Speciation of selenium in cells by HPLC-ICP-MS after (on-chip) magnetic solid phase extraction

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Supplementary material.

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Apparatus. A KQ 5200DE model ultrasonicator (Shumei Instrument Factory, Kunshan, China) was used to disperse the nanoparticles in the solution for the MSPE. An Nd-Fe-B magnet (15.0 cm × 6.0 cm × 1.6 cm) was used for magnetic separation. The Vortex-5 vortex mixer was purchased from Kylin-Bell Lab Instruments Co., Ltd. (Haimen, China). An inverted fluorescence microscope (Nikon, TE2000-U, Kanagawa, Japan) coupled with a CCD (Q-imaging Retiga 2000R) was used for *in situ* observations of the microfluidic chip. A KW-4A spin coater (Siyouyen Electronic Technology Co., Ltd., Beijing, China) and PDC-M plasma cleaner (Mingheng Science and Technology Development Co., Ltd., Chengdu, China) were used for the preparation of microfluidic chips. TS2-60 syringe pumps (Baoding Longer Precision Pump Co., Ltd., Baoding, China) and sterile syringes were applied for sample introduction in the microfluidic chip.

AT 20 microbalance (Mettler Toledo Instruments Co., Ltd., Shanghai, China) and BS110S electronic balance (Beijing Sartorius Instrument Systems, Inc., Beijing China) were used for reagent weighing. A Mettler Toledo 320-s pH meter (Mettler Toledo Instruments Co., Ltd., Shanghai, China) with a combined electrode was used to adjust the pH. The instruments applied for characterization include 170SX FT-IR (Nicolet, USA), scanning electron micrograph (SEM) (Hitachi modal X-650, Tokyo, Japan), JEM-100 CXM transmission electron microscope (TEM) (JEOL, Tokyo, Japan), and S4 Pioneer X-ray spectrometer (Bruker AXS, Germany). Gaussmeter (LZ-630 model, Linkjoin Technology, Inc., Hunan, China) was purchased from a local market (Wuhan, China).

Reagents. 1-Butyl-3-methylimidazolium tetrafluroborate ([BMIM]BF_4, 97%) was purchased from Hangzhou Chemer Chemical Co., Ltd. (Hangzhou, China). Polydimethylsilicone (PDMS) was prepared by mixing oligomers (component A) and crosslinking agents (component B) (GE RTV 615, Momentive Performance Materials, NY, USA) with a certain ratio. Sodium carbonate was obtained from Beijing Chemical Works (Beijing, China). Ammonium acetate used was chromatographically pure (TEDIA, USA). Acetic acid, styrene (99%), ammonium persulfate (95%), and sodium dodecyl benzene sulfonate (90%) was obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Divinylbenzene was purchased from Guangfu Fine Chemical Research Institute of Tianjin (Tianjin, China). Sodium styrene sulfonate (90%) was obtained from Aladdin Reagent Inc. (Shanghai, China). Emulsifier OP-10 (polyoxyethylene nonylphenol ether, 99%, imported) was purchased from Wuhan Tongxin Chemical Plant (Wuhan,

China). Pronase (isolated from *Streptomyces griseus*, lyophilized powder) was obtained from Roche (Roche Diagnostics GmbH, Germany).

Fabrication of Microfluidic Devices for On-chip MSPE. A transparent mask patterned with a high-resolution laser printer was used to create a master on a silicon wafer with AZ 9260 photoresist. The master was exposed to trimethylchlorosilane vapor for 3 min before PDMS casting to avoid adhesion between PDMS and silicon wafer. For the upper thick layer with flow channels, GE RTV 615 (PDMS) components A and B were mixed at a ratio of 10:1 and cast on the master after air bubbles disappeared. Two permanent magnets (2.5 × 0.6 × 0.3 cm, the magnetic flux density was ca. 500 mT, as determined by a Gausmeter) were equidistantly placed on each side of the central microchannel to generate a uniform magnetic field. The distance between the permanent magnet edge and the microchannel edge was fixed at 340 μm (the magnetic flux density of ca. 350 mT in the middle of central micro-channel). The solidified PDMS was peeled off and drilled on demand after incubation at 80 °C for 1.5 h. GE RTV 615 (PDMS) components A and B were mixed at a ratio of 20:1 for the lower thin layer with controlling channels. The mixture was spin-coated onto the master (~30 μm thick) after air bubbles disappeared, and then incubated at 80 °C for 30 min. Afterward, these two layers were treated with oxygen plasma, and the upper layer was aligned onto the lower layer and bonded at 80 °C for 30 min. The ensemble and a clean glass were exposed to oxygen plasma and then bonded together after the ensemble was peeled off and the holes for the valves were drilled.

A MATLAB interface and a pressure controller were used to control the opening/closure of the different microvalves. The thin PDMS membrane between the upper and lower channels was deflected to switch off the upper flow channels when sufficient air pressure was exerted to the lower controlling channels. Consequently, the thin PDMS membrane was returned to the original state and the upper flow channel can be switched on when pressure was removed.

Sample Pretreatment. Certified Reference Material (SELM-1) was extracted by methane sulfonic acid. [23] Briefly, 0.1 g of SELM-1 powder was placed in a round bottom flask and then 10 mL 4 mol L^{-1} methane sulfonic acid was added. The solution was refluxed for 16 h. Afterward, the extract was transferred into a 100 mL volumetric flask and diluted to calibrate with high-purity water. The solution was diluted 1000 times and adjusted to pH 3.5 before the analysis.

For on-chip MSPE, the selenium-enriched yeast cells were counted and pre-digested.

Approximately 2 mL 0.05 mol L^{-1} EDTA and 60 μL β-mercaptoethanol were added into the sample after the yeast cells were washed with 0.05 mol L^{-1} EDTA. The mixture was shaken for 30 min at 30 °C in the dark before the sample was washed by 1 mol L^{-1} sorbitol. Afterward, 1 mL of 40 mg mL^{-1} snailase, 1 mL of 40 mg mL^{-1} cellulase, and 60 μL of β-mercaptoethanol were added into the sample. The mixture was again shaken for 30 min at 30 °C in darkness. The sample was washed with 1 mol L^{-1} sorbitol twice and re-dispersed in 1.5 mL of high-purity water prior to analysis.

For MSPE, the selenium-enriched yeast cells were lyophilized. Up to 10 mL high-purity water was added to a 0.05 g lyophilized sample in a 15 mL centrifuge tube, and then the tube was placed in a boiling water bath for 1 h. The mixture was shaken well every 15 min. Afterward, the samples were centrifuged at 4000 rpm for 30 min and then filtered. The supernatant was diluted 100 times, and the pH was adjusted to 3.5 prior to analysis.

For total selenium analysis, 0.01 g samples were weighed into the crucibles. About 4 mL of concentrated HNO_3 (65%) was added, and then the samples were placed on the ECH-1 temperature control heating panel (Sineo Microwave Chemistry Technology Co., Ltd., Shanghai, China) at 150 °C for digestion. The digest was filtrated and transferred into a 100 mL flask and diluted to the calibration with high-purity water prior to pneumatic nebulization (PN)-ICP-MS detection.

Optimization of MSPE. *Effect of Sample Volume and Extraction Time.* By fixing the amount of each target selenium species at 20 ng, the effect of varying sample volume in the range of 1 mL to 20 mL on the extraction efficiency of the target selenium species was investigated. Figure S-5 shows that the sample volume in the investigated range had no obvious influence on the extraction of the target selenium species. Although a larger sample volume would result in higher enrichment factors, considering that biological samples are always limited, 5 mL sample solution was employed for further experiments.

The effect of extraction time on the extraction efficiency of the five target selenium species was studied with the extraction time varying from 2 min to 20 min. The experimental results in Figure S-6 indicated that the extraction efficiency of the five target selenium species increased with the increase of extraction time from 2 min to 8 min, and almost remained constant with further increase of the extraction time from 8 min to 20 min. Therefore, an extraction time of 12

min was adopted for simultaneous extraction of the five target selenium species.

Effect of Desorption Time and Volume. The effect of desorption time in the range of 2 min to 20 min on the desorption of the five target selenium species was studied, and the results are shown in Figure S-7. The signal intensity of the five target selenium species increased with the increase of desorption time from 2 min to 10 min, and then remained almost constant with further increase of the desorption time. As a result, a desorption time of 10 min was selected for subsequent experiments.

By fixing the concentration of the Na_2CO_3 solution at 0.5 mol L^{-1}, desorption volumes of 15, 20, 50, 80, and 100 μL (with the injection volume fixed at 10 μL for HPLC-ICP-MS analysis) were used to investigate the effect on the desorption of the five target selenium species. Figure S-8 shows that with the increase of the desorption volume, the signal intensity of the five target selenium species decreased gradually, probably due to the dilution effect. Thus, 15 μL was selected as the desorption volume.

Optimization of HPLC Conditions. Perfluorocarboxylic acids, such as trifluoroacetic acid (TFA) and heptafluorobutanoic acid (HFBA), are often used as mobile phase additives for ion-pair HPLC separation of different selenium species. [26, 27] The function of room temperature ionic liquid (RTIL) used as the mobile phase additive in HPLC-ICP-MS for the speciation of inorganic selenium and selenoamino acids were studied in our previous work, [28] and it was found that the addition of RTILs in mobile phase could adjust the retention behavior of selenium species to achieve good separation in a relatively short time. Based on this observation, under the same conditions in which the mobile phase contained 50 mmol L^{-1} acetic acid/ammonium acetate (pH 4.0) and 3% (v/v) methanol with flow rate of 1 mL min^{-1} and column temperature of 30 °C, a comparison of the HPLC separation by employing 0.4% (v/v) $[BMIM]BF_4$ as mobile phase additive with that by applying 0.1% (v/v) TFA as ion-pair reagent was performed. Figs. S-12 (a) and (b) show that the retention behaviors of $SeCys_2$, MeSeCys, SeMet, and SeEt did not obviously change when either 0.4% (v/v) $[BMIM]BF_4$ or 0.1% (v/v) TFA was used as mobile phase additive. However, the retention time of GluMeSeCys changed from 252 s using 0.1% (v/v) TFA to 464 s using 0.4% (v/v) $[BMIM]BF_4$, indicating that better resolution can be obtained by employing 0.4% (v/v) $[BMIM]BF_4$ as mobile phase additive. Setting $[BMIM]BF_4$ as mobile phase additive, the separation conditions were further studied. The optimized HPLC-ICP-MS conditions were as

follows: mobile phase, 50 mmol L^{-1} acetic acid/ammonium acetate (pH 4.0) + 3% (v/v) methanol + 0.4% (v/v) [BMIM]BF_4; flow rate, 1 mL min^{-1}; and column temperature, 50 °C. Under optimized conditions, five target selenium species can be baseline separated within 8 min, and the retention times for $SeCys_2$, MeSeCys, SeMet, GluMeSeCys, and SeEt were 169, 199, 255, 311, and 427 s, respectively.

Table S1 The elemental composition of Fe_3O_4@PSS MNPs obtained by X-ray spectrometer

Elements	CH	O	S	Fe
A.W.	13	16	32	56
Mass ratio	9.32%	24.60%	1.29%	64.50%
Molar ratio	20.80%	44.61%	1.17%	33.42%

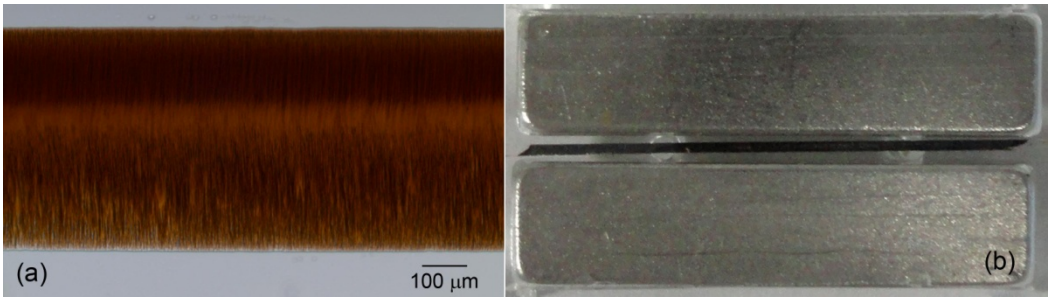

Fig. S1 Micrograph (a) and photograph (b) of the self-assembly MNPs packed-column on chip.

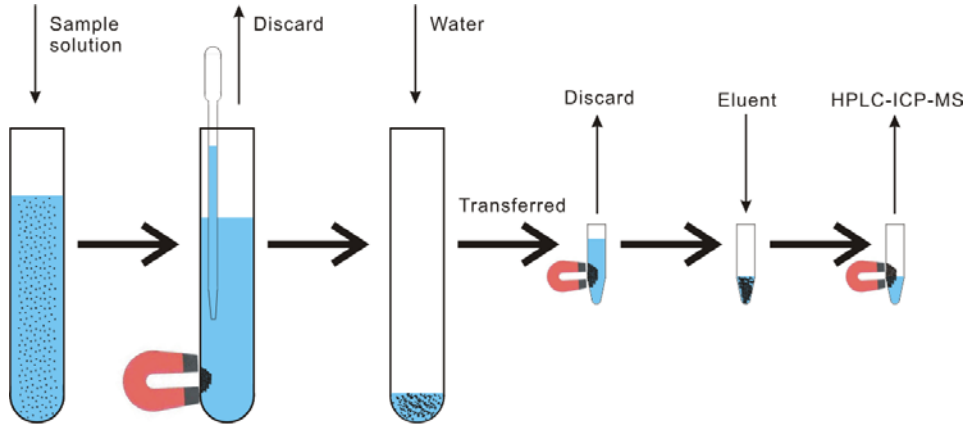

Fig. S2 Procedure for magnetic solid phase extraction.

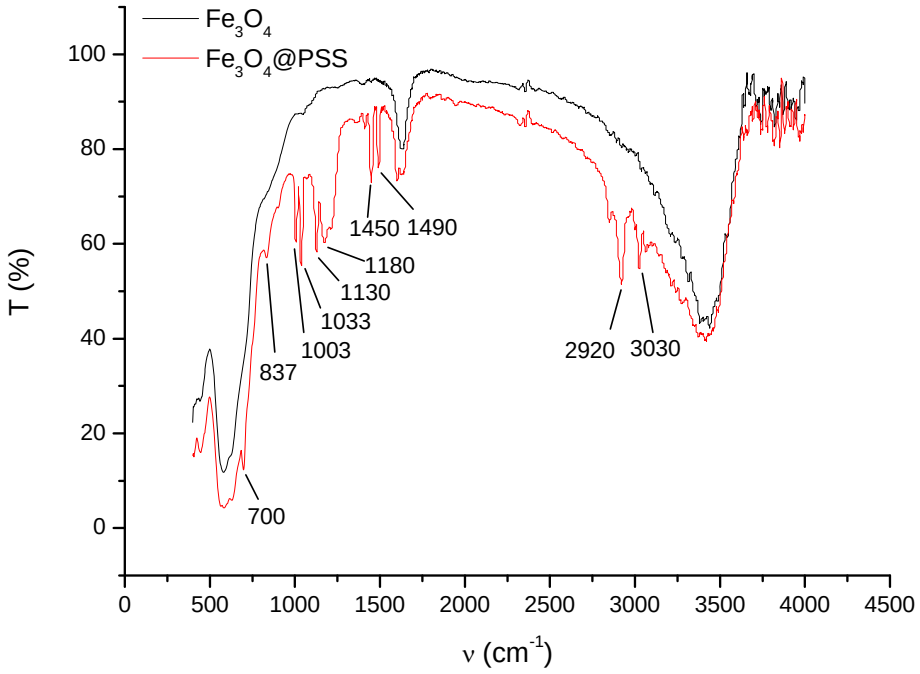

Fig. S3 FT-IR of Fe_3O_4@PSS MNPs.

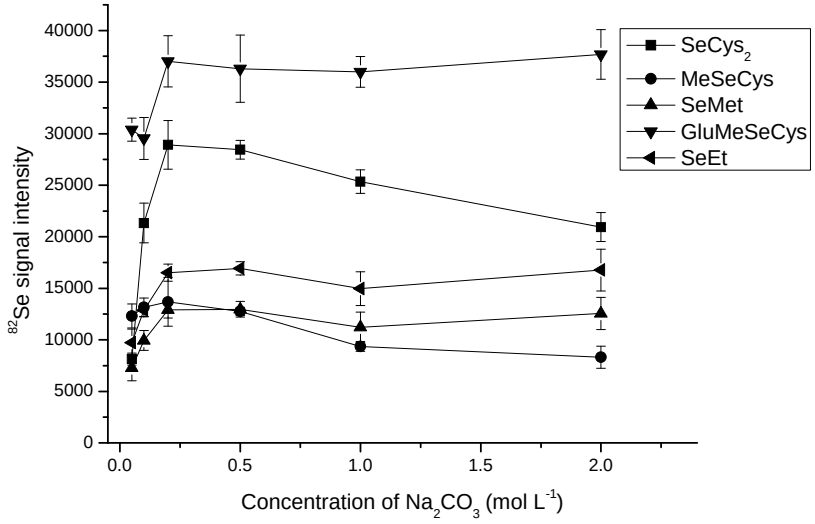

Fig. S4 Effect of the concentration of Na_2CO_3 solution on the desorption of five target selenium species by MSPE. (Conditions: Sample volume: 5 mL; Sample pH: 3.5; Extraction time: 12 min; Desorption volume: 50 μL; Desorption time: 10 min; Error bars represent the standard deviation of triplicate experiments.)

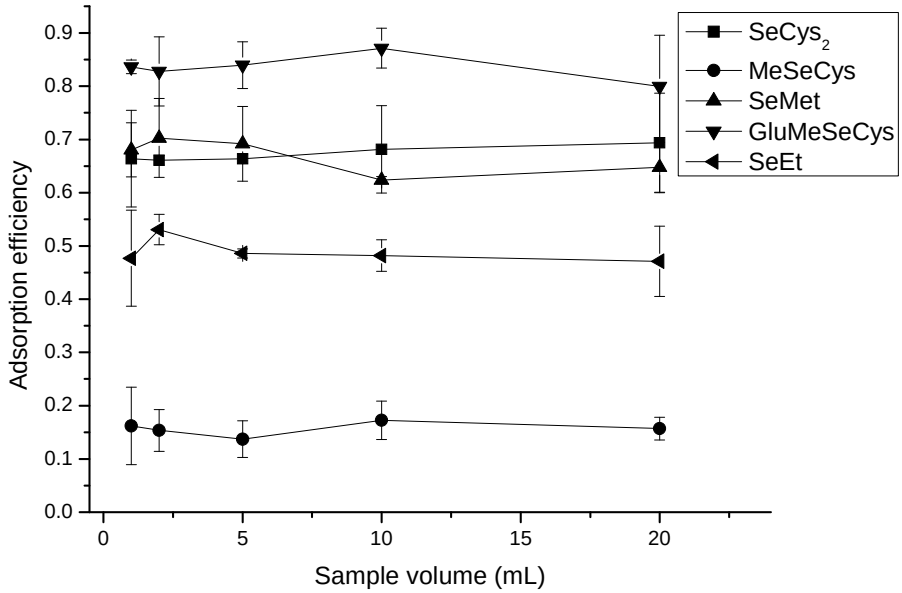

Fig. S5 Effect of the sample volume on the extraction of five target selenium species by MSPE. (Conditions: Sample pH: 3.5; Extraction time: 12 min; Error bars represent the standard deviation of triplicate experiments.)

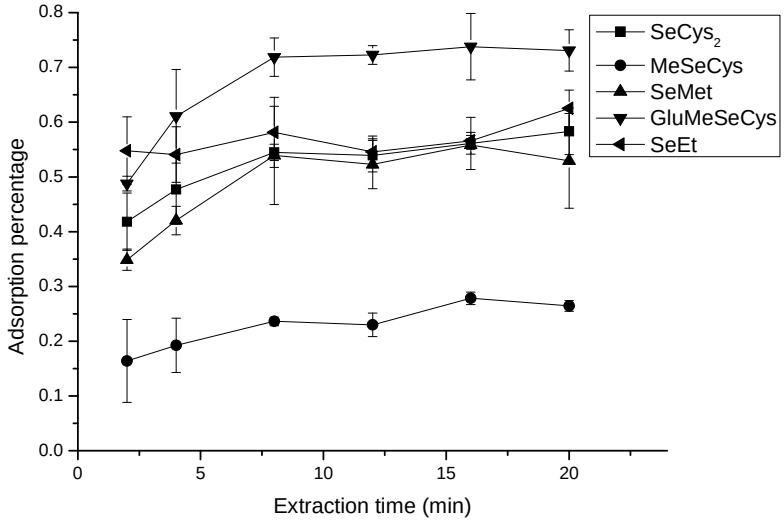

Fig. S6 Effect of the extraction time on the extraction of five target selenium species by MSPE. (Conditions: Sample volume: 5 mL; Sample pH: 3.5; Error bars represent the standard deviation of triplicate experiments.)

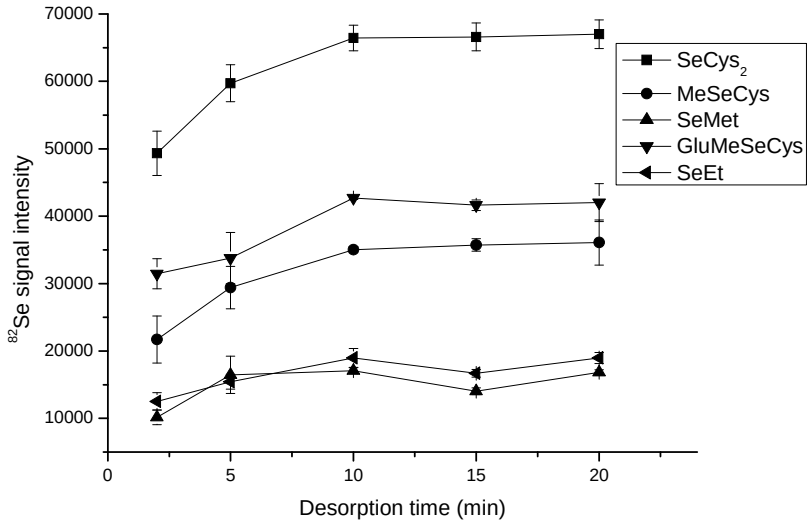

Fig. S7 Effect of the desorption time on the desorption of five target selenium species by MSPE. (Conditions: Sample volume: 5 mL; Sample pH: 3.5; Extraction time: 12 min; Eluent: 0.5 mol L^{-1} Na_2CO_3; Desorption volume: 50 μL; Error bars represent the standard deviation of triplicate experiments.)

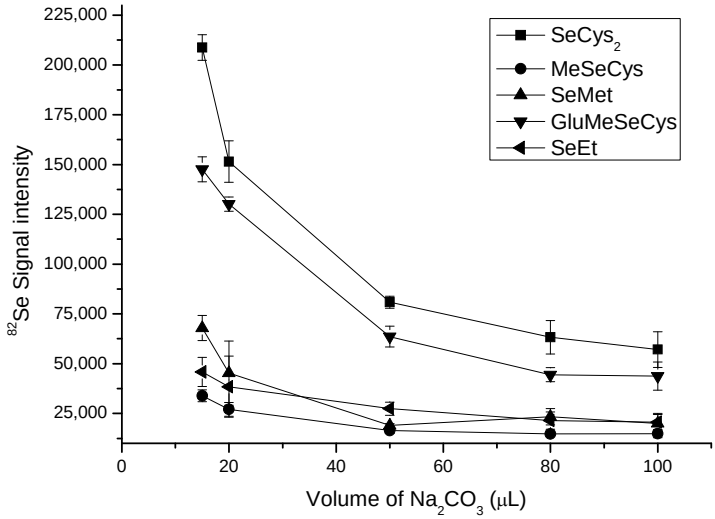

Fig. S8 Effect of the volume of Na_2CO_3 on the desorption of five target selenium species by MSPE. (Conditions: Sample volume: 5 mL; Sample pH: 3.5; Extraction time: 12 min; Eluent: 0.5 mol L^{-1} Na_2CO_3; Desorption time: 10 min; Error bars represent the standard deviation of triplicate experiments.)

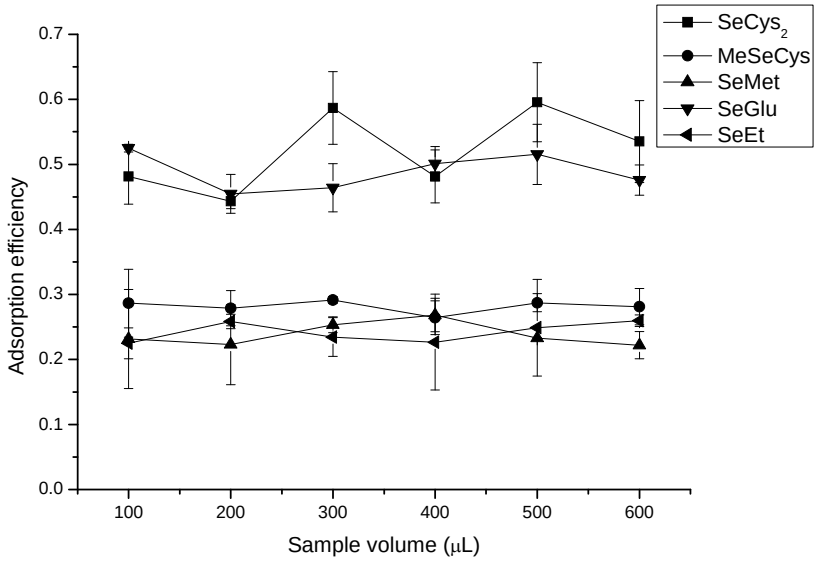

Fig. S9 Effect of the sample volume on the extraction of five target selenium species by on-chip MSPE. (Conditions: Sample pH: 3.5; Sample flow rate: 3 μL min^{-1}; Error bars represent the standard deviation of triplicate experiments.)

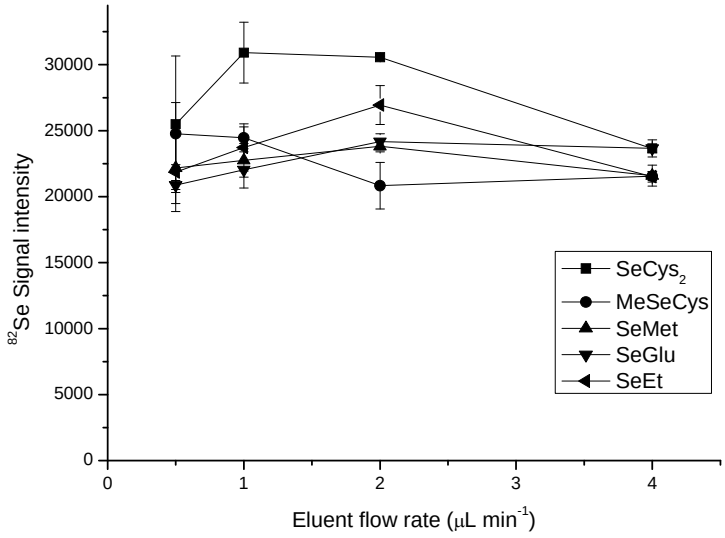

Fig. S10 Effect of the eluent flow rate on the desorption of five target selenium species by on-chip MSPE. (Conditions: Sample pH: 3.5; Sample volume: 300 μL; Sample flow rate: 3 μL min^{-1}; Eluent: 0.5 mol L^{-1} Na_2CO_3; Eluent volume: 10 μL; Error bars represent the standard deviation of triplicate experiments.)

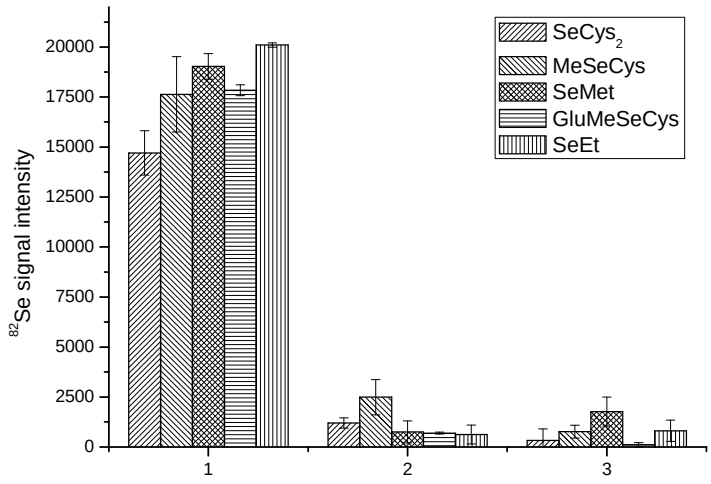

Fig. S11 Effect of the eluent volume on the desorption of five target selenium species by on-chip MSPE. (Conditions: Sample pH: 3.5; Sample volume: 300 µL; Sample flow rate: 3 µL min^{-1}; Eluent: 0.5 mol L^{-1} Na_2CO_3; Eluent flow rate: 2 µL min^{-1}; Error bars represent the standard deviation of triplicate experiments.)

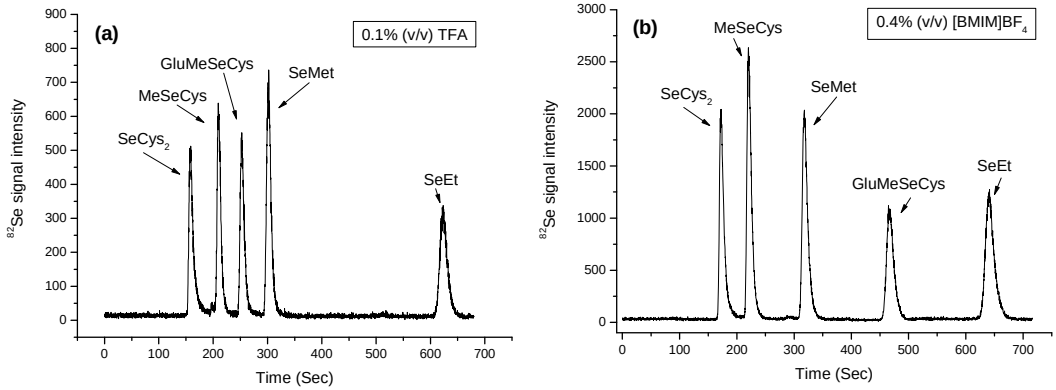

Fig. S12 Chromatogram of target selenium species obtained by RP-HPLC-ICP-MS by using (a) 0.1% (v/v) TFA (25 µg L^{-1} for each target selenium species) and (b) 0.4% (v/v) $[BMIM]BF_4$ (100 µg L^{-1} for each target selenium species) as mobile phase additive, respectively. (mobile phase: 50 mmol L^{-1} acetic acid/ammonium acetate (pH 4.0) + 3% (v/v) methanol, 1 mL min^{-1}, column temperature: 30°C)