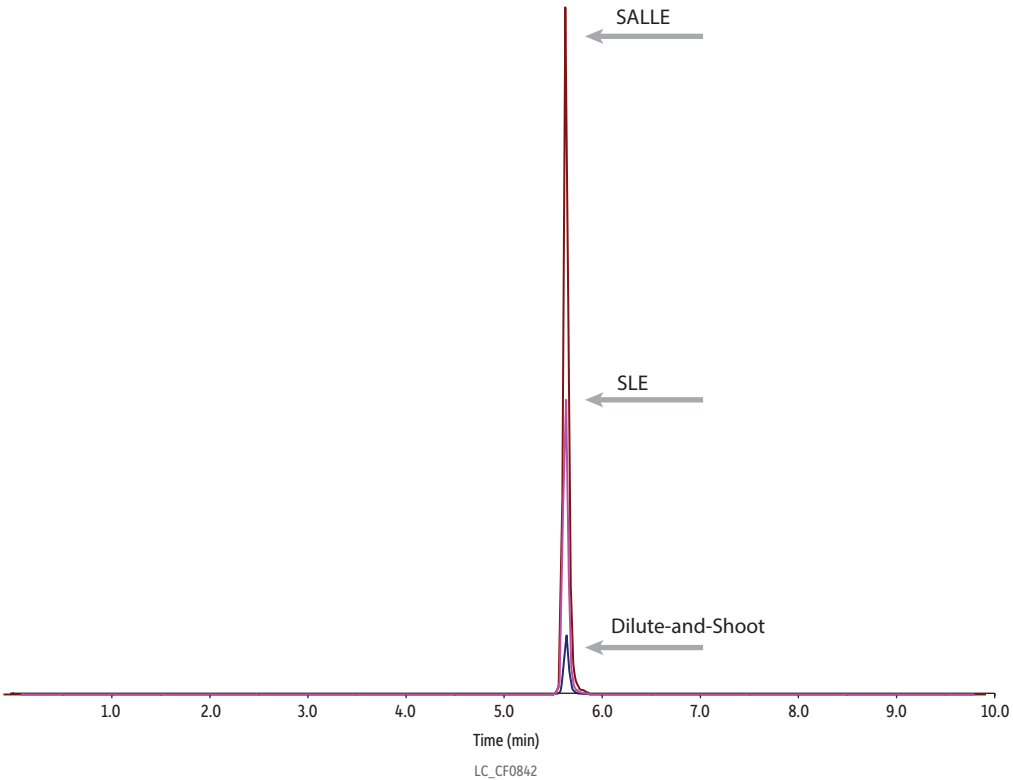
Comparison of Sample Preparation Techniques on Mitragynine in Oral Fluid



Peaks	tR (min)	Conc. (ng/mL)	Precursor Ion	Product Ion
1. Mitragynine	5.78	100	399.25	159.00

Column Raptor Biphenyl (cat.# 9309A52)
Dimensions: 50 mm x 2.1 mm ID
Particle Size: 2.7 µm
Pore Size: 90 Å
Guard Column: Raptor Biphenyl EXP guard column cartridge 5 mm, 2.1 mm ID, 2.7 µm (cat.# 9309A0252)
Temp.: 40 °C
Standard/Sample
Diluent: 90% Water, 0.1 % formic acid:10% methanol, 0.1% formic acid
Inj. Vol.: 5 µL
Mobile Phase
A: Water, 0.1% formic acid
B: Methanol, 0.1% formic acid

Time (min)	Flow (mL/min)	%A	%B
0.00	0.5	85	15
1.00	0.5	80	20
2.00	0.5	80	20
4.00	0.5	50	50
6.00	0.5	40	60
8.00	0.5	0	100
9.00	0.5	0	100
9.01	0.5	85	15
10.00	0.5	85	15

Max Pressure: 310 bar
Detector SCIEX 4500 MS/MS
Ion Mode: ESI+
Instrument Shimadzu Nexera X2
Sample Preparation In this example, three sample preparation techniques were compared: dilute-and-shoot; supported liquid extraction (SLE); and salt-assisted liquid-liquid extraction (SALLE).

Dilute-and-shoot: One milliliter of prepared calibrator or QC sample was added to 3 mL of Quantisal buffer to simulate real-world ratios. Then, 100 µL of the oral fluid/buffer mixture was added to a 2 mL microcentrifuge tube, 20 µL of internal standard was added, and the sample was vortexed for 10 seconds. Next, 460 µL of 90:10 0.1% formic acid in water:0.1% formic acid in methanol was added. Samples were vortexed again, and an aliquot was transferred to a 50 µL vial insert (cat.# 24513) in a 2.0 mL, amber, short-cap vial (cat.# 21142) and capped with a 9 mm short cap (cat.# 24497), then moved to the LC-MS/MS for analysis.

SLE: One milliliter of prepared calibrator or QC sample was added to 3 mL of Quantisal buffer to simulate real-world ratios. Then, 100 µL of the oral fluid/buffer mixture was added to a 2 mL microcentrifuge tube along with 100 µL of 5% ammonium hydroxide and 20 µL of the internal standard. The sample was then vortexed, and 200 µL was loaded into a 200 mg Resprep SLE cartridge (cat.# 28302). Light vacuum was applied for a few seconds to initiate loading the sample into the sorbent bed. The sample was then allowed to absorb into the sorbent over a period of 5 minutes. After 5 minutes, the sample was eluted with two 500 µL aliquots of 95:5 dichloromethane: isopropanol. The sample was evaporated to dryness and then reconstituted in 100 µL of 90:10 0.1% formic acid in water:0.1% formic acid in methanol. Samples were vortexed and added to a 50 µL vial insert (cat.# 24513) in a 2.0 mL, amber, short-cap vial (cat.# 21142) and capped with a 9 mm short cap (cat.# 24497), then moved to the LC-MS/MS for analysis.

SALLE: One milliliter of prepared calibrator or QC sample was added to 3 mL of Quantisal buffer to simulate real-world ratios. Then, 100 µL of the oral fluid/buffer mixture was added to a 2 mL microcentrifuge tube along with 20 µL of internal standard. The sample was vortexed for 10 seconds. Next, 100 µL of a saturated NaCl solution was added to the tube, and the sample was vortexed again for 10 seconds. Next, 280 µL microliters of acetonitrile was added to the sample. The sample was vortexed for 10 seconds and then centrifuged at 3700 rpm for 10 minutes. Two hundred microliters of the organic layer was aliquoted to a test tube, and the sample was evaporated to dryness under nitrogen. The sample was then reconstituted in 50 µL of 90:10 0.1% formic acid in water:0.1% formic acid in methanol. Samples were vortexed and added to a 50 µL vial insert (cat.# 24513) in a 2.0 mL, amber, short-cap vial (cat.# 21142) and capped with a 9 mm short cap (cat.# 24497), then moved to the LC-MS/MS for analysis.