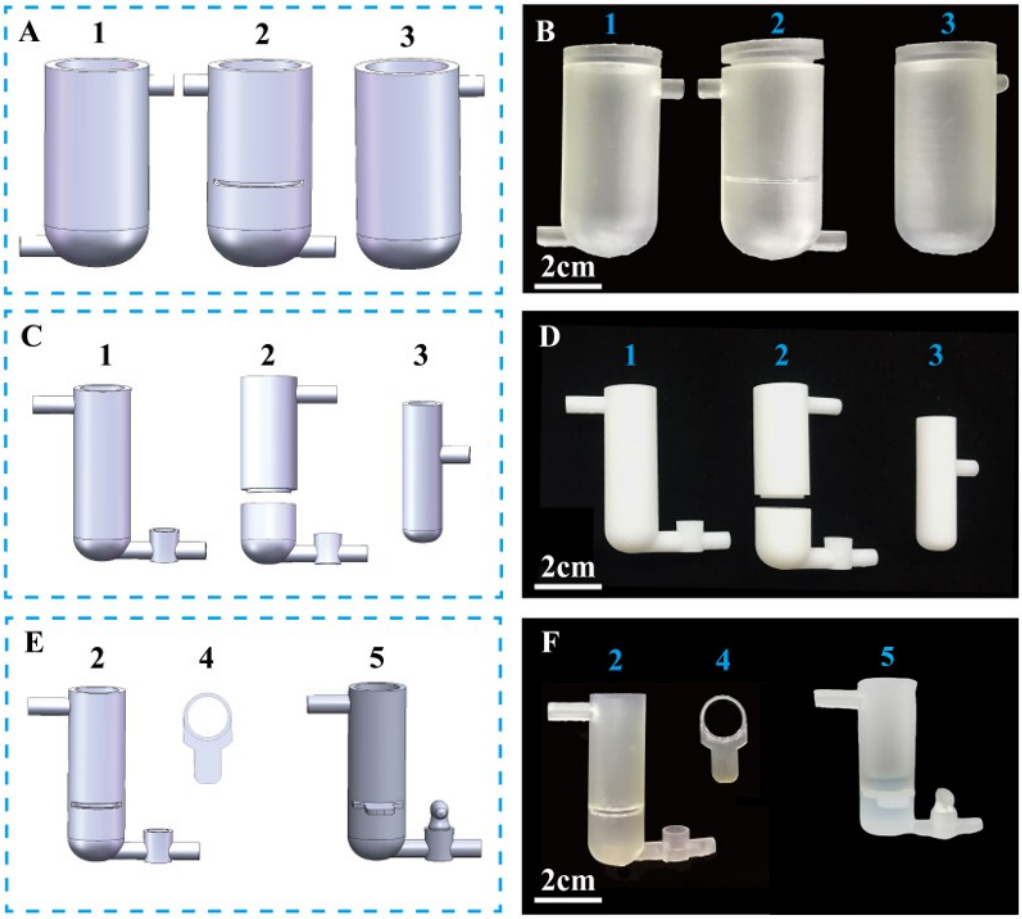


Figure S3. Optimization of the modular design. 3D models (**A, C, E**) and digital photos (**B, D, F**) of the modules with different configurations. Module: **1**, Extraction module; **2**, Filtration module; **3**, Sample collection module; **4**, Filter holder; **5**, Assemblies of the filtration module and filter holder. Depending on the sample volume, the volume of each module could be approximately 22 mL (**A** and **B**), 6 mL (**C** and **D**), and 2 mL (see Figure 1). For the filtration module, a special holder (**4**) was designed to place the filter membrane and it could be exactly inserted into the groove in the filtration module as shown in **E** and **F**.

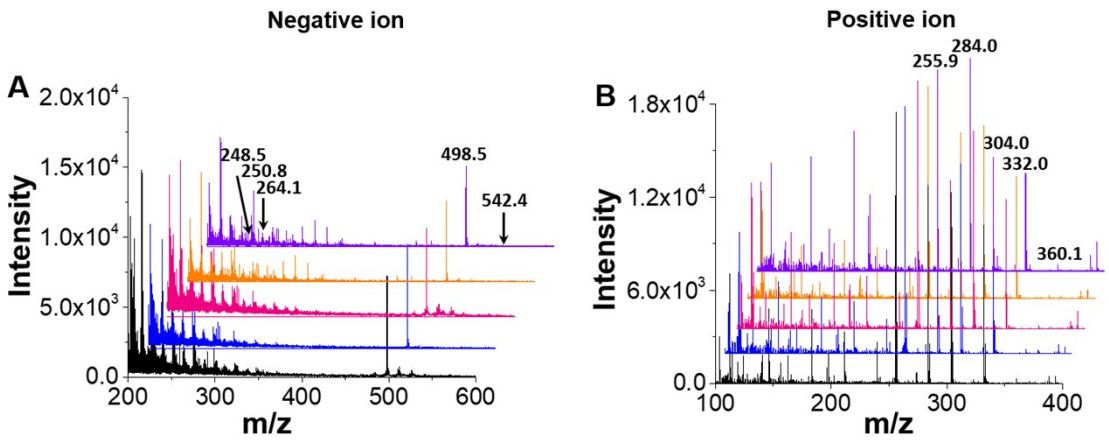


Figure S4. MALDI-TOF mass spectra with the MSPS obtained in the five channels in detection of ten typical toxic chemicals (BPS, BDE-47, PCP, PFOS, TBBPA, HDBAC, TDBAC, DDBAC, CTAB, TTAB) in a single drop of spiked whole blood. (**A**) negative ion and (**B**) positive ion mode. Spike concentration: TBBPA, 0.83 μg/mL; PFOS, 0.20 μg/mL; PCP, 0.83 μg/mL; BDE-47, 0.28 μg/mL; and BPS, 0.083 μg/mL; HDBAC, 4.2 μg/mL; TDBAC, 4.2 μg/mL; DDBAC, 4.2 μg/mL; CTAB, 0.83 μg/mL; and TTAB, 0.83 μg/mL. The five mass spectra in different colors represent the results from the five parallel channels with the same sample.

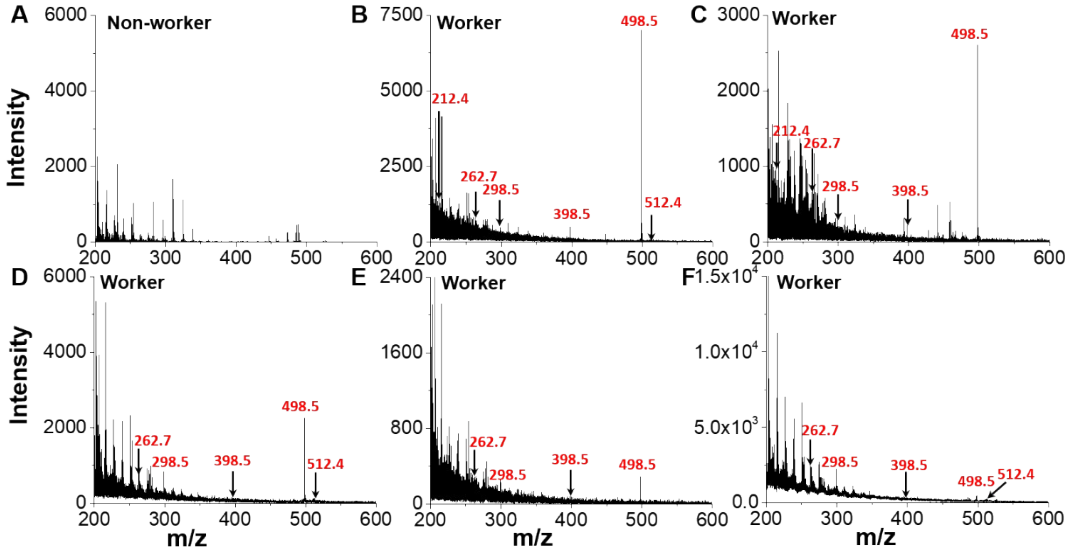


Figure S5. Rapid screening of toxic chemicals in a single drop of human whole blood samples collected from workers in a perfluorochemical plant (Wuhan, China) with the MSPS. Results showed the mass spectra of (A) blank sample from non-workers and (B-F) five samples collected from five different workers. Figure S5A showed that none of toxic chemicals could be detected in healthy adult whole blood samples. Six perfluorochemicals were identified at *m*/*z* 212.4, 262.7, 298.5, 398.5, 498.5 and 512.4, which were assigned to the peaks of $[M - H]^-$ of perfluorobutyric acid (PFBA), perfluorovaleric acid (PFPeA), perfluorobutanesulfonic acid (PFBS), perfluorohexanesulfonate (PFHxS), perfluorooctane sulfonate (PFOS), and perfluorodecanoic acid (PFDA), respectively.

4. Captions for Supporting Movies

Movie S1. Assembly of modules into one channel for the MSPS.

Movie S2. Assembly of five-channel MSPS.

Movie S3. The whole sample purification procedures with the MSPS.

Movie S4. Disassembly of the MSPS.

5. Supporting .STL Files for 3D Printing

6. References for SI

1. D. Wang, X. Huang, J. Li, B. He, Q. Liu, L. Hu and G. Jiang, Chem. Commun., 2018, **54**, 2723-2726.
2. X. Huang, Q. Liu, J. Fu, Z. Nie, K. Gao and G. Jiang, *Anal. Chem.*, 2016, **88**, 4107-4113.