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National Measurement Institute

Proficiency Test Final Report AQA 24-02 MDMA/Methamphetamine

October 2024

ACKNOWLEDGMENTS

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I would like to thank the management and staff of the participating laboratories for supporting the study. It is only through widespread participation that we can provide an effective service to laboratories.

The assistance of the following NMI staff members in the planning, conduct and reporting of the study is acknowledged.

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Accredited for compliance with ISO/IEC 17043

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SUMMARY

AQA 24-02 MDMA/Methamphetamine commenced in February 2024. Sample sets each containing two 3,4-methylenedioxymethamphetamine (MDMA) samples and two methamphetamine samples were sent to 31 laboratories, with one laboratory requesting two sets of test samples to be analysed by different analysts. All participants returned results.

Samples were prepared at the NMI Sydney laboratory. Samples S1 and S2 were prepared from MDMA hydrochloride and Samples S3 and S4 were prepared from methamphetamine hydrochloride, all supplied by the Australian Federal Police.

The assigned values for all samples were the reference values as determined by quantitative nuclear magnetic resonance (qNMR) spectroscopy, with maleic acid (NMI certified reference material QNMR010) as the internal standard.

Traceability: The reference values are traceable to the SI through Australian Standards for mass via balance calibration certificates and the purity of the NMI maleic acid certified reference material QNMR010 (Batch No.: 10-Q-02).

The outcomes of the study were assessed against the aims as follows:

- *Assess the proficiency of laboratories measuring MDMA and methamphetamine in samples typical of a routine seizure.*

Of 116 *z*-scores, 94 (81%) returned $|z| \leq 2.0$, indicating an acceptable performance.

Of 116 E_n-scores, 96 (83%) returned $|E_n| < 1.0$, indicating agreement of the participant's results with the assigned value within their respective expanded uncertainties.

Laboratories **4**, **5**, **8**, **9**, **10**, **14**, **22**, **23**, **24**, **25** and **33** returned acceptable *z*-scores and E_n-scores for all four samples.

- *Develop a practical application of traceability and measurement uncertainty, and provide participants with information that will assist uncertainty estimates.*

Of 116 numeric results, 110 (95%) were reported with an associated expanded measurement uncertainty. The magnitudes of uncertainties were within the range 0.1% to 72% relative.

- *Test the ability of participants to identify cutting agents commonly found in controlled drug preparation.*

Sample S1 was cut with glucose, Samples S2 and S3 were cut with niacinamide, and Sample S4 was cut with dimethyl sulfone. Twenty-six participants (81%) reported on the identity of at least one cutting agent in the samples.

Laboratories **4**, **8**, **16**, **21**, **24**, **26** and **30** correctly identified all cutting agents in this study.

- *Produce materials that can be used in method validation and as control samples.*

The samples produced for this study are homogeneous and are well characterised. Surplus of these samples is available for purchase and can be used for quality control and for method validation purposes.

1 INTRODUCTION

1.1 NMI Proficiency Testing Program

The National Measurement Institute (NMI) is responsible for Australia's national measurement infrastructure, providing a range of services including a chemical proficiency testing program.

Proficiency testing (PT) is the 'evaluation of participant performance against pre-established criteria by means of interlaboratory comparisons'.[1] NMI PT studies target chemical testing in areas of high public significance such as trade, environment, law enforcement and food safety. NMI offers studies in:

- pesticide residues in soil, water, fruit, vegetables and herbs;
- petroleum hydrocarbons in soil and water;
- per- and polyfluoroalkyl substances in water, soil, biosolid, food and biota;
- inorganic analytes in soil, water, filters, food and pharmaceuticals;
- controlled drug assay, drugs in wipes, and clandestine laboratory; and
- allergens in food.

1.2 Study Aims

The aims of the study were to:

- assess the proficiency of laboratories measuring 3,4-methylenedioxymethamphetamine (MDMA) and methamphetamine in samples typical of a routine seizure;
- develop a practical application of traceability and measurement uncertainty, and provide participants with information that will assist uncertainty estimates;
- test the ability of participants to identify cutting agents commonly found in controlled drug preparation; and
- produce materials that can be used in method validation and as control samples.

The choice of the test method was left to the participating laboratories.

1.3 Study Conduct

The conduct of NMI PT studies is described in the NMI Study Protocol for Proficiency Testing.[2] The statistical methods used are described in the NMI Chemical Proficiency Testing Statistical Manual.[3] These documents have been prepared with reference to ISO/IEC 17043 and The International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories.[1,4]

NMI is accredited by the National Association of Testing Authorities, Australia (NATA) to ISO/IEC 17043:2023 as a provider of proficiency testing schemes.[1] This controlled drug PT study is within the scope of NMI's accreditation.

2 STUDY INFORMATION

2.1 Study Timetable

The timetable of the study was:

Invitations sent	5/02/2024
Samples sent	15/04/2024
Results due	2/08/2024
Interim Report	5/08/2024
Preliminary Report	6/08/2024

Due to significant sample delivery delays to some international participants, the project timeline was extended.

2.2 Participation and Laboratory Code

Thirty-one laboratories registered to participate, with one laboratory requesting two sets of samples to be analysed independently by different analysts (total of 32 participants). Each participant was assigned a confidential laboratory code number for this study. All participants submitted results.

2.3 Test Material Specification

Four test samples were prepared in February 2024. Samples S1 and S2 contained MDMA hydrochloride, and Samples S3 and S4 contained methamphetamine hydrochloride. The starting materials were supplied by the Australian Federal Police.

Glucose purchased from a local pharmacy, and niacinamide and dimethyl sulfone purchased from Sigma-Aldrich, were used as cutting agents. Sample S1 was cut with glucose, Samples S2 and S3 were cut with niacinamide, and Sample S4 was cut with dimethyl sulfone.

The MDMA and methamphetamine were ground and sieved through a 180 µm sieve. The cutting agents were processed similarly. Test samples were prepared by mixing a known mass of sieved drug with known amounts of sieved cutting agent in a tumbler overnight. Portions of 150 mg of each of the test samples were weighed into labelled glass vials.

Sample S1 was prepared to contain approximately 21.9% MDMA base (m/m).

Sample S2 was prepared to contain approximately 40.1% MDMA base (m/m).

Sample S3 was prepared to contain approximately 39.7% methamphetamine base (m/m).

Sample S4 was prepared to contain approximately 69.7% methamphetamine base (m/m).

2.4 Test Sample Homogeneity and Stability

The preparation of homogeneous test samples is an important part of a PT study. Given the small (< 150 mg) test portions normally used for controlled substance analysis, the particle size must be sufficiently small and uniformly distributed to ensure minimal influence on analytical precision.

No homogeneity testing was conducted in this PT study. Samples were prepared using the same procedure as previous controlled drug PT studies, which has been demonstrated to produce sufficiently homogeneous samples. Results returned by the participants gave no reason to question the homogeneity of the test samples.

To assess the stability of the samples, results returned by participants were compared to the dates of analysis (Section 6.7). The results gave no reason to question the samples' stability.

2.5 Sample Dispatch and Receipt

A set of four test samples, with each sample containing approximately 150 mg of test material, was dispatched to each participant on 15 April 2024.

The following items were also sent with the samples:

- a letter with instructions for participants; and
- a form for participants to confirm the receipt of the test samples.

An Excel spreadsheet for the electronic reporting of results was emailed to participants.

2.6 Instructions to Participants

Participants were instructed as follows:

- Analyse each sample for the amount of drug by your routine test method. It is recommended to thoroughly mix the contents of each vial before taking a test portion for analysis.
- For each sample report % m/m drug as base. Report this figure as if reporting to a client.
- For each result report an estimate of your expanded uncertainty as % m/m drug as base.
- Report the identity of cutting agents in all samples if this is within your normal scope of analysis.
- Give brief details of your:
    - basis of uncertainty estimate (e.g. uncertainty budget, repeatability precision)
    - analytical method (e.g. sample treatment, instrument type, calibration method)
    - reference standard (e.g. source, purity)

  as requested by the results sheet.
- A result spreadsheet will be emailed to you. Please complete the results spreadsheet and return by email to jenny.xu@measurement.gov.au.
- Results are to be returned by 27 May 2024.

The results due date was later changed to 2 August 2024. This was to accommodate for significant sample delivery delays to some international participants.

2.7 Interim Report and Preliminary Report

An Interim Report was emailed to all participants on 5 August 2024.

A Preliminary Report was emailed to all participants on 6 August 2024. This report included a summary of the results reported by participants, assigned values, performance coefficients of variation (PCVs), *z*-scores and E_n-scores for each analyte in this study. After the release of the Preliminary Report, Laboratory **19** reported that their results were in units of % hydrochloride salt (m/m) instead of % base (m/m). Their results have been excluded from all statistical calculations in this Final Report, resulting in some slight changes from the Preliminary Report for the summary statistics. All assigned values (reference values), *z*-scores and E_n-scores remain unchanged.

3 PARTICIPANT LABORATORY INFORMATION

3.1 Test Methods Reported by Participants

Participants were requested to provide information about their test methods. Responses received are presented in Table 1. Some responses may be modified so that the participant cannot be identified.

Table 1 Summary of Participants' Test Methods

Lab. Code	Analyte	Extraction Solvent	Internal Standard	Calib. Points	Technique	Detector	Column
1	MDMA	ACN/MeOH/H2O	Analog of MDMA	7	UPLC	MS/MS	C-18 column
	Methamphetamine		Analog of methamphetamine				
2	Methamphetamine	Dissolution in acetonitrile/water	Methoxyphenamine HCl	3	HPLC	DAD	Alltima C-18
3	All	Methanol	2,4,6-trimethylpyridine	6	GC	FID	RTX-5-Amine
4	All	acetonitrile/water (80/20)	external standard	2	HPLC	DAD	C8
5	All	Purified Water	Phentermine	1	UPLC	DAD	Agilent Zorbax SB-C8
6	All	Methanol	Propylparaben	3	UPLC	PDA	ACQUITY C-18
7	MDMA	Acetonitrile	/	4	HPLC	UV/Vis	C8H125
8	All	methanol	strychnine	6	UPLC	DAD	Phenyl
9	All	Eluent: Acetonitrile, ammonium acetate, diethylamine and water	N/A	4	UHPLC	UV-VIS	RP18
10	All	Ethyl Acetate	Diphenylamine	5	GC	FID	HP1
11	All	Chloroform Hexane/triethylamine + TFA derivatisation	Bupivacaine		GC	MS	COLUMN BP1 0.25UM, 12M X 0.22MM ID
12	All	methanol	NO	1	HPLC	DAD	zorbax eclipse XDB-C18 (4.6x 1500mm
13	MDMA	Deuterium Oxide	Dimethylsulfone and Maleic Acid	3	QNMR		
	Methamphetamine	Water:Acetonitrile (90:10) (0.1%TFA)	N/A	5	HPLC	DAD	Xselect CSH, C18 3.5micron, 4.6X100mm
14	All	Methanol:KOH buffer (50:50)	Methoxyphenamine	3	UPLC	PDA	Aquity BEH C18

Lab. Code	Analyte	Extraction Solvent	Internal Standard	Calib. Points	Technique	Detector	Column
15	All	Purified water	none	4	HPLC	DAD	Zorbax RX-SIL
16	MDMA	acetic acid, acetonitrile, water	No IS	4	HPLC	DAD	Poroshell 120 EC-C18
	Methamphetamine						Poroshell 120 EC-C19
18	All	Methanol	Selegilin	4	UPLC	DAD	C18
19	All	Methanol	None	4	HPLC	DAD	Zorbax C18
20	All	Methanol	none	5	HPLC	DAD	Phenomenex C-18-XB
21	MDMA	water	N/A	7	HPLC	UV/Vis	Phenomenex PFP (2) Luna 3u Narrow Bore 100mm
	Methamphetamine		ortho-methoxyphenamine	3	UPLC		Acquity UPLC BEH C18 1.7µm 2.1x100mm
22	All	Acetonitrile/Water 20:80	N/A	3	HPLC	DAD	Luna 2.5um C18(2) HIST 100A
23	All	Methanol	Diazepam	6	GC	FID	128-5512 DB-5ms
24	All	Isooctane with ammonium hydroxide	Dodecane	3	GC	FID	HP1-MS
25	All	Methanol	N/A	6	HPLC	UV/Vis	Luna C-18
26	MDMA	Ethanol	Propylparaben	8	UPLC	DAD	BEH shield RP 18
	Methamphetamine			7			
27	MDMA	Methanol	Methadone	5	GC	FID	Rxi-5ms
28	All	Ethanol	Eicosane	6	GC	FID	
29	MDMA	buffer phosphate pH 3 / methanol (70/30)	none	3	HPLC	DAD	C18
30	All	D2O	Maleic acid		QNMR		NA
31	All	D2O	Maleic Acid		QNMR		
32	All	water	nil	1	UPLC	UV/Vis	Acquity UPLC BEH C18 1.7um 2.1x100mm
33	All	Water	Phentermine	1	UPLC	DAD	Agilent Zorbax SB-C8

3.2 Reported Basis of Participants' Measurement Uncertainty Estimates

Participants were requested to provide information about their basis of measurement uncertainty (MU). Responses received are presented in Table 2. Some responses may be modified so that the participant cannot be identified.

Table 2 Reported Basis of Uncertainty Estimate

Lab. Code	Approach to Estimating MU	Information Sources for MU Estimation*		Guide Document for Estimating MU
		Precision	Method Bias	
1	Top Down - precision and estimates of the method and laboratory bias Coverage factor not reported			
2	Bottom Up (ISO/GUM, fishbone/cause and effect diagram) k = 2	Duplicate analysis	Instrument calibration Masses and volumes Matrix effects Recoveries of SS Standard purity	ISO/GUM
3	Top Down - precision and estimates of the method and laboratory bias Coverage factor not reported	Standard deviation from PT studies only		
4	Coverage factor not reported	Control samples - RM Duplicate analysis	Instrument calibration	Eurachem/CITAC Guide
5	Top Down - precision and estimates of the method and laboratory bias k = 2	Control samples - SS Duplicate analysis	Laboratory bias from PT studies Recoveries of SS	Eurachem/CITAC Guide
6	Top Down - precision and estimates of the method and laboratory bias k = 3	Control samples - CRM Duplicate analysis	Instrument calibration Homogeneity of sample Masses and volumes Standard purity	NATA GAG Estimating and Reporting Measurement Uncertainty of chemical test results
7	Standard deviation of replicate analyses multiplied by 2 or 3 k = 2	Control samples - RM	Instrument calibration	ISO/GUM
8	Bottom Up (ISO/GUM, fish bone/cause and effect diagram) k = 2	Control samples - CRM Duplicate analysis	Instrument calibration Homogeneity of sample Masses and volumes Laboratory bias from PT studies	ISO/GUM
9	Budget Method Coverage factor not reported	Control samples - RM Duplicate analysis	Instrument calibration Masses and volumes Standard purity	Internal SOP
10	Top Down - precision and estimates of the method and laboratory bias Coverage factor not reported	Control samples - RM	Standard purity	ISO/GUM

Lab. Code	Approach to Estimating MU	Information Sources for MU Estimation*		Guide Document for Estimating MU
		Precision	Method Bias	
11	Statistical assessment of measurement uncertainty not undertaken (Analysis undertaken to confirm ID only)			
12	Coverage factor not reported	Control samples - CRM Duplicate analysis	Instrument calibration Laboratory bias from PT studies	
13	Top Down - precision and estimates of the method and laboratory bias Coverage factor not reported	Duplicate analysis		
14	Top Down - precision and estimates of the method and laboratory bias Coverage factor not reported	Control samples - RM Duplicate analysis	Homogeneity of sample	Eurachem/CITAC Guide
15	Top Down - precision and estimates of the method and laboratory bias k = 2	Control samples - Sample from case	Laboratory bias from PT studies	Nordtest Report TR537
16	Top Down - precision and estimates of the method and laboratory bias k = 2	Control samples - SS	Recoveries of SS	accuracy profile - based on intermediate precision and repeatability
18	Top Down - precision and estimates of the method and laboratory bias k = 1	Control samples - authentic powders Duplicate analysis	Instrument calibration Homogeneity of sample Matrix effects Laboratory bias from PT studies Recoveries of SS Standard purity	EA-04/16 EA guidelines on the expression of uncertainty in quantitative testing.
19	Bottom Up (ISO/GUM, fish bone/cause and effect diagram) Coverage factor not reported			ISO/GUM
20	Standard deviation of replicate analyses multiplied by 2 or 3 k = 3	Control samples - RM Duplicate analysis		
21	Top Down - precision and estimates of the method and laboratory bias k = 2	Control samples - In house controls Duplicate analysis	Instrument calibration Masses and volumes	ISO/GUM
22	Top Down - precision and estimates of the method and laboratory bias Coverage factor not reported	Control samples - Retained known purity samples	Recoveries of SS	ISO/GUM
23	Estimating Measurement Uncertainty by black box with pairs of values k = 2	Standard deviation from PT studies only		ISO/GUM ENAC G 09 or ISO 21748

Lab. Code	Approach to Estimating MU	Information Sources for MU Estimation*		Guide Document for Estimating MU
		Precision	Method Bias	
24	Bottom Up (ISO/GUM, fish bone/cause and effect diagram) k = 2	Control samples - CRM Duplicate analysis	Instrument calibration Homogeneity of sample Masses and volumes Matrix effects Recoveries of SS Standard purity	Eurachem/CITAC Guide
25	Top Down - precision and estimates of the method and laboratory bias k = 3	Control samples - RM Duplicate analysis		Eurachem/CITAC Guide
26	Coverage factor not reported			
27	Standard deviation of replicate analyses multiplied by 2 or 3 k = 2	Duplicate analysis	Masses and volumes	ISO/GUM
28	Top Down - precision and estimates of the method and laboratory bias k = 2	Control samples - RM Duplicate analysis	Laboratory bias from PT studies	ISO/GUM
29	Coverage factor not reported	Standard deviation from PT studies only		
30	Top Down - precision and estimates of the method and laboratory bias k = 2	Control samples - previously analysed real seizure samples Duplicate analysis	Homogeneity of sample Masses and volumes Matrix effects Standard purity	Eurachem/CITAC Guide
31	Standard deviation of replicate analyses multiplied by 2 or 3 Coverage factor not reported	Control samples - RM		ISO/GUM
32	Validation study k = 2	Duplicate analysis		
33	Top Down - precision and estimates of the method and laboratory bias k = 2	Control samples - SS Duplicate analysis	Laboratory bias from PT studies Recoveries of SS	Eurachem/CITAC Guide

* CRM = Certified Reference Material; RM = Reference Material; SS = Spiked Samples.

3.3 Details of Participants' Calibration Standards

Participants were requested to provide information about their calibration standards. Responses as received are presented in Table 3. Responses may be modified so that the participant cannot be identified.

Table 3 Participant Calibration Standard

Lab. Code	MDMA		Methamphetamine	
	Reference Standard	Purity (%)	Reference Standard	Purity (%)
1		99	Sigma Aldrich	100
2			NMI	99.8

Lab. Code	MDMA		Methamphetamine	
	Reference Standard	Purity (%)	Reference Standard	Purity (%)
3	Lipomed	99.95	Lipomed	99.987
4	LGC	83.8	NMIA	99.8
5	Lipomed	99.950±0.050	Lipomed	99.950±0.050
6	NMI	99.8	NMI	99.8
7	Lipomed	99.95		
8	NMI	99.8	NMI	99.8
9	Lipomed	99.95	NMI	99.8
10	Lipomed	99.95	Lipomed	99.005
11	Sigma Aldrich	99.5	Sigma Aldrich	99.5
12				
13	Sigma	99.65	Lipomed	79.8
14	NMI	99.8	NMI	99.8
15	Internal	100	Sigma	100
16	Lipomed	99.95	Lipomed	99.95
18	Lipomed	99.950 +/- 0.050	Lipomed	99.005 +/- 0.027
19	NMI	99.8	NMI	99.8
20	Chiron	99.4	Sigma	99.9
21	NMI	99.8	NMI	99.8
22	National Measurement Institute	99.8	National Measurement Institute	99.8
23	Lipomed HCl MDMA	83.7	Lipomed HCl Methamphetamine	79.5
24	NMI	99.8±0.3	NMI	99.5±1.2
25	NMI	99.8	NMI	99.8
26	NMI	97.5	NMI	99.8
27	LGC	1.016		
28	Lipomed	99.81	Lipomed	99.005
29	Lipomed/Euromedex	99.95		
30	NA	NA	NA	NA
31				
32	In-house synthesis	1000.8	In-house synthesis	99.8
33	Lipomed	99.950 ± 0.05	Lipomed	99.950 ± 0.05

3.4 Participants' Comments

Participants were invited to comment on the samples, their methodology, the PT study in general and suggestions for future PT studies. Such feedback allows for the improvement of future studies. Participants' comments are presented in Table 4, along with the study coordinator's response where appropriate. Responses may be modified so that the participant cannot be identified.

Table 4 Participants' Comments

Lab. Code	Participants' Comments	Study Coordinator's Response
2	Methamphetamine Methodology: Linear regression	
9	This laboratory does not have a nicotinamide standard. Therefore UHPLC detection and potential interfering peaks using this method for nicotinamide has not been carried-out. Note uncertainty rounded (up) to 1.d.p. as would normally be reported in this laboratory.	
11	Purity determination of MDMA/Methylamphetamine not undertaken - Identification only methods accredited to ISO:17025 standard. Methodology: Qualitative (ID only)	
12	Quantitative analysis is based on the use of a historical value obtained from different batches of Certified reference material Methodology: External Standard	
13	Methodology: Validation in progress	
15	We have not accreditation for quantification of MDMA.	
16	Methodology: 5,20,60,100	
21	Questions 'MDMA: Conversion to free base? MA Conversion to free base?' are slightly ambiguous, please provide more clarification in future templates	Thank you for your feedback, we will update our future results sheets.
22	Methodology: Each sample was run in duplicate	
24	MDMA Methodology: 400uL of ammonium hydroxide was added for every 10mL of isooctane Methamphetamine Methodology: 200uL of ammonium hydroxide was added for every 5mL of isooctane	
26	Precursor present in S1 and S2: PMK	
27	Methamphetamine is not tested at our laboratory for purity however we do routinely test for Amphetamine purity, could there be a variety of analytes sent to include amphetamine in future rounds of PT? Also, the samples submitted are labelled with their analyte type - is there a possibility this could be removed as we also use the samples for proficiency of ID work	We have included amphetamine in previous PT studies and intend to include it in future studies, pending sourcing of material. This PT study is not intended to be a qualitative study. All participants are informed what analyte they are assessing for, on the sample label as well as the dispatch letter sent with the samples and the results sheet.
29	it is requested to warn the laboratories before the registration, on the distribution of the type of samples included in the circuit (amphetamine, MDMA, Methamphetamine) because we only do the quantification of MDMA.	The invitation letter issued for the study specified which analytes were included. Our distributors have also confirmed that this information was passed on to potential participants. The staff involved with enrolment should confirm that the analytes in the study are relevant to that laboratory.
30	Methodology: No reference standard involved	
31	Maleic Acid: Origin: Sigma Aldrich; Purity: > 99.93 %; Ref: 92816; Lot: BCCK2148	
32	Methodology: purity estimate analysis	

4 PRESENTATION OF RESULTS AND STATISTICAL ANALYSIS

4.1 Results Summary

Participant results are listed in Tables 5 to 8 with resultant summary statistics: robust average, median, mean, number of numeric results (N), maximum (Max), minimum (Min), robust standard deviation (Robust SD) and robust coefficient of variation (Robust CV). Bar charts of results and performance scores are presented in Figures 2 to 5. An example chart with interpretation guide is shown in Figure 1.

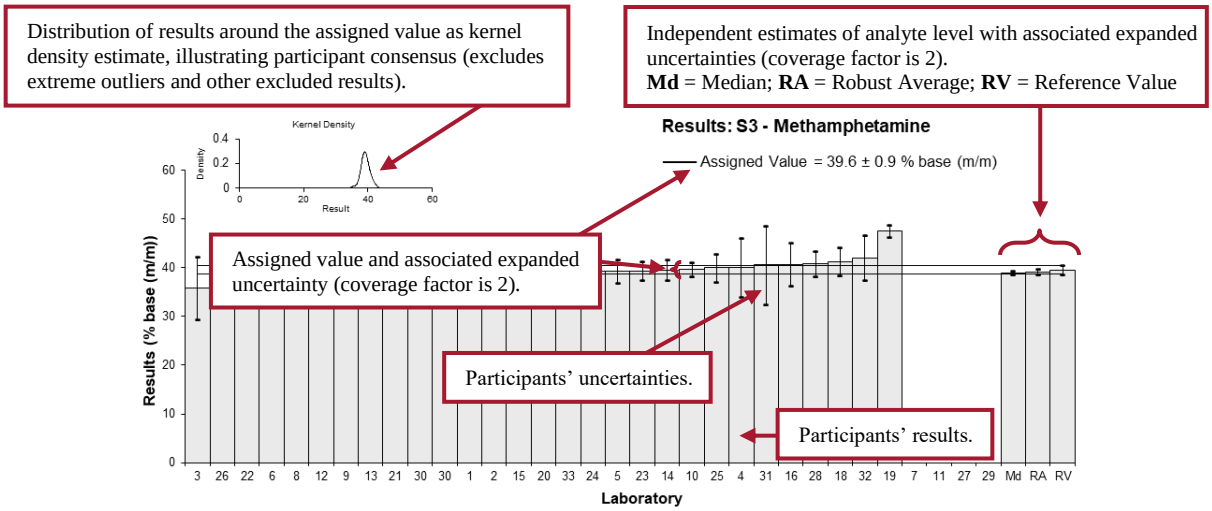


Figure 1 Guide to Presentation of Results

4.2 Outliers, Extreme Outliers and Other Excluded Results

Outliers were any result less than 50% and greater than 150% of the robust average, and these were removed before the calculation of the assigned value (if by consensus).[3,4] Extreme outliers were any obvious blunders, e.g. results with incorrect units, or for a different analyte or sample, and such results were removed before the calculation of all summary statistics.[3]

After the release of the Preliminary Report, Laboratory **19** reported that their results were in units of % hydrochloride salt (m/m) instead of % base (m/m) as requested for this study. Their results have been excluded from all statistical calculations.

4.3 Assigned Value

The assigned value is defined as the 'value attributed to a particular property or characteristic of a proficiency test item'.[1] In this PT study, the property is the % drug base (m/m) in the samples. The assigned values for all samples in this study were reference values determined by quantitative nuclear magnetic resonance (qNMR) spectroscopy (Appendix 1).

4.4 Robust Average and Robust Between-Laboratory Coefficient of Variation

Robust averages and associated expanded MUs, and robust CVs (a measure of the variability of participants' results) were calculated as described in ISO 13528.[5]

4.5 Performance Coefficient of Variation (PCV)

The PCV is a measure of the between-laboratory variation that in the judgement of the study coordinator would be expected from participants, given the analyte levels present. The PCV is set by the study coordinator, and it is not the CV of participants' results. The PCV is based on the mass fraction of the analytes and experience from previous studies, and is also supported by mathematical models such as the Thompson-Horwitz equation.[6] By setting a fixed and realistic value for the PCV, a participant's performance does not depend on other participants' performances.

4.6 Target Standard Deviation for Proficiency Assessment

The target SD for proficiency assessment (σ) is the product of the assigned value (X) and the PCV, as presented in Equation 1. This value is used for calculation of *z*-scores.

$$\sigma = X \times PCV \qquad \textit{Equation 1}$$

4.7 z-Score

For each participant's result, a *z*-score is calculated according to Equation 2.

$$z = \frac{(\chi - X)}{\sigma} \qquad \textit{Equation 2}$$

where:

z is *z*-score

χ is a participant's result

X is the assigned value

σ is the target standard deviation for proficiency assessment from Equation 1

For the absolute value of a *z*-score:

- $|z| \leq 2.0$ is acceptable;
- $2.0 < |z| < 3.0$ is questionable; and
- $|z| \geq 3.0$ is unacceptable.

4.8 E_n-Score

The E_n-score is complementary to the *z*-score in assessment of laboratory performance. The E_n-score includes measurement uncertainty and is calculated according to Equation 3.

$$E_n = \frac{(\chi - X)}{\sqrt{U_\chi{}^2 + U_X{}^2}} \qquad \textit{Equation 3}$$

where:

E_n is E_n-score

χ is a participant's result

X is the assigned value

U_χ is the expanded uncertainty of the participant's result

U_X is the expanded uncertainty of the assigned value

For the absolute value of an E_n-score:

- $|E_n| < 1.0$ is acceptable; and
- $|E_n| \geq 1.0$ is unacceptable.

4.9 Traceability and Measurement Uncertainty

Laboratories accredited to ISO/IEC 17025 must establish and demonstrate the traceability and measurement uncertainty associated with their test results.[7]

Guidelines for quantifying uncertainty in analytical measurement are described in the Eurachem/CITAC Guide.[8]

5 TABLES AND FIGURES

Table 5

Sample Details

Sample No.	S1
Matrix	Powder
Analyte	MDMA
Unit	% base (m/m)

Participant Results

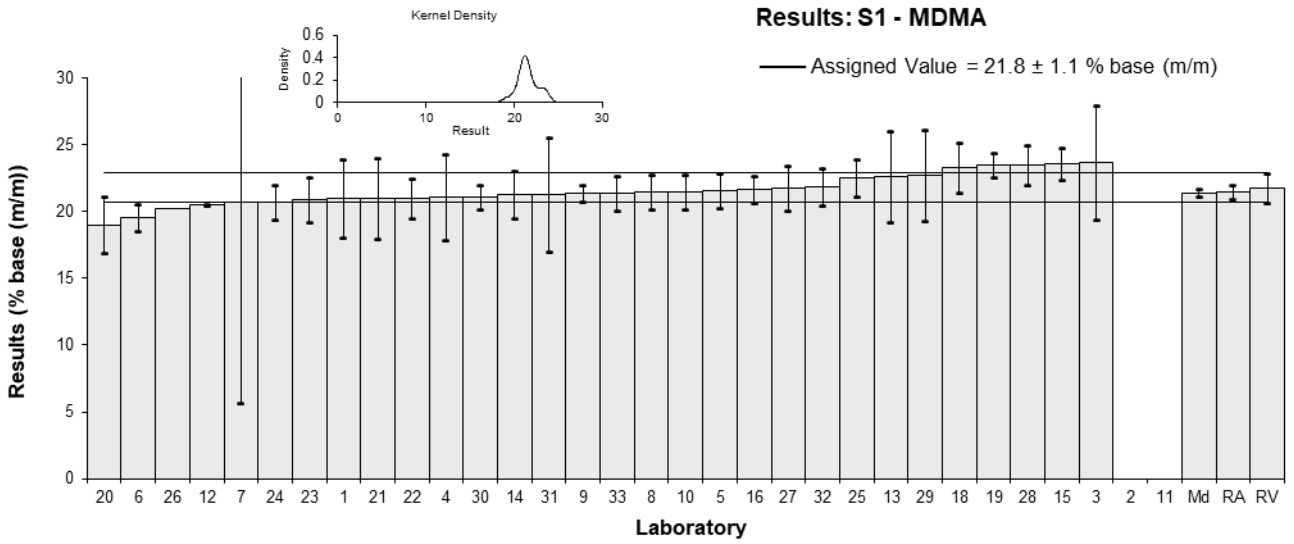
Lab. Code	Result	Uncertainty	z	E_n
1	21	2.9	-1.22	-0.26
2	NR	NR		
3	23.7	4.3	2.91	0.43
4	21.1	3.2	-1.07	-0.21
5	21.6	1.3	-0.31	-0.12
6	19.6	1	-3.36	-1.48
7	20.7	15	-1.68	-0.07
8	21.5	1.3	-0.46	-0.18
9	21.4	0.6	-0.61	-0.32
10	21.51	1.3	-0.44	-0.17
11	NR	NR		
12	20.5	0.03	-1.99	-1.18
13	22.66	3.40	1.31	0.24
14	21.3	1.8	-0.76	-0.24
15	23.6	1.2	2.75	1.11
16	21.7	1	-0.15	-0.07
18	23.3	1.9	2.29	0.68
19**	23.5	0.9	2.60	1.20
20	19	2.1	-4.28	-1.18
21	21.0	3.00	-1.22	-0.25
22	21	1.46	-1.22	-0.44
23	20.9	1.7	-1.38	-0.44
24	20.7	1.3	-1.68	-0.65
25	22.5	1.4	1.07	0.39
26	20.2	NR	-2.45	-1.45
27	21.78	1.7	-0.03	-0.01
28	23.5	1.5	2.60	0.91
29	22.77	3.41	1.48	0.27
30	21.1	0.9	-1.07	-0.49
31	21.3	4.3	-0.76	-0.11
32	21.9	1.4	0.15	0.06
33	21.4	1.3	-0.61	-0.23

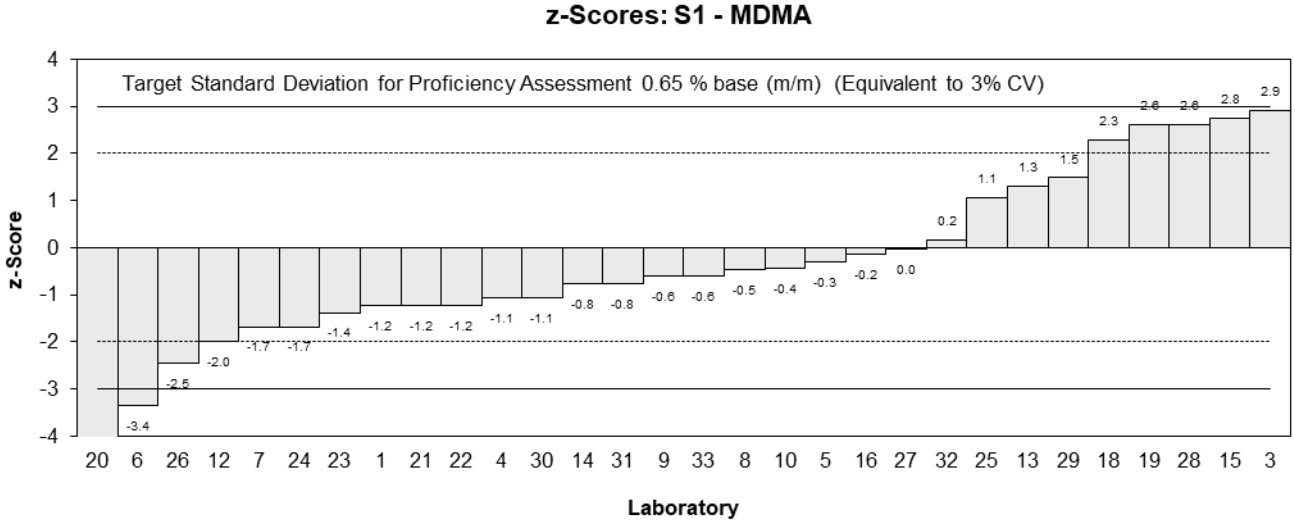
** Excluded Result, see Section 4.2

Statistics

Assigned Value	21.8	1.1
Reference Value	21.8	1.1
Robust Average	21.5	0.5
Median	21.4	0.3
Mean	21.5	
N	29	
Max	23.7	
Min	19	
Robust SD	1.1	
Robust CV	5%	

Assigned value is the reference value as determined by qNMR spectroscopy.


Results: S1 - MDMA
Assigned Value = 21.8 ± 1.1 % base (m/m)
Kernel Density
Density
Result
Results (% base (m/m))
Laboratory
20 6 26 12 7 24 23 1 21 22 4 30 14 31 9 33 8 10 5 16 27 32 25 13 29 18 19 28 15 3 2 11 Md RA RV


z-Scores: S1 - MDMA
Target Standard Deviation for Proficiency Assessment 0.65 % base (m/m) (Equivalent to 3% CV)
z-Score
Laboratory
20 6 26 12 7 24 23 1 21 22 4 30 14 31 9 33 8 10 5 16 27 32 25 13 29 18 19 28 15 3

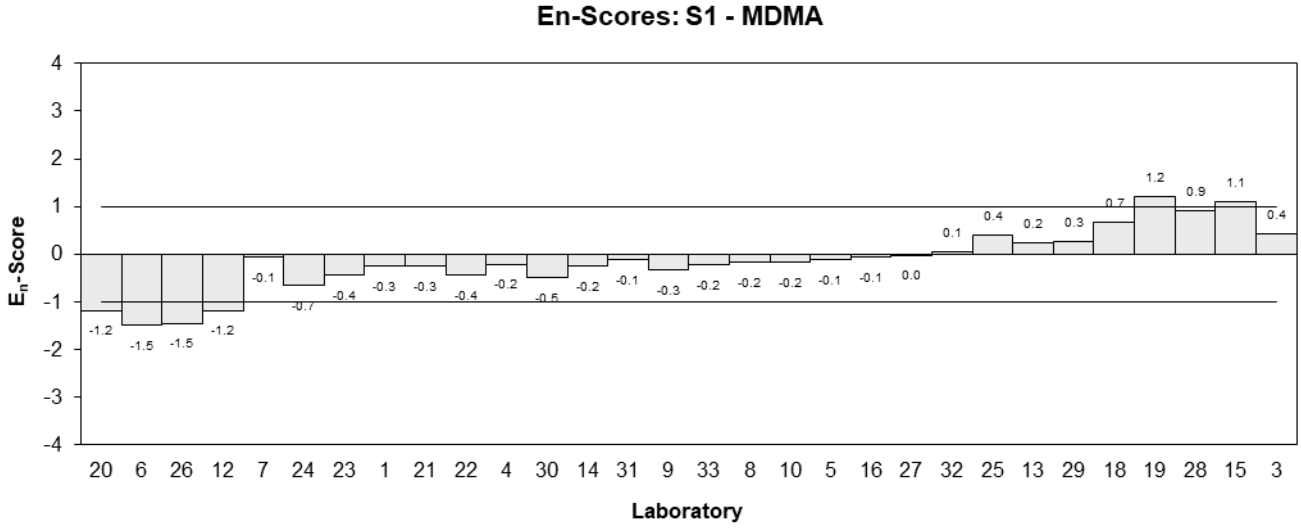

En-Scores: S1 - MDMA
En-Score
Laboratory
20 6 26 12 7 24 23 1 21 22 4 30 14 31 9 33 8 10 5 16 27 32 25 13 29 18 19 28 15 3

Figure 2

Table 6

Sample Details

Sample No.	S2
Matrix	Powder
Analyte	MDMA
Unit	% base (m/m)

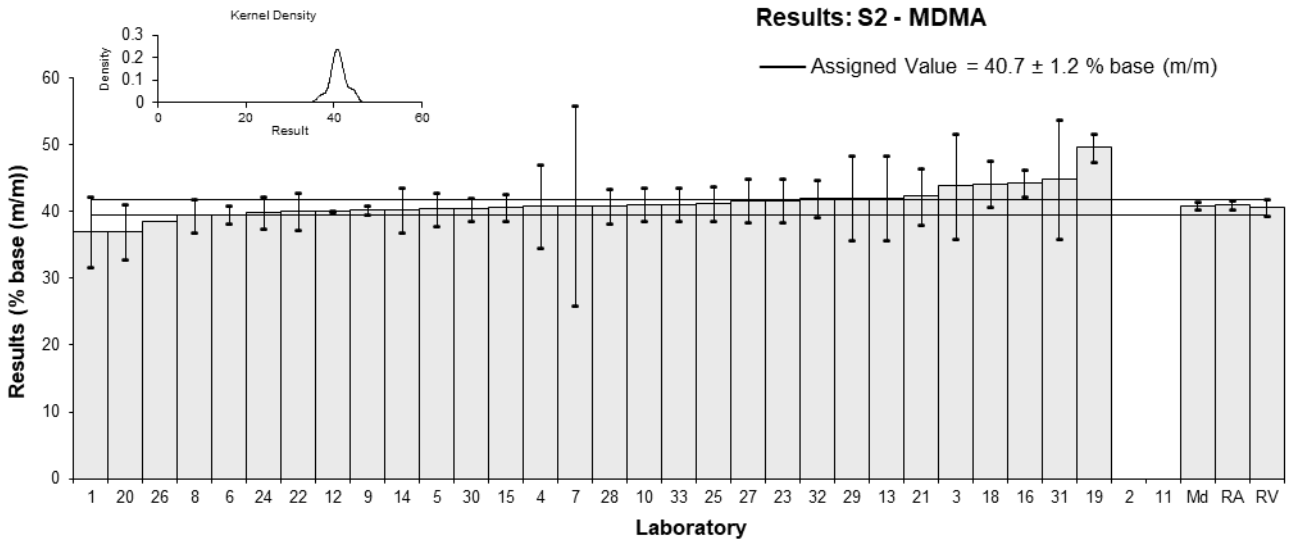
Participant Results

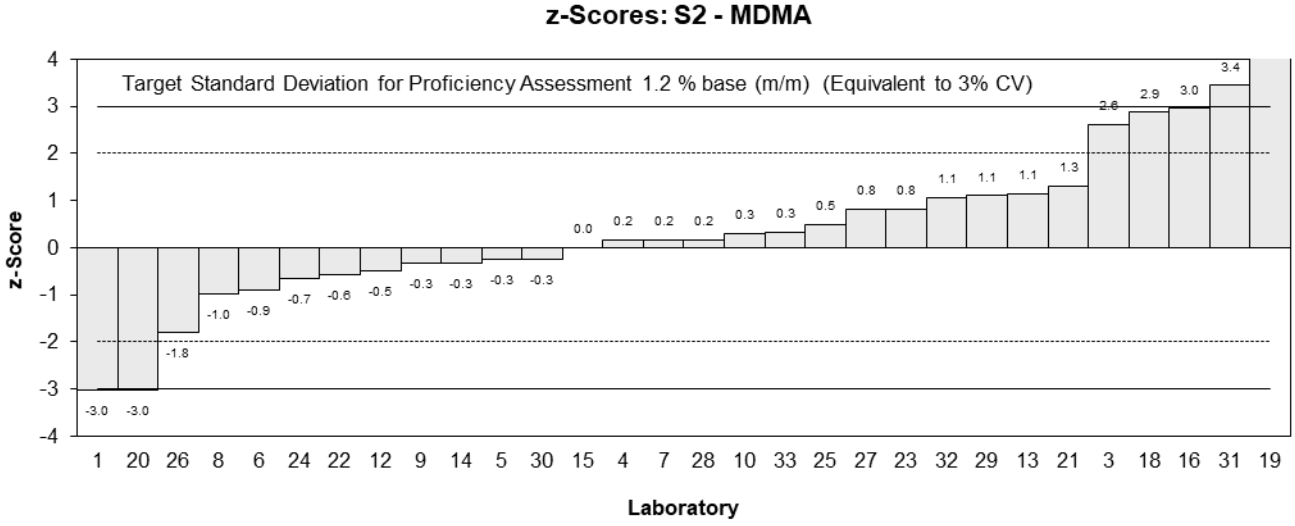
Lab. Code	Result	Uncertainty	z	E_n
1	37	5.2	-3.03	-0.69
2	NR	NR		
3	43.9	7.9	2.62	0.40
4	40.9	6.2	0.16	0.03
5	40.4	2.5	-0.25	-0.11
6	39.6	1.3	-0.90	-0.62
7	40.9	15	0.16	0.01
8	39.5	2.5	-0.98	-0.43
9	40.3	0.7	-0.33	-0.29
10	41.07	2.5	0.30	0.13
11	NR	NR		
12	40.1	0.06	-0.49	-0.50
13	42.09	6.31	1.14	0.22
14	40.3	3.3	-0.33	-0.11
15	40.7	2.0	0.00	0.00
16	44.3	2.1	2.95	1.49
18	44.2	3.5	2.87	0.95
19**	49.6	2.1	7.29	3.68
20	37	4.1	-3.03	-0.87
21	42.3	4.23	1.31	0.36
22	40	2.78	-0.57	-0.23
23	41.7	3.3	0.82	0.28
24	39.9	2.4	-0.66	-0.30
25	41.3	2.6	0.49	0.21
26	38.5	NR	-1.80	-1.83
27	41.69	3.26	0.81	0.28
28	40.9	2.6	0.16	0.07
29	42.04	6.31	1.10	0.21
30	40.4	1.7	-0.25	-0.14
31	44.9	9	3.44	0.46
32	42.0	2.7	1.06	0.44
33	41.1	2.5	0.33	0.14

** Excluded Result, see Section 4.2

Statistics

Assigned Value	40.7	1.2	Assigned value is the reference value as determined by qNMR spectroscopy.
Reference Value	40.7	1.2	
Robust Average	41.0	0.7	
Median	40.9	0.6	
Mean	41.0		
N	29		
Max	44.9		
Min	37		
Robust SD	1.6		
Robust CV	3.8%		


Results: S2 - MDMA
Assigned Value = 40.7 ± 1.2 % base (m/m)
Kernel Density
Density
Result
Results (% base (m/m))
Laboratory
1 20 26 8 6 24 22 12 9 14 5 30 15 4 7 28 10 33 25 27 23 32 29 13 21 3 18 16 31 19 2 11 Md RA RV


z-Scores: S2 - MDMA
Target Standard Deviation for Proficiency Assessment 1.2 % base (m/m) (Equivalent to 3% CV)
z-Score
Laboratory
1 20 26 8 6 24 22 12 9 14 5 30 15 4 7 28 10 33 25 27 23 32 29 13 21 3 18 16 31 19

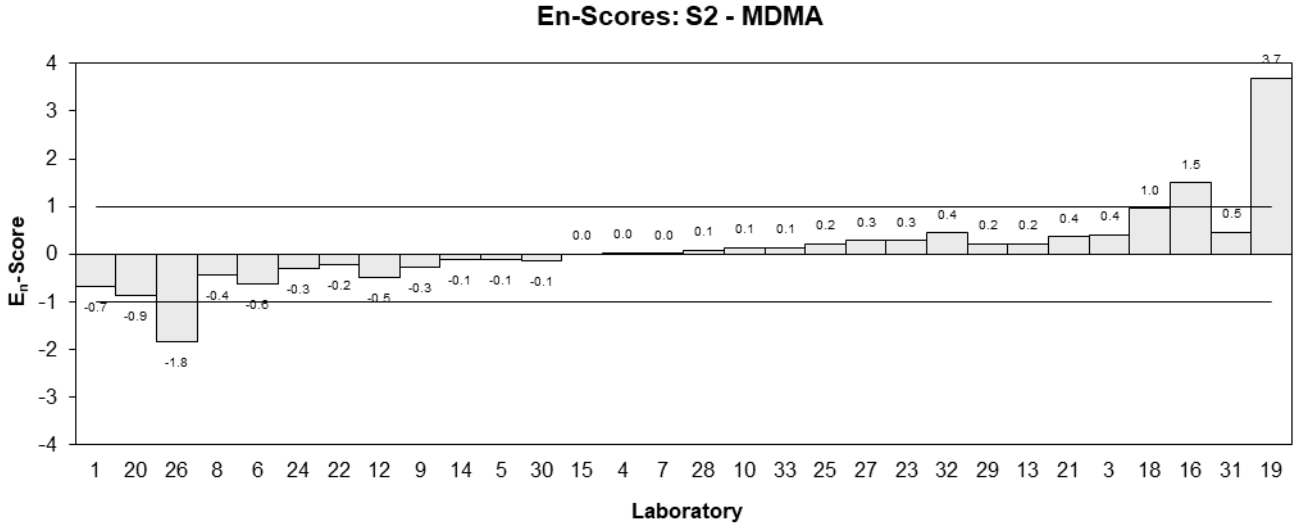

En-Scores: S2 - MDMA
En-Score
Laboratory
1 20 26 8 6 24 22 12 9 14 5 30 15 4 7 28 10 33 25 27 23 32 29 13 21 3 18 16 31 19

Figure 3

Table 7

Sample Details

Sample No.	S3
Matrix	Powder
Analyte	Methamphetamine
Unit	% base (m/m)

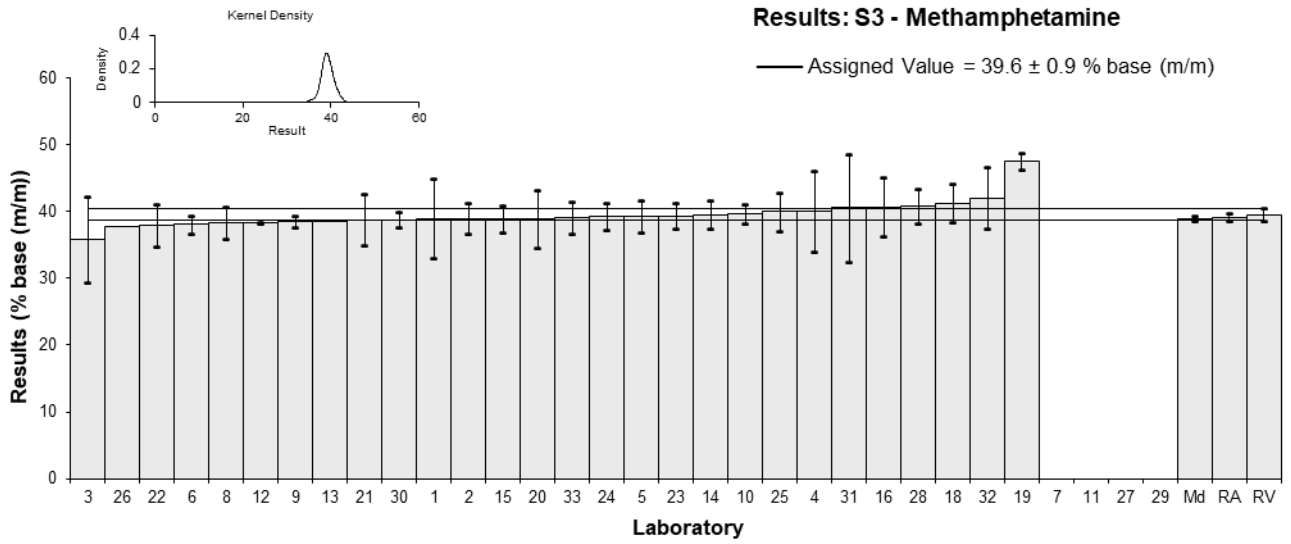
Participant Results

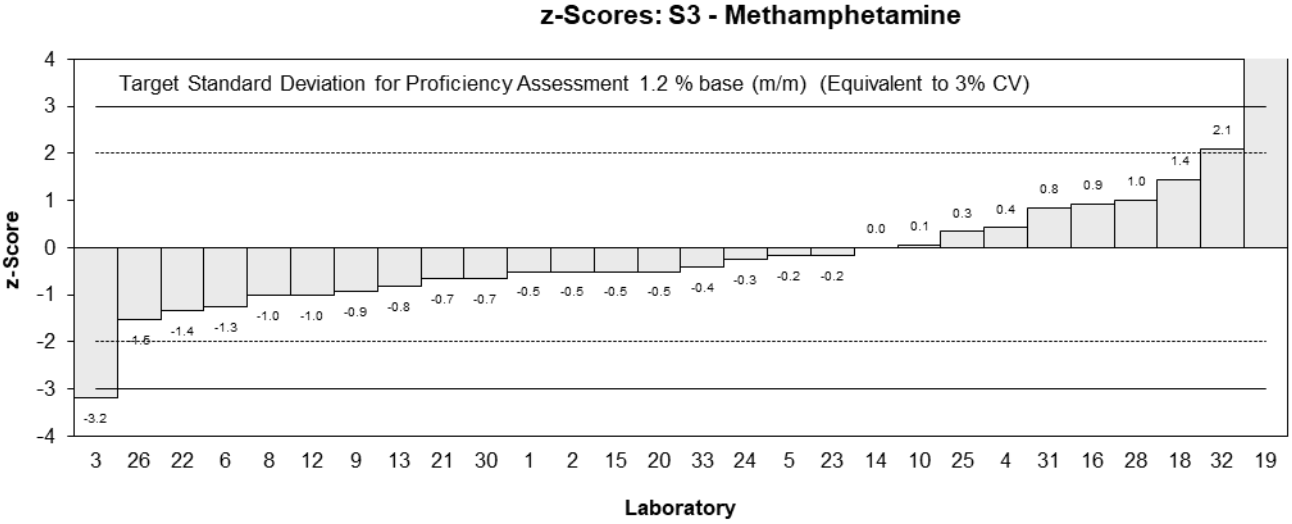
Lab. Code	Result	Uncertainty	z	E_n
1	39	5.9	-0.51	-0.10
2	39.0	2.3	-0.51	-0.24
3	35.8	6.4	-3.20	-0.59
4	40.1	6.1	0.42	0.08
5	39.4	2.4	-0.17	-0.08
6	38.1	1.4	-1.26	-0.90
7	NR	NR		
8	38.4	2.4	-1.01	-0.47
9	38.5	0.9	-0.93	-0.86
10	39.66	1.4	0.05	0.04
11	NR	NR		
12	38.4	0.06	-1.01	-1.33
13	38.627	NR	-0.82	-1.08
14	39.6	2.1	0.00	0.00
15	39.0	2.0	-0.51	-0.27
16	40.7	4.4	0.93	0.24
18	41.3	2.9	1.43	0.56
19**	47.6	1.2	6.73	5.33
20	39	4.3	-0.51	-0.14
21	38.8	3.88	-0.67	-0.20
22	38	3.12	-1.35	-0.49
23	39.4	2.0	-0.17	-0.09
24	39.3	2.0	-0.25	-0.14
25	40	2.8	0.34	0.14
26	37.8	NR	-1.52	-2.00
27	NR	NR		
28	40.8	2.6	1.01	0.44
29	NR	NR		
30	38.8	1.2	-0.67	-0.53
31	40.6	8.1	0.84	0.12
32	42.1	4.7	2.10	0.52
33	39.1	2.4	-0.42	-0.20

** Excluded Result, see Section 4.2

Statistics

Assigned Value	39.6	0.9	Assigned value is the reference value as determined by qNMR spectroscopy.
Reference Value	39.6	0.9	
Robust Average	39.2	0.5	
Median	39.0	0.4	
Mean	39.2		
N	27		
Max	42.1		
Min	35.8		
Robust SD	1.1		
Robust CV	2.8%		


Results: S3 - Methamphetamine
Assigned Value = 39.6 ± 0.9 % base (m/m)
Kernel Density
Density
Result
Results (% base (m/m))
Laboratory
3 26 22 6 8 12 9 13 21 30 1 2 15 20 33 24 5 23 14 10 25 4 31 16 28 18 32 19 7 11 27 29 Md RA RV


z-Scores: S3 - Methamphetamine
Target Standard Deviation for Proficiency Assessment 1.2 % base (m/m) (Equivalent to 3% CV)
z-Score
Laboratory
3 26 22 6 8 12 9 13 21 30 1 2 15 20 33 24 5 23 14 10 25 4 31 16 28 18 32 19

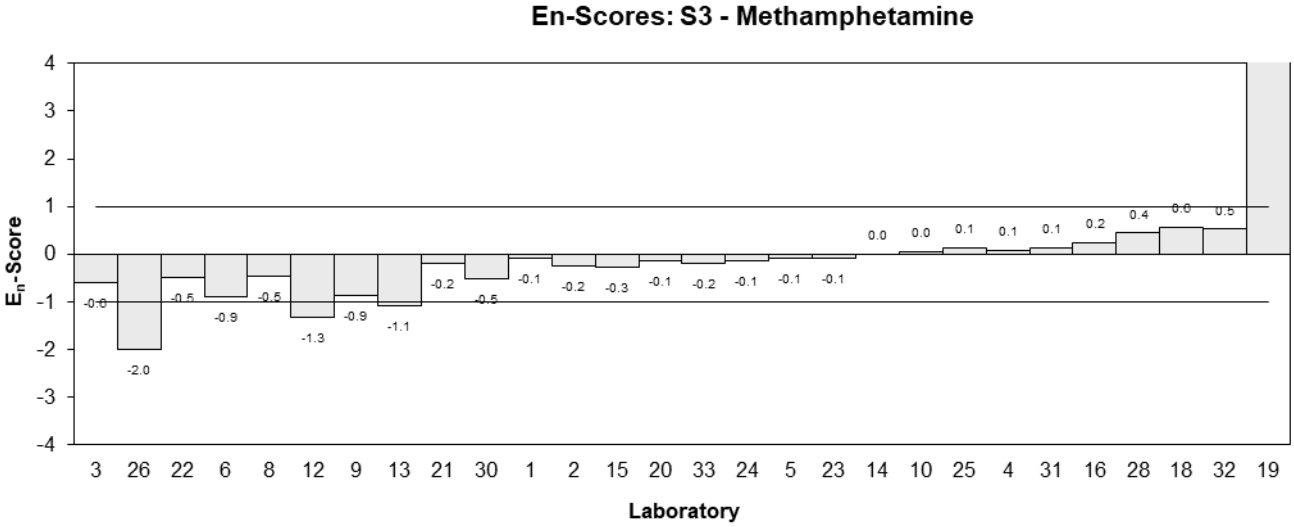

En-Scores: S3 - Methamphetamine
En-Score
Laboratory
3 26 22 6 8 12 9 13 21 30 1 2 15 20 33 24 5 23 14 10 25 4 31 16 28 18 32 19

Figure 4

Table 8

Sample Details

Sample No.	S4
Matrix	Powder
Analyte	Methamphetamine
Unit	% base (m/m)

Participant Results

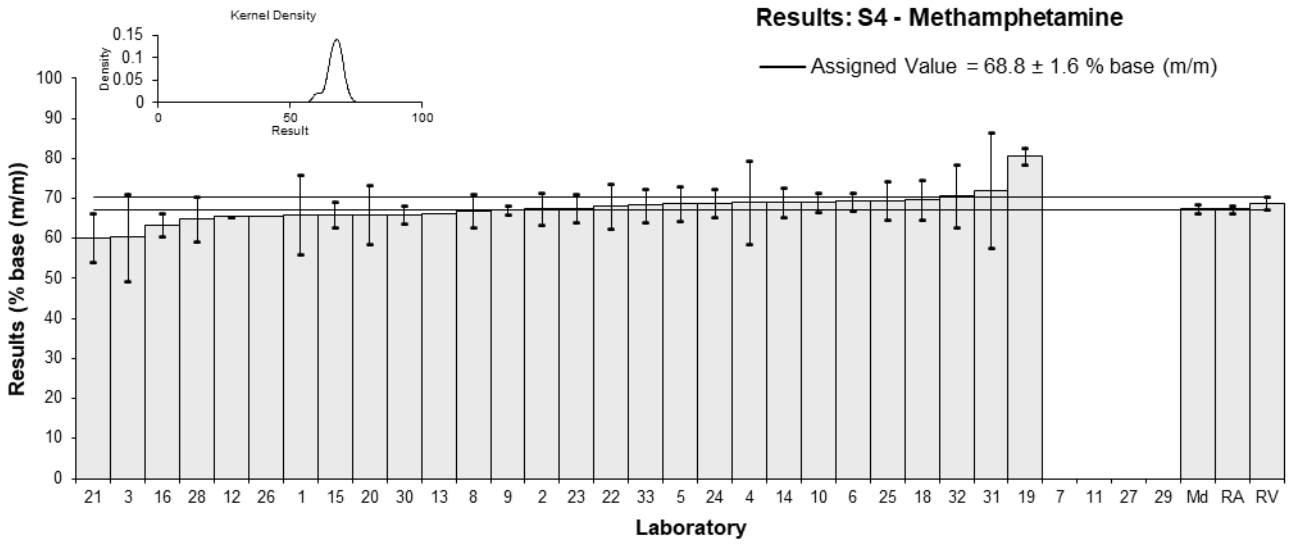
Lab. Code	Result	Uncertainty	z	E_n
1	66	9.9	-1.36	-0.28
2	67.4	4.0	-0.68	-0.32
3	60.3	10.9	-4.12	-0.77
4	69	10.4	0.10	0.02
5	68.7	4.2	-0.05	-0.02
6	69.3	2.2	0.24	0.18
7	NR	NR		
8	66.9	4.2	-0.92	-0.42
9	67.1	1.0	-0.82	-0.90
10	69.01	2.5	0.10	0.07
11	NR	NR		
12	65.4	0.09	-1.65	-2.12
13	66.099	NR	-1.31	-1.69
14	69.0	3.6	0.10	0.05
15	66.0	3.3	-1.36	-0.76
16	63.4	2.9	-2.62	-1.63
18	69.6	4.9	0.39	0.16
19**	80.7	2.1	5.77	4.51
20	66	7.3	-1.36	-0.37
21	60.2	6.02	-4.17	-1.38
22	68	5.59	-0.39	-0.14
23	67.6	3.4	-0.58	-0.32
24	68.8	3.5	0.00	0.00
25	69.5	4.9	0.34	0.14
26	65.4	NR	-1.65	-2.12
27	NR	NR		
28	64.8	5.6	-1.94	-0.69
29	NR	NR		
30	66	2.1	-1.36	-1.06
31	72	14.4	1.55	0.22
32	70.6	7.9	0.87	0.22
33	68.3	4.1	-0.24	-0.11

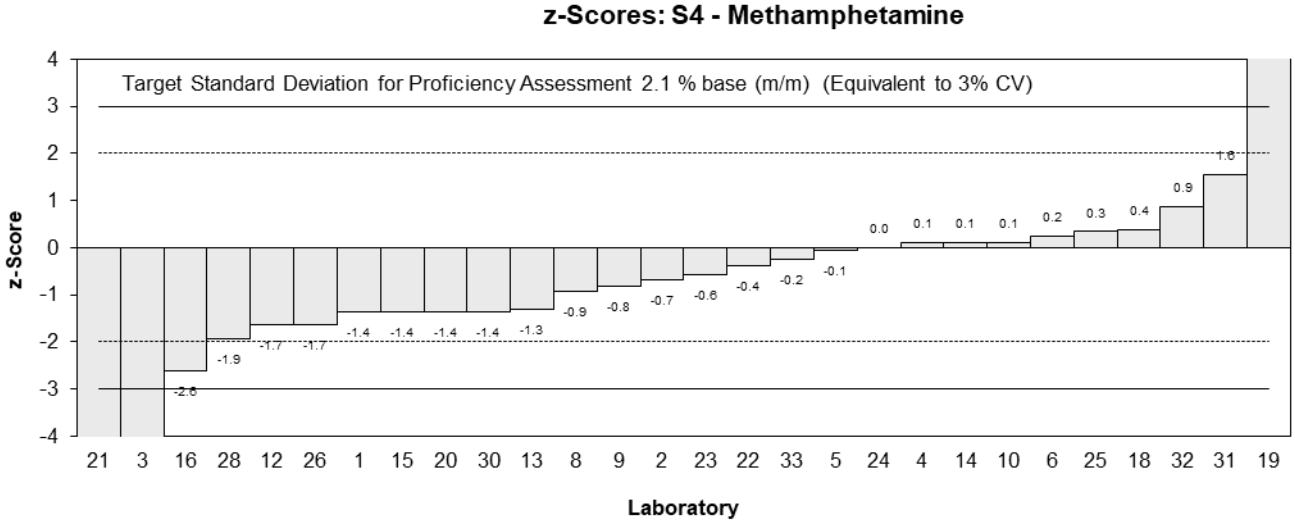
** Excluded Result, see Section 4.2

Statistics

Assigned Value	68.8	1.6
Reference Value	68.8	1.6
Robust Average	67.3	1.1
Median	67.4	1.1
Mean	67.1	
N	27	
Max	72	
Min	60.2	
Robust SD	2.3	
Robust CV	3.5%	

Assigned value is the reference value as determined by qNMR spectroscopy.


Kernel Density
Density
Result
Results: S4 - Methamphetamine
Assigned Value = 68.8 ± 1.6 % base (m/m)
Results (% base (m/m))
Laboratory
21 3 16 28 12 26 1 15 20 30 13 8 9 2 23 22 33 5 24 4 14 10 6 25 18 32 31 19 7 11 27 29 Md RA RV


z-Scores: S4 - Methamphetamine
Target Standard Deviation for Proficiency Assessment 2.1 % base (m/m) (Equivalent to 3% CV)
z-Score
Laboratory
21 3 16 28 12 26 1 15 20 30 13 8 9 2 23 22 33 5 24 4 14 10 6 25 18 32 31 19

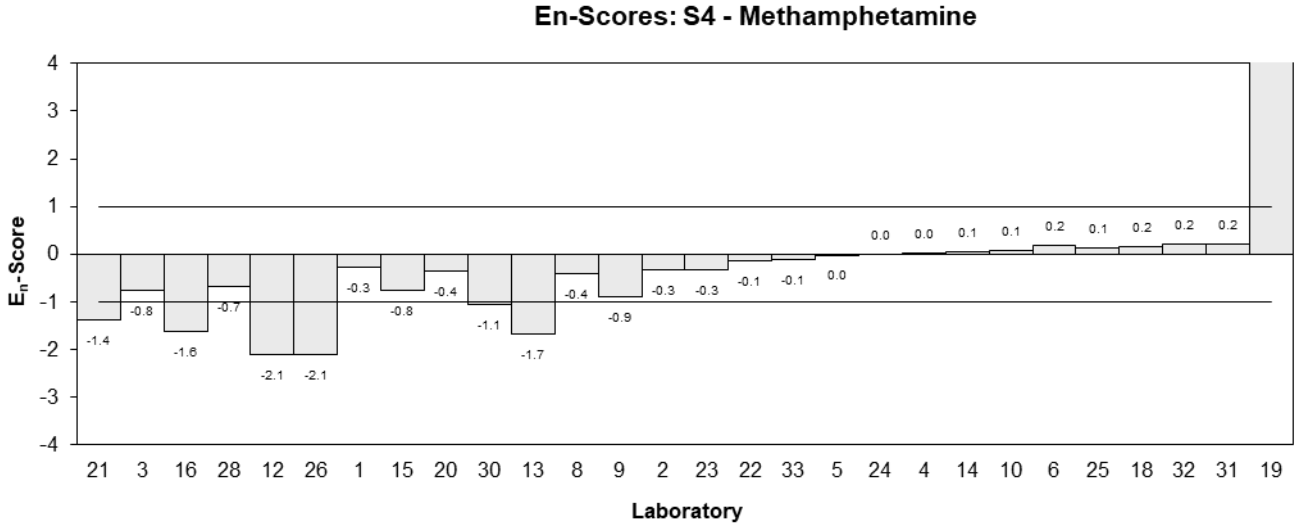

En-Scores: S4 - Methamphetamine
En-Score
Laboratory
21 3 16 28 12 26 1 15 20 30 13 8 9 2 23 22 33 5 24 4 14 10 6 25 18 32 31 19

Figure 5

Table 9 Participants' Identification of Cutting Agents*

Lab. Code	Cutting Agents			
	S1	S2	S3	S4
Preparation	Glucose	Niacinamide	Niacinamide	Dimethyl sulfone
1	none	none	none	none
2			Nicotinamide	
3		Niacinamide	Niacinamide	
4	Dextrose	Nicotinamide	Nicotinamide	Dimethylsulfone
5	Nil	Nicotinamide	Dimethyl sulfone, Nicotinamide	Dimethyl sulfone
6				
7		Nicotinamide	Nicotinamide	
8	glucose	nicotinamide	dimethylsulfone, nicotinamide	dimethylsulfone
9	None detected	Nicotinamide (indicated - not confirmed by a standard)	Nicotinamide (indicated - not confirmed by a standard)	Dimethylsulfone (indicated - not confirmed by a standard)
10	Sugars	Nicotinamide	Nicotinamide	Dimethyl sulfone
11	no adulterants detected	Niacinamide	Niacinamide	no adulterants detected
12		Niacinamide	Niacinamide	
13				
14		Niacinamide indicated.	Niacinamide indicated.	Methylsulfonylmethane (MSM) indicated.
15				
16	Glucose	Nicotinamide	Nicotinamide	Methane sulfonyl-bis
18		Nicotinamide	Nicotinamide	Dimethylsulfone
19	Saccharides	Niacinamide	Niacinamide	Dimethylsulfone
20			nicotinamide	
21	Dextrose	Niacinamide	Niacinamide	Dimethylsulfone
22	Dextrose	Niacinamide	Niacinamide	
23		NIACINAMIDE	NIACINAMIDE	
24	Glucose	Nicotinamide	Nicotinamide	Dimethyl sulfone
25	Not Determined	Not Determined	Not Determined	Not Determined
26	Glucose : 57.2 %	Niacinamide	Dimethyl sulfone Niacinamide	Dimethyl sulfone
27	Glucose	nicotinamide		
28				
29	splenda	nicotinamide	nicotinamide	
30	Glucose	niacinamide	niacinamide, dimethylsulfone	dimethylsulfone

Lab. Code	Cutting Agents			
	S1	S2	S3	S4
31	/	Niacinamide	Niacinamide, dimethylsulfone	Dimethylsulfone
32	other sub/s	nicotinamide	nicotinamide	
33	Nil	Nicotinamide	Dimethyl sulfone, Nicotinamide	Dimethyl sulfone

* Some responses may be modified so that the participant cannot be identified.

6 DISCUSSION OF RESULTS

6.1 Assigned Value

The reference values obtained using qNMR spectroscopy were used as the assigned values for all samples. Maleic acid (NMI CRM QNMR010) was used as the internal standard. The uncertainty of the reference value was estimated in accordance with the ISO GUM.[9] Additional details are given in Appendix 1.

Traceability: The measurements of the reference values were made using qNMR and are traceable to the SI through Australian Standards for mass via balance calibration certificates and the purity of the NMI maleic acid CRM (QNMR010, Batch No.: 10-Q-02).

6.2 Measurement Uncertainty Reported by Participants

Participants were asked to report an estimate of the expanded measurement uncertainty associated with their results and the basis of this uncertainty estimate. It is a requirement of ISO/IEC 17025 that laboratories have procedures to estimate the uncertainty of chemical measurements and to report this uncertainty in specific circumstances, including when the client's instruction so requires.[7]

Of 116 numeric results, 110 (95%) were reported with an associated expanded uncertainty. Participants used a wide variety of procedures to estimate their uncertainties (Table 2). One participant reported using the NATA GAG Estimating and Reporting MU as their guide; NATA no longer publishes this document.[10]

Laboratory **13** reported uncertainties for Samples S1 and S2 MDMA, however did not report uncertainties for Samples S3 and S4 methamphetamine; this participant reported that they were not accredited. Laboratory **26** did not report any uncertainties; this participant did not report if they were accredited or not.

For this PT study, participants were instructed to report uncertainties as % drug as base (m/m). Laboratory **7** reported all their uncertainties as '15'; this participant may have reported relative uncertainties instead.

The magnitudes of reported uncertainties were within the range 0.1% to 72% relative to the reported result. In general, an expanded uncertainty of less than 3% relative may be unrealistically small for a routine measurement, while an expanded uncertainty of over 10% relative may be too large and not fit for purpose. Of the 110 expanded MUs reported, ten were less than 3% relative, while 30 were greater than 10% relative.

Participants were also requested to report the coverage factor associated with their uncertainties (Table 2). Of the participants reporting coverage factors, most reported k = 2 at approximately 95% confidence level (14 participants). Three participants reported k = 3 at approximately 99% confidence level. One participant, Laboratory **18**, reported k = 1 at approximately 68% confidence level; therefore this participant's uncertainties were reported as standard uncertainty rather than expanded uncertainty as requested for this PT study.

Uncertainties associated with results returning an acceptable *z*-score but an unacceptable E_n-score may have been underestimated.

In some cases, results were reported with an inappropriate number of significant figures. Including too many significant figures may inaccurately reflect the precision of measurements. The recommended format is to write the uncertainty to no more than two significant figures and then to write the result with the corresponding number of decimal places. For example, instead of 42.09 ± 6.31%, it is recommended to report 42.1 ± 6.3%.[8]

6.3 z-Score

Target SDs equivalent to 3% PCV were used to calculate *z*-scores for all samples. The CVs predicted by the Thompson-Horwitz equation,[6] between-laboratory CVs and target SDs (as PCV) obtained in this study are presented for comparison in Table 10.

Table 10 Comparison of Thompson-Horwitz CVs, Between-Laboratory CVs and Target SDs

Sample	Analyte	Assigned Value (% base (m/m))	Thompson-Horwitz CV (%)	Between-Laboratory CV* (%)	Target SD (as PCV) (%)
S1	MDMA	21.8	2.1	5.0	3
S2	MDMA	40.7	1.6	3.8	3
S3	Methamphetamine	39.6	1.6	2.8	3
S4	Methamphetamine	68.8	1.2	3.5	3

* Robust between-laboratory CV with outliers removed, if applicable.

Of 116 results for which *z*-scores were calculated, 94 (81%) returned a *z*-score of $|z| \leq 2.0$, indicating an acceptable performance.

Eighteen participants: **2** (methamphetamine only), **4**, **5**, **7** (MDMA only), **8**, **9**, **10**, **12**, **13**, **14**, **22**, **23**, **24**, **25**, **27** (MDMA only), **29** (MDMA only), **30** and **33** returned acceptable *z*-scores for all reported numeric results.

Laboratory **11** did not report any numeric results for this study and therefore did not receive any *z*-scores.

Thirteen participants returned at least one questionable or unacceptable *z*-score. Laboratory **3** returned questionable or unacceptable *z*-scores for all samples (positive bias for MDMA and negative bias for methamphetamine). Laboratory **19** returned questionable or unacceptable *z*-scores for all samples (positive bias for all); after the release of the Preliminary Report, this participant reported that they had reported their results were in units of % hydrochloride salt (m/m) instead of % base (m/m).

The dispersal of participants' *z*-scores is presented graphically in Figure 6.

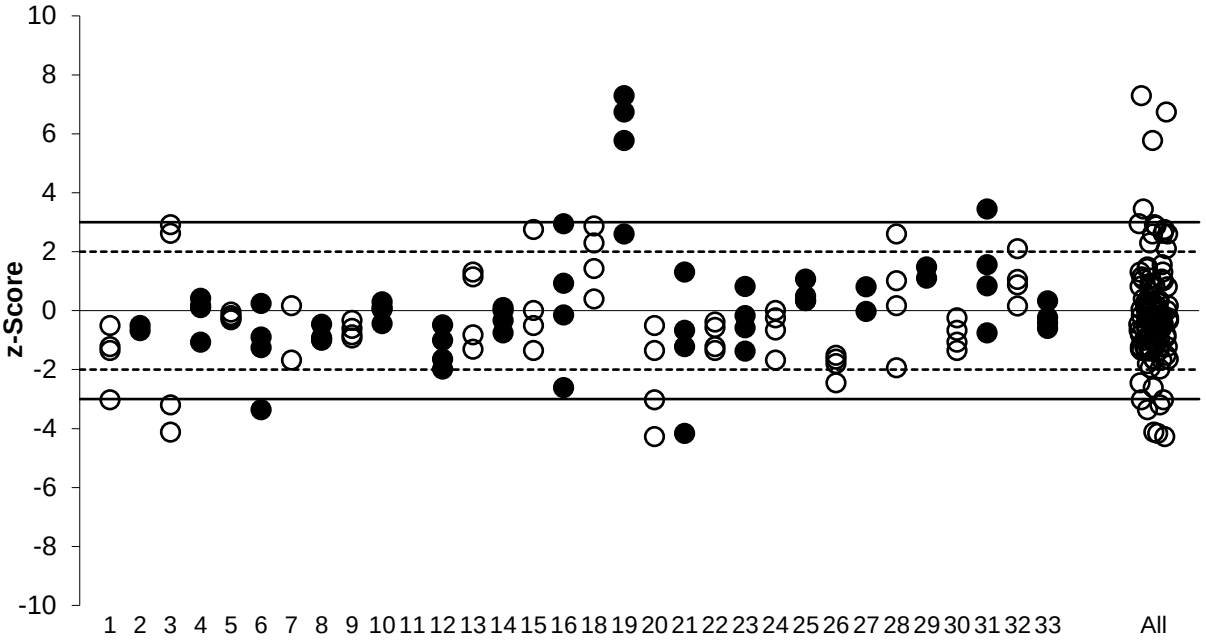
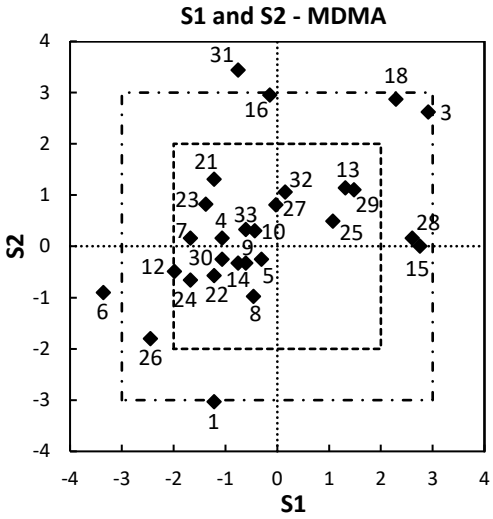


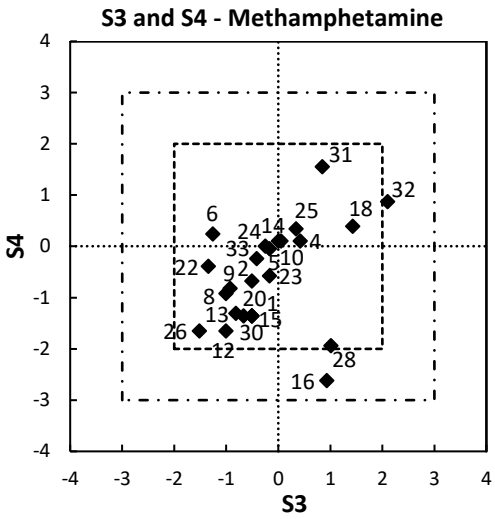
Figure 6 *z*-Score Dispersal by Laboratory

Scatter plots of *z*-scores for MDMA in Samples S1 and S2, and methamphetamine in Samples S3 and S4, are presented in Figures 7 and 8 respectively. Scores are predominantly in the upper right and lower left quadrants, indicating that laboratory bias is the major contributor to the variability of results. Points close to the diagonal axis demonstrate excellent repeatability, while points close to the zero demonstrate excellent repeatability and accuracy.



Laboratories **19** and **20** are off-scale.

Figure 7 *z*-Score Scatter Plot – MDMA



Laboratories **3**, **19** and **20** are off-scale.

Figure 8 *z*-Score Scatter Plot – Methamphetamine

6.4 E_n-Score

E_n-scores can be interpreted in conjunction with *z*-scores, as an unacceptable E_n-score can either be caused by issues with measurement, or uncertainty, or both. Where a participant did not report an expanded uncertainty with a result, an expanded uncertainty of zero (0) was used to calculate the E_n-score.

Of 116 results for which E_n-scores were calculated, 96 (83%) returned an E_n-score of $|E_n| < 1.0$, indicating agreement of the participant's result with the assigned value within their respective uncertainties.

Twenty-one participants: **1**, **2** (methamphetamine only), **3**, **4**, **5**, **7** (MDMA only), **8**, **9**, **10**, **14**, **18**, **22**, **23**, **24**, **25**, **27** (MDMA only), **28**, **29** (MDMA only), **31**, **32** and **33** returned acceptable E_n-scores for all reported numeric results.

Ten participants returned at least one unacceptable E_n-score. Laboratories **19** and **26** returned unacceptable E_n-scores for all samples.

The dispersal of participants' E_n-scores is presented graphically in Figure 9.

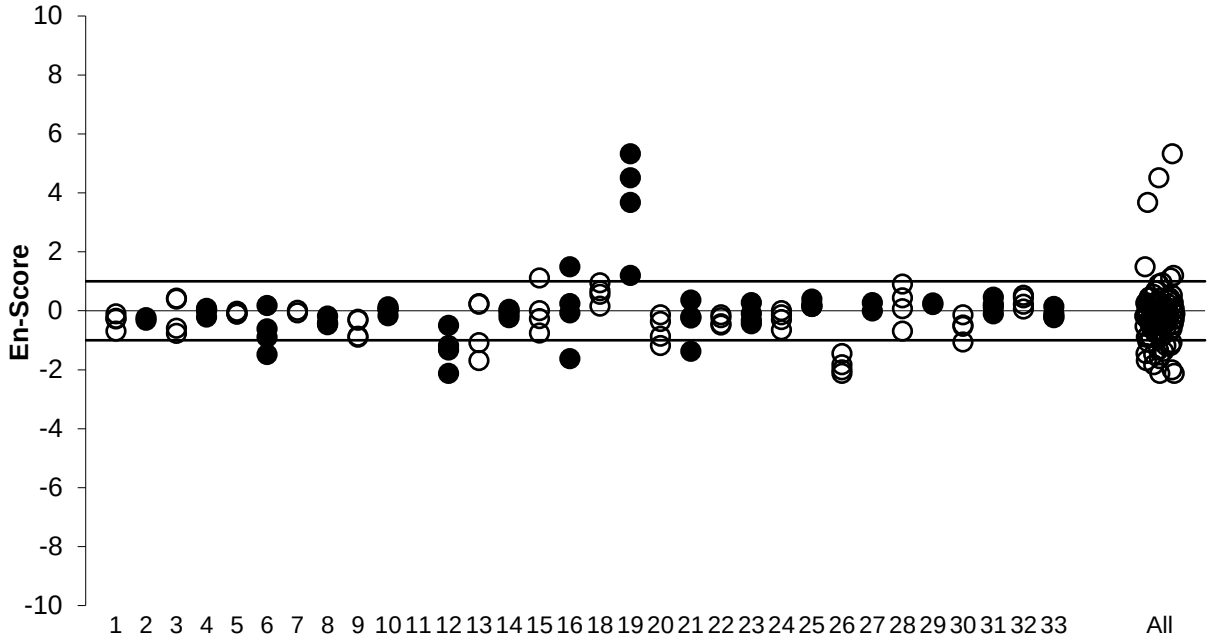


Figure 9 E_n-Score Dispersal by Laboratory

6.5 Identification of Cutting Agents

Samples S1 and S2 were prepared by adding glucose and niacinamide respectively to MDMA hydrochloride. Samples S3 and S4 were prepared by adding niacinamide and dimethyl sulfone respectively to methamphetamine hydrochloride.

Participants were requested to identify the cutting agents in the samples if part of their routine analysis, and the results reported are presented in Table 9.

Twenty-six participants (81%) reported on the identity of at least one cutting agent in the samples.

Laboratories **4**, **8**, **16**, **21**, **24**, **26** and **30** correctly identified all cutting agents in this study.

For Sample S1, 9 participants correctly identified glucose as the cutting agent (Laboratories **4**, **8**, **16**, **21**, **22**, **24**, **26**, **27** and **30**). Laboratories **10** and **19** reported the more general 'sugars' and 'saccharides' respectively as the cutting agent. Laboratory **29** reported 'splenda', which contains sucralose, a compound structurally related to glucose.

For Samples S2 and S3, most participants were able to correctly identify niacinamide as the cutting agent: Laboratories **2** (S3 only), **3**, **4**, **5**, **7**, **8**, **9**, **10**, **11**, **12**, **14**, **16**, **18**, **19**, **20** (S3 only), **21**, **22**, **23**, **24**, **26**, **27** (S2 only), **29**, **30**, **31**, **32** and **33**.

Also, for Sample S3, six participants (Laboratories **5**, **8**, **26**, **30**, **31** and **33**) reported dimethyl sulfone as a cutting agent. This may have been a small impurity in the original methylamphetamine matrix.

For Sample S4, 15 participants correctly identified dimethyl sulfone as the cutting agent (Laboratories **4**, **5**, **8**, **9**, **10**, **14**, **16**, **18**, **19**, **21**, **24**, **26**, **30**, **31** and **33**).

6.6 Participants' Analytical Methods

Participants were requested to analyse the samples using their routine test methods and to report a single result for each sample as they would normally report to a client. Results reported in this way reflect the true variability of results reported to laboratory clients. The method descriptions provided by participants are presented in Table 1.

A summary of accreditation status, participants' methods and reference standards' sources is presented in Table 11.

Table 11 Summary of Participants' Analytical Methods

		Lab. Code
Accreditation	Yes to ISO/IEC 17025	1, 2 (meth), 5, 6, 7 (MDMA), 8, 9, 11 (ID only), 14, 15 (meth), 16 (ID only), 18, 21, 22, 23, 24, 25, 27 (MDMA), 30, 32, 33
	Not accredited / NR	3, 4, 10, 12, 13, 15 (MDMA), 19, 20, 26, 28, 29 (MDMA), 31
Average Sample Mass Used (mg)	< 20	2 (meth), 11, 13, 21 (meth), 22, 23, 24, 25, 29 (MDMA), 30, 32 (meth)
	20 – 30	4, 5, 6, 7 (MDMA), 10, 15 (MDMA), 16, 20, 21 (MDMA), 27 (MDMA), 28, 33
	31 – 50	1, 9, 12, 14, 15 (meth), 18, 19, 26, 31
	51 – 100	8
	> 100	32 (MDMA)
	NR	3
Instrument Used for Quantification	HPLC-DAD	2 (meth), 4, 12, 13 (meth), 15, 16, 19, 20, 22, 29 (MDMA)
	HPLC-UV/Vis	7 (MDMA), 21 (MDMA), 25
	UPLC-DAD	5, 6, 8, 14, 18, 26, 33
	UPLC-UV/Vis	9, 21 (meth), 32
	UPLC-MS/MS	1
	GC-FID	3, 10, 23, 24, 27 (MDMA), 28
	GC-MS	11
	QNMR	13 (MDMA), 30, 31
Solvent	Acetonitrile(/Water/Other)	1, 2 (meth), 4, 7 (MDMA), 9, 13 (meth), 16, 22
	Methanol	3, 6, 8, 12, 18, 19, 20, 23, 25, 27 (MDMA)
	Ethanol	26, 28
	Water	5, 15, 21, 32, 33
	Other	10, 11, 13 (MDMA), 14, 24, 29 (MDMA), 30, 31
Sources of Calibration Standard (MDMA)	NMI Australia	6, 8, 14, 19, 21, 22, 24, 25, 26
	Lipomed	3, 5, 7, 9, 10, 16, 18, 23, 28, 29, 33
	LGC	4, 27
	Sigma Aldrich	11, 13
	Other / NR	1, 12, 15, 20, 30, 31, 32
Sources of Calibration Standard (methamphetamine)	NMI Australia	2, 4, 6, 8, 9, 14, 19, 21, 22, 24, 25, 26
	Lipomed	3, 5, 10, 13, 16, 18, 23, 28, 33
	Sigma Aldrich	1, 11, 15, 20
	Other / NR	12, 30, 31, 32

Plots of the *z*-scores versus various parameters are presented in Figures 10 to 13 (results removed from statistical calculations in Section 5 have not been included). A variety of methodologies were used by participants in this study, and no significant trends were observed.

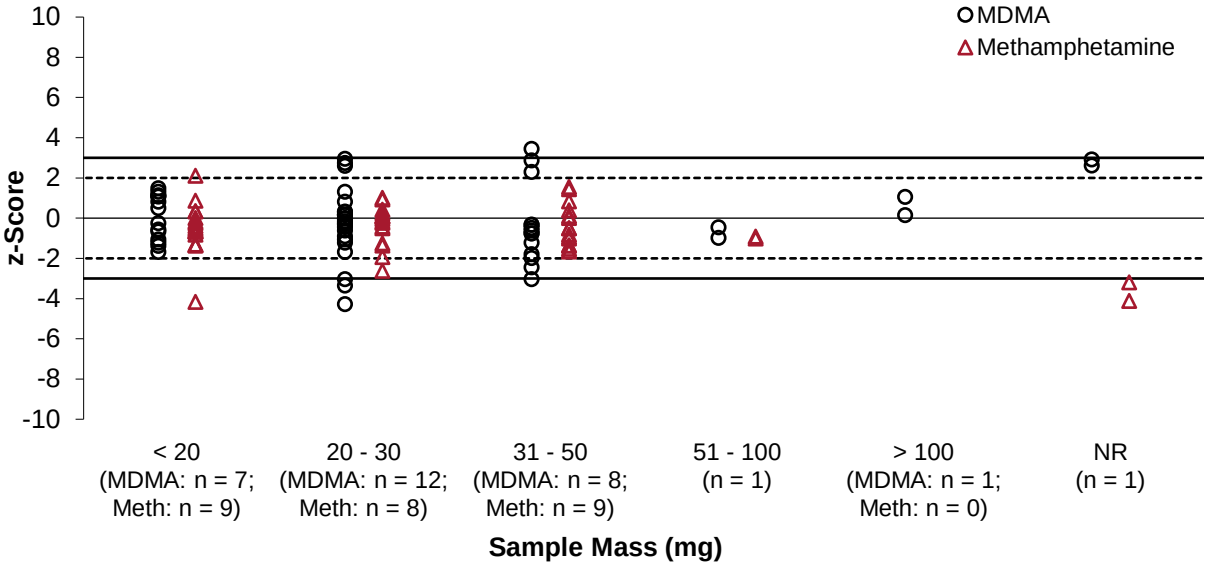


Figure 10 *z*-Score vs Sample Mass Used for Analysis

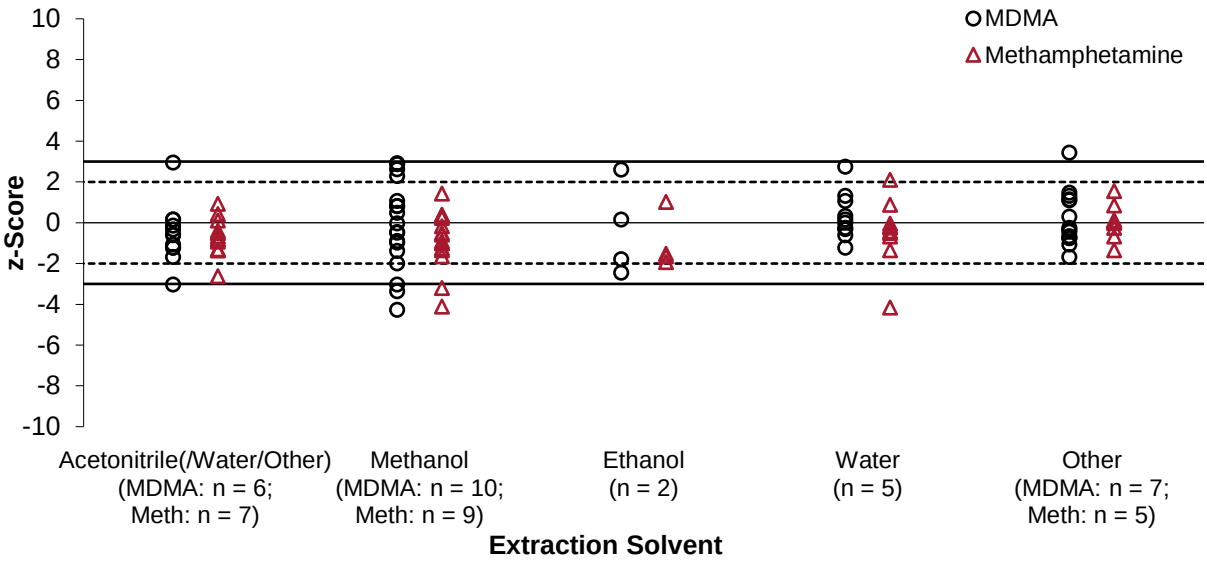


Figure 11 *z*-Score vs Extraction Solvent

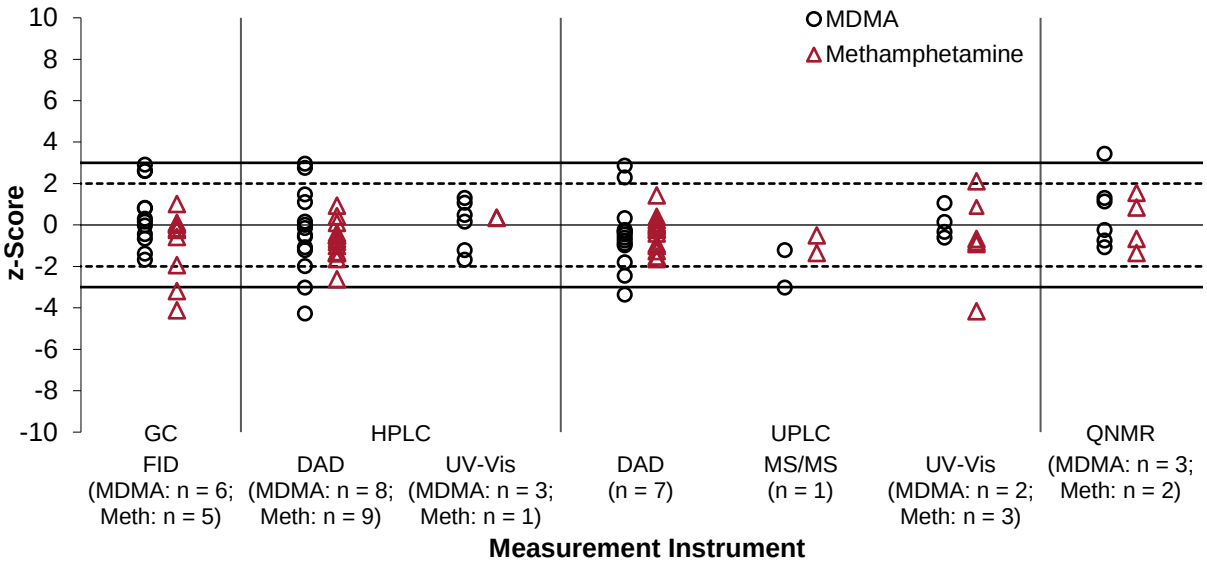


Figure 12 *z*-Score vs Measurement Instrument

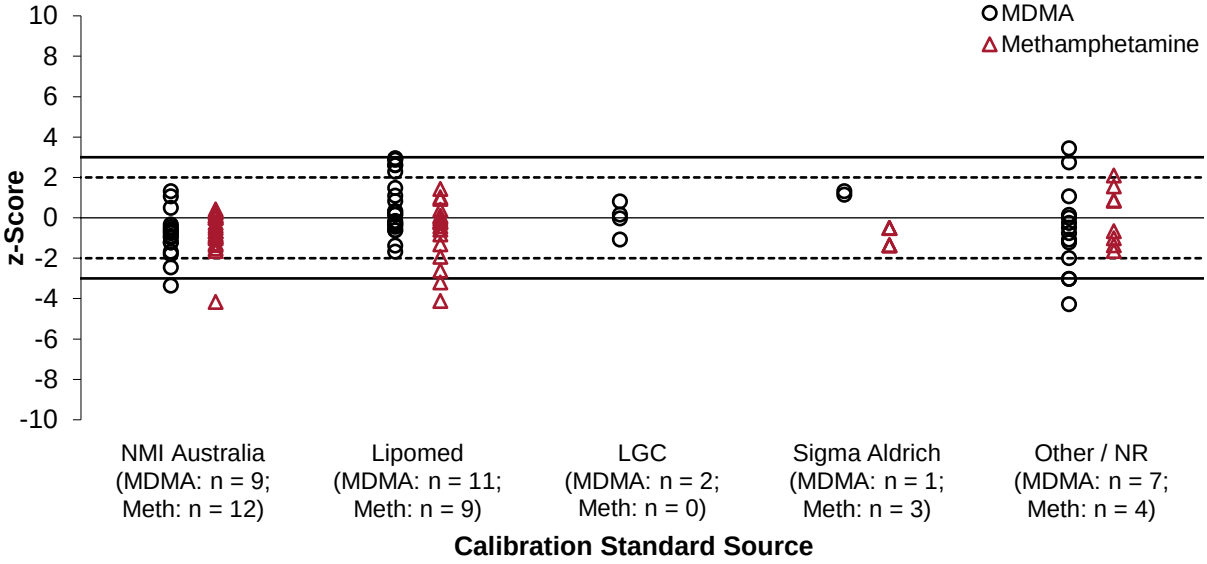


Figure 13 *z*-Score vs Calibration Standard Source

6.7 Comparison of Results and Date of Analysis

As there were delays with sample delivery to some participants, the samples were analysed by participants over approximately 3.5 months. No trend was found between when the samples were analysed and the results obtained (Figure 14; results removed from statistical calculations in Section 5 have not been included).

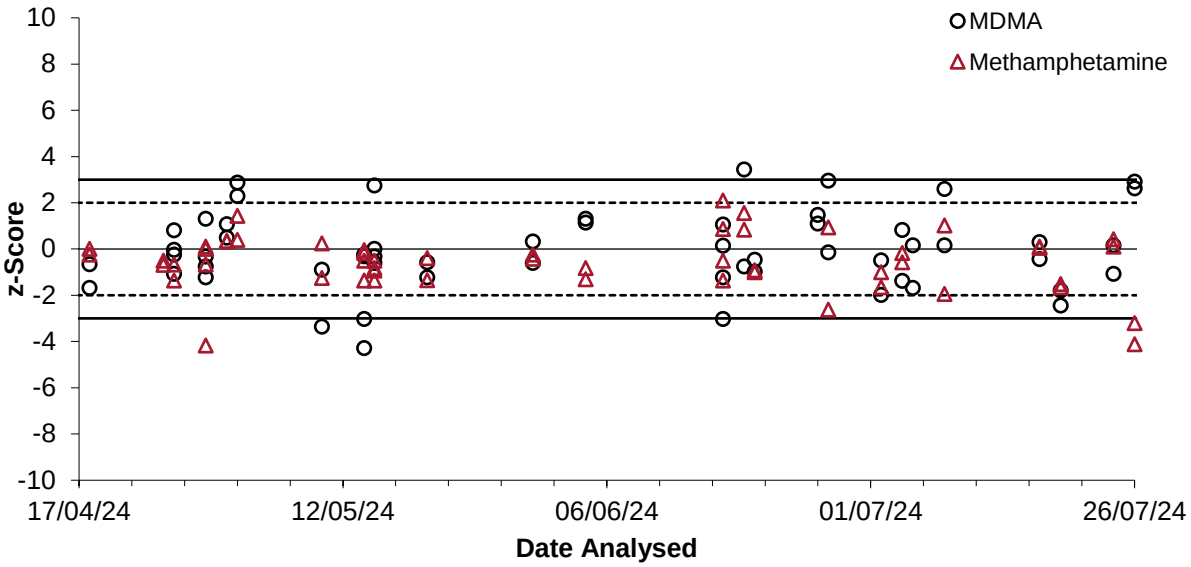


Figure 14 *z*-Score vs Sample Analysis Date

6.8 Comparison with Previous PT Studies

A summary of the acceptable performance, presented as a percentage of the total number of scores, obtained by PT study participants for MDMA from 2002 – 2024 (last ten studies with MDMA) is presented in Figure 15. The average proportion of acceptable *z*-scores and E_n-scores over this period is 77% and 71% respectively.

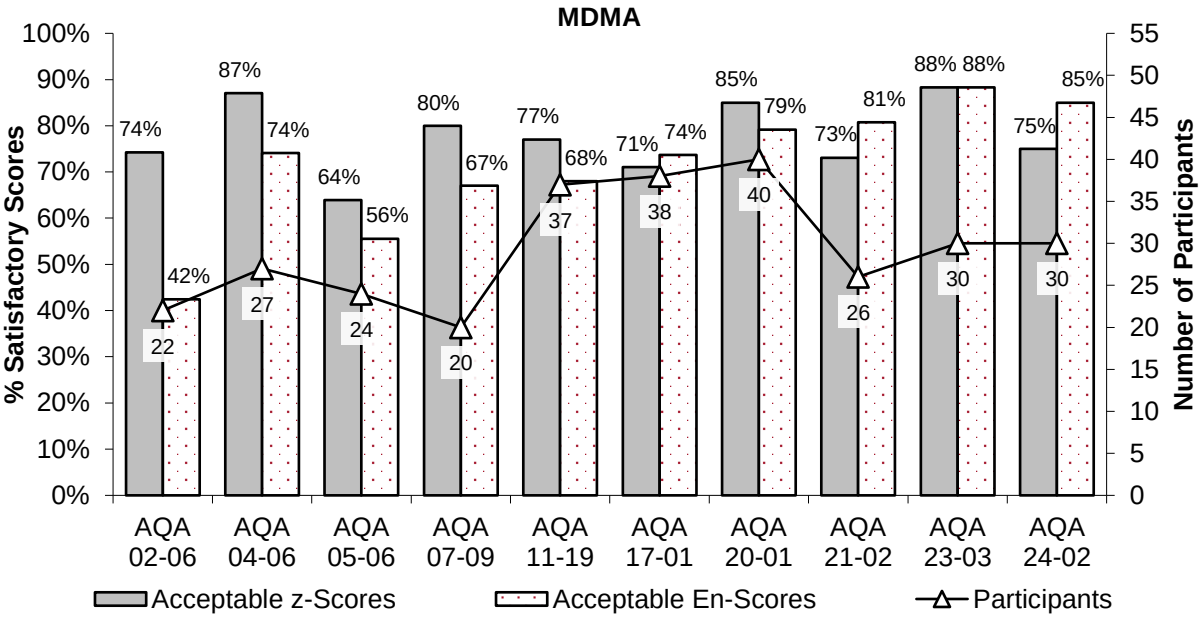
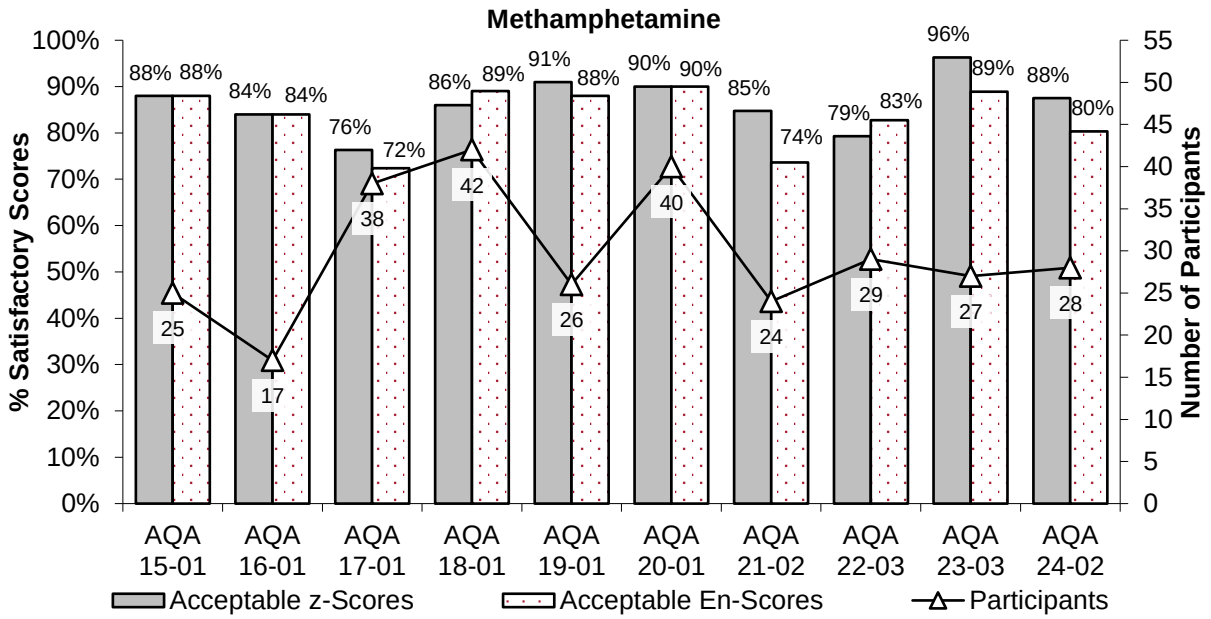


Figure 15 Summary of Participants' Performance in NMI MDMA PT Studies

A summary of the acceptable performance, presented as a percentage of the total number of scores, obtained by PT study participants for methamphetamine from 2015 – 2024 (last ten studies with methamphetamine) is presented in Figure 16. The average proportion of acceptable *z*-scores and E_n-scores over this period is 86% and 84% respectively. Overall, participants' performance with methamphetamine quantitation has been better than for MDMA.



One sample in AQA 23-03 was scored using 5% PCV; all other samples included in this chart were scored using 3% PCV.

Figure 16 Summary of Participants' Performance in NMI Methamphetamine PT Studies

A number of participants have consistently participated in NMI MDMA and methamphetamine PT studies, and individual performance history reports are emailed to each participant at the end of every PT study. The consideration of *z*-scores for an analyte over time provides much more useful information than a single *z*-score. Over time, laboratories should expect at least 95% of their scores to lie within the range $|z| \leq 2.0$. Scores in the range $2.0 < |z| < 3.0$ can occasionally occur, however, these should be interpreted in conjunction with the other scores obtained by that laboratory. For example, a trend of *z*-scores on one side of the zero line is an indication of method or laboratory bias.

A summary of individual laboratory's performances over the last six NMI MDMA PT studies is presented in Figures 17 and 18 for Australian and international laboratories respectively. Three Australian and three international laboratories have achieved acceptable *z*-scores across all MDMA samples in PT studies participated in over this period.

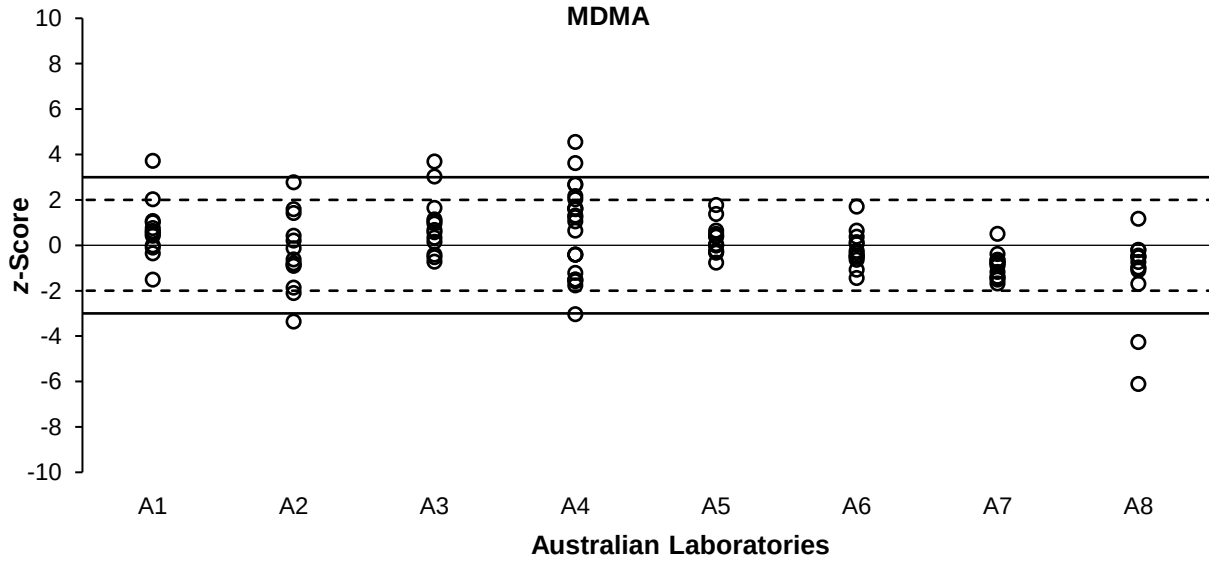
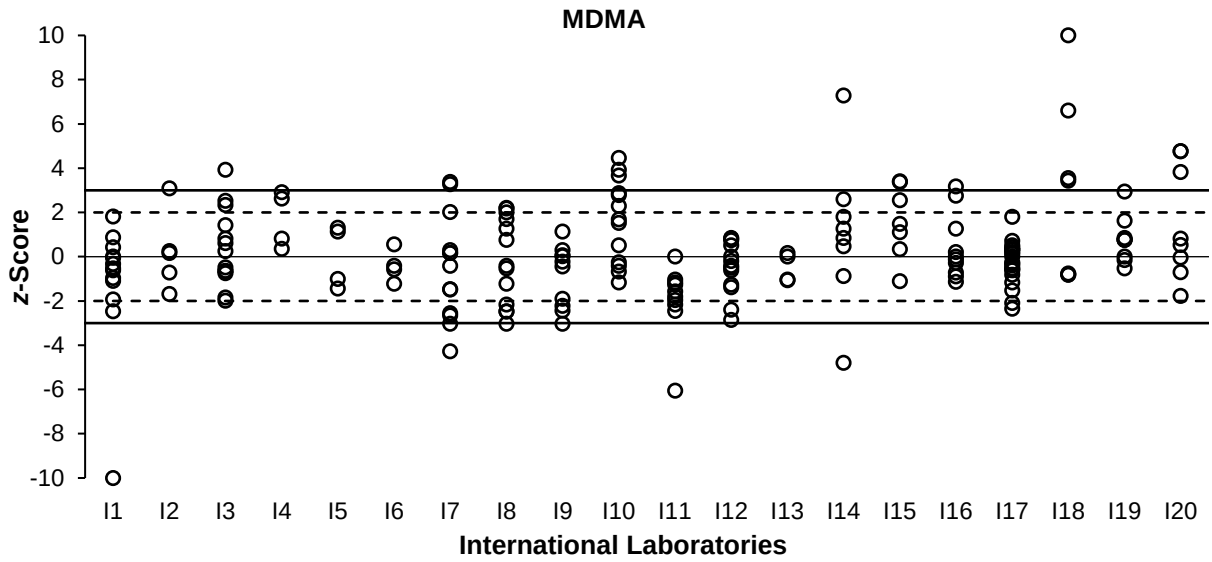


Figure 17 Summary of Australian Participants' *z*-Scores in NMI MDMA PT Studies



z-Scores greater than 10.0 or less than -10.0 have been plotted at 10.0 or -10.0 respectively.

Figure 18 Summary of International Participants' *z*-Scores in NMI MDMA PT Studies

A comparison of all results from Australian and international laboratories in NMI MDMA PT studies over the last six studies is presented in Figure 19. Overall, Australian laboratories have achieved a higher proportion of acceptable *z*-scores over this period.

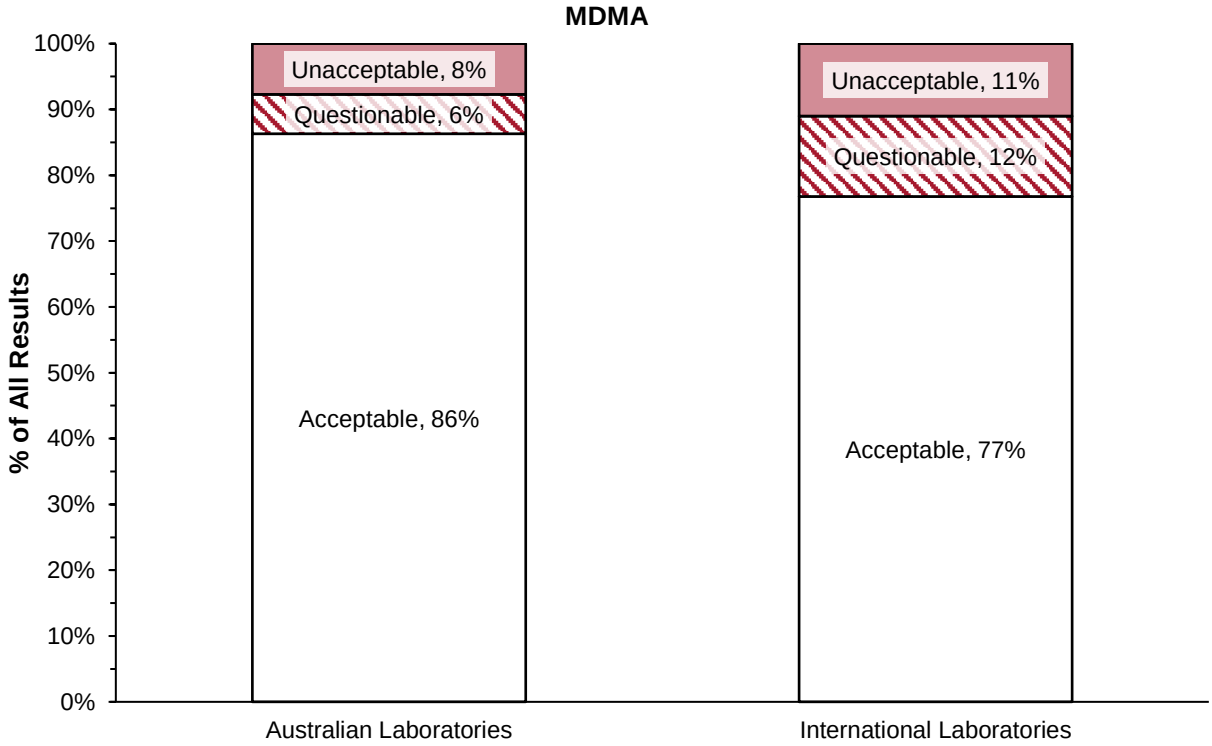


Figure 19 Comparison of Australian and International Laboratories in NMI MDMA PT Studies

A summary of individual laboratory's performances over the last ten NMI methamphetamine PT studies is presented in Figures 20 and 21 for Australian and international laboratories respectively (laboratory identifiers are not the same as for Figures 17 and 18). Four Australian and three international laboratories have achieved acceptable *z*-scores across all methamphetamine samples in PT studies participated in over this period.

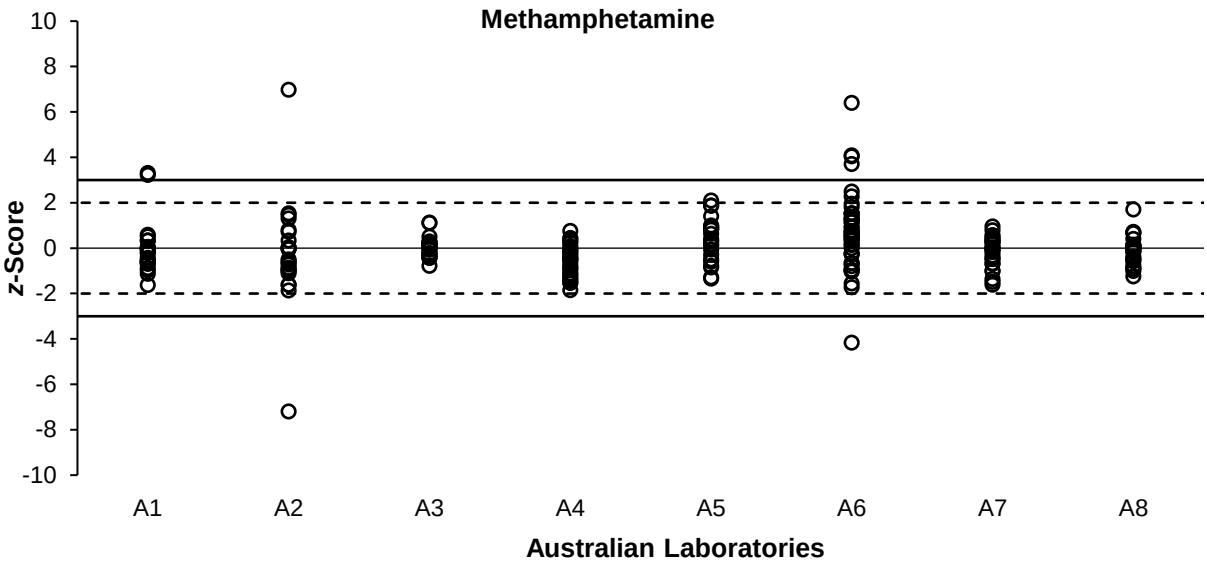


Figure 20 Summary of Australian Participants' *z*-Scores in NMI Methamphetamine PT Studies

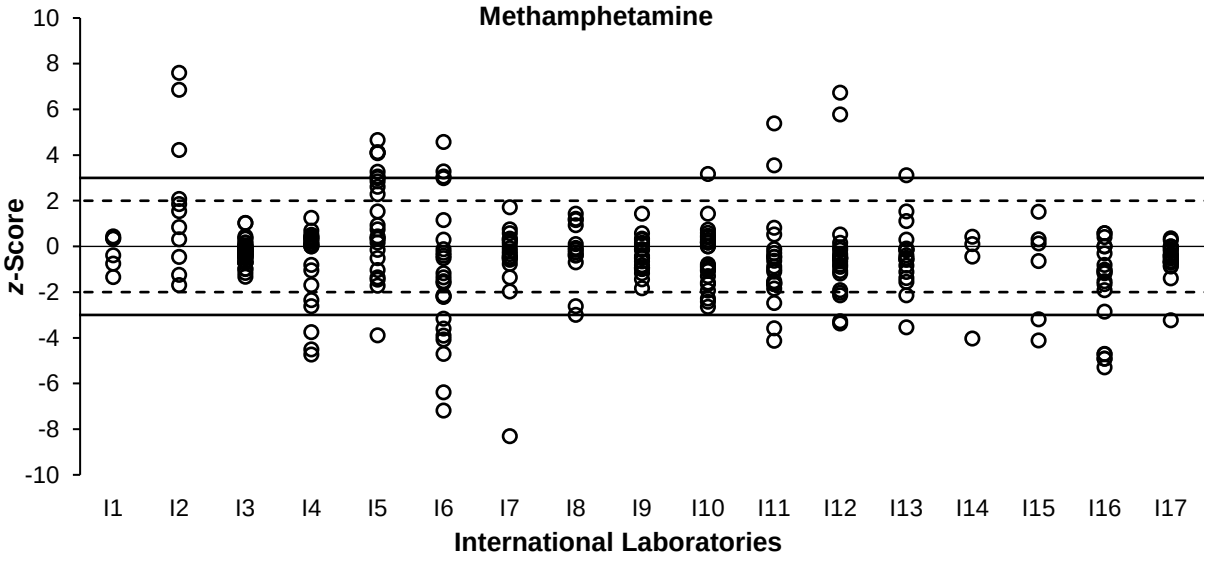
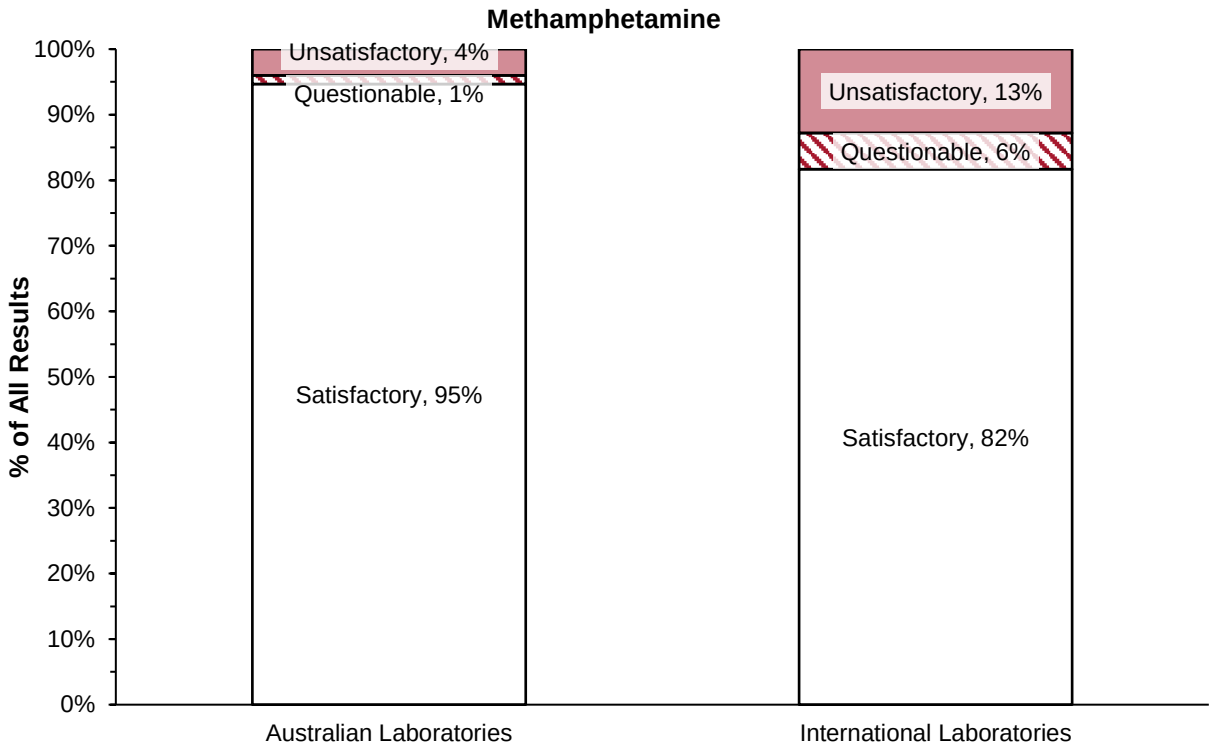


Figure 21 Summary of International Participants' *z*-Scores in NMI Methamphetamine PT Studies

A comparison of all results from Australian and international laboratories in NMI methamphetamine PT studies over the last ten studies is presented in Figure 22. Overall, Australian participants have performed well, and also have had a higher proportion of acceptable *z*-scores over this period.



Percentages have been rounded to the nearest whole number and therefore may not total 100%.

Figure 22 Comparison of Australian and International Laboratories in NMI Methamphetamine PT Studies

7 REFERENCES

Please note that for all undated references, the latest edition of the referenced document (including any amendments) applies.

[1] ISO/IEC 17043, *Conformity assessment — General requirements for the competence of proficiency testing providers*.

[2] Commonwealth of Australia, Department of Industry, Science and Resources, NMI, 2024, *Study Protocol for Proficiency Testing*, viewed September 2024, <https://www.industry.gov.au/sites/default/files/2020-10/cpt_study_protocol.pdf>.

[3] Commonwealth of Australia, Department of Industry, Science and Resources, NMI, 2024, *Chemical Proficiency Testing Statistical Manual*, viewed September 2024, <https://www.industry.gov.au/sites/default/files/2019-07/cpt_statistical_manual.pdf>.

[4] Thompson, M., Ellison, S.L.R. and Wood, R., 2006, 'The International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories', *Pure Appl. Chem.*, vol. 78, pp. 145-196.

[5] ISO 13528, *Statistical methods for use in proficiency testing by interlaboratory comparison*.

[6] Thompson, M., 2000, 'Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing', *Analyst*, vol. 125, pp. 385-386.

[7] ISO/IEC 17025, *General requirements for the competence of testing and calibration laboratories.*

[8] Eurachem/CITAC Guide CG 4, QUAM:2012.P1, *Quantifying Uncertainty in Analytical Measurement*, 3rd edition, viewed September 2024, <http://www.eurachem.org/images/stories/Guides/pdf/QUAM2012_P1.pdf>.

[9] ISO/IEC Guide 98-3, *Uncertainty of measurement – Part 3: Guide to the expression of uncertainty in measurement (GUM:1995)*.

[10] NATA, 2020, *Update to Measurement Uncertainty resources*, viewed September 2024, <https://nata.com.au/news/update-to-measurement-uncertainty-resources/>

APPENDIX 1 REFERENCE VALUES

Three sample vials from each of Samples S1, S2, S3 and S4 were analysed in duplicate for the purpose of assigning reference values. Measurements were made using qNMR spectroscopy with maleic acid as the internal standard. A maleic acid CRM was obtained from NMI, Chemical Reference Materials. The purity data supplied with the material is shown in Table 12 and is traceable to the SI unit for mass, the kilogram (kg). Internal standard solutions were prepared gravimetrically in D_2O.

Table 12 Maleic Acid CRM Details

Supplier	Catalogue No.	Batch No.	Purity (95% confidence)
NMI, Chemical Reference Materials	QNMR010	10-Q-02	98.8 ± 0.12%

Samples were prepared gravimetrically, by accurately weighing approximately 20 mg of sample and dissolving this in 900 µL of internal standard solution which was also accurately weighed. Samples were analysed on a Bruker Ascend 500 MHz NMR spectrometer, using a qNMR relaxation time of 25 s. The mass fraction of MDMA and methamphetamine was determined from the NMR response at around 1.24 ppm and 1.30 ppm respectively.

The averages of the mass fractions determined for the different vials of each sample (Tables 13 to 16) were used as the reference values and the assigned values. The standard uncertainty on the mass fraction reference value was estimated in accordance with the ISO GUM,[9] by combining standard uncertainty terms for method precision, sample homogeneity, weighing of sample, preparation and addition of standard solution, the very small uncertainties in molecular weights, an estimate of potential interference bias made by comparing the results from different NMR signals, and the between-batch variation. A coverage factor, k, was calculated using effective degrees of freedom derived from the Welch-Satterthwaite equation.

The measured reference values for all samples were in agreement with the robust averages of participants' results, within their respective associated uncertainties.

Table 13 Reference Value for Sample S1

Vial No.	MDMA (% base (m/m))	
	Replicate 1	Replicate 2
115	21.8	21.3
124	22.0	21.6
132	21.6	22.6
Average	21.8	
CV	2.0%	

Sample S1 Reference Value: 21.8 ± 1.1% MDMA base (m/m)

The uncertainty is an expanded uncertainty at 95% confidence level (k = 2.3).[9]

Table 14 Reference Value for Sample S2

Vial No.	MDMA (% base (m/m))	
	Replicate 1	Replicate 2
206	40.8	41.0
213	40.6	40.9
227	40.2	40.3
Average	40.7	
CV	0.83%	

Sample S2 Reference Value: 40.7 ± 1.2% MDMA base (m/m)

The uncertainty is an expanded uncertainty at 95% confidence level (k = 2.2).[9]

Table 15 Reference Value for Sample S3

Vial No.	Methamphetamine (% base (m/m))	
	Replicate 1	Replicate 2
309	39.5	39.6
314	40.2	39.5
333	39.3	39.5
Average	39.6	
CV	0.80%	

Sample S3 Reference Value: 39.6 ± 0.9% methamphetamine base (m/m)

The uncertainty is an expanded uncertainty at 95% confidence level (k = 2.1).[9]

Table 16 Reference Value for Sample S4

Vial No.	Methamphetamine (% base (m/m))	
	Replicate 1	Replicate 2
405	68.5	68.8
422	68.9	69.4
432	68.2	68.9
Average	68.8	
CV	0.59%	

Sample S4 Reference Value: 68.8 ± 1.6% methamphetamine base (m/m)

The uncertainty is an expanded uncertainty at 95% confidence level (k = 2.1).[9]

APPENDIX 2 ROBUST AVERAGE AND ASSOCIATED UNCERTAINTY, z-SCORE AND E_n-SCORE CALCULATIONS

A2.1 Robust Average and Associated Uncertainty

Robust averages were calculated using the procedure described in ISO 13528.[5] The associated uncertainties were estimated as according to Equation 4.

$$u_{rob\ av} = \frac{1.25 \times S_{rob\ av}}{\sqrt{p}} \quad \textit{Equation 4}$$

where:

$u_{rob\ av}$ is the standard uncertainty of the robust average

$S_{rob\ av}$ is the standard deviation of the robust average

p is the number of results

The expanded uncertainty ($U_{rob\ average}$) is the standard uncertainty multiplied by a coverage factor of two at approximately 95% confidence level.

A worked example is set out below in Table 17.

Table 17 Uncertainty of Robust Average of MDMA in Sample S2

No. results (p)	29
Robust Average	41.0% base (m/m)
$S_{rob\ average}$	1.6% base (m/m)
$u_{rob\ average}$	0.37% base (m/m)
k	2
$U_{rob\ average}$	0.74% base (m/m)

Therefore, the robust average for Sample S2 MDMA is 41.0 ± 0.7% base (m/m).

A2.2 z-Score and E_n-Score Calculations

For each participant's result, a z-score and E_n-score are calculated according to Equations 2 and 3 respectively (Section 4).

A worked example is set out below in Table 18.

Table 18 z-Score and E_n-Score for Sample S1 MDMA Result Reported by Laboratory 1

Participant Result (% base (m/m))	Assigned Value (% base (m/m))	Target Standard Deviation	z-Score	E_n-Score
21 ± 2.9	21.8 ± 1.1	3% as PCV, or: 0.03 × 21.8 = 0.654% base (m/m)	$z = \frac{21 - 21.8}{0.654} = -1.22$	$E_n = \frac{21 - 21.8}{\sqrt{2.9^2 + 1.1^2}} = -0.26$

APPENDIX 3 ACRONYMS AND ABBREVIATIONS

CITAC	Cooperation on International Traceability in Analytical Chemistry
CRM	Certified Reference Material
CV	Coefficient of Variation
DAD	Diode Array Detection
EA	European Accreditation
FID	Flame Ionisation Detection
GAG	General Accreditation Guidance (NATA)
GC	Gas Chromatography
GUM	Guide to the expression of Uncertainty in Measurement
HPLC	High Performance Liquid Chromatography
IEC	International Electrotechnical Commission
ISO	International Organization for Standardization
k	Coverage factor
Max	Maximum
Md	Median
MDMA	3,4-Methylenedioxymethamphetamine
Min	Minimum
MS	Mass Spectrometry
MS/MS	Tandem Mass Spectrometry
MU	Measurement Uncertainty
N	Number of numeric results
NATA	National Association of Testing Authorities, Australia
NMI	National Measurement Institute, Australia
NMR	Nuclear Magnetic Resonance
NR	Not Reported
PCV	Performance Coefficient of Variation
PDA	Photodiode Array
PT	Proficiency Testing
qNMR	Quantitative NMR
RA	Robust Average
RM	Reference Material
RV	Reference Value
SD	Standard Deviation
SI	International System of Units
SS	Spiked Samples
UPLC	Ultra Performance Liquid Chromatography
UV/Vis	Ultraviolet/Visible spectroscopy

END OF REPORT