Confirmation and Quantitation of THC and THCA by LC/MS/MS

Miami Valley Regional Crime Laboratory

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Summary of Validation

The toxicology and drug chemistry sections together validated a LC/MS/MS method for quantitating delta-9 tetrahydrocannabinol in plant material. The new method included a liquid extraction procedure of the plant material followed by quantitative analysis by LC/MS/MS. Two compounds were targeted: delta-9 tetrahydrocannabinol (THC) and delta-9 tetrahydrocannabinolic acid (THCA). Calibrators, controls, and interference studies were prepared from NIST-Traceable reference standards with certificates of analysis. The validation plan previously prepared was followed for the validation.

The method was validated using hops matrix-based calibrators and controls, as well as a purchased hemp sample with accompanying certificate of analysis. The drug chemistry work was performed by Jennifer Watson, Hillary Crosley, Meredith Goebel, and Todd Yoak. The toxicology work was performed by Treena Wiebe, Kialee Bowles, Quinton Carter, Elizabeth Kiely, and Brian Simons. All data was reviewed by Matthew Juhascik.

During the validation, it was determined that the plant material from casework had to be homogenized with a metal grinder prior to analysis.

This validation started on September 30, 2019 and ended on October 4, 2019. LC/MS/MS2 and LC/MS/MS3 were used during the method validation and all data was combined. All analysts' analytical balances were also used to weigh out the plant material used during the validation.

The method was determined to be acceptable for the quantitative determination of THC and THCA from plant material.

The validated parameters are shown below:

Parameter	**Acceptance Criteria**	**Results**
Bias (Trueness)	Maximum of +/- 20%	All biases (within and between day) were less than 14%.
Carryover	A negative specimen following the highest calibrator is a true negative	No carryover was seen in negative specimens following the highest calibrator or in specimens following a case with a large amount of drug present.
Interference	No interfering signal from matrix or drugs used in assay	Delta-8 THC, a structural analog of delta-9 THC, was injected each day of the validation and had a retention time greater than 0.1 minutes from delta-9 THC. The following common cannabinoids were also analyzed and found to not interfere with the analysis:

		Cannabinol Cannabichromene Cannabichromenic Acid Cannabidiol Cannabidiolic Acid Cannabidivarin Cannabigerol Cannabigerolic Acid Cannabicyclol Cannabicyclolic Acid Tetrahydrocannabivarin Tetrahydrocannabivarinic Acid No interference from the hops matrix or from actual case samples was observed. Large concentrations of THCA may produce wide peaks; these cases may be diluted and reinjected to obtain sharper peaks.
Limit of Detection/Limit of Quantitation	At least 0.1% by weight	Calibrator level 1 was set at 0.1% and achieved acceptable chromatography and analytical response. 0.1% will be administratively set as the limit of detection and quantitation.
Linearity	N/A	Both THC and THCA were linear between 0.1% and 1%. Weighted quadratic regression (1/x) with the origin ignored was used for both drugs. Acceptable r^2 for all curves (>0.99) was achieved for each run.
Precision	The % coefficient of variation for controls (within and between runs) not to exceed 15%	The % coefficient of variation for all controls (within and between runs) was less than 11%.
Stability on the autosampler	N/A	Samples were stable on the autosampler (< 20% change) for 1 day after the initial extraction.
Recovery	N/A	Recovery from the plant matrix is assumed to be 100%. If the recovery is less than 100%, the calculated quantitative value will be less than the actual quantitative value.
Ruggedness	N/A	Ruggedness was not evaluated as this assay will only be performed at this laboratory's location.
Robustness	N/A	The robustness of the analysis was confirmed by: 1. Using four drug chemists in the preparation of the plant material for validation;

		2. Using five toxicologists in the extraction of the plant material; 3. Using two different LC/MS/MS instruments.
Uncertainty of Measurement	N/A	The UOM for THC and THCA was estimated using the precision of the positive control data, the uncertainty of the reference standards, the precision of the pipets used to prepare the calibrators and control, and the uncertainty of the balances used to weigh out the plant material. THC – 23% THCA – 20%