Ultra-Sensitive Measurements of 11-Nor-Δ^9-Tetrahydrocannabinol-9-Carboxylic Acid in Oral Fluid by Microflow LC-MS/MS Using Benchtop Quadrupole/Orbitrap Mass Spectrometer

Xiang He*, Marta Kozak and Subodh Nimkar
ThermoFisher Scientific, 355 River Oaks Parkway, San Jose, CA 95134, USA
Kevin.he@thermofisher.com, Phone: 408-965-6432 Fax: 408-965-6140

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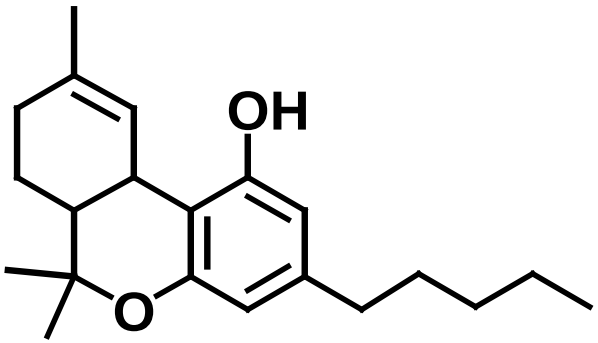
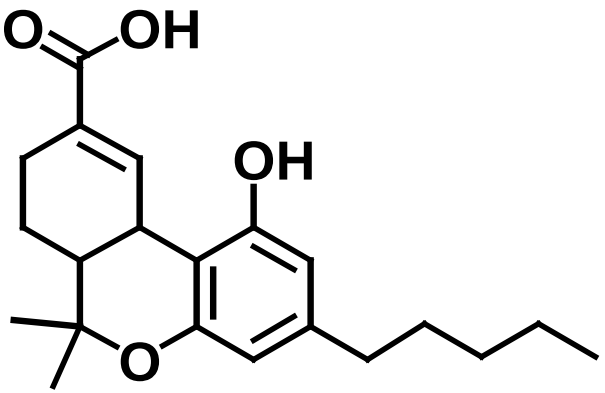
Figure S-1. Structures of THC and THCA.



THC



THCA

Protocol 1: Sample preparation Procedures for LLE and SPE

- LLE. 0.4 mL Oral fluid sample in preservation buffer was added into a silanized glass tube (16×150 mm). 2 mL extraction solvent (hexanes:ethyl acetate:acetic acid, 90:10:3, v/v/v) was added and the mixture was vigorously vortexed for 2 min. The mixture was stored in -30°C freezer for 20 min and the upper organic layer was decanted to a clean silanized glass tube. The extracts were dried under nitrogen stream at 37°C for 8 min and reconstituted with 200 mL 30% methanol in water. The solution was centrifuged at 17,000 g for 5 min at room temperature (RT) and transferred to glass autosampler vials for LC-MS/MS anslysis.

- SPE. C18 SPE cartridge was first pre-conditioned with 1 mL hexanes:ethyl acetate:acetic acid (90:10:3, v/v/v), methanol and 20% methanol in water. 0.4 mL Oral fluid sample in preservation buffer was loaded to the cartridge and the cartridge was washed with 1 mL 60% methanol in water. The SPE cartridge was dried under vacuum for 20 min before elution was performed with hexanes:ethyl acetate:acetic acid (90:10:3, v/v/v, 0.5 mL×2). Pressure inside the vacuum manifold was kept at 18 mmHg during all SPE steps. The eluate was dried under N_2 stream at 37°C for 8 min, and reconstituted with 200 mL 30% methanol in water. The solution was centrifuged at 17,000 *g* for 5 min at RT and transferred to glass autosampler vials for LC-MS/MS analysis.

Figure S-2. Valve switching diagram for online sample cleanup using trapping column.

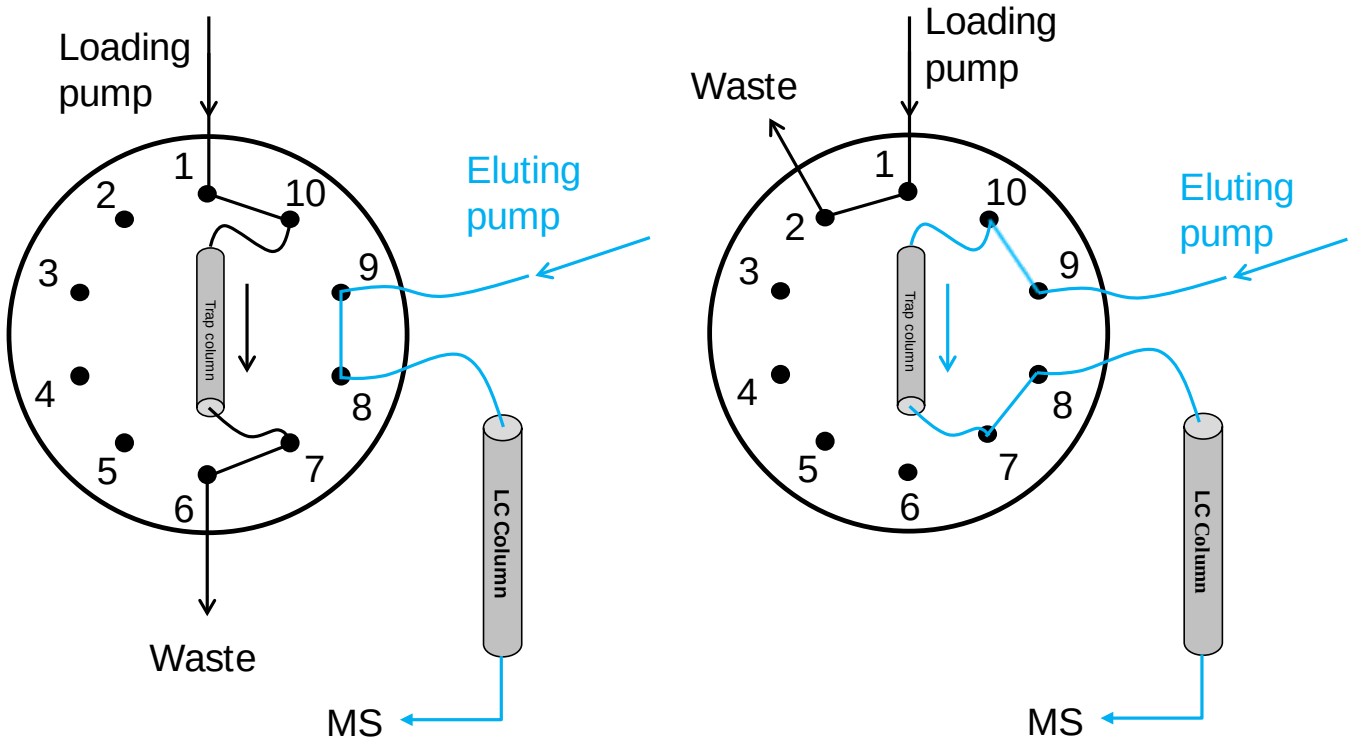


Table S-1. LC gradient.

Step	Time (min)	Valve position	Loading Flow (μL/min)	LMPA (%)	LMPB (%)	Eluting Flow (μL/min)	EMPA (%)	EMPB (%)
1	0.00	Load	40	95	5	20	80	20
2	1.00	Load	40	95	5	20	80	20
3	1.05	Load	40	62.5	37.5	20	80	20
4	3.00	Load	40	62.5	37.5	20	80	20
5	3.05	Elute	40	95	5	20	80	20
6	4.00	Elute	40	95	5	20	80	20
7	4.10	Elute	40	95	5	20	50	50
8	6.00	Elute	40	95	5	20	50	50
9	9.50	Elute	40	95	5	20	2	98
10	9.55	Elute	40	95	5	40	2	98
11	10.50	Elute	40	95	5	40	2	98
12	10.55	Elute	40	95	5	20	80	20
13	11.00	Load	40	95	5	20	80	20
14	12.50	Load	40	95	5	20	80	20

LPMA: 20% MeOH in water; LPMB: ACN; EPMA: 5 mM NH_4Ac in water; EPMB: 5 mM NH_4Ac in MeOH.

Table S-2. List of parent and product ions used in negative ion mode for THCA, THCA-d9, THC and THC-d3.

	THC	THC-d3	THCA	THCA-d9
Parent (*m/z*)	313.2167	316.2353	343.1909	352.2465
Product ion 1 (*m/z*)	245.1547	248.1735	299.2011	308.2567
Product ion 2 (*m/z*)	191.1075		245.1544	
Product ion 3 (*m/z*)			191.1075	

Figure S-3. Extracted ion chromatograms (m/z 343.2→299.2011) of THCA spiked in OFNC samples at 30 pg/mL. The samples underwent three different sample preparation procedures: (A) LLE, (B) SPE, (C) DRUF. Data was acquired in negative ion mode.

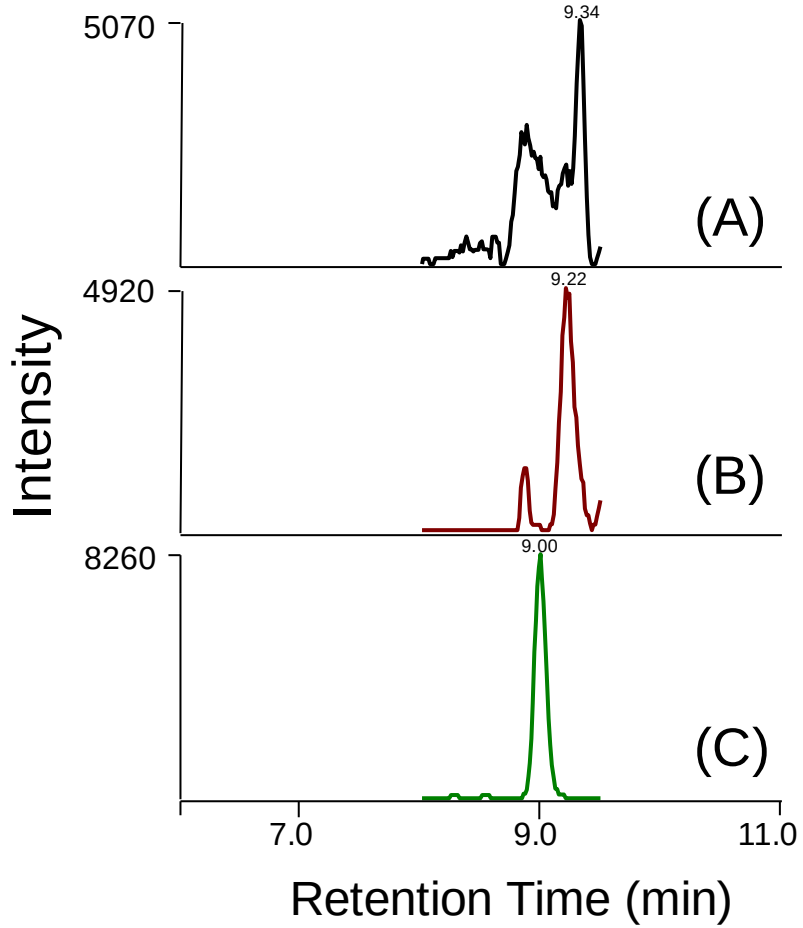


Figure S-4. Targeted-MS2 spectra of THCA.

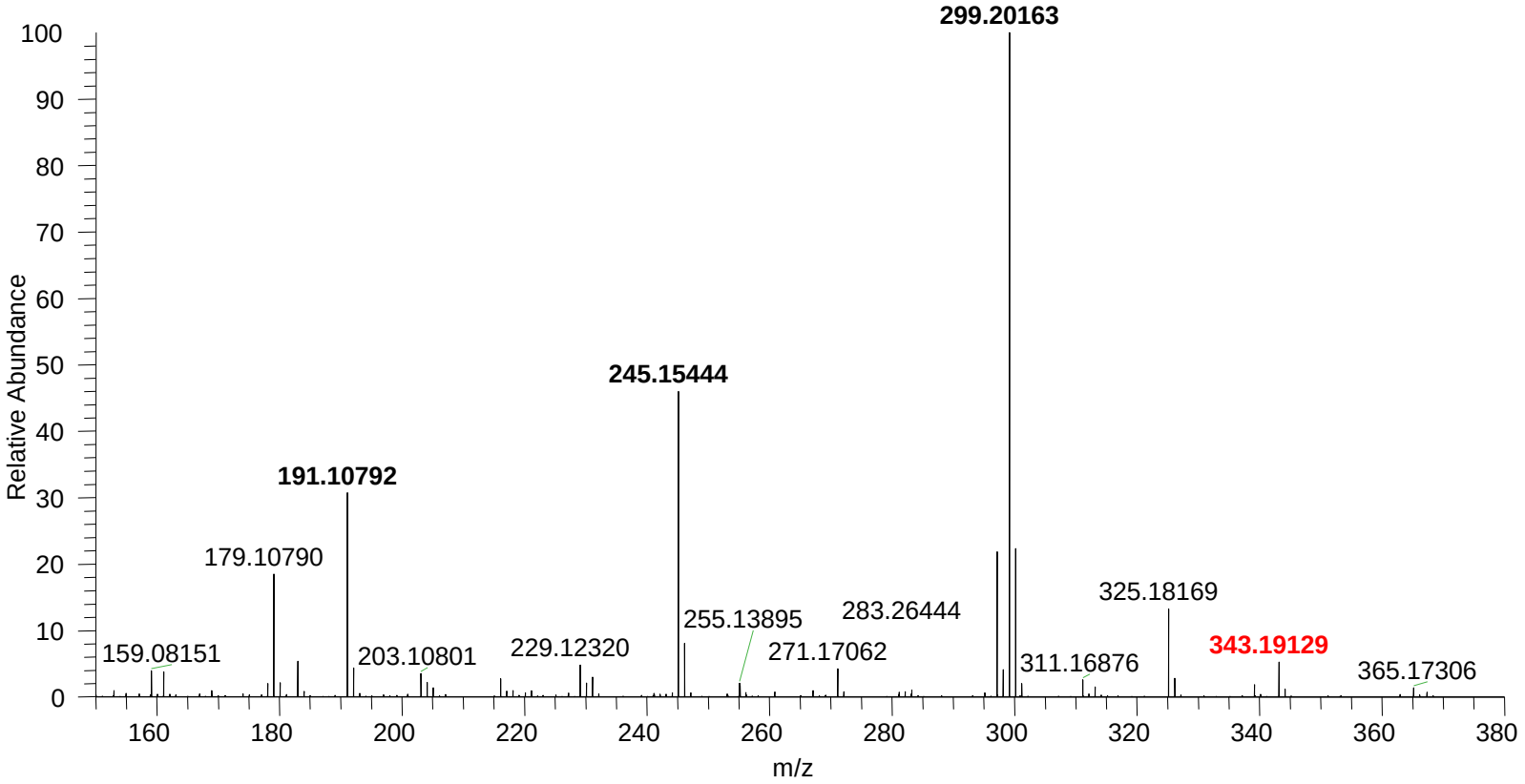


Table S-3. Linearity, accuracy and LLOQ determination for THCA in OFNC samples.

Specified (pg/mL)	Measured (pg/mL, n=3)	Standard Deviation (pg/mL, n=3)	Accuracy (%, n=3)	Precision (%, n=3)
7.5	8.7	1.1	116.3	13.0
15	14.6	1.1	97.1	7.3
30	27.0	1.4	89.9	5.3
150	137.7	1.8	91.8	1.3
300	314.5	8.1	104.8	2.6

Table S-4. Summary of precision data for THCA and THC in OFNC samples.

THCA		Batch 1	Batch 2	Batch 3	Inter-batch
		n=5	n=5	n=5	n=15
Low (24 pg/mL)	Precision (%)	9.3	7.7	6.9	8.4
	Accuracy (%)	107.9	99.8	100.3	102.7
High (120 pg/mL)	Precision (%)	3.9	6.9	3.3	5.3
	Accuracy (%)	95.9	97.2	91.5	94.9
THC		Batch 1	Batch 2	Batch 3	Inter-batch
		n=5	n=5	n=5	n=15
Low (0.64 ng/mL)	Precision (%)	5.6	3.4	5.3	5.3
	Accuracy (%)	107.2	104.0	100.2	103.8
High (2.0 ng/mL)	Precision (%)	1.6	2.9	3.9	2.8
	Accuracy (%)	95.4	96.2	96.6	96.1