Supporting Information

On-line multi-residue analysis of fluoroquinolones and amantadine based on integrated microfluidic chip coupled to triple quadrupole mass spectrometry

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1. Microchip fabrication

The microfluidic device consisted of six parallel filtration units and micro-SPE columns in an array. The microfluidic channel designed for filtration with a dimension of 19 mm in length, 8 mm in width and 100 μm in height, was fabricated from PDMS by standard photolithography techniques[1](Figure S1) . Briefly, a design on a photomask with designed micro-channels was first transferred to the SG-2506 borosilicate glass using a 30 s of UV exposure, followed by 1 min of 0.5 % (w/v) NaOH solution soaking before another 10 min of soaking in dechroming liquid. After washing with ultrapure water, the micro-channels were generated in the glass substrate in a well-stirred bath containing $HF/NH_4F/HNO_3$ at 37 °C for 90 min. The resulting glass molds were then rinsed with ultrapure water, dried at 80 °C and stored for PDMS molding. Mix the PDMS prepolymer and curing agent in 10:1 proportion. The mixture was degassed the mixture in a vacuum chamber and poured onto the mold. After curing in a 80 °C oven for 30 min, the PDMS was cut from the mold and carefully peeled off. The PDMS sheet was treated with oxygen plasma for 1 min and then sealed with a glass slip immediately, through which the filtration microchannel device was formed. The fabrication of micro-SPE with a dimension of 10 mm in length, 1.8mm in width and 100μm in height, was similar to the filtration units. To avoid the leakage of SPE particles, the height of each column was about 100μm and each dam with 20 μm height was fabricated into micro-scale channel. The end of micro-SPE column was protected by 1 mm wide adhesive tape before the first etching step. After 90 min etching, remove the protected adhesive tape, etch another 10 min in the same condition to form the dam. The HLB particles were suspended in methanol and then injected into the microchannel with a Hamilton syringe. The HLB particles can be effectively immobilized into micro-SPE columns by these dams.

2. Method optimization

Method optimization consisted of a 2-fold process. The first step was to optimize the array spacing of filtration units which was determined by the size of impurity particles. To prevent impurity particles plugging filtration units, the reduced micropillar array spacing filter from 200μm to 40μm was adopted in order to get higher efficiency and better filtration effect. Subsequently, upon completion of the filtration operation, the second step was to maximize the performance of the microfluidic chip system with respect to SPE phases, loading time, washing time, elution time, solvent choices, solvent compositions, and flow rates.

C18 and HLB particles which were most commonly used were evaluated in this work by the other conditions remaining constant. The standard solution (100 ng/mL) with identical microfluidic chip conditions were evaluated in order to select the optimal SPE particles with regards to signal intensity and recovery studies. Results conclusively demonstrated that the HLB particles were the optimal phase choice for this method with recovery from 91.7% to 98.5% (Figure S4). Most of the analytes of interest got higher recovery with the HLB particles in comparison to the C18 particles because HLB phase is not easy to be dry once wetted. The packing amount of HLB in the SPE micro-column was investigated from 1 mg to 7 mg with the same size of the micro-column. The absorption ability increased with the packing amount. But the resistance in the micro-channels increased. The amount of SPE particles (6 mg of HLB/column) was adjusted to provide enough efficiency for preconcentration of the diluted sample without blocking the channels.

Further research was conducted using different flow rates in order to maximize the microfluidic

chip system with respect to analytes recovery. Solvent blanks were also evaluated directly after the analysis of standards to ascertain if any residual carryover was present. The flow rate of 80 μL/min was chosen considering the effects of filtration and absorption. The washing rate of 80 μL/min was selected. The flow rate of elution could not be too fast as the elution efficiency would decrease. The flow rate of elution was investigated from 20 μL/min to 60 μL/min. The proper flow rate of 25 μL/min was selected for later use. When the flow rate of elution has been fixed, the elution time will influence the elution efficiency. If the elution time is short, the sample could not be eluted completely. Too long time would result in low sensitivity. The elution time from 1min to 5 min was studied in this research. At last elution time was fixed at 4 min.

References for SI

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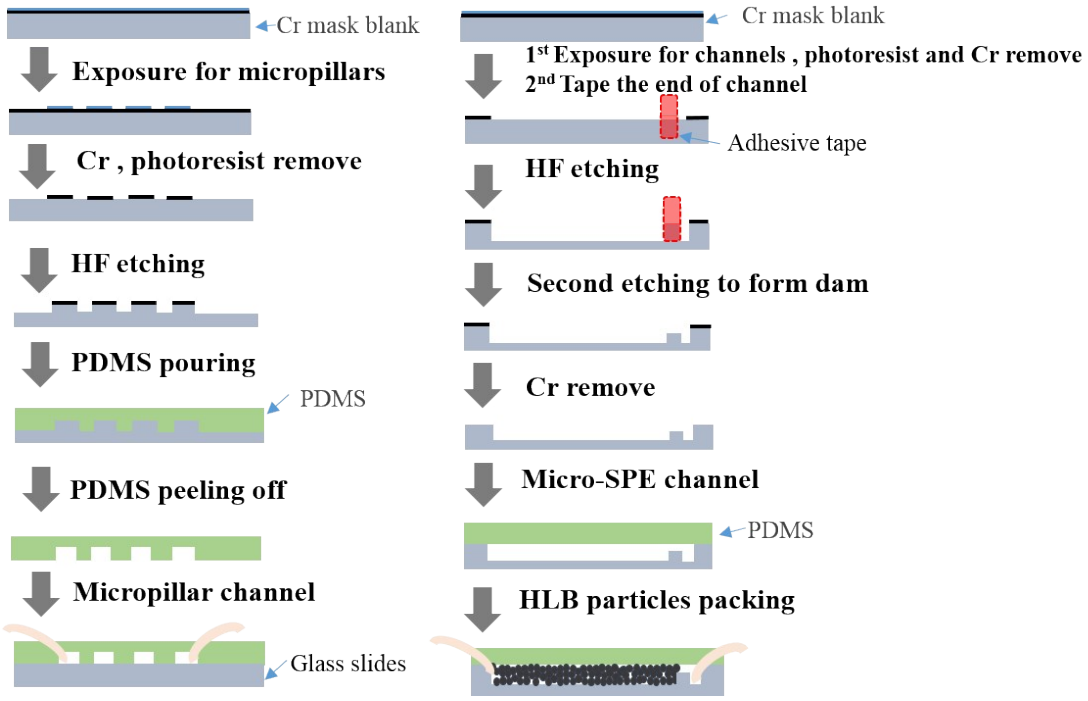

Figure S1 Microfluidic chip fabrication by photolithographic and wet chemical etching techniques.

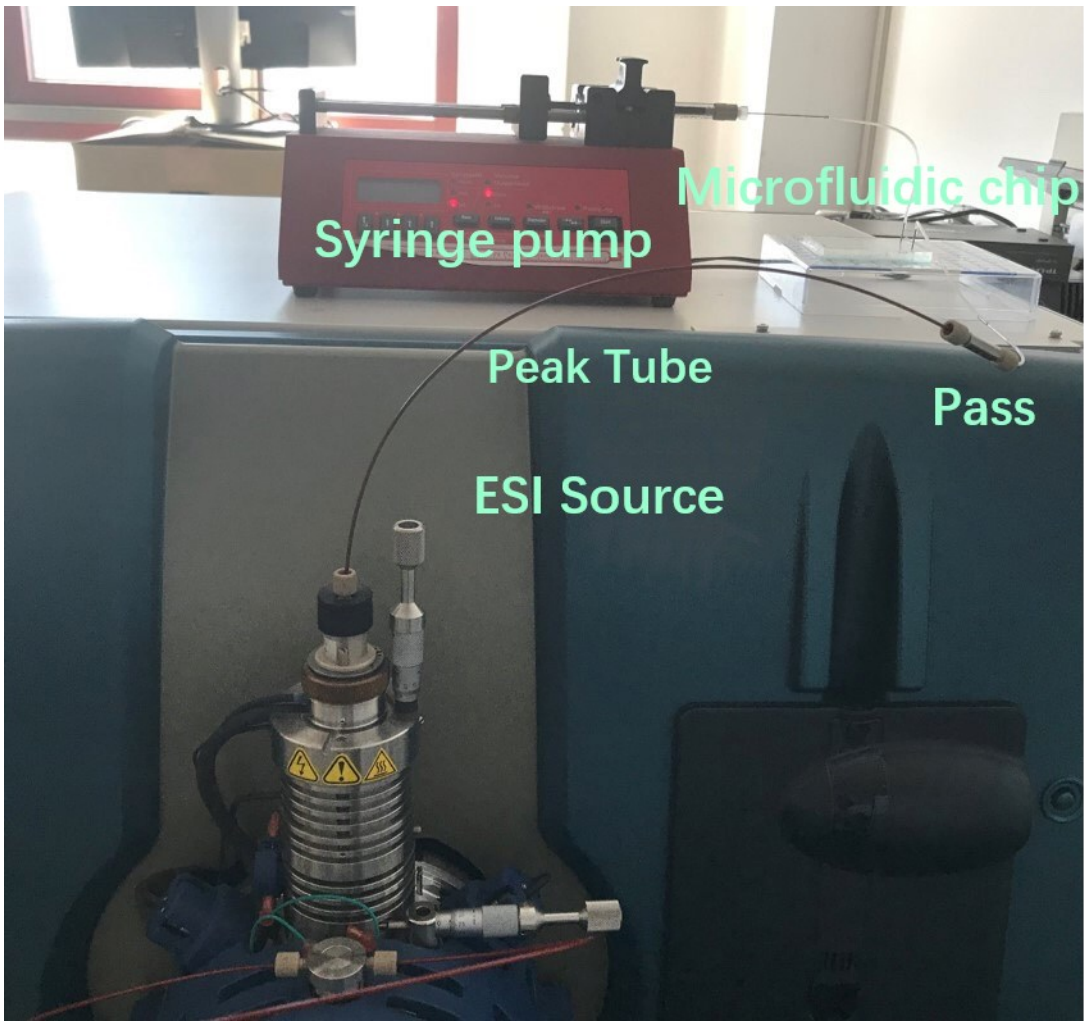

Figure S2 Microfluidic chip combined with ESI-mass spectrometry for Chip-ESI-MS system.

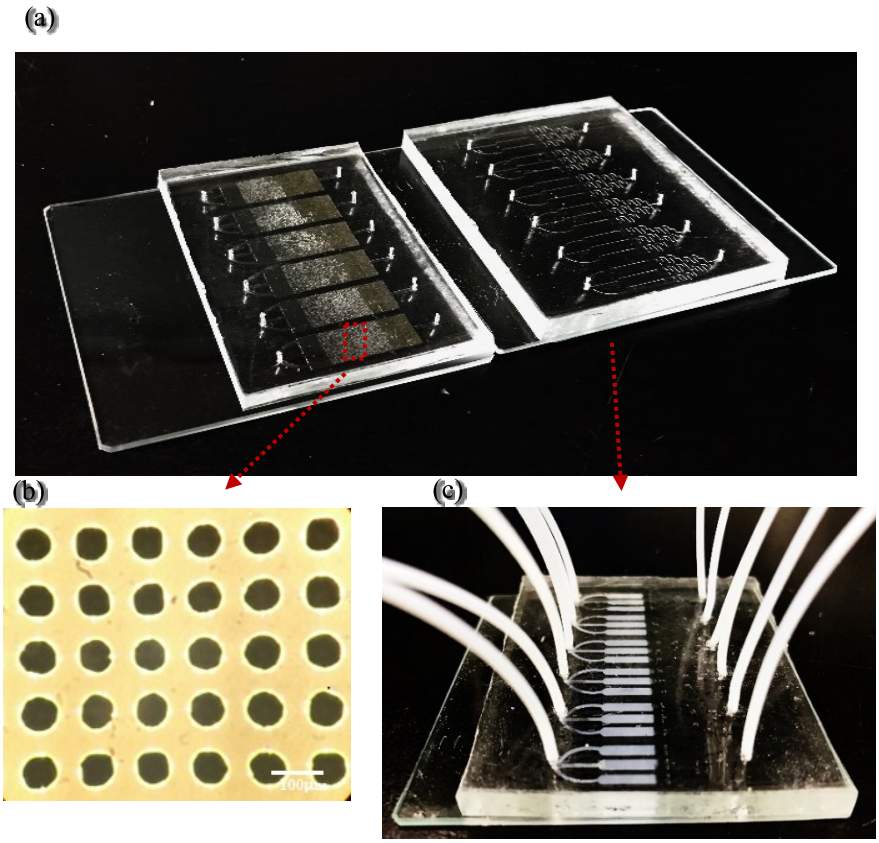

Figure S3 (a) Photographic image of the fabricated microfluidic chip with filtration and micro-SPE. The size of the device is 126 mm×63 mm. (b) Optical micrograph of a portion of micropillar-array microfiltration in a channel (×20). (c) Photographic image of micro-SPE packed with HLB particles.

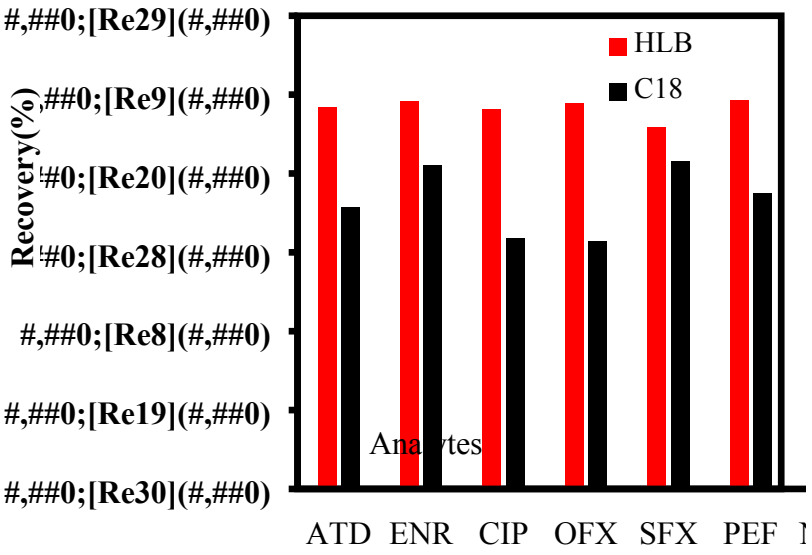

Figure S4 Effects of different particles packed in micro- SPE on recovery of analytes

Table S1 Maximum permitted tolerances for relative ion intensities using a range of mass spectrometric techniques

Relative intensity	Tolerance
＞50%	±20%
20% to 50%	±25%
10% to 20%	±30%
≤10%	±50%

Table S2 Comparison of the performances for QNs and ATD detection based on different approaches

Detection techniques	Sample extraction and purification	Limit of detection	Time in total	Major cost source	Detection targets	Ref.
LC-MS	Ultrasound-assisted extraction, dispersive SPE	0.2-0.6 μg/kg	About 60min	Large amount of reagents, labor costs	multiple drugs: FQs and β-lactam	[2]
LC-fluorescence	SPE	5ng/L	About 3-4h	Large amount of reagents, labor costs	Single class drug: FQs	[3]
FAAS[a]	HLLE[c] and DLLME[d]	human plasma: 1.8ng/mL urine: 1.1ng/mL	About 1-2h	Large amount of reagents, labor costs	Single class drug :ATD	[4]
UHPLC-Qtrap-MS[b]	SPE	0.2 μg/kg	About 3-4h	Large amount of reagents, labor costs	Single class antiviral drug: ATD and rimantadine	[5]
Chip-MS	TCA, immunoaffinity magnetic enrichment	0.47μg/kg-4.90μg/kg	30min	MB, antibody, SG-2506 borosilicate	Single class drug: FQs	[6]
FSPE-chip QQQ-MS	On line filtration and micro-SPE enrichment	0.018-0.043μg/kg	Less than 30min	SG-2506 borosilicate glass	multiple drug: FQs and ATD	Current Work

[a] FAAS: Flame atomic absorption spectrometry

[b] UHPLC-Qtrap-MS : Ultra-high-performance liquid chromatography-triple quadrupole linear ion trap mass spectrometry

[c] HLLE: homogenous liquid–liquid extraction

[d] DLLME: dispersive liquid–liquid micro-extraction