DEPARTMENT OF FORENSIC SCIENCE
METHOD VALIDATION SUMMARY FORM

Section: Controlled Substances

Method: Quantitation of Methamphetamine Hydrochloride by UV-Vis Spectroscopy

Results recorded? ☑

Procedure documented? ☑

Method fit for use? ☑

Approved by: Robyn Weimer Date: 03/25/2026

Executive Validation Summary: “Quantitation of Methamphetamine Hydrochloride by UV-Vis Spectroscopy”

Recommended Uses:

This validation indicates that the UV-Vis quantitative method is a viable option within an analytical scheme for evaluating the purity of methamphetamine HCl within a linear range of 1.0 mg/mL to 5.0 mg/mL.

Limitations:

Methamphetamine HCl samples that contain cutting agents that do not dissolve in water, have a known interferent such as caffeine, cocaine HCl, α-benzyl-N-methylphenethylamine (B-compound), or phenethylamine, are non-white methamphetamine HCl samples, or have low methamphetamine HCl concentrations are not suitable for quantitation by UV-Vis.

Information Provided:

- Summary of Results and Conclusions
- References

Studies Performed:

- Selectivity
- Linearity
- Sensitivity
- Reproducibility/Repeatability
- Accuracy (bias) and Precision
- Stability
- Evaluation of Sampling Plan
- Interlaboratory Study
- Robustness
- Evaluation of Cuvette Type

RBW

03/25/2026

M**emo To:** Robyn Weimer, Chemistry Program Manager
From: Sarah Blake and Bryana Parlock, Forensic Scientists
CC: Alka Lohmann, Technical Services Director
Date: March 19, 2026
Re: Validation Summary: Quantitation of Methamphetamine Hydrochloride by UV-Vis Spectroscopy

Validation Summary: Quantitation of Methamphetamine Hydrochloride by UV-Vis Spectroscopy

A method for the quantitative analysis of methamphetamine HCl (meth) using Ultraviolet-visible spectroscopy (UV-Vis) was validated. The Drug Enforcement Administration (DEA) Office of Forensic Sciences provided the Virginia Department of Forensic Science (DFS) with a Quantitative Method Validation Final Report entitled "Quantitation of Methamphetamine HCl by UV-Vis Spectroscopy" (Method Name DEA 503). This method was adapted to meet the requirements of DFS.

The instrumental method employed an Agilent Technologies Cary 60 UV-Vis Spectrometer with both an 18-cell cuvette chamber and a fiber optic dip probe. The fiber optic dip probe was not used during this validation. Data was collected at a wavelength of 267 nm. A stock solution of approximately 5.0 mg/mL was prepared using a methamphetamine HCl certified reference material (CRM) and deionized water. Deionized water was also used as the solvent blank. The calibrators, 4.5 mg/mL high quality control (QC) check standard and 1.5 mg/mL low QC check standard, were prepared using either Class A volumetric flasks or calibrated pipettes and were stored in the refrigerator. Samples were dissolved in the appropriate amount of deionized water using a calibrated pipette to ensure the concentration would fall within the working range of the method. The samples were transferred to either a plastic or quartz cuvette using a pipette until approximately 80% full.

The working range for methamphetamine quantitation using the 18-cell cuvette chamber was determined to be 1.0 mg/mL to 5.0 mg/mL. The working range was established based on the results from the three-point linearity study. The limit of quantitation was determined to be 0.78835 mg/mL for plastic cuvettes and 0.55334 mg/mL for quartz cuvettes. The limit of quantitation was determined using linear regression analysis.

The validation for the quantitative analysis of methamphetamine HCl using UV-Vis spectroscopy was performed herein pursuant to the Quality Manual (Qualtrax Revision 35) and Controlled Substances Procedures Manual (Qualtrax Revision 24) and included the following studies:

1. Selectivity
2. Linearity
3. Sensitivity
4. Reproducibility/Repeatability
5. Accuracy (bias) and Precision
6. Stability

7. Evaluation of Sampling Plan
8. Interlaboratory Study
9. Robustness
10. Evaluation of Cuvette Type
11. References

Validation experiments were performed on an Agilent Technologies Cary 60 UV-Vis spectrophotometer with an 18-cell cuvette chamber. The instrumental parameters from the DEA 503 method were followed, as applicable. This included the use of a detection wavelength at 267 nm, which was selected during the DEA validation as it has a larger linear range compared to the λ_{max} at 255 nm.

1) Selectivity

Methamphetamine HCl and multiple potential interferents were evaluated. These included all compounds evaluated for interferents within the DEA 503 method validation and additional compounds relevant to analysis at DFS. Compounds included were dimethyl sulfone ($DMSO_2$), lactose, caffeine, cocaine HCl, creatine, inositol, mannitol, sodium chloride (NaCl), α-benzyl-N-methylphenethylamine (B-Compound), phenethylamine, polyethylene glycol (PEG) 3350, and bis(2,2,6,6-tetramethyl-4-piperidyl) sebacate (BTMPS). Solutions of individual compounds at a concentration of 2.0 mg/mL were prepared and analyzed in both plastic and quartz cuvettes. In addition, a 50:50 mixture of methamphetamine HCl CRM and potential interferant compound, at a concentration of 2.0 mg/mL for each compound in the mixture, were prepared and analyzed in both cuvette types. UV-Vis spectra were collected by scanning the individual compounds and mixtures from 200-300 nm. The absorbance (A) at 267 nm was evaluated to determine if the absorption measured >2%, 5%, 7%, or 10% relative to the absorption of methamphetamine HCl, thus indicating the compound was an interferent. The acceptance criterion used to determine interferents in the DEA 503 method was >2% relative absorption of methamphetamine HCl in both individual compounds and compounds in 50:50 mixtures (Equations 1 and 2).

Equation 1 DEA's acceptance criterion for evaluating if an individual compound is an interferent at 267 nm

$$Interferent = A\ of\ individual\ compound\ > (0.02 \cdot A\ of\ meth\ HCl\ std)$$

Equation 2 DEA's acceptance criterion for evaluating if a compound in a 50:50 mixture is an interferent at 267 nm

$$Interferent = |A\ of\ 50{:}50\ mixture - A\ of\ meth\ HCl\ std| > (0.02 \cdot A\ of\ meth\ HCl\ std)$$

Upon analysis, some compounds were found to be an interferent when present in a 50:50 mixture with methamphetamine HCl, but not when the compound was analyzed individually. These compounds were $DMSO_2$, mannitol, lactose, inositol, NaCl, PEG, and BTMPS. Not all results could be compared to the DEA validation results as the only consistent 50:50 mixture compounds were methamphetamine HCl/$DMSO_2$

and 50:50 methamphetamine HCl/NaCl. The results were further investigated by preparing a second replicate, ensuring the solution was fully dissolved, and then analyzing the mixture alongside a methamphetamine HCl standard. The previously mentioned 50:50 mixtures were analyzed in both plastic and quartz cuvettes and were still indicating potential interferents. The equation to evaluate interferents for the 50:50 mixtures used the absolute value of the absorbance of the 50:50 mixture at 267 nm subtracted from the absorbance of the methamphetamine HCl standard at 267 nm to see if this is >2% of the methamphetamine HCl standard's absorbance. This requires the absorbance of methamphetamine HCl in the 50:50 mixture to be compared to a single absorbance value of methamphetamine HCl analyzed prior to the 50:50 mixture. Slight variations in sample preparation can cause variations in absorbances which could cause these values to be outside of the 2% acceptance window when the methamphetamine HCl standard's absorbance was subtracted from the absorbance of the methamphetamine HCl in the 50:50 mixture. In addition, when analyzing the solvent blank, there was a higher baseline for plastic than quartz, which led to more plastic 50:50 mixtures being interferents as the solvent blank's absorbance was subtracted from the absorbance of the 50:50 mixture. To mitigate these factors causing variability, expanded acceptance criteria of 5%, 7%, and 10% were evaluated for determining potential interferents.

With the exception of creatine, all known interferents listed within the DEA method validation report were determined to be interferents at the 2% acceptance window and were still reported as interferents at the 10% acceptance window, both when analyzed individually and in a 50:50 mixture (Equations 3 and 4 and Table 1). These were caffeine, cocaine HCl, B-compound, and phenethylamine. In this validation, creatine was not noted as an interferent when using either plastic or quartz cuvettes. BTMPS did not readily dissolve in deionized water so the 50:50 mixture of BTMPS and methamphetamine HCl was filtered before being analyzed for a second time. This produced similar results to the first analysis and compounds that do not readily dissolve in deionized water were determined to be a limitation for this method. Typically, DFS does not receive case samples containing BTMPS in methamphetamine mixtures and therefore this should not inhibit the ability of this method to be used in casework.

Equation 3 Highest percentage acceptance criterion used during selectivity study for evaluating if a single compound is a potential interferent at 267 nm

$$Interferent = A\ of\ individual\ compound > (0.1 \cdot\ A\ of\ meth\ HCl\ std)$$

Equation 4 Highest percentage acceptance criterion used during selectivity study for evaluating if a compound in a 50:50 mixture is a potential interferent at 267 nm

$$Interferent = |A\ of\ 50{:}50\ mixture - A\ of\ meth\ HCl\ std| > (0.1\ \cdot\ A\ of\ meth\ HCl\ std)$$

Table 1 Compounds and mixtures evaluated for selectivity and the highest percentage identified as an interferent

Compound	DEA Result	Plastic Cuvette	Quartz Cuvette
$DMSO_2$	No	No	No
Lactose	No	No	No
Caffeine	Yes	10%	10%
Cocaine HCl	Yes	10%	10%
Creatine	Yes	No	No
Inositol	No	No	No
Mannitol	No	No	No
NaCl	No	No	No
B-Compound*	Yes	10%	10%
Phenethylamine	Yes	10%	10%
PEG	N/A	No	No
BTMPS	N/A	No	No
50:50 Meth HCl/$DMSO_2$	No	2%	2%**
50:50 Meth HCl/Lactose	N/A	2%	No
50:50 Meth HCl/Caffeine	N/A	10%	10%
50:50 Meth HCl/Cocaine HCl	N/A	10%	10%
50:50 Meth HCl/Creatine	N/A	No	No
50:50 Meth HCl/Inositol	N/A	2%	No
50:50 Meth HCl/Mannitol	N/A	5%**	2%**
50:50 Meth HCl/NaCl	No	2%**	2%
50:50 Meth HCl/B-Compound*	N/A	10%	10%
50:50 Meth HCl/Phenethylamine	N/A	10%	10%
50:50 Meth HCl/PEG	N/A	2%**	No
50:50 Meth HCl/BTMPS	N/A	10%**	7%**

* alpha-benzyl-N-methylphenethylamine

** Mixtures were analyzed twice and produced conflicting results at the reported percentage as being an interferent or not an interferent. These were also analyzed following the procedure in the accuracy study and the percent bias was calculated.

The originally prepared 2.0 mg/mL 50:50 mixtures that resulted in potentially being considered as interferents were evaluated using the ±5% bias acceptance criterion to demonstrate if each compound in the 50:50 mixture had an impact on the quantitative analysis of methamphetamine HCl (Equations 5 and 6).

Equation 5

$$\%\ Purity = \frac{Concentration \cdot Volume}{Weigh} \cdot 100$$

Equation 6

$$\%\ Bias = \frac{Measured\ concentration - Theoretica\ \ concentration}{Theoretica\ \ concentration} \cdot 100$$

Pure methamphetamine HCl was included as a control, while the caffeine mixture was included to provide a known interferent result. When the percent bias was calculated for methamphetamine HCl in each of the 2.0 mg/mL 50:50 mixtures, the percent biases were within ±5% for both plastic and quartz cuvettes (Tables 2 and 3). Therefore, these were not interferents within the method, whether individually or in 50:50 mixtures.

Table 2 Compounds evaluated for percent bias using plastic cuvettes

Compound	Measured Meth HCl Conc. (mg/mL)	Measured Meth HCl Purity (%)	% Bias
Meth HCl	1.9746	98.36	-1.59
50:50 Meth HCl/Caffeine	9.4313	235.49	371.21
50:50 Meth HCl/$DMSO_2$	1.9943	49.61	-1.22
50:50 Meth HCl/Lactose	1.9764	49.38	-1.13
50:50 Meth HCl/Creatine	2.0245	50.11	-0.10
50:50 Meth HCl/Inositol	1.9958	49.62	-1.03
50:50 Meth HCl/Mannitol	2.0002	49.97	-0.06
50:50 Meth HCl/NaCl	2.0619	49.86	-0.46
50:50 Meth HCl/PEG	2.0184	49.81	-1.01
50:50 Meth HCl/BTMPS	2.0958	51.27	2.66

Table 3 Compounds evaluated for percent bias using quartz cuvettes

Compound	Measured Meth HCl Conc. (mg/mL)	Measured Meth HCl Purity (%)	% Bias
Meth HCl	2.0073	99.99	0.04
50:50 Meth HCl/Caffeine	10.1976	254.62	409.50
50:50 Meth HCl/$DMSO_2$	2.0187	50.22	-0.01
50:50 Meth HCl/Lactose	2.0068	50.14	0.39
50:50 Meth HCl/Creatine	2.0573	50.92	1.52
50:50 Meth HCl/Inositol	2.0281	50.42	0.58
50:50 Meth HCl/Mannitol	2.0281	50.67	1.33
50:50 Meth HCl/NaCl	2.0848	50.42	0.64
50:50 Meth HCl/PEG	2.0447	50.46	0.28
50:50 Meth HCl/BTMPS	2.1185	51.83	3.77

2) Linearity

Linearity was established using nine calibrators. A stock solution of approximately 5.0 mg/mL was prepared in a Class A volumetric flask using methamphetamine HCl CRM and deionized water. The sequential calibrators were prepared using calibrated pipettes to perform the dilution using deionized water. The target concentration and prepared calibrator concentrations used in the experiment are shown in Table 4. The prepared concentration was the calibrator concentration corrected for the purity and mass of the standard. Five lots of the nine calibrators were prepared by five different examiners for the evaluation of linearity.

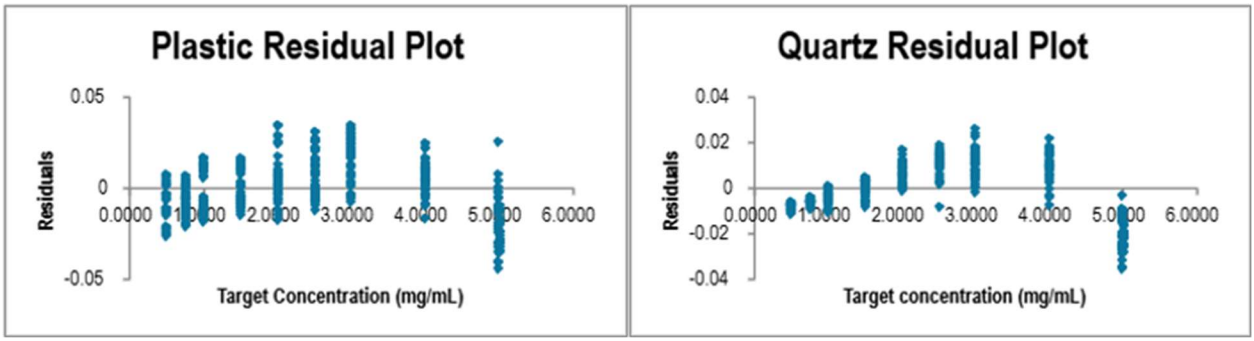
Table 4 Linearity experiment calibrator concentrations

Calibrator Level	Target Conc (mg/mL)	Prepared Conc Lot 1 (mg/mL)	Prepared Conc Lot 2 (mg/mL)	Prepared Conc Lot 3 (mg/mL)	Prepared Conc Lot 4 (mg/mL)	Prepared Conc Lot 5 (mg/mL)
1	5.00	4.9961	4.9977	5.0017	4.9993	4.9977
2	4.00	3.9969	3.9982	4.0014	3.9994	3.9982
3	3.00	2.9977	2.9986	3.0010	2.9996	2.9986
4	2.50	2.4981	2.4988	2.5008	2.4996	2.4988
5	2.00	1.9984	1.9991	2.0007	1.9997	1.9991
6	1.50	1.4988	1.4993	1.5005	1.4998	1.4993
7	1.00	0.9992	0.9995	1.0003	0.9999	0.9995
8	0.75	0.7494	0.7497	0.7503	0.7499	0.7497
9	0.50	0.4996	0.4998	0.5002*	0.4999	0.4998

*Outside ±5% acceptance range on plastic cuvettes

All calibrators were analyzed in nine replicates to establish the regression model. Residual plots were produced using data from all five lots (Figure 1). The residual plots produced an overall “u” shape. However, all the calibrators except one were within the acceptance range, even with this slight bias, and a linear regression model was used for this validation.

Figure 1 Residual plot of all nine calibrators analyzed nine times in plastic and quartz cuvettes from all five lots

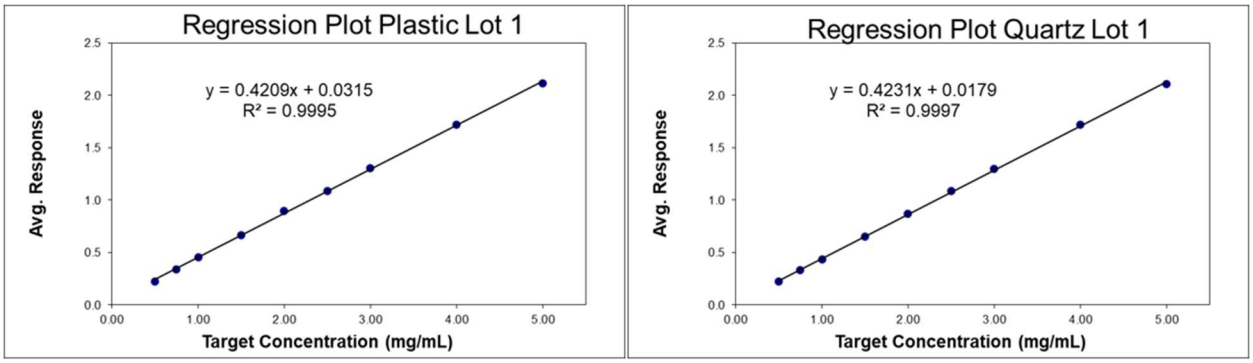


Sensitivity of the calibrators were evaluated using Equation 7. The sensitivity of each calibrator was compared to the average sensitivity of the nine calibrators and allowed a ±5% acceptance range. In the plastic cuvettes, four out of the five lots were within the ±5% acceptance range for all nine calibrators. After removing the nonconforming calibrator in Lot 3, the eight calibrators were within the ±5% acceptance range. In the quartz cuvettes, all five lots were within the ±5% acceptance range for all nine calibrators. The correlation coefficient, R^2, for each regression plot in both plastic and quartz cuvettes was ≥0.995 (Figure 2). All five lots produced similar results and Lot 1 was selected for demonstrative purposes.

Equation 7

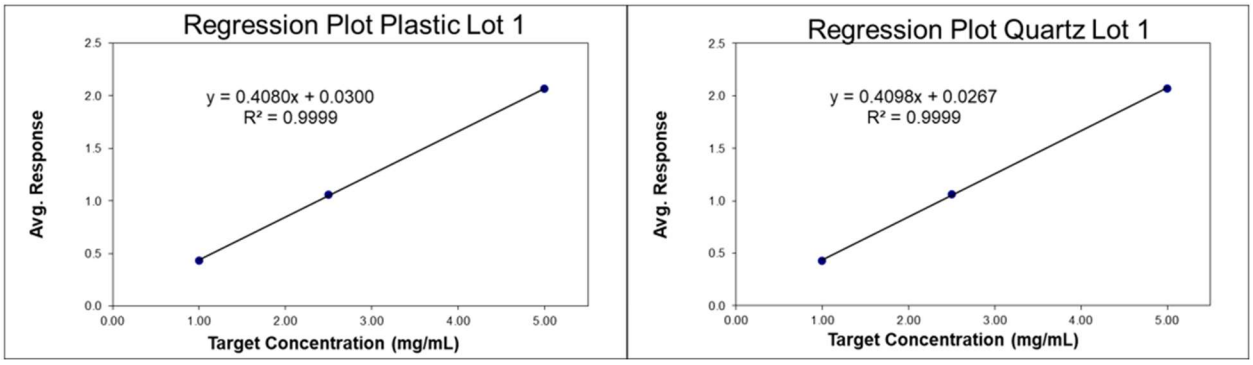
$$Sensitivity = \frac{Avg.\ Absorbance\ Response}{Theoretical\ concentration}$$

Figure 2 Regression plot using nine calibrators in plastic and quartz cuvettes from Lot 1



Additionally, three calibrators from the nine-point linearity study (5.0, 2.5, and 1.0 mg/mL) were reevaluated to assess the feasibility of a three-point calibration curve for use in the remaining studies in the validation and future casework for both plastic and quartz cuvettes. All five lots for both plastic and quartz cuvettes were within ±5% bias on their regression plots and had correlation coefficients ≥0.995, demonstrating feasibility for casework (Figure 3). This produced a linear range of 1.0 mg/mL to 5.0 mg/mL. All five lots produced similar results and Lot 1 was selected for demonstrative purposes.

Figure 3 Regression plot using three calibrators in plastic and quartz cuvettes from Lot 1



3) Sensitivity

The limit of quantitation (LOQ) was validated using the data obtained from the linearity study. Linear regression analysis was utilized in the LOQ determination. The limit of quantitation was calculated using Equation 8.

Equation 8

$$LOQ = \frac{10\sigma}{m}$$

Where σ is the standard deviation of the y-intercept and *m* is the slope of the linearity function.

The validation determined a LOQ of 0.78835 mg/mL for plastic cuvettes and 0.55334 mg/mL for quartz cuvettes using the 18-cell cuvette chamber.

4) Reproducibility/Repeatability

The nine calibrators were analyzed in nine replicates to determine the percent relative standard deviation (%RSD) (Equation 9). Additionally, three calibrators (5.0 mg/mL, 2.5 mg/mL, and 1.0 mg/mL) from the original nine calibrators were re-analyzed in nine replicates to demonstrate effectiveness of a three-point calibration curve for casework and to determine the %RSD. Both plastic and quartz cuvettes had %RSD values <10% which showed limited variability (Tables 5-8).

Equation 9

$$\%RSD = \left(\frac{Std\ Dev}{Average}\right) \cdot 100$$

Table 5 Results of %RSD for nine-point calibrators using 45 scans per calibrator for each lot in plastic cuvettes

Target Conc (mg/mL)	Lot 1 RSD (%)	Lot 2 RSD (%)	Lot 3 RSD (%)	Lot 4 RSD (%)	Lot 5 RSD (%)
5.00	0.52	0.52	0.24	0.34	0.55
4.00	0.63	0.59	0.34	0.31	0.38
3.00	0.69	0.56	0.35	0.25	0.34
2.50	0.60	0.36	0.33	0.30	0.44
2.00	0.59	0.53	0.39	0.40	0.28
1.50	0.85	0.54	0.25	0.49	0.42
1.00	0.87	0.62	0.25	0.33	0.46
0.75	1.03	0.73	0.34	0.52	0.71
0.50	0.49	0.83	0.97	0.66	0.55

Table 6 Results of %RSD for nine-point calibrators using 45 scans per calibrator for each lot in quartz cuvettes

Target Conc (mg/mL)	Lot 1 RSD (%)	Lot 2 RSD (%)	Lot 3 RSD (%)	Lot 4 RSD (%)	Lot 5 RSD (%)
5.00	0.45	0.38	0.19	0.20	0.36
4.00	0.34	0.38	0.17	0.20	0.31
3.00	0.44	0.48	0.14	0.20	0.33
2.50	0.35	0.32	0.12	0.66	0.16
2.00	0.32	0.34	0.16	0.28	0.32
1.50	0.26	0.37	0.14	0.18	0.33
1.00	0.25	0.44	0.13	0.19	0.26
0.75	0.24	0.40	0.13	0.18	0.34
0.50	0.28	0.55	0.38	0.27	0.49

Table 7 Results of RSD for three-point calibrators using 45 scans per calibrator for each lot in plastic cuvettes

Target Conc (mg/mL)	Lot 1 RSD (%)	Lot 2 RSD (%)	Lot 3 RSD (%)	Lot 4 RSD (%)	Lot 5 RSD (%)
5.00	0.46	0.36	0.38	0.45	0.31
2.50	0.34	0.34	0.39	0.41	0.38
1.00	0.26	0.53	0.48	0.48	0.63

Table 8 Results of %RSD for three-point calibrators using 45 scans per calibrator for each lot in quartz cuvettes

Target Conc (mg/mL)	Lot 1 RSD (%)	Lot 2 RSD (%)	Lot 3 RSD (%)	Lot 4 RSD (%)	Lot 5 RSD (%)
5.00	0.23	0.18	0.14	0.23	0.17
2.50	0.14	0.19	0.15	0.21	0.08
1.00	0.15	0.15	0.24	0.21	0.12

The QC check standards were analyzed multiple times to show that the percent bias was maintained within ±5% for both plastic and quartz cuvettes (Tables 9 and 10).

Table 9 Percent bias of QC Standards for plastic cuvettes

Study and examiner	Theoretical Conc. (mg/mL)	Measured Conc. (mg/mL)	% Bias
Unknown 3 Examiner 1 QC Low	1.51	1.5797	4.60
Unknown 3 Examiner 2 QC Low	1.51	1.5657	3.67
Unknown 2 Examiner 1 QC Low	1.51	1.5248	0.96
Unknown 2 Examiner 2 QC Low	1.51	1.5230	0.84
Unknown 3 Examiner 1 QC High	4.54	4.6675	2.81
Unknown 3 Examiner 2 QC High	4.54	4.6660	2.78
Unknown 2 Examiner 1 QC High	4.54	4.5694	0.65
Unknown 2 Examiner 2 QC High	4.54	4.5671	0.60
CTS 19-505-1 Examiner 1 QC Low	1.05	1.0210	-3.17
CTS 19-505-1 Examiner 2 QC Low	1.05	1.0174	-3.52
CTS 21-5051-2 Examiner 1 QC Low	1.05	1.0248	-2.82
CTS 21-5051-2 Examiner 2 QC Low	1.05	1.0220	-3.08
CTS 19-505-1 Examiner 1 QC High	5.01	5.0528	0.94
CTS 19-505-1 Examiner 2 QC High	5.01	5.0522	0.93
CTS 21-5051-2 Examiner 1 QC High	5.01	5.0717	1.32
CTS 21-5051-2 Examiner 2 QC High	5.01	5.0461	0.81

Note: The 1.50 and 4.50 mg/mL QC check standards were selected for casework since they were not at the linear range limits. Two other unknowns (unknown 1 and unknown 2) were also prepared and analyzed throughout the validation. These both were found to be invalid upon analysis of the data and unknown 3 was subsequently prepared. For unknown 1, the concentration values for all six samples for both examiners were below the linear range for the method. For unknown 2, the percent difference between the two methods was calculated to be outside of the ±5% acceptance criterion. This was most likely due to poor homogenization prior to sampling for quantitation.

Table 10 Percent bias of QC Standards for quartz cuvettes

Study and examiner	Theoretical Conc. (mg/mL)	Measured Conc. (mg/mL)	% Bias
Unknown 3 Examiner 1 QC Low	1.51	1.5608	3.35
Unknown 3 Examiner 2 QC Low	1.51	1.5619	3.42
Unknown 2 Examiner 1 QC Low	1.51	1.5267	1.09
Unknown 2 Examiner 2 QC Low	1.51	1.5286	1.21
Unknown 3 Examiner 1 QC High	4.54	4.6675	2.81
Unknown 3 Examiner 2 QC High	4.54	4.6715	2.90
Unknown 2 Examiner 1 QC High	4.54	4.5829	0.95
Unknown 2 Examiner 2 QC High	4.54	4.5830	0.95
CTS 19-505-1 Examiner 1 QC Low	1.05	1.0337	-1.97
CTS 19-505-1 Examiner 2 QC Low	1.05	1.0373	-1.63
CTS 21-5051-2 Examiner 1 QC Low	1.05	1.0374	-1.62
CTS 21-5051-2 Examiner 2 QC Low	1.05	1.0400	-1.37
CTS 19-505-1 Examiner 1 QC High	5.01	5.1272	2.43
CTS 19-505-1 Examiner 2 QC High	5.01	5.1319	2.52
CTS 21-5051-2 Examiner 1 QC High	5.01	5.1417	2.72
CTS 21-5051-2 Examiner 2 QC High	5.01	5.1444	2.77

Note: The 1.50 and 4.50 mg/mL QC check standards were selected for casework since they were not at the linear range limits. Two other unknowns (unknown 1 and unknown 2) were also prepared and analyzed throughout the validation. These both were found to be invalid upon analysis of the data and unknown 3 was subsequently prepared. For unknown 1, the concentration values for all six samples for both examiners were below the linear range for the method. For unknown 2, the percent difference between the two methods was calculated to be outside of the ±5% acceptance criterion. This was most likely due to poor homogenization prior to sampling for quantitation.

Previously evaluated proficiency test samples from Collaborative Testing Services, Inc. (CTS) containing methamphetamine HCl were selected based on the absence of known interferents: CTS 19-505-1 and CTS

21-5051-2. The two proficiency test samples were analyzed on the UV-Vis by each examiner and compared to the expected results from the CTS published summary report. The percent bias for both plastic and quartz cuvettes was within ±5% of the expected results for both proficiency tests (Tables 11 and 12).

Table 11 Proficiency test results for CTS 19-505-1, reported value 89%

	Examiner 1 Plastic	Examiner 2 Plastic	Examiner 1 Quartz	Examiner 2 Quartz
% Bias for UV-Vis	1.00	1.42	2.02	2.44

Table 12 Proficiency test results for CTS 21-5051-2, reported value 77%

	Examiner 1 Plastic	Examiner 2 Plastic	Examiner 1 Quartz	Examiner 2 Quartz
% Bias for UV-Vis	0.98	0.77	2.14	1.86

5) Accuracy (bias) and Precision

Mixtures of methamphetamine HCl and $DMSO_2$ were prepared at different purity levels (20%, 40%, 60%, 80%, and 100%) and then diluted to make different concentration levels (approximately 4.5, 2.5, and 1.5 mg/mL). The percent bias was calculated and was within ±5% of the expected concentration for both plastic and quartz cuvettes (Tables 13 and 14)

Table 13 Accuracy results for 100%, 80%, 60%, 40%, and 20% purity levels mixed with $DMSO_2$ at 4.5, 2.5, and 1.5 mg/mL concentrations using plastic cuvettes

100%	1A	1B	1C
Meth HCl (mg)	125.17		
$DMSO_2$ (mg)	N/A		
Calculated Meth HCl Purity (%)*	99.95		
Total Volume (mL)	10	10	10
Dilution	9:10	5:10	3:10
Measured Meth HCl Conc. (mg/mL)	4.5538	2.5684	1.4976
Measured Meth HCl Purity (%)	101.06	102.60	99.70
% Bias	1.11	2.65	-0.25
80%	3A	3B	3C
Meth HCl (mg)	125.51		
$DMSO_2$ (mg)	31.51		
Calculated Meth HCl Purity (%)*	79.89		
Total Volume (mL)	10	10	10
Dilution	9:10	5:10	3:10
Measured Meth HCl Conc. (mg/mL)	4.5392	2.5353	1.4979
Measured Meth HCl Purity (%)	80.30	80.73	79.50
% Bias	0.51	1.05	-0.50

60%	5A	5B	5C
Meth HCl (mg)	125.02		
$DMSO_2$ (mg)	83.43		
Calculated Meth HCl Purity (%)*	59.95		
Total Volume (mL)	10	10	10
Dilution	9:10	5:10	3:10
Measured Meth HCl Conc. (mg/mL)	4.4908	2.4974	1.4756
Measured Meth HCl Purity (%)	59.84	59.90	58.99
% Bias	-0.17	-0.07	-1.59
40%	7A	7B	7C
Meth HCl (mg)	125.38		
$DMSO_2$ (mg)	188.29		
Calculated Meth HCl Purity (%)*	39.95		
Total Volume (mL)	10	10	10
Dilution	9:10	5:10	3:10
Measured Meth HCl Conc. (mg/mL)	4.5827	2.5527	1.5002
Measured Meth HCl Purity (%)	40.58	40.69	39.86
% Bias	1.58	1.85	-0.24
20%	9A	9B	9C
Meth HCl (mg)	125.26		
$DMSO_2$ (mg)	501.29		
Calculated Meth HCl Purity (%)*	19.98		
Total Volume (mL)	10	10	10
Dilution	9:10	5:10	3:10
Measured Meth HCl Conc. (mg/mL)	4.5632	2.5145	1.4631
Measured Meth HCl Purity (%)	20.23	20.07	19.46
% Bias	1.24	0.42	-2.61

*Considering purity of Meth HCl certified reference material (99.95%)

Table 14 Accuracy results for 100%, 80%, 60%, 40%, and 20% purity levels mixed with $DMSO_2$ at 4.5, 2.5, and 1.5 mg/mL concentrations using quartz cuvettes

100%	1A	1B	1C
Meth HCl (mg)	125.17		
$DMSO_2$ (mg)	N/A		
Calculated Meth HCl Purity (%)*	99.95		
Total Volume (mL)	10	10	10
Dilution	9:10	5:10	3:10
Measured Meth HCl Conc. (mg/mL)	4.5373	2.5263	1.4975
Measured Meth HCl Purity (%)	100.69	100.91	99.70
% Bias	0.74	0.97	-0.25

80%	3A	3B	3C
Meth HCl (mg)	125.51		
$DMSO_2$ (mg)	31.51		
Calculated Meth HCl Purity (%)*	79.89		
Total Volume (mL)	10	10	10
Dilution	9:10	5:10	3:10
Measured Meth HCl Conc. (mg/mL)	4.5517	2.5334	1.4997
Measured Meth HCl Purity (%)	80.52	80.67	79.59
% Bias	0.79	0.97	-0.38
60%	5A	5B	5C
Meth HCl (mg)	125.02		
$DMSO_2$ (mg)	83.43		
Calculated Meth HCl Purity (%)*	59.95		
Total Volume (mL)	10	10	10
Dilution	9:10	5:10	3:10
Measured Meth HCl Conc. (mg/mL)	4.4602	2.4951	1.4746
Measured Meth HCl Purity (%)	59.44	59.85	58.95
% Bias	-0.85	-0.16	-1.66
40%	7A	7B	7C
Meth HCl (mg)	125.38		
$DMSO_2$ (mg)	188.29		
Calculated Meth HCl Purity (%)*	39.95		
Total Volume (mL)	10	10	10
Dilution	9:10	5:10	3:10
Measured Meth HCl Conc. (mg/mL)	4.5751	2.5485	1.5069
Measured Meth HCl Purity (%)	40.52	40.62	40.03
% Bias	1.41	1.68	0.21
20%	9A	9B	9C
Meth HCl (mg)	125.26		
$DMSO_2$ (mg)	501.29		
Calculated Meth HCl Purity (%)*	19.98		
Total Volume (mL)	10	10	10
Dilution	9:10	5:10	3:10
Measured Meth HCl Conc. (mg/mL)	4.5850	2.5355	1.4778
Measured Meth HCl Purity (%)	20.33	20.23	19.66
% Bias	1.73	1.26	-1.64

*Considering purity of Meth HCl certified reference material (99.95%)

Two CTS samples were analyzed using two analytical techniques: Gas Chromatography-Flame Ionization Detection (GC-FID) and UV-Vis using both cuvette types. The results from these methods were compared with each other. The percent bias of both cuvette types and the GC-FID were within the ±5% acceptance

window and the percent difference for both cuvette types compared to the GC-FID results were within the ±5% acceptance window (Equation 10 and Tables 15 and 16).

Equation 10

$$\%Difference = \frac{|Purity\ 1 - Purity\ 2|}{((Purity 1 + Purit\quad)/2)} \cdot 100$$

Table 15 Comparison of results from GC-FID to UV-Vis methods for CTS 19-505-1, reported value 89%

Examiner and cuvette type	GC-FID Purity (%)	UV-Vis Purity (%)	% Difference between methods	% Bias for UV-Vis	% Bias for GC-FID
Examiner 1 Plastic	87.02	89.89	3.24	1.00	-2.22
Examiner 2 Plastic	87.59	90.26	3.00	1.42	-1.58
Examiner 1 Quartz	87.02	90.79	4.24	2.02	-2.22
Examiner 2 Quartz	87.59	91.17	4.00	2.44	-1.58

Table 16 Comparison of results from GC-FID to UV-Vis methods for CTS 21-5051-2, reported value 77%

Examiner and cuvette type	GC-FID Purity (%)	UV-Vis Purity (%)	% Difference between methods	% Bias for UV-Vis	% Bias for GC-FID
Examiner 1 Plastic	75.20	77.76	3.34	0.98	-2.34
Examiner 2 Plastic	74.81	77.59	3.64	0.77	-2.84
Examiner 1 Quartz	75.20	78.65	4.48	2.14	-2.34
Examiner 2 Quartz	74.81	78.43	4.72	1.86	-2.84

An unknown mixture was prepared by a third examiner and analyzed on both instruments by examiners 1 and 2. The percent bias and percent difference for both cuvette types were within the ±5% acceptance window (Table 17).

Table 17 Comparison of results from GC-FID to UV-Vis methods for the unknown mixture calculated value 66.42%

Examiner and cuvette type	GC-FID Purity (%)	UV-Vis Purity (%)	% Difference between methods	% Bias for UV-Vis	% Bias for GC-FID
Examiner 1 Plastic	64.81	65.90	1.67	-0.79	-2.42
Examiner 2 Plastic	64.48	65.85	2.10	-0.87	-2.93
Examiner 1 Quartz	64.81	65.64	1.27	-1.18	-2.42
Examiner 2 Quartz	64.48	65.95	2.25	-0.72	-2.93

The precision was evaluated by calculating the variability of calibrators and QC check standards over multiple analyses. The three-point calibrators had a %RSD <2% over multiple analyses, satisfying the criterion of a %RSD of <10% (see Tables 7 and 8). The QC check standards had precision within ±3% and a %RSD <2% over multiple analyses, satisfying the criterion of a %RSD of <10%. This included thirteen analyses for plastic and five analyses for quartz during the study before the standard was outside the ±5% percent bias range (Equation 11 and Tables 18 and 19).

Equation 11

$$\%Precision = \frac{Std\ Dev}{Concentration} \cdot 100$$

Table 18 Results of precision and %RSD of the QC check standards listed in Tables 9 and 10

QC conc and cuvette type	Precision (%)	RSD (%)
QC Low 1.5 mg/mL plastic	1.90	1.86
QC High 4.5 mg/mL plastic	1.25	1.23
QC Low 1.0 mg/mL plastic	0.29	0.30
QC High 5.0 mg/mL plastic	0.22	0.22
QC Low 1.5 mg/mL quartz	1.29	1.26
QC High 4.5 mg/mL quartz	1.10	1.08
QC Low 1.0 mg/mL quartz	0.25	0.25
QC High 5.0 mg/mL quartz	0.16	0.16

Table 19 Results of precision and %RSD of the stability study

QC conc and cuvette type	Precision (%)	RSD (%)
QC Low 1.0 mg/mL plastic	1.82	1.81
QC High 5.0 mg/mL plastic	1.74	1.71
QC Low 1.0 mg/mL quartz	1.48	1.47
QC High 5.0 mg/mL quartz	1.07	1.04

6) Stability

Short-term stability of the calibrators was evaluated during the linearity study. After preparation, the calibrators were analyzed and stored in the refrigerator overnight. On subsequent days of analysis, the calibrators were removed from the refrigerator and allowed to come to room temperature before analysis. The %RSD was calculated to be <10% for both plastic and quartz (Tables 5, 6, 7, and 8) showing limited variability within the calibrators over the short-term.

For long-term stability, two calibrator standards from Lot 5 (1.0 mg/mL and 5.0 mg/mL) were selected and analyzed against the existing calibration curve. After analysis, the standards were stored in the refrigerator. The standards were removed from the refrigerator after one week and remained at room temperature throughout the day without analysis on the UV-Vis. They were returned to the refrigerator for an additional week. After the second week, the standards were removed from the refrigerator and allowed to come to room temperature before analysis on the UV-Vis.

This two-week cycle continued for five months. The calibrators in Lot 5 were first analyzed on October 7, 2024, for the linearity study and the stability study began May 1, 2025. The percent bias was outside of the ±5% acceptance range on July 10, 2025, for the quartz cuvettes and October 8, 2025, for the plastic cuvettes. This demonstrated a three-to-five-month stability range of active standard use, with a six-month storage period.

7) Evaluation of Sampling Plan

To evaluate the sampling plan, 20 mg of material in 10 mL of deionized water (similar to an example provided within the DEA method) was used. Following the DEA method, one sample was prepared, scanned five times, and assessed using their %RSD acceptance window of <2% RSD. To apply this method using DFS criteria, six independent samples were prepared and scanned both one time and five times, using the DFS acceptance window of a %RSD <10%. The high and low QC check standards were scanned the same number of times as the samples. This experiment was repeated three times. Based on the data collected, all samples were within the predetermined acceptance criteria for %RSD for all sampling and scanning methods. However, based on the data needed for uncertainty of measurement calculations and purity determination, the method using six samples scanned a total of five times was chosen (Tables 20 and 21).

Table 20 Comparison of sampling plan methods for plastic cuvettes

Sampling plan method	Average absorbance	Average Conc. (mg/mL)	Average Purity (%)	% RSD of Purity
6 samples 5 scans set 1	0.8875	2.0935	102.07	0.18
6 samples 5 scans set 2	0.8288	2.0450	101.46	0.67
6 samples 5 scans set 3	0.8472	2.0925	103.88	0.74
6 samples 1 scan set 1	0.8951	2.1117	102.96	0.33
6 samples 1 scan set 2	0.8268	2.0399	101.21	0.83
6 samples 1 scan set 3	0.8434	2.0828	103.39	0.70
1 sample 5 scans set 1	0.8727	2.0572*	101.29*	---
1 sample 5 scans set 2	0.8315	2.0519*	101.08*	---
1 sample 5 scans set 3	0.8578	2.1198*	103.97*	---

*No average was calculated since it was a single sample.

Table 21 Comparison of sampling plan methods for quartz cuvettes

Sampling plan method	Average absorbance	Average Conc. (mg/mL)	Average Purity (%)	% RSD of Purity
6 samples 5 scans set 1	0.8876	2.1152	103.08	0.23
6 samples 5 scans set 2	0.8306	2.0645	102.38	0.91
6 samples 5 scans set 3	0.8445	2.1005	104.22	0.67
6 samples 1 scan set1	0.8914	2.1247	103.54	0.20
6 samples 1 scan set 2	0.8261	2.0529	101.80	0.98
6 samples 1 scan set 3	0.8406	2.0905	103.72	0.72
1 sample 5 scans set 1	0.8785	2.0928*	102.99*	---
1 sample 5 scans set 2	0.8366	2.0802*	102.42*	---
1 sample 5 scans set 3	0.8536	2.1241*	104.12*	---

*No average was calculated since it was a single sample.

Upon establishing the sampling plan, the amount of sample weight was adjusted above and below 20 mg of material. For this study, three sample sets of the tested weights: 10 mg, 20 mg, and 30 mg were prepared and scanned five times. Decreasing the mass weighed to 10 mg lowered the calculated concentration to the bottom of the linear range increasing the variability in the calculated concentration compared to the purity obtained when weighing 20 mg of material. (Tables 22 and 23). Increasing to 30 mg maintained a calculated concentration in the middle of the linear range keeping the purity values more consistent to the 20 mg purity values.

Table 22 Weight adjustments for sampling plan using plastic cuvettes

Sample Weight (mg)	Concentration (mg/mL)	Purity (%)	% Bias
Set 1 10	1.0425	98.02	-1.98
Set 2 10	1.0231	97.30	-2.70
Set 3 10	1.0347	100.80	0.80
Set 1 20	2.0693	101.89	1.89
Set 2 20	2.0546	101.21	1.21
Set 3 20	2.1161	103.78	3.78
Set 1 30	3.0859	102.23	2.23
Set 2 30	3.0955	103.17	3.17
Set 3 30	3.1341	103.69	3.69

Table 23 Weight adjustments for sampling plan using quartz cuvettes

Sample Weight (mg)	Concentration (mg/mL)	Purity (%)	% Bias
Set 1 10	1.0573	99.37	-0.58
Set 2 10	1.0359	98.52	-1.48
Set 3 10	1.0492	102.16	2.21
Set 1 20	2.0921	102.96	3.01
Set 2 20	2.0758	102.25	2.25
Set 3 20	2.1229	104.06	4.12
Set 1 30	3.1367	103.86	3.92
Set 2 30	3.0794	102.63	2.63
Set 3 30	3.1410	103.87	3.92

8) Interlaboratory Study

A powder sample and a liquid sample were prepared and distributed to each of the four DFS laboratories. The powder sample was used to evaluate the variability of the entire sample and analysis process. The liquid sample was used to assess instrumental variability. Each laboratory prepared their own 1.0 mg/mL, 2.5 mg/mL, and 5.0 mg/mL calibrators and high and low QCs of 4.5 mg/mL and 1.5 mg/mL from their own methamphetamine HCl CRM. The results from both the powder and liquid samples were within ±5% percent bias and <10% RSD (Tables 24 and 25). This showed consistent instrument response and little variability among examiner preparation.

Table 24 Results from interlaboratory study for the powder sample, true value 69.99% pure

DFS laboratory and examiner	Purity (%) Plastic Cuvette	% Bias Plastic Cuvette	Purity (%) Quartz Cuvette	% Bias Quartz Cuvette
W1	69.39	-0.86	69.19	-1.14
W2	67.23	-3.94	66.82	-4.53
N1	70.61	0.89	70.72	1.04
N2	70.25	0.37	71.29	1.86
E1	69.87	-0.17	70.20	0.30
E2	70.56	0.81	69.76	-0.33
C1	69.83	-0.23	71.64	2.36
C2	66.72	-4.67	69.15	-1.20
Average	69.31	-0.98	69.85	-0.21
%RSD	2.17	---	2.18	---

Table 25 Results from interlaboratory study for the liquid sample, true value 85.00% pure

DFS laboratory and examiner	Purity (%) Plastic Cuvette	% Bias Plastic Cuvette	Purity (%) Quartz Cuvette	% Bias Quartz Cuvette
W1	84.27	-0.86	84.09	-1.07
W2	83.42	-1.86	84.52	-0.56
N1	85.13	0.15	83.87	-1.33
N2	84.72	-0.33	84.50	-0.59
E1	84.86	-0.16	84.66	-0.40
E2	84.56	-0.52	85.19	0.22
C1	84.68	-0.38	87.14	2.52
C2	84.90	-0.12	87.54	2.99
Average	84.57	-0.51	85.19	0.22
%RSD	0.62	---	1.63	---

9) Robustness

During the linearity study, five different examiners prepared both a stock solution (5.0 mg/mL) and nine calibrators (Table 4).

Two examiners performed a methamphetamine HCl quantitation using the UV-Vis with three different sources: two CTS proficiency test sources and an unknown methamphetamine HCl mixture prepared by a third examiner. The difference between the two examiners for the CTS source and unknown mixture were within ±5% (Tables 26-28).

Table 26 Comparison of CTS proficiency test 19-505-1 between examiners

Instrument	Examiner 1 Purity (%)	Examiner 2 Purity (%)	% Difference
GC-FID	87.02	87.59	0.65
UV-Vis Plastic cuvette	89.89	90.26	0.42
UV-Vis Quartz cuvette	90.79	91.17	0.41

Table 27 Comparison of CTS proficiency test 21-5051-2 between examiners

Instrument	Examiner 1 Purity (%)	Examiner 2 Purity (%)	% Difference
GC-FID	75.20	74.81	0.52
UV-Vis Plastic cuvette	77.76	77.59	0.22
UV-Vis Quartz cuvette	78.65	78.43	0.27

Table 28 Comparison of the unknown mixture between examiners

Instrument	Examiner 1 Purity (%)	Examiner 2 Purity (%)	% Difference
GC-FID	64.81	64.48	0.51
UV-Vis Plastic cuvette	65.90	65.85	0.08
UV-Vis Quartz cuvette	65.64	65.95	0.47

Robustness was also evaluated in the interlaboratory study. Two individuals from each of the four DFS laboratories performed quantitation on a powder sample and a pre-prepared liquid sample. The evaluation showed consistent instrument response and little variability among examiner preparation (Tables 24 and 25).

10) Evaluation of Cuvette Type

Disposable plastic and reusable quartz cuvettes were used for each study within the validation. The method validation studies indicated no difference between the two different types of cuvette material.

To evaluate the cleanliness of the reusable quartz cuvettes, the cuvette was filled with deionized water and analyzed using the scan application prior to sample analysis. The absorbance data at 267 nm was evaluated throughout several studies in the validation and 0.1 absorbance was chosen to be the limit to determine a clean quartz cuvette.

11) References

Drug Enforcement Administration. Methamphetamine Quantitation via UV/Vis- General Overview [PowerPoint slides].

Drug Enforcement Administration Office of Forensic Sciences. Analysis of Drugs Manual. 03/22/2021.

Drug Enforcement Administration Office of Forensic Sciences Quantitative Method Validation Final Report. Quantitation of Methamphetamine HCl by UV/Vis Spectroscopy, Method #DEA 503. 12/02/2022.

Drug Enforcement Administration Office of Forensic Sciences Validated Quantitative Methods. DEA 503-Quantiation of Methamphetamine Hydrochloride by UV/Vis Spectroscopy. 12/7/2022.

Virginia Department of Forensic Science Controlled Substances Procedures Manual (Qualtrax Revision 24). 2025.

Virginia Department of Forensic Science Quality Manual (Qualtrax Revision 35). 2026