Electronic Supplementary Material (ESI) for Analyst.

ELECTRONIC SUPPLEMENTARY INFORMATION:

A Direct Mass Spectrometry Method for Cannabinoid Quantitation in Urine and Oral Fluid Utilizing Reactive Paper Spray Ionization

Authors:

Scott A. Borden[1,2], Armin Saatchi[1], Jan Palaty[3], Chris G. Gill[1,2,4,5]*

Author Affiliations:

[1] Applied Environmental Research Laboratories, Department of Chemistry, Vancouver Island University, Nanaimo, BC, Canada

[2] Department of Chemistry, University of Victoria, Victoria, BC, Canada

[3] LifeLabs Medical Laboratories, Burnaby, BC, Canada

[4] Department of Chemistry, Simon Fraser University, Burnaby, BC, Canada

[5] Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, WA, USA

***Address correspondence to:**

Professor Chris G. Gill, Ph.D., P. Chem.
Co-Director, Applied Environmental Research Laboratories (AERL)
Department of Chemistry
Vancouver Island University
900 Fifth Street, Nanaimo, BC, Canada, V9R 5S5

Ph: 250-753-3245
Chris.Gill@viu.ca

Table S1 Paper spray mass spectrometry analyte-independent parameters.

Parameter	Value
Ionization polarity	Positive
Spray voltage (V)	4000
Q1 FWHM resolution	0.7
Q3 FWHM resolution	1.2
Dwell time (ms)	26.3
Cycle time (sec)	1
Data points per peak	60
Argon CID gas pressure (mTorr)	2
Sweep gas (Arb)	0
Ion transfer tube (°C)	300

Table S2 Mass-dependent selected reaction monitoring (SRM) transition parameters of underivatized cannabinoids and isotopically labelled internal standards. The first ion listed is the quantifier ion, and the reported ion ratio is between the first and second ions listed.

Compound	Precursor Ion (*m/z*)	Product Ion (*m/z*)	Tube Lens (V)	Collision Energy (eV)	Source Fragmentation (V)
THC	315.3	123.1	107	32.30	10.0
	315.3	193.1	107	22.99	10.0
THC-d_3	318.3	123.1	107	32.67	10.0
THC-COOH	345.2	299.2	128	19.90	33.0
	345.2	304.1	128	8.96	33.0
THC-COOH-d_3	348.2	302.2	126	20.04	33.0
CBD	315.2	193.1	154	22.06	27.8
	315.2	283.2	154	5.29	27.8
CBD-d_9	324.3	123.1	148	33.14	34.3
CBN	311.2	55.1	125	19.24	0
	311.2	87.2	125	11.70	0
CBN-d_3	314.3	195.1	159	26.61	47.3

Table S3 Mass-dependent selected reaction monitoring (SRM) transition parameters of derivatized cannabinoids and isotopically labelled internal standards. The first ion listed is the quantifier ion, and the reported ion ratio is between the first and second ions listed.

Compound (Derivatized)	Precursor Ion (*m/z*)	Product Ion (*m/z*)	Tube Lens (V)	Collision Energy (eV)	Source Fragmentation (V)
THC	483.3	157.0	115	37.10	23.0
	483.3	361.2	115	25.18	23.0
THC-d_3	486.3	157.2	115	37.39	23.0
THC-COOH	513.3	338.1	153	23.74	42.4
	513.3	310.2	153	27.37	42.4
THC-COOH-d_3	516.3	341.2	153	24.21	42.4
CBD	483.2	157.1	121	35.40	20.2
	483.2	298.1	121	27.49	20.2
CBD-d_9	492.3	157.1	121	36.76	20.2
CBN	479.2	222.2	130	52.43	24.0
	479.2	236.1	130	53.86	24.0
CBN-d_3	482.3	325.1	130	20.70	24.0

Table S4 Paper spray mass spectrometry solvent dispense conditions.

Rewet Solvent Dispense (10 µL)	
Aliquot #	Delay (s)
1	1
2	1
Spray Solvent Dispense (10 µL)	
Aliquot #	Delay (s)
1	1
2	1
3	1
4	1
5	3
6	3
7	5
8	5
9	5
10	5
11	5
12	7
13	7
14	7

Table S5 Mass spectrometry time-dependent voltage parameters.

Time (min)	**Spray Voltage (V)**
0	0
0.1	4000
1.1	0

Table S6 PS-MS calibrations for underivatized cannabinoids in methanol (n = 4, 8 levels [2,5,10,20,40,100,250,500 ng/mL], [internal standard] = 200 ng/mL)) using acetonitrile with 0.1% formic acid as spray solvent.

Analyte	Internal standard	Slope (×10^2)	y-intercept	R^2	LOD[a] (ng/mL)	LLOQ[b] (ng/mL)
THC	THC-d_3	0.268	0.070	0.902	25.2	250
THC-COOH	THC-COOH-d_3	0.560	0.305	0.971	47.4	250

[a] LOD defined as 3.3 × standard deviation of lowest calibrator / slope

[b] LLOQ defined as the lowest calibrator that meets the acceptance criteria: intra-assay %CV < 15, %bias within ± 20%, ion ratio within ± 20%, and S/N > 4

Table S7 Reactive PS-MS calibrations for derivatized cannabinoids in methanol (n = 4, 8 levels [2,5,10,20,40,100,250,500 ng/mL], [internal standard] = 200 ng/mL) using acetonitrile with 0.1% formic acid as spray solvent.

Analyte	Internal standard	Slope (×10^2)	y-intercept	R^2	LOD[a] (ng/mL)	LLOQ[b] (ng/mL)
THC	THC-d_3	1.06	0.118	0.997	1.6	10
THC-COOH	THC-COOH-d_3	0.84	0.0322	0.994	1.4	10
CBD	CBD-d_9	2.01	0.00785	0.990	1.6	10
CBN	CBN-d_3	1.27	0.0129	0.999	0.89	10

[a] LOD defined as 3.3 × standard deviation of lowest calibrator / slope

[b] LLOQ defined as the lowest calibrator that meets the acceptance criteria: intra-assay %CV < 15, %bias within ± 20%, ion ratio within ± 20%, and S/N > 4

Table S8 Cut-off values for drug testing of THC in oral fluid and THC-COOH in urine recommended by regulatory bodies.

Analyte	Matrix	Screening (ng/mL)	Confirmatory (ng/mL)	Source
THC-COOH	Urine	50	15	Cann-Amm Occupational testing services[1]
THC-COOH	Urine	50	15	US Department of Transportation[2]
THC-COOH	Urine	50	15	Substance Abuse and Mental Health Services Administration (SAMHSA)[3]
THC-COOH	Urine	50	15	European Workplace Drug Testing Society[4]
THC-COOH	Urine	50	25	The Australian Standard (AS/NZ 4308:2008)[5]
THC	Oral fluid	4	2	Cann-Amm Occupational testing services[1]
THC	Oral fluid	25	-	Canadian Society of Forensic Sciences – Drugs and Driving Committee[6]
THC	Oral fluid	4	2	Substance Abuse and Mental Health Services Administration (SAMSHA)[3]
THC	Oral fluid	10	2	European Workplace Drug Testing Society[7]
THC	Oral fluid	15	5	The Australian Standard (AS/NZ 4760:2019)[8]

Table S9 Preparation of protein-free artificial urine in deionized water, pH = 6.0 (adjusted using 1.0 M hydrochloric acid).

Chemical	Concentration (mM)
Urea	170
Sodium chloride	90
Ammonium chloride	25
Sodium bicarbonate	25
Sodium sulfate	10
Potassium dihydrogen phosphate	7.0
Calcium chloride	2.5
Magnesium sulfate	2.0
Citric acid	2.0
Lactic acid	1.1

Table S10 Comparison of reactive PS-MS and LC-MS for the analysis of prepared human urine samples for THC-COOH content. PS-MS results averaged from 6 replicates, LC-MS results from single analysis.

[THC-COOH] (ng/mL)	PS-MS Average (ng/mL)	%CV	Avg. PS-MS Ion Ratio[a]	LC-MS Result (ng/mL)	PS-MS %Bias	LC-MS %Bias	%Difference
15	14.5	12.8	0.65	15.0	-2.8	-0.26	-2.6
25	25.8	9.5	0.70	23.4	3.3	-6.4	9.8
50	47.4	5.0	0.65	46.3	-5.2	-7.4	2.4
50	49.0	7.8	0.66	45.2	-2.1	-9.6	8.0
50	53.0	4.1	0.64	49.4	5.9	-1.2	6.9
125	121.3	3.9	0.58	118.0	-3.0	-5.6	2.8
300	271.8	2.6	0.53	321.7	-9.4	7.2	-16.8

[a] Target ion ratio = 0.661 ± 20% (0.529 – 0.793)

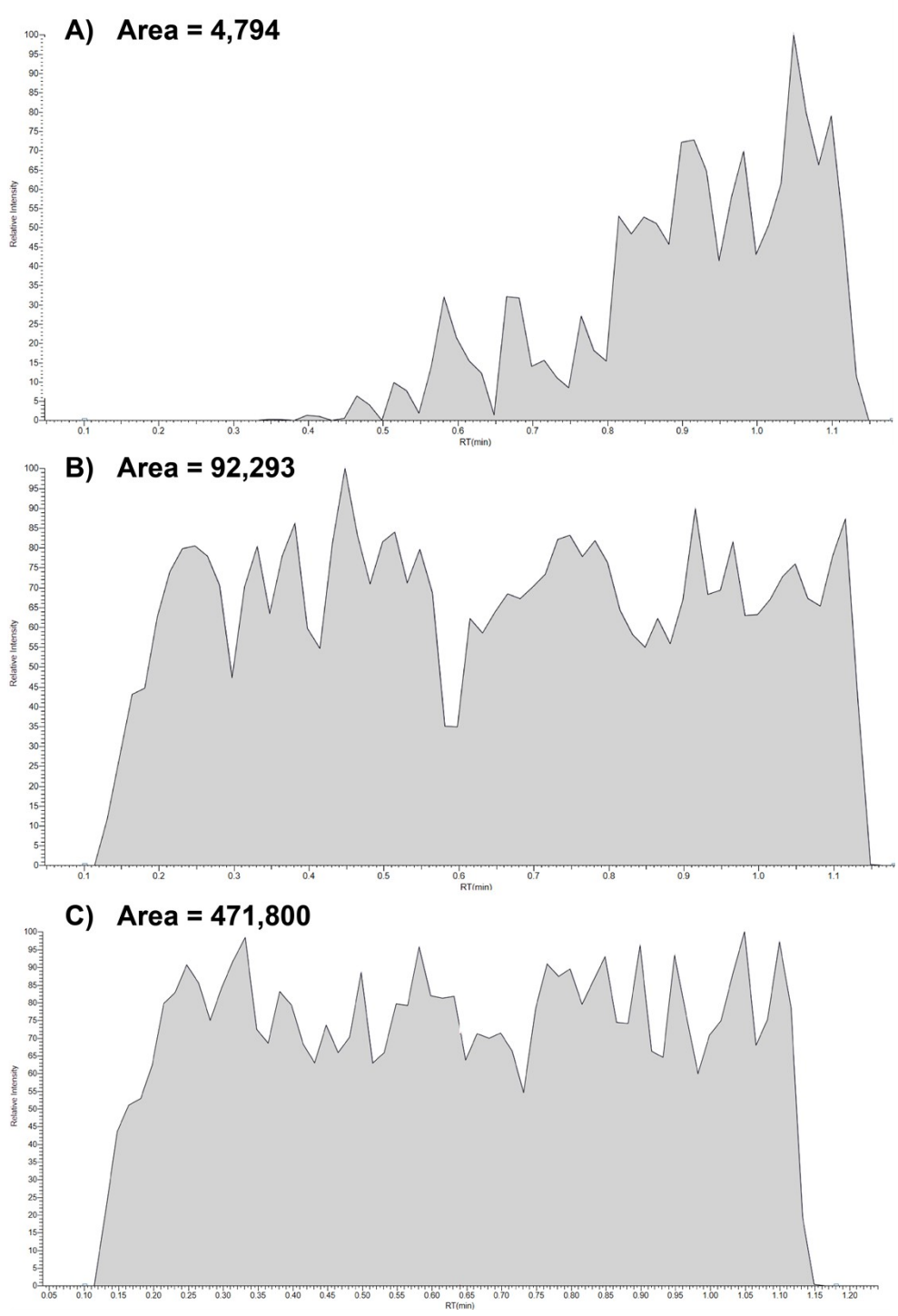

Fig. S1 Reactive PS-MS signal chronograms for 250 ng/mL of derivatized THC-COOH in **A)** undiluted human urine using 90/9.9/0.1% acetonitrile/water/formic acid as spray solvent, **B)** human urine diluted with methanol (70/30 v/v%) using 90/9.9/0.1 acetonitrile/water/formic acid as spray solvent, and **C)** human urine diluted with methanol (70/30 v/v%) using acetonitrile with 0.1% formic acid as a spray solvent and application of two 10 µL aliquots of DCM to the dried sample spot prior to measurement.

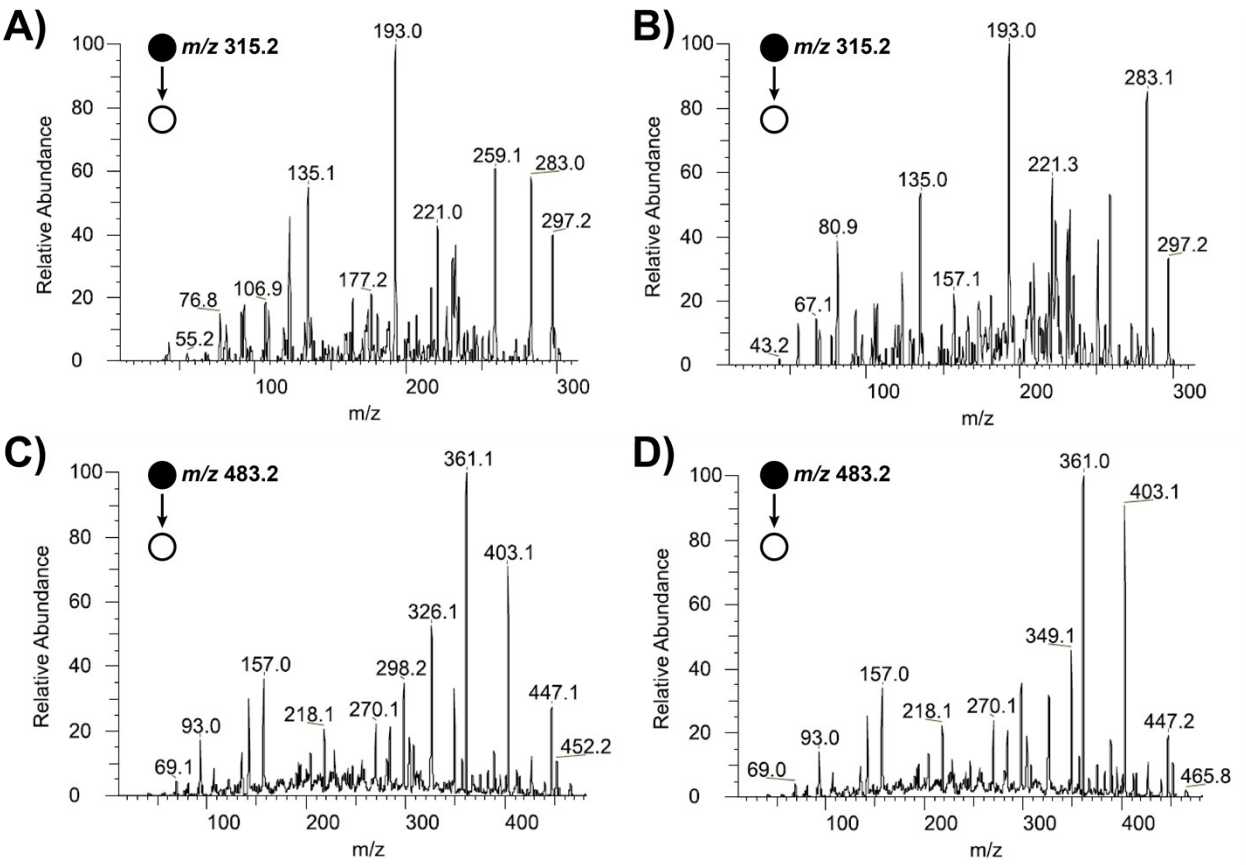

Fig. S2 Direct infusion electrospray ionization product ion MS/MS spectra of **A)** THC, **B)** CBD, **C)** derivatized THC, and **D)** derivatized CBD.

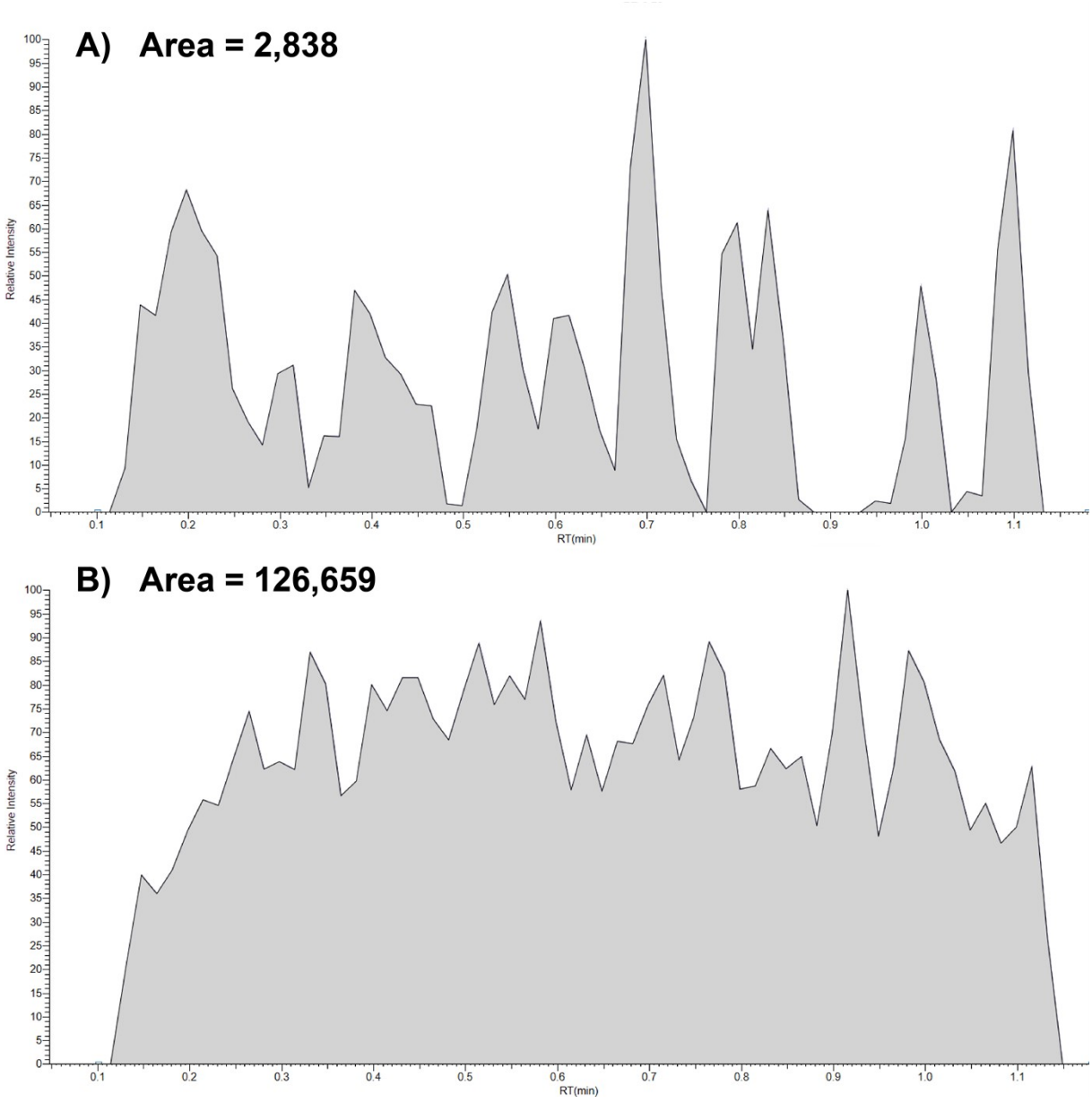

Fig S3 Reactive PS-MS signal chronograms for 200 ng/mL of derivatized THC-COOH using acetonitrile with 0.1% formic acid as spray solvent in **A)** artificial urine spiked with 0.14 µg/mL bovine serum albumin, and **B)** protein-free artificial urine.

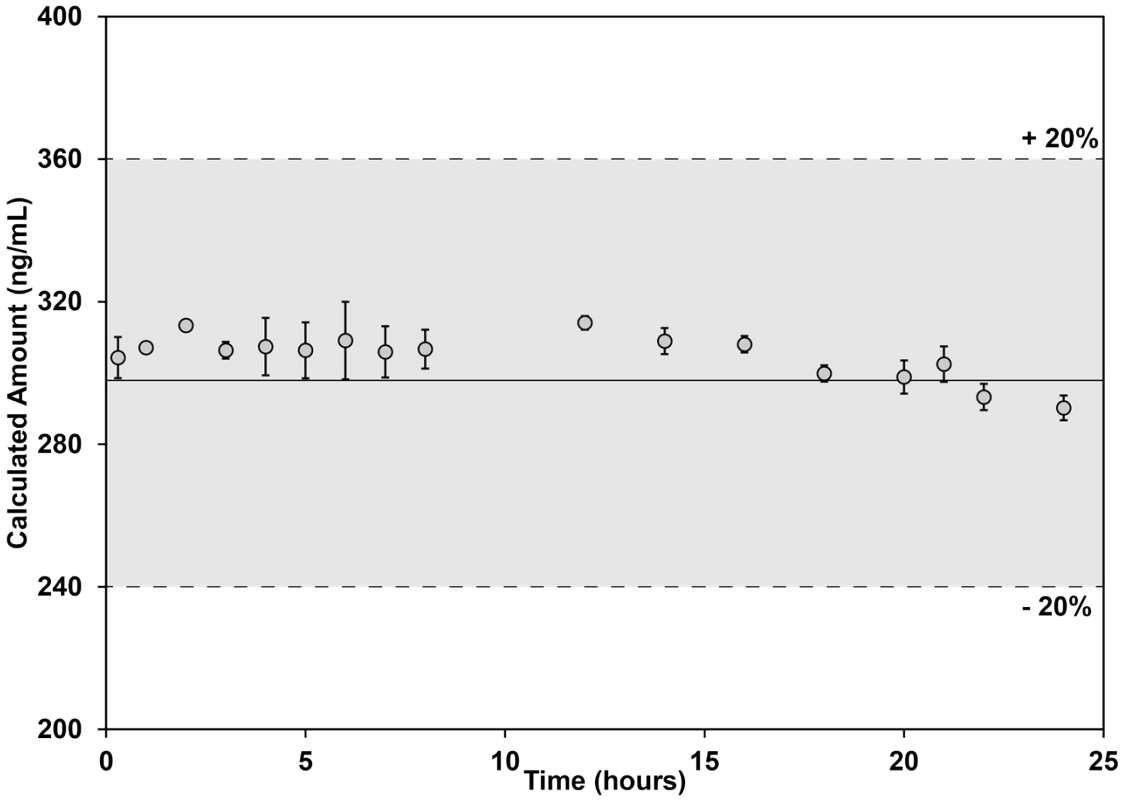

Fig. S4 Measured THC-COOH levels from reactive paper spray measurements (n=3) for prepared urine samples (300 ng/mL) measured at various intervals using PS-MS sample strips prepared in advance with pre-deposited derivatization reagent. 300 ng/mL is represented by the solid line, with ± 20% bias represented by the dashed lines and shaded grey box. Error bars represent ± one standard deviation.

The stability of the dried derivatization reagent on the PS-MS paper substrate was evaluated over the course of 24 hours. In this study, Fast Red RC was spotted on the PS-MS sample strips and allowed to dry at ambient temperatures. Prepared urine sample was then spotted periodically (followed by reactive PS-MS analysis) over the next 24 hours at prescribed intervals.

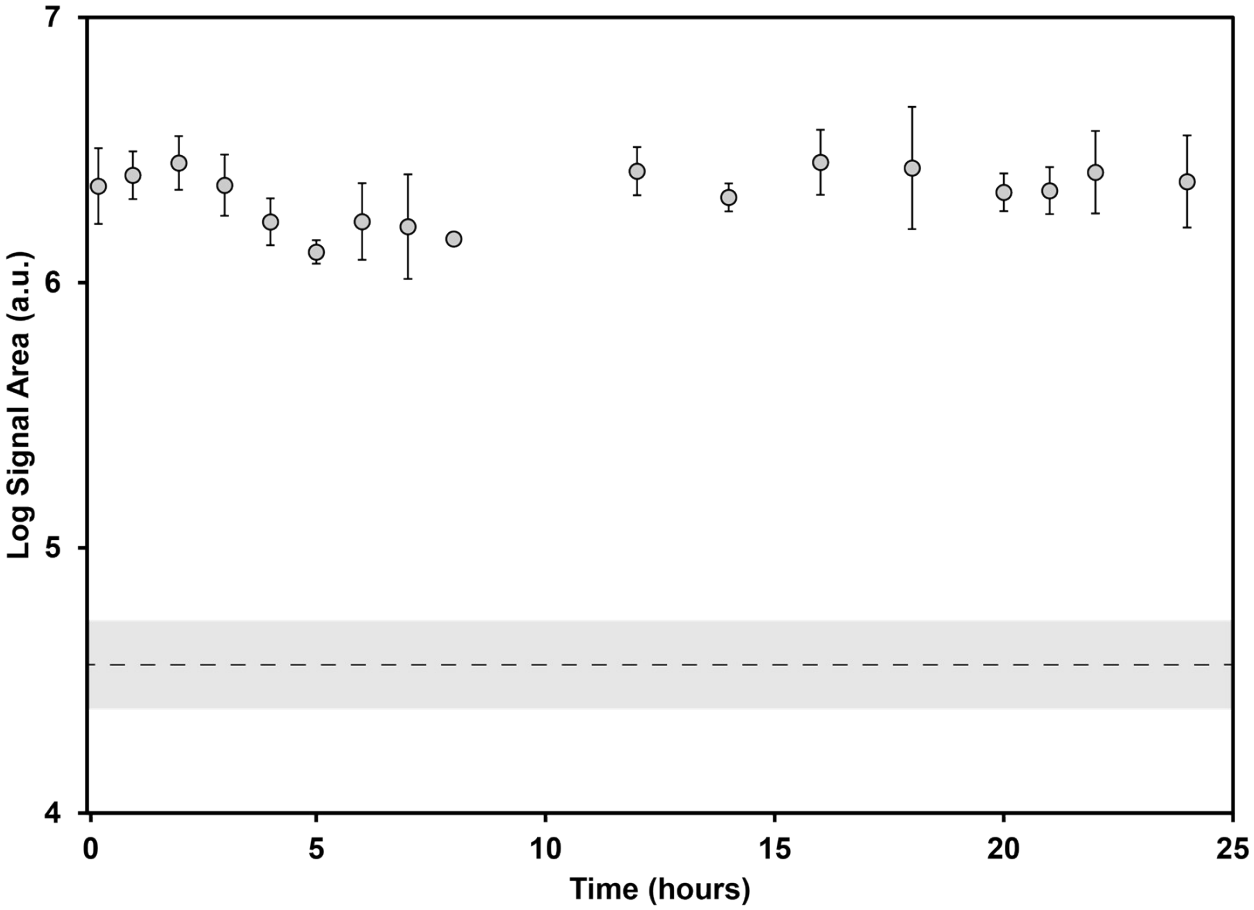

Fig. S5 Log signal area intensities of derivatized THC-COOH from reactive paper spray measurements (n=3) of prepared urine samples (300 ng/mL THC-COOH) obtained at various intervals using PS-MS sample strips prepared in advance with pre-deposited derivatization reagent. The dashed line indicates the mean signal intensity of blank measurements (n=6) with the shaded grey box representing ± one standard deviation. Error bars represent ± one standard deviation.

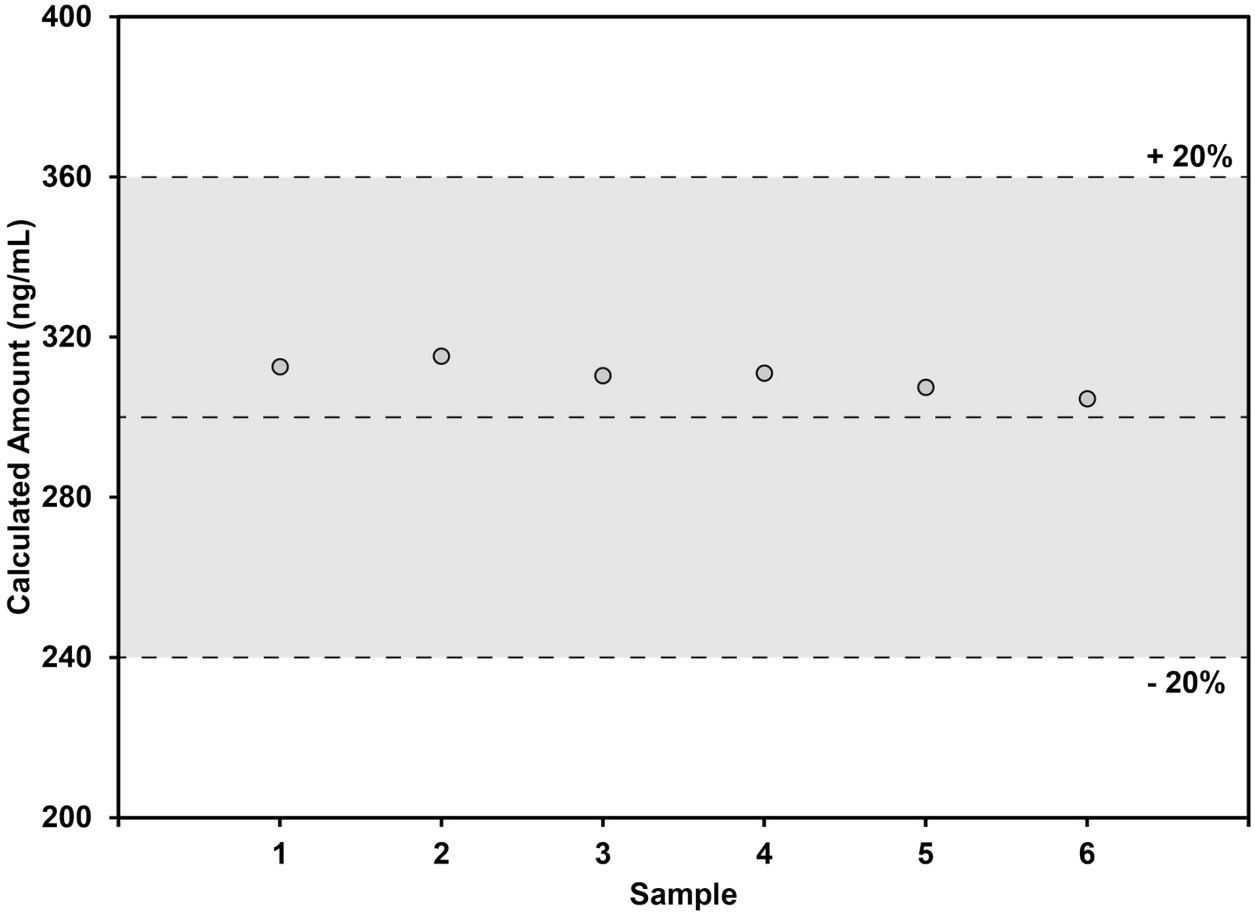

Fig. S6 Measured THC-COOH levels for reactive PS-MS measurements of prepared urine samples (300 ng/mL) using a 60°C oven for two 1.5-minute drying steps (after Fast Red RC spotting, and after urine spotting). The shaded grey box represents ± 20% bias. The 6 samples were prepared on 6 unique PS-MS sample plates and measured at different times.

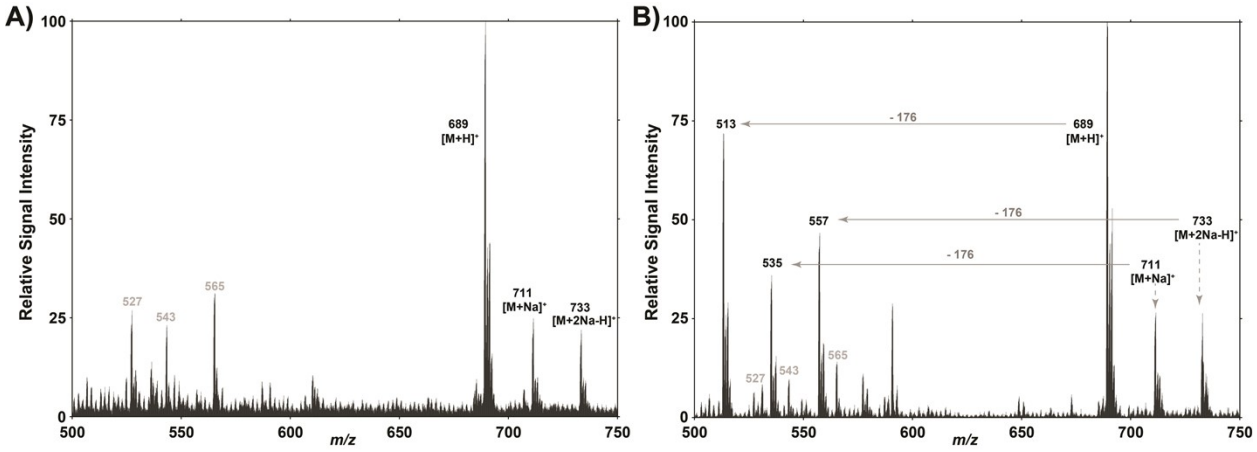

Fig. S7 Direct infusion electrospray ionization full scan mass spectra (500-750 *m/z*) of **A)** THC-COOH glucuronide derivatized with Fast Red RC at a source fragmentation of 0V, and **B)** THC-COOH glucuronide derivatized with Fast Red RC at a source fragmentation of 100V*

*Spectra from Fig. S7 were collected on a different triple quadrupole mass spectrometer due to instrument availability:

TSQ Altis QqQ (Thermo Fisher Scientific, San Jose, CA)

Parameter	**Value**
Ionization polarity	Positive
Spray voltage (V)	4000
Q1 FWHM resolution	0.7
Scan Rate (Da/s)	1000
Averaged from (#scans)	20
Ion transfer tube (°C)	300

References

1. Cann-Amm Occupational Testing Services. (Jun 3, 2019). "What are cut-off levels and what do they mean?" Retrieved from: https://www.cannamm.com/news/what-are-cut-off-levels-and-what-do-they-mean
2. U.S. Department of Transportation. (Apr 15, 2020). “DOT Rule 49 CFD part 40 Section 40.87. Subpart F – Drug Testing Laboratories.” Retrieved from: https://www.transportation.gov/odapc/part40/40-87
3. Department of Health and Human Services: Substance Abuse and Mental Health Services Administration (SAMHSA). (Jan 23, 2017). “Mandatory Guidelines for Federal Workplace Drug Testing Programs.” Retrieved from: https://www.govinfo.gov/content/pkg/FR-2017-01-23/pdf/2017-00979.pdf
4. European Workplace Drug Testing Society. (Nov 1, 2015). “European Guidelines for Workplace Drug Testing in Urine. Version 2.0.” Retrieved from: http://www.ewdts.org/data/uploads/documents/ewdts-urine-guideline-2015-11-01-v2.0.pdf
5. Standards Australia. (2008). AS/NZA 4308:2008. “Procedures for specimen collection and the detection and quantitation of drugs of abuse in urine.” Retrieved from: https://www.standards.org.au/standards-catalogue/sa-snz/health/ch-039/as-slash-nzs--4308-colon-2008
6. Canadian Society of Forensic Science: Drugs and Driving Committee. (Oct 2018). “Report on Drug Screening Equipment – Oral Fluid.” Retrieved from: https://www.csfs.ca/wp-content/uploads/2018/10/Report-on-Drug-Screening-Equipment-%E2%80%93-Oral-Fluid.pdf
7. European Workplace Drug Testing Society. (Nov 1, 2015). “European Guidelines for Workplace Drug Testing in Oral Fluid. Version 2.0.” Retrieved from: http://www.ewdts.org/data/uploads/documents/ewdts-oral-fluid-2015-11-01-v2.0.pdf
8. Standards Australia. (2019). AS/NZA 4760:2019. “Procedure for specimen collection and the detection and quantification of drugs in oral fluid.” Retrieved from: https://www.standards.org.au/standards-catalogue/sa-snz/health/ch-039/as-slash-nzs--4760-colon-2019