Electronic Supporting Information

Coupling Liquid Chromatography/Mass Spectrometry Detection with Microfluidic Droplet Array for Label-Free Enzyme Inhibition Assay

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Chemicals and Materials.

All chemicals were of analytical reagent grade unless otherwise stated and demineralized water (Milli Q, Millipore, Billerica, USA) was used throughout. 3-(Trimethoxysilyl)propyl methacrylate and lauryl methacrylate (LMA) were purchased from Acros Organics (Fair Lawn, USA). Ethylene glycol dimethacrylate (EDMA) was obtained from Aldrich (Milwaukee, USA). 2,2-Azobisisobutyronitrile (AIBN, Shanghai Fourth Reagent Plant, Shanghai, China) was used as initiator for polymerization of LMA and EDMA. 1-Propanol and 1,4-butanediol (Sinopharm Chemical Reagent Co., Shanghai, China) were used as porogenic solvents for polymerization of LMA and EDMA. Human liver microsomes,

glucose-6-phosphate, $NADP^+$, glucose-6-phosphate dehydrogenase, magnesium chloride, potassium dihydrogen phosphate, dipotassium hydrogen phosphate, mineral oil, quercetin, miconzole, cimetidime, chlorpheniramine, quinidine, angiotensin I, and angiotensin II were obtained from Sigma (St. Louis, USA). Phenacetin, acetaminophen, and erythromycin were purchased from TCI chemical industry Development Co. (Shanghai, China). Chlorpropamide, sulfameter, and reserpine were obtained from Fluka (Buchs, Switzerland).

Stock solution of cytochrome P450 1A2 (CYP1A2) substrate phenacetin was prepared in methanol (20 mM for traditional enzymes inhibition assay, 60 mM for droplet-based enzyme inhibition assay). The internal standard chlorpropamide was prepared by dilution of the stock solution (20 mM in acetonitrile) to a concentration of 20 μM with acetonitrile or 50% acetonitrile solution. Test compounds were dissolved in methanol and the final assay concentrations were 20 μM. For inhibitor α-naphthoflavone, the final assay concentration range of 0.01-100 μM was adopted to obtain an inhibition curve.

Procedure of Enzyme Inhibition Assay.

For droplet-based enzyme inhibition assay, 268 nL of droplets containing 0.75 mg/mL human liver microsomes, NADPH generating system (4.95 mM glucose-6-phosphate, 1.95 mM of $NADP^+$, 4.95 mM magnesium chloride, and 0.6 U/ml glucose-6-phosphate dehydrogenase), and test compounds with different concentrations were generated, respectively. The droplet array chip was first incubated for 15 min at 37 °C. Then, 134 nL of 300 μM phenacetin solution was added to each droplet, and the droplet array chip was allowed to react for 1 h set at 37 °C. Finally, the reactions in droplets were quenched by adding 200 nL of 50% ice-cold acetonitrile solution containing 20 μM chlorpropamide to each droplet.

In the comparison experiment, for traditional inhibition assay of human cytochrome P450 isoform CYP1A2, 100 μL of reactionmixtures were prepared which contained 0.1 M potassium phosphate buffer (pH 7.4), 0.5 mg/mL human liver microsomes, NADPH-generating system (3.3 mM glucose-6-

phosphate, 1.3 mM of $NADP^+$, 3.3 mM magnesium chloride, and 0.4 U/ml glucose-6-phosphate dehydrogenase), and test compounds with different concentrations, respectively. Enzyme reactions were initiated by adding the substrate phenacetin for CYP1A2 after a 15 min pre-incubation at 37 °C. The incubation operation was carried out in a shaking water bath at 37 °C for 1 h and terminated by the addition of 50 μL of ice-cold acetonitrile containing 20 μM chlorpropamide as internal standard to the reaction mixture, and then placed it in an ice bath. The experiments for each condition were performed in triplicate.

The enzyme inhibition assay was based on the inhibition of CYP1A2 with inhibitor, impeding the conversion of substrate phenacetin to acetaminophen. The intensities of acetaminophen and internal standard chlorpropamide in the quenched reaction mixtures were measured with the ESI-MS instrument. The acetaminophen formed in the reaction was quantitatively measured using an acetaminophen calibration curve.

Z factor for evaluating the reliability of screening assay is defined as followed equation:[1]

$$Z = 1.0 - \frac{(3.0\sigma_{C+} + 3.0\sigma_{C+})}{\left|\mu_{C+} - \mu_{C-}\right|}$$

where σ_{c+} is the standard deviation of the response of a positive control (with inhibitor), σ_{c-} is the standard deviation of the response of a negative control (no inhibitor), μ_{c+} and μ_{c-} is the mean signal of positive and negative controls, respectively.

Reference

1 J. H. Zhang, T. D. Y. Chung and K. R. Oldenburg, *J. Biomol. Screen.*, 1999, **4**, 67–73.

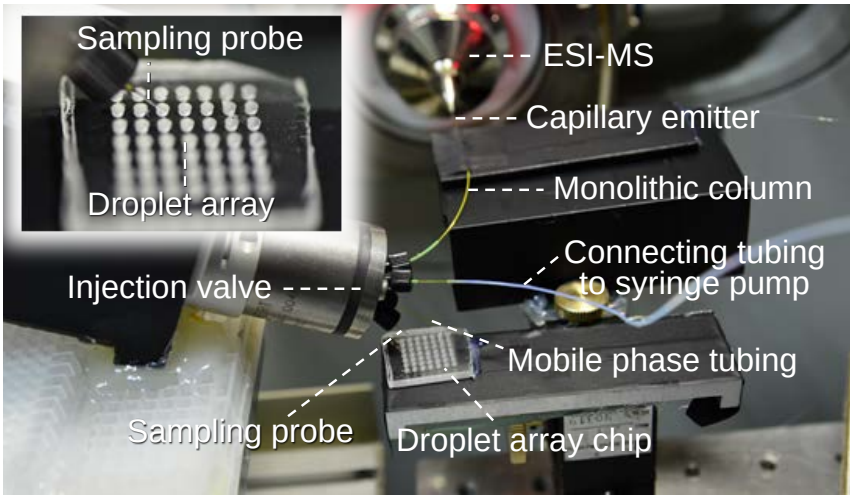

Figure S1. Setup of the droplet array-LC/MS system.

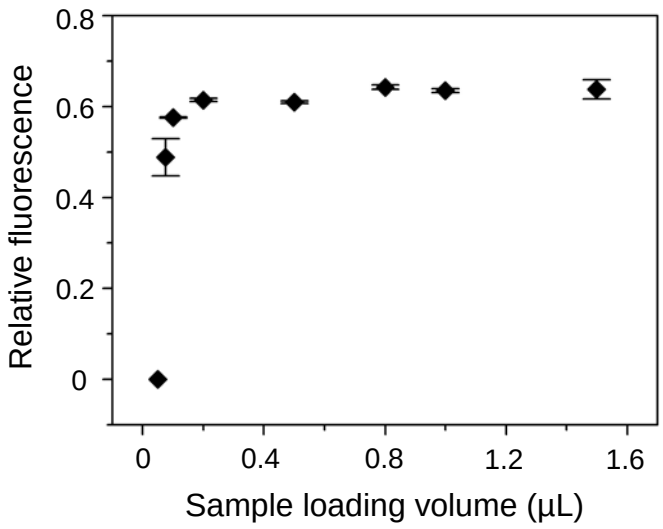

Figure S2. The effect of sample loading volume to the sample injection.

Model sample, 5 µM fluorescein solution; sample aspirating flow rate, 150 nL/min.