

Elements of Measurement Uncertainty

Analysis:	CONFIRMATION OF CANNABINOIDS BY LIQUID CHROMATOGRAPHY – TANDEM MASS SPECTROMETRY
Define the measurand:	The concentration of THC and carboxy-THC in a blood sample
Traceability for the measurement is established through the use of:	<p>Calibrated equipment (pipettes, glassware), with that calibration traceable to SI units of measurement</p> <p>Use of certified reference materials from manufacturers that provide traceability to SI units of measurement</p>
Equipment and Instrumentation Used:	<p>Calibrated, adjustable piston pipettes</p> <p>Volumetric glassware</p> <p>Liquid Chromatograph – Tandem Mass Spectrometer</p>
All uncertainty components considered:	<p>$u(r)$ – experimental uncertainty arising from random effects</p> <p>$u(cal)$ – uncertainty in the preparation of working standard solution used to prepare calibrators</p> <p>$u(ctl)$ – uncertainty in the preparation of working control standard solution used to prepared positive controls</p> <p>$u(IS)$ – uncertainty in the preparation of the stock and/or working internal standard solutions</p> <p>$u(cur)$ – uncertainty arising from use of a linear least-squares regression curve</p> <p>$u(CRM)$ – uncertainty in the concentration of the certified reference material used in preparation of working standard and working control standard solutions</p> <p>$u(Rm)$ – uncertainty associated with the evaluation of systematic error (bias)</p> <p>$u(sp)$ – uncertainty arising from the process of sample preparation</p> <p>$u(calprep)$ – uncertainty arising from the process of spiking calibrators with working standard and prepared dilutions of the working standard</p> <p>$u(ctlprep)$ – uncertainty arising from the process of spiking positive controls with working control standard and prepared dilutions of the working control standard</p> <p>$u(ISprep)$ – uncertainty arising from the addition of internal standard during sample preparation</p> <p>$u(inst)$ – uncertainty associated with the use of the liquid chromatograph - tandem mass spectrometer for sample analysis (instrument precision)</p> <p>$u(environ)$ – uncertainty associated with the impact of environmental factors on the results</p>
All uncertainty components of significance and how they were evaluated:	<p>$u(r)$, $u(cur)$, $u(Ctl)$, $u(Cal)$, $u(CRM)$, $u(SP)$, $u(R_m)$ - see uncertainty budget</p>

Excluded components and reason for exclusion:	<p><i>u(IS)</i> – The deuterated internal standard is added to all tubes in extraction, with quantification of the target compound based on the response ratio of target compound to internal standard. The preparation and use of internal standard can be accounted for in the reproducibility data, as it represents test batches prepared by different analysts and data acquired on different instruments over the lifetime of the assay.</p> <p><i>u(Rm)</i> – Excluded from carboxy-THC only. Specific bias studies were performed for THC at the legal specification limit. Specific bias studies were not performed for carboxy-THC.</p> <p><i>u(calprep)</i> – This is reflected in the reproducibility data, as each test batch includes a set of calibrators spiked by the analyst performing testing. Reproducibility data represents different calibration curves, different analysts, different instruments and difference preparations (lot numbers) of working standard.</p> <p><i>u(ctlprep)</i> – This is reflected in the reproducibility data, which represents test batches extracted by all certified analysts and data acquired on all LC-MSMS instruments approved for use with this assay and includes different preparations (lot numbers) of working control standard.</p> <p><i>u(ISprep)</i> – This is reflected in the reproducibility data, as the same amount of internal standard is added to all members of the test batch (except blank matrices). Quantification of the target compound is based on the response ratios of target compound to internal standard.</p> <p><i>u(inst)</i> – This is reflected in the reproducibility data, as all members of the batch are run on the same instrument.</p> <p><i>u(environ)</i> – This is reflected in the reproducibility data, which represents test batches extracted by all certified analysts and data acquired on all LC-MSMS instruments approved for use, over the lifetime of the assay.</p>
Data used to estimate repeatability and/or reproducibility:	Positive Control (Low, Mid, High) data collected over the lifetime of the method by all certified analysts using data acquired on all LC-MSMS instruments approved for use.
All calculations performed:	See Excel spreadsheets
Combined Standard Uncertainty (THC):	8.65%
Coverage Factor (THC):	k=3
Coverage Probability (THC):	99.70%
Resulting Expanded Uncertainty (THC):	25.96%
Combined Standard Uncertainty (carboxy-THC):	7.22%
Coverage Factor (carboxy-THC):	k=2
Coverage Probability (carboxy-THC):	95.45%
Resulting Expanded Uncertainty (carboxy-THC):	14.45%
Schedule to review and/or recalculate measurement uncertainty:	Reviewed annually. Re-evaluation and recalculation as warranted.