

**DEPARTMENT OF FORENSIC SCIENCE
METHOD VALIDATION SUMMARY FORM**

Section: Controlled Substances

Method: Addition of delta-7- and cis-delta-9-Tetrahydrocannabinol (THC) Isomers to
Existing Semi-Quantitative Gas Chromatography-Flame Ionization Detection-
Mass Spectrometry (GC-FID/MS) Method

Results recorded?

Procedure documented?

Method fit for use?

Approved by:  Date: 09/26/2024

Executive Validation Summary: “GC-FID Semi-Quantitative Screen for Additional THC Isomers”

Recommended Uses:

This validation indicates that the existing GC-FID semi-quantitative screen method, including the gas chromatography-flame ionization detection/mass spectrometry and gas chromatography-flame ionization detection instruments, are viable options for evaluating the below (-)-trans- Δ^9 -tetrahydrocannabinol (THC) isomers. Two administratively established decision points (1% and 2%) were evaluated for the ratio of THC isomers using testosterone as an internal standard. The validation included the following compounds:

- 9(R)- Δ^7 -Tetrahydrocannabinol
- 9(S)- Δ^7 -Tetrahydrocannabinol
- (\pm)-cis- Δ^9 -Tetrahydrocannabinol

Limitations:

The validation observed co-elution of isomers with (\pm)-cis- Δ^9 -THC with Δ^8 -THC and $\Delta^{(6a,10a)}$ -THC on the DB-35 analytical column. Although co-elution was observed, the method provides an evaluation above or below a total THC administrative threshold which is not impacted by the presence of multiple co-eluting isomers rather has the potential to impact the individual identification of isomers within the method. There was no baseline resolution observed between the two Δ^7 -THC isomers on all columns evaluated.

Information Provided:

Validation Summary: Addition of Δ^7 -Tetrahydrocannabinol (THC) and (\pm)-cis- Δ^9 -THC Isomers to Existing Semi-Quantitative Gas Chromatography-Flame Ionization Detection/Mass Spectrometry Method

Studies Performed:

- Selectivity
- Reproducibility
- Evaluation of Processed Matrices (Matrix Variability)
- Round Robin Study of Isomers
- Ratio Stability Study

RRW
09/26/24
AJR
09/26/2024

Memo To: Robyn Weimer, Chemistry Program Manager
 From: Amanda Pau, Forensic Scientist Senior and Jessica Belton, Forensic Scientist Senior
 CC: Alka Lohmann, Technical Services Director
 Date: June 7, 2024
 RE: Validation Summary: Addition of Δ^7 -Tetrahydrocannabinol (THC) and (\pm)-cis- Δ^9 -THC Isomers to Existing Semi-Quantitative Gas Chromatography-Flame Ionization Detection/Mass Spectrometry Method

Validation Summary - Addition of Δ^7 -Tetrahydrocannabinol (THC) and (\pm)-cis- Δ^9 -THC Isomers to Existing Semi-Quantitative Gas Chromatography-Flame Ionization Detection/Mass Spectrometry (GC-FID/MS) Method

The existing GC-FID/MS and GC-FID semi-quantitative methods previously validated for (-)-trans- Δ^9 -tetrahydrocannabinol (trans- Δ^9 -THC), Δ^8 -THC, $\Delta^{6a,10a}$ -THC and Δ^{10} -THC, were validated to add Δ^7 -THC and (\pm)-cis- Δ^9 -THC. The additional compounds validated are listed in Table 1 with approximate retention times. It shall be noted that the 9(R)- Δ^7 -THC and 9(S)- Δ^7 -THC stereoisomers nearly co-elute on all columns investigated.

Table 1: Target compounds within the analytical method

Compound	Abbreviation	Retention Time (min)		
		DB-35MS	DB-5MS	HP-1MS
(\pm)-cis- Δ^9 -tetrahydrocannabinol	cis- Δ^9 -THC	4.330	3.043	3.626
9(R)- Δ^7 -tetrahydrocannabinol	9(R)- Δ^7 -THC	3.776	2.820	3.465
9(S)- Δ^7 -tetrahydrocannabinol	9(S)- Δ^7 -THC	3.815	2.847	3.490

The matrices evaluated in the validation of these compounds included cannabis plant material and alternative matrices. The alternative matrices included tincture, waxes and electronic cigarette cartridge contents. The validation incorporated the following experiments:

1. Selectivity
2. Reproducibility
3. Evaluation of Processed Matrices (Matrix Variability)
4. Round Robin Study of Isomers
5. Ratio Stability Study
6. References

Plant material samples evaluated during the validation were prepared using the procedure delineated in Section 6.7.2 of the Controlled Substances Procedures Manual (Qualtrax Revision 21). Cannabis extracts and products were prepared using the modification listed in Section 7.2.3.1 of the Controlled Substances Procedures Manual (Qualtrax Revision 21). Both 4-androstene-3,17-dione (androstenedione) and testosterone were evaluated as internal standards during this validation with the exception of the round robin study. This change was prompted when androstenedione was removed as an internal standard option from the Controlled Substances Procedures Manual (Qualtrax Revision 22) while the validation study was in progress. In addition to multiple matrices and internal standards, a 1% and 2% THC threshold was evaluated. Samples were extracted and evaluated with both the dual column GC-FID/MS and the single column GC-FID methods.

1. Selectivity

To evaluate the selectivity of the existing GC-FID/MS and GC-FID methods, the individual target compounds and other related isomers previously validated for these methods were evaluated without the addition of internal standard. Table 2 delineates the retention times of the cannabinoids evaluated.

Table 2: Cannabinoid selectivity evaluation

Compound	Abbreviation	Retention Time (min)		
		DB-35MS	DB-5MS	HP-1MS
(±)-cis- Δ^9 -tetrahydrocannabinol	cis- Δ^9 -THC	4.330	3.043	3.626
(-)-trans- Δ^9 -tetrahydrocannabinol	trans- Δ^9 -THC	4.658	3.327	3.970
(6aR,9R)- Δ^{10} -tetrahydrocannabinol	9(R)- Δ^{10} -THC	4.243	3.153	3.857
(6aR,9S)- Δ^{10} -tetrahydrocannabinol	9(S)- Δ^{10} -THC	4.767	3.465	4.132
9(R)- Δ^7 -tetrahydrocannabinol	9(R)- Δ^7 -THC	3.776	2.820	3.465
9(S)- Δ^7 -tetrahydrocannabinol	9(S)- Δ^7 -THC	3.815	2.847	3.490
$\Delta^{(6a,10a)}$ -tetrahydrocannabinol	$\Delta^{(6a,10a)}$ -THC	4.348	3.251	3.964
Δ^8 -tetrahydrocannabinol	Δ^8 -THC	4.334	3.139	3.802

During the evaluation and verification of another GC-FID/MS instrument, the individual target compounds and related isomers were re-evaluated along with additional commercially available cannabinoid derivatives. Table 2 DB-5MS and HP1-MS retention times are from this new instrument evaluation. Table 3 delineates the retention times of the additional derivatives.

Table 3: Phytocannabinoid selectivity evaluation

Compound	Retention Time (min)	
	DB-5MS	HP-1MS
exo-THC	2.987	3.617
(-)-trans- Δ^9 -THC-O-acetate	2.928	3.645
Δ^9 -tetrahydrocannabivarin (THCV)	2.117	2.642
Δ^8 -THC-O-acetate	2.807	3.521
Δ^9 -tetrahydrocannabihexol (THCH)	4.200	4.602
Δ^9 -tetrahydrocannabiorcol (THCO)	1.492	1.935
Δ^9 -tetrahydrocannabibutol (THCB)	2.647	3.230
Δ^9 -tetrahydrocannabiphorol (THCP)	4.796	5.035

In addition to the individual standards, mixtures of 9(S)- Δ^7 -THC, 9(R)- Δ^7 -THC, cis- Δ^9 -THC, Δ^8 -THC, 9(S)- Δ^{10} -THC and 9(R)- Δ^{10} -THC with either trans- Δ^9 -THC or $\Delta^{(6a,10a)}$ -THC were also evaluated at both a 2% and 1% concentration per standard to evaluate potential isomer co-elution on both methods. Previous validation studies indicated co-elution of Δ^8 -THC and 9(R)- Δ^{10} -THC on a DB-5MS column. Additional co-elutions were noted with trans- Δ^9 -THC and $\Delta^{(6a,10a)}$ -THC on a HP-1MS column, and Δ^8 -THC and $\Delta^{(6a,10a)}$ -THC on a DB-35MS column. Within this validation, it was determined that 9(R)- Δ^7 -THC and 9(S)- Δ^7 -THC co-elute on a HP-1MS column and produce a split peak on a DB-5MS

and a DB-35MS column. It was also determined that cis- Δ^9 -THC co-elutes with Δ^8 -THC and $\Delta^{(6a,10a)}$ -THC on a DB-35MS column.

Evaluation of the cis- Δ^9 -THC isomer indicated a small peak at the trans- Δ^9 -THC retention time. A directly proportional relationship between time and peak abundance was observed at the trans- Δ^9 -THC retention time, indicating isomerization of the standard over time. Additionally, the purity of the solution, received in either methyl acetate or methanol, was $\geq 95\%$. The vendor’s standard mass spectrum also shows a minor second peak present, indicating the possible presence of the trans- Δ^9 -THC in the initial standard.

2. Reproducibility

To establish the reproducibility of the methods, several commercially available infused products were evaluated. Two (2) “dabs” products, four (4) plant material flower products, one (1) tincture and two (2) electronic cigarette cartridges were evaluated alongside one (1) Soxhlet extract (from plant material) and one (1) butane honey oil (BHO) winterization wax material (shatter) obtained from the Eastern Laboratory Controlled Substances Section (EDX). Each sample was extracted and evaluated with twelve (12) replicated injections over a total of four (4) days on each instrumental method.

The ratioed responses of the twelve (12) injections were averaged and utilized to calculate the standard deviation and percent coefficient of variation (%CV) for each isomer, or combination of isomers, observed in each sample evaluated using the HP-1MS column with testosterone as the internal standard. The results are delineated in Table 4. As indicated, the data within the table represents the average instrumental response ratio obtained and does not take into account the tenfold dilution performed prior to analysis. Δ^7 -THC was not detected in any of the samples evaluated.

Table 4: Extracted sample reproducibility using HP-1MS column (testosterone internal standard)

Extracted Sample Reproducibility (HP-1MS)			
Average Ratio \pm Standard Deviation (%CV)			
Item	cis- Δ^9 -THC	trans- Δ^9 -THC	Δ^8 -THC
PharmaCBD Δ^8 -THC Blueberry OG Dabs*	Not Detected (ND)	0.38998 \pm 0.01834 (4.7)	6.70369 \pm 0.36125 (5.4)
3Chi Δ^8 -THC London Pound Cake Dabs Sauce*	ND	0.30902 \pm 0.00527 (1.7)	9.90859 \pm 0.35653 (3.6)
PharmaCBD Δ^8 -THC Infused Bubba Kush Moonrocks	ND	0.37896 \pm 0.00701 (1.9)	2.69799 \pm 0.17751 (6.6)
PharmaCBD Δ^8 -THC Bubble Gum Tincture	ND	ND	2.12804 \pm 0.05497 (2.6)
Δ^8 -THC Infused Sour Space Candy Flower	0.4278 \pm 0.0045 (1.0)	1.71335 \pm 0.03882 (2.3)	13.81667 \pm 0.83009 (6.0)
Δ^8 -THC Infused Blackberry Haze Flower	ND	0.76419 \pm 0.02471 (3.2)	3.04623 \pm 0.16899 (5.5)
Δ^{10} -THC Caviar Flower Mint Cookies	ND	2.08134 \pm 0.10325 (5.0)	8.96511 \pm 0.55710 (6.2)
URB Δ^8 -THC Resin Gremlin Glue Cartridge*	ND	0.38211 \pm 0.01202 (3.1)	9.16677 \pm 0.38401 (4.2)
URB Δ^8 -THC Glue Berry Infinity Cartridge*	ND	0.29900 \pm 0.00571 (1.9)	9.24652 \pm 0.36971 (4.0)
Shatter (BHO winterization wax extract)*	ND	5.18998 \pm 0.24312 (4.7)	ND
Soxhlet Extract (from plant material)*	ND	1.96711 \pm 0.06117 (3.1)	0.27000 \pm 0.00459 (1.7)

*Samples were diluted tenfold prior to analysis based on standard dilution procedure for oils and waxes.

The variability (%CV) was less than or equal to 6.6% for all compounds identified in Table 4. The samples were each compared to a decision point of 1% (ratio of analyte to internal standard when compared to a known 1% standard run the same day on the same method and instrument) for each isomer detected. Reporting guidelines in the Controlled Substances Procedures Manual (Qualtrax Revision 22) state samples with a ratio greater than the known standard for trans- Δ^9 -THC would be reported as “marijuana”. Samples with a ratio less than the known trans- Δ^9 -THC standard would be investigated further for additional THC isomers prior to reporting as either “marijuana” or “cannabis”. If the total THC administrative threshold is reached or exceeded with the assessment of a single or subset of THC isomers (e.g., trans- Δ^9 -THC), there is no need to assess the contribution from all THC isomers that may be present.

All samples containing trans- Δ^9 -THC except for the Blackberry Haze Flower and the Bubba Kush Moonrocks had a ratio greater than the known standard and would therefore be reported as “marijuana”. All samples containing Δ^8 -THC had a ratio greater than the known standard and would also be reported as “marijuana”. The Sour Space Candy Flower was the only sample to contain cis- Δ^9 -THC, producing a ratio less than the known cis- Δ^9 -THC standard. Since the Sour Space Candy Flower also contained both trans- Δ^9 -THC and Δ^8 -THC, the presence of the cis- Δ^9 -THC would be noted, but not considered in the determination of reporting “marijuana”.

Table 5 shows the average ratio, standard deviation and percent coefficient of variation for each isomer observed using the DB-35MS column and testosterone as the internal standard. As indicated, the data within the table represents the average instrumental response ratio obtained and does not take into account the tenfold dilution performed prior to analysis.

Table 5: Extracted sample reproducibility using DB-35MS column (testosterone internal standard)

Extracted Sample Reproducibility (DB-35MS)			
Average Ratio \pm Standard Deviation (%CV)			
Item	cis- Δ^9 -THC/ Δ^8 -THC	Δ^7 -THC	trans- Δ^9 -THC
PharmaCBD Δ^8 -THC Blueberry OG Dabs*	6.76041 \pm 0.24653 (3.6)	ND	0.41788 \pm 0.06708 (16)
3Chi Δ^8 -THC London Pound Cake Dabs Sauce*	9.67788 \pm 0.17136 (1.8)	ND	0.30940 \pm 0.00561 (1.8)
PharmaCBD Δ^8 -THC Infused Bubba Kush Moonrocks	2.70877 \pm 0.18614 (6.9)	ND	0.38978 \pm 0.01156 (3.0)
PharmaCBD Δ^8 -THC Bubble Gum Tincture	2.15255 \pm 0.02552 (1.2)	ND	ND
Δ^8 -THC Infused Sour Space Candy Flower	13.92832 \pm 0.35488 (2.5)	ND	1.20269 \pm 0.03560 (3.0)
Δ^8 -THC Infused Blackberry Haze Flower	3.25238 \pm 0.10277 (3.2)	ND	0.74895 \pm 0.04035 (5.4)
Δ^{10} -THC Caviar Flower Mint Cookies	9.97589 \pm 0.39276 (3.9)	ND	0.79654 \pm 0.00633 (0.79)
URB Δ^8 -THC Resin Gremlin Glue Cartridge*	8.81428 \pm 0.08107 (0.92)	ND	0.36987 \pm 0.00248 (0.67)
URB Δ^8 -THC Glue Berry Infinity Cartridge*	8.92735 \pm 0.10366 (1.2)	ND	0.29221 \pm 0.00388 (1.3)
Shatter (BHO winterization wax extract)*	ND	ND	5.10119 \pm 0.16129 (3.2)
Soxhlet Extract (from plant material)*	2.08993 \pm 0.01949 (0.93)	ND	0.55972 \pm 0.06779 (12)

*Samples were diluted tenfold prior to analysis based on standard dilution procedure for oils and waxes.

For the ratioed responses evaluated on the DB-35MS column, the variability (%CV) was less than or equal to 16% for all compounds identified in Table 5.

For samples containing trans- Δ^9 -THC, all of the extract sample replicates (dabs, dabs sauce, cartridges, shatter and Soxhlet extract) and the Sour Space Candy Flower sample replicates had a ratio greater than the known standard and would be reported as “marijuana”. The Moonrocks, Tincture, Blackberry Haze Flower and Mint Cookies Caviar Flower samples all had trans- Δ^9 -THC ratios less than the known standard and would be investigated further for additional isomers before reporting as either “marijuana” or “cannabis”. All samples containing cis- Δ^9 -THC and/or Δ^8 -THC had a ratio greater than the known standards and would be reported as “marijuana”.

Several of these products were marketed as being infused with Δ^8 -THC. Table 6 describes the concentrations listed in the manufacturer-provided certificates of analysis from a third party testing laboratory for each commercially available product.

Table 6: Reported cannabinoid concentration of commercially available products

Item	Concentration Denoted on Certificate		
	trans- Δ^9 -THC	Δ^8 -THC	Δ^{10} -THC
PharmaCBD Δ^8 -THC Blueberry OG Dabs	Not detected (ND)	84.22 wt%	
3Chi Δ^8 -THC London Pound Cake Dabs Sauce	ND	$\geq 99\%$	
PharmaCBD Δ^8 -THC Infused Bubba Kush Moonrocks	ND	30.3 wt%	
PharmaCBD Δ^8 -THC Bubble Gum Tincture	0.23%	2.48%	
Δ^8 -THC Infused Sour Space Candy Flower	ND	31.1 wt%	
Δ^8 -THC Infused Blackberry Haze Flower	ND	13.44 wt%	
Δ^{10} -THC Caviar Flower Mint Cookies	ND	21.66 wt%	1.16 wt%
URB Δ^8 -THC Resin Gremlin Glue Cartridge	0.264 wt%	96.3 wt%	ND
URB Δ^8 -THC Glue Berry Infinity Cartridge	ND	89.69 wt%	

Considering the tenfold dilution in the majority of the samples, the ratio obtained from the semi-quantitative method is consistent with the concentration delineated on the certificate of analysis. Plant material samples have the largest discrepancy between the semi-quantitative value and the concentration from the certificate of analysis. For example, the certificate of analysis for the Bubba Kush Moonrocks states that the sample contains 30.3 wt% Δ^8 -THC with no detectable trans- Δ^9 -THC. The HP-1MS ratio of the Δ^8 -THC to testosterone was 2.69799 ± 0.17751 , which is approximately 2.698% Δ^8 -THC content without considering the mass of the plant material sampled. The Δ^8 -THC ratio from the DB-35MS column was consistent with that of the HP-1MS (2.70877 ± 0.18614). While the ratios should not typically be used to establish a quantitative concentration, considering the semi-quantitative nature of the method, the ratio for this sample appears to indicate that the concentration is bias by an order of magnitude from the reported concentration on the certificate of analysis.

Several factors may impact the resultant discrepancy for the Moonrocks, including sampling, sample homogeneity and source (lot number). The semi-quantitative method requires a single sample of approximately 50 mg of plant material to be plucked from the bulk of the available material, whereas typical full quantitative procedures require homogenization of a larger bulk of plant material prior to multiple samples being taken for analysis. There may also be a difference in reporting the results of a single sample replicate run and the results of multiple samples being run once each. In addition, the Δ^8 -THC was marketed as being *infused* into the plant material, and not a genetic modification of the plant itself. The infusion process could have caused variability across the sample.

Additionally, most of the samples according to the provided certificates did not have Δ^9 -THC detected and quantitated at the time of third-party testing. However, the presence of Δ^9 -THC was observed, and a ratio calculated for all samples except for Bubble Gum Tincture. It should also be noted that certificates of multiple commercial products state that the values reported relate only to the sample that was tested, which may indicate that the value cannot be applied to all samples within the lot the certificate is being used for. This shows that even with access to ISO accredited testing laboratories and their provided certificates, obtaining an accurate certificate of analysis for cannabinoid containing products may be a limitation when comparing results of internal method development and validation.

In addition to the analysis of commercially available cannabis products, individual 1% and 2% concentration standards of 9(S)- Δ^7 -THC and cis- Δ^9 -THC were prepared in triplicate and evaluated for a total of twelve (12) injections over four (4) days (36 total injections per isomer per percentage) on both instrumental methods. The average ratio, standard deviation and percent coefficient of variation were calculated for each isomer at each percentage. Only the 9(S)- Δ^7 -THC isomer was used in this part of the validation due to the inability to achieve baseline resolution of 9(S)- Δ^7 -THC and 9(R)- Δ^7 -THC on either analytical column.

The previously established acceptance criteria for a 1% trans- Δ^9 -THC threshold control was 0.85316-1.26463 for the HP-1MS column, GC-FID/MS method, and 0.85669-1.33295 for the DB-35MS column, GC-FID method (Controlled Substances Procedures Manual (Qualtrax Revision 21)). The average ratio results for 9(S)- Δ^7 -THC and cis- Δ^9 -THC were compared to these ranges for acceptability of trans- Δ^9 -THC.

Table 7 shows the average results obtained for each isomer at a 1% concentration on the HP-1MS column. Table 8 shows the average results obtained for each isomer at a 1% concentration on the DB-35MS column.

Table 7: 1% isomer evaluation (HP-1MS column)

Individual Isomer Evaluation (HP-1MS)		
Average Ratio \pm Standard Deviation (%CV)		
Compound	Androstenedione	Testosterone
(\pm)-cis- Δ^9 -tetrahydrocannabinol	1.06025 \pm 0.04540 (4.3)	1.07366 \pm 0.04868 (4.5)
9(S)- Δ^7 -tetrahydrocannabinol	1.16508 \pm 0.04916 (4.2)	1.18611 \pm 0.05758 (4.9)

Table 8: 1% isomer evaluation (DB-35MS column)

Individual Isomer Evaluation (DB-35MS)		
Average Ratio \pm Standard Deviation (%CV)		
Compound	Androstenedione	Testosterone
(\pm)-cis- Δ^9 -tetrahydrocannabinol	1.07111 \pm 0.01053 (0.98)	1.08671 \pm 0.01000 (0.92)
9(S)- Δ^7 -tetrahydrocannabinol	1.17040 \pm 0.01668 (1.4)	1.19886 \pm 0.01823 (1.5)

The 1% cis- Δ^9 -THC and 9(S)- Δ^7 -THC isomer solutions were both evaluated with 36 replicate analyses. All individual analyses of the cis- Δ^9 -THC average ratios were within the predetermined acceptance range for trans- Δ^9 -THC. All but five (5) of the individual analyses of the 9(S)- Δ^7 -THC average ratios were also within the predetermined

acceptance range for the trans- Δ^9 -THC. The five replicates that fell outside of this range were all observed on the HP-1MS column and were all biased high, with the largest ratio being 1.28981.

The established acceptance criteria for a 2% trans- Δ^9 -THC solution, obtained from the *Validation Summary: Evaluation of Alternative Cannabis Matrices using Semi-quantitative Gas Chromatography-Flame Ionization Detection/Mass Spectrometry* (page 16; range = average \pm 2 standard deviation units), are 1.52232-2.84182 for the HP-1MS column and 1.67770-2.81008 for the DB-35MS column. The average ratio results for 9(S)- Δ^7 -THC and cis- Δ^9 -THC were compared to these ranges for acceptability of trans- Δ^9 -THC.

Table 9 shows the average results obtained for each isomer at a 2% concentration on the HP-1MS column. Table 10 shows the average results obtained for each isomer at a 2% concentration on the DB-35MS column.

Table 9: 2% isomer evaluation (HP-1MS column)

Individual Isomer Evaluation (HP-1MS)		
Average Ratio \pm Standard Deviation (%CV)		
Compound	Androstenedione	Testosterone
(\pm)-cis- Δ^9 -tetrahydrocannabinol	2.12604 \pm 0.09438 (4.4)	2.15935 \pm 0.09939 (4.6)
9(S)- Δ^7 -tetrahydrocannabinol	2.36503 \pm 0.10483 (4.4)	2.40341 \pm 0.13043 (5.4)

Table 10: 2% isomer evaluation (DB-35MS column)

Individual Isomer Evaluation (DB-35MS)		
Average Ratio \pm Standard Deviation (%CV)		
Compound	Androstenedione	Testosterone
(\pm)-cis- Δ^9 -tetrahydrocannabinol	2.11768 \pm 0.03191 (1.5)	2.15489 \pm 0.03255 (1.5)
9(S)- Δ^7 -tetrahydrocannabinol	2.36404 \pm 0.05405 (2.3)	2.39104 \pm 0.05256 (2.2)

The 2% cis- Δ^9 -THC and 9(S)- Δ^7 -THC isomer solutions were both evaluated with 36 replicate analyses. All individual analyses of all compounds and average ratios with both internal standards were within the predetermined acceptance range for trans- Δ^9 -THC.

3. Evaluation of Process Matrices (Matrix Variability)

Given the limited number of plant material samples and alternative matrices encompassing all compounds within the method, an evaluation of processed matrices was also performed. Two (2) plant material samples, two (2) electronic cigarette cartridges (e-liquids), one (1) shatter sample, and one (1) tincture were extracted and fortified with various concentrations of the target compounds after extraction (post-extraction fortified). For each sample, 0.5 mL of extract was fortified at concentrations representing 0.3%, 1%, 2%, and 5% (0.03 mg/mL, 0.1 mg/mL, 0.2 mg/mL, and 0.5 mg/mL, respectively) of each target compound.

Each matrix sample was extracted and evaluated in triplicate at each concentration. Neat standards were also prepared at the same concentrations to enable a comparison of post-extraction fortified samples and neat

standards. In addition to these samples, a non-fortified sample of each matrix (matrix blank) was extracted to evaluate the naturally occurring isomer concentration(s) in the matrix.

The bias and precision were evaluated for each matrix with each instrumental method. The bias at each concentration for each compound was calculated using Equation 1.

Equation 1

$$\text{Bias (\% Concentration)}_x = \left(\frac{\text{Mean of Calculated Concentration}_x - \text{Expected Concentration}_x}{\text{Expected Concentration}_x} \right) \times 100\%$$

Furthermore, precision was calculated using the percent coefficient of variation (%CV) as shown in Equation 2.

Equation 2

$$\text{Precision (\%CV)} = \left(\frac{\text{Standard Deviation of Mean}}{\text{Calculated Mean}} \right) \times 100\%$$

The predetermined acceptance criterion delineates an appropriate identification conclusion (“marijuana” or “cannabis”) for all samples. Additionally, the variability between the ratioed results for the post-extraction fortified samples shall not exceed a %CV of greater than $\pm 20\%$.

Matrix samples that were not post-extraction fortified were evaluated to determine the presence of isomers (“matrix blanks”). The Blackberry Haze Flower and both cartridges contained cis- Δ^9 -THC and Δ^8 -THC. On the HP-1MS column, these isomers are resolved from one another, however on the DB-35MS column, they co-elute. The Sour Space Candy Flower did not have detectable cis- Δ^9 -THC; however, it did contain Δ^8 -THC. Therefore, a peak was observed at the cis- Δ^9 -THC/ Δ^8 -THC retention time on the DB-35MS column.

The peak area of the matrix blank was subtracted from the instrumental response associated with each post-extraction fortified sample prior to calculating the bias and precision of each sample. The results are delineated in the subsequent instrument specific sections. As 9(S)- Δ^7 -THC and 9(R)- Δ^7 -THC have the same retention time, the 9(R)- Δ^7 -THC isomer was selected for this evaluation.

GC-FID/MS Analysis (HP-1MS Column)

Each compound was evaluated for bias and precision using androstenedione and testosterone as the internal standards. Instrumental analysis was performed on a GC-FID/MS with a HP-1MS column. Table 11 shows the average bias results for 9(R)- Δ^7 -THC using androstenedione.

Table 11: 9(R)- Δ^7 -THC bias with androstenedione

Matrix Evaluation (9(R)- Δ^7 -THC)				
FID (HP-1MS) Average Bias \pm Standard Deviation (%)				
Matrix	0.03 mg/mL spike	0.1 mg/mL spike	0.2 mg/mL spike	0.5 mg/mL spike
Plant Material 1	-19.86 \pm 1.18	-16.11 \pm 3.41	-19.01 \pm 4.09	-16.63 \pm 0.42
Plant Material 2	-12.36 \pm 2.86	-14.07 \pm 0.91	-14.18 \pm 0.60	-11.47 \pm 7.65
e-Liquid 1	-33.30 \pm 0.78	-43.77 \pm 1.36	-48.55 \pm 5.21	-47.39 \pm 1.95
e-Liquid 2	-17.37 \pm 0.21	-32.93 \pm 0.67	-38.63 \pm 1.34	-39.44 \pm 1.09
Shatter	-6.85 \pm 1.03	-11.63 \pm 1.61	-14.58 \pm 1.45	-12.29 \pm 1.25
Tincture	2.77 \pm 1.31	1.32 \pm 0.76	-0.65 \pm 0.95	-2.04 \pm 1.50

The tincture demonstrated the least bias of the matrices evaluated. The e-liquid samples, with the exception of e-liquid 2 at 0.03 mg/mL, had a bias greater than $\pm 20\%$ for all post-extraction fortified samples. Table 12 shows the average bias results for 9(R)- Δ^7 -THC using testosterone.

Table 12: 9(R)- Δ^7 -THC bias with testosterone

Matrix Evaluation (9(R)- Δ^7 -THC)				
FID (HP-1MS) Average Bias \pm Standard Deviation (%)				
Matrix	0.03 mg/mL spike	0.1 mg/mL spike	0.2 mg/mL spike	0.5 mg/mL spike
Plant Material 1	-6.06 \pm 1.30	-2.73 \pm 1.98	-4.13 \pm 2.60	-2.27 \pm 1.00
Plant Material 2	-0.50 \pm 3.15	-2.54 \pm 0.68	-3.13 \pm 0.55	-4.55 \pm 1.09
e-Liquid 1	32.04 \pm 0.93	10.88 \pm 0.82	4.59 \pm 1.02	3.51 \pm 0.27
e-Liquid 2	39.96 \pm 0.54	13.86 \pm 0.33	4.40 \pm 0.75	3.26 \pm 1.08
Shatter	0.72 \pm 2.28	-3.78 \pm 2.49	-3.87 \pm 0.99	-5.25 \pm 3.10
Tincture	3.62 \pm 1.21	1.88 \pm 0.65	-0.05 \pm 0.77	-1.47 \pm 1.21

Using testosterone, only the 0.03 mg/mL post-extraction fortified samples for the e-liquids had a bias greater than $\pm 20\%$ indicating testosterone as a more appropriate internal standard than androstenedione. Table 13 shows the average bias results for cis- Δ^9 -THC using androstenedione.

Table 13: cis- Δ^9 -THC average bias with androstenedione

Matrix Evaluation (cis- Δ^9 -THC)				
FID (HP-1MS) Average Bias \pm Standard Deviation (%)				
Matrix	0.03 mg/mL spike	0.1 mg/mL spike	0.2 mg/mL spike	0.5 mg/mL spike
Plant Material 1	-4.23 \pm 29.98	-15.55 \pm 0.91	-15.66 \pm 1.62	-14.95 \pm 1.32
Plant Material 2	42.55 \pm 5.92	2.87 \pm 1.14	-6.48 \pm 2.11	-8.65 \pm 8.75
e-Liquid 1	-51.79 \pm 2.15	-51.62 \pm 4.52	-52.35 \pm 3.47	-48.03 \pm 2.60
e-Liquid 2	-43.57 \pm 2.21	-43.35 \pm 3.03	-43.17 \pm 0.70	-40.89 \pm 1.87
Shatter	-12.10 \pm 3.31	-12.94 \pm 2.99	-16.69 \pm 1.15	-12.93 \pm 2.03
Tincture	6.97 \pm 1.19	1.13 \pm 2.61	-0.40 \pm 0.21	0.60 \pm 1.42

Plant material 1, shatter, and tincture samples had a bias within $\pm 20\%$ for all concentrations evaluated. Plant material 2 at a concentration of 0.03 mg/mL and both e-liquids at all three concentrations demonstrated a bias greater than $\pm 20\%$. Table 14 shows the average bias results for cis- Δ^9 -THC using testosterone.

Table 14: cis- Δ^9 -THC average bias with testosterone

Matrix Evaluation (cis- Δ^9 -THC)				
FID (HP-1MS) Average Bias \pm Standard Deviation (%)				
Matrix	0.03 mg/mL spike	0.1 mg/mL spike	0.2 mg/mL spike	0.5 mg/mL spike
Plant Material 1	12.99 \pm 29.47	-2.28 \pm 1.39	0.08 \pm 0.12	-0.23 \pm 1.87
Plant Material 2	61.92 \pm 6.52	16.69 \pm 1.67	5.57 \pm 2.08	-1.57 \pm 0.73
e-Liquid 1	-3.63 \pm 5.42	-4.46 \pm 3.27	-1.05 \pm 10.04	2.22 \pm 0.85
e-Liquid 2	-1.39 \pm 3.65	-2.39 \pm 3.79	-2.64 \pm 1.60	1.16 \pm 1.85
Shatter	-4.94 \pm 4.35	-5.20 \pm 3.78	-6.24 \pm 1.20	-5.94 \pm 3.87
Tincture	7.86 \pm 1.40	1.69 \pm 2.80	0.20 \pm 0.19	1.18 \pm 1.15

Plant material 1, e-liquid 1, e-liquid 2, shatter, and tincture samples were within $\pm 20\%$ bias for all concentrations evaluated. Plant material 2 at a concentration of 0.03 mg/mL had a bias greater than $\pm 20\%$ (61.92% \pm 6.52%). Regardless of which internal standard was used, plant material 1 had large standard deviation at the 0.3 mg/mL post-extraction fortified concentration. This could be the result of the low volume of the standard spike used. Therefore, larger deviations are more likely to be observed across replicate samples, especially when only three samples are used for the calculation.

Additionally, each ratio replicate was compared to a ratio of known 1% and 2% standards. When comparing samples to the known 1% standard ratio, the 0.03 mg/mL samples were expected to produce a ratio less than the standard ratio producing a result of "cannabis". The 0.2 mg/mL and 0.5 mg/mL samples were expected to produce a ratio greater than the standard ratio to produce a result of "marijuana". When comparing the known 2% standard ratio to the samples, the 0.03 mg/mL and 0.1 mg/mL samples were expected to produce a ratio less than the standard ratio producing a result of "cannabis". The 0.5 mg/mL samples were expected produce a ratio greater than the standard ratio producing a result of "marijuana". All sample replicates evaluated, and concentrations produced the expected results for both 9(R)- Δ^7 -THC and cis- Δ^9 -THC isomers.

Precision was evaluated alongside bias for 9(R)- Δ^7 -THC and cis- Δ^9 -THC. As 9(S)- Δ^7 -THC and 9(R)- Δ^7 -THC have the same retention time, the 9(R)- Δ^7 -THC isomer was selected for this evaluation. The average ratio, standard deviation, and %CV for each concentration were tabulated for both compounds with each internal standard. Table 15 and Table 16 represent the precision for 9(R)- Δ^7 -THC using androstenedione and testosterone as internal standards, respectively.

Table 15: 9(R)- Δ^7 -THC precision with androstenedione

Matrix Evaluation (9(R)- Δ^7 -THC)				
FID (HP-1MS) Average Ratio \pm Standard Deviation (%CV)				
Matrix	0.3% Ratio	1.0% Ratio	2.0% Ratio	5.0% Ratio
Plant Material 1	0.27884 \pm 0.00330 (1.2)	0.96760 \pm 0.03297 (3.4)	1.78391 \pm 0.07292 (4.1)	4.61565 \pm 0.01953 (0.42)
Plant Material 2	0.30492 \pm 0.00873 (2.9)	0.99108 \pm 0.00903 (0.91)	1.89024 \pm 0.01137 (0.60)	4.90162 \pm 0.37514 (7.7)
e-Liquid 1	0.31445 \pm 0.00245 (0.78)	0.84717 \pm 0.01150 (1.4)	1.51333 \pm 0.07883 (5.2)	3.87588 \pm 0.07549 (1.9)
e-Liquid 2	0.38958 \pm 0.00082 (0.21)	1.01060 \pm 0.00675 (0.67)	1.80526 \pm 0.02412 (1.3)	4.46189 \pm 0.04874 (1.1)
Shatter	0.41523 \pm 0.00428 (1.0)	1.29009 \pm 0.02072 (1.6)	2.39423 \pm 0.03483 (1.5)	6.20742 \pm 0.07729 (1.2)
Tincture	0.45812 \pm 0.00599 (1.3)	1.47915 \pm 0.01119 (0.76)	2.78458 \pm 0.02641 (0.95)	6.93332 \pm 0.10398 (1.5)

Table 16: 9(R)- Δ^7 -THC precision with testosterone

Matrix Evaluation (9(R)- Δ^7 -THC)				
FID (HP-1MS) Average Ratio \pm Standard Deviation (%CV)				
Matrix	0.3% Ratio	1.0% Ratio	2.0% Ratio	5.0% Ratio
Plant Material 1	0.31736 \pm 0.00412 (1.3)	1.08550 \pm 0.02154 (2.0)	2.04244 \pm 0.05300 (2.6)	5.27089 \pm 0.05289 (1.0)
Plant Material 2	0.33614 \pm 0.01060 (3.2)	1.08757 \pm 0.00744 (0.68)	2.06369 \pm 0.01135 (0.55)	5.14788 \pm 0.05630 (1.1)
e-Liquid 1	0.50107 \pm 0.00468 (0.93)	1.34819 \pm 0.01102 (0.82)	2.47533 \pm 0.02531 (1.0)	6.15557 \pm 0.01682 (0.27)
e-Liquid 2	0.53115 \pm 0.00286 (0.54)	1.38440 \pm 0.00453 (0.33)	2.47089 \pm 0.01864 (0.75)	6.14063 \pm 0.06645 (1.1)
Shatter	0.38747 \pm 0.00885 (2.3)	1.21346 \pm 0.03027 (2.5)	2.32989 \pm 0.02299 (1.0)	5.83064 \pm 0.18085 (3.1)
Tincture	0.39865 \pm 0.00481 (1.2)	1.28474 \pm 0.00839 (0.65)	2.42256 \pm 0.01869 (0.77)	6.06332 \pm 0.07318 (1.2)

The precision when applying either internal standard to 9(R)- Δ^7 -THC was within the predetermined acceptance criteria of $\pm 20\%$ CV. The largest variability observed was plant material 2 sample at the 5.0% ratio (7.7%) using androstenedione as the internal standard. Table 17 and Table 18 represent the precision results for cis- Δ^9 -THC using androstenedione and testosterone as internal standards, respectively.

Table 17: cis- Δ^9 -THC precision with androstenedione

Matrix Evaluation (cis- Δ^9 -THC)				
FID (HP-1MS) Average Ratio \pm Standard Deviation (%CV)				
Matrix	0.3% Ratio	1.0% Ratio	2.0% Ratio	5.0% Ratio
Plant Material 1	0.30660 \pm 0.09191 (30)	0.92169 \pm 0.00834 (0.91)	1.80470 \pm 0.02925 (1.6)	4.63272 \pm 0.06098 (1.3)
Plant Material 2	0.45639 \pm 0.02700 (5.9)	1.12277 \pm 0.01279 (1.1)	2.00119 \pm 0.04215 (2.1)	4.97611 \pm 0.43530 (8.7)
e-Liquid 1	0.21770 \pm 0.00468 (2.2)	0.71316 \pm 0.03226 (4.5)	1.37781 \pm 0.04778 (3.5)	3.77839 \pm 0.09818 (2.6)
e-Liquid 2	0.25486 \pm 0.00562 (2.2)	0.83506 \pm 0.02531 (3.0)	1.64306 \pm 0.01153 (0.70)	4.29774 \pm 0.08056 (1.9)
Shatter	0.37063 \pm 0.01228 (3.3)	1.23065 \pm 0.03678 (3.0)	2.29686 \pm 0.02645 (1.2)	6.04662 \pm 0.12250 (2.0)
Tincture	0.45101 \pm 0.00536 (1.2)	1.42962 \pm 0.03730 (2.6)	2.74582 \pm 0.00570 (0.21)	6.98647 \pm 0.09946 (1.4)

Table 18: cis- Δ^9 -THC precision with testosterone

Matrix Evaluation (cis- Δ^9 -THC)				
FID (HP-1MS) Average Ratio \pm Standard Deviation (%CV)				
Matrix	0.3% Ratio	1.0% Ratio	2.0% Ratio	5.0% Ratio
Plant Material 1	0.35117 \pm 0.10349 (29)	1.03186 \pm 0.01436 (1.4)	2.07123 \pm 0.00240 (0.12)	5.29398 \pm 0.09897 (1.9)
Plant Material 2	0.50321 \pm 0.03279 (6.5)	1.23212 \pm 0.02052 (1.7)	2.18481 \pm 0.04547 (2.1)	5.22303 \pm 0.03827 (0.73)
e-Liquid 1	0.35035 \pm 0.01898 (5.4)	1.13640 \pm 0.03719 (3.3)	2.30210 \pm 0.23115 (10)	5.99935 \pm 0.05128 (0.85)
e-Liquid 2	0.35847 \pm 0.01309 (3.7)	1.16100 \pm 0.04402 (3.8)	2.26503 \pm 0.03613 (1.6)	5.93755 \pm 0.10994 (1.9)
Shatter	0.34589 \pm 0.01503 (4.3)	1.15762 \pm 0.04376 (3.8)	2.23523 \pm 0.02684 (1.2)	5.68011 \pm 0.21982 (3.9)
Tincture	0.39247 \pm 0.00551 (1.4)	1.24177 \pm 0.03475 (2.8)	2.38886 \pm 0.00446 (0.19)	6.10982 \pm 0.07036 (1.2)

The precision when applying either internal standard to cis- Δ^9 -THC was within the predetermined acceptance criteria of $\pm 20\%$ CV, except for two samples as highlighted above. Plant material 1 at the 0.3% ratio for both internal standards failed this criterion as the variability observed was 30% and 29%, for androstenedione and testosterone, respectively. As with the bias, this failure is likely due to the low volume standard spike used for these samples.

GC-FID Analysis (DB-35MS Column)

Each compound was evaluated for bias and precision using androstenedione and testosterone as the internal standards. Instrumental analysis was performed on a GC-FID using a DB-35MS (or equivalent) column. Table 19 shows the average bias results for 9(R)- Δ^7 -THC using androstenedione.

Table 19: 9(R)- Δ^7 -THC bias with androstenedione

Matrix Evaluation (9(R)- Δ^7 -THC)				
FID (DB-35MS) Average Bias \pm Standard Deviation (%)				
Matrix	0.03 mg/mL spike	0.1 mg/mL spike	0.2 mg/mL spike	0.5 mg/mL spike
Plant Material 1	16.99 \pm 7.62	-0.16 \pm 1.10	-4.89 \pm 1.88	-6.10 \pm 1.29
Plant Material 2	-7.78 \pm 2.40	-7.78 \pm 0.42	-8.15 \pm 0.28	-9.10 \pm 1.47
e-Liquid 1	-5.96 \pm 0.98	-1.30 \pm 0.95	-2.58 \pm 0.71	-0.08 \pm 0.58
e-Liquid 2	-2.44 \pm 0.48	-1.00 \pm 0.65	-4.37 \pm 1.08	-1.82 \pm 1.10
Shatter	-3.85 \pm 4.91	-1.90 \pm 0.52	-6.17 \pm 7.79	1.67 \pm 4.47
Tincture	76.86 \pm 11.29	25.36 \pm 0.92	14.04 \pm 0.37	5.98 \pm 0.83

All matrices had a bias within $\pm 20\%$ with the exception of the tincture post-extraction fortified samples at 0.03 mg/mL and 0.1 mg/mL. Table 20 shows the average bias results for 9(R)- Δ^7 -THC using testosterone.

Table 20: 9(R)- Δ^7 -THC bias with testosterone

Matrix Evaluation (9(R)- Δ^7 -THC)				
FID (DB-35MS) Average Bias \pm Standard Deviation (%)				
Matrix	0.03 mg/mL spike	0.1 mg/mL spike	0.2 mg/mL spike	0.5 mg/mL spike
Plant Material 1	18.25 \pm 7.74	1.35 \pm 1.28	-3.39 \pm 1.73	-3.12 \pm 1.18
Plant Material 2	-6.21 \pm 2.44	-5.35 \pm 0.52	-5.56 \pm 0.15	-5.40 \pm 1.55
e-Liquid 1	-7.99 \pm 0.97	-2.89 \pm 0.80	-3.65 \pm 0.70	-0.59 \pm 0.40
e-Liquid 2	-4.91 \pm 0.56	-2.83 \pm 0.42	-5.60 \pm 0.92	-2.68 \pm 1.22
Shatter	-1.88 \pm 6.14	-1.34 \pm 1.00	-0.56 \pm 5.57	-1.17 \pm 10.46
Tincture	75.12 \pm 11.02	23.74 \pm 0.88	12.77 \pm 0.18	5.12 \pm 0.61

All of the samples evaluated demonstrated similar results using testosterone as an internal standard as they did with the androstenedione. Table 21 shows the average bias results for cis- Δ^9 -THC using androstenedione.

Table 21: cis- Δ^9 -THC average bias with androstenedione

Matrix Evaluation (cis- Δ^9 -THC)				
FID (DB-35MS) Average Bias \pm Standard Deviation (%)				
Matrix	0.03 mg/mL spike	0.1 mg/mL spike	0.2 mg/mL spike	0.5 mg/mL spike
Plant Material 1	91.42 \pm 88.61	-51.55 \pm 78.69	-22.91 \pm 17.25	-13.68 \pm 12.92
Plant Material 2	68.71 \pm 97.97	134.04 \pm 51.00	-10.78 \pm 15.28	10.45 \pm 19.57
e-Liquid 1	287.50 \pm 84.49	19.83 \pm 33.14	214.82 \pm 154.23	-17.99 \pm 42.56
e-Liquid 2	73.38 \pm 80.85	6.52 \pm 124.74	-2.71 \pm 48.68	-4.05 \pm 7.83
Shatter	-0.70 \pm 2.72	-0.43 \pm 1.17	-6.45 \pm 8.03	0.93 \pm 4.36
Tincture	631.50 \pm 2.55	192.66 \pm 0.76	98.04 \pm 0.38	41.00 \pm 1.00

Only the shatter samples demonstrated a bias within $\pm 20\%$ for all post-extraction fortified concentrations. Plant material 1 had a bias within $\pm 20\%$ at the 0.5 mg/mL concentration. Plant material 2 was within $\pm 20\%$ bias for the 0.2 mg/mL and 0.5 mg/mL post-extraction fortified concentrations. Further, e-liquid 1 was within $\pm 20\%$ bias for the 0.1 mg/mL and 0.5 mg/mL post-extraction fortified concentrations. E-Liquid 2 had post-extraction fortified samples within $\pm 20\%$ for all concentrations with the exception of the 0.03 mg/mL concentration while the tincture had no post-extraction fortified sample concentrations within $\pm 20\%$ bias. Table 22 shows the average bias results for cis- Δ^9 -THC using testosterone.

Table 22: cis- Δ^9 -THC average bias with testosterone

Matrix Evaluation (cis- Δ^9 -THC)				
FID (DB-35MS) Average Bias \pm Standard Deviation (%)				
Matrix	0.03 mg/mL spike	0.1 mg/mL spike	0.2 mg/mL spike	0.5 mg/mL spike
Plant Material 1	94.75 \pm 88.30	-52.13 \pm 80.31	-22.72 \pm 18.02	-11.03 \pm 12.76
Plant Material 2	72.77 \pm 98.50	141.18 \pm 50.52	-7.95 \pm 14.83	15.17 \pm 19.64
e-Liquid 1	211.46 \pm 103.34	11.46 \pm 4.25	196.23 \pm 161.20	-26.19 \pm 50.51
e-Liquid 2	85.00 \pm 72.48	3.49 \pm 110.83	-5.14 \pm 57.03	-8.66 \pm 6.14
Shatter	1.31 \pm 3.52	0.15 \pm 2.39	-0.82 \pm 6.90	-1.86 \pm 10.74
Tincture	624.51 \pm 2.56	188.88 \pm 0.74	95.85 \pm 0.19	39.86 \pm 0.88

As with the androstenedione internal standard, only the shatter samples reliably demonstrated a bias within $\pm 20\%$. Of the remaining samples, e-liquid 1 (0.1 mg/mL) and e-liquid 2 (0.5 mg/mL) were the only samples that had a bias within $\pm 20\%$.

Each ratio replicate was compared to a ratio of known 1% and 2% standards. When comparing samples to the known 1% standard ratio, the 0.03 mg/mL samples were expected to produce a ratio less than the standard ratio producing a result of "cannabis". The 0.2 mg/mL and 0.5 mg/mL samples were expected to produce a ratio greater than the standard ratio to produce a result of "marijuana". When comparing the known 2% standard ratio to the samples, the 0.03 mg/mL and 0.1 mg/mL samples were expected to produce a ratio less than the standard ratio producing a result of "cannabis". The 0.5 mg/mL samples were expected produce a ratio greater than the standard ratio producing a result of "marijuana".

When compared to the 1% known standard, all sample replicates produced the appropriate results for 9(R)- Δ^7 -THC with both internal standards. The shatter sample replicates also produced appropriate results for cis- Δ^9 -THC with both internal standards. However, the presence of Δ^8 -THC and its resulting co-elution with cis- Δ^9 -THC on the DB-35MS column appears to cause data skewing, which affected the results for cis- Δ^9 -THC. The plant material 1 sample replicates produced appropriate results with the exception of the 0.3% ratio (0.03 mg/mL) samples with both internal standards. Some of the plant material 2 (2%, 5%), e-liquid 2 (0.3%, 5%) and tincture (2%, 5%) sample replicates produced appropriate results. Only the 5% samples for e-liquid 1 produced appropriate results with both internal standards. When compared to the 2% known standard, all sample replicates produced the appropriate results for 9(R)- Δ^7 -THC with both internal standards. The shatter sample replicates also produced appropriate results for cis- Δ^9 -THC with both internal standards. Some plant material 2 (0.3%, 5%), e-liquid 2 (0.3%, 5%) and tincture (5%) sample replicates produced appropriate results. Only the 1% samples for e-liquid 1 produced appropriate results with androstenedione as the internal standard. None of the e-liquid 1 sample replicates produced appropriate results with testosterone as the internal standard.

Precision was evaluated alongside bias for 9(R)- Δ^7 -THC and cis- Δ^9 -THC. The average ratio, standard deviation, and %CV for each concentration were tabulated for both compounds with each internal standard. Table 23 and Table 24 represent the precision for 9(R)- Δ^7 -THC with androstenedione and testosterone, respectively.

Table 23: 9(R)- Δ^7 -THC precision with androstenedione

Matrix Evaluation (9(R)- Δ^7 -THC)				
FID (DB-35MS) Average Ratio \pm Standard Deviation (%CV)				
Matrix	0.3% Ratio	1.0% Ratio	2.0% Ratio	5.0% Ratio
Plant Material 1	0.42125 \pm 0.03210 (7.6)	1.15781 \pm 0.01269 (1.1)	2.11438 \pm 0.03984 (1.9)	5.27975 \pm 0.06789 (1.3)
Plant Material 2	0.33206 \pm 0.00796 (2.4)	1.06940 \pm 0.00446 (0.42)	2.04204 \pm 0.00580 (0.28)	5.11116 \pm 0.07523 (1.5)
e-Liquid 1	0.46034 \pm 0.00453 (1.0)	1.53674 \pm 0.01456 (0.95)	2.96965 \pm 0.02112 (0.71)	7.66102 \pm 0.04446 (0.58)
e-Liquid 2	0.47758 \pm 0.00227 (0.48)	1.54141 \pm 0.01006 (0.65)	2.91505 \pm 0.03150 (1.1)	7.52813 \pm 0.08276 (1.1)
Shatter	0.47528 \pm 0.02333 (4.9)	1.56526 \pm 0.00807 (0.52)	2.88172 \pm 0.22450 (7.8)	7.86672 \pm 0.35147 (4.5)
Tincture	0.87429 \pm 0.9875 (11)	2.00022 \pm 0.01834 (0.92)	3.50228 \pm 0.01307 (0.37)	8.19982 \pm 0.06829 (0.83)

Table 24: 9(R)- Δ^7 -THC precision with testosterone

Matrix Evaluation (9(R)- Δ^7 -THC)				
FID (DB-35MS) Average Ratio \pm Standard Deviation (%CV)				
Matrix	0.3% Ratio	1.0% Ratio	2.0% Ratio	5.0% Ratio
Plant Material 1	0.42427 \pm 0.03285 (7.7)	1.16135 \pm 0.01482 (1.3)	2.11587 \pm 0.03663 (1.7)	5.30977 \pm 0.06246 (1.2)
Plant Material 2	0.33649 \pm 0.00821 (2.4)	1.08463 \pm 0.00569 (0.52)	2.06839 \pm 0.00307 (0.15)	5.18436 \pm 0.08039 (1.6)
e-Liquid 1	0.36807 \pm 0.00357 (1.0)	1.22720 \pm 0.00985 (0.80)	2.36991 \pm 0.01664 (0.70)	6.11222 \pm 0.02435 (0.40)
e-Liquid 2	0.38038 \pm 0.00212 (0.56)	1.22792 \pm 0.00520 (0.42)	2.32174 \pm 0.02129 (0.92)	5.98364 \pm 0.07317 (1.2)
Shatter	0.39938 \pm 0.02451 (6.1)	1.29372 \pm 0.01300 (1.0)	2.49758 \pm 0.13921 (5.6)	6.22416 \pm 0.65129 (10.5)
Tincture	0.71279 \pm 0.07852 (11)	1.62262 \pm 0.01422 (0.88)	2.83263 \pm 0.00524 (0.18)	6.61995 \pm 0.04051 (0.61)

The precision when applying either internal standard to 9(R)- Δ^7 -THC was within the predetermined acceptance criteria of $\pm 20\%$ CV. The largest variability observed (11%) was in the tincture sample at the 0.3% ratio, regardless of which internal standard was used. Tables 25 and 26 list the precision results for cis- Δ^9 -THC for androstenedione and testosterone, respectively.

Table 25: cis- Δ^9 -THC precision with androstenedione

Matrix Evaluation (cis- Δ^9 -THC)				
FID (DB-35MS) Average Ratio \pm Standard Deviation (%CV)				
Matrix	0.3% Ratio	1.0% Ratio	2.0% Ratio	5.0% Ratio
Plant Material 1	0.64086 \pm 0.56785 (89)	0.53501 \pm 0.42100 (79)	1.66853 \pm 0.28781 (17)	4.80591 \pm 0.62111 (13)
Plant Material 2	0.56481 \pm 0.55335 (98)	2.58459 \pm 1.31823 (51)	1.93109 \pm 0.29507 (15)	6.14973 \pm 1.20337 (20)
e-Liquid 1	1.82495 \pm 1.54198 (84)	1.83225 \pm 0.60727 (33)	9.42178 \pm 14.53085 (154)	6.21229 \pm 2.64401 (43)
e-Liquid 2	0.81655 \pm 0.66019 (81)	1.62885 \pm 2.03176 (125)	2.91171 \pm 1.41734 (49)	7.26810 \pm 0.56920 (7.8)
Shatter	0.47741 \pm 0.01301 (2.7)	1.55547 \pm 0.01812 (1.2)	2.83739 \pm 0.22774 (8.0)	7.71809 \pm 0.33637 (4.4)
Tincture	3.51693 \pm 0.08967 (2.5)	4.57180 \pm 0.03481 (0.76)	6.00650 \pm 0.02301 (0.38)	10.78232 \pm 0.10773 (1.0)

Table 26: cis- Δ^9 -THC precision with testosterone

Matrix Evaluation (cis- Δ^9 -THC)				
FID (DB-35MS) Average Ratio \pm Standard Deviation (%CV)				
Matrix	0.3% Ratio	1.0% Ratio	2.0% Ratio	5.0% Ratio
Plant Material 1	0.64939 \pm 0.57339 (88)	0.52232 \pm 0.57339 (80)	1.64751 \pm 0.29687 (18)	4.82832 \pm 0.61623 (13)
Plant Material 2	0.57612 \pm 0.56747 (98)	2.63137 \pm 1.32943 (51)	1.96250 \pm 0.29106 (15)	6.24964 \pm 1.22742 (20)
e-Liquid 1	1.19827 \pm 1.23825 (103)	1.38313 \pm 0.05881 (4.3)	7.15296 \pm 11.53026 (161)	4.48336 \pm 2.26458 (51)
e-Liquid 2	0.71175 \pm 0.51590 (72)	1.28426 \pm 1.42336 (111)	2.29051 \pm 1.30617 (57)	5.54806 \pm 0.34071 (6.1)
Shatter	0.40105 \pm 0.01412 (3.5)	1.28579 \pm 0.03071 (2.4)	2.46027 \pm 0.16979 (6.9)	6.10812 \pm 0.65576 (10.7)
Tincture	2.86793 \pm 0.07348 (2.6)	3.70873 \pm 0.02733 (0.74)	4.85803 \pm 0.00909 (0.19)	8.70491 \pm 0.07681 (0.88)

The average ratio for cis- Δ^9 -THC was inflated compared to the expected result based on the post-extraction fortified concentration for the analyte. This is possibly due to the co-elution between cis- Δ^9 -THC and Δ^8 -THC on the DB-35MS column. In addition, neither e-liquid sample was diluted 10:1 prior to spiking. Therefore, any responses would be minimal when compared to the Δ^8 -THC response. The shatter was diluted 10:1 and results were observed as expected.

4. Round Robin Study

A round robin study was performed to include all four laboratories. A total of 18 samples were prepared at one location, dispersed, and analyzed by each laboratory. The samples included THC isomer standards, THC isomer standard mixtures, and matrix sample extracts. They were accompanied with instructions regarding the number of injections and the number of days over which the analysis was to be conducted. Table 27 lists the sample number, sample contents, and instrumental injection parameters for each sample.

Table 27: Round robin sample preparations

Sample	Sample Name	Instrumental Injections
1	(-)-trans- Δ^9 -tetrahydrocannabinol	Inject 1x
2	Δ^8 -tetrahydrocannabinol	Inject 1x
3	$\Delta^{(6a,10a)}$ -tetrahydrocannabinol	Inject 1x
4	(6aR,9R)- Δ^{10} -tetrahydrocannabinol	Inject 1x
5	(6aR,9S)- Δ^{10} -tetrahydrocannabinol	Inject 1x
6	(\pm)-cis- Δ^9 -tetrahydrocannabinol	Inject 1x
7	9(R)- Δ^7 -tetrahydrocannabinol	Inject 1x
8	9(S)- Δ^7 -tetrahydrocannabinol	Inject 1x
9	Testosterone	Inject 1x
10	9(S)- Δ^7 -THC/9(R)- Δ^7 -THC mix	Inject 1x
11	cis- Δ^9 -THC/ $\Delta^{(6a,10a)}$ -THC/ Δ^8 -THC mix	Inject 1x
12	trans- Δ^9 -THC/ Δ^8 -THC 2% mix	10 injections over 4 days
13	cis- Δ^9 -THC/9(R)- Δ^7 -THC 2% mix	10 injections over 4 days
14	trans- Δ^9 -THC/ Δ^8 -THC 1% mix	10 injections over 4 days
15	cis- Δ^9 -THC/9(R)- Δ^7 -THC 1% mix	10 injections over 4 days
16	Δ^8 -THC Infused Sour Space Candy Flower	10 injections over 4 days
17	3Chi Δ^8 -THC London Pound Cake Dabs Sauce*	10 injections over 4 days
18	PharmaCBD Δ^8 -THC Blueberry OG Dabs*	10 injections over 4 days

In addition to the above listed samples, the preparation and analysis of 2% and 1% THC standard mixtures was required from each laboratory. The preparation of these standards is shown in Table 28.

Table 28: Independent laboratory sample preparations

Sample	Compounds Included	Sample Preparation
2%	trans- Δ^9 -THC; Δ^8 -THC	Spike 100 μ L of each 1.0 mg/mL (10 μ L of 10 mg/mL) standard; dry down and reconstitute with 0.5 mL provided internal standard
1%	trans- Δ^9 -THC; Δ^8 -THC	Spike 100 μ L of each 1.0 mg/mL (10 μ L of 10 mg/mL) standard; dry down and reconstitute with 0.5 mL provided internal standard
2%	cis- Δ^9 -THC; 9(R)- Δ^7 -THC	Spike 100 μ L of each 1.0 mg/mL (10 μ L of 10 mg/mL) standard; dry down and reconstitute with 0.5 mL provided internal standard
1%	cis- Δ^9 -THC; 9(R)- Δ^7 -THC	Spike 100 μ L of each 1.0 mg/mL (10 μ L of 10 mg/mL) standard; dry down and reconstitute with 0.5 mL provided internal standard

The testosterone internal standard was prepared by one laboratory and shipped to each laboratory with the samples listed in Table 27. The internal standard was thus the same lot number for each laboratory. The minimum expectation was that each laboratory would analyze each sample one (1) time each day for a total of four (4) days. Only testosterone was used as an internal standard during the round robin portion of the study due to procedural changes at the laboratory. As 9(S)- Δ^7 -THC and 9(R)- Δ^7 -THC have the same retention time, the 9(R)- Δ^7 -THC isomer was selected for this evaluation.

The data obtained from the pre-prepared standards, standard mixtures and sample extracts, and the individually prepared standard mixtures were combined and evaluated to assess the overall variation of each target compound within the method. This evaluation was performed for both instrumental methods. The average ratio, standard deviation, percent coefficient of variation (%CV) and number of injections was compiled for each isomer. Table 29 shows the combined results obtained for the 1% and 2% standard data obtained from the GC-FID/MS method using a HP-1MS column or equivalent.

Table 29: GC-FID/MS round robin combined 1% and 2% data with Testosterone

GC-FID/MS Data (HP-1MS or equivalent)				
Data Summary	1% Standard with Testosterone		2% Standard with Testosterone	
	cis- Δ^9 -THC	9(R)- Δ^7 -THC	cis- Δ^9 -THC	9(R)- Δ^7 -THC
Average \pm Std Dev	1.05416 \pm 0.05676	1.14348 \pm 0.06814	2.09775 \pm 0.11285	2.23120 \pm 0.12226
%CV	5.4%	6.0%	5.4%	5.5%
Count (n)	64	64	64	64

All compounds had a %CV of 6.0% or less for both the 1% and 2% standards. The largest variability (%CV) observed was at 6.0%, associated with 9(R)- Δ^7 -THC in the 1% data.

The same samples were evaluated using the GC-FID method that employed a DB-35MS or equivalent column. The average ratio, standard deviation, percent coefficient of variation (%CV) and number of injections was compiled for each isomer. Tables 30 shows the combined results obtained for the 1% and 2% standard data obtained from the GC-FID method using a DB-35MS column or equivalent.

Table 30: GC-FID round robin combined 1% and 2% data with Testosterone

GC-FID Data (DB-35MS or equivalent)				
Data Summary	1% Standard with Testosterone		2% Standard with Testosterone	
	cis- Δ^9 -THC	9(R)- Δ^7 -THC	cis- Δ^9 -THC	9(R)- Δ^7 -THC
Average \pm Std Dev	1.04137 \pm 0.05329	1.10185 \pm 0.07248	2.05145 \pm 0.08813	2.14635 \pm 0.10636
%CV	5.1%	6.6%	4.3%	5.0%
Count (n)	64	64	64	64

All compounds had a %CV of 6.6% or less with both the 1% and 2% standards. The largest variability (%CV) observed was 6.6%, associated with 9(R)- Δ^7 -THC in the 1% data. The DB-35MS GC-FID method at a 2% concentration had the lowest observed variability across all samples and data.

5. Ratio Stability Study

The variability of the ratio of target compounds to internal standard in a 1% and 2% standard solution mixture was evaluated. Each compound was assessed in a mixture containing both cis- Δ^9 -THC and 9(S)- Δ^7 -THC to determine the ratio stability.

Samples at a 1% and 2% concentration were evaluated with a total of twelve (12) injections over four (4) days using both the GC-FID/MS and GC-FID methods. The data was evaluated using both androstenedione and testosterone as internal standards. The average ratio, standard deviation, percent coefficient of variation (%CV) and number of injections was compiled for each isomer. These were then compared to the previously established acceptance criteria for trans- Δ^9 -THC. As previously listed on page 6, these criteria for the 1% standard with testosterone as an internal standard are 0.85316-1.26463 on the HP-1MS column and 0.85669-1.33295 on the DB-35MS column. As previously listed on page 7, the criteria for the 2% standard with testosterone as an internal standard are 1.52232-2.84182 for the HP-1MS column and 1.67770-2.81008 for the DB-35MS column. When using androstenedione as an internal standard on the HP-1MS column, the 1% range is 0.77073-1.25664 (Controlled Substances Procedures Manual (Qualtrax Revision 21)) and the 2% range is 1.43604-2.70200 (Supplemental Information: Validation Summary: Plant Material Screen for THC using GC-FID).

Table 31 lists the values obtained from the GC-FID/MS analysis of the 1% standard mixtures using an HP-1MS for each internal standard.

Table 31: GC-FID/MS stability of 1% standard with internal standard

GC-FID/MS Data (HP-1MS)				
Data Summary	1% Standard with Androstenedione		1% Standard with Testosterone	
	cis- Δ^9 -THC	9(S)- Δ^7 -THC	cis- Δ^9 -THC	9(S)- Δ^7 -THC
Average \pm Std Dev	1.06263 \pm 0.04437	1.20555 \pm 0.05751	1.08180 \pm 0.05426	1.21933 \pm 0.06519
%CV	4.2%	4.8%	5.0%	5.3%
Count (n)	36	36	36	36

The largest %CV observed was 5.3%. This was observed when analyzing 9(S)- Δ^7 -THC with testosterone as an internal standard. When comparing to the previously established range for trans- Δ^9 -THC, thirteen (13) of the 36 total replicates exceeded the high end of this established acceptance range for trans- Δ^9 -THC. The 9(S)- Δ^7 -THC also had eight (8) of the 36 total replicates that exceeded the high end of the established criteria for trans- Δ^9 -THC when androstenedione was the internal standard. All of the cis- Δ^9 -THC replicate analyses were within the previously established range for trans- Δ^9 -THC. Table 32 lists the values obtained from the GC-FID/MS analysis of the 2% standard mixtures using an HP-1MS column for each internal standard.

Table 32: GC-FID/MS stability of 2% standard with internal standard

GC-FID/MS Data (HP-1MS)				
Data Summary	2% Standard with Androstenedione		2% Standard with Testosterone	
	cis- Δ^9 -THC	9(S)- Δ^7 -THC	cis- Δ^9 -THC	9(S)- Δ^7 -THC
Average \pm Std Dev	2.18319 \pm 0.11288	2.41596 \pm 0.14206	2.22972 \pm 0.12631	2.44816 \pm 0.16791
%CV	5.2%	5.9%	5.7%	6.9%
Count (n)	36	36	36	36

The largest %CV observed was 6.9% with 9(S)- Δ^7 -THC with testosterone as an internal standard. Only one (1) of the individual replicate analyses was outside of the previously established range for trans- Δ^9 -THC. All other

compounds and replicates for both internal standards were found to be within the previously established range for trans- Δ^9 -THC.

The same samples that were used to generate the data for Tables 31-32 were used to generate the data for Tables 33-34. Table 33 lists the values obtained from the GC-FID analysis of the 1% standard mixtures using a DB-35MS column for each internal standard.

Table 33: GC-FID stability of 1% standard with internal standard

GC-FID Data (DB-35MS)				
Data Summary	1% Standard with Androstenedione		1% Standard with Testosterone	
	cis- Δ^9 -THC	9(S)- Δ^7 -THC	cis- Δ^9 -THC	9(S)- Δ^7 -THC
Average \pm Std Dev	1.07988 \pm 0.02024	1.19415 \pm 0.01882	1.10823 \pm 0.01721	1.22562 \pm 0.02274
%CV	1.9%	1.6%	1.6%	1.9%
Count (n)	36	36	36	36

The largest %CV observed was 1.9%. All compounds and replicates for both internal standards were found to be within the previously established range for trans- Δ^9 -THC. Table 34 lists the values obtained from the GC-FID analysis of the 2% standard mixtures using a DB-35MS column for each internal standard.

Table 34: GC-FID stability of 2% standard with internal standard

GC-FID Data (DB-35MS)				
Data Summary	2% Standard with Androstenedione		2% Standard with Testosterone	
	cis- Δ^9 -THC	9(S)- Δ^7 -THC	cis- Δ^9 -THC	9(S)- Δ^7 -THC
Average \pm Std Dev	2.16537 \pm 0.05584	2.35120 \pm 0.05818	2.22425 \pm 0.04352	2.41554 \pm 0.06254
%CV	2.6%	2.5%	2.0%	2.6%
Count (n)	36	36	36	36

The largest %CV observed was 2.6% with both the 9(S)- Δ^7 -THC with testosterone and the cis- Δ^9 -THC with androstenedione. All compounds and replicates for both internal standards were found to be within the previously established range for trans- Δ^9 -THC.

In addition to the abovementioned samples, all standard solutions from the validation experiments, including round robin study samples, were combined to evaluate the overall variability of the isomers. This evaluation was performed for both instrumental methods for the 9(R)- Δ^7 -THC, 9(S)- Δ^7 -THC and cis- Δ^9 -THC isomers. Due to the inability to baseline separate the 9(R)- and 9(S)- Δ^7 -THC isomers, they were not used together in the various portions of the validation study which resulted in fewer injections of both isomers. The average, standard deviation, %CV, and number of injections was compiled for each THC isomer. Since the round robin samples only used testosterone as the internal standard, androstenedione was not assessed in the combined data. Table 35 lists the combined results for the 1% standard data obtained from the GC-FID/MS method using a HP-1MS column or equivalent.

Table 35: GC-FID/MS combined 1% data with testosterone internal standard

GC-FID/MS Data (HP-1MS or equivalent)			
1% Standard with Testosterone			
Data Summary	cis- Δ^9 -THC	9(R)- Δ^7 -THC	9(S)- Δ^7 -THC
Average \pm Std Dev	1.07111 \pm 0.05897	1.14805 \pm 0.06952	1.20272 \pm 0.06331
%CV	5.5%	6.1%	5.3%
Count (n)	145	73	72

The 9(R)- Δ^7 -THC isomer data showed the most variability in analysis with a %CV of 6.1%. In addition to the 1% standards, 2% standards were also evaluated with testosterone as the internal standard. The GC-FID/MS combined results for the 2% standards are listed in Table 36.

Table 36: GC-FID/MS combined 2% data with testosterone internal standard

GC-FID/MS Data (HP-1MS or equivalent)			
2% Standard with Testosterone			
Data Summary	cis- Δ^9 -THC	9(R)- Δ^7 -THC	9(S)- Δ^7 -THC
Average \pm Std Dev	2.15313 \pm 0.12582	2.23590 \pm 0.12285	2.42579 \pm 0.15097
%CV	5.8%	5.5%	6.2%
Count (n)	145	73	72

As shown, the 9(S)- Δ^7 -THC isomer data had the largest variability with a %CV of 6.2%.

The same samples were evaluated using the GC-FID method that employed a DB-35MS or equivalent column. The average, standard deviation, %CV, and number of injections were compiled for each THC isomer. Table 37 lists the combined results for the 1% standard data obtained from the GC-FID method.

Table 37: GC-FID combined 1% data with testosterone internal standard

GC-FID Data (DB-35MS or equivalent)			
1% Standard with Testosterone			
Data Summary	cis- Δ^9 -THC	9(R)- Δ^7 -THC	9(S)- Δ^7 -THC
Average \pm Std Dev	1.07807 \pm 0.05895	1.11489 \pm 0.08188	1.21224 \pm 0.02450
%CV	5.5%	7.3%	2.0%
Count (n)	145	73	72

The 9(R)- Δ^7 -THC isomer data showed the most variability in analysis with a %CV of 7.3%. The 9(S)- Δ^7 -THC isomer %CV was noticeably lower than the others (2.0). This difference may be due to the 9(S)- Δ^7 -THC isomer data being collected using one instrument at one location, rather than multiple instruments at multiple locations.

In addition to the 1% standards, 2% standards were also evaluated with testosterone as an internal standard. The GC-FID combined results for the 2% standards are listed in Table 38.

Table 38: GC-FID combined 2% data with testosterone internal standard

GC-FID Data (DB-35MS or equivalent)			
2% Standard with Testosterone			
Data Summary	cis- Δ^9 -THC	9(R)- Δ^7 -THC	9(S)- Δ^7 -THC
Average \pm Std Dev	2.13503 \pm 0.10962	2.16940 \pm 0.12721	2.40329 \pm 0.05867
%CV	5.1%	5.9%	2.4%
Count (n)	145	73	72

The 9(R)- Δ^7 -THC isomer data showed the most variability in analysis with a %CV of 5.9%. The 9(S)- Δ^7 -THC isomer %CV was again noticeably lower than the others due to the 9(S)- Δ^7 -THC isomer data being collected using one instrument at one location, rather than multiple instruments at multiple locations.

The combined average ratios obtained for each isomer were evaluated against the previously validated acceptance criteria established for trans- Δ^9 -THC using testosterone as an internal standard. As stated previously, the range established for the 1% standard on the GC-FID/MS (HP-1MS) method was 0.85316-1.26463 and the range established for the 2% standard on the same method was 1.52232-2.84182. The range established for the 1% standard on the GC-FID (DB-35MS) method was 0.85669-1.33295 and the range established for the 2% standard on the same method was 1.6770-2.81008. Each sample was evaluated against its respective acceptance criteria range.

When evaluating the 1% standard data for each isomer using the GC-FID/MS method, several samples and/or replicates were outside of the accepted trans- Δ^9 -THC range for both of the Δ^7 -THC isomers, more so with the 9(S)- Δ^7 -THC isomer than the 9(R)- Δ^7 -THC isomer. The samples and replicates of cis- Δ^9 -THC all fell within the accepted trans- Δ^9 -THC range. When evaluating the 2% standard data for each isomer, none of the samples or replicates for any of the isomers fell outside of the accepted trans- Δ^9 -THC range. The average ratios for both of the Δ^7 -THC isomers were always higher than the trans- Δ^9 -THC average ratio. This slight high bias was presumably significant enough to shift several samples and/or replicates outside of the acceptance criteria range for the 1% samples, but not significant enough to do the same for the 2% samples or replicates. The same evaluation was performed for the samples and replicates using the DB-35MS column, with none of the replicates falling outside of the accepted trans- Δ^9 -THC range for either the 1% or 2% standards.

To establish the acceptance criteria for the 9(R)- Δ^7 -THC, 9(S)- Δ^7 -THC and cis- Δ^9 -THC isomers, the two and three standard deviation unit ranges (Table 39 and 40, respectively) were calculated for each isomer at the 1% and 2% standard concentrations for both the GC-FID/MS (HP-1MS) and GC-FID (DB-35MS) methods using testosterone as an internal standard.

Table 39 lists the ranges when applying two standard deviations to the average ratio measurements. Table 40 lists the ranges when applying three standard deviations to the average ratio measurements.

Table 39: Acceptance criteria when using two standard deviation range and testosterone as internal standard

Ratio acceptance criteria			
Two Standard Deviation Range			
Sample	cis- Δ^9 -THC	9(R)- Δ^7 -THC	9(S)- Δ^7 -THC
1% GC-FID/MS	0.95317-1.18905	1.00901-1.28709	1.07609-1.32935
2% GC-FID/MS	1.90150-2.40476	1.99020-2.48159	2.12385-2.72773
1% GC-FID	0.96017-1.19597	0.95112-1.27865	1.16324-1.26124
2% GC-FID	1.91578-2.35428	1.91498-2.42382	2.28595-2.52063

Table 40: Acceptance criteria when using three standard deviation range and testosterone as internal standard

Ratio acceptance criteria			
Three Standard Deviation Range			
Sample	cis- Δ^9 -THC	9(R)- Δ^7 -THC	9(S)- Δ^7 -THC
1% GC-FID/MS	0.89420-1.24802	0.93949-1.35661	1.01278-1.39266
2% GC-FID/MS	1.77569-2.53058	1.86735-2.60444	1.97288-2.87870
1% GC-FID	0.90122-1.25492	0.86924-1.36053	1.13874-1.28574
2% GC-FID	1.80616-2.46391	1.78778-2.55103	2.22728-2.57930

The range determined for trans- Δ^9 -THC was the two standard deviation range previously determined during the alternative matrix validation study, which overlapped the calculated Δ^7 -THC and cis- Δ^9 -THC two standard deviation ranges. The individual cis- Δ^9 -THC isomer replicates all fell within the established range for trans- Δ^9 -THC. Approximately 15% of the individual Δ^7 -THC isomer replicates fell outside of the trans- Δ^9 -THC established range. As a result, it is recommended that new acceptance criteria be established for the Δ^7 -THC isomers for these methods.

6. References

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