Cannabinoids Confirmation by LC-MSMS - Procedure TCc12727 - Components of Measurement Uncertainty Considered

Measurement process:
The concentration of THC in blood using cannabinoids confirmation test method TCc12727.
The concentration of carboxy-THC in blood using cannabinoids confirmation test method TCc12727.

Traceability:
Established through use of calibrated equipment (pipettes, glassware) and certified reference materials, with all traceable to SI units of measurement

Equipment and instrumentation used:
Calibrated, adjustable piston pipettes, calibrated volumetric glassware for quantitative standard materials, liquid chromatograph - tandem mass spectrometer

Components	Evaluation
Scientists Performing Testing	
Variation amongst scientists performing testing	Represented in Type A data from controls analyzed during validation on all instruments
Training/experience of testing scientists	Represented in Type A data from controls analyzed during validation on all instruments
Calibrator (Working Standard Solution) Preparation	
CRM	Type B Evaluation (uncertainty from COA for CRMs)
Volumetric flasks and piptettes used to prepare standard solutions	Type B Evaluation (uncertainty from calibration certificates)
Solvent (methanol) used in preparation of standard solutions	Represented in Type A data from controls analyzed during validation on all instruments

Quality Control (Working Control Standard Solution) Preparation	
CRM	Represented in Type A data from controls analyzed during validation on all instruments
Volumetric flasks and piptettes used to prepare standard solutions	Represented in Type A data from controls analyzed during validation on all instruments
Solvent (methanol) used in preparation of standard solutions	Represented in Type A data from controls analyzed during validation on all instruments
Internal Standard Preparation	
CRM	Deuterated internal standards used, COA includes purity; volume of internal standard added is accounted for as precision of pipette used to deliver in sample preparation process
Volumetric flasks and piptettes used to prepare standard solutions	Internal standard is relative, with same amount added to each member of a testing batch (except blank matrix); precision of pipette used to deliver internal standard in testing batch is included as uncertainty component
Solvent (methanol) used in preparation of standard solutions	Represented in Type A data from controls analyzed during validation on all instruments
Sample Preparation (Calibrators, Controls, Specimens)	
Specimen homogeneity	Not determined as a significant component for measurement uncertainty evaluation; multiple matrices included in method validation (precision, ion suppression)
Environmental conditions (day to day variation in temperature when testing performed, humidity, and time blank blood and specimens are at room temperature)	Represented in Type A data from controls analyzed during validation on all instruments
Calibrator spike	Type B Evaluation (uncertainty from pipette calibration certificates)
Control spike	Type B Evaluation (uncertainty from pipette calibration certificates)
Internal standard spike	Type B Evaluation - maximum variability (precision) for pipette used to deliver internal standard included in MU budget.
Specimen volume	Type B Evaluation - maximum variability (precision) for pipette used to deliver blank blood and specimen sample included in MU budget.
Equipment (piston pipettes, vials/caps) - variation in use by multiple personnel	Represented in Type A data from controls analyzed during validation on all instruments

Instrumental Analysis	
LC-MSMS test method instrument parameters	Represented by the evaluation for Type A data; reproducibility data from controls analyzed during method valiation on all instruments
Interference from other compounds	Evaluated during method validation; not considered an uncertainty component
Interference from reagents	Blanks and negative controls included in each testing batch; negative matrices are certified prior to being used to produce calibrators and controls for casework batches
Interference from the matrix (ion suppression/enhancement)	Evaluated during method validation, with comparable results for target compounds and deuterated internal standards; not a component included in final MU budget
Variation within batch (systematic)	Specimens are bracketed with positive controls, positive control run at end of batch
Instrument precision	Represented in Type data from controls analyzed during validation on all instruments
Data Analysis/Instrument Software	
Calibration model/range	Represented in Type A data from controls analyzed during validation on all instruments
Integration parameters - instrument software	Represented in Type A data from controls analyzed during validation on all instruments
Software data processing algorithms	Represented in Type A data from controls analyzed during validation on all instruments

OB 5-5-25

Measurement Uncertainty Budget - Cannabinoids LC-MSMS Confirmation

$$u_{comb} = \sqrt{u(r)^2 + u(crm)^2 + u(pip)^2 + u(vol)^2 + u(cal_{sp})^2 + u(ctl_{sp})^2 + u(IS_{sp})^2 + u(samp)^2}$$

$$U = u_{comb} \times k$$

Component	Source Type	Description	Relative MU	Relative MU %	Distribution	k factor	Divisor	Relative Standard Uncertainties %
Reproducibility u(r)	Type A	% RSD for QC level from test batches	see table	see table	Normal	1	1	see table
Working Standard Preparation u(ws)	Type B	Uncertainty in reference value, mL u(crm)	see table	see table	Normal	2	2	see table
		Uncertainty in pipette calibration, µL u(pip)	0.99/1000	0.0990	Normal	2	2	0.0495
		Uncertainty in volumetric flask calibration, mL u(vol)	0.016/50	0.0320	Normal	2	2	0.0160
Calibrator Spike $u(cal_{sp})$	Type B	Uncertainty in pipette calibration, µL	2.0/100	2.0000	Normal	2	2	1.0000
Control Spike $u(ctl_{sp})$	Type B	Uncertainty in pipette calibration, µL	2.0/100	2.0000	Normal	2	2	1.0000
Internal Standard Spike $u(IS_{sp})$	Type B	Max imprecision for piston pipette or repeater, µL	2/100	2.0000	Rectangular	1	√3	1.1547
Test Item Aliquot u(samp)	Type B	Max imprecision for piston pipette, µL	20/1000	2.0000	Rectangular	1	√3	1.1547

Bias u(d)	% Bias for QC level from test batches	see table	Rectangular	1	√3

	^Cerilliant COA MU (mg/mL) k=2	Amount Added µL	Pipette Nominal µL	Pipette MU µL, k=2	WS/QC Pipette Relative MU %	WS/QC Pipette Relative Std Uncert %	Lipomed COA MU (mg/mL) k=2	Combined Std Low MU k=1, %	Combined Std Mid MU k=1, %	Combined Std High MU k=1, %	Combined Std MU Including Bias	Expanded Combined Std MU k=2, %	Expanded Combined Std MU k=3, %
THC	0.018	500	1000	0.99	0.099	0.0495	0.004	8.188	7.956	7.025	n/a	**16.786**	**25.875**
carboxy-THC	0.006	500	1000	0.99	0.099	0.0495	0.007	6.385	7.259	5.321	n/a	**14.881**	**22.939**
THC (k=1)	0.009					COOH (k=1)	0.0035						

From Validation/Reprocessed Ctls

	Low % RSD	Low % Bias	Mid % RSD	Mid % Bias	High % RSD	High % Bias	Std MU Bias u(d)
THC	7.898	-5.017	7.657	-6.510	6.684	-2.610	n/a
carboxy-THC	6.008	-3.146	6.930	-3.208	4.863	-0.117	n/a

largest variation - used in expanded combined MU calc

largest bias > comb std MU - NONE

No change to MU for THC - current is 26%.
Update COOH MU to 23% (currently 21.66%)

AB 1/31/2025

	Vol Flask (mL) 25	Vol Flask (mL) 50
largest MU	0.014	0.016
k factor	2	2

2024 COA Review

	Cerilliant COA MU (mg/mL) k=2	Lipomed COA MU (mg/mL) k=2
THC	0.018	0.004
carboxy-THC	0.006	0.007

n for reproducibility
99, 98, 97

degrees of freedom
98, 97, 96

k value for 95.45%
2.05

k value for 99.73%
3.16

largest MU at k=2 for Rainin or Sartorius pipettes used in prep of WS (from cal certs)

calibrator spike volumes are 10, 20, 25, 50 and 100 µL; largest MU from 100, 200 and 250 µL nominal used (Rainin, Sartorius, Gilson)
control spike volumes are 20, 30 and 80 µL; largest MU from 100, 200 and 250 µL nominal used (Rainin, Sartorius, Gilson)
internal standard spike volume is 100 µL; max imprecision for repeater-pipette used

blank blood, test item aliquot volume is 1000 µL; max imprecision for piston pipette used (Rainin, Sartorius, Gilson)

*(u_{comb}) multiplied by k value; ref. JCGM 100:2008 Table G.2 (pg 78), corresponding to, *p* =% and *v* degrees of freedom

JMF 4-30-2025
OB 5-5-25