Validation of a Microsampling-Compatible LC-MS/MS Method for Cannabinoid Quantitation in Whole Blood

Supplementary Data

Supplementary Table S1. Published methods for cannabinoid quantitation in whole blood, plasma, or serum. The table summarizes sample volume, extraction procedure, analytical technology, and reported lower limits of quantitation (LLOQ) for Δ9-tetrahydrocannabinol (THC), 11-nor-9-carboxy-THC (THC-COOH), 11-hydroxy-THC (11-OH-THC), cannabinol (CBN), cannabigerol (CBG), and cannabidiol (CBD). References for each method are listed at the end of the Supplementary Data.

Reference	Sample (µL)	Extraction Procedure	Analytical Technology	LLOQ (ng/mL)					
				THC	THC-COOH	11-OH-THC	CBN	CBG	CBD
Proença et al., 2023 [1]	100 WB	Protein precipitation	LC-MS/MS	0.5	0.5	0.5	-	-	-
Hädener et al., 2016 [2]	100 WB	Protein precipitation	LC-MS/MS	-	5	-	-	-	-
Hubbard et al., 2020 [3]	200 WB	SPE	LC-MS/MS	0.5	1	1	0.5	1	0.5
Scheidweiler et al., 2016 [4]	200 WB	SPE	LC-MS/MS	0.5	0.5	0.5	0.5	1	0.5
Orfanidis et al., 2020 [5]	200 WB	QuEChERS (dSPE)	LC-MS/MS	18.54	3.31	-	-	-	-
Palazzoli et al., 2018 [6]	200 WB	Protein precipitation	LC-MS/MS	1	1	1	-	-	0.5
Fernandez et al., 2008 [7]	250 WB	LLE	LC-MS/MS	0.5	2	1	-	-	-
Chan-Hosokawa et al., 2022 [8]	250 WB	LLE	LC-MS/MS	0.5	5	1	-	-	-
Frei et al., 2022 [9]	250 WB	SPE	GC-MS/MS	0.3	3	0.3	0.2	-	0.3
Thomas et al., 2007 [10]	500 WB	LLE	GC-MS/MS	0.5	2.5	0.5	-	-	-

Supplementary Table S1 Continued

Reference	Sample (µL)	Extraction Procedure	Analytical Technology	LLOQ (ng/mL)					
				THC	THC-COOH	11-OH-THC	CBN	CBG	CBD
Simoes et al., 2011 [11]	500 WB	SPE	LC-MS/MS	0.5	0.5	0.5	-	-	-
Schwope et al., 2011 [12]	500 WB	SPE	LC-MS/MS	1	1	1	1	-	1
Holland et al., 2011 [13]	500 WB	SPE	GC-MS	0.5	0.5	0.5	-	-	-
Castro et al., 2018 [14]	500 WB	SPE	GC-MS/MS	0.93	0.99	0.87	-	-	-
Ferrari et al., 2022 [15]	500 WB	QuEChERS (dSPE)	LC-MS/MS	4	10	-	-	-	-
Joye et al., 2020 [16]	1000 WB	LLE	LC-MS/MS	0.4	2.5	0.4	-	-	0.4
Andrews et al., 2012 [17]	1000 WB	LLE	GC-MS	0.25	0.5	0.25	0.25	-	0.5
Reber et al., 2022 [18]	1000 WB	SPE	LC-MS/MS	1	5	-	-	-	-
Teixeira et al., 2007 [19]	1000 WB	SPE	LC-MS	2	2	25	-	-	-
Giroud et al., 2001 [20]	1000 WB	SPE	GC-MS/MS	1	5	1	-	-	-
Hoffman et al., 2020 [21]	2000 WB	SPE	GC-MS	1	1	-	-	-	-
da Silva et al., 2020 [22]	100 P	LLE	LC-MS/MS	0.5	1	0.5	0.5	-	0.5
Manca et al., 2022 [23]	100 P	Protein precipitation	LC-MS/MS	5	5	-	-	-	-

Supplementary Table S1 Continued

Reference	Sample (µL)	Extraction Procedure	Analytical Technology	LLOQ (ng/mL)					
				THC	THC-COOH	11-OH-THC	CBN	CBG	CBD
Sempio et al., 2021 [24]	200 P	Protein precipitation	LC-MS/MS	0.78	0.78	3.13	0.78	0.78	0.78
Rosado et al. 2017 [25]	250 P	MEPS (SPE)	GC-MS/MS	0.1	0.1	0.1	-	-	-
Andrenyak et al., 2017 [26]	1000 P	LLE	GC-MS/MS	0.1	0.5	0.1	-	-	0.25
Álvarez-Freire et al., 2023 [27]	1000 P	LLE	GC-MS/MS	50	80	-	-	-	-
Pichini et al., 2020 [28]	100 S	LLE	LC-MS/MS	0.12	0.19	0.12	-	-	0.13

WB – whole blood; P – plasma; S – serum; LLE – liquid-liquid extraction; SPE – solid phase extraction; dSPE – dispersive SPE; LC-MS/MS – liquid chromatography-tandem mass spectrometry; GC-MS/MS – gas chromatography-tandem mass spectrometry; MEPS - microextraction by packed sorbent.

Supplementary Table S2. Optimized mass transitions of analytes and internal standards.

Compound	RT	Type	Precursor Ion	Product Ion	CE	Polarity
THC	3.86	Quantifier	315.2	193.0	24	Positive
		Qualifier	315.2	123.0	37	Positive
THC-d_3	3.85	Quantifier	318.3	196.1	30	Positive
		Qualifier	318.3	123.0	40	Positive
CBN	3.57	Quantifier	311.2	223.0	24	Positive
		Qualifier	311.2	293.2	16	Positive
CBN-d_3	3.56	Quantifier	314.2	296.1	20	Positive
		Qualifier	314.2	223.1	20	Positive
CBG	2.78	Quantifier	317.3	193.1	20	Positive
		Qualifier	317.3	123.0	20	Positive
CBG-d_3	2.77	Quantifier	320.3	196.0	14	Positive
		Qualifier	320.3	123.0	36	Positive
CBD	2.77	Quantifier	315.2	193.1	34	Positive
		Qualifier	315.2	123.0	20	Positive
CBD-d_3	2.76	Quantifier	318.2	196.1	20	Positive
		Qualifier	318.2	123.2	30	Positive
11-OH-THC	2.56	Quantifier	331.2	313.2	14	Positive
		Qualifier	331.2	193.1	5	Positive
11-OH-THC-d_3	2.56	Quantifier	334.2	316.2	10	Positive
		Qualifier	334.2	196.1	20	Positive
THC-COOH	2.81	Quantifier	343.2	299.0	20	Negative
		Qualifier	345.2	299.1	20	Negative
THC-COOH-d_3	2.80	Quantifier	348.2	302.2	20	Positive
		Qualifier	348.2	330.2	10	Positive

Supplementary Table S3. List of 98 common impairing drugs analyzed for exogenous interference study.

Category	**Compounds Tested**
Alcohol	ethanol
Opioids	6-acetylmorphine, buprenorphine, carfentanil, codeine, EDDP, etodesnitazene, fentanyl, hydrocodone, hydromorphone, isotonitazene, meperidine, methadone, mitragynine, morphine, norfentanyl, ortho-methylfentanyl, ortho-fluorofentanyl, oxcodone, para-fluorofentanyl, protonitazene, tramadol
CNS Stimulants	amphetamine, benzoylecgonine, cocaethylene, cocaine, MDA, MDMA, methamphetamine
Dissociative Anesthetics	dextromethorphan, ketamine, phencyclidine
Benzodiazepines	7-aminoclonazepam, 7-aminoflunitrazepam, 7-aminonitrazepam, 8-aminoclonazolam, alprazolam, bromazolam, chlordiazepoxide, chlorpheniramine, clonazepam, desalkylflurazepam, desalkylgidazepam, deschloroetizolam, diazepam, etizolam, flualprazolam, flubromazepam, flunitrazepam, lorazepam, nitrazepam, nordiazepam, oxazepam, temazepam, zolpidem, zopiclone
Antidepressants	amitriptyline, bupropion, citalopram, clomipramine, desipramine, doxepin, fluoxetine, hydroxybupropion, imipramine, mirtazapine, didesmethylcitalopram, norsertraline, nortriptyline, o-desmethylvenlafaxine, paroxetine, sertraline, trazodone, venlafaxine
Anticonvulsants	carbamazepine, gabapentin, lamotrigine, levetiracetam, phenytoin, topiramate
Antipsychotics	aripiprazole, chlorpromazine, clozapine, haloperidol, loxapine, olanzapine, quetiapine, risperidone, ziprasidone,
Antihistamines	cetirizine, cyclobenzaprine, diphenhydramine, doxylamine,
Hallucinogen	LSD, psilocin
Other	medetomidine, naloxone, xylazine

Supplementary Table S4. Stability of extracted cannabinoid samples at the low and high quality control levels following storage in the autosampler at 8ºC for 24, 48, and 72 hours.

	24 h autosampler (% difference, n = 3)		48 h autosampler (% difference, n = 3)		72 h autosampler (% difference, n = 3)	
Analyte	**Low**	**High**	**Low**	**High**	**Low**	**High**
THC	-15.4	-12.0	-19.7	-17.8	-13.2	-16.9
CBN	-17.2	-16.4	-18.9	-24.0	-17.4	-19.5
CBG	-11.7	-19.8	-19.9	-26.5	-35.6	-18.1
CBD	-0.4	-8.2	-14.0	-19.7	-27.3	-8.4
11-OH-THC	-13.6	-11.8	-17.1	-21.8	-18.4	-15.8
THC-COOH	-17.7	-1.9	-24.1	-16.2	-15.4	-12.1

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