Supporting Information

Highly Efficient Three-Phase Single Drop Microextraction Technique for Sample Preconcentration

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Experimental

1. Identification of analyst in HPLC/Q-TOF-MS: Five statins were confirmed by their retention time and the identification of precursor ions and were quantified on the basis of the peak area using the quantitative ions.

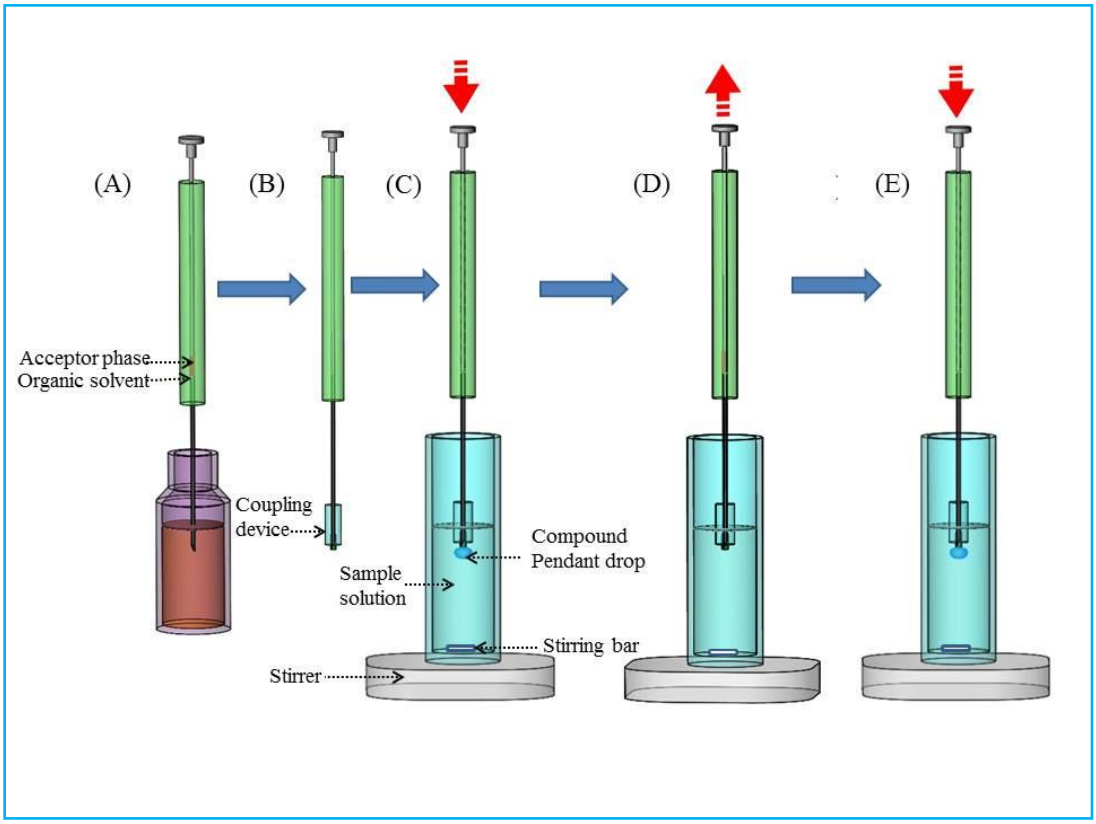
Supplementary Table S2. Retention times and Precursor ions of five statins

Analyte	t_R (min)	Precursor ion (m/z)	Quantitative ion (m/z)
Lovastatin	13.482	$[M+H^+]$: 404.2563	404.2563
Simvastatin	15.229	$[M+H^+]$: 418.2719	418.2719
Fluvastatin	11.131	$[M+H^+]$: 412.1919	412.1919
Atrorvastatin	10.494	$[M+H^+]$: 559.2603	559.2603
Mevastatin	12.171	$[M+H^+]$: 391.2479	391.2479

2. LC/MS Analysis

The microextraction optimizations were carried out using an Agilent model 1290 Ultra Performance LC coupled to an Agilent model 6230 accurate mass time-of-flight (TOF) MS (Agilent Technologies, Santa Clara, CA), equipped with an Agilent Jet Stream (AJS) electrospray ionization (ESI) source system. The liquid chromatography was equipped with a binary pump, on-line degasser, thermostatic dual 54-well plate autosampler, a thermostatic column compartment and a Zorbax XDB C18 reverse phase column (4.6x150 mm, 5 μm, particle size, Agilent Technologies, Santa Clara, CA) temperature-controlled at 30 ℃. The mobile phase consisted of A (0.1% v/v ammonium formate in water) and B (0.1% v/v ammonium formate in acetonitrile). The gradient conditions of the mobile phase were as follows: 0 min, 60% B; 10 min, 100% B; 16 min, 100% B; 16.10 min, 60% B; and 20 min, 60% B. The flow rate of the mobile phase was 0.2 mL/min, and the injection volume was 10 μL. The mass spectrometer was operated in the positive ion mode with the following parameters: capillary voltage, 4000 V; the skimmer voltage, 65 V; nozzle voltage, 800 V; fragmentor voltage, 135 V. Nitrogen was used as the drying (8 L/min, 325 ℃), sheath (11 L/min, 350 ℃) and nebulizer gas (35 psi). Data was saved in centroid mode using Agilent Mass Hunter Workstation Data Acquisition Software (revision B.04). Normalized data was generated by using calibration curves.

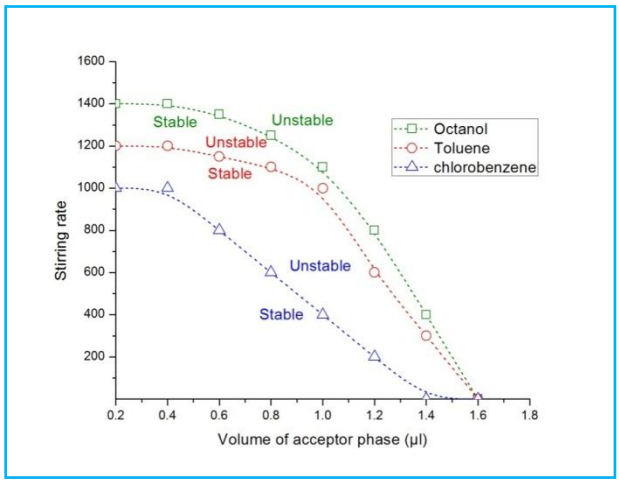
3. Supplementary Figure S1. Schematic illustration of liquid-liquid compound droplet microextraction (A) acceptor phase and organic solvent were withdrawn into the microsyringe sequentially; (B) the capillary was fixed with coupling device; (C) the microsyringe plunger was slowly depressed to initialize a compound drop at the tip of the capillary; (D) at specific time interval, the acceptor solvent was picked up into the microsyringe by pulling the syringe plunger; (E) immediately, it was inflated with acceptor phase and repeated the steps (D) and (E) at given time interval up to certain period. From step (A) to (C) were same for static and iterative droplet formation mode, and (D) and (E) were additional step for the iterative mode.



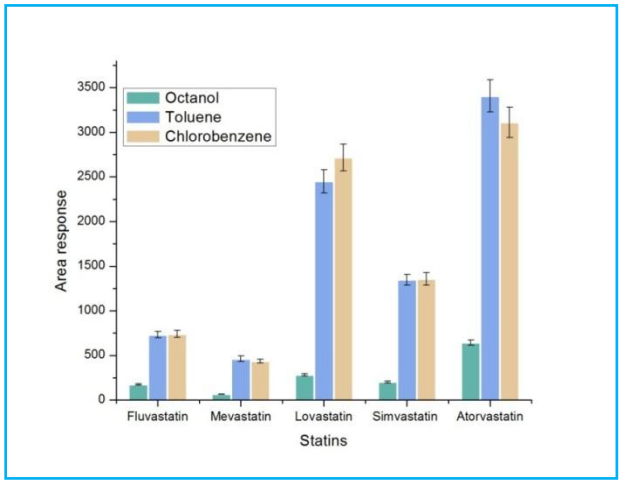
Result and discussion

4. Supplementary Figure S2. Stability of organic-aqueous droplet

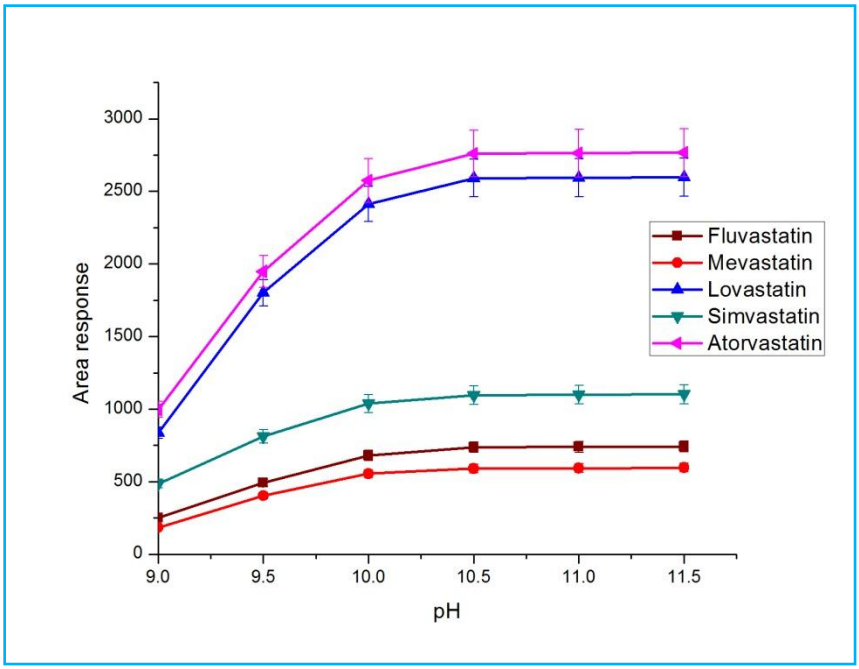
Organic-aqueous droplet is a complex fluid system in which drop stability is influenced by its volume, shape, surface and interface properties. The relation between the shape of droplet and stability were very well illustrated in previous literatures, and for the sake of simplicity, we just investigated the influence of organic solvents (octanol, toluene, chlorobenzene), volume of acceptor phase (0.2-1.6 µL) and stirring rate (200-1600 rpm) on the stability of droplet.



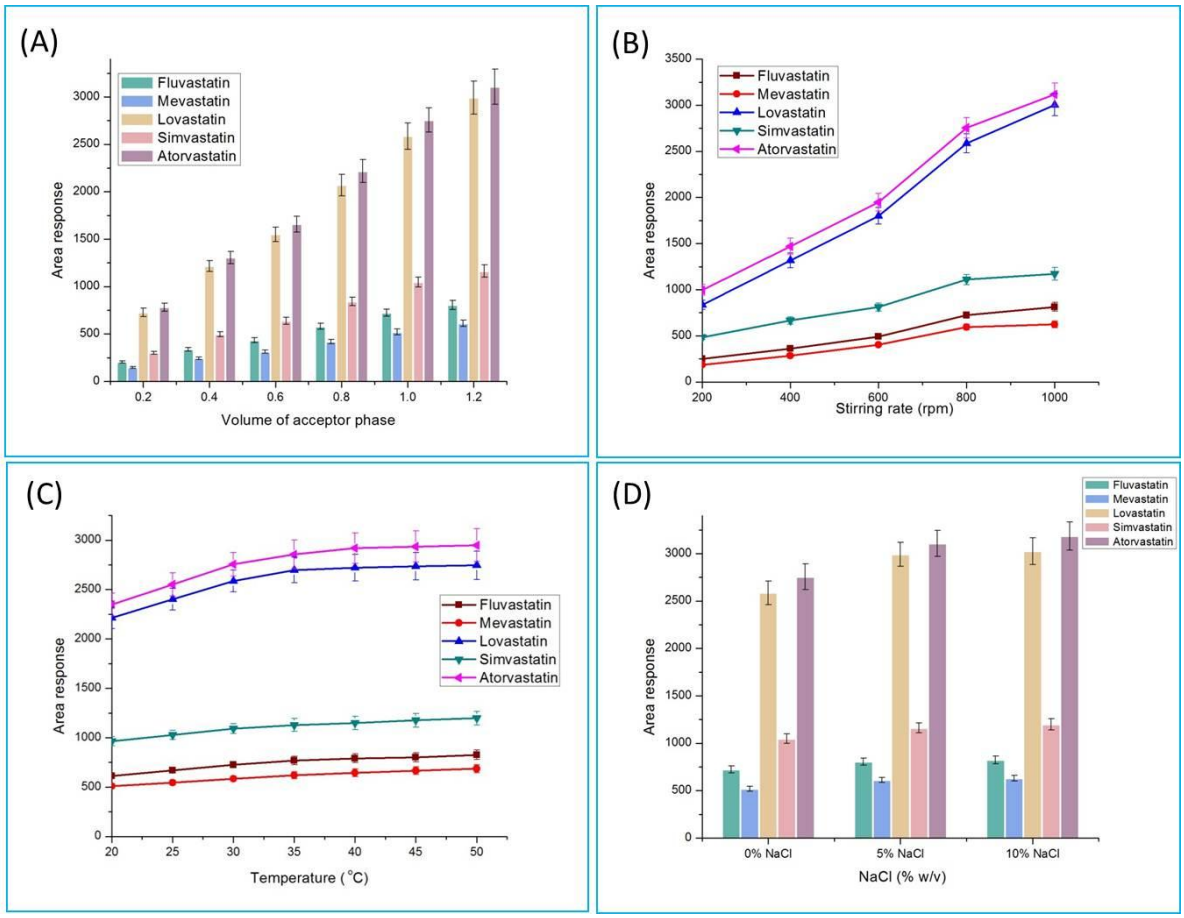
5. Supplementary Figure S3. Influence of organic solvent on extraction. Experimental conditions: 5 ml sample solution (5% NaCl in 5 mM HCl) containing 100 ng L^{-1} of each statin, 1 µL acceptor phase, 0.2 µL organic filter phase, 10 minute, 1000 rpm and 25 ºC (n=6).



6. Supplementary Figure S4. Influence of pH of acceptor phase on extraction. Experimental conditions: 5 ml donor phase (5 mM HCl solution) containing 100 ng L^{-1} of each statin, 1 µL acceptor phase, 0.2 µL organic filter phase (toluene), 10 minute, 1000 rpm and 25 ºC (n=6, RSD < 6).



7. Supplementary Figure S5. (A) **Effect of volume of acceptor phase:** Extraction efficiency was studied in the range of 0.2 µL to 1.2 µL. (B) **Influence of agitation speed:** Agitation speed was examined over the range of 200 to 1000 rpm. It was observed that the peak area responses of five statins gradually augmented by increasing the stirring rate from 200 to 600 rpm, and sharply risen from 600 to 1000 rpm. (C) **Influence of temperature:** Investigation was done in the range of 20 ^{o}C to 50 ^{o}C. There was a little improvement in area responses with high temperature. No significant enhancement was noticed in response area above 40 ^{o}C. It was also observed that air bubbles were sometimes generated in the sample solution at higher temperature (above 50 ^{o}C), which had an adverse effect on the extraction. (D) **Effect of salt concentration:** The salt effect was explored by adding NaCl to donor phase in range of 5 to 20% (w/v). The addition of 5% and 10% salt exhibited a slight improvement of area response and the increased density of NaCl showed additional buoyancy, and the droplet was more vulnerable to breakup at the concentration of 10%. [Experimental conditions: 5 ml sample solution (5 mM HCl) containing 100 ng L^{-1} of each statin, acceptor phase (0.01 M liquid ammonia), 0.2 µL organic filter phase (toluene), 10 minute extraction time (n=6).]



8. Relative recovery studies and Accuracy validation

Relative recoveries of the extraction were calculated as the ratio of the response after extraction from real samples and the ultra-pure water following the same condition. [W. Gao et al. *Talanta*, 83 (2011) 1673–1679]

Relative recovery = (RA_x / RA_k) x100

The student's *t* test was also applied to the results demonstrating acceptable accuracy within the 95% confidence level according to the following equation for river water and serum separately.

$$t=\pm(\bar{x} - \mu)/(s/\sqrt{N})$$

Where, "$\bar{x}$" is Mean of experimental value, "μ" is Known value, "s" is Standard deviation and "N" is Number of measurements.

9. Supplementary Figure S6. Chromatography spectrum of river water (A) and human serum sample (B) spiked with 100 ng L^{-1} of each statin with Iterative mode of three-phase SDME. Peak identification: (1) Atorvastatin, (2) Fluvastatin, (3) Mevastatin, (4) Lovastatin, (5) Simvastatin. Experimental conditions: 5 ml sample solution (5% NaCl in 5 mM M HCl solution), 1 µL acceptor phase (0.01 M liquid ammonia), 0.2 µL organic filter phase (toluene), 4 minute (1 min interval in iterative microextraction method), 1000 rpm and 40 ºC.