Australian Government
Department of Industry, Science, Energy and Resources

National Measurement Institute

Proficiency Test Final Report AQA 21-02 Methamphetamine and MDMA

August 2021

ACKNOWLEDGMENTS

This study was conducted by the National Measurement Institute (NMI). Support funding was provided by the Australian Government Department of Industry, Science, Energy and Resources.

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.

Jenny Xu

Lesley Johnston

Raluca Iavetz

Manager, Chemical Reference Values

105 Delhi Rd, North Ryde, NSW 2113, Australia

Phone: +61 2 9449 0178

Email: raluca.iavetz@measurement.gov.au

Accredited for compliance with ISO/IEC 17043

TABLE OF CONTENTS

THIS PAGE IS INTENTIONALLY BLANK

SUMMARY

AQA 21-02 Methamphetamine and MDMA commenced in February 2021. Sample sets each containing three methamphetamine samples and one 3,4-methylenedioxymethamphetamine (MDMA) sample were sent to 26 laboratories, with two laboratories 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, S2 and S3 were prepared from methamphetamine hydrochloride, approximately 80% base (m/m), supplied by the Australian Federal Police. Sample S4 was prepared from MDMA hydrochloride, approximately 84% base (m/m), synthesised by NMI Chemical Reference Materials.

The assigned values for Samples S1, S3 and S4 were the reference values 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 value for Sample S2 was the robust average 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 methamphetamine and MDMA in samples typical of a routine seizure.*

Of 98 z-scores, 80 (82%) returned $|z| \leq 2.0$, indicating a satisfactory performance.

Of 98 E_n-scores, 74 (76%) returned $|E_n| \leq 1.0$, indicating agreement of the participant's results with the assigned value within their respective expanded uncertainties.

Laboratories **2**, **4**, **10**, **13**, **14**, **24**, **25** and **27** 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 98 numeric results, 93 (95%) were reported with an associated expanded measurement uncertainty. Laboratory **17** did not report any uncertainties; this laboratory was not accredited. Laboratory **21** did not report an uncertainty for one result as it was an approximation.

The magnitudes of reported uncertainties were within the range 0.9% to 20% relative.

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

Sample S1 was cut with dimethyl sulfone, Sample S2 was cut with paracetamol, Sample S3 was cut with levamisole, and Sample S4 was cut with glucose. Twenty-six participants (93%) reported on the identity of at least one cutting agent in the samples.

Laboratories **9**, **13**, **18** and **19** correctly identified all cutting agents added to the samples.

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

The test 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 fruit and vegetables, soil and water;
- petroleum hydrocarbons in soil and water;
- PFAS 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 methamphetamine and 3,4-methylenedioxymethamphetamine (MDMA) 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 issued	4 February 2021
Samples dispatched	12 April 2021
Results due	26 July 2021
Interim report issued	27 July 2021

2.2 Participation and Laboratory Code

Twenty-six laboratories registered to participate, with two laboratories requesting two sets of samples to be analysed independently by different analysts. Each participant was assigned a confidential laboratory code number. All participants submitted results, with one participant returning qualitative results only.

2.3 Test Material Specification

Four test samples were prepared in March 2021. The starting material for Samples S1, S2 and S3 was methamphetamine hydrochloride, approximately 80% base (m/m), supplied by the Australian Federal Police. The starting material for Sample S4 was MDMA hydrochloride, approximately 84% base (m/m), synthesised by NMI Chemical Reference Materials.

Dimethyl sulfone and 4-acetamidophenol (paracetamol) purchased from Sigma-Aldrich, levamisole hydrochloride purchased from ACROS, and glucodin (glucose) purchased from a local pharmacy were used as cutting agents. Sample S1 was cut with dimethyl sulfone, Sample S2 was cut with paracetamol, Sample S3 was cut with levamisole hydrochloride, and Sample S4 was cut with glucose.

The methamphetamine and MDMA 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 material with a known mass 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 68% methamphetamine base (m/m).

Sample S2 was prepared to contain approximately 21% methamphetamine base (m/m).

Sample S3 was prepared to contain approximately 44% methamphetamine base (m/m).

Sample S4 was prepared to contain approximately 47% MDMA 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, S3 and S4 is described in Appendix 1. Samples were demonstrated to be sufficiently homogeneous for the purpose of this PT study. Sample S2 was prepared using the same procedure and the results returned by participants 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 12 April 2021.

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 asked to analyse the samples using their routine quantitative method and were instructed as follows:

- Analyse each sample for the amount of drug by your routine test method.
- 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 Thursday 3 June 2021. Late results may not be included in the study report.

The results due date was changed from 3 June 2021 to 26 July 2021. Due to the exceptional international circumstances occurring over the course of this study, sample delivery to some participants was delayed. Therefore, the results turnaround time was extended by approximately two months.

2.7 Interim Report

An interim report was emailed to all participants on 27 July 2021.

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	Meth	ACN/MeOH/ H2O	Analog of methamphetamine	7	UPLC	MS/MS	C-18 column
	MDMA		Analog of MDMA				
2	All	Methanol	N/A	6	HPLC	UV/Vis	Luna C-18
3	All	methanol	External Standard	1	HPLC	DAD	zorbax eclipse XDB-C18 (4.6x1500 mm)
4	All	Purified Water	Phentermine	1	UPLC	DAD	Agilent Zorbax SB-C8
5	All	Ethyl Acetate	Diphenylamine	5	GC	FID	HP1
6	All	Methanol	Diazepam	6	GC	FID	128-5512 DB-5ms
7	MDMA	Methanol	PPA	5	GC	FID	Agilent HP-5 30mx0.32mm x0.25µm
8	All	Qualitative analysis only carried out by Marquis Colour test and GCMS					
9	All	D2O	Maleic acid		QNMR		NA
10	Meth	S1 and S3: water S2: 1-chlorobutane	S1 and S3: NA S2: isopropylamphetamine HCl	S1 and S2: 4 S3: 1	S1 and S3: LC S2: GC	S1 and S3: PDA S2: FID	S1 and S3: C18 BondaPak S2: HP1
	MDMA	water	N/A	4	LC	PDA	C18 BondaPak
11	Meth	S1 and S2: Dissolution in acetonitrile/ water S3: Chloroform	S1 and S2: Methoxyphenamine HCl S3: Hexacosane (C26)	S1 and S2: 3 S3: 4	S1 and S2: HPLC S3: GC	S1 and S2: DAD S3: FID	S1 and S2: Alltima C-18 S3: HP-1
12	All	Water	none	4	HPLC	DAD	Zorbax RX-SIL
13	All	Iso-octane with ammonium hydroxide	Dodecane	3	GC	FID	HP-1MS
14	All	Purified water	Phentermine	1	UPLC	DAD	Agilent Zorbax SB-C8
15	MDMA	Acetonitrile	None	4	HPLC	UV	PROTECOL C8 H 5UM 150X4.6MM
16	All	Water/ Acetonitrile (50/50)	None	7	HPLC	MS/MS	Acclaim RSLS 120 C18

Lab. Code	Analyte	Extraction Solvent	Internal Standard	Calib. Points	Technique	Detector	Column
17	All	Ethanol	Propyl Paraben	8	UPLC	DAD	BEH Shield RP18
18	All	Water	N/A	6	UPLC	PDA	Acquity UPLC BEH C18 1.7 µm 2.1 x 100 mm
19	All	deuterium oxide	maleic acid		QNMR	Bruker AVIII 600	N/A
20	All	Acetonitrile, ammonium acetate, diethylamine & water	n/a	4	HPLC	DAD	LiChrospher 100-5 RP18
21	All	Methanol	Strychnine	6	UPLC	FID	BEH Phenyl 1.8um, 2.1x100mm
22	MDMA	phosphate buffer pH 3 / methanol (70/30)	none	3	HPLC	DAD	C18
23	All	Methanol		5	HPLC	DAD	C18 column
24	All	methanol	propylparaben	3	UPLC	PDA	ACQUITY C-18
25	All	Methanol	None	5	HPLC	DAD	Phenomenex C-18-XB
26	Meth	Methanol	Procaine	4	HPLC	DAD	C18
	MDMA		Selegiline				
27	All	Methanol: KOH buffer (50:50)	Methoxyphenamine	3	UPLC	PDA	Waters Acquity UPLC BEH C18
28	All	Water	n/a	6	UPLC	PDA	Acquity UPLC BEH 1.7um 2.1 x 100mm

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			
2	Top Down - precision and estimates of the method and laboratory bias	Control samples - RM Duplicate analysis		Eurachem/CITAC Guide

Lab. Code	Approach to Estimating MU	Information Sources for MU Estimation*		Guide Document for Estimating MU
		Precision	Method Bias	
3		Control samples - CRM Duplicate analysis	Instrument calibration Laboratory bias from PT studies Standard purity	
4	Top Down - precision and estimates of the method and laboratory bias	Control samples - SS Duplicate analysis	Laboratory bias from PT studies Recoveries of SS	Eurachem/CITAC Guide
5	Top Down - precision and estimates of the method and laboratory bias	Control samples - RM	Standard purity	ISO/GUM
6	Estimating Measurement Uncertainty by black box by pairs of values	Standard deviation from PT studies only		ISO 21748
7	Standard deviation of replicate analyses multiplied by 2 or 3	Duplicate analysis	Masses and volumes	ISO/GUM
8	Qualitative analysis only			
9	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
10				
11	Bottom Up (ISO/GUM, fish bone/cause and effect diagram)	Duplicate analysis	Instrument calibration (S1 and S2) Masses and volumes Matrix effects (S1 and S2) Recoveries of SS (S1 and S2) Standard purity	ISO/GUM
12	Top Down - precision and estimates of the method and laboratory bias	Control samples - Sample from case Duplicate analysis	Laboratory bias from PT studies	Nordtest Report TR537
13	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	Eurachem/CITAC Guide
14	Top Down - precision and estimates of the method and laboratory bias	Control samples - SS Duplicate analysis	Laboratory bias from PT studies Recoveries of SS	Eurachem/CITAC Guide
15	Standard deviation of replicate analyses multiplied by 2 or 3	Control samples - RM		
16	Under determination. Fixed at 20% (relative).	Control samples - RM		ISO/GUM
17				
18	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	NATA Technical Note 33

Lab. Code	Approach to Estimating MU	Information Sources for MU Estimation*		Guide Document for Estimating MU
		Precision	Method Bias	
19	Bottom Up (ISO/GUM, fish bone/cause and effect diagram)	Control samples - CRM Duplicate analysis	Instrument calibration Masses and volumes Standard purity	NATA GAG Estimating and Reporting Measurement Uncertainty of Chemical Test Results
20	Uncertainty Budget Method	Control samples - RM Duplicate analysis	Instrument calibration Masses and volumes Standard purity	Internal SOP Document
21	Top Down - precision and estimates of the method and laboratory bias	Control samples - RM Duplicate analysis	Instrument calibration Masses and volumes Matrix effects Recoveries of SS	ISO/GUM
22	Bottom Up (ISO/GUM, fish bone/cause and effect diagram)	Standard deviation from PT studies only		ISO 11352
		Control samples - RM		
23	Bottom Up (ISO/GUM, fish bone/cause and effect diagram)	Control samples	Instrument calibration Masses and volumes Laboratory bias from PT studies	ISO/GUM
24	Top Down - precision and estimates of the method and laboratory bias	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
25				
26	Top Down - precision and estimates of the method and laboratory bias	Control samples - Authentic samples	Instrument calibration Homogeneity of sample Masses and volumes Matrix effects Laboratory bias from PT studies Recoveries of SS Standard purity	EA-4/16: 2003 and ILAC G-17:2002
27	Top Down - precision and estimates of the method and laboratory bias	Control samples - RM Duplicate analysis	Standard purity	Eurachem/CITAC Guide
28	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	Nata Technical Note 33

* 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	Methamphetamine		MDMA	
	Reference Standard	Purity (%)	Reference Standard	Purity (%)
1	Sigma Aldrich	100		99
2	In house	100	NMI	99.4
3				
4	Lipomed	99.950 ± 0.050	Lipomed	99.950 ± 0.050
5	Lipomed	99.987	Lipomed	99.95
6	Lipomed HCl Methamphetamine	79.4	Lipomed HCl MDMA	83.7
7	NT		LGC	97.7
8	Qualitative analysis only			
9	No reference standard involved			
10	in house std	100.6	in house std	96.8
11	NMI	99.8		
12	Sigma	100	Internal	100
13	NMI	99.8±0.9	NMI	99.4±1.5
14	Lipomed	99.950 +/- 0.050	Lipomed	99.950 +/- 0.050
15	NT		MDM-94-HC-100	83.7
16	Sigma	100	Lipomed	99.95
17	Lipomed	99.5	NMI	99.4
18	NMI	99.3	NMI	100
19	Sigma Aldrich	99.98	Sigma Aldrich	99.98
20	Lipomed	99.987+/-0.002	Lipomed	99.95+/-0.050
21	NMI	99.8	NMI	99.4
22	NT		Lipomed	99.95
23	NMI	99.4	NMI	99.8
24	NMI	99.80	NMI	99.40
25	Sigma	99.9	Chiron	99.4
26	Lipomed	99.467 +/- 0.015	Lipomed	99.95 +/- 0.050
27	NMI	99.8	NMI	99.4
28	NMI	99.3	NMI	100

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 Co-ordinator's Response
3	Quantitative analysis is based on the use of a historical value obtained from different batches of Certified reference material	
5	Accreditation for MDMA, not for methamphetamine	
7	MDMA accredited to ISO17025. Methamphetamine method still under development.	
8	Qualitative analysis only carried out by Marquis Colour test and GCMS	
10	insufficient sample if analysis needs repeating	Most participants use less than 50 mg for each analysis. For security and accountability reasons, NMI PT studies are conducted using the minimum practical amount of controlled substance.
11	Levamisole was seldom encountered as adulterant in local illicit seizures of methamphetamine hydrochloride ("ICE"). Methamphetamine Methodology: Linear regression	A variety of cutting agents are selected in NMI PT studies to cater for different participant laboratories.
13	For S3 - The analysis conducted does not distinguish between the two substances levamisole and dexamisole or a mixture of the two, known as tetramisole. Methamphetamine Methodology: 200uL of ammonium hydroxide for every 5mL of iso-octane MDMA Methodology: 400uL of ammonium hydroxide for every 10mL of iso-octane	
16	Uncertainty: method under validation	
19	Methodology: Simultaneous observation of analyte and IS peaks in 1H NMR spectrum acquired using QNMR conditions	
21	Sample S3 had an interfering peak in the quantitation analysis. Reporting here is approximate only.	
22	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 for this study specified details for the samples, including the analytes in the study and how many samples of each analyte. For participants enrolling through distributors, we will also confirm with them that they are passing this information on to participants.

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.

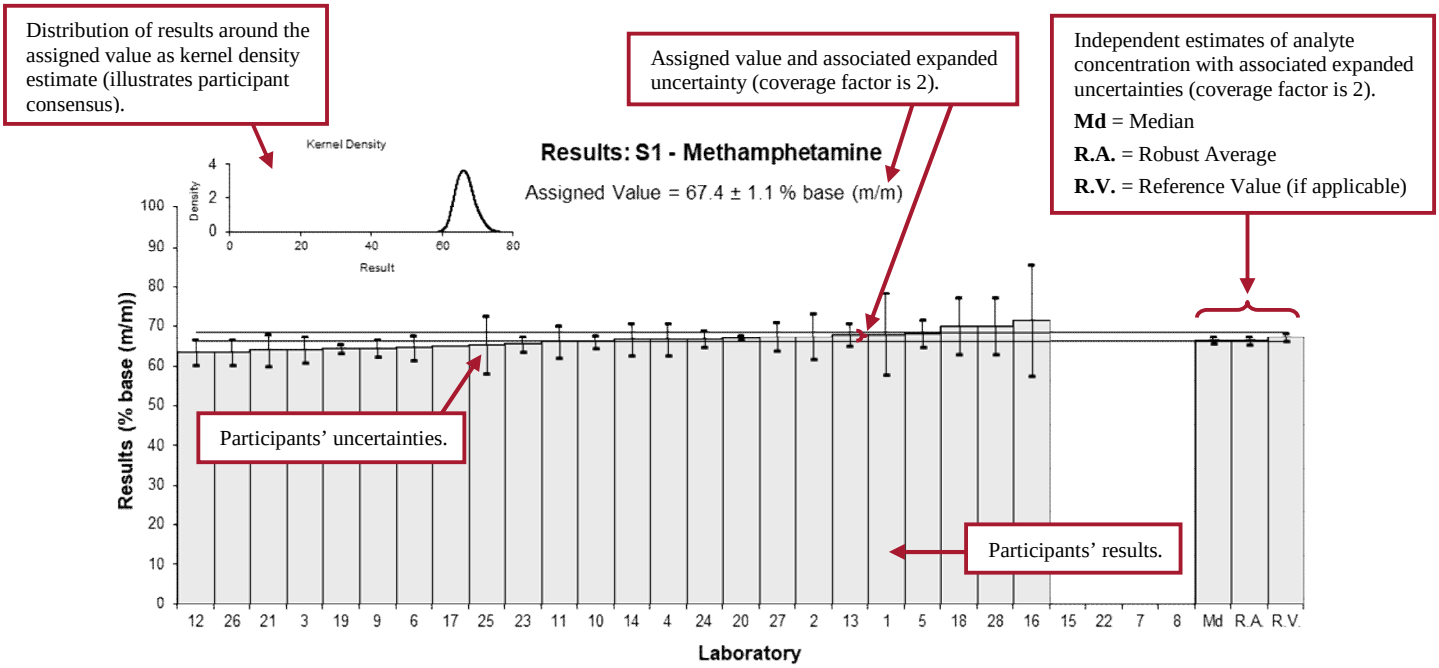


Figure 1 Guide to Presentation of Results

4.2 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 % methamphetamine or MDMA base (m/m) in the samples. The assigned values for Samples S1, S3 and S4 were reference values determined by quantitative nuclear magnetic resonance (qNMR) spectroscopy (Appendix 1). The assigned value for Sample S2 was the robust average of participants' results, and the expanded uncertainty was estimated from the associated robust SD (Appendix 2).

4.3 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]

4.4 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 levels of analytes 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.

4.5 Target Standard Deviation

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.

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

4.6 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;
- $|z| \geq 3.0$ is unsatisfactory.

4.7 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;
- $|E_n| > 1.0$ is unsatisfactory.

4.8 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	Methamphetamine
Units	% base (m/m)

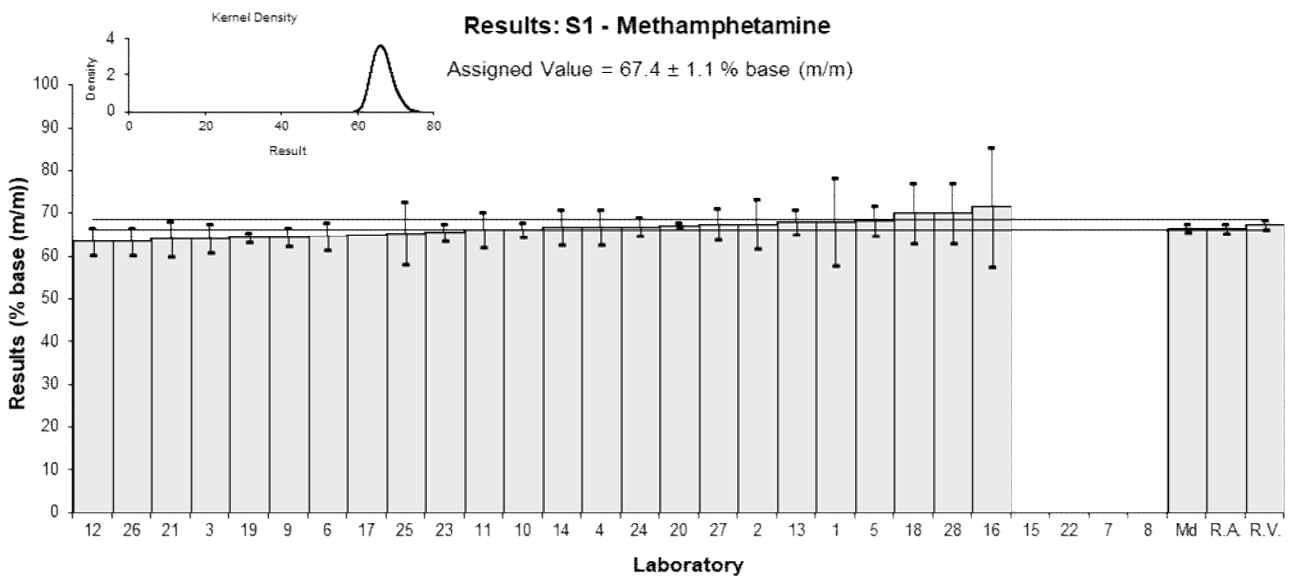
Participant Results

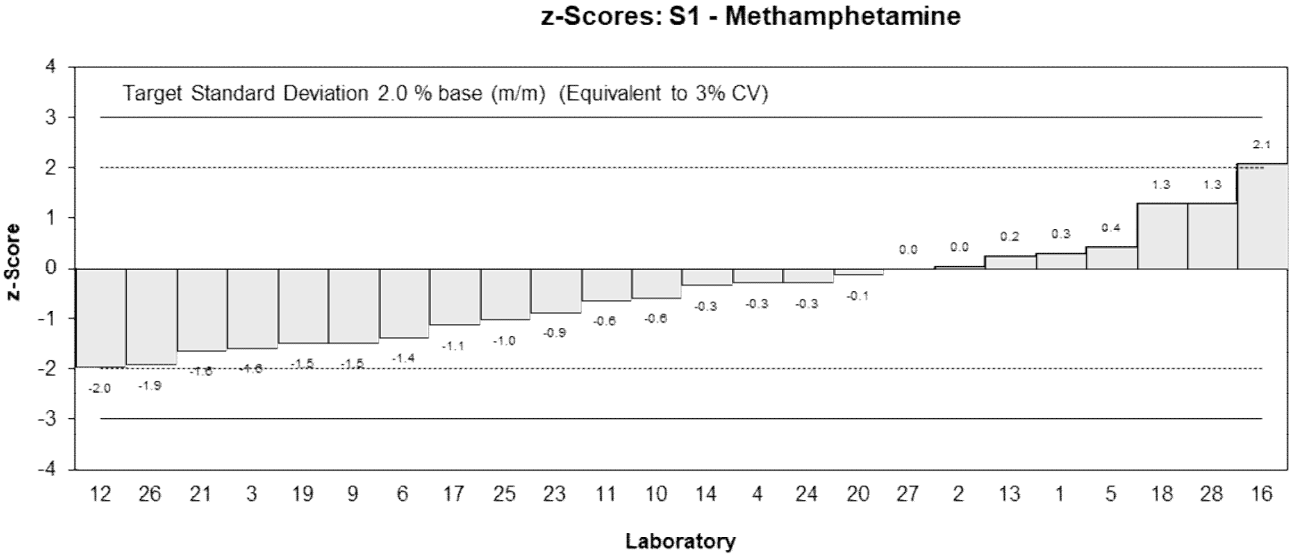
Lab. Code	Result	Uncertainty	z-Score	E_n-Score
1	68	10.2	0.30	0.06
2	67.5	5.7	0.05	0.02
3	64.2	3.2	-1.58	-0.95
4	66.8	4.1	-0.30	-0.14
5	68.25	3.4	0.42	0.24
6	64.6	3.2	-1.38	-0.83
7	NR	NR		
8	NR	NR		
9	64.4	2.1	-1.48	-1.27
10	66.2	1.6	-0.59	-0.62
11	66.1	4	-0.64	-0.31
12	63.4	3.2	-1.98	-1.18
13	67.9	2.8	0.25	0.17
14	66.7	4.1	-0.35	-0.16
15	NR	NR		
16	71.6	14	2.08	0.30
17	65.1	NR	-1.14	-2.09
18	70	7	1.29	0.37
19	64.4	1.0	-1.48	-2.02
20	67.1	0.6	-0.15	-0.24
21	64.1	4	-1.63	-0.80
22	NR	NR		
23	65.6	1.9	-0.89	-0.82
24	66.81	2.10	-0.29	-0.25
25	65.3	7.2	-1.04	-0.29
26	63.5	3.2	-1.93	-1.15
27	67.4	3.6	0.00	0.00
28	70	7.0	1.29	0.37

Statistics

Assigned Value*	67.4	1.1
Reference Value	67.4	1.1
Robust Average	66.4	1.1
Median	66.5	0.9
Mean	66.5	
N	24	
Max.	71.6	
Min.	63.4	
Robust SD	2.2	
Robust CV	3.3%	

* Assigned value is the reference value, determined by qNMR spectroscopy.


Kernel Density
Density
Result
Results: S1 - Methamphetamine
Assigned Value = 67.4 ± 1.1 % base (m/m)
Results (% base (m/m))
Laboratory
12 26 21 3 19 9 6 17 25 23 11 10 14 4 24 20 27 2 13 1 5 18 28 16 15 22 7 8 Md R.A. R.V.


z-Scores: S1 - Methamphetamine
Target Standard Deviation 2.0 % base (m/m) (Equivalent to 3% CV)
z-Score
Laboratory
12 26 21 3 19 9 6 17 25 23 11 10 14 4 24 20 27 2 13 1 5 18 28 16

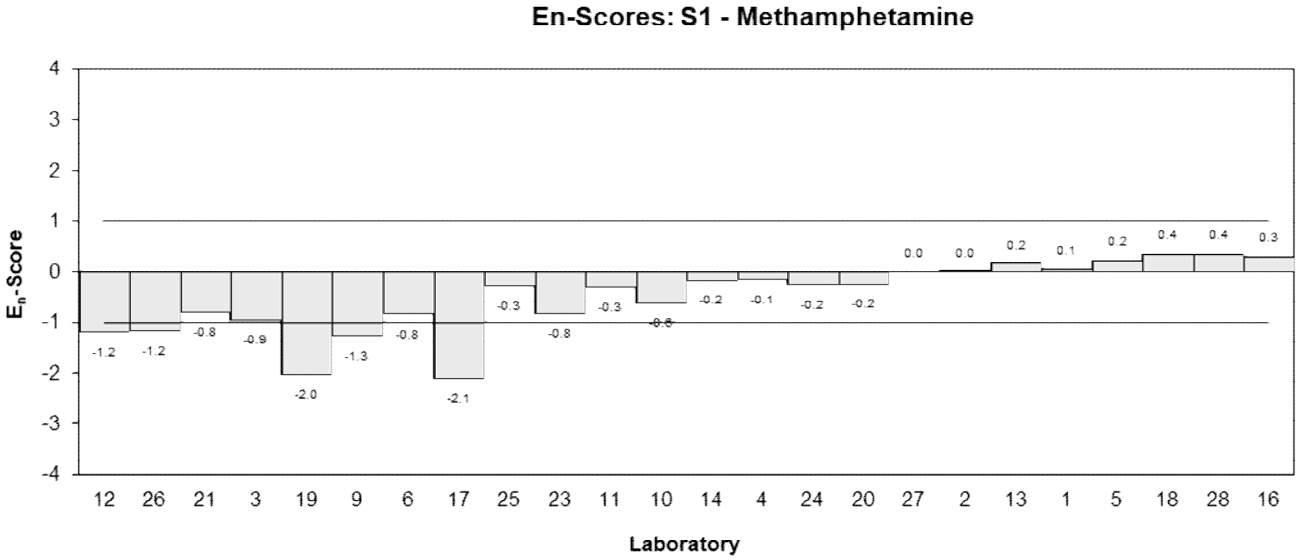

En-Scores: S1 - Methamphetamine
En-Score
Laboratory
12 26 21 3 19 9 6 17 25 23 11 10 14 4 24 20 27 2 13 1 5 18 28 16

Figure 2

Table 6

Sample Details

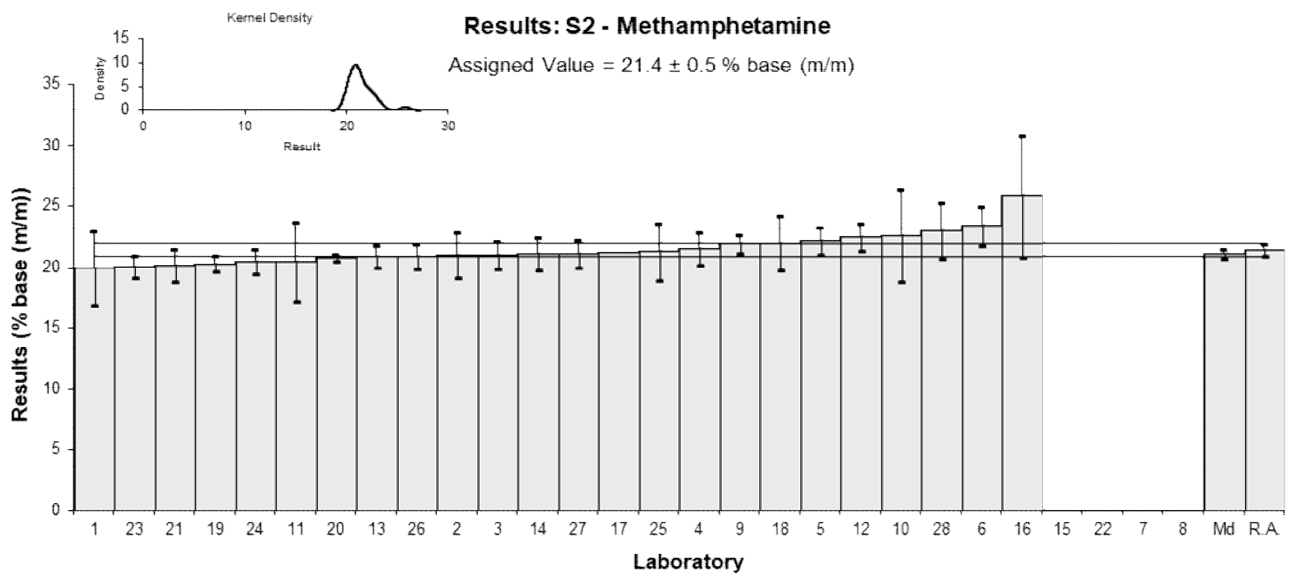
Sample No.	S2
Matrix	Powder
Analyte	Methamphetamine
Units	% base (m/m)

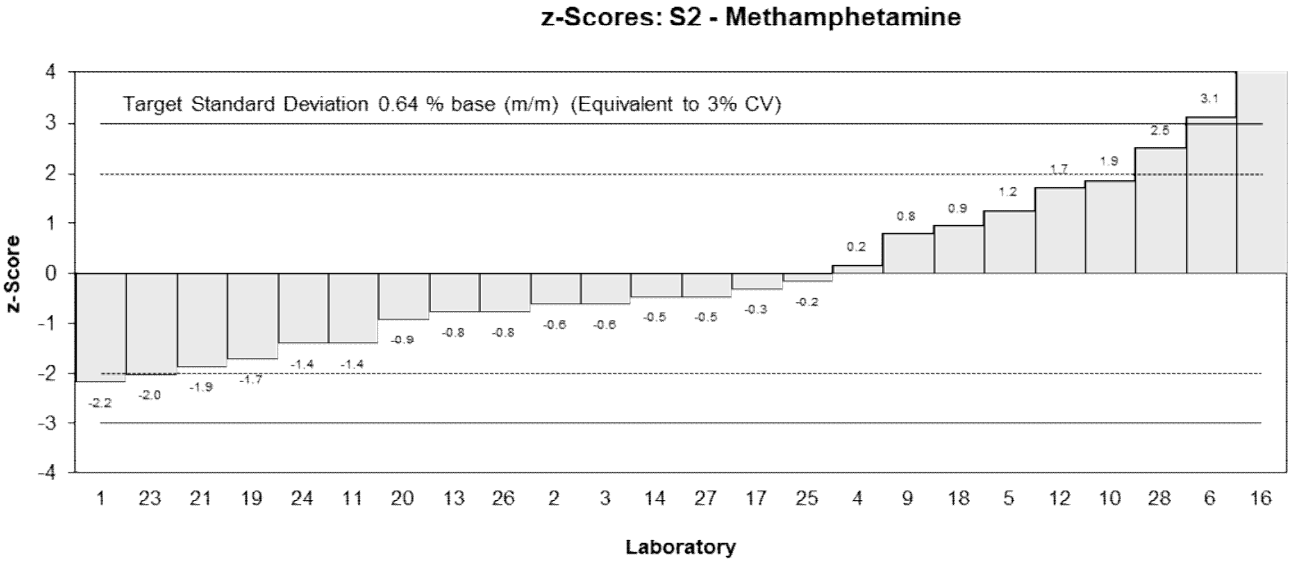
Participant Results

Lab. Code	Result	Uncertainty	z-Score	E_n-Score
1	20	3	-2.18	-0.46
2	21	1.8	-0.62	-0.21
3	21.0	1.1	-0.62	-0.33
4	21.5	1.3	0.16	0.07
5	22.2	1.1	1.25	0.66
6	23.4	1.6	3.12	1.19
7	NR	NR		
8	NR	NR		
9	21.9	0.7	0.78	0.58
10	22.6	3.7	1.87	0.32
11	20.5	3.2	-1.40	-0.28
12	22.5	1.1	1.71	0.91
13	20.9	0.9	-0.78	-0.49
14	21.1	1.3	-0.47	-0.22
15	NR	NR		
16	25.8	5	6.85	0.88
17	21.2	NR	-0.31	-0.40
18	22	2.2	0.93	0.27
19	20.3	0.6	-1.71	-1.41
20	20.8	0.3	-0.93	-1.03
21	20.2	1.3	-1.87	-0.86
22	NR	NR		
23	20.1	0.9	-2.02	-1.26
24	20.49	1.00	-1.42	-0.81
25	21.3	2.3	-0.16	-0.04
26	20.9	1	-0.78	-0.45
27	21.1	1.1	-0.47	-0.25
28	23	2.3	2.49	0.68

Statistics

Assigned Value	21.4	0.5
Robust Average	21.4	0.5
Median	21.1	0.4
Mean	21.5	
N	24	
Max.	25.8	
Min.	20	
Robust SD	1.1	
Robust CV	5.0%	


Kernel Density
Density
Result
Results: S2 - Methamphetamine
Assigned Value = 21.4 ± 0.5 % base (m/m)
Results (% base (m/m))
Laboratory
1 23 21 19 24 11 20 13 26 2 3 14 27 17 25 4 9 18 5 12 10 28 6 16 15 22 7 8 Md R.A.


z-Scores: S2 - Methamphetamine
Target Standard Deviation 0.64 % base (m/m) (Equivalent to 3% CV)
z-Score
Laboratory
1 23 21 19 24 11 20 13 26 2 3 14 27 17 25 4 9 18 5 12 10 28 6 16
-2.2 -2.0 -1.9 -1.7 -1.4 -1.4 -0.9 -0.8 -0.8 -0.6 -0.6 -0.5 -0.5 -0.3 -0.2 0.2 0.8 0.9 1.2 1.7 1.9 2.5 3.1

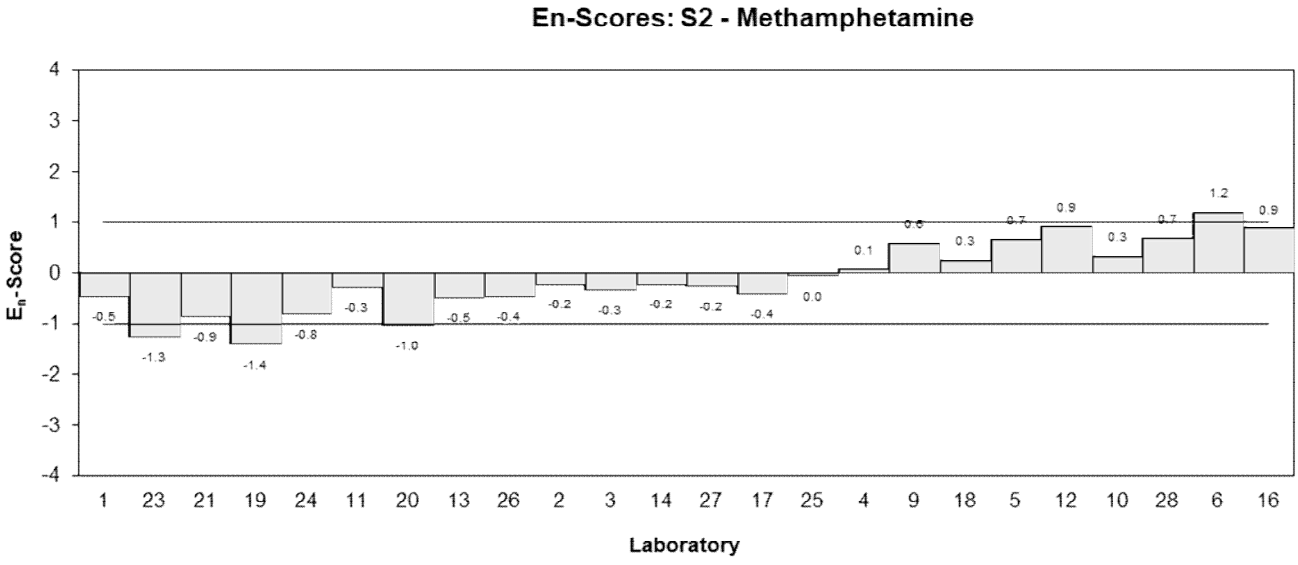

En-Scores: S2 - Methamphetamine
En-Score
Laboratory
1 23 21 19 24 11 20 13 26 2 3 14 27 17 25 4 9 18 5 12 10 28 6 16
-0.5 -1.3 -0.9 -1.4 -0.8 -0.3 -1.0 -0.5 -0.4 -0.2 -0.3 -0.2 -0.2 -0.4 0.0 0.1 0.6 0.3 0.7 0.9 0.3 0.7 1.2 0.9

Figure 3

Table 7

Sample Details

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

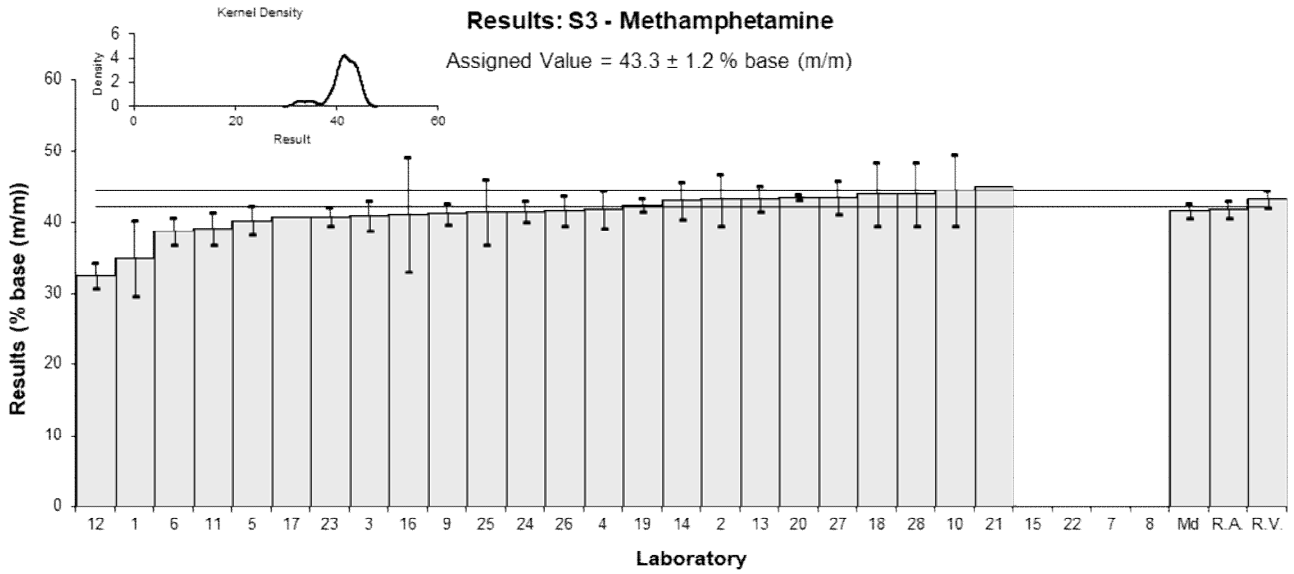
Participant Results

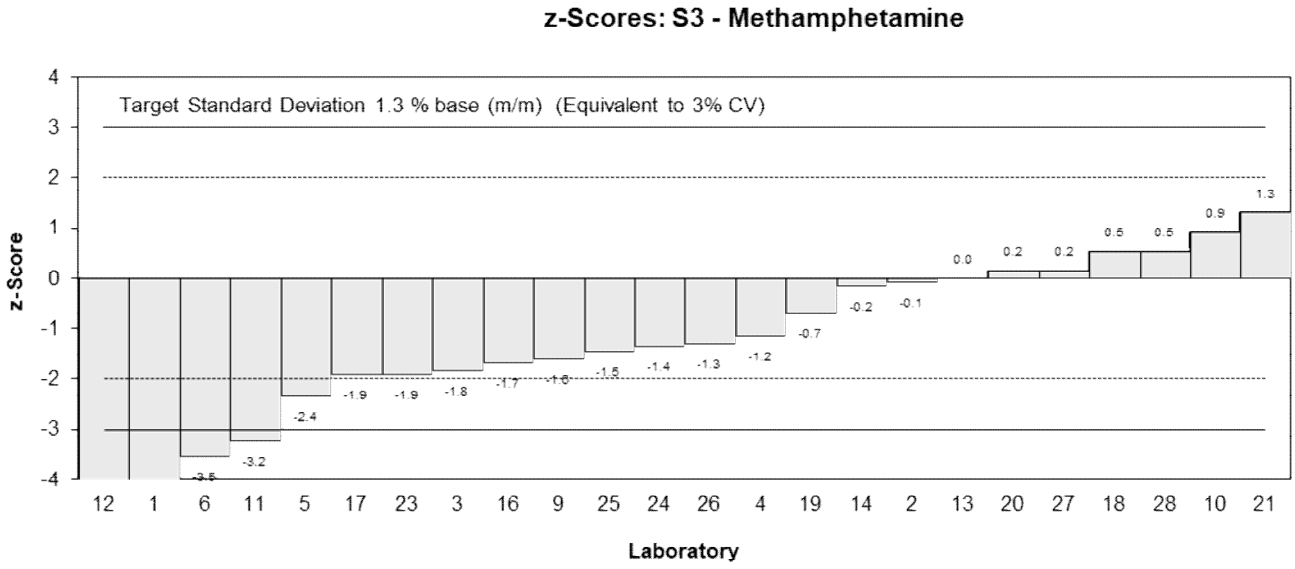
Lab. Code	Result	Uncertainty	z-Score	E_n-Score
1	35	5.3	-6.39	-1.53
2	43.2	3.6	-0.08	-0.03
3	40.9	2	-1.85	-1.03
4	41.8	2.6	-1.15	-0.52
5	40.24	2	-2.36	-1.31
6	38.7	1.9	-3.54	-2.05
7	NR	NR		
8	NR	NR		
9	41.2	1.4	-1.62	-1.14
10	44.5	5.0	0.92	0.23
11	39.1	2.2	-3.23	-1.68
12	32.5	1.8	-8.31	-4.99
13	43.3	1.8	0.00	0.00
14	43.1	2.6	-0.15	-0.07
15	NR	NR		
16	41.1	8	-1.69	-0.27
17	40.8	NR	-1.92	-2.08
18	44	4.4	0.54	0.15
19	42.4	0.9	-0.69	-0.60
20	43.5	0.4	0.15	0.16
21	45	NR	1.31	1.42
22	NR	NR		
23	40.8	1.3	-1.92	-1.41
24	41.53	1.40	-1.36	-0.96
25	41.4	4.6	-1.46	-0.40
26	41.6	2.1	-1.31	-0.70
27	43.5	2.3	0.15	0.08
28	44	4.4	0.54	0.15

Statistics

Assigned Value*	43.3	1.2
Reference Value	43.3	1.2
Robust Average	41.8	1.1
Median	41.6	1.0
Mean	41.4	
N	24	
Max.	45	
Min.	32.5	
Robust SD	2.2	
Robust CV	5.2%	

* Assigned value is the reference value, determined by qNMR spectroscopy.


Kernel Density
Density
Result
Results: S3 - Methamphetamine
Assigned Value = 43.3 ± 1.2 % base (m/m)
Results (% base (m/m))
Laboratory
12 1 6 11 5 17 23 3 16 9 25 24 26 4 19 14 2 13 20 27 18 28 10 21 15 22 7 8 Md R.A. R.V.


z-Scores: S3 - Methamphetamine
Target Standard Deviation 1.3 % base (m/m) (Equivalent to 3% CV)
z-Score
Laboratory
12 1 6 11 5 17 23 3 16 9 25 24 26 4 19 14 2 13 20 27 18 28 10 21

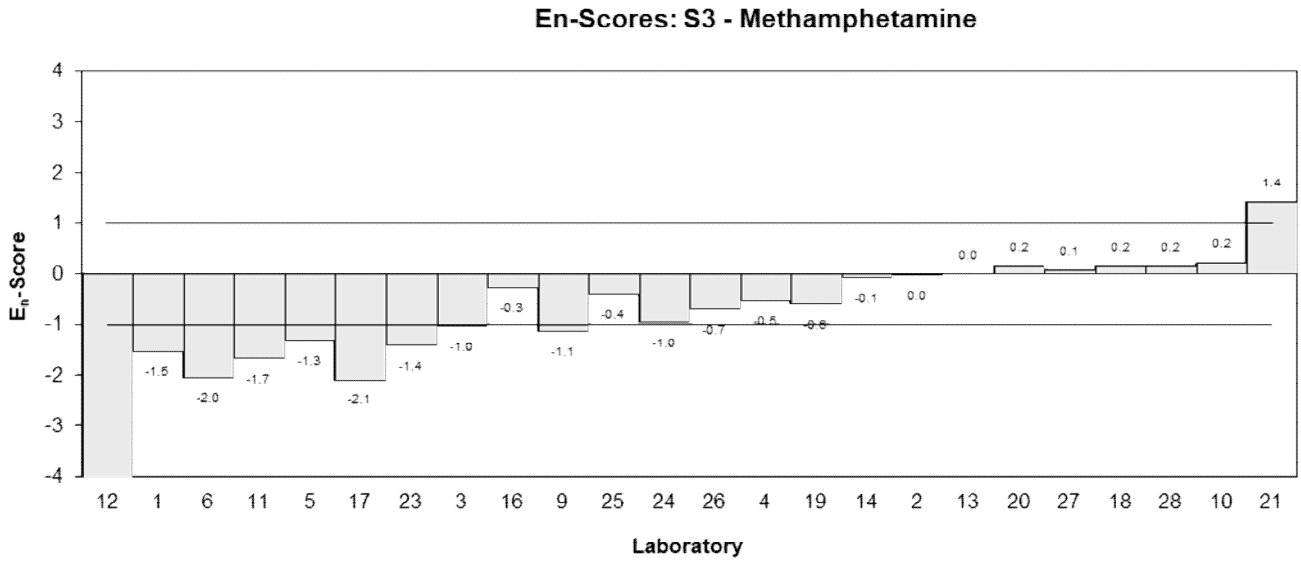

En-Scores: S3 - Methamphetamine
En-Score
Laboratory
12 1 6 11 5 17 23 3 16 9 25 24 26 4 19 14 2 13 20 27 18 28 10 21

Figure 4

Table 8

Sample Details

Sample No.	S4
Matrix	Powder
Analyte	MDMA
Units	% base (m/m)

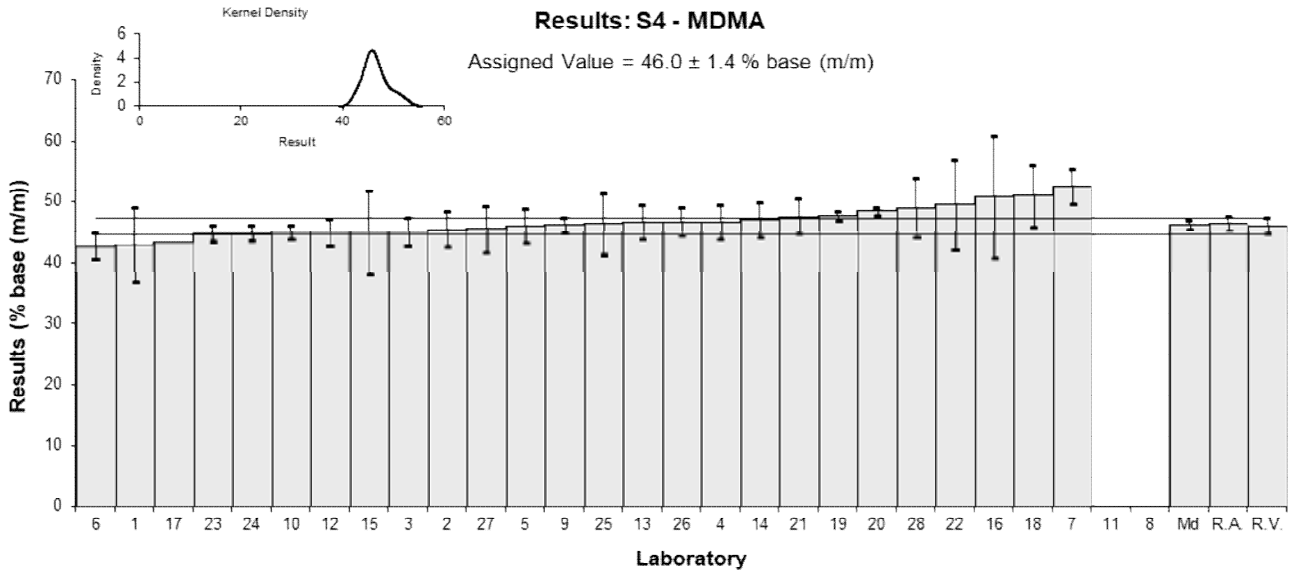
Participant Results

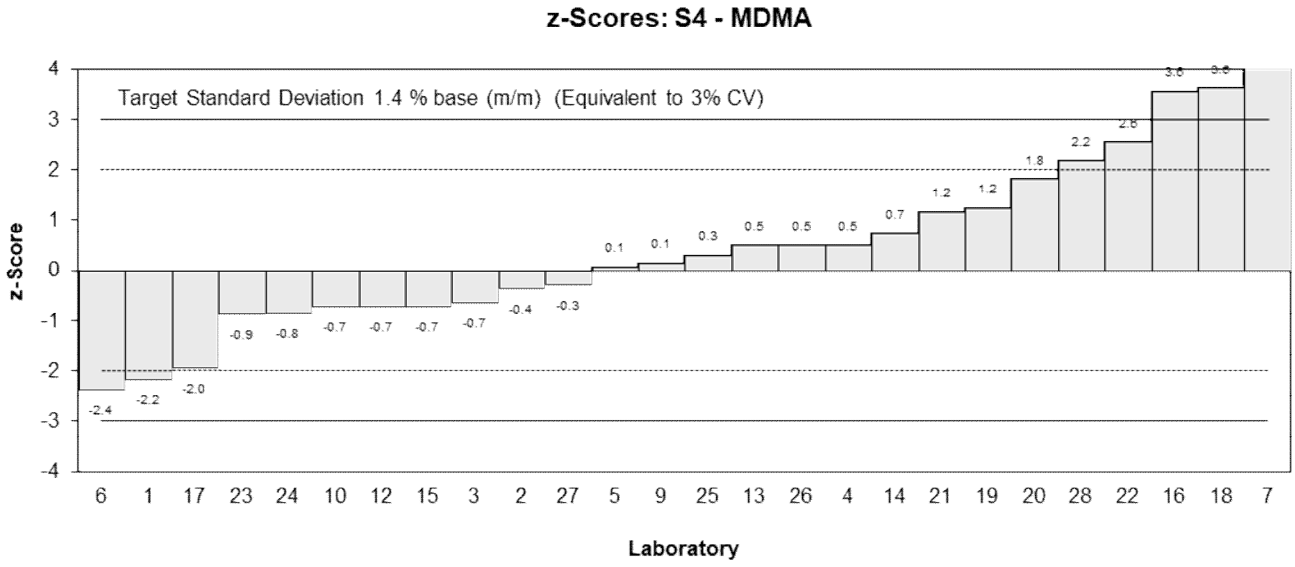
Lab. Code	Result	Uncertainty	z-Score	E_n-Score
1	43	6	-2.17	-0.49
2	45.5	2.9	-0.36	-0.16
3	45.1	2.3	-0.65	-0.33
4	46.7	2.8	0.51	0.22
5	46.09	2.8	0.07	0.03
6	42.7	2.1	-2.39	-1.31
7	52.58	2.77	4.77	2.12
8	NR	NR		
9	46.2	1.2	0.14	0.11
10	45.0	1.1	-0.72	-0.56
11	NR	NR		
12	45	2.3	-0.72	-0.37
13	46.7	2.9	0.51	0.22
14	47.0	2.9	0.72	0.31
15	45	6.75	-0.72	-0.15
16	50.9	10	3.55	0.49
17	43.3	NR	-1.96	-1.93
18	51	5.1	3.62	0.95
19	47.7	0.8	1.23	1.05
20	48.5	0.6	1.81	1.64
21	47.6	3	1.16	0.48
22	49.52	7.43	2.55	0.47
23	44.8	1.4	-0.87	-0.61
24	44.83	1.30	-0.85	-0.61
25	46.4	5.1	0.29	0.08
26	46.7	2.3	0.51	0.26
27	45.6	3.8	-0.29	-0.10
28	49	4.9	2.17	0.59

Statistics

Assigned Value*	46.0	1.4
Reference Value	46.0	1.4
Robust Average	46.5	1.2
Median	46.3	0.8
Mean	46.6	
N	26	
Max.	52.58	
Min.	42.7	
Robust SD	2.4	
Robust CV	5.2%	

* Assigned value is the reference value, determined by qNMR spectroscopy.


Kernel Density
Density
Result
Results: S4 - MDMA
Assigned Value = 46.0 ± 1.4 % base (m/m)
Results (% base (m/m))
Laboratory
6 1 17 23 24 10 12 15 3 2 27 5 9 25 13 26 4 14 21 19 20 28 22 16 18 7 11 8 Md R.A R.V.


z-Scores: S4 - MDMA
Target Standard Deviation 1.4 % base (m/m) (Equivalent to 3% CV)
z-Score
Laboratory
6 1 17 23 24 10 12 15 3 2 27 5 9 25 13 26 4 14 21 19 20 28 22 16 18 7

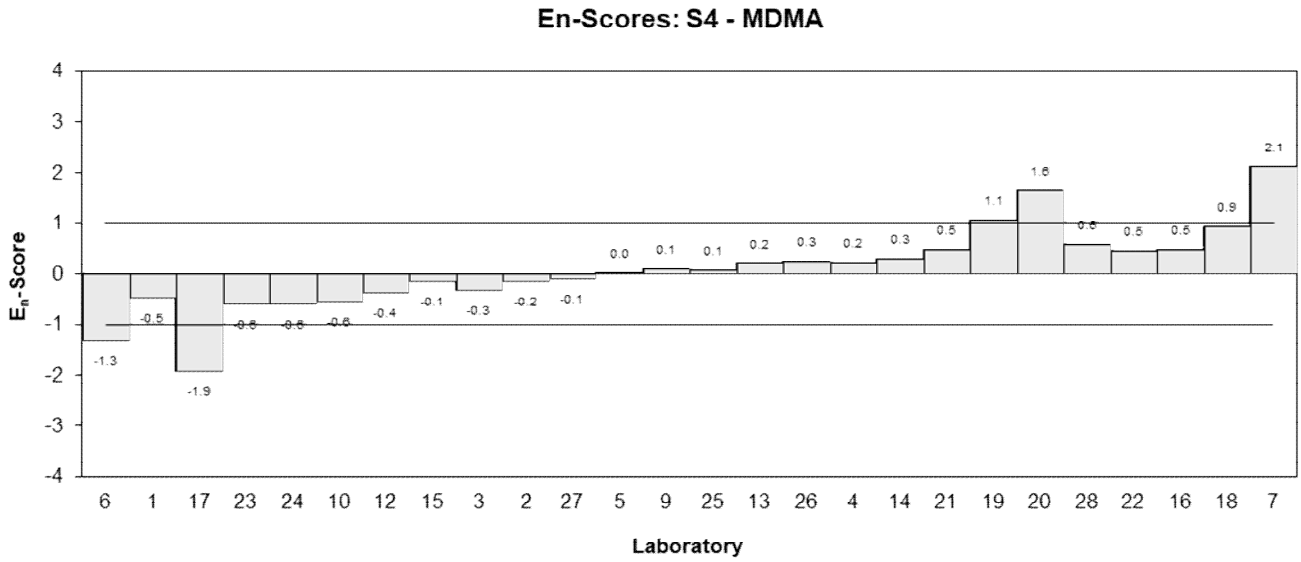

En-Scores: S4 - MDMA
En-Score
Laboratory
6 1 17 23 24 10 12 15 3 2 27 5 9 25 13 26 4 14 21 19 20 28 22 16 18 7

Figure 5

Table 9 Participants' Identification of Cutting Agents

Lab. Code	Cutting Agents*			
	S1	S2	S3	S4
Preparation	Dimethyl sulfone	Paracetamol	Levamisole	Glucose
1	none	paracetamol	levamisole	none
2				
3		Paracetamol	Levamisole	
4	Dimethyl Sulfone	Paracetamol	Tetramisole / Levamisole (specific isomer not determined)	-
5	Dimethyl sulfone (not quantified)	Paracetamol (not quantified)	Levamisole (not quantified)	Sugars (not quantified)
6		Acetaminophen	Levamisole	
7	None identified	Paracetamol	Tetramisole	Glucose
8	Methylamphetamine (Qualitative analysis only)	Methylamphetamine (Qualitative analysis only)	Methylamphetamine and levamisole (Qualitative analysis only)	MDMA (Qualitative analysis only)
9	dimethylsulfone	paracetamol	levamisole	glucose
10	Not determined	paracetamol	phenylimidothiazole	Not determined
11		Paracetamol	Levamisole	
12		Paracetamol	Levamisole/Tetramisole	Glucose
13	Dimethylsulfone	Paracetamol	Levamisole	Glucose
14	Dimethyl sulfone	Paracetamol	Tetramisole/Levamisole (specific isomer not determined)	-
15				
16	Dimethylsulfone	Acetaminophen	Levamisole	
17	Dimethylsulfone	Acetaminophen : 73.2 %	Levamisole : 39.2 %	
18	Dimethyl sulfone	Paracetamol	Levamisole/Dexamisole	Glucose
19	Dimethylsulfone	Paracetamol	Levamisole	Glucose
20	dimethylsulfone	paracetamol	levamisole	
21		Paracetamol	Levamisole	
22	none	paracetamol	levamisole	glucose
23	Dimethyl sulfone	Acetaminophen	Levamisole (or Tetramisole)	–
24	Dimethyl sulfone	Paracetamol	Levamisole	
25	None	Paracetamol	Levamisole	None
26	Dimethylsulfone	Paracetamol	Levamisole	
27	Methylsulfonylmethane	Paracetamol	Levamisole	-
28	Not detected	Paracetamol	Levamisole/Dexamisole	Glucose

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

6 DISCUSSION OF RESULTS

6.1 Assigned Value

Reference values obtained using the qNMR measurement method described in Appendix 1 were used as the assigned values for Samples S1, S3 and S4. Maleic acid (NMI CRM QNMR010) was used as the internal standard. The measured reference values for these samples were in agreement with their gravimetric preparation value and the robust average of participants' results within their respective associated uncertainties. The uncertainty of the 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.

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 value for Sample S2 was the robust average of participants' results. The robust average 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 S2 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).

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 98 numeric results, 93 (95%) were reported with an associated expanded uncertainty. Laboratory **17** did not report any uncertainties; this participant was not accredited. Laboratory **21** did not report an uncertainty for Sample S3; this participant reported that there was an interference in their quantitation analysis for this sample and therefore their result was approximate.

The magnitudes of reported uncertainties were within the range 0.9% to 20% relative to the reported result. Of the 93 expanded measurement uncertainties reported, 63 (68%) were between 3% and 10% relative. Laboratories reporting uncertainties smaller than 3% or larger than 10% relative may wish to consider whether these estimates are realistic for routine measurements or fit for purpose.

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 66.81 ± 2.10%, it is recommended to report 66.8 ± 2.1%.[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, and between-laboratory CVs 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	Methamphetamine	67.4	1.2	3	3.3
S2	Methamphetamine	21.4	2.2	3	5.0
S3	Methamphetamine	43.3	1.5	3	5.2
S4	MDMA	46.0	1.5	3	5.2

Of 98 results for which z-scores were calculated, 80 (82%) returned a z-score of $|z| \leq 2.0$, indicating a satisfactory performance.

Sixteen participants: **2**, **3**, **4**, **9**, **10**, **13**, **14**, **15** (one result submitted only), **17**, **19**, **20**, **21**, **24**, **25**, **26** and **27** returned satisfactory z-scores for all samples. Eleven participants returned at least one questionable or unsatisfactory z-score. Laboratory **8** did not submit numeric results for any samples as they performed qualitative analysis only.

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

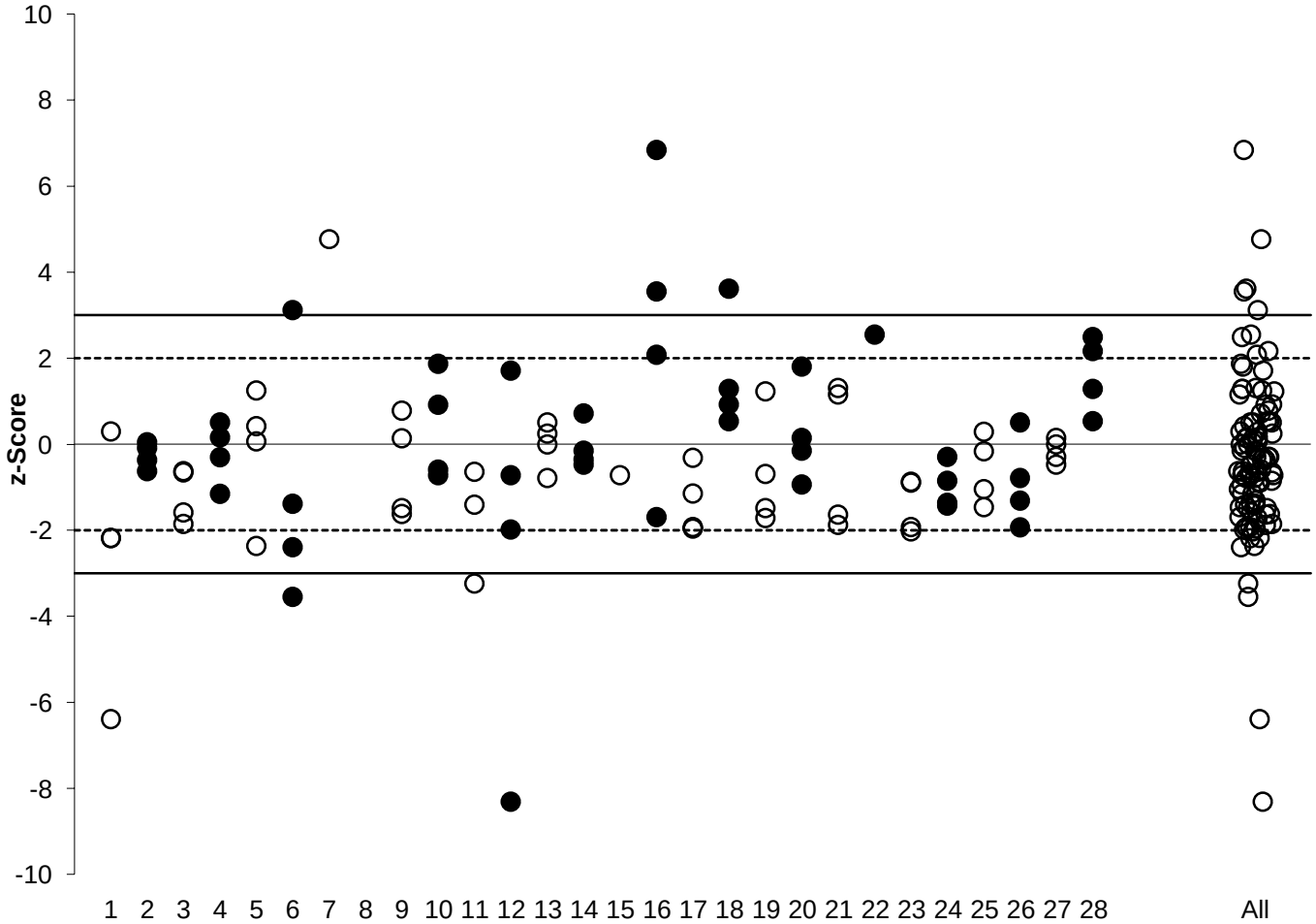


Figure 6 z-Score Dispersal by Laboratory

6.4 E_n-Score

If 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 98 results for which E_n-scores were calculated, 74 (76%) 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.

Thirteen participants: **2**, **4**, **10**, **13**, **14**, **15** (one result submitted only), **16**, **18**, **22** (one result submitted only), **24**, **25**, **27** and **28** returned satisfactory E_n-scores for all samples. Fourteen participants returned at least one unsatisfactory E_n-score.

The dispersal of participants' E_n-scores is presented graphically in Figure 7.

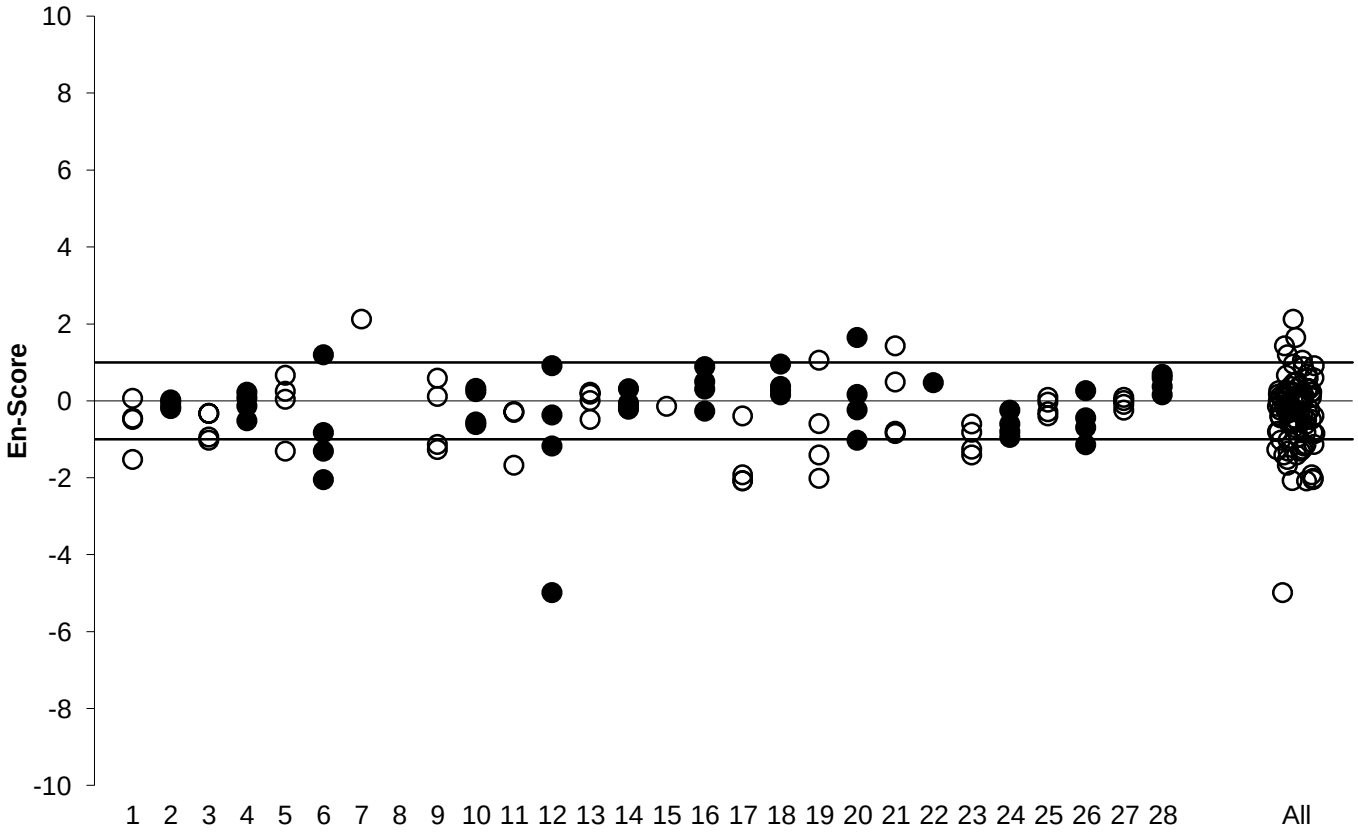


Figure 7 E_n-Score Dispersal by Laboratory

6.5 Identification of Cutting Agents

A number of cutting agents were added to the samples in this study: dimethyl sulfone in Sample S1, paracetamol in Sample S2, levamisole in Sample S3, and glucose in Sample S4. Participants were requested to identify the cutting agent(s) in the samples if part of their routine analysis, and results reported are presented in Table 9.

Twenty-six participants (93%) reported on the identity of at least one cutting agent in the samples. Laboratory **9**, **13**, **18** and **19** correctly identified all cutting agents in this study.

For Sample S1, all 14 participants reporting on the cutting agent correctly identified dimethyl sulfone. For Sample S2, all 25 participants reporting on the cutting agent correctly identified paracetamol. Sample S3 had the highest proportion of cutting agent results reported, and all 26 reported results correctly identified levamisole. For Sample S4, nine participants reported on the identity of the cutting agent; of these, eight participants correctly identified glucose, with another participant reported unspecified 'sugars'.

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, 4, 5 (MDMA), 6, 7 (MDMA), 9, 10, 11 (methamphetamine), 12 (methamphetamine) 13, 14, 18, 20, 21, 24, 26, 27, 28
	Not accredited / Not reported	3, 5 (methamphetamine), 7 (methamphetamine), 11(MDMA), 12 (MDMA), 15, 16, 17, 19, 22, 23, 25
Average Sample Mass Used (mg)	5 – 19	2, 6, 7 (MDMA), 9, 11 (methamphetamine), 13, 16, 19, 22 (MDMA)
	20 – 30	4, 5, 14, 15 (MDMA), 18, 24, 25, 28
	31 – 50	1, 3, 12, 17, 20, 23, 26, 27
	51 – 100	10, 21
Conversion to Base?	Yes	3, 5, 6, 9, 10, 11 (methamphetamine Sample S3) 12, 13, 15 (MDMA), 16, 17, 20, 22 (MDMA), 23, 26, 27
	No	2, 4, 7 (MDMA), 11 (methamphetamine Samples S1 and S2), 14, 18, 19, 24, 25, 28
	Not reported	1, 21
Instrument Used for Quantification	HPLC-DAD	3, 10 (methamphetamine Samples S1 and S3; MDMA), 11 (methamphetamine Samples S1 and S2), 12, 20, 22 (MDMA), 23, 25, 26
	HPLC-UV/Vis	2, 15 (MDMA)
	HPLC-MS/MS	16
	UPLC-DAD	4, 14, 17, 18, 24, 27, 28
	UPLC-FID	21
	UPLC-MS/MS	1
	GC-FID	5, 6, 7 (MDMA), 10 (methamphetamine Sample S2), 11 (methamphetamine Sample S3), 13
	QNMR	9, 19
Solvent	Water	4, 10 (methamphetamine Samples S1 and S3; MDMA), 12, 14, 18, 28
	Methanol	2, 3, 6, 7 (MDMA), 21, 23, 24, 25, 26
	Other / Not reported	1, 5, 9, 10 (methamphetamine Sample S2), 11 (methamphetamine), 13, 15 (MDMA), 16, 17, 19, 20, 22 (MDMA), 27
Sources of Calibration Standard (Methamphetamine)	NMI Australia	11, 13, 18, 21, 23, 24, 27, 28
	Lipomed	4, 5, 6, 14, 17, 20, 26
	Sigma Aldrich	1, 12, 16, 19, 25
	Other	2, 10
	Not Reported	3, 9

		Lab. Code
Sources of Calibration Standard (MDMA)	NMI Australia	2, 13, 17, 18, 21, 23, 24, 27, 28
	Lipomed	4, 5, 6, 14, 15, 16, 20, 22, 26
	Other	7, 10, 12, 19, 25
	Not Reported	1, 3, 9

Plots of the z-score versus various methodology parameters are presented in Figures 8 to 12. A variety of methodologies were used by participants in this study. It was observed in this study that methamphetamine results obtained by extracting with methanol and then analysing using HPLC-DAD were in general slightly biased low.

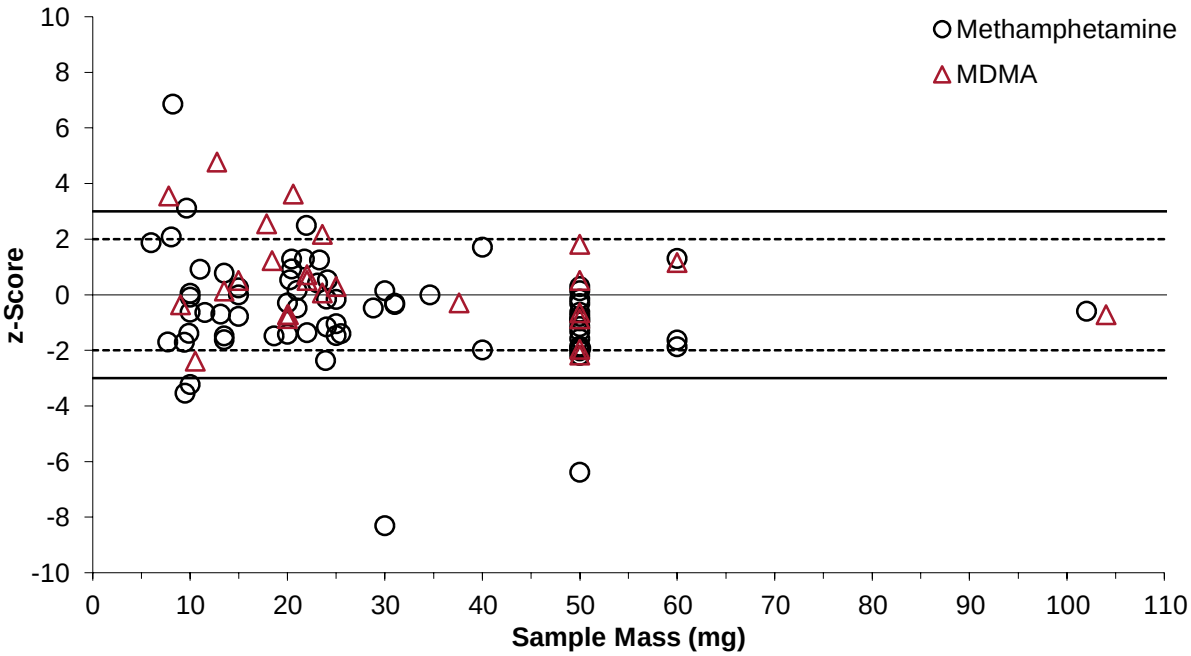


Figure 8 z-Score vs Sample Mass Used for Analysis

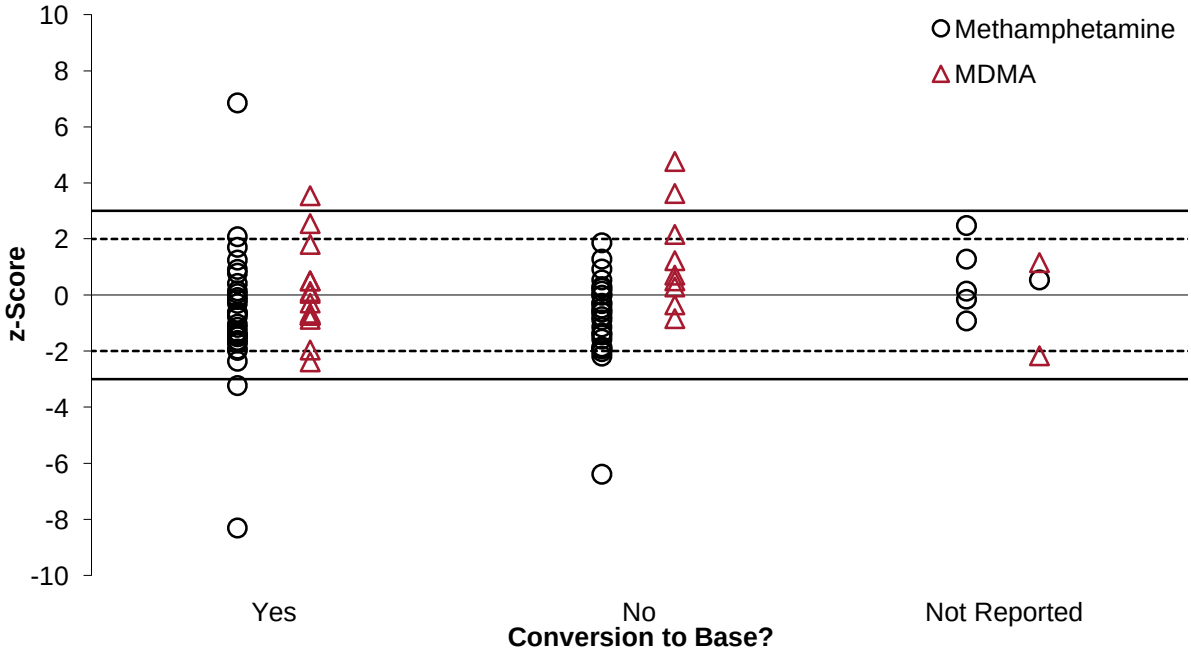


Figure 9 z-Score vs Sample Processing

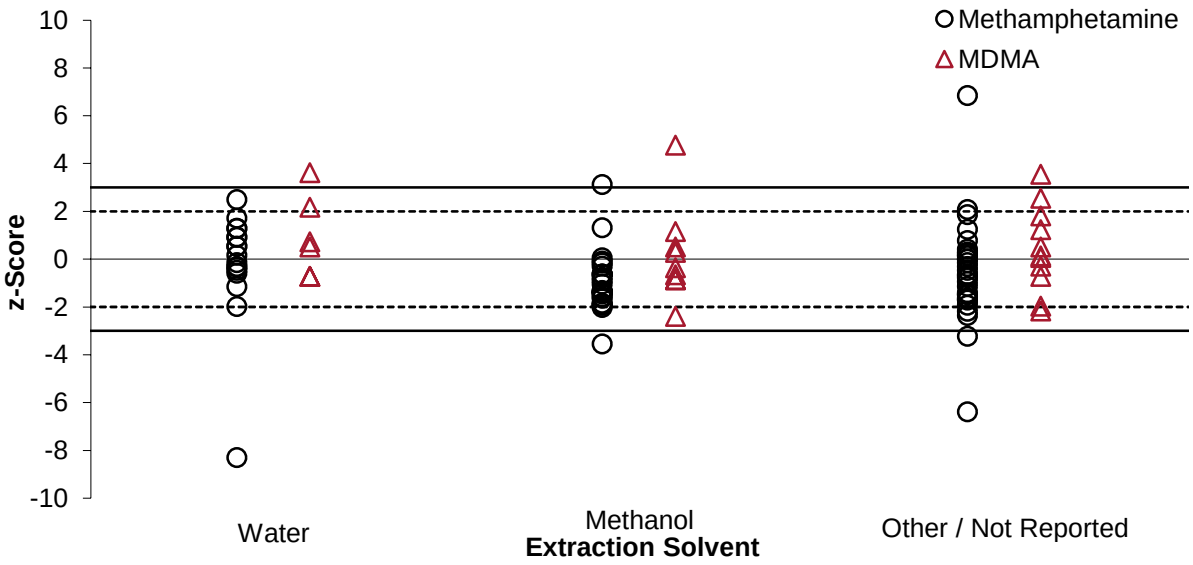


Figure 10 z-Score vs Extraction Solvent

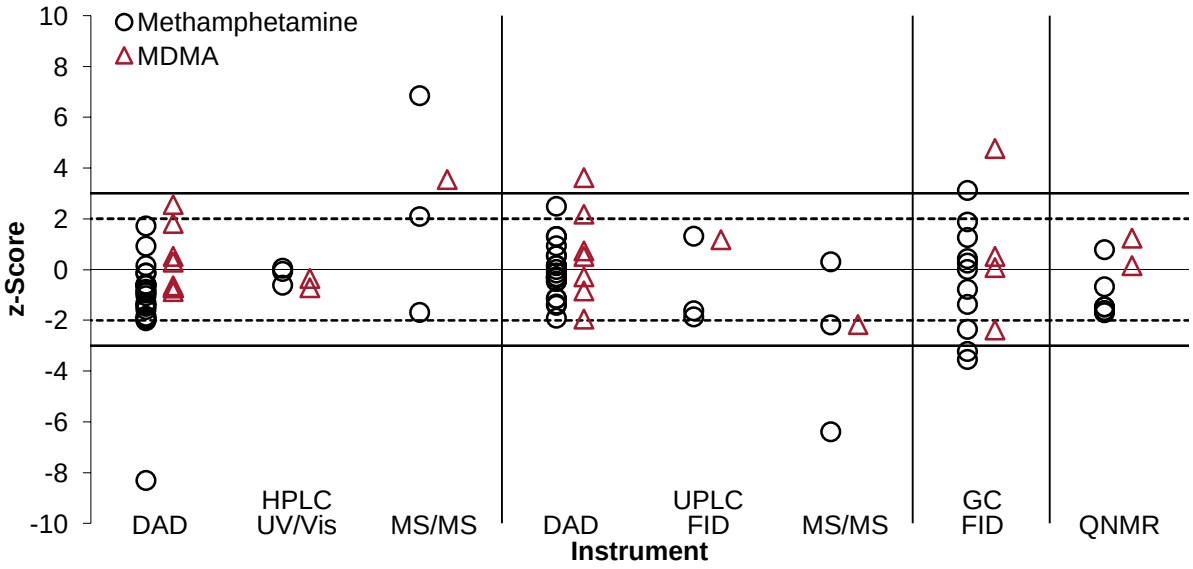


Figure 11 z-Score vs Measurement Instrument

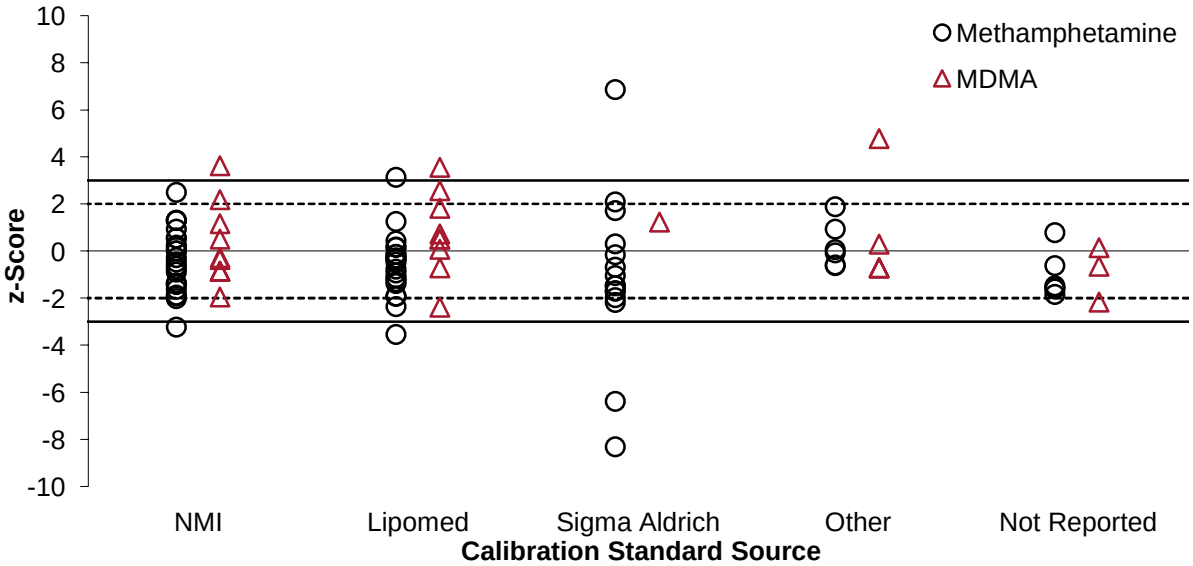


Figure 12 z-Score vs Calibration Standard Source

6.7 Comparison with Previous Methamphetamine and MDMA PT Studies

To enable direct comparison with previous Methamphetamine and MDMA PT studies, 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 2010 – 2021 (last ten studies with methamphetamine) is presented in Figure 13. The average proportion of satisfactory z-scores and E_n-scores over this period is 85% and 79% respectively.

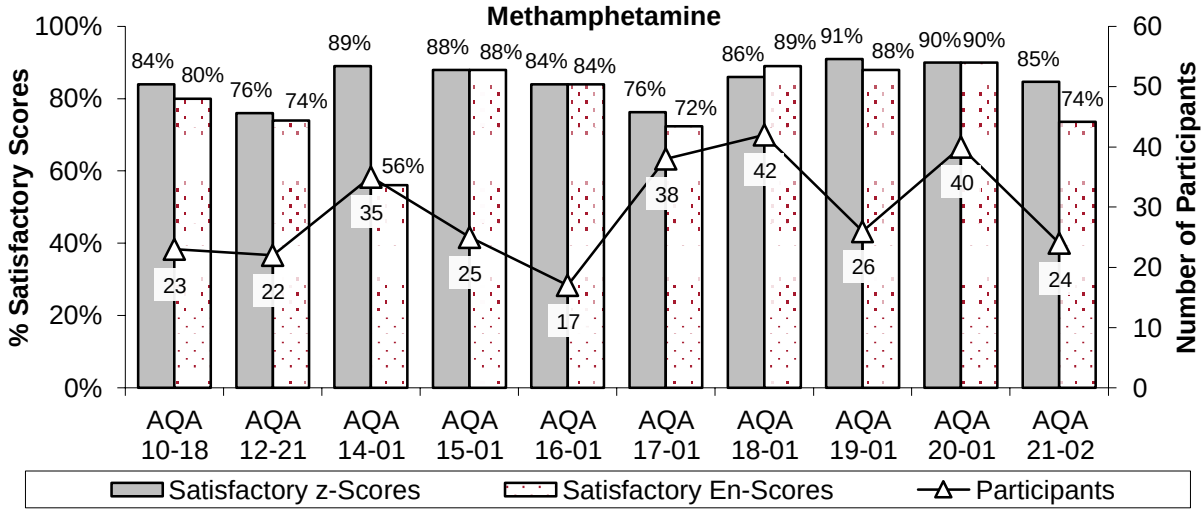


Figure 13 Summary of Participants' Performance in Methamphetamine PT Studies

A summary of the satisfactory performance, presented as a percentage of the total number of scores, obtained by PT study participants for MDMA from 2001 – 2021 (last nine studies with MDMA) is presented in Figure 14. The average proportion of satisfactory z-scores and E_n-scores over this period is 75% and 64% respectively.

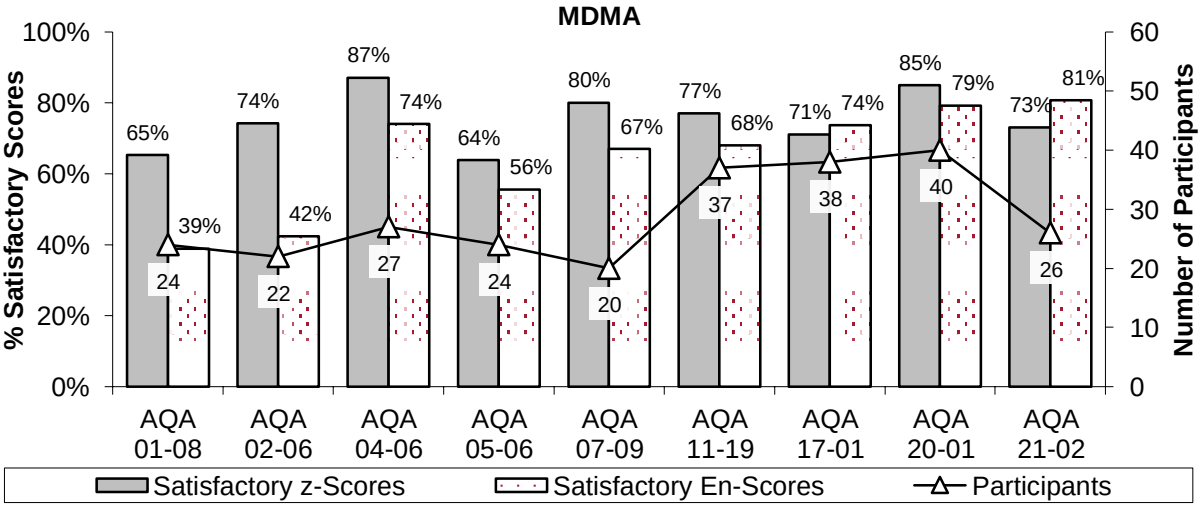


Figure 14 Summary of Participants' Performance in MDMA PT Studies

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.

7 REFERENCES

[1] ISO/IEC 17043:2010, *Conformity assessment – General requirements for proficiency testing*.

[2] NMI, 2021, *Study Protocol for Proficiency Testing*, viewed August 2021, <https://www.industry.gov.au/sites/default/files/2020-10/cpt_study_protocol.pdf>.

[3] NMI, 2021, *Chemical Proficiency Testing Statistical Manual*, viewed August 2021, <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 13528:2015, *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 17025:2017, *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 August 2021, <http://www.eurachem.org/images/stories/Guides/pdf/QUAM2012_P1.pdf>.

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

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

APPENDIX 1 – REFERENCE VALUES

Five sample vials from each of Samples S1, 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 in a final total volume of 900 µL of internal standard solution, with accurate weighing of the internal standard solution added. Samples were analysed on a Bruker 500 MHz Ascend NMR spectrometer, using a qNMR relaxation time of 25 s.

For Samples S1 and S3, the mass fraction of methamphetamine was determined from the NMR response at 1.25 ppm. For Sample S4, the mass fraction of MDMA was determined from the NMR response at 1.26 ppm. The averages of the mass fractions determined for the different vials of Samples S1, S3 and S4 (Tables 13 to 15) were used as the reference values and the assigned values for this PT study. The standard uncertainties on the mass fraction reference values were 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, S3 and S4 were in agreement with both the gravimetric preparation value and the robust average of participants' results, within their respective associated uncertainties.

Homogeneity checks were based on that described by Thompson and Fearn,[10] 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 of 3%.

Table 13 Reference Value for Sample S1

Vial No.	Methamphetamine (% base (m/m))	
	Replicate 1	Replicate 2
114	67.4	67.4
125	67.6	68.0
127	67.1	67.2
132	67.5	67.1
135	67.3	67.6
Mean	67.4	
CV	0.41%	

Thompson and Fearn Homogeneity Tests[10]

Test	Value	Critical	Result
Cochran	0.38	0.84	**Pass**
S_{an}/σ	0.10	0.5	**Pass**
s^2_{sam}	0.037	0.96	**Pass**

Sample S1 Reference Value: 67.4 ± 1.1% methamphetamine 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 = 2.04).[9]

Table 14 Reference Value for Sample S3

Vial No.	Methamphetamine (% base (m/m))	
	Replicate 1	Replicate 2
317	43.5	42.3
319	42.8	42.6
336	44.0	43.5
339	43.6	43.2
344	43.8	43.8
Mean	43.3	
CV	1.3%	

Thompson and Fearn Homogeneity Tests[10]

Test	Value	Critical	Result
Cochran	0.76	0.84	**Pass**
S_{an}/σ	0.33	0.5	**Pass**
s^2_{sam}	0.151	0.76	**Pass**

Sample S3 Reference Value: 43.3 ± 1.2% methamphetamine 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 = 2.13).[9]

Table 15 Reference Value for Sample S4

Vial No.	MDMA (% base (m/m))	
	Replicate 1	Replicate 2
403	45.4	45.6
417**	46.7	45.4
424	46.2	46.2
440	45.9	45.7
443	46.2	46.5
Mean	46.0	
CV	1.0%	

Thompson and Fearn Homogeneity Tests[10]

Test	Value	Critical	Result
Cochran	0.53	0.91	**Pass**
S_{an}/σ	0.11	0.5	**Pass**
s^2_{sam}	0.138	0.51	**Pass**

Sample S4 Reference Value: 46.0 ± 1.4% MDMA 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 = 2.09).[9]

** Results from vial 417 were not included in the test for homogeneity, being identified as Cochran outliers due to the difference between replicates.[10]

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.

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

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 16.

Table 16 Uncertainty of Robust Average of Methamphetamine in Sample S2

No. results (p)	24
Robust Average	21.35% base (m/m)
$S_{rob\ average}$	1.07% base (m/m)
$u_{rob\ average}$	0.27% base (m/m)
k	2
$U_{rob\ average}$	0.54% base (m/m)

Therefore, the robust average for Sample S2 is 21.4 ± 0.5% 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 (Sections 4.6 and 4.7).

A worked example is set out below in Table 17.

Table 17 z-Score and E_n-Score for Sample S1 Methamphetamine Result Reported by Laboratory 1

Participant Result (% base (m/m))	Assigned Value (% base (m/m))	Target Standard Deviation	z-Score	E_n-Score
68 ± 10.2	67.4 ± 1.1	3% as PCV, or: 0.03 × 67.4 = 2.022% base (m/m)	z-Score = $\frac{68-67.4}{2.022}$ = 0.30	E_n-Score = $\frac{68-67.4}{\sqrt{10.2^2+1.1^2}}$ = 0.06

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 Detector
EA	European Accreditation
FID	Flame Ionisation Detector
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
ILAC	International Laboratory Accreditation Cooperation
ISO	International Organization for Standardization
LC	Liquid Chromatography
Max.	Maximum value
Md	Median value
MDMA	3,4-Methylenedioxymethamphetamine
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
NT	Not Tested
PCV	Performance Coefficient of Variation
PDA	Photodiode Array
PT	Proficiency Test
qNMR	Quantitative Nuclear Magnetic Resonance
R.A.	Robust Average

R.V.	Reference Value
RM	Reference Material
SD	Standard Deviation
SI	International System of Units
SS	Spiked Samples
UPLC	Ultra Performance Liquid Chromatography
UV/Vis	Ultraviolet/Visible spectroscopy

END OF REPORT