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National Measurement Institute

Proficiency Test Final Report AQA 22-03 Amphetamine and Methamphetamine

August 2022

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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NATA WORLD RECOGNISED ACCREDITATION Accredited for compliance with ISO/IEC 17043

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SUMMARY

AQA 22-03 Amphetamine and Methamphetamine commenced in January 2022. Sample sets each containing two amphetamine samples and two methamphetamine samples were sent to 31 laboratories, with two laboratories requesting two sets of test samples to be analysed by different analysts. Thirty-two participants returned results.

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

The assigned values for Samples S1 and S2 were the reference value 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 assigned values for Samples S3 and S4 were the robust averages of participants' results.

Traceability: The consensus of participants' results is not traceable to any external reference, so although expressed in SI units, metrological traceability has not been established.

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

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

Of 114 *z* scores, 81 (71%) returned $|z| \leq 2.0$, indicating a satisfactory performance.

Of 114 E_n scores, 86 (75%) returned $|E_n| \leq 1.0$, indicating agreement of the participant's results with the assigned value within their respective expanded uncertainties.

Laboratories **3**, **9**, **10**, **14**, **16**, **17**, **24**, **26**, **29**, **31** and **33** returned satisfactory *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 114 numeric results, 106 (93%) were reported with an associated expanded measurement uncertainty. The magnitudes of uncertainties were within the range 0.7% to 41% relative.

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

Samples S1 and S2 were cut with niacinamide, Sample S3 was cut with caffeine, niacinamide and phenacetin, and Sample S4 was cut with phenacetin. Thirty-one participants (97%) reported on the identity of at least one cutting agent in the samples.

Laboratories **5**, **6**, **7**, **12**, **13**, **16**, **20**, **21**, **26**, **29**, **31** and **33** correctly identified all cutting agents in all four samples.

- *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 comparison'.[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, 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 amphetamine 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

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

The conduct of NMI proficiency tests 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]

2 STUDY INFORMATION

2.1 Study Timetable

The timetable of the study was:

Invitation sent	17/01/2022
Samples dispatched	31/03/2022
Results due	17/06/2022
Interim report sent	22/06/2022

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

2.2 Participation and Laboratory Code

Thirty-one laboratories registered to participate, with two laboratories requesting two sets of samples to be analysed independently by different analysts (total of 33 participants). Each participant was assigned a confidential laboratory code number for this study. Thirty-two participants submitted results.

2.3 Test Material Specification

Four test samples were prepared in February 2022. Samples S1 and S2 contained amphetamine sulfate, and Samples S3 and S4 contained methamphetamine hydrochloride. The starting materials were supplied by the Australian Federal Police.

Niacinamide, caffeine and phenacetin purchased from Sigma-Aldrich were used as cutting agents. Samples S1 and S2 were blind duplicates cut with niacinamide, Sample S3 was cut with caffeine, niacinamide and phenacetin, and Sample S4 was cut with phenacetin.

The amphetamine 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(s) in a tumbler overnight. Portions of 150 mg of each of the test samples were weighed into labelled glass vials.

The mass fraction of the amphetamine sulfate starting material was not known to an accurate degree, and therefore there is no preparation value for Samples S1 and S2.

Sample S3 was prepared to contain approximately 20% methamphetamine base (m/m).

Sample S4 was prepared to contain approximately 75% methamphetamine base (m/m).

2.4 Test Sample Homogeneity

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.

The homogeneity testing of Samples S1 and S2 is described in Appendix 1. Samples were demonstrated to be sufficiently homogeneous for the purpose of this PT study. Samples S3 and S4 were prepared using the same procedure which has been previously demonstrated to produce homogeneous samples; the results returned by participants also gave no reason to question the homogeneity of the test samples.

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 31 March 2022.

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 content 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.
- Report the diluent(s)/adulterant(s) 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.
- Please complete the results spreadsheet and return by email to jenny.xu@measurement.gov.au.
- Results are to be returned by 27 May 2022.

The results due date was changed from 27 May 2022 to 17 June 2022. This was to accommodate for significant sample delivery delays to some international participants caused by customs clearance and distributor delays.

2.7 Interim Report

An interim report was emailed to all participants on 22 June 2022.

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	Methamphetamine	Purified Water	Phentermine	1	UPLC	DAD	Agilent Zorbax SB-C8
2	All	water	none	3	HPLC	Diode Array	Shimpack XR-ODS
3	All	D2O	Maleic acid		QNMR		NA
4	All	H_2O	nil	4	HPLC	PDA	C18 µbondapak stainless steel, 10µmPS, 3.9x150mm
5	All	Water	None	6	UPLC	UV/Vis	BEH C18 1.7um 2.1 x 100 mm (Part No. 186002352)
6	All	Methanol	2,4,6-trimethylpyridine	6	GC	FID	RTX-5-Amine
7	All	CH3CN/H2O (80/20)	No internal standard	2	HPLC	DAD	C8
8	Methamphetamine	Purified Water	Phentermine	1	UPLC	DAD	Agilent Zorbax SB-C8
9	All	Acetonitrile/Water 20:80	N/A	3	HPLC	DAD	Luna 2.5um C18(2) HST 100A
10	All	purified water	none	5	HPLC	UV-DAD	Zorbax Sil (150mm x 4.6mm, 5micron)
11	Methamphetamine	Dissolution in acetonitrile/water	Methoxyphenamine HCl	3	HPLC	DAD	Alltima C-18
12	Amphetamine	Water / Ethanol	Propyl Paraben	7	UPLC	DAD	BEH Shield RP 18
	Methamphetamine	Ethanol					
13	All	methanol	NO (External Standard)	1	HPLC	DAD	zorbax eclipse XDB-C18 (4.6x1500mm)
14	All	Methanol:KOH Buffer (50:50)	Methoxyphenamine	3	UPLC	DAD	Acquity BEH C18

Lab. Code	Analyte	Extraction Solvent	Internal Standard	Calib. Points	Technique	Detector	Column
15	Amphetamine	hexane	C14	4	GC	FID	HP-1
	Methamphetamine	D2O	calcium formate		NMR (proton)		
16	All	Isooctane	Dodecane	3	GC	FID	HP1-MS
17	All	Water	none	4	HPLC	DAD	Zorbax RX-SIL
19	Methamphetamine	Methanol		5	HPLC	DAD	C18
20	All	Water	N/A	6	UPLC	DAD	BEH C18 1.7 um 2.1x 100mm
21	Amphetamine	Acetonitrile, water, ammonium acetate & diethylamine	N/A	3	HPLC	DAD	LiChrospher 100-5 RP18
	Methamphetamine			4			
22	All	Methanol	Propylparaben	3	UPLC	PDA	ACQUITY C-18
23	Amphetamine	Water/ACN	N/A	5	HPLC	UV	Kinetex 5um C18 100A 200x4.6mm
24	All	Methanol	N/A	6	HPLC	UV/Vis	Luna C-18
25	Amphetamine	ACN/MeOH/H2O	Analog amphetamine	7	UPLC	MS/MS	C-18 Column
	Methamphetamine		Analog methamphetamine				
26	All	Methanol	Diazepam	6	GC	FID	128-5512 DB-5ms
27	All	Water/Acetonitrile (50/50)	None	7	HPLC	MS/MS	Acclaim RSLC 120 C18
28	Amphetamine	Methanol	Amphetamine D11	5	GC	MS	Rxi-5Sil MS (30m)
	Methamphetamine		N/A	N/A			
29	All	Methanol	Strychnine	6	UPLC	PDA	Phenyl
30	Amphetamine	Water	None	3	HPLC	DAD	ODS2 Inertpak
31	All	Methanol	Selegiline	4	UPLC	DAD	C18
32	All	Methanol	None	5	HPLC	DAD	Phenomenex C-18-XB
33	All	Ethyl Acetate	Diphenylamine	5	GC	FID	HP1

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	Duplicate analysis	Laboratory bias from PT studies Recoveries of SS	Eurachem/CITAC Guide
2	Standard deviation of replicate analyses multiplied by 2 or 3 UoM determined from 3 x std deviation of multiple injections expanded by professional judgement.	Control samples - CRM Duplicate analysis	Standard purity	ISO/GUM
3	Top Down - precision and estimates of the method and laboratory bias	Control samples - previously analysed real seizure samples Duplicate analysis	Instrument calibration Homogeneity of sample Masses and volumes Matrix effects	Eurachem/CITAC Guide
4	Validation (k=2)			
5	Top Down - precision and estimates of the method and laboratory bias	Control samples - RM Duplicate analysis	Instrument calibration Homogeneity of sample Masses and volumes Standard purity	ISO/GUM
6	Top Down - precision and estimates of the method and laboratory bias	Standard deviation from PT studies only		ISO/GUM
7	Global approach	Control samples		Eurolab Technical Report No1/2007
8	Top Down - precision and estimates of the method and laboratory bias	Duplicate analysis	Laboratory bias from PT studies Recoveries of SS	Eurachem/CITAC Guide
9	Top Down - precision and estimates of the method and laboratory bias	Duplicate analysis	Recoveries of SS	Eurachem/CITAC Guide

Lab. Code	Approach to Estimating MU	Information Sources for MU Estimation*		Guide Document for Estimating MU
		Precision	Method Bias	
10	repeatability, sample heterogeneity	Control samples - RM Duplicate analysis	Homogeneity of sample	Eurachem/Citac, ENFSI documents
11	Bottom Up (ISO/GUM, fish bone/cause and effect diagram)	Duplicate analysis	Instrument calibration Masses and volumes Matrix effects Recoveries of SS Standard purity	ISO/GUM
12				
13		Duplicate analysis	Instrument calibration Laboratory bias from PT studies	
14	Top Down - precision and estimates of the method and laboratory bias	Control samples - RM Duplicate analysis	Standard purity	Eurachem/CITAC Guide
15	Top Down - precision and estimates of the method and laboratory bias Expanded uncertainty = 95% confidence level	Control samples - RM Duplicate analysis		Internal document based on Eurachem/CITAC Guide, ISO/GUM
16	Bottom Up (ISO/GUM, fish bone/cause and effect diagram)	Control samples - CRM Duplicate analysis	Instrument calibration Homogeneity of sample Masses and volumes Matrix effects Recoveries of SS Standard purity	Eurachem/CITAC Guide
17	Top Down - precision and estimates of the method and laboratory bias	Control samples - Samples from case Duplicate analysis	Laboratory bias from PT studies	Nordtest Report TR537
19	Bottom Up (ISO/GUM, fish bone/cause and effect diagram)			ISO/GUM
20	Top Down - precision and estimates of the method and laboratory bias	Control samples - In House Control Duplicate analysis	Homogeneity of sample Masses and volumes Standard purity	ISO/GUM

Lab. Code	Approach to Estimating MU	Information Sources for MU Estimation*		Guide Document for Estimating MU
		Precision	Method Bias	
21	Uncertainty Budget Method	Control samples - RM Duplicate analysis	Instrument calibration Masses and volumes Standard purity	Internal SOP Document: "Uncertainty of Measurement in Drugs Analysis"
22	Top Down - precision and estimates of the method and laboratory bias	Control samples - CRM Duplicate analysis	Instrument calibration Homogeneity of sample Masses and volumes	NATA GAG Estimating and Reporting Measurement Uncertainty of Chemical Test Results
23	Top Down - precision and estimates of the method and laboratory bias	Duplicate analysis	Instrument calibration Homogeneity of sample Masses and volumes Matrix effects Standard purity	Eurachem/CITAC Guide
24	Top Down - precision and estimates of the method and laboratory bias	Control samples - RM Duplicate analysis		Eurachem/CITAC Guide
25	Top Down - precision and estimates of the method and laboratory bias	Control samples - RM		
26	Estimating Measurement Uncertainty by black box by pairs of values	Standard deviation from PT studies only		ISO/GUM Guide ENAC G 09 or ISO 21748
27	Under determination. Fixed at 20% (relative)	Control samples - RM		ISO/GUM
28	Standard deviation of replicate analyses multiplied by 2 or 3	Duplicate analysis	Masses and volumes	ISO/GUM
29	Bottom Up (ISO/GUM, fish bone/cause and effect diagram)	Control samples - RM Duplicate analysis	Instrument calibration Homogeneity of sample Masses and volumes Matrix effects Recoveries of SS Standard purity	ISO/GUM
30	Standard deviation of replicate analyses multiplied by 2 or 3	Control samples - SS	Instrument calibration Masses and volumes Recoveries of SS	ISO/GUM

Lab. Code	Approach to Estimating MU	Information Sources for MU Estimation*		Guide Document for Estimating MU
		Precision	Method Bias	
31	Top Down - precision and estimates of the method and laboratory bias	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.
32	Standard deviation of replicate analyses multiplied by 2 or 3	Duplicate analysis	Matrix effects Recoveries of SS	
33	Top Down - precision and estimates of the method and laboratory bias	Control samples - RM	Standard purity	ISO/GUM

* 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	Amphetamine		Methamphetamine	
	Reference Standard	Purity (%)	Reference Standard	Purity (%)
1	NS		Lipomed	99.950 +/- 0.050
2	NMIA	99.2	Sigma Aldrich	>98
3	No reference standard involved			
4	K&K Labs	>98	In-house synthesis	100.6
5	NMI	98.7	NMI	99.8
6	Lipomed	99.95	Lipomed	99.987
7	NMI	99.2	NMI	99.8
8	NS		Lipomed	99.950 ± 0.050

Lab. Code	Amphetamine		Methamphetamine	
	Reference Standard	Purity (%)	Reference Standard	Purity (%)
9	LGC (MM0741)	99.8	Sigma Aldrich (M8750-25g)	100
10	Chiron	99.9	Chiron	99.4
11	NT		NMI	99.8
12	NMI	99.8	NMI	99.8
13				
14	NMI	98.7	NMI	99.8
15	Fagron	99.8	N/A	
16	NMI	99.2	NMI	99.8
17	Chiron	94.3	Sigma	100
19	NT		NMI	99.8
20	NMI	98.7	NMI	99.8
21	Lipomed	99.950+/-0.050	Lipomed	99.005+/-0.027
22	NMI	98.70	NMI	99.80
23	Sigma	100	NT	
24	NMI	99.2	In house	100
25	Unikem	99.8	Sigma Aldrich	100
26	Lipomed Amphetamine.HCl	78.2	Lipomed Methamphetamine.HCl	79.4
27	Lipomed	99.95	Sigma	100
28	LGC	99.30	Sigma	100
29	NMI	99.2	NMI	99.8
30	LGC (Mikromol)	99.98	NT	
31	Lipomed	99.972 +/- 0.007	Lipomed	99.99
32				
33	Lipomed	99.867	Lipomed	99.987

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	No analysis carried out for inert bulking agents	
4	Insufficient sample if analysis requires repeating.	Most participants use less than 50 mg for each analysis, and 150 mg of each sample is provided to participants. For security and accountability reasons, NMI PT studies are conducted using the minimum practical amount of controlled substance.
7	We have Co-eluted Peaks with both Amphetamine and Methamphetamine by applying our routine method	
9	Amphetamine and Methamphetamine Methodology: Each sample was run as duplicate	
11	Methamphetamine Methodology: Linear regression	
13	Quantitative analysis is based on the use of a historical value obtained from different batches of Certified reference material.	
16	Amphetamine and Methamphetamine Methodology: Ammonium hydroxide added to convert to the free base	
21	Amphetamine Methodology: This quantitation method is unvalidated for amphetamine. Uncertainty: Uncertainty not reported for amphetamine as the quantitation method is unvalidated for amphetamine. The amphetamine quantitation aspect of this proficiency test was completed for our information purposes only.	
23	Sample results above have been affected by an instrument problem - the results above would not have been reported if they were casework samples as the batch did not have a satisfactory system suitability or IRM (other checks were satisfactory) however as we have been unable to resolve the instrument fault on time we are using these results as we wish to participate in this round.	

Lab. Code	Participants' Comments	Study Coordinator's Response
28	Laboratory only holds accreditation for Amphetamine ID and purity and Methamphetamine ID, not for Methamphetamine purity The samples for this study have been delayed in transit - perhaps at customs. Although an extended due date was provided it would be useful to have a larger time window for completion and submission of test results. Methamphetamine Methodology: ID Only	There was significant sample delivery delays to some international participants due to customs clearance, and the due date was extended for all participants. There was an additional distributor delay for this participant, and so a further extension was provided for this participant, and they advised that they would be able to report results by the new due date. In future we will consider providing for a larger time window for participants affected by exceptional circumstances to submit results.
30	Uncertainty: The reported result (in routine case samples) is defined as the average of the individual results multiplied by the uncertainty correction factor and is rounded down to the nearest whole number (unless <1%w/w). The uncertainty correction factor is defined as (mean-2SD)/mean expressed as a percentage using the relevant standard control chart. e.g. a result of 36.0% would give a reported result of 36.0*0.9790 = 35.24 therefore rounded down to 35%.	
32	Methamphetamine quantitative analysis does currently not hold an accreditation.	
33	Accreditation for amphetamine but not for methamphetamine	

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.

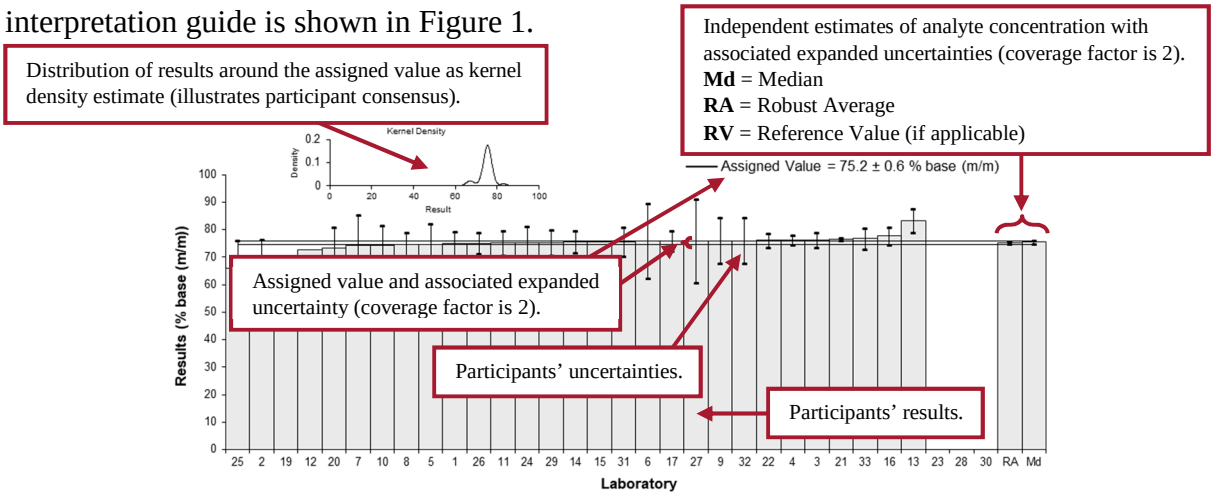


Figure 1 Guide to Presentation of Results

4.2 Outliers and Gross Errors

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] Gross errors 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]

4.3 Assigned Value

The assigned value is defined as the 'value attributed to a particular property of a proficiency test item'.[1] In this PT study, the property is the % amphetamine or methamphetamine base (m/m) in the samples. The assigned values for Samples S1 and S2 were reference values determined by quantitative nuclear magnetic resonance (qNMR) spectroscopy (Appendix 1). The assigned values for Samples S3 and S4 were the robust averages of participants' results, and the expanded uncertainties were estimated from the associated robust SDs (Appendix 2).

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 13528:2015.[5]

4.5 Performance Coefficient of Variation (PCV)

The performance coefficient of variation (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 and can be compared from study to study.

4.6 Target Standard Deviation for Proficiency Assessment

The target standard deviation (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 from Equation 1

For the absolute value of a z score:

- $|z| \leq 2.0$ is satisfactory;
- $2.0 < |z| < 3.0$ is questionable; and
- $|z| \geq 3.0$ is unsatisfactory.

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:

- $|E_n| \leq 1.0$ is satisfactory; and
- $|E_n| > 1.0$ is unsatisfactory.

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	S1
Analyte	Amphetamine
Matrix	Powder
Unit	% base (m/m)

Participant Results

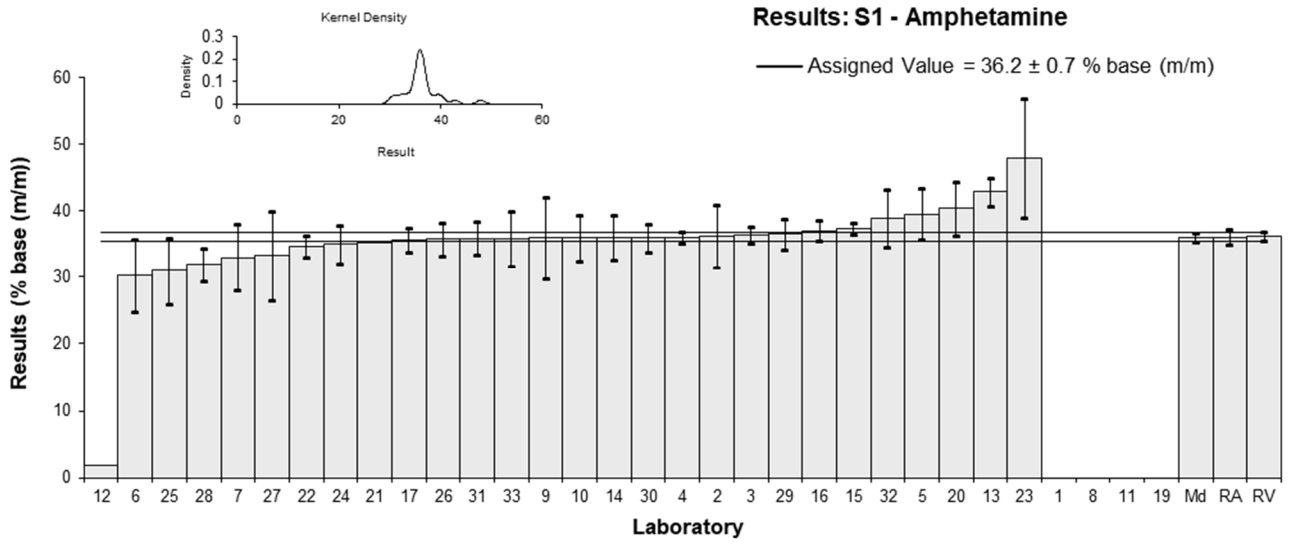
Lab. Code	Result	U	z	E_n
1	NS	NS		
2	36.29	4.69	0.08	0.02
3	36.4	1.3	0.18	0.14
4	36.1	0.9	-0.09	-0.09
5	39.6	3.9	3.13	0.86
6	30.3	5.5	-5.43	-1.06
7	33	5	-2.95	-0.63
8	NS	NS		
9	36	6.12	-0.18	-0.03
10	36.0	3.5	-0.18	-0.06
11	NR	NR		
12**	1.8	NR	-31.68	-49.14
13	42.9	2.1	6.17	3.03
14	36.0	3.4	-0.18	-0.06
15	37.4	0.9	1.10	1.05
16	37.1	1.5	0.83	0.54
17	35.7	1.8	-0.46	-0.26
19	NR	NR		
20	40.4	4	3.87	1.03
21	35.3	NR	-0.83	-1.29
22	34.75	1.60	-1.34	-0.83
23	47.97	8.97	10.84	1.31
24	35	2.8	-1.10	-0.42
25	31	5.0	-4.79	-1.03
26	35.8	2.5	-0.37	-0.15
27	33.3	6.7	-2.67	-0.43
28	32	2.5	-3.87	-1.62
29	36.6	2.3	0.37	0.17
30	36.0	2.1	-0.18	-0.09
31	35.9	2.5	-0.28	-0.12
32	39	4.3	2.58	0.64
33	35.91	4.1	-0.27	-0.07

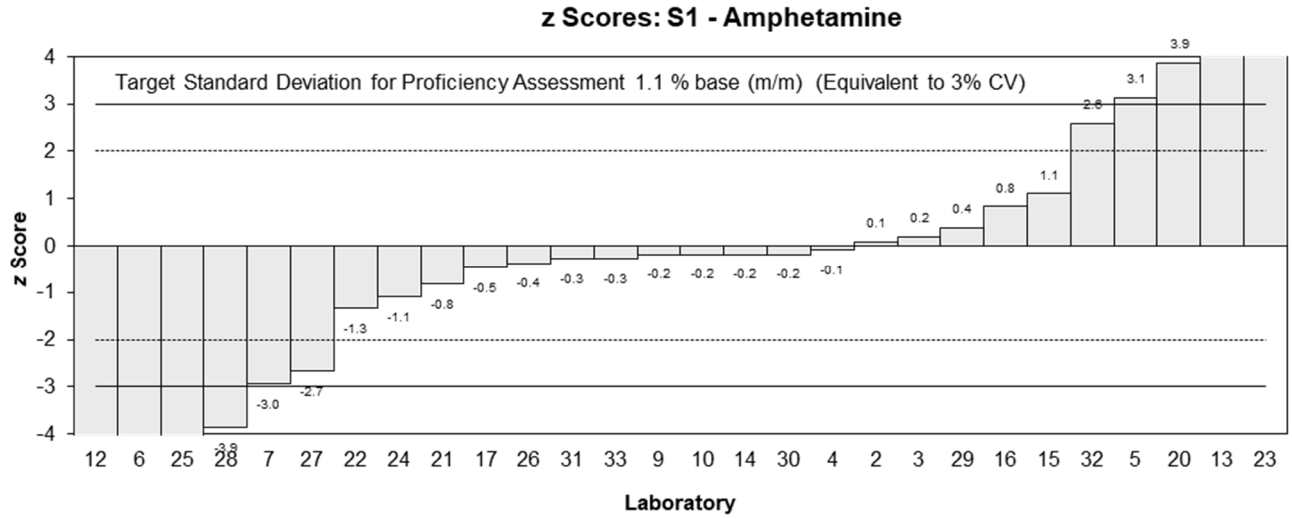
** Gross Error

Statistics

Assigned Value*	36.2	0.7
Reference Value	36.2	0.7
Robust Average	36.1	1.2
Median	36.0	0.7
Mean	36.4	1.4
N	27	
Max	47.97	
Min	30.3	
Robust SD	2.6	
Robust CV (%)	7.2	

* Assigned value is the reference value for duplicate Samples S1 and S2, as determined by qNMR spectroscopy.


Results: S1 - Amphetamine
Assigned Value = 36.2 ± 0.7 % base (m/m)
Kernel Density
Density
Result
Results (% base (m/m))
Laboratory
12 6 25 28 7 27 22 24 21 17 26 31 33 9 10 14 30 4 2 3 29 16 15 32 5 20 13 23 1 8 11 19 Md RA RV


z Scores: S1 - Amphetamine
Target Standard Deviation for Proficiency Assessment 1.1 % base (m/m) (Equivalent to 3% CV)
z Score
Laboratory
12 6 25 28 7 27 22 24 21 17 26 31 33 9 10 14 30 4 2 3 29 16 15 32 5 20 13 23

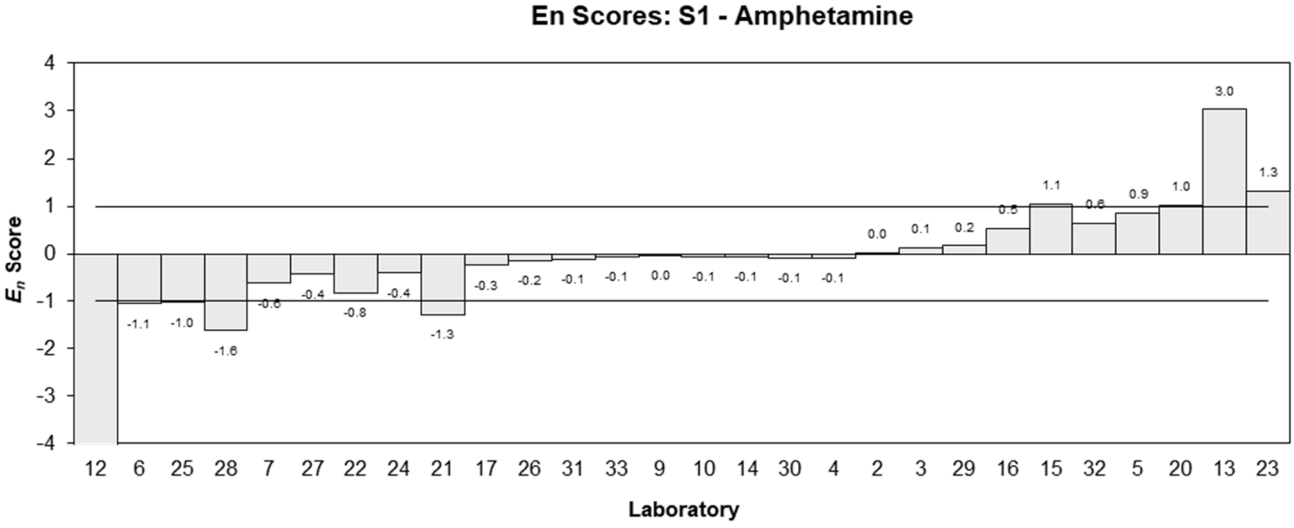

En Scores: S1 - Amphetamine
En Score
Laboratory
12 6 25 28 7 27 22 24 21 17 26 31 33 9 10 14 30 4 2 3 29 16 15 32 5 20 13 23

Figure 2

Table 6

Sample Details

Sample	S2
Analyte	Amphetamine
Matrix	Powder
Unit	% base (m/m)

Participant Results

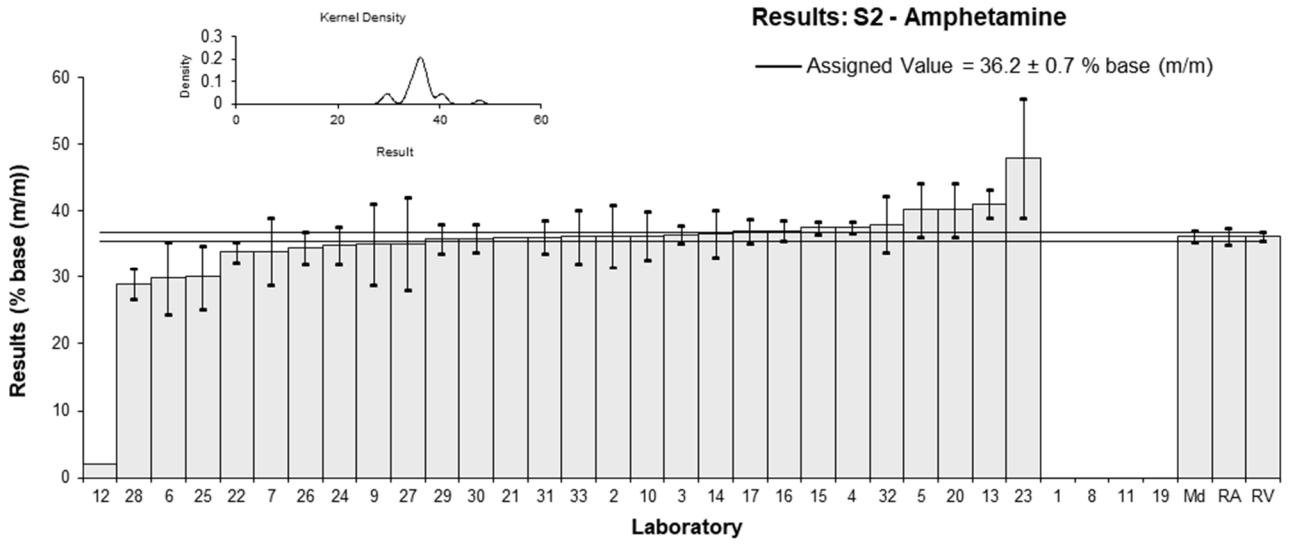
Lab. Code	Result	U	z	E_n
1	NS	NS		
2	36.19	4.68	-0.01	0.00
3	36.5	1.3	0.28	0.20
4	37.6	0.9	1.29	1.23
5	40.2	4.0	3.68	0.99
6	29.9	5.4	-5.80	-1.16
7	33.9	5.1	-2.12	-0.45
8	NS	NS		
9	35	6.12	-1.10	-0.19
10	36.3	3.6	0.09	0.03
11	NR	NR		
12**	2.0	NR	-31.49	-48.86
13	41.1	2.1	4.51	2.21
14	36.6	3.5	0.37	0.11
15	37.5	0.9	1.20	1.14
16	37.1	1.5	0.83	0.54
17	37	1.9	0.74	0.40
19	NR	NR		
20	40.2	4	3.68	0.99
21	36.1	NR	-0.09	-0.14
22	33.87	1.60	-2.15	-1.33
23	47.92	8.96	10.79	1.30
24	34.9	2.8	-1.20	-0.45
25	30	4.8	-5.71	-1.28
26	34.5	2.4	-1.57	-0.68
27	35.1	7	-1.01	-0.16
28	29	2.3	-6.63	-2.99
29	35.9	2.2	-0.28	-0.13
30	35.9	2.1	-0.28	-0.14
31	36.1	2.5	-0.09	-0.04
32	38	4.2	1.66	0.42
33	36.15	4.1	-0.05	-0.01

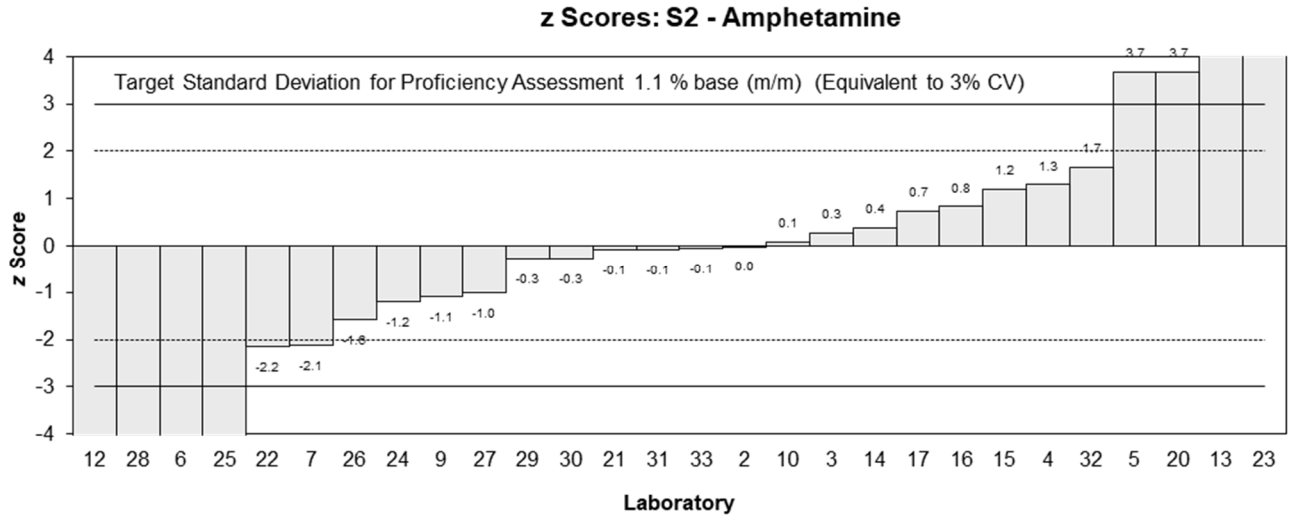
** Gross Error

Statistics

Assigned Value	36.2	0.7
Reference Value	36.2	0.7
Robust Average	36.2	1.2
Median	36.2	0.9
Mean	36.2	1.4
N	27	
Max	47.92	
Min	29	
Robust SD	2.4	
Robust CV (%)	6.7	

* Assigned value is the reference value for duplicate Samples S1 and S2, as determined by qNMR spectroscopy.


Kernel Density
Density
Result
Results: S2 - Amphetamine
Assigned Value = 36.2 ± 0.7 % base (m/m)
Results (% base (m/m))
Laboratory
12 28 6 25 22 7 26 24 9 27 29 30 21 31 33 2 10 3 14 17 16 15 4 32 5 20 13 23 1 8 11 19 Md RA RV


z Scores: S2 - Amphetamine
Target Standard Deviation for Proficiency Assessment 1.1 % base (m/m) (Equivalent to 3% CV)
z Score
Laboratory
12 28 6 25 22 7 26 24 9 27 29 30 21 31 33 2 10 3 14 17 16 15 4 32 5 20 13 23

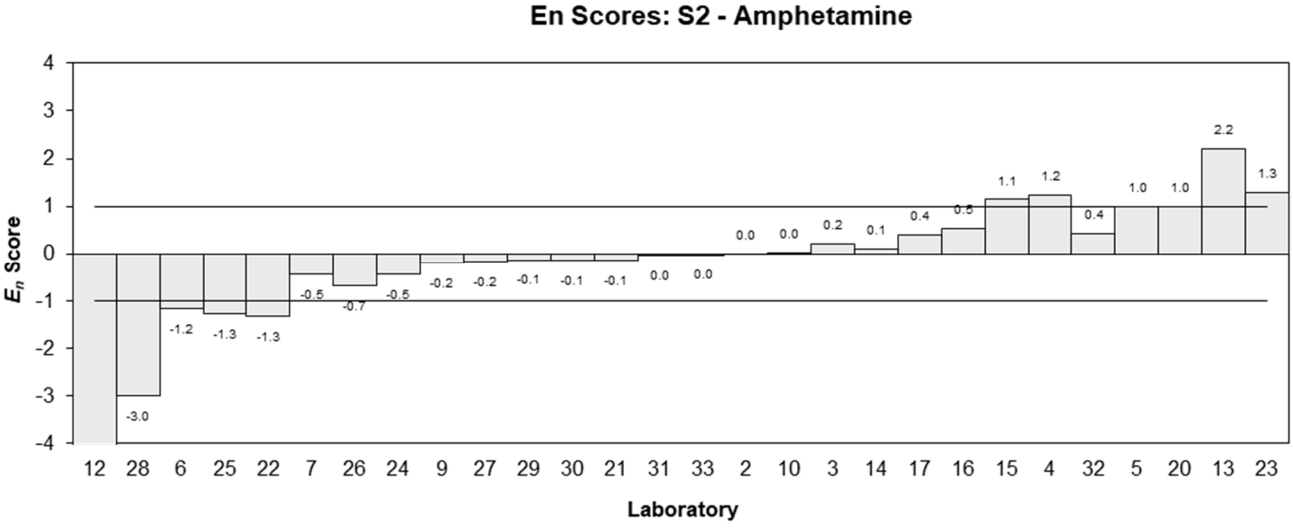

En Scores: S2 - Amphetamine
E_n Score
Laboratory
12 28 6 25 22 7 26 24 9 27 29 30 21 31 33 2 10 3 14 17 16 15 4 32 5 20 13 23

Figure 3

Table 7

Sample Details

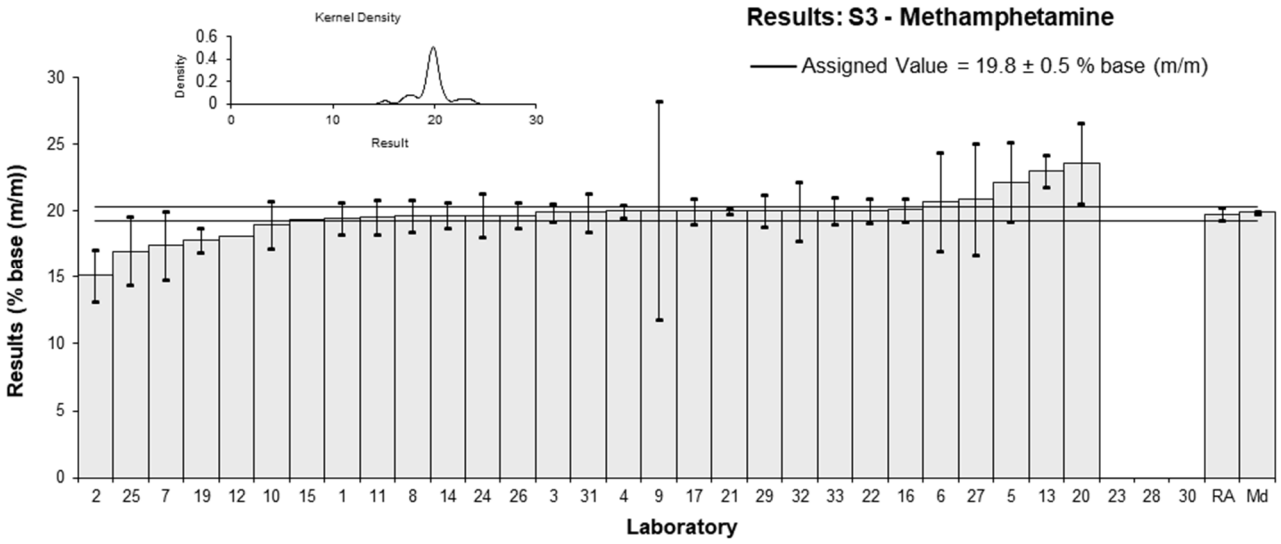
Sample	S3
Analyte	Methamphetamine
Matrix	Powder
Unit	% base (m/m)

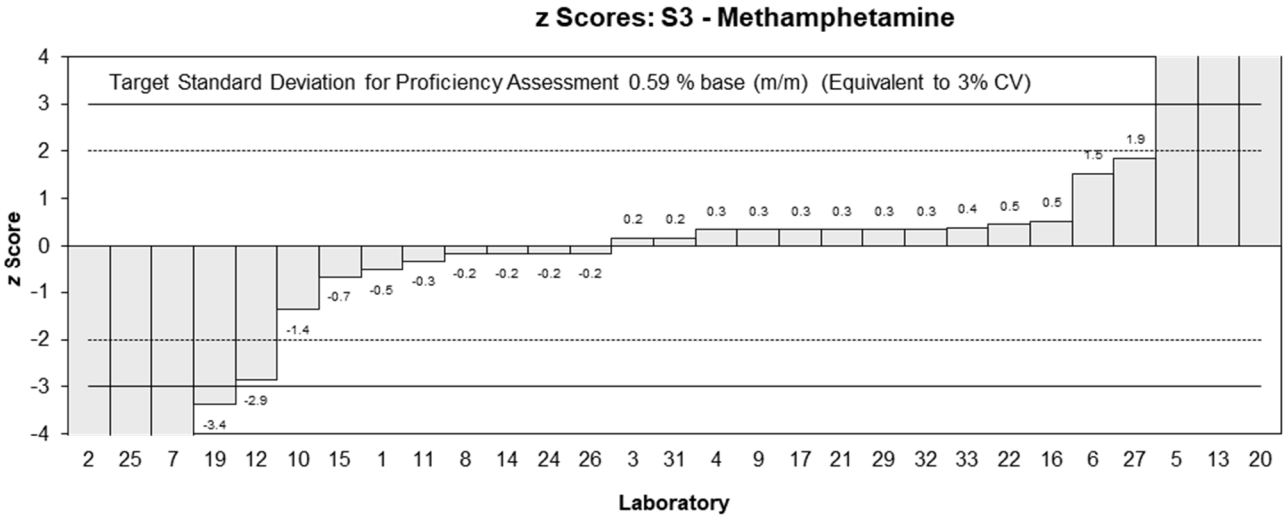
Participant Results

Lab. Code	Result	U	z	E_n
1	19.5	1.2	-0.51	-0.23
2	15.14	1.96	-7.85	-2.30
3	19.9	0.7	0.17	0.12
4	20.0	0.5	0.34	0.28
5	22.2	3.0	4.04	0.79
6	20.7	3.7	1.52	0.24
7	17.4	2.6	-4.04	-0.91
8	19.7	1.2	-0.17	-0.08
9	20	8.22	0.34	0.02
10	19.0	1.8	-1.35	-0.43
11	19.6	1.3	-0.34	-0.14
12	18.1	NR	-2.86	-3.40
13	23.0	1.2	5.39	2.46
14	19.7	1.0	-0.17	-0.09
15	19.4	NR	-0.67	-0.80
16	20.1	0.9	0.51	0.29
17	20	1.0	0.34	0.18
19	17.8	0.9	-3.37	-1.94
20	23.6	3	6.40	1.25
21	20.0	0.2	0.34	0.37
22	20.07	0.90	0.45	0.26
23	NR	NR		
24	19.7	1.6	-0.17	-0.06
25	17	2.6	-4.71	-1.06
26	19.7	1	-0.17	-0.09
27	20.9	4.2	1.85	0.26
28	NR	NR		
29	20	1.2	0.34	0.15
30	NR	NR		
31	19.9	1.4	0.17	0.07
32	20	2.2	0.34	0.09
33	20.03	1	0.39	0.21

Statistics

Assigned Value	19.8	0.5
Robust Average	19.8	0.5
Median	19.9	0.1
Mean	19.7	0.6
N	29	
Max	23.6	
Min	15.14	
Robust SD	0.98	
Robust CV (%)	5.0	


Results: S3 - Methamphetamine
Assigned Value = 19.8 ± 0.5 % base (m/m)
Kernel Density
Density
Result
Results (% base (m/m))
Laboratory
2 25 7 19 12 10 15 1 11 8 14 24 26 3 31 4 9 17 21 29 32 33 22 16 6 27 5 13 20 23 28 30 RA Md


z Scores: S3 - Methamphetamine
Target Standard Deviation for Proficiency Assessment 0.59 % base (m/m) (Equivalent to 3% CV)
z Score
Laboratory
2 25 7 19 12 10 15 1 11 8 14 24 26 3 31 4 9 17 21 29 32 33 22 16 6 27 5 13 20

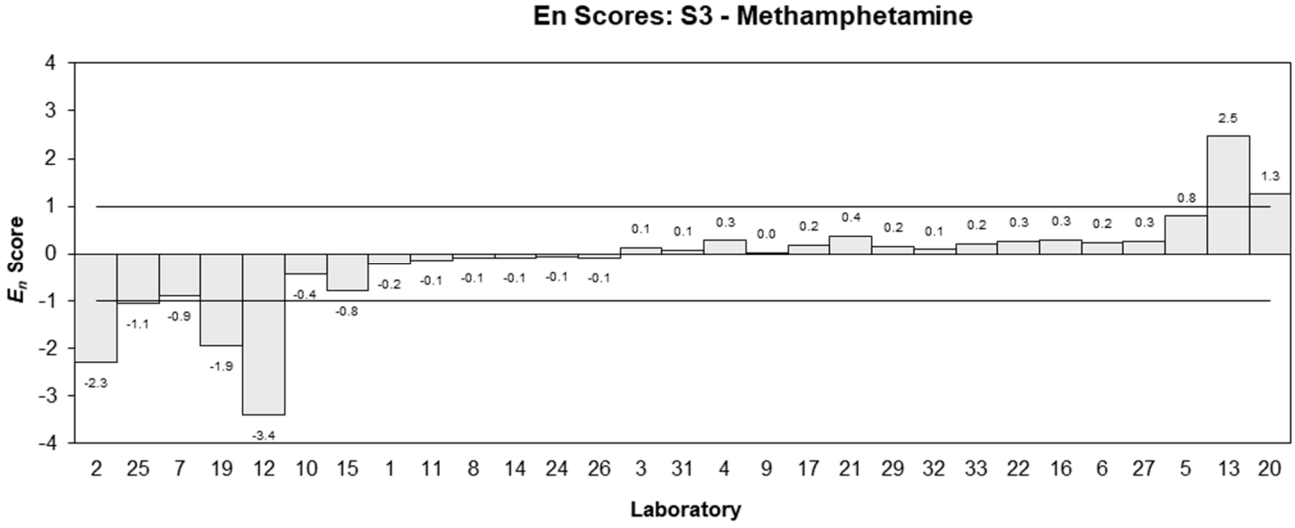

En Scores: S3 - Methamphetamine
En Score
Laboratory
2 25 7 19 12 10 15 1 11 8 14 24 26 3 31 4 9 17 21 29 32 33 22 16 6 27 5 13 20

Figure 4

Table 8

Sample Details

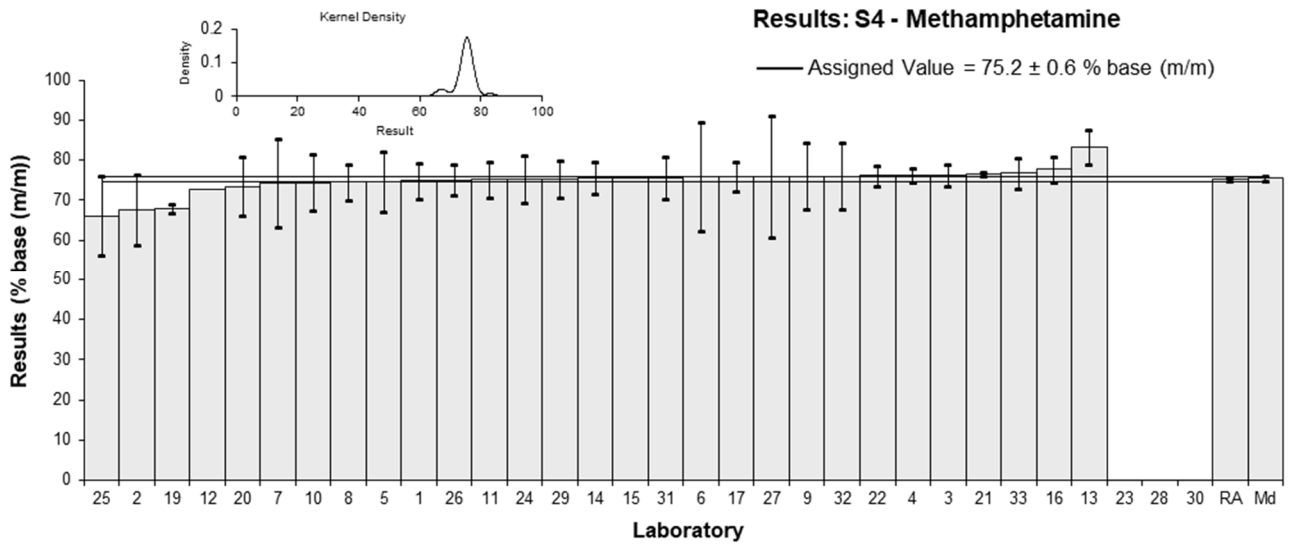
Sample	S4
Analyte	Methamphetamine
Matrix	Powder
Unit	% base (m/m)

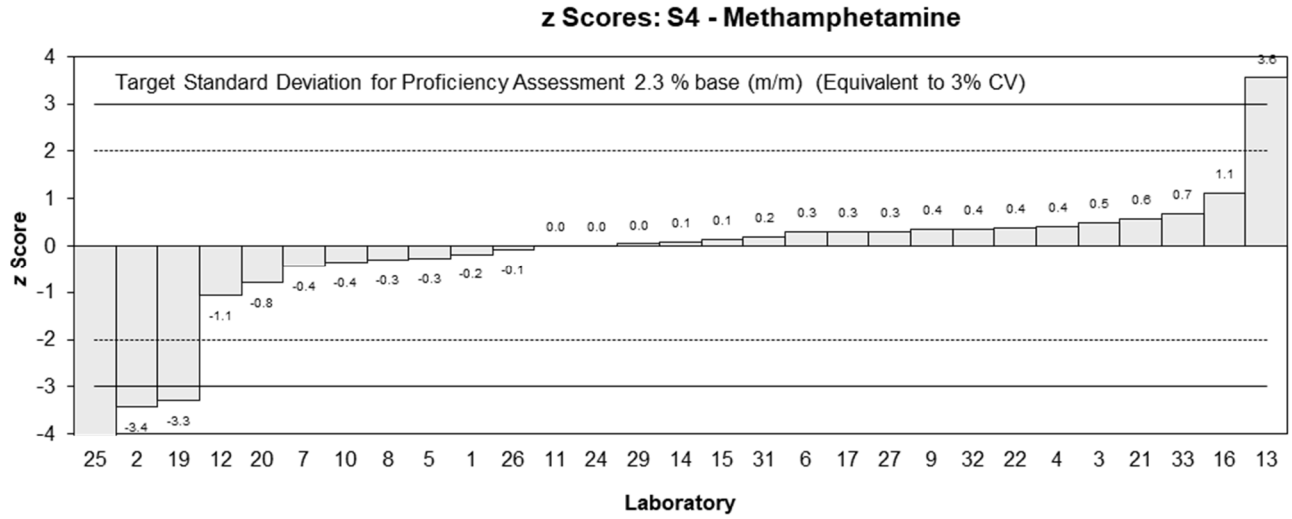
Participant Results

Lab. Code	Result	U	z	E_n
1	74.8	4.5	-0.18	-0.09
2	67.48	8.72	-3.42	-0.88
3	76.3	2.7	0.49	0.40
4	76.1	1.8	0.40	0.47
5	74.6	7.4	-0.27	-0.08
6	75.9	13.7	0.31	0.05
7	74.2	11.1	-0.44	-0.09
8	74.5	4.5	-0.31	-0.15
9	76	8.22	0.35	0.10
10	74.4	7.1	-0.35	-0.11
11	75.2	4.5	0.00	0.00
12	72.8	NR	-1.06	-4.00
13	83.2	4.2	3.55	1.89
14	75.4	4.0	0.09	0.05
15	75.5	NR	0.13	0.50
16	77.7	3.2	1.11	0.77
17	75.9	3.8	0.31	0.18
19	67.8	1.1	-3.28	-5.91
20	73.4	7.3	-0.80	-0.25
21	76.5	0.5	0.58	1.66
22	76.08	2.50	0.39	0.34
23	NR	NR		
24	75.2	5.9	0.00	0.00
25	66	9.9	-4.08	-0.93
26	75	3.8	-0.09	-0.05
27	75.9	15.2	0.31	0.05
28	NR	NR		
29	75.3	4.7	0.04	0.02
30	NR	NR		
31	75.6	5.3	0.18	0.07
32	76	8.4	0.35	0.09
33	76.76	3.8	0.69	0.41

Statistics

Assigned Value	75.2	0.6
Robust Average	75.2	0.6
Median	75.4	0.5
Mean	74.8	1.2
N	29	
Max	83.2	
Min	66	
Robust SD	1.4	
Robust CV (%)	1.8	


Results: S4 - Methamphetamine
Assigned Value = 75.2 ± 0.6 % base (m/m)
Kernel Density
Density
Result
Results (% base (m/m))
Laboratory
25 2 19 12 20 7 10 8 5 1 26 11 24 29 14 15 31 6 17 27 9 32 22 4 3 21 33 16 13 23 28 30 RA Md


z Scores: S4 - Methamphetamine
Target Standard Deviation for Proficiency Assessment 2.3 % base (m/m) (Equivalent to 3% CV)
z Score
Laboratory
25 2 19 12 20 7 10 8 5 1 26 11 24 29 14 15 31 6 17 27 9 32 22 4 3 21 33 16 13

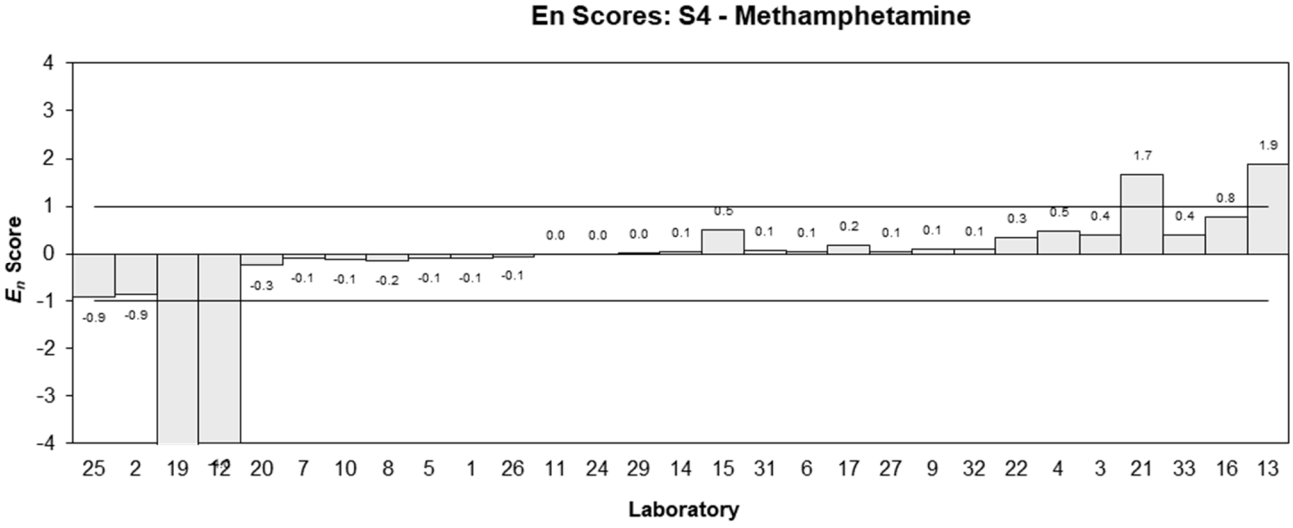

En Scores: S4 - Methamphetamine
En Score
Laboratory
25 2 19 12 20 7 10 8 5 1 26 11 24 29 14 15 31 6 17 27 9 32 22 4 3 21 33 16 13

Figure 5

Table 9 Participants' Identification of Cutting Agents

Lab. Code	Cutting Agents			
	S1	S2	S3	S4
Preparation	Niacinamide	Niacinamide	Caffeine, Niacinamide, Phenacetin	Phenacetin
1	NS		Nicotinamide, Phenacetin, Caffeine	Phenacetin
2	Niacinamide	Niacinamide	caffeine, phenacetin	phenacetin
3	nicotinamide	nicotinamide	phenacetin, caffeine	phenacetin
4	other substance(s)	other substance(s)	caffeine, phenacetin	phenacetin
5	Niacinamide	Niacinamide	Caffeine, phenacetin, niacinamide	Phenacetin
6	Niacinamide	Niacinamide	Niacinamide / Caffeine / Phenacetin	Phenacetin
7	Nicotinamide	Nicotinamide	Caffeine/Nicotinamide/Phenacetin	Phenacetin
8	NS		Nicotinamide, Phenacetin, Caffeine	Phenacetin
9	Niacinamide	Niacinamide	Niacinamide, Phenacetin, Caffeine	N/A
10	niacinamide	niacinamide	phenacetin, caffeine	phenacetin
11			Caffeine, phenacetin	
12	Niacinamide	Niacinamide	Caffeine : 54.8% - Phenacetin : 10.1% - Niacinamide	Phenacetin : 6.2 %
13	Nicotinamide	Nicotinamide	Nicotinamide+caffeine+phenacetin	phenacetin
14			Caffeine	
15	nicotinamide	nicotinamide	caffeine, phenacetin	phenacetin
16	Nicotinamide	Nicotinamide	Nicotinamide, Phenacetin, Caffeine	Phenacetin
17	Nicotinamide	Nicotinamide	Caffeine, Phenacetin	Phenacetin
19			Caffeine	Dimethyl sulfone
20	Niacinamide	Niacinamide	Phenacetin Caffeine Niacianamide	Phenacetin
21	Nicotinamide	Nicotinamide	Caffeine, Phenacetin, Nicotinamide	Phenacetin
22	Nicotinamide	Nicotinamide	Caffeine, Phenacetin	
23	Niacinamide	Niacinamide	Phenacetin, Caffeine	
24				
25	none	none	caffeine, phenacetin	phenacetin
26	niacinamide	niacinamide	niacinamide, phenacetin, caffeine	phenacetin
27	Nicotinamide	Nicotinamide	Phenacetin / Caffeine	Phenacetin
28	Nicotinamide	Nicotinamide	Caffeine, Phenacetin	Phenacetin
29	Nicotinamide	Nicotinamide	Caffeine, Phenacetin, Nicotinamide	Phenacetin
30	None	None	Caffeine	None
31	nicotinamide	nicotinamide	caffeine, nicotinamide, phenacetin	phenacetin
32	(Nicotinamide)	(Nicotinamide)	Caffeine, phenacetin	phenacetin
33	Nicotinamide (not quantified)	Nicotinamide (not quantified)	Nicotinamide (not quantified), phenacetin (10.14 w%), caffeine (52.48 w%)	Phenacetin (5.73 w%)

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

6 DISCUSSION OF RESULTS

6.1 Assigned Value

The reference value obtained using qNMR spectroscopy was used as the assigned values for duplicate Samples S1 and S2. 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).

The assigned values for Samples S3 and S4 were the robust averages of participants' results. The robust averages and associated expanded uncertainties were calculated using the procedure described in ISO 13528:2015.[5] The calculation procedure for the expanded uncertainty for robust averages is presented in Appendix 2, using Sample S4 as an example.

Traceability: The consensus of participants' results is not traceable to any external reference, so although expressed in SI units, metrological traceability has not been established.

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 (Table 2). One participant reported using the NATA GAG Estimating and Reporting MU as their guide; NATA no longer publishes this document.[10]

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] From 1 July 2012 this is also a requirement of ANAB-ASCLD/LAB accreditation program.

Of 114 numeric results, 106 (93%) were reported with an associated expanded uncertainty. Laboratory **12** did not report any uncertainties; this participant did not report if they were accredited or not. Laboratory **15** did not report uncertainties for methamphetamine (Samples S3 and S4); this participant was not accredited to ISO/IEC 17025 for methamphetamine quantitation. Laboratory **21** did not report uncertainties for amphetamine (Samples S1 and S2); this participant reported that their quantitation method was unvalidated for amphetamine.

The magnitudes of reported uncertainties were within the range 0.7% to 41% 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 106 expanded MUs reported, nine were less than 3% relative, while 34 were greater than 10% relative.

Uncertainties associated with results returning a satisfactory *z* score but an unsatisfactory 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 67.48 ± 8.72%, it is recommended to report 67.5 ± 8.7%.[8]

6.3 *z* Score

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

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

Sample	Analyte	Assigned Value (% base (m/m))	Thompson-Horwitz CV (%)	Target SD (as PCV) (%)	Between-Laboratory CV* (%)
S1	Amphetamine	36.2	1.7	3	7.2
S2					6.7
S3	Methamphetamine	19.8	2.2	3	5.0
S4	Methamphetamine	75.2	1.2	3	1.8

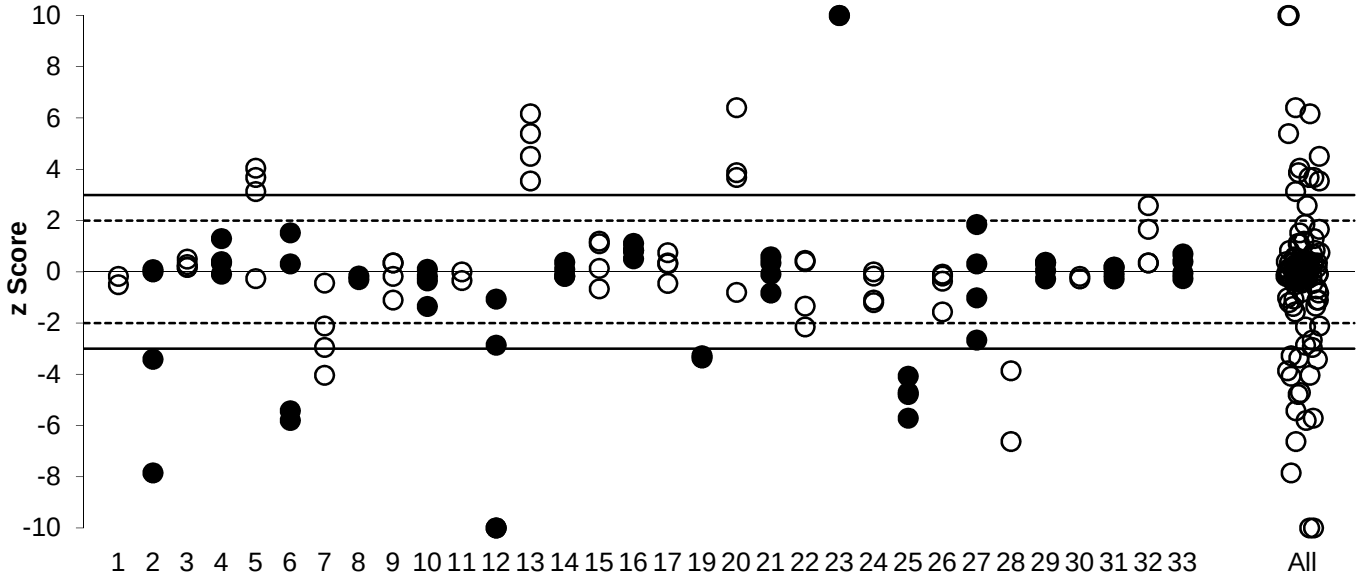
* Robust between-laboratory CV with outliers removed, if applicable.

Of 114 results for which *z* scores were calculated, 81 (71%) returned a *z* score of $|z| \leq 2.0$, indicating a satisfactory performance.

Eighteen participants: **1** (methamphetamine only), **3**, **4**, **8** (methamphetamine only), **9**, **10**, **11** (methamphetamine only), **14**, **15**, **16**, **17**, **21**, **24**, **26**, **29**, **30** (amphetamine only), **31** and **33** returned satisfactory *z* scores for all reported numeric results.

Fourteen participants returned at least one questionable or unsatisfactory *z* score. Laboratories **13** and **23** returned unsatisfactory *z* scores for all reported results, with all being higher than the assigned value (positive bias). Laboratories **19**, **25** and **28** returned unsatisfactory *z* scores for all reported results, with all being lower than the assigned value (negative bias). These participants may need to investigate the source of these biases. It is possible that participants with a positive bias may have reported results as % salt (m/m) instead of % base (m/m) as requested for this PT study.

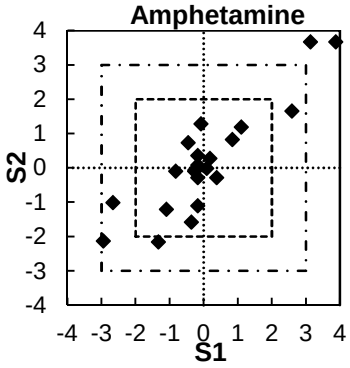
The dispersal of participants' *z* scores is presented graphically in Figure 6.



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

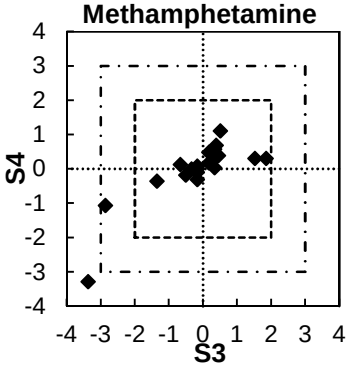
Figure 6 *z* Score Dispersal by Laboratory

Scatter plots of *z* scores for amphetamine 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 6, 12, 13, 23, 25 and 28 are off-scale.

Figure 7 *z* Score Scatter Plot – Amphetamine



Laboratories 2, 5, 7, 13, 20 and 25 are off-scale.

Figure 8 *z* Score Scatter Plot – Methamphetamine

6.4 E_n Score

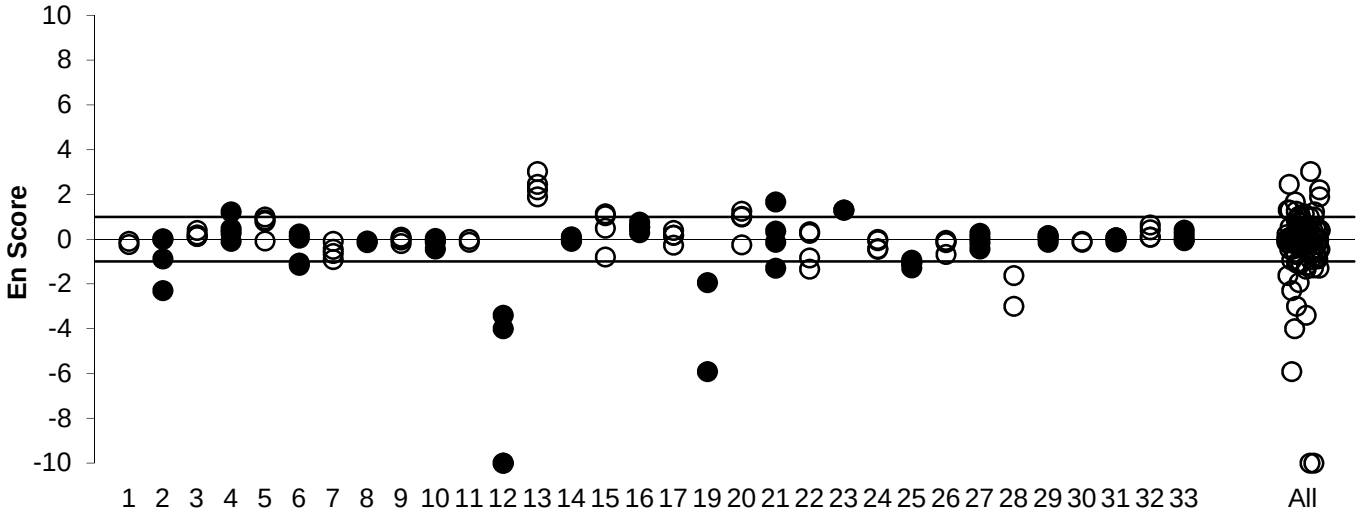
Where a participant did not report an expanded uncertainty with a result, an expanded uncertainty of zero (0) was used to calculate the E_n score.

Of 114 results for which E_n scores were calculated, 86 (75%) returned an E_n score of $|E_n| \leq 1.0$, indicating agreement of the participant's result with the assigned value within their respective uncertainties.

Nineteen participants: **1** (methamphetamine only), **3**, **5**, **7**, **8** (methamphetamine only), **9**, **10**, **11** (methamphetamine only), **14**, **16**, **17**, **24**, **26**, **27**, **29**, **30** (amphetamine only), **31**, **32** and **33** returned satisfactory E_n scores for all reported numeric results.

Thirteen participants returned at least one unsatisfactory E_n score. Laboratories **12**, **13**, **19**, **23** and **28** returned unsatisfactory E_n scores for all reported results.

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



E_n scores less than -10.0 have been plotted at -10.0.

Figure 9 E_n Score Dispersal by Laboratory

6.5 Identification of Cutting Agents

The test samples were prepared by adding a number of cutting agents to amphetamine sulfate (Samples S1 and S2) and methamphetamine hydrochloride (Samples S3 and S4) starting materials. For Samples S1 and S2, niacinamide (nicotinamide) was added. For Sample S3, caffeine, niacinamide and phenacetin were added. For Sample S4, phenacetin was added.

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

Thirty-one participants (97%) reported on the identity of at least one cutting agent in the samples. Laboratories **5**, **6**, **7**, **12**, **13**, **16**, **20**, **21**, **26**, **29**, **31** and **33** correctly identified all cutting agents in this study. Laboratories **1**, **8** only analysed Samples S3 and S4, and correctly identified all cutting agents in these samples.

All participants reporting the cutting agent in Samples S1 and S2 (23) correctly identified niacinamide, except for one participant who only reported ‘other substance(s)’.

All participants reporting on the cutting agent(s) in Sample S3 (31) correctly reported caffeine, which was the major cutting agent prepared at around 56% (w/w). Phenacetin and niacinamide were added at lower levels, being around 10% (w/w) and 9% (w/w) respectively. Thirteen participants correctly reported phenacetin in addition to caffeine. A further 15 participants correctly reported both phenacetin and niacinamide in addition to caffeine.

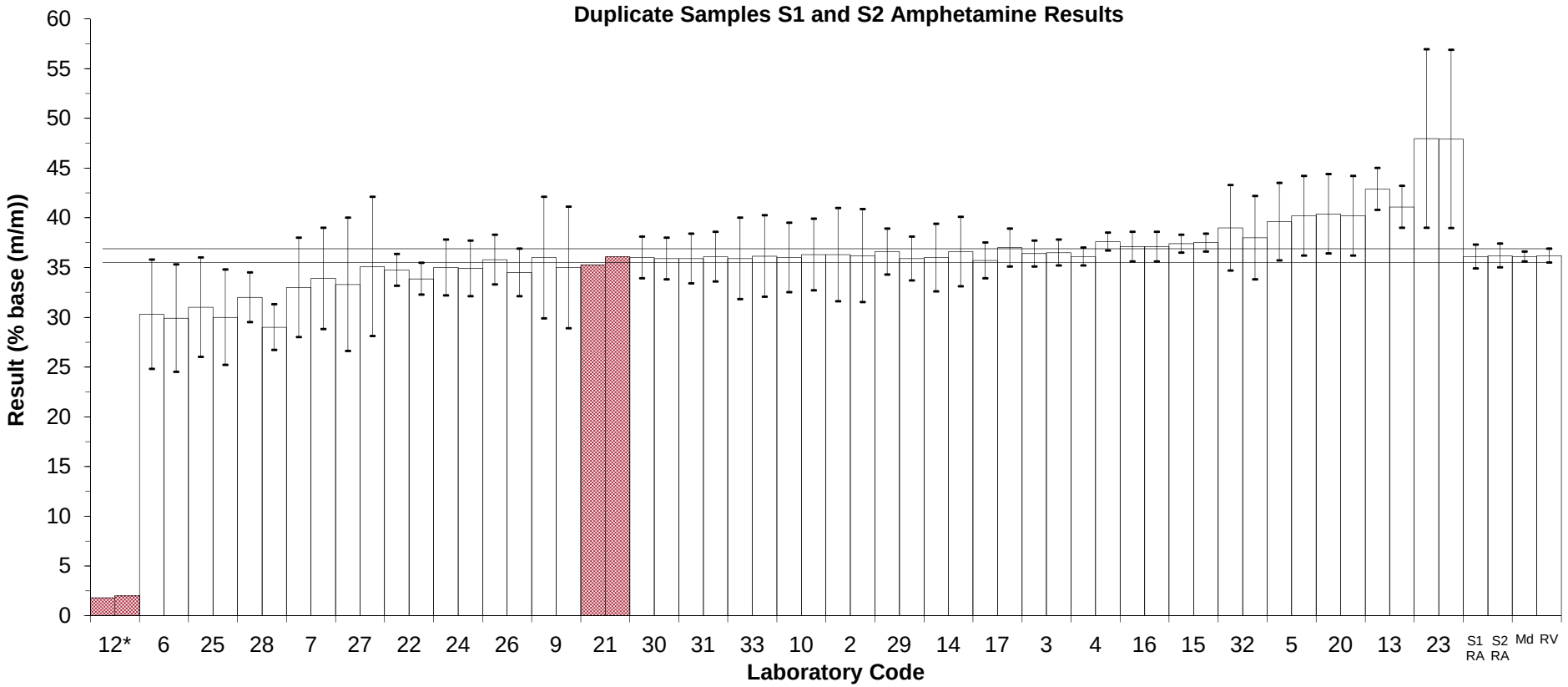
For Sample S4, 24 participants correctly reported phenacetin as the cutting agent. Phenacetin was added to Sample S4 at around 6% (w/w), which was lower than for Sample S3; there were four participants who reported phenacetin in Sample S3 but not in Sample S4. One participant reported dimethyl sulfone in Sample S4; participants should take care to avoid any potential cross-contamination at their laboratory.

6.6 Duplicate Samples S1 and S2

Samples S1 and S2 were blind duplicate amphetamine samples. Participants' results for these samples, along with the robust averages, median and reference value, are presented in Figure 10.

The majority of participants' results for these samples were in agreement with each other within their reported expanded uncertainties.

Laboratories **12** and **21** duplicate results were not in agreement, as these participants did not report any uncertainties with their results.



* Gross error; not included for statistical calculations.
Horizontal lines are the assigned value ± U. Participants' results which are not in agreement with each other within reported uncertainties are shaded.

Figure 10 Results for Blind Duplicate Samples S1 and S2 Amphetamine

6.7 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 (MA), 2, 3, 4, 5, 8 (MA), 9, 10, 11 (MA), 14, 15 (A) 16, 17, 20, 21, 22, 24, 25, 26, 28 (ID and quantification for A; ID only for MA), 29, 30 (A), 31, 32, 33 (A)
	Not accredited / NR	6, 7, 12, 13, 15 (MA), 19 (MA), 23 (A), 27, 33 (MA)
Average Sample Mass Used (mg)	< 20	3, 6, 9 (MA), 24, 26, 27, 28 (A)
	20 – 30	1 (MA), 5, 7, 9 (A), 11 (MA), 12 (A), 15 (MA), 16, 20, 22, 29, 32, 33
	31 – 50	2, 8 (MA), 10, 12 (MA), 13, 14, 15 (A), 17, 21 (A), 23 (A), 25, 30 (A), 31
	51 – 100	19 (MA), 21 (MA)
	> 101	4
Conversion to Base?	Yes	3, 4, 5, 6, 7, 9, 10, 13, 14, 15 (A), 16, 17, 19 (MA), 20, 21, 23 (A), 26, 27, 30 (A), 31, 33
	No	1 (MA), 2, 8 (MA), 11 (MA), 15 (MA), 22, 24, 28, 29, 32
	NR	12, 25
Instrument Used for Quantification	HPLC-DAD	2, 4, 7, 9, 10, 11 (MA), 13, 17, 19 (MA), 21, 30 (A), 32
	HPLC-UV/Vis	23 (A), 24
	HPLC-MS/MS	27
	UPLC-DAD	1 (MA), 8 (MA), 12, 14, 20, 22, 29, 31
	UPLC-UV/Vis	5
	UPLC-MS/MS	25
	GC-FID	6, 15 (A), 16, 26, 33
	GC-MS	28 (qualitative only for MA)
	QNMR	3, 15 (MA)
Solvent	Acetonitrile/Water(/Other)	7, 9, 11 (MA), 21, 23 (A), 25, 27
	Methanol	6, 13, 19 (MA), 22, 24, 26, 28, 29, 31, 32
	Water	1 (MA), 2, 4, 5, 8 (MA), 10, 17, 20, 30 (A)
	Other	3, 12, 14, 15, 16, 33
Sources of Calibration Standard (A)	NMI Australia	2, 5, 7, 12, 14, 16, 20, 22, 24, 29
	Lipomed	6, 21, 26, 27, 31, 33
	Chiron	10, 17
	LGC	9, 28, 30
	Other	4, 15, 23, 25
	NR	13, 32

		Lab. Code
Sources of Calibration Standard (MA)	NMI Australia	5, 7, 11, 12, 14, 16, 19, 20, 22, 29
	Lipomed	1, 6, 8, 21, 26, 31, 33
	Sigma Aldrich	2, 9, 17, 25, 27, 28
	Other	4, 10, 24
	NR	13, 15, 32

*A = Amphetamine; MA = Methamphetamine

Plots of the *z* score versus various parameters are presented in Figures 11 to 15 (gross errors have been removed, and *z* scores greater than 10.0 have been plotted at 10.0). A variety of methodologies were used by participants in this study, and no significant trends were observed.

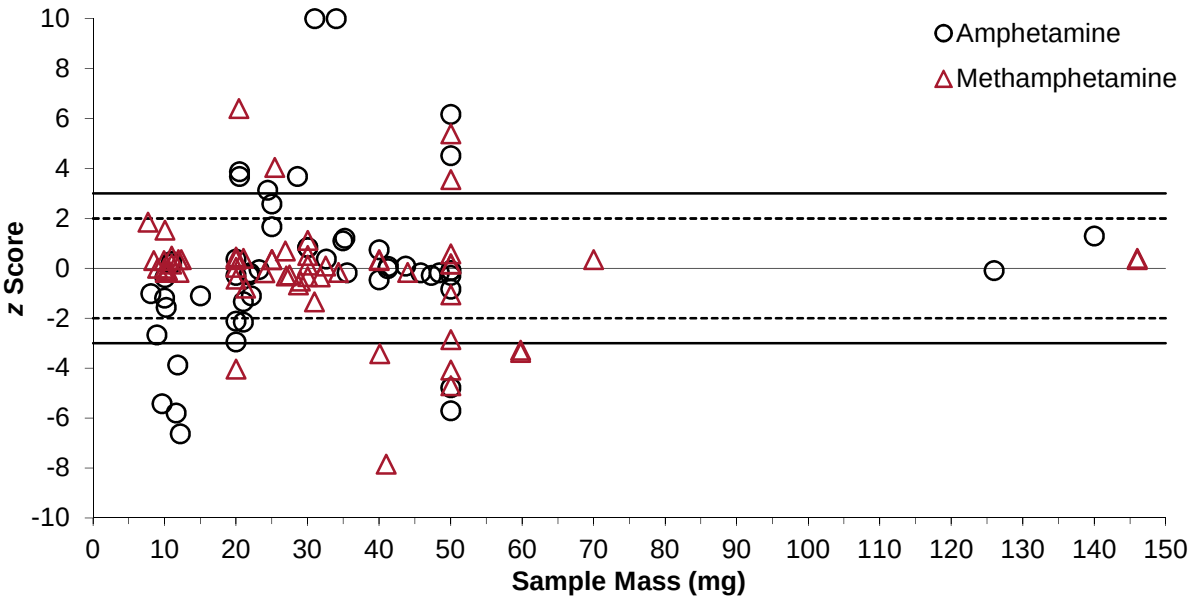


Figure 11 *z* Score vs Sample Mass Used for Analysis

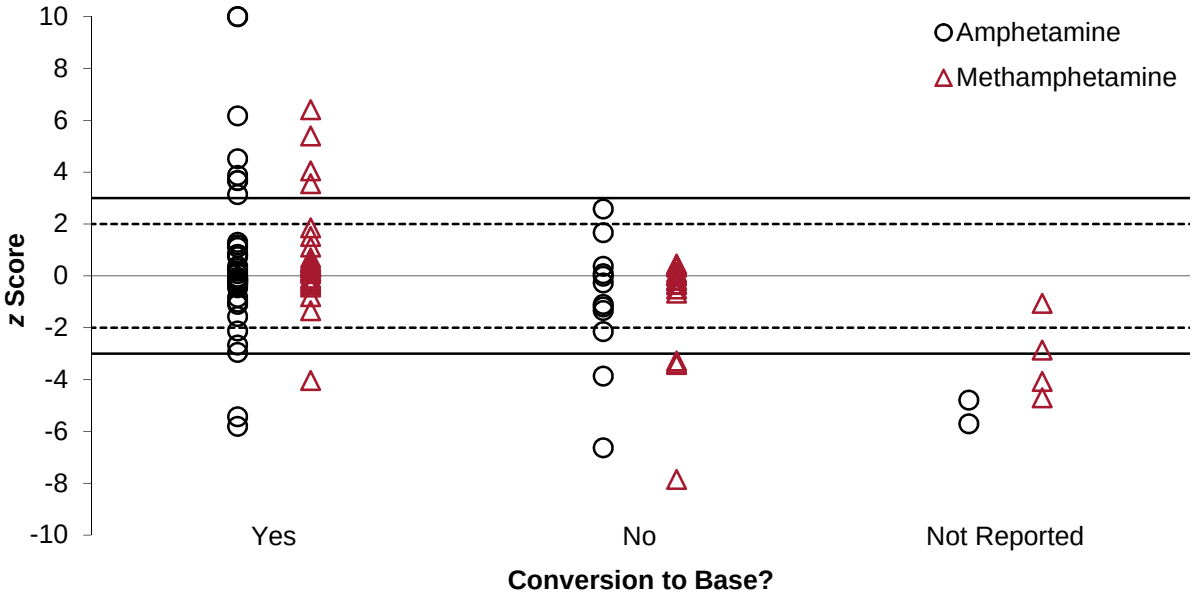


Figure 12 *z* Score vs Sample Processing

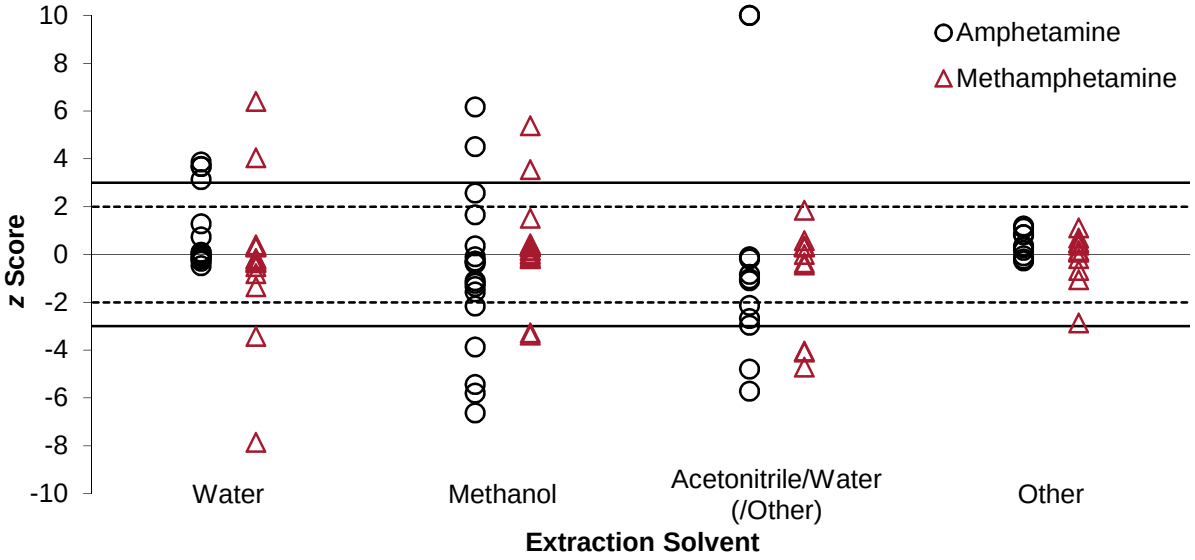


Figure 13 *z* Score vs Extraction Solvent

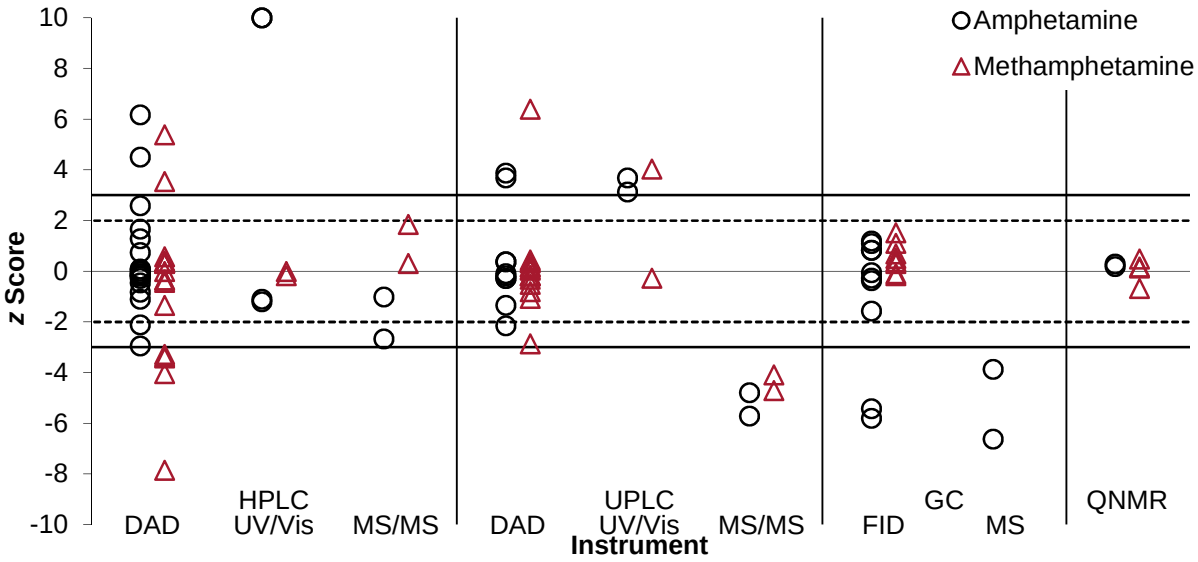


Figure 14 *z* Score vs Measurement Instrument

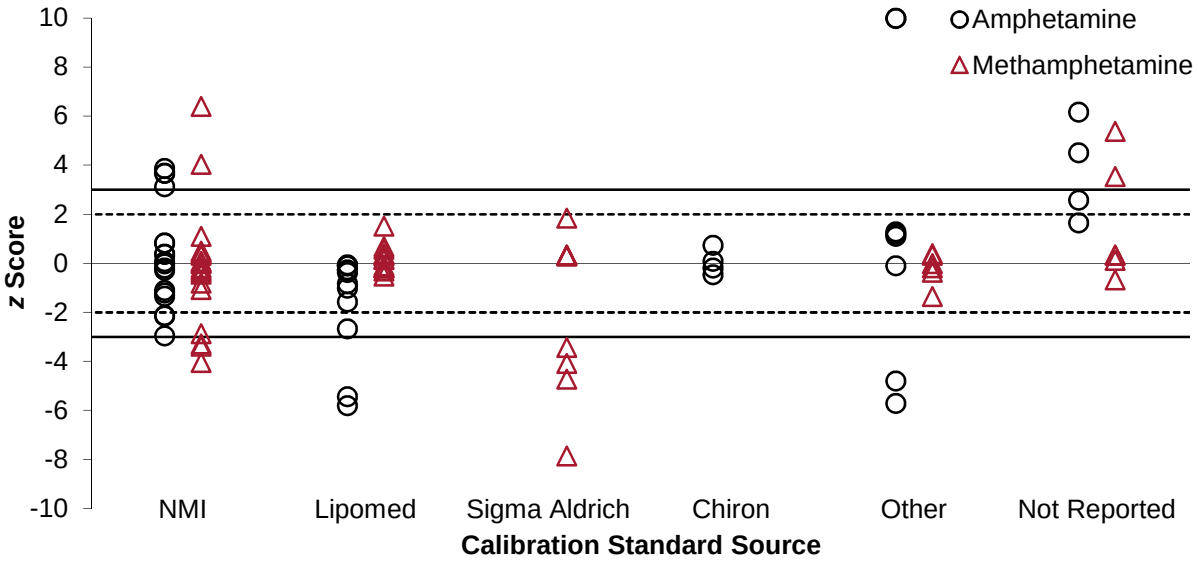
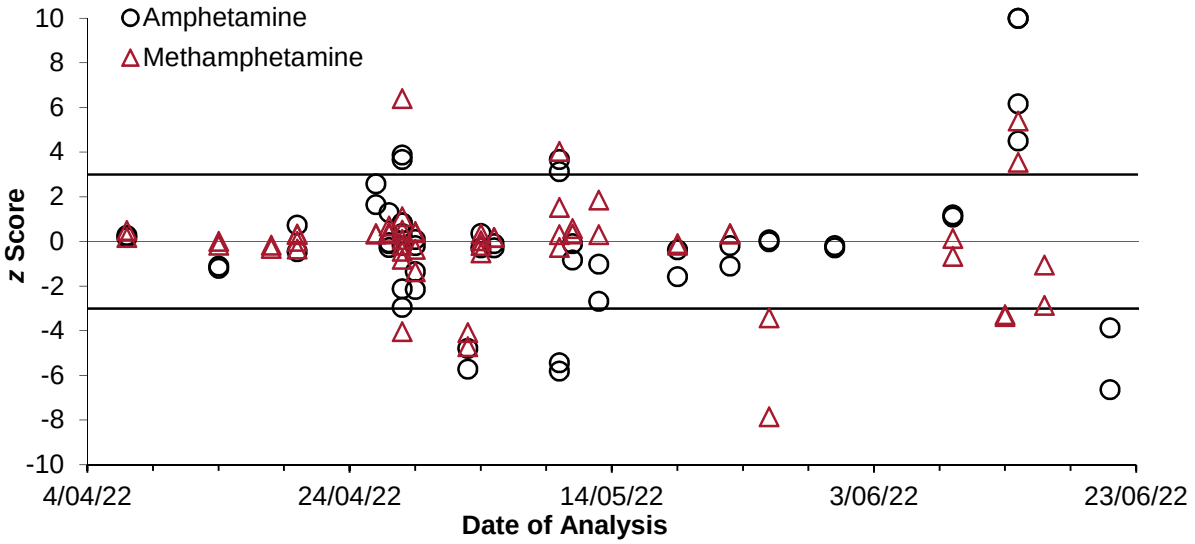


Figure 15 *z* Score vs Calibration Standard Source

6.8 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 2.5 months. No trend was found between when the samples were analysed and the results obtained (Figure 16; gross errors have been removed).



z Scores greater than 10.0 have been plotted at 10.0

Figure 16 *z* Score vs Sample Analysis Date

6.9 Comparison with Previous PT Studies

This is the first NMI PT study which has included amphetamine, and therefore no comparison with previous studies is available for this analyte.

To enable direct comparison with previous PT studies for methamphetamine, the target SD used to calculate *z* scores has been kept constant at 3% PCV.

A summary of the satisfactory performance, presented as a percentage of the total number of scores, obtained by PT study participants for methamphetamine from 2012 – 2022 (last ten studies with methamphetamine) is presented in Figure 17. The average proportion of satisfactory *z* scores and E_n scores over this period is 84% and 80% respectively.

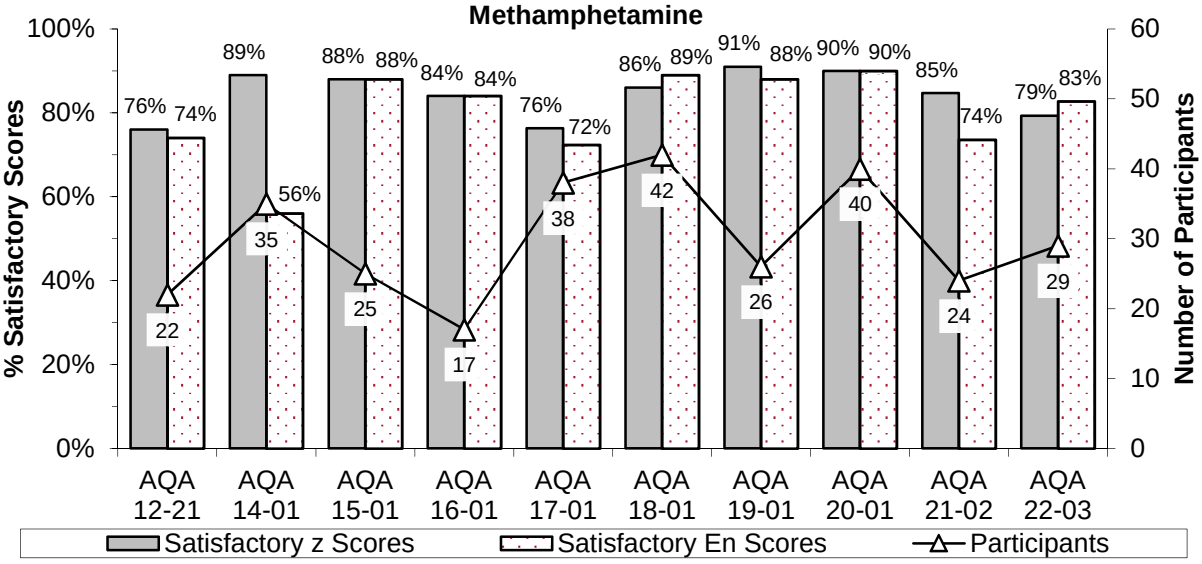


Figure 17 Summary of Participants' Performance in NMI Methamphetamine PT Studies

A number of participants have consistently participated in NMI 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 ten NMI Methamphetamine PT studies is presented in Figures 18 and 19 for Australian and international laboratories respectively. Three Australian and one international laboratories have achieved satisfactory *z* scores across all methamphetamine samples in PT studies participated in over this period.

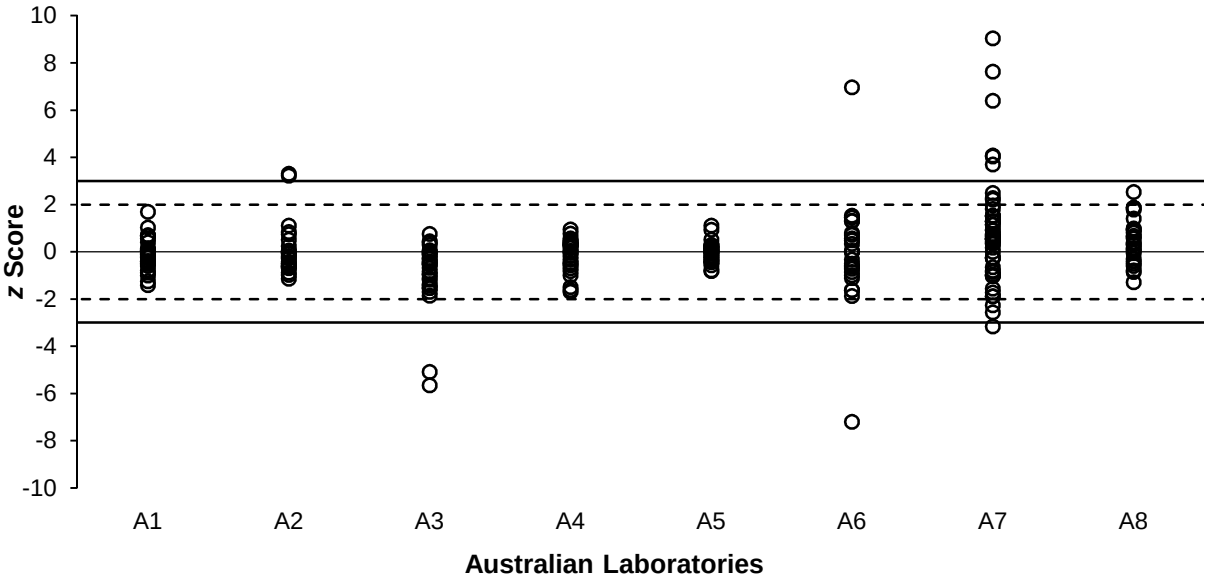


Figure 18 Summary of Australian Participants' *z* Scores in NMI Methamphetamine PT Studies

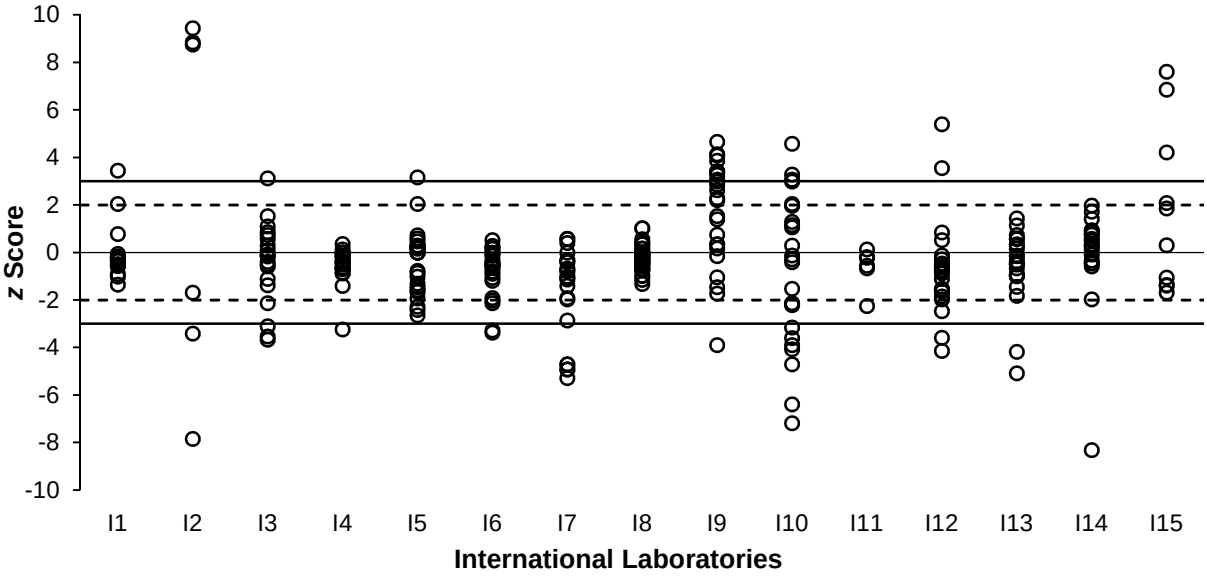


Figure 19 Summary of International Participants' *z* Scores in NMI Methamphetamine PT Studies

A comparison of all results from Australian and international laboratories in NMI Methamphetamine PT studies over the last ten years is presented in Figure 20. Overall, Australian participants have performed well, with a higher proportion of satisfactory *z* scores over this period.

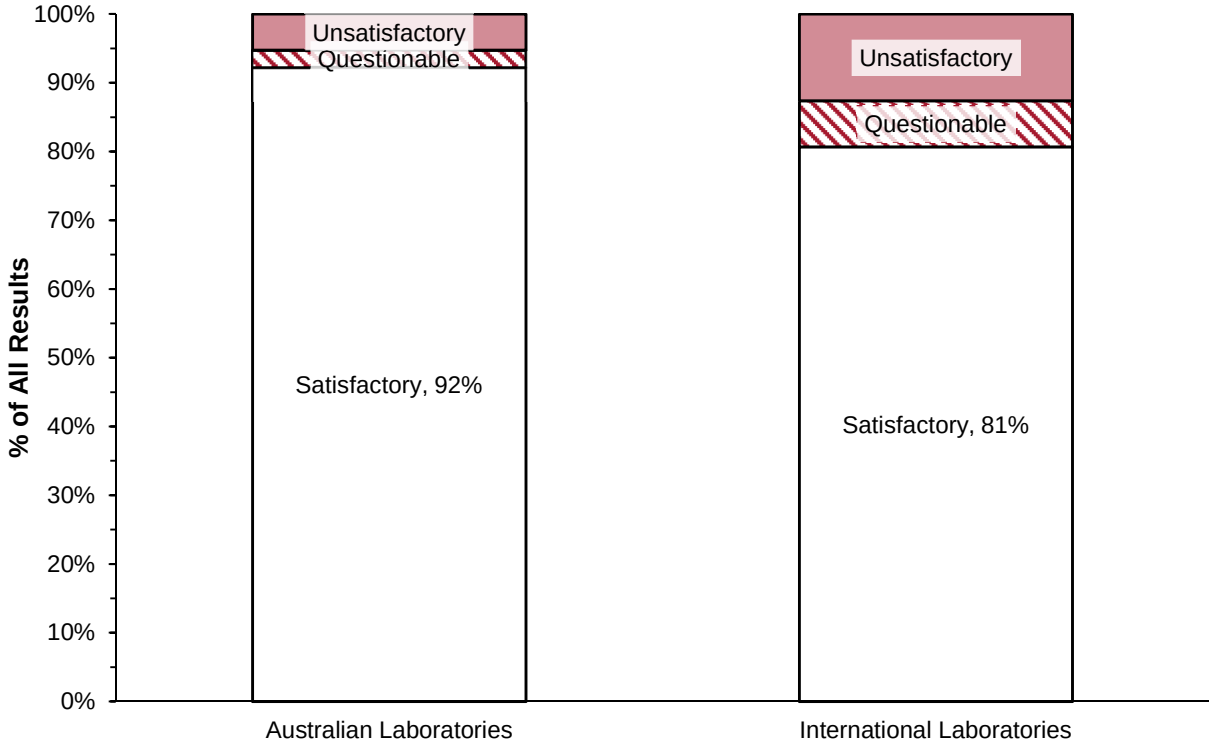


Figure 20 Comparison of Australian and International Laboratories in NMI Methamphetamine PT Studies

7 REFERENCES

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[9] ISO/IEC Guide 98-3:2008, *Uncertainty of measurement – Part 3: Guide to the expression of uncertainty in measurement (GUM:1995).*

[10] NATA, 2020, *Update to Measurement Uncertainty resources*, viewed July 2022, <https://nata.com.au/news/update-to-measurement-uncertainty-resources/>

[11] Fearn, T. and Thompson, M., 2001, 'A new test for 'sufficient homogeneity'', *Analyst*, vol. 126, pp. 1414-1417.

APPENDIX 1 REFERENCE VALUES

Seven sample vials across duplicate Samples S1 and S2 were analysed in duplicate for the purpose of assigning a reference value for the mass fraction of amphetamine. 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 400 MHz NMR spectrometer, using a qNMR relaxation time of 25 s. The mass fraction of amphetamine was determined from the NMR response at 1.31 ppm. The average of the mass fractions determined for the different vials of Samples S1 and S2 (Table 13) was used as the reference value and the assigned value. 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.

The measured reference value for Samples S1 and S2 was in agreement with the robust averages of participants' results, within their respective associated uncertainties.

Homogeneity checks were performed based on that described by Thompson and Fearn,[11] which is also the procedure described in the International Protocol.[4] Samples were found to be sufficiently homogeneous for use in a PT study with a target SD (as PCV) of 3%.

Table 13 Reference Value for Samples S1 and S2

Vial No.	Amphetamine (% base (m/m))	
	Replicate 1	Replicate 2
109	35.9	36.1
119	36.1	36.0
127	36.5	35.9
146	36.1	36.2
209	36.4	36.5
229	36.7	36.6
241	36.0	36.5
Average	36.2	
CV	0.74%	

Thompson and Fearn Homogeneity Tests[11]

Test	Value	Critical	Result
Cochran	0.43	0.73	**Pass**
S_{an}/σ	0.20	0.5	**Pass**
s^2_{sam}	0.028	0.29	**Pass**

Samples S1 and S2 Reference Value: 36.2 ± 0.7% amphetamine base (m/m)*

* The uncertainty is an expanded uncertainty at 95% confidence level. A coverage factor k was calculated using the effective degrees of freedom derived from the Welch-Satterthwaite equation (k = 2.2).[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 13528:2015.[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 14.

Table 14 Uncertainty of Robust Average of Methamphetamine in Sample S4

No. results (p)	29
Robust Average	75.2% base (m/m)
$S_{rob\ average}$	1.4% base (m/m)
$u_{rob\ average}$	0.3% base (m/m)
k	2
$U_{rob\ average}$	0.6% base (m/m)

Therefore, the robust average for Sample S4 is 75.2 ± 0.6% 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 15.

Table 15 *z* Score and E_n Score for Sample S1 Amphetamine Result Reported by Laboratory 2

Participant Result (% base (m/m))	Assigned Value (% base (m/m))	Target Standard Deviation	*z* Score	E_n Score
36.29 ± 4.69	36.2 ± 0.7	3% as PCV, or: 0.03 × 36.2 = 1.1% base (m/m)	$z = \frac{36.29-36.2}{1.1}$ = 0.08	$E_n = \frac{36.29-36.2}{\sqrt{4.69^2+0.7^2}}$ = 0.02

APPENDIX 3 ACRONYMS AND ABBREVIATIONS

ANAB	ANSI (American National Standards Institute) National Accreditation Board
ASCLD/LAB	American Society of Crime Laboratory Directors/Laboratory Accreditation Board
CITAC	Cooperation on International Traceability in Analytical Chemistry
CRM	Certified Reference Material
CV	Coefficient of Variation
DAD	Diode Array Detection
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
Max	Maximum value
Md	Median value
Min	Minimum value
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
NR	Not Reported
NS	Not Supplied
NT	Not Tested
PCV	Performance Coefficient of Variation
PDA	Photodiode Array
PT	Proficiency Testing
qNMR	Quantitative Nuclear Magnetic Resonance
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