Supplementary Information

Utilizing Venturi Effect for Automated High-Throughput Droplet-MS From Well Plates

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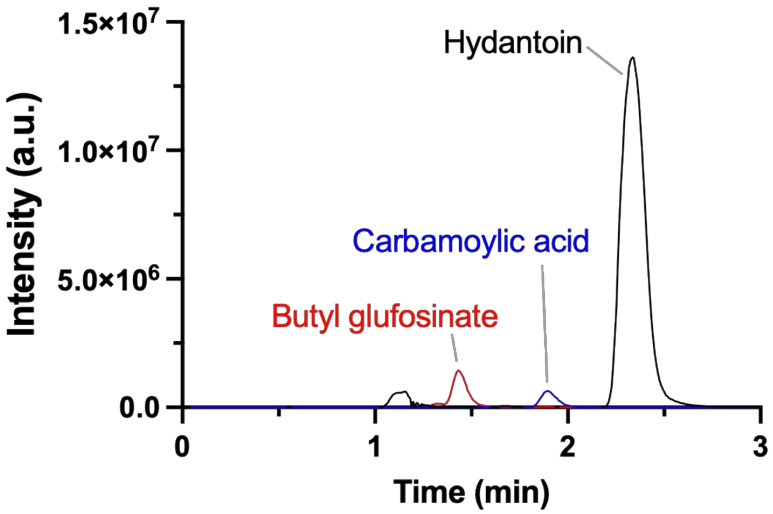

Fig. S1. Extracted ion chromatograms for glufosinate (red), carbamoylic acid (blue), and hydantoin (black) signal from a hydantoinase variant and carbamoylase cascade reaction acquired by achiral LC-MS. Flow rate was 0.2 mL/min and mobile phase was 20% B, where mobile phase A was 95/5 water/acetonitrile + 0.1% formic acid and B was 5/95 water/acetonitrile + 0.1% formic acid.

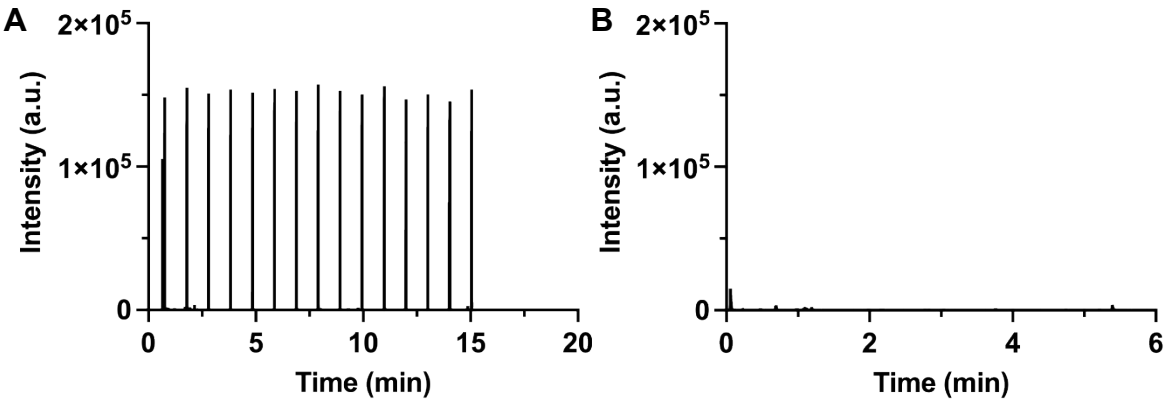

Fig. S2. Effect of nebulizing pressure and sheath flow rate on droplet-MS/MS signals using Venturi effect for sampling and infusion. (A) Resulting MS/MS trace for detection of 2 µM butyl glufosinate in 20/80 MeOH/water containing 0.1% formic acid. 1000 nL droplets flowing at 40 µL/min were created and infused with nebulizing pressure = 20 psi and sheath flow rate = 0 mL/min. Sustained droplet generation and infusion was achieved using these conditions, evidenced by the repeated droplet signal. (B) Resulting MS/MS trace for same sample as (A) created and infused with nebulizing pressure = 10 psi and sheath flow rate = 0.1 mL/min. Sustained droplet generation and infusion was not achieved under these conditions, evidenced by the lack of droplet signal. Fused silica capillary (150 i.d. x 70 cm long) was used for droplet generation and infusion.

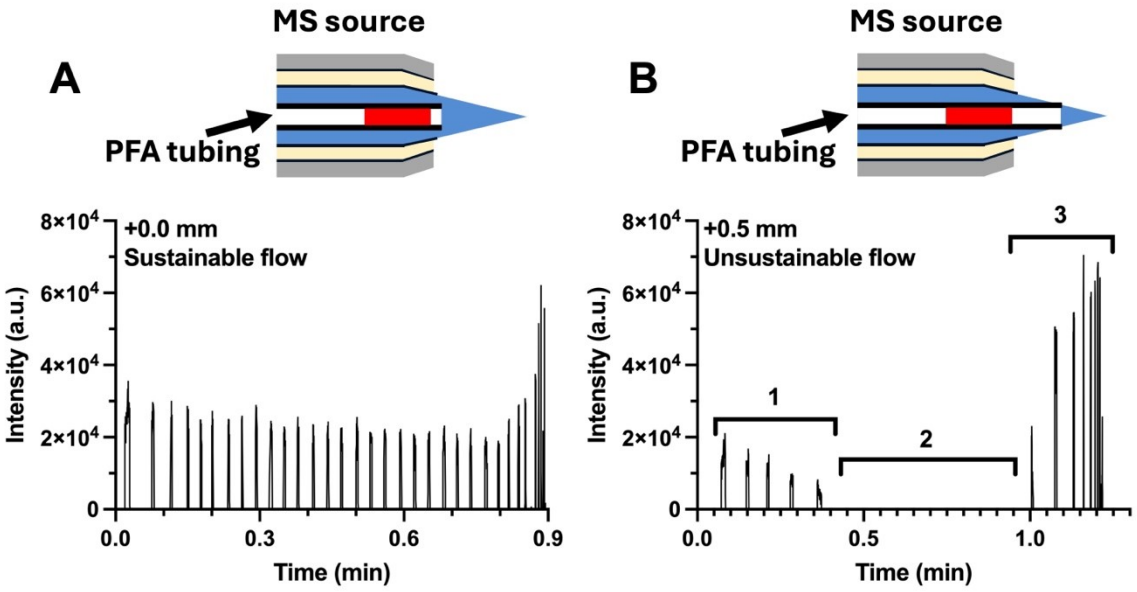

Fig. S3. Effect of tubing placement within sheath flow source on droplets created and infused by Venturi effect. (A) MS/MS signal for droplets generated with tubing positioned ~ 0.1 mm beyond the source with illustration shown above plot. Sustained droplet generation and infusion is achieved. (B) MS/MS signal for droplets collected with tubing positioned 0.5 mm beyond the source with illustration shown above plot. The trace is divided into three regions, labeled 1, 2, and 3, and each region represents a different flow condition. In region 1, droplet generation and infusion is temporarily achieved with droplets appearing at regular intervals (roughly every 0.1 min). In region 2, no droplet signal is detected, indicating that flow has stopped. In region 3, droplet flow is re-achieved after moving the tubing back to 0.1 mm. Sample was 2.5 µM acetylcholine in diluted reaction matrix. Nebulizing pressure was 60 psi, and PFA tubing was 150 µm i.d. x 70 cm long. Relative tubing extensions between panels (A) and (B) are to scale but other elements of illustration are not.

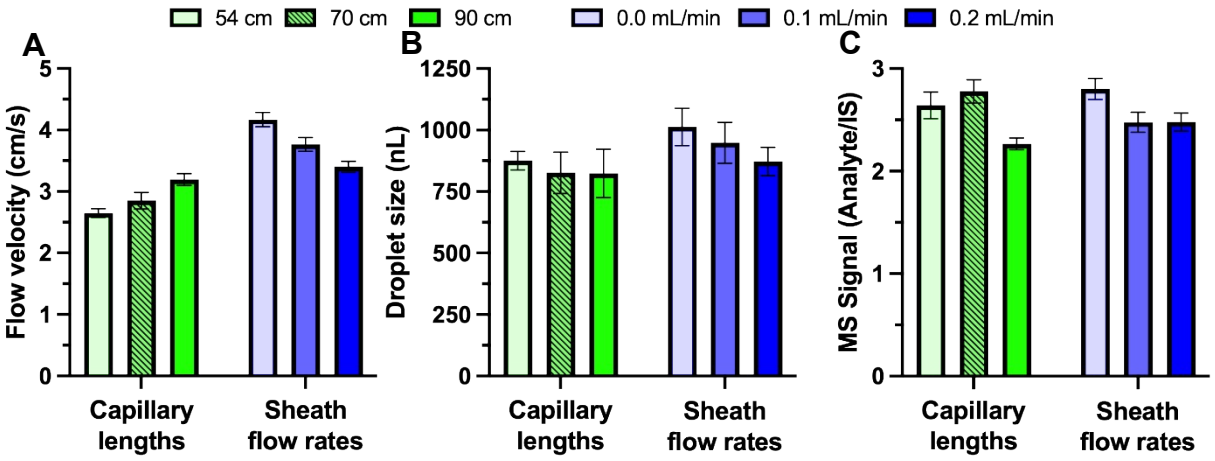

Fig. S4. Effect of capillary length and sheath liquid flow rate on (A) flow velocity, (B) droplet size, and (C) resulting MS/MS signal of droplets generated and infused using Venturi effect. Y axis in (C) represents butyl glufosinate divided by acetylcholine signal. Sample was 2 µM butyl glufosinate, 2 µM acetylcholine, and 2 µM glufosinate in 20/80 MeOH/water containing 1% blue food dye. Flow velocity values are plotted as average of n = 5 droplets, volumes are average of n = 10, and MS signals are average of n = 25. Error bars represent ± 1 SD. One droplet was in tubing at a time. Fused silica capillary (150 µm i.d. x 70 cm long) was used to generate and infuse droplets. Nebulizing gas pressure was 20 psi.

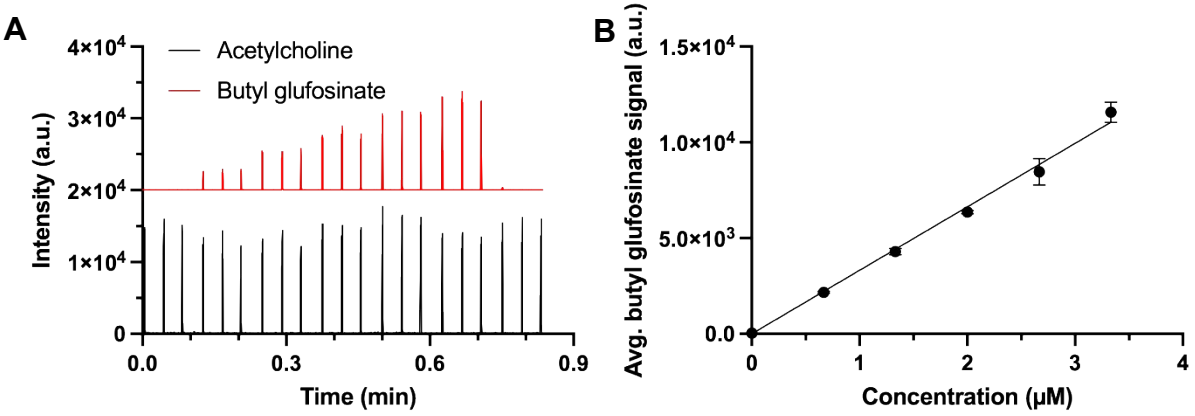

Fig. S5. Calibration curve generation with droplets created and infused using the Venturi effect. (A) Droplet-MS/MS trace of blanks and calibration standards infused in triplicate covering the following butyl glufosinate concentrations in diluted reaction matrix: 0.0, 0.7, 1.3, 2.0, 2.7, and 3.3 µM. The black trace represents acetylcholine and the red trace represents butyl glufosinate. The red trace is offset for better viewing of the data. Blanks (n = 3) were infused before and after the infusion of calibration standards. Droplets were 380 nL, flowed at 330 µL/min, and contained 2.5 µM acetylcholine. (B) The calibration curve generated from the droplets infused in (A). Points are plotted as the average of n = 3 and the error bars represent ± 1 SD. Some error bars are too small to be seen. PFA transfer tubing was 150 µm i.d. x 70 cm long. Nebulizing gas pressure was 60 psi.

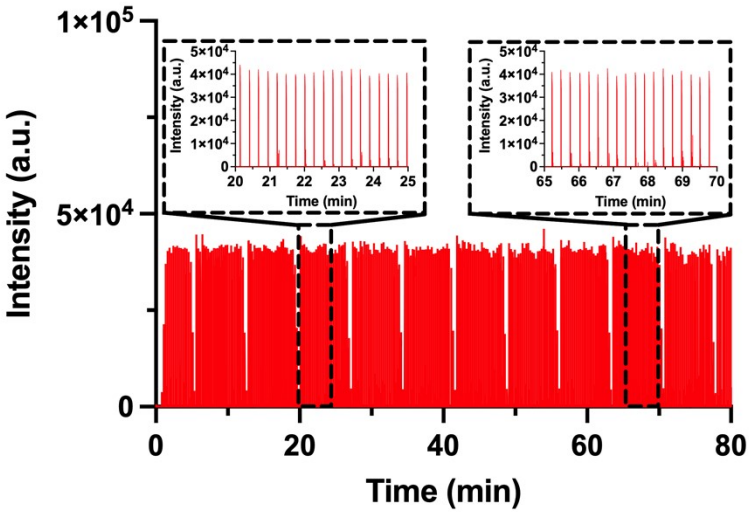

Fig. S6. Droplet-MS/MS trace of a mock 96-well plate screen. Wells contained 3.3 µM butyl glufosinate (red trace) standard dissolved in the screening matrix. A total of 96 butyl glufosinate wells were sampled in triplicate. The insets show a zoomed in view of the MS trace at two different time periods during infusion. Fused silica capillary (250 µm i.d. x 70 cm long) was used to generate and infuse droplets. Nebulizing gas was 15 psi. Periodic dips in signal occurring every ~8 min are due to wash droplets that were infused. Butyl glufosinate signal RSD = 7%.

Table S1. Dimensionless droplet lengths for droplets generated and infused with dwell time in aqueous = 0.0 s and air = 0.2 s. These settings resulted in flow stoppage. Droplet composition was 2.7 µM acetylcholine in 20/80 methanol/water containing 0.1 % formic acid and 5 % (v/v) food dye. 23 out of 42 expected droplets were generated. The rest of the droplets were not generated because flow stopped. PFA tubing was 150 µm i.d. x 70 cm long. Sheath was 20/80 methanol/water containing 0.1 % formic acid and flowed at 0.1 mL/min. Nebulizing gas pressure was 60 psi.

Droplet number	Dimensionless plug length
1	688
2	285
3	206
4	199
5	163
6	128
7	114
8	99
9	88
10	90
11	74
12	62
13	53
14	45
15	41
16	38
17	22
18	23
19	18
20	15
21	5
22	3
23	0.7