# DROPLET ANALYSIS SYSTEM USING LIQUID CHROMATOGRAPHY AND MASS SPECTROMETRY FOR ENZYME INHIBITION ASSAY

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**ABSTRACT**

In this work, the combination of droplet array chip with LC/MS was firstly achieved, for providing a high-separation-resolution and high-information-content detection method for analysis of complex compositions in nanoliter droplets. A novel interface for capillary LC and droplet array chip with outstanding flexibility in accurate addressing of interesting droplets on demand was developed, with which multistep operations including enzyme inhibition reaction in nanoliter droplet, 4-nL sample injection, high-resolution separation with HPLC, and label-free detection with ESI-MS were successfully realized. The present system was further applied in the enzyme inhibition assay to demonstrate its potential feasibility in low-consumption drug screening.

**KEYWORDS**

Droplet-based microfluidics, LC/MS, enzyme inhibition assay.

## INTRODUCTION

Droplet-based microfluidics is an attractive technique for performing miniaturized chemical and biological reactions ranging from picoliter to nanoliter volumes without dilution,[1] and has been successfully applied in enzyme inhibition assay [2], and protein crystallization [3]. Various analytical techniques for analyzing contents of droplets have been developed, most of which adopt fluorescence or absorbance technique [4]. These methods cannot analyze droplets with complex compositions. High-resolution separation techniques playing a prominent role in proteomics, genomics, metabolomics, and other biochemical fields are attractive analytical methods for droplets. In recent years, some high-resolution-separation methods have been developed to analyze contents of droplets, such as electrospray ionization mass spectrometry (ESI-MS) [5], and capillary electrophoresis (CE) [6]. Liquid chromatography/mass spectrometry (LC/MS) is by far the most widely used analysis technique in routine analysis. The use of microscale LC coupled with MS for droplet-based microfluidic analysis will significantly broaden the applications of droplet microfluidics. The coupling of microscale LC with droplet-based systems has been achieved. However, in most of these systems, droplets were used as a novel way to collect fractions from capillary LC [7, 8],there is no report on using capillary LC to read the contents of droplets.

In most of the droplet-based microfluidic systems, the droplets were usually manipulated in microchannels. Such a close mode is difficult to accurately address interesting droplets on demand and extract specific droplets for further analysis. In addition, the volumes of droplets in most of reported systems are in the nanoliter range or smaller, while the sample injection volumes in conventional LC systems are often in the range of several microliters. Therefore, it is a significant challenge to realize nanoliter sample injection of droplet into LC valve.

In this work, we developed a semi-closed droplet array system for high-resolution separation and detection of complex components in droplets using LC/MS. A novel interface for capillary LC and droplet array chip with outstanding flexibility in accurate addressing of interesting droplets for different samples on demand was developed. Sample injection for LC was achieved benefiting from the direct droplet access and manipulation in the semi-closed droplet-array system under off-line mode. The present system was further applied in screening for inhibitors of CYP 1A2.

## EXPERIMENT

The schematic diagram of the setup of the droplet analysis system based on LC/MS is shown in Figure 1. It consisted of a microchip, two HPLC pumps, a sampling probe, a 4-nL injection valve, a syringe pump, a monolithic column, a MS emitter, and an ion trap mass spectrometer as detector. The sample loading was performed by immersing the sampling probe in the droplet filled in the well through mineral oil under the loading mode of the valve, and aspirating definite volumes of the sample solution into the 4-nL quantification loop of the valve by switching the syringe pump ON for a definite time. In the injection stage, the 4-nL introduced sample solution was injected into the LC monolithic column for separation, and detected by the MS detector. The sample changing was conducted by moving the microchip to switch the next sample well to the sampling probe to aspirate the new sample into the valve.



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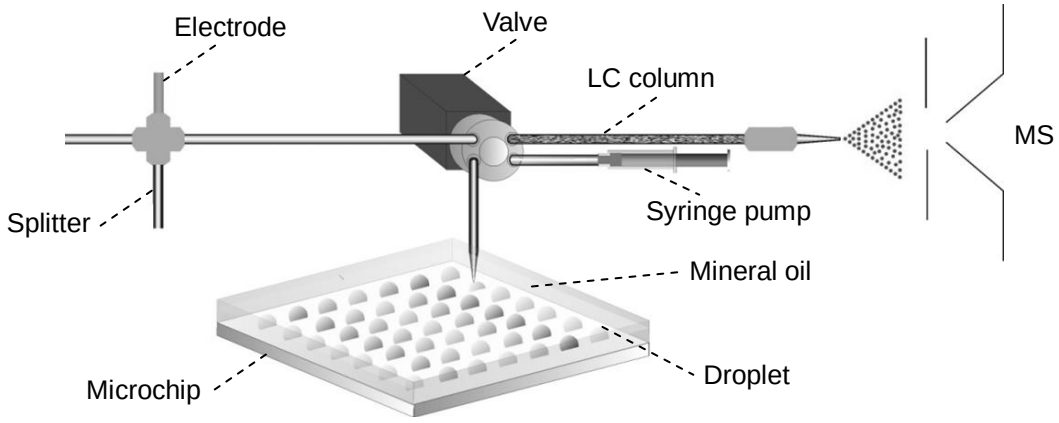


*Figure 1. Schematic diagram of the setup of droplet analysis system based on LC/MS (not to scale).*

## RESULTS AND DISCUSSION

We evaluated the influences of sample loading volume and withdraw flow rate. Under optimized conditions, a sample loading volume of 100 nL was sufficient to perform the sample injection. Angiotensin I and angiotensin II were utilized as model samples to test the performance of the droplet analysis system. The results are shown in Figure 2. The repeatabilities of retention time and peak area for angiotensin I were 2.8% and 2.7% (RSD, n=4), and for angiotensin II were 1.5% and 7.5% (RSD, n=4), respectively.

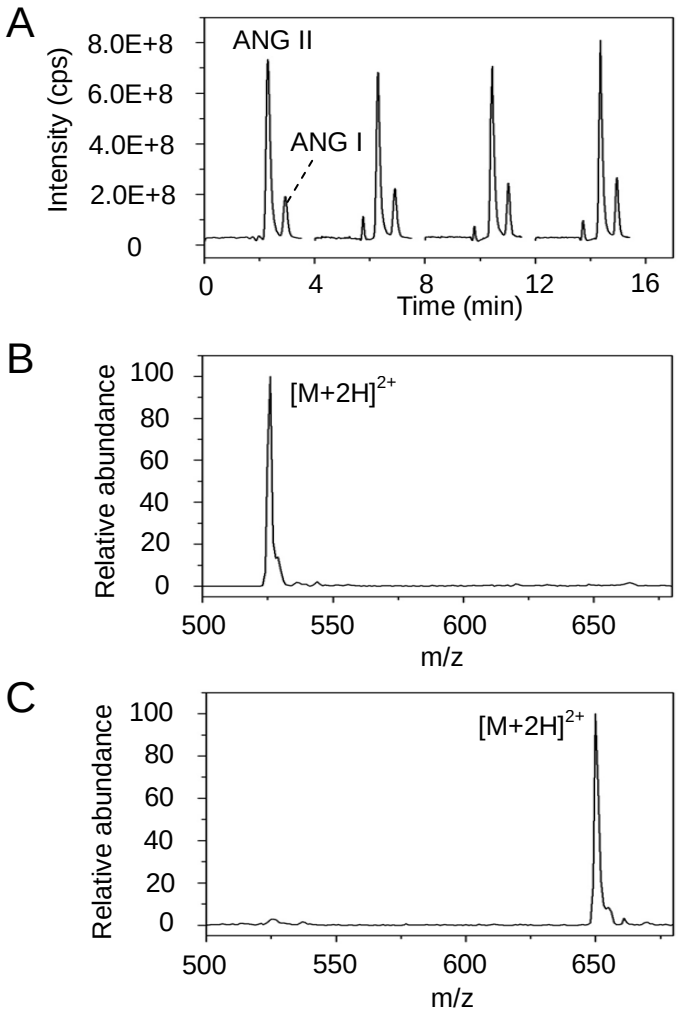


*Figure 2. (A) Total ion current (TIC) chronogram from replicate sampling of angiotensin I and angiotensin II mixture. (B) Mass spectra obtained from the apex of the angiotensin II peak labeled ANG II. (C) Mass spectra obtained from the apex of the angiotensin I peak labeled ANG I.*

The present system was further used to perform the screening for CYP 1A2 inhibitors. Nine compounds were

tested, and the results are shown in Figure 3. α-Naphthoflavone shows strong inhibition on CYP 1A2, and others show no evident effects. These results agree with those obtained with previous-reported multiwell plate-based CYP 1A2 inhibition screening systems.

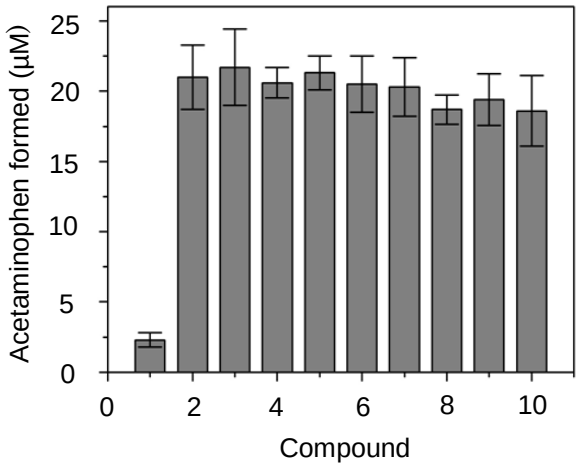


*Figure 3. Quantification of acetaminophen formed in each sample droplet. Compounds: α-naphthoflavone, negative control, sulfateme, quercetin, erythromycin, miconzole, cimetidime, chlorpheniramine, quinidine, reserpine.*

## CONCLUSIONS

We developed a droplet array-LC/MS system capable of performing high-separation-resolution and high-information-content detection for enzyme reaction system with complex compositions. A novel interface was proposed for coupling LC/MS with droplet chip based on off-line mode. The use of the semi-closed droplet array and off-line interfacing mode endows the present system an outstanding flexibility in droplet indexing, sampling, and LC/MS analysis on demand. The successful application in the screening for inhibitors of CYP 1A2 demonstrate its potential in high throughput screening with rare and expensive samples and reagents.

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