Australian Government
Department of Industry, Science, Energy and Resources

National Measurement Institute

Proficiency Test Final Report
AQA 20-12
Heroin

January 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.

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TABLE OF CONTENTS

SUMMARY

AQA 20-12 Heroin commenced in July 2020. Sets of heroin hydrochloride, each containing three test samples, were sent to thirty laboratories, with two laboratories requesting two sample sets to be analysed independently by different analysts. Thirty-one participants returned results.

Samples were prepared at the NMI laboratory in Sydney using heroin hydrochloride approximately 75% base (m/m) supplied by the Australian Federal Police.

The assigned values in this study were 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 heroin in samples typical of a routine seizure;*

Laboratory performance was assessed by z-score and E_n-score.

Of 93 results for which z-scores were calculated, 74 (80%) returned a z-score with $|z| \leq 2.0$, indicating a satisfactory performance.

Of 93 results for which E_n-scores were calculated, 77 (83%) returned a satisfactory E_n-score of $|E_n| \leq 1.0$, indicating agreement of the participant's result with the assigned value within their respective expanded uncertainties.

Laboratories **1**, **2**, **4**, **6**, **8**, **10**, **13**, **14**, **15**, **17**, **18**, **19**, **21**, **24**, **25**, **28**, **31** and **32** returned satisfactory z- and E_n-scores for all results.

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

Of 93 reported results, 84 (90%) were reported with an associated expanded MU.

The magnitude of reported uncertainties was within the range 1.2% to 15% relative.

- *test the ability of participants to identify a cutting agent commonly found in controlled drug preparation*

Sample S1 was cut with quinine, Sample S2 was cut with procaine hydrochloride, and Sample S3 was cut with caffeine.

Thirty participants (97%) reported on the identity of the cutting agents. Laboratories **1**, **3**, **4**, **5**, **6**, **8**, **9**, **10**, **12**, **13**, **14**, **15**, **16**, **17**, **18**, **19**, **20**, **21**, **24**, **27**, **28**, **31** and **32** correctly reported all cutting agents used.

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, biota and food;
- inorganic analytes in soil, water, food and pharmaceuticals;
- controlled drug assay and clandestine laboratory; and
- allergens in food.

1.2 Study Aims

The aims of the study were to:

- assess the proficiency of laboratories measuring heroin 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; and
- test the ability of participants to identify cutting agents commonly found in controlled drug preparation.

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[1] as a provider of proficiency testing schemes. This controlled drug proficiency test is within the scope of NMI's accreditation.

The conduct of NMI proficiency tests is described in the NMI Chemical Proficiency Testing Study Protocol.[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[1] and The International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories.[4]

2 STUDY INFORMATION

2.1 Study Timetable

The timetable of the study was:

Invitation issued: 13 July 2020

Samples dispatched: 10 September 2020

Results due: 14 December 2020

Interim report issued: 4 January 2021

2.2 Participation

Thirty laboratories registered to participate, with two laboratories requesting two sets of samples to be analysed independently by different analysts. Thirty-one participants returned results by the due date.

2.3 Test Material Specification

Three test samples were prepared in July 2020. The starting material was heroin hydrochloride, approximately 75% base (m/m) supplied by the Australian Federal Police (AFP). Quinine and caffeine purchased from Sigma-Aldrich, and procaine hydrochloride purchased from Ajax Finechem were used as cutting agents. Sample S1 was cut with quinine, Sample S2 was cut with procaine hydrochloride, and Sample S3 was cut with caffeine.

The heroin was 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 61% heroin base (m/m).

Sample S2 was prepared to contain approximately 36% heroin base (m/m).

Sample S3 was prepared to contain approximately 31% heroin base (m/m).

2.4 Laboratory Code

Each participant was assigned a confidential laboratory code.

2.5 Test Sample Homogeneity

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

The procedure for the preparation of the study samples has been validated in previous studies. No homogeneity testing was conducted in this proficiency study. Results returned by the participants gave no reason to question the homogeneity of the test samples.

2.6 Sample Dispatch and Receipt

Sets of three test samples, with each sample containing approximately 150 mg of material, were dispatched to participants on 10 September 2020.

The following items were packaged with the samples:

- a covering 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 e-mailed to participants.

2.7 Instructions to Participants

Participants were asked to analyse the samples using their routine quantitative method and return the following information:

- one result for each sample as % heroin base (m/m);
- an estimate of the expanded uncertainty associated with the result as % heroin base (m/m) at the 95% confidence level;
- brief detail on how the uncertainty was calculated e.g. uncertainty budget method;
- the identity of the cutting agents in all three samples, if part of routine analysis;
- origin and stated purity of the analytical reference standard used;
- brief summary of the quantitative method used;
- the completed results sheet by 2 November 2020, as late results may not be included in the report; and
- any other comment.

The due date for the results was extended to 14 December 2020 for all participants, due to significant delivery delays to some participants.

2.8 Interim Report

An interim report was emailed to all participants on 4 January 2021. The interim report release for this study was delayed due to further delivery issues affecting a participant and the NMI end-of-year shut down period.

3 PARTICIPANT LABORATORY INFORMATION

3.1 Test Methods Reported by Participants

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

Table 1 Summary of Participants' Test Methods

Lab. Code	Extraction Solvent	Internal Standard	Calib. Points	Technique	Detector	Column
1	acetonitrile/H20 (80/20)	External standard	2	HPLC	DAD	NH2
2	Methanol	None	3	HPLC	Diode Array	Silica Column (1500mm x 4.6mm)
3	Acetonitrile	N/A	6	UPLC	UV	BEH C18, 1.7um 2.1 X 100mm
4	Methanol	none	5	HPLC	DAD	Kinetex C-18-XB
5	Methanol	Diazepam	6	GC	FID	128-5512 DB-5ms
6	Methanol	NO	7	UPLC	DAD	Poroshell 120 EC-C18 (4.6x150mm, 2.7 microns pore size)
7	d6-DMSO	1,2,4,5-tetrachloro-3-nitrobenzene		NMR	NMR	-
8	Acetonitrile/Methanol (95:5)	Pholcodine 1mg/ml	3	UPLC	PDA	ACQUITY C-18
9	Ethanol	Triphenylacetophenone (TPAP)	3	GC	FID	HP1-MS
10	acetonitrile / water	none	1	HPLC	UV	Kromasil
11	Dichloromethane	Tetracosane	7	GC	FID	Equity 5
12	water/acetonitrile/2.5M sulfuric acid 90:10:1	None	3	HPLC	Diode array	Shimpack XR-ODS
13	Acetonitrile: Water (75:25)	Benzocaine	3	UPLC	DAD	BEH C18 1.7 µm (2.1x100mm)
14	ethanol:dimethylformamide (9:1)	tribenzylamine	6	GC	FID	HP1
15	Methanol	Alprazolam	1	LC	DAD	Hypersil-5-ODS
16	Methanol	Mepivacaine	4	HPLC	DAD	C18
17	Chloroform	Octacosane	5	GC	FID	HP5
18	chloroform-d	dimethylterephthalate		1H QNMR	Bruker AVIII600 with BBFO probe	N/A
19	Ethanol	Propyl Paraben	8	UPLC	PDA	BEH Shield RP18
20	Acetonitrile	N/A	6	UPLC	PDA	Acquity UPLC BEH C18 1.7um 2.1 x 100mm
21	Acetonitrile	Strychnine	6	GC	FID	HP1

Lab. Code	Extraction Solvent	Internal Standard	Calib. Points	Technique	Detector	Column
22	ACN/MeOH/H2O	Analog of heroin	7	UPLC	MSMS	C-18 Column
23	Chloroform	octacosane	5	GC	MS	Rxi-5Sil-MS
24	Acetonitrile, acetic acid, water	NO ISTD	4	HPLC	UV DAD	Poroshell 120 EC-18
25	Methanol	Methadone	4	GC	FID	Hp-5 30mx0.32mm 0.25um
26	Acetonitrile / water / PicA	/	4	HPLC	UV	NH2
27	Water/ACN	N/A	5	HPLC	UV	Kinetex 5u C18
28	Chloroform	2,2,2-triphenylacetophenone	4	GC	FID	HP1
30	METHANOL	LOXAPINE	5	HPLC	DAD	XTERRA C18
31	acetonitrile/water (86/14)	none	4	HPLC	UV	NH2
32	Methanol	Alprazolam	1	LC	DAD	Hypersil-5-ODS

3.2 Reported Basis of Participants' Measurement Uncertainty Estimates

Participants were requested to provide information about their basis of measurement uncertainty (MU). Responses are presented in Tables 2 and 3. Some responses have been 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		Duplicate analysis	Instrument calibration Standard purity	ISO/GUM
2	Standard deviation of replicate analyses multiplied by 2 or 3	Control samples - CRM	Instrument calibration Masses and volumes Recoveries of SS	ISO/GUM
3	Standard deviation of replicate analyses multiplied by 2 or 3	Control samples - RM Duplicate analysis	Instrument calibration Homogeneity of sample Masses and volumes Standard purity	NATA GAG Estimating and Reporting Measurement Uncertainty of Chemical Test Results
4		Control samples - RM Duplicate analysis	Instrument calibration Masses and volumes Recoveries of SS Standard purity	Eurachem/CITAC Guide
5	Estimating Measurement Uncertainty by black box with pairs of values	Standard deviation from PT studies only		ISO/GUM (ENAC G 09 or ISO 21748)
6	Top Down - precision and estimates of the method and laboratory bias	Control samples - CRM Duplicate analysis	Instrument calibration Homogeneity of sample Masses and volumes Laboratory bias from PT studies Standard purity	Eurachem/CITAC Guide, Measurement Uncertainty for weight Determination in Seized Drug Analysis

Lab. Code	Approach to Estimating MU	Information Sources for MU Estimation*		Guide Document for Estimating MU
		Precision	Method Bias	
7	Top Down - precision and estimates of the method and laboratory bias	Control samples - RM Duplicate analysis	Laboratory bias from PT studies	Nordtest Report TR537
8	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
9	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
10	Standard deviation of replicate analyses multiplied by 2 or 3	Control samples - RM		ISO/GUM
11	Top Down - precision and estimates of the method and laboratory bias	Standard deviation from PT studies only		
		Control samples - CRM	Instrument calibration Homogeneity of sample Masses and volumes Matrix effects Laboratory bias from PT studies Standard purity	
12	Standard deviation of replicate analyses multiplied by 2 or 3	Control samples - RM Duplicate analysis	Instrument calibration Standard purity	ISO/GUM
13	Top Down - precision and estimates of the method and laboratory bias	Control samples - RM Duplicate analysis	Masses and volumes Standard purity	Eurachem/CITAC Guide
14	Top Down - precision and estimates of the method and laboratory bias	Control samples - RM	Standard purity	
15	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
16	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 ILAG G-17-2002
17	Top Down - precision and estimates of the method and laboratory bias	Control samples - previously analysed police seizures Duplicate analysis	Instrument calibration Homogeneity of sample Masses and volumes Matrix effects Standard purity	Eurachem/CITAC Guide

Lab. Code	Approach to Estimating MU	Information Sources for MU Estimation*		Guide Document for Estimating MU
		Precision	Method Bias	
18	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
19				
20	Standard deviation of replicate analyses multiplied by 2 or 3	Control samples - RM Duplicate analysis	Instrument calibration Homogeneity of sample Masses and volumes Standard purity	NATA GAG Estimating and Reporting Measurement Uncertainty of Chemical Test Results
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	Top Down - precision and estimates of the method and laboratory bias	Control samples - RM		
23	Top Down - precision and estimates of the method and laboratory bias	Control samples - RM	Laboratory bias from PT studies	ISO/GUM
24	Accuracy profile _ based on intermediate precision and repeatability	Control samples - RM	Standard purity	ISO 5725-2 years- and ISO/TS 21748
25	Standard deviation of replicate analyses multiplied by 2 or 3	Duplicate analysis	Masses and volumes	ISO/GUM
26				
27	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
28	Validation			
30				
31	Top Down - precision and estimates of the method and laboratory bias	Control samples - RM	Laboratory bias from PT studies Standard purity	ISO 11352 and NF V03-110
32	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

*SS = Spiked Samples, RM = Reference Material, CRM = Certified Reference Material

Table 3 Uncertainty Comments

Lab. Code	Participants' Uncertainty Comments
2	The reported result (in routine case samples) is defined by the average of the individual results multiplied by the certainty 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. Eg. a result of 59.3% would give a reported result of 59.3*0.959561=56.69 therefore rounded down to 56%.
12	MuM determined from multiple injections of reference material. 3x(Std Dev/mean)x100. no analysis undertaken for inert bulking agents
26	Our method hasn't been validated yet.

3.3 Details of Participant Calibration Standards

Participants were requested to provide information about their calibration standard used. Responses are presented in Table 4. Some responses have been modified so that the participant cannot be identified.

Table 4 Participant Calibration Standard

Lab. Code	Reference Standard	Purity (%)
1	NMI	99.4
2	Johnson Matthew	99.7
3	NMI	99.4
4	LGC	99.7
5	Lipomed	99.1
6	LIPOMED	99.95
7	d6-DMSO	99.5
8	NMI	99.40
9	NMI	99.4
10	Lipomed	99.6
11	Lipomed	99.827, 87.7 free base
12	LGC	99.7
13	NMI	99.4
14	LGC	99.4
15	Lipomed	99.091±0.079
16	Lipomed	99.6 +/- 0.020
17	NMI	99.4
18	Sigma Aldrich	99.95
19	NMI	99.4
20	NMI	99.4
21	NMI	99.3
22	Lipomed	100
23	Lipomed	99.09
24	Lipomed	99.6

Lab. Code	Reference Standard	Purity (%)
25	LGC	100
26		
27	Johnson Matthey	99.5
28	In house	99.14
30	LIPOMED	>98
31	Lipomed	99.801
32	Lipomed	99.091±0.079

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 5, along with the study coordinator's response where appropriate.

Table 5 Participant Comments

Lab. Code	Participants' Comments	Study Coordinator's Response
6	Qualitative analysis was carried out by GC-MS Methodology: External Standard	
9	Methodology: Dichloromethane (30mL/L of ethanol) was used to dissolve the TPAP.	
13	Heroin, acetylcodeine and monoacetylmorphine detected	
18	Methodology: Simultaneous observation of analyte and IS peaks in 1H NMR spectrum acquired using QNMR conditions	
24	Methodology: 0 ; 5 ; 20 ; 100 mg/l	
31	we would like to receive 3 samples of very different concentration for example 2%, 30% and 60%	A range of drug purities are selected to cater for the needs of different participant laboratories. In this study, the samples were prepared to contain approximately 61%, 36% and 31% heroin base (m/m).

4 PRESENTATION OF RESULTS AND STATISTICAL ANALYSIS

4.1 Results Summary

Participant results are listed in Tables 6 to 8 with resultant summary statistics: robust average, median, mean, number of numerical 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 4.

An example chart with interpretation guide is shown in Figure 1.

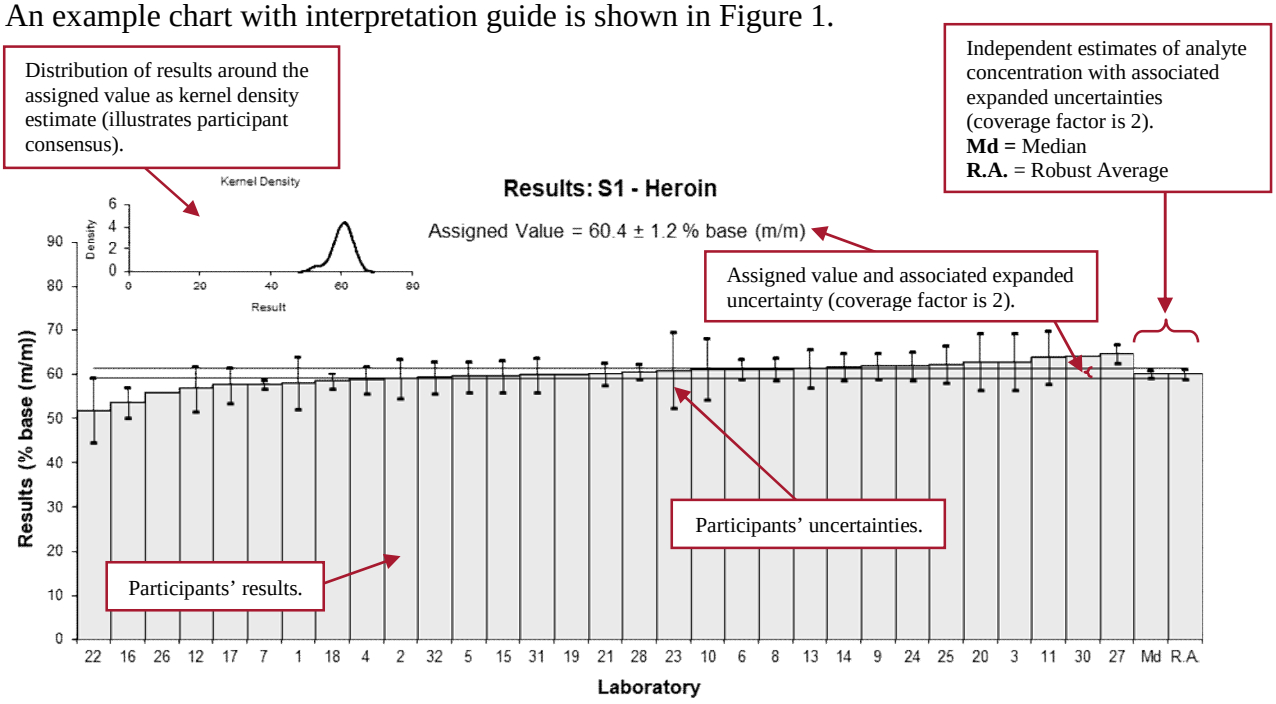


Figure 1 Guide to Presentation of Results

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 study, the property is the concentration of the analyte in the test samples. Assigned values were the robust averages of participants' results and the expanded uncertainties were estimated from the associated robust SDs (Appendix 1).

4.3 Robust Average and Robust Standard Deviation

The robust averages and associated expanded MUs, and robust SDs (a measure of the variability of participants' results), were calculated using the procedure described in ISO 13528:2015.[5]

4.4 Performance Coefficient of Variation (PCV)

The performance coefficient of variation (PCV) is a fixed measure of the between laboratories variation that in the judgement of the study coordinator would be expected from participants given the levels of analytes present, and is supported by mathematical models such as the Thompson-Horwitz equation.[6] It is important to note that this is a performance measure set by the study coordinator; it is not the robust CV of participants' results. 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 participant z-scores.

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

4.6 z-Score

For each participant result a z-score is calculated according to Equation 2 below.

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

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|):

- $|z| \leq 2.0$ 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. E_n-score includes measurement uncertainty and is calculated according to Equation 3 below.

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

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 ($|E_n|$):

- $|E_n| \leq 1.0$ 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 6

Sample Details

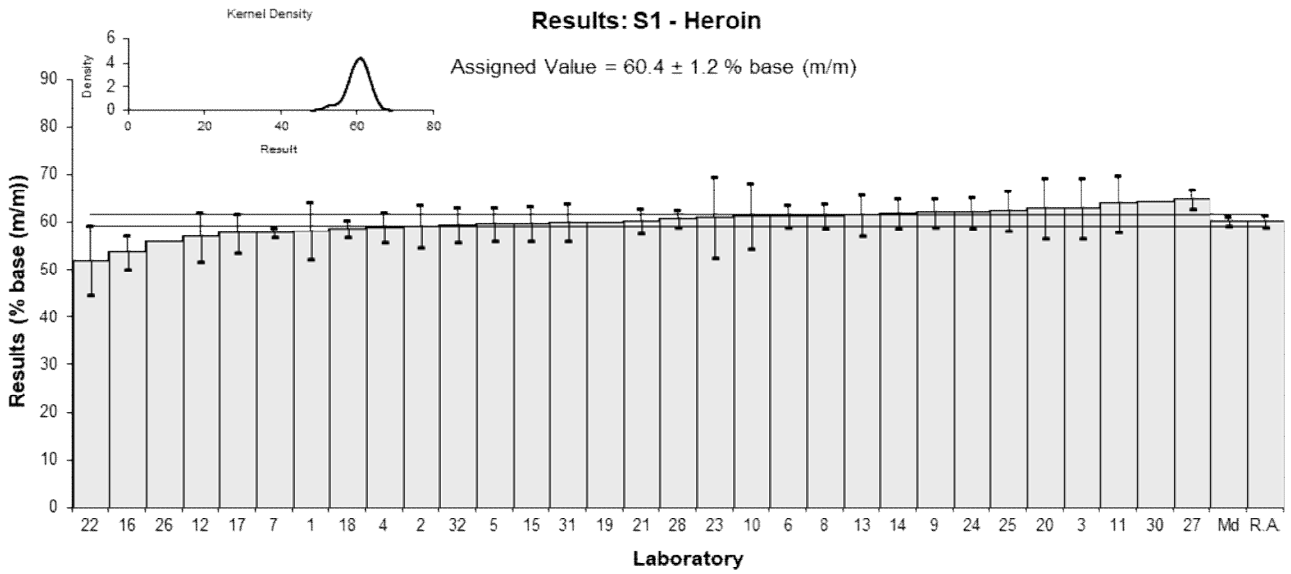
Sample No.	S1
Matrix	Powder
Analyte	Heroin
Units	% base (m/m)

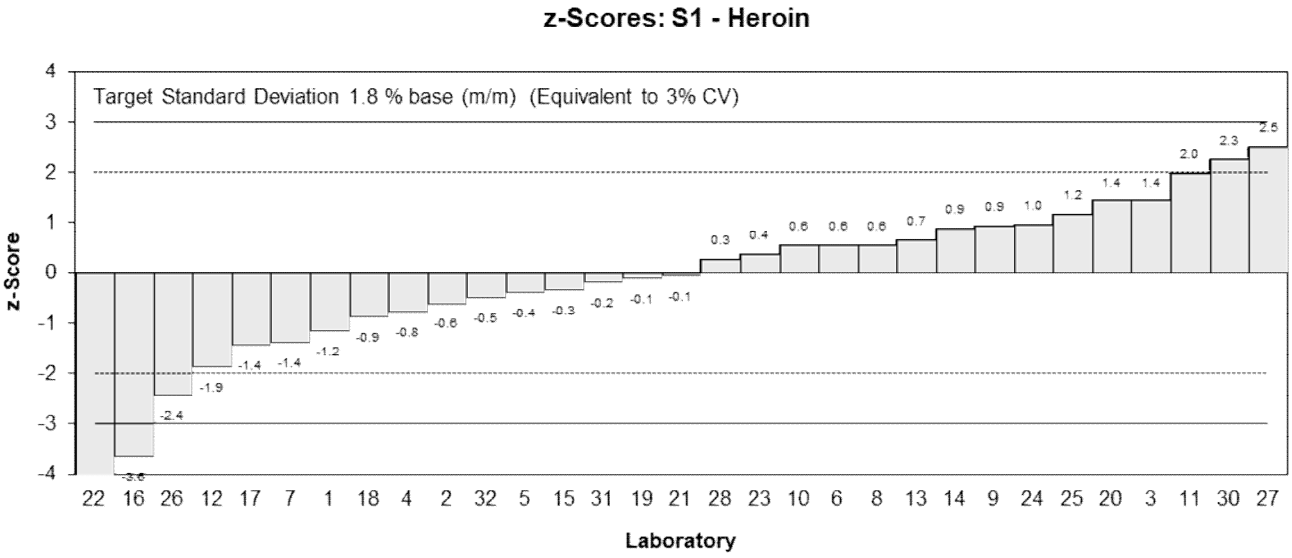
Participant Results

Lab. Code	Result	Uncertainty	z-Score	E_n-Score
1	58.3	5.9	-1.16	-0.35
2	59.3	4.39	-0.61	-0.24
3	63	6.3	1.43	0.41
4	59	3.0	-0.77	-0.43
5	59.7	3.6	-0.39	-0.18
6	61.4	2.4	0.55	0.37
7	57.9	0.94	-1.38	-1.64
8	61.40	2.50	0.55	0.36
9	62.1	3.0	0.94	0.53
10	61.4	6.8	0.55	0.14
11	64	6	1.99	0.59
12	57	5.13	-1.88	-0.65
13	61.6	4.4	0.66	0.26
14	62	3.1	0.88	0.48
15	59.8	3.6	-0.33	-0.16
16	53.8	3.5	-3.64	-1.78
17	57.8	4	-1.43	-0.62
18	58.8	1.8	-0.88	-0.74
19	60.2	NR	-0.11	-0.17
20	63	6.3	1.43	0.41
21	60.3	2.6	-0.06	-0.03
22	52	7.3	-4.64	-1.14
23	61.1	8.5	0.39	0.08
24	62.16	3.29	0.97	0.50
25	62.5	4.15	1.16	0.49
26	56	NR	-2.43	-3.67
27	64.95	1.95	2.51	1.99
28	60.9	1.8	0.28	0.23
30	64.48	NR	2.25	3.40
31	60.05	4.02	-0.19	-0.08
32	59.5	3.6	-0.50	-0.24

Statistics

Assigned Value	60.4	1.2
Robust Average	60.4	1.2
Median	60.3	0.9
Mean	60.2	
N	31	
Max.	64.95	
Min.	52	
Robust SD	2.6	
Robust CV	4.3%	


Results: S1 - Heroin
Kernel Density
Density
Result
Assigned Value = 60.4 ± 1.2 % base (m/m)
Results (% base (m/m))
22 16 26 12 17 7 1 18 4 2 32 5 15 31 19 21 28 23 10 6 8 13 14 9 24 25 20 3 11 30 27 Md R.A.
Laboratory


z-Scores: S1 - Heroin
Target Standard Deviation 1.8 % base (m/m) (Equivalent to 3% CV)
z-Score
22 16 26 12 17 7 1 18 4 2 32 5 15 31 19 21 28 23 10 6 8 13 14 9 24 25 20 3 11 30 27
Laboratory

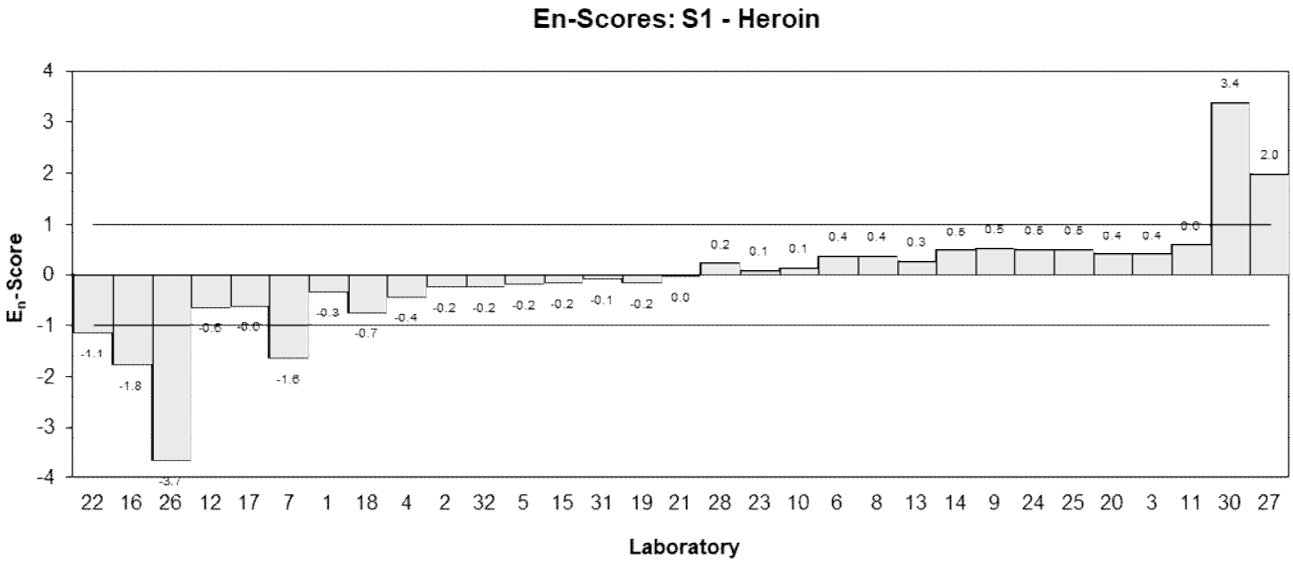

En-Scores: S1 - Heroin
En-Score
22 16 26 12 17 7 1 18 4 2 32 5 15 31 19 21 28 23 10 6 8 13 14 9 24 25 20 3 11 30 27
Laboratory

Figure 2

Table 7

Sample Details

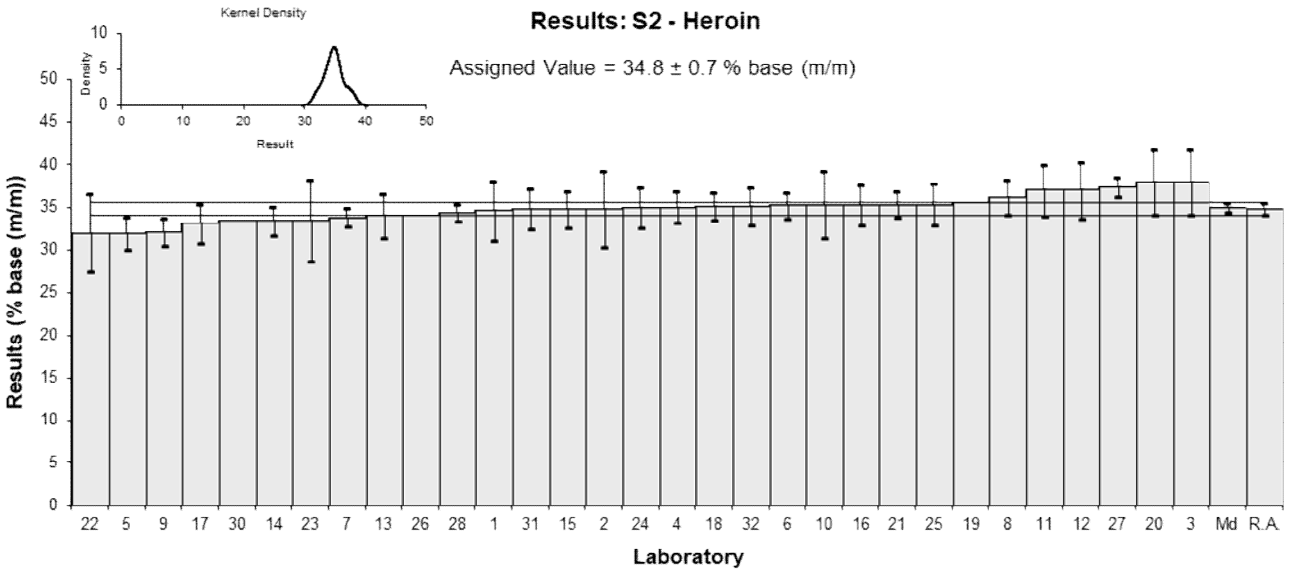
Sample No.	S2
Matrix	Powder
Analyte	Heroin
Units	% base (m/m)

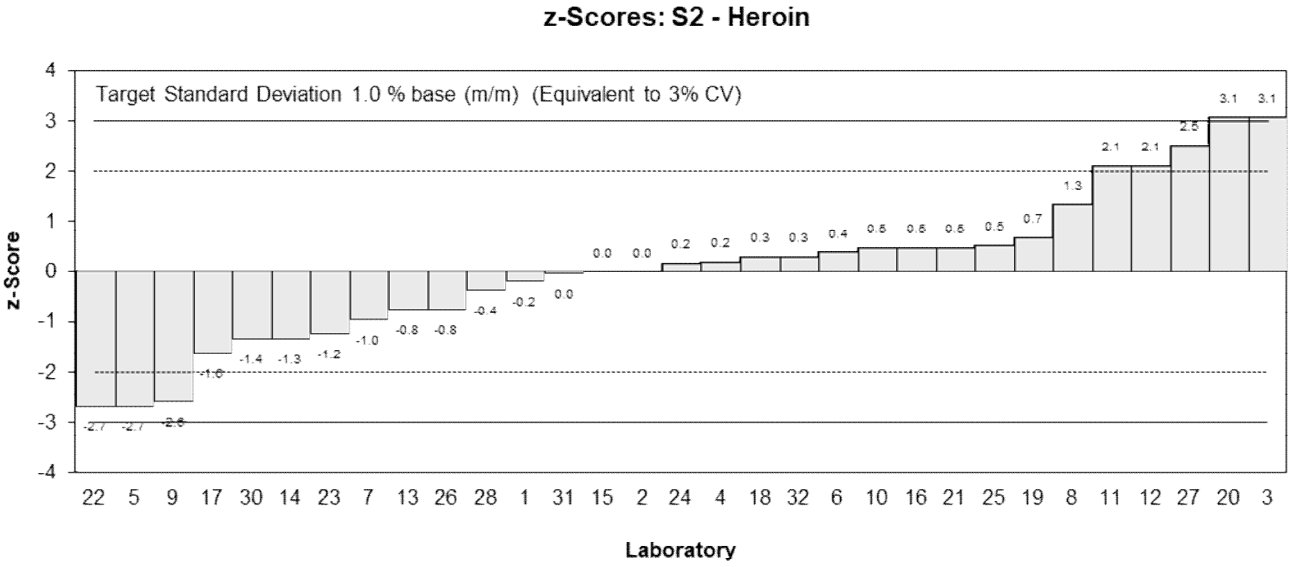
Participant Results

Lab. Code	Result	Uncertainty	z-Score	E_n-Score
1	34.6	3.5	-0.19	-0.06
2	34.8	4.39	0.00	0.00
3	38	3.8	3.07	0.83
4	35	1.8	0.19	0.10
5	32.0	1.9	-2.68	-1.38
6	35.2	1.5	0.38	0.24
7	33.8	1.06	-0.96	-0.79
8	36.20	2.00	1.34	0.66
9	32.1	1.6	-2.59	-1.55
10	35.3	3.9	0.48	0.13
11	37	3	2.11	0.71
12	37	3.33	2.11	0.65
13	34.0	2.5	-0.77	-0.31
14	33.4	1.7	-1.34	-0.76
15	34.8	2.1	0.00	0.00
16	35.3	2.3	0.48	0.21
17	33.1	2.3	-1.63	-0.71
18	35.1	1.6	0.29	0.17
19	35.5	NR	0.67	1.00
20	38	3.8	3.07	0.83
21	35.3	1.5	0.48	0.30
22	32	4.5	-2.68	-0.61
23	33.5	4.7	-1.25	-0.27
24	34.97	2.33	0.16	0.07
25	35.34	2.35	0.52	0.22
26	34	NR	-0.77	-1.14
27	37.43	1.12	2.52	1.99
28	34.4	1.0	-0.38	-0.33
30	33.39	NR	-1.35	-2.01
31	34.76	2.33	-0.04	-0.02
32	35.1	2.2	0.29	0.13

Statistics

Assigned Value	34.8	0.7
Robust Average	34.8	0.7
Median	35.0	0.5
Mean	34.9	
N	31	
Max.	38	
Min.	32	
Robust SD	1.6	
Robust CV	4.5%	


Results: S2 - Heroin
Assigned Value = 34.8 ± 0.7 % base (m/m)
Kernel Density
Density
Result
Results (% base (m/m))
22 5 9 17 30 14 23 7 13 26 28 1 31 15 2 24 4 18 32 6 10 16 21 25 19 8 11 12 27 20 3 Md R.A.
Laboratory


z-Scores: S2 - Heroin
Target Standard Deviation 1.0 % base (m/m) (Equivalent to 3% CV)
z-Score
22 5 9 17 30 14 23 7 13 26 28 1 31 15 2 24 4 18 32 6 10 16 21 25 19 8 11 12 27 20 3
Laboratory

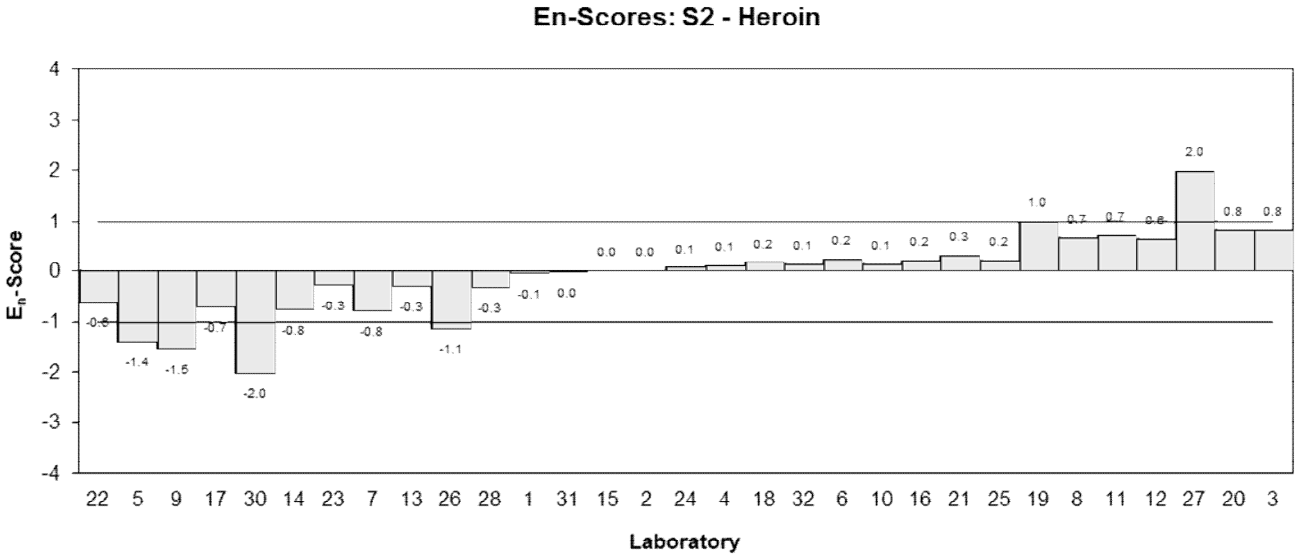

En-Scores: S2 - Heroin
En-Score
22 5 9 17 30 14 23 7 13 26 28 1 31 15 2 24 4 18 32 6 10 16 21 25 19 8 11 12 27 20 3
Laboratory

Figure 3

Table 8

Sample Details

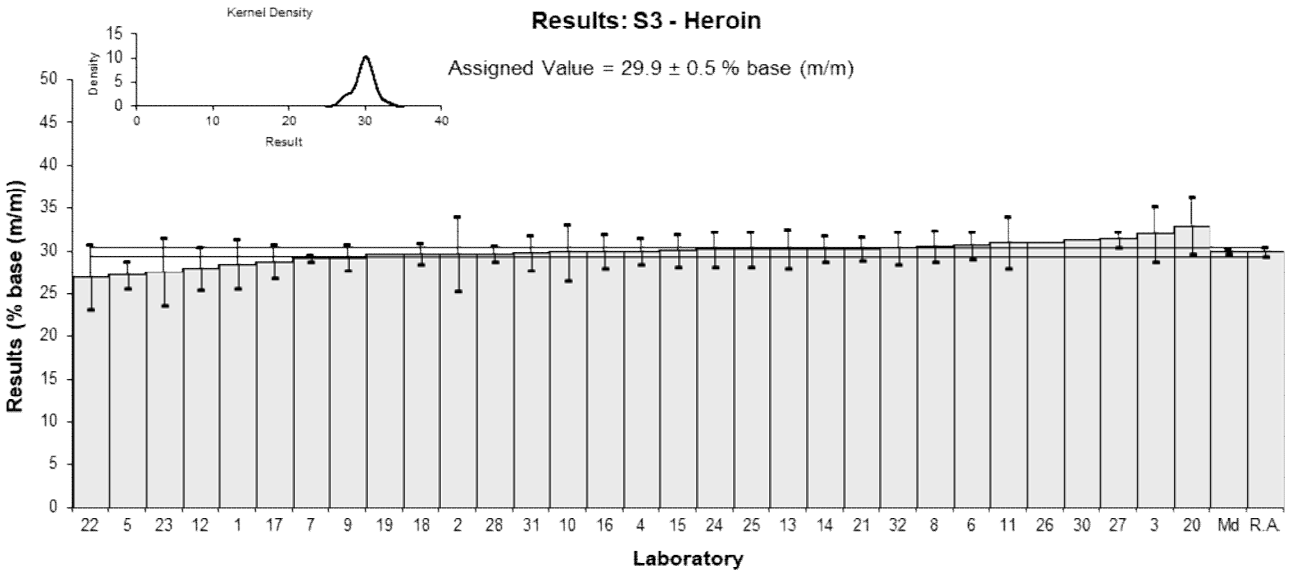
Sample No.	S3
Matrix	Powder
Analyte	Heroin
Units	% base (m/m)

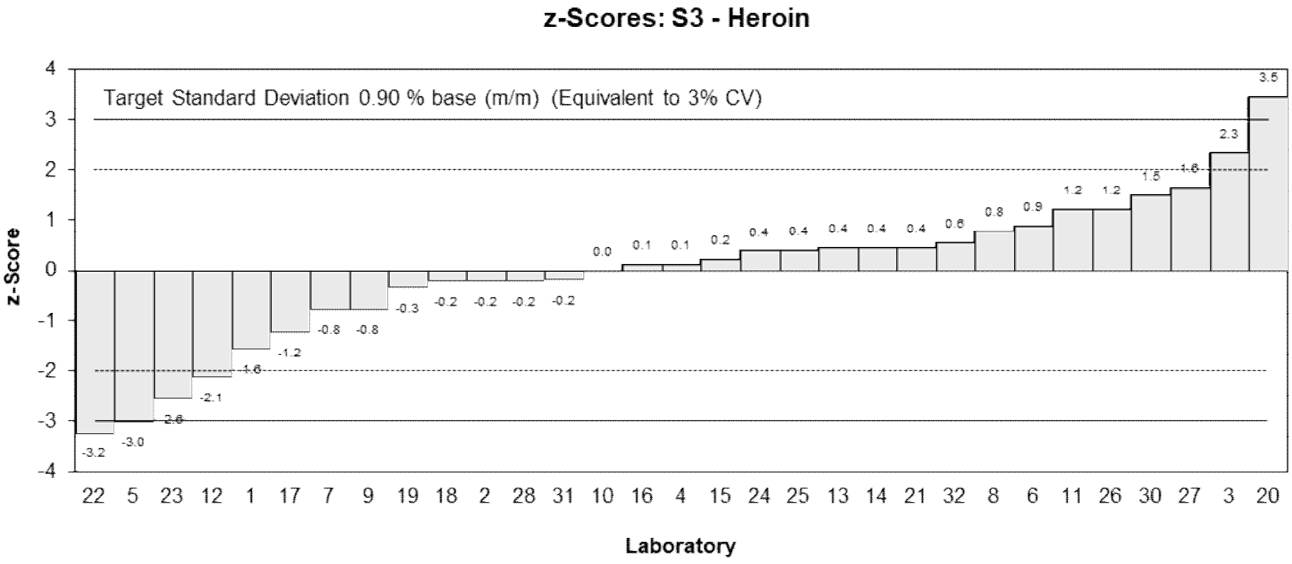
Participant Results

Lab. Code	Result	Uncertainty	z-Score	E_n-Score
1	28.5	2.9	-1.56	-0.48
2	29.7	4.39	-0.22	-0.05
3	32	3.2	2.34	0.65
4	30	1.5	0.11	0.06
5	27.2	1.6	-3.01	-1.61
6	30.7	1.6	0.89	0.48
7	29.2	0.36	-0.78	-1.14
8	30.60	1.80	0.78	0.37
9	29.2	1.5	-0.78	-0.44
10	29.9	3.3	0.00	0.00
11	31	3	1.23	0.36
12	28	2.52	-2.12	-0.74
13	30.3	2.2	0.45	0.18
14	30.3	1.5	0.45	0.25
15	30.1	1.9	0.22	0.10
16	30.0	2.0	0.11	0.05
17	28.8	2	-1.23	-0.53
18	29.7	1.2	-0.22	-0.15
19	29.6	NR	-0.33	-0.60
20	33	3.3	3.46	0.93
21	30.3	1.3	0.45	0.29
22	27	3.8	-3.23	-0.76
23	27.6	3.9	-2.56	-0.58
24	30.25	2.02	0.39	0.17
25	30.26	2.01	0.40	0.17
26	31	NR	1.23	2.20
27	31.38	0.94	1.65	1.39
28	29.7	0.9	-0.22	-0.19
30	31.25	NR	1.51	2.70
31	29.74	1.99	-0.18	-0.08
32	30.4	1.9	0.56	0.25

Statistics

Assigned Value	29.9	0.5
Robust Average	29.9	0.5
Median	30.0	0.3
Mean	29.9	
N	31	
Max.	33	
Min.	27	
Robust SD	1.1	
Robust CV	3.8%	


Results: S3 - Heroin
Kernel Density
Density
Result
Assigned Value = 29.9 ± 0.5 % base (m/m)
Results (% base (m/m))
22 5 23 12 1 17 7 9 19 18 2 28 31 10 16 4 15 24 25 13 14 21 32 8 6 11 26 30 27 3 20 Mid R.A.
Laboratory


z-Scores: S3 - Heroin
Target Standard Deviation 0.90 % base (m/m) (Equivalent to 3% CV)
z-Score
22 5 23 12 1 17 7 9 19 18 2 28 31 10 16 4 15 24 25 13 14 21 32 8 6 11 26 30 27 3 20
Laboratory

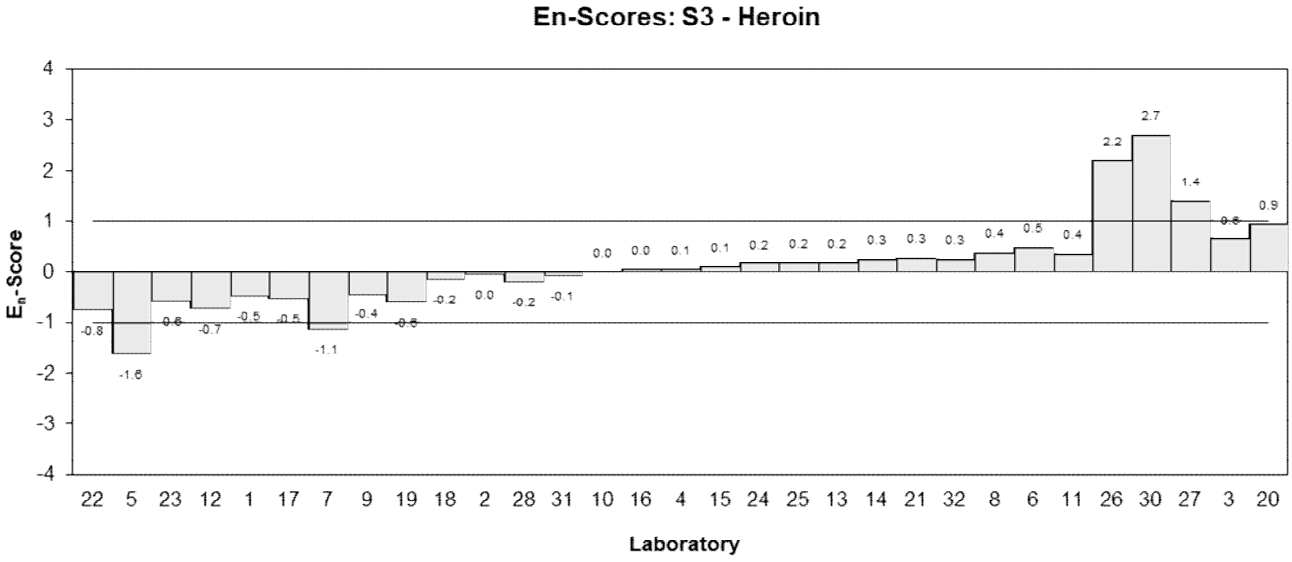

En-Scores: S3 - Heroin
En-Score
22 5 23 12 1 17 7 9 19 18 2 28 31 10 16 4 15 24 25 13 14 21 32 8 6 11 26 30 27 3 20
Laboratory

Figure 4

Table 9 Reported Cutting Agents*

Lab. Code	S1	S2	S3
Preparation	Quinine	Procaine hydrochloride	Caffeine
1	Quinine	Procaine	Caffeine
2	None	Procaine	Caffeine
3	Acetylcodeine (8%) and Quinine	Acetylcodeine(5%) and Procaine	Acetylcodeine (4%) and Caffeine
4	MAM, acetylcodeine, quinine	MAM, acetylcodeine, procaine	Caffeine, acetylcodeine
5	Acetylcodeine, 6-Monoacetylmorphine and quinine	Acetylcodeine, 6-Monoacetylmorphine and procaine	Acetylcodeine, 6-Monoacetylmorphine and caffeine
6	Quinine	Procaine	Caffeine
7	None	Procaine	Caffeine
8	Also indicated to contain quinine	Also indicated to contain procaine	caffeine
9	Quinine	Procaine	Caffeine
10	quinine	procaine	caffeine
11	Acetylcodeine	Acetylcodeine, Procaine	Acetylcodeine, Caffeine
12	quinine	procaine	caffeine
13	Quinine indicated	Procaine indicated	Caffeine indicated
14	quinine (not quantified)	procaine (not quantified)	caffeine (61.4%)
15	Quinine	Procaine	Caffeine
16	Quinine	Procaine	Caffeine
17	quinine	procaine	caffeine
18	Quinine ~18%	Procaine	Caffeine ~59%
19	Quinine	Procaine : 43.8 %	Caffeine : 59.5 %
20	Quinine	Procaine	Caffeine
21	Acetylcodeine, Quinine	Acetylcodeine, Procaine, 6-MAM	Acetylcodeine, 6-MAM, caffeine
22	none	Procaine	Caffeine
23	None	Procaine	Caffeine
24	Quinine	Procaine	Caffeine
25	Unidentified material similar in nature to quinine	Procaine	Caffeine
26			
27	Quinine	Procaine	Caffeine
28	quinine	procaine	caffeine
30	HYDROQUINIDINE	PROCAINE	CAFFEINE
31	acetylcodeine, MAM, quinine	procaine, acetylcodeine	acetylcodeine, MAM, morphine, codeine, caffeine
32	Quinine	Procaine	Caffeine

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

6 DISCUSSION OF RESULTS

6.1 Assigned Value

The assigned values in this study are the robust averages of the results reported by the participants. The robust averages and associated expanded uncertainties were calculated using the procedure described in ISO 13528:2015.[5] If results less than 50% and greater than 150% of the robust average were reported, these were removed before the calculation of the assigned value.[3,4] The calculation procedure for the expanded uncertainty of the robust average, using Sample S1 as an example, is presented in Appendix 1.

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 MU 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 ASCLD/LAB accreditation program.

Of 93 reported results, 84 (90%) were reported with an associated expanded MU. Laboratories **19**, **26** and **30** did not report uncertainties; these participants reported that they were not accredited.

The magnitude of reported uncertainties was within the range 1.2% to 15% relative. Of 84 expanded MUs, 64 (76%) were between 3% and 10% relative to the reported result. Participants reporting uncertainties smaller than 3% or larger than 10% relative may need to reconsider whether these estimates are realistic or fit for purpose.

Laboratories having a satisfactory z-score and an unsatisfactory E_n-score are likely to have underestimated the expanded uncertainty associated with the result.

In some cases, results were reported with an inappropriate number of significant figures. 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 reporting 60.05 ± 4.02%, the recommended format is 60.1 ± 4.0%).[8]

6.3 z-Score

A target SD equivalent to 3% PCV was used to calculate z-scores for all samples. The CVs predicted by the Thompson-Horwitz equation,[6] target SDs and between-laboratory CVs obtained in this study are presented in Table 10.

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

Sample	Analyte	Assigned value (% base (m/m))	Thompson-Horwitz CV (%)	Target SD (as PCV) (%)	Between-Laboratories CV (%)
S1	Heroin	60.4	1.3	3	4.3
S2	Heroin	34.8	1.7	3	4.5
S3	Heroin	29.9	1.8	3	3.8

Of 93 results for which z-scores were calculated, 74 (80%) returned a z-score with $|z| \leq 2.0$, indicating a satisfactory performance.

Nineteen participants: **1**, **2**, **4**, **6**, **7**, **8**, **10**, **13**, **14**, **15**, **17**, **18**, **19**, **21**, **24**, **25**, **28**, **31** and **32** returned satisfactory z-scores for all three samples. Twelve participants returned at least one questionable or unsatisfactory z-score.

Laboratory **22** returned questionable or unsatisfactory z-scores for all reported results. All results were lower than the assigned value (negative bias). This laboratory may need to investigate the source of this bias.

The dispersal of participants' z-scores is presented graphically in Figure 5.

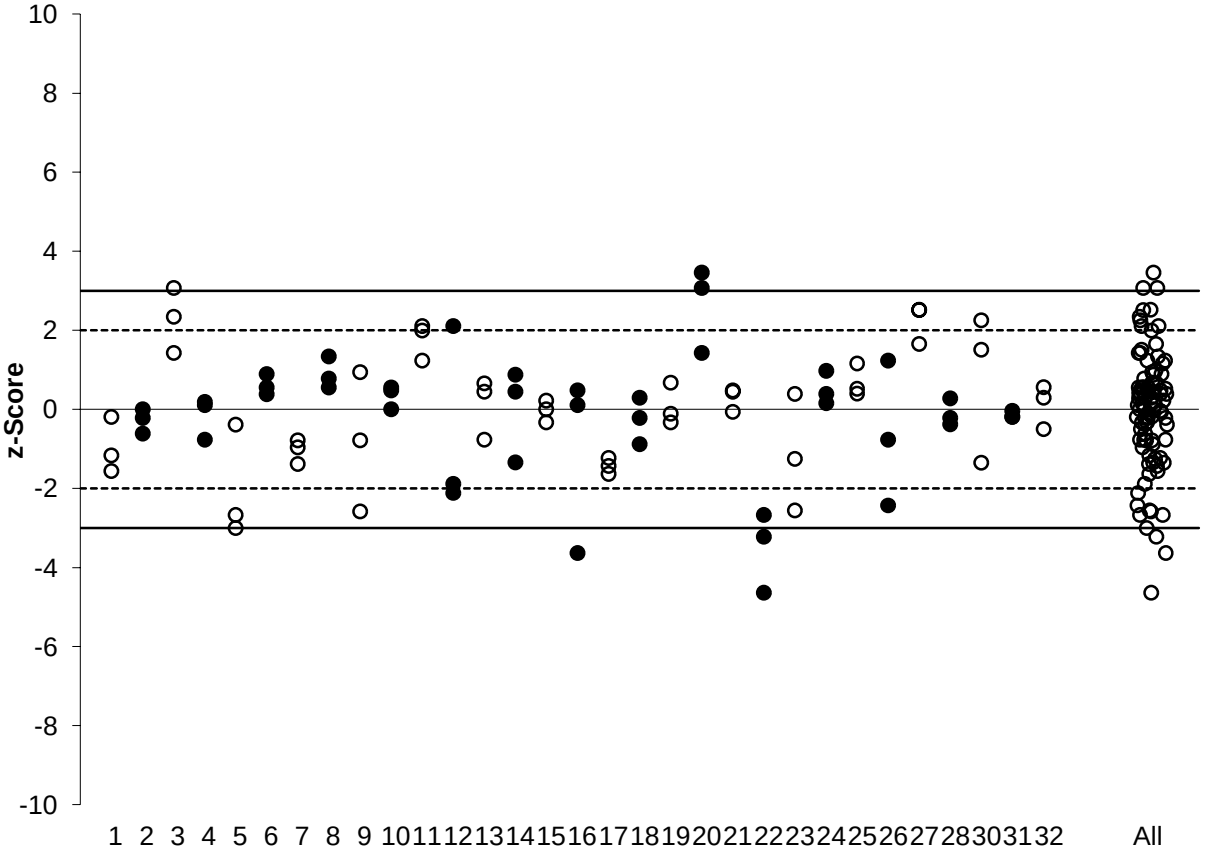


Figure 5 z-Score Dispersal by Laboratory

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 93 results for which E_n-scores were calculated, 77 (83%) returned a satisfactory E_n-score of $|E_n| \leq 1.0$, indicating agreement of the participant's result with the assigned value within their respective expanded uncertainties.

Twenty-three laboratories: **1**, **2**, **3**, **4**, **6**, **8**, **10**, **11**, **12**, **13**, **14**, **15**, **17**, **18**, **19**, **20**, **21**, **23**, **24**, **25**, **28**, **31** and **32** returned satisfactory E_n-scores for all three samples. Eight laboratories returned at least one unsatisfactory E_n-score.

Laboratories **26**, **27** and **30** returned unsatisfactory E_n-scores for all samples.

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

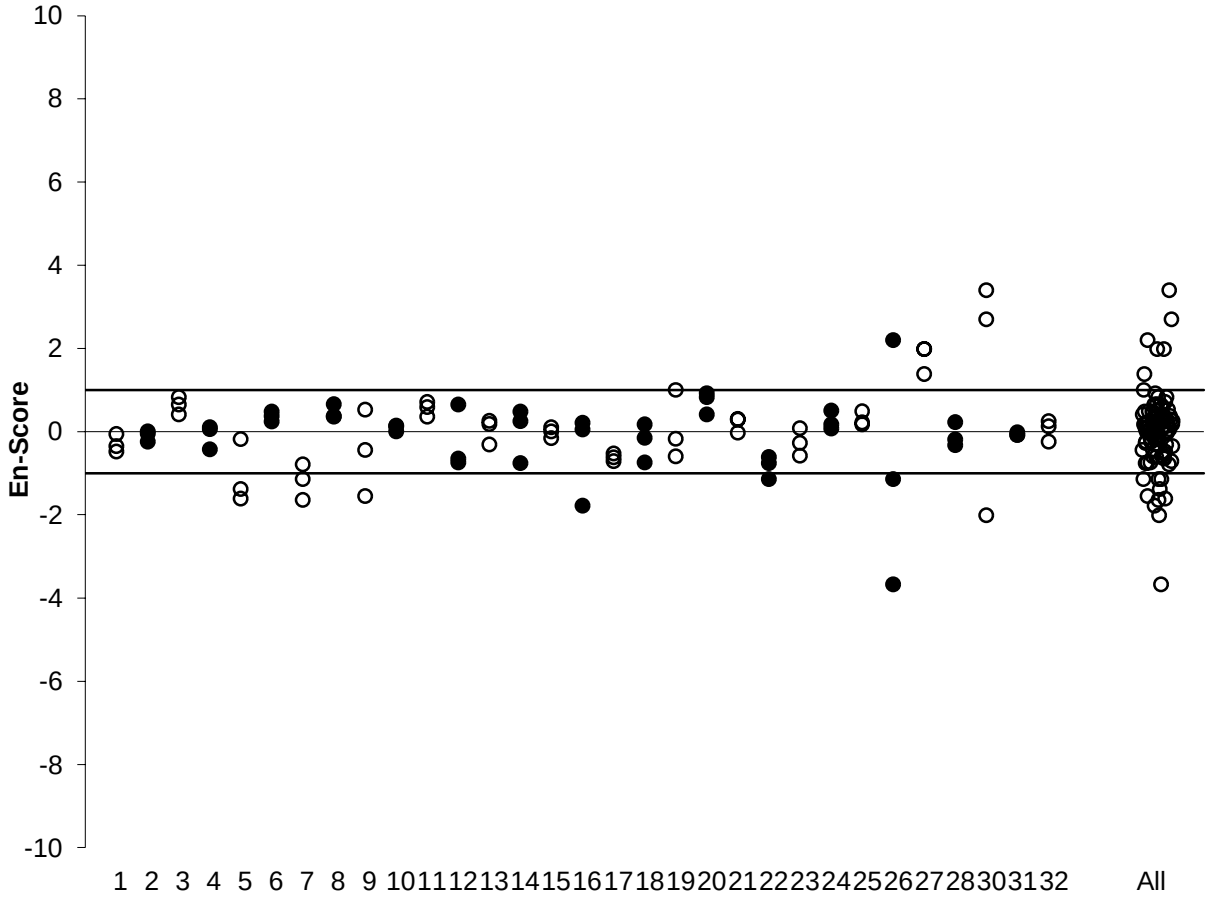


Figure 6 E_n-Score Dispersal by Laboratory

6.5 Identification of Cutting Agent

Samples were prepared by cutting heroin hydrochloride with quinine (Sample S1), procaine hydrochloride (Sample S2) and caffeine (Sample S3).

Thirty participants (97%) reported on the identity of at least one sample's cutting agent. Results reported by participants are presented in Table 9.

Laboratories **1**, **3**, **4**, **5**, **6**, **8**, **9**, **10**, **12**, **13**, **14**, **15**, **16**, **17**, **18**, **19**, **20**, **21**, **24**, **27**, **28**, **31** and **32** correctly reported all cutting agents used.

For Sample S1, 23 participants correctly reported quinine as the cutting agent. One participant reported that the cutting agent was hydroquinidine, a compound closely related to quinine. Another participant reported that the cutting agent was an unidentified material, though similar to quinine.

For Samples S2 and S3, all participants reporting on the cutting agents correctly identified procaine and caffeine respectively.

6.6 Participants' Analytical Methods

Participants were requested to analyse the samples using their normal 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 methodology descriptions provided by participants are presented in Table 1.

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

Table 11 Summary of Participants' Analytical Methods

		Lab. Code
Accreditation	Yes to ISO/IEC 17025	2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 20, 21, 22, 25, 28, 31, 32
	Not accredited / Not reported	1, 18, 19, 23, 24, 26, 27, 30
Average Sample Mass Used per Analysis (mg)	5 – 10	5, 10, 30
	11 – 30	1, 2, 3, 4, 6, 7, 8, 9, 11, 12, 13, 14, 15, 17, 18, 20, 21, 23, 24, 25, 26, 27*, 31, 32
	31 – 50	16, 22
	51 – 100	19
	101 – 150	28
Conversion to base?	Yes	2, 5, 6, 7, 11, 14, 16, 17, 23, 24, 26, 27, 28, 30
	No	3, 4, 8, 9, 10, 12, 13, 15, 18, 19, 20, 21, 22, 25, 31, 32
	Not reported	1
Instrument Used for Quantification	HPLC-DAD	1, 2, 4, 12, 16, 24, 30
	HPLC-UV	10, 26, 27, 31
	UPLC-DAD	6, 8, 13, 19, 20
	UPLC-UV	3
	UPLC-MS/MS	22
	LC-DAD	15, 32
	GC-FID	5, 9, 11, 14, 17, 21, 25, 28
	GC-MS	23
	QNMR	7, 18
Solvent	Acetonitrile/Other	1, 8, 10, 12, 13, 22, 24, 26, 27, 31
	Acetonitrile	3, 20, 21
	Methanol	2, 4, 5, 6, 15, 16, 25, 30, 32
	Ethanol(/Other)	9, 14, 19
	Chloroform	17, 23, 28
	Other / Not reported	7, 11, 18
Source of Calibration Standard	NMI Australia	1, 3, 8, 9, 13, 17, 19, 20, 21
	Lipomed	5, 6, 10, 11, 15, 16, 22, 23, 24, 30, 31, 32
	LGC	4, 12, 14, 25
	Johnson Matthey	2, 27
	Other	18, 28
	Not reported	7, 26

* Assumed; masses appear to be reported in g instead of mg.

Plots of z-scores vs various methodology parameters are presented in Figures 7 to 11. No trends were observed when more than one participant used a particular parameter.

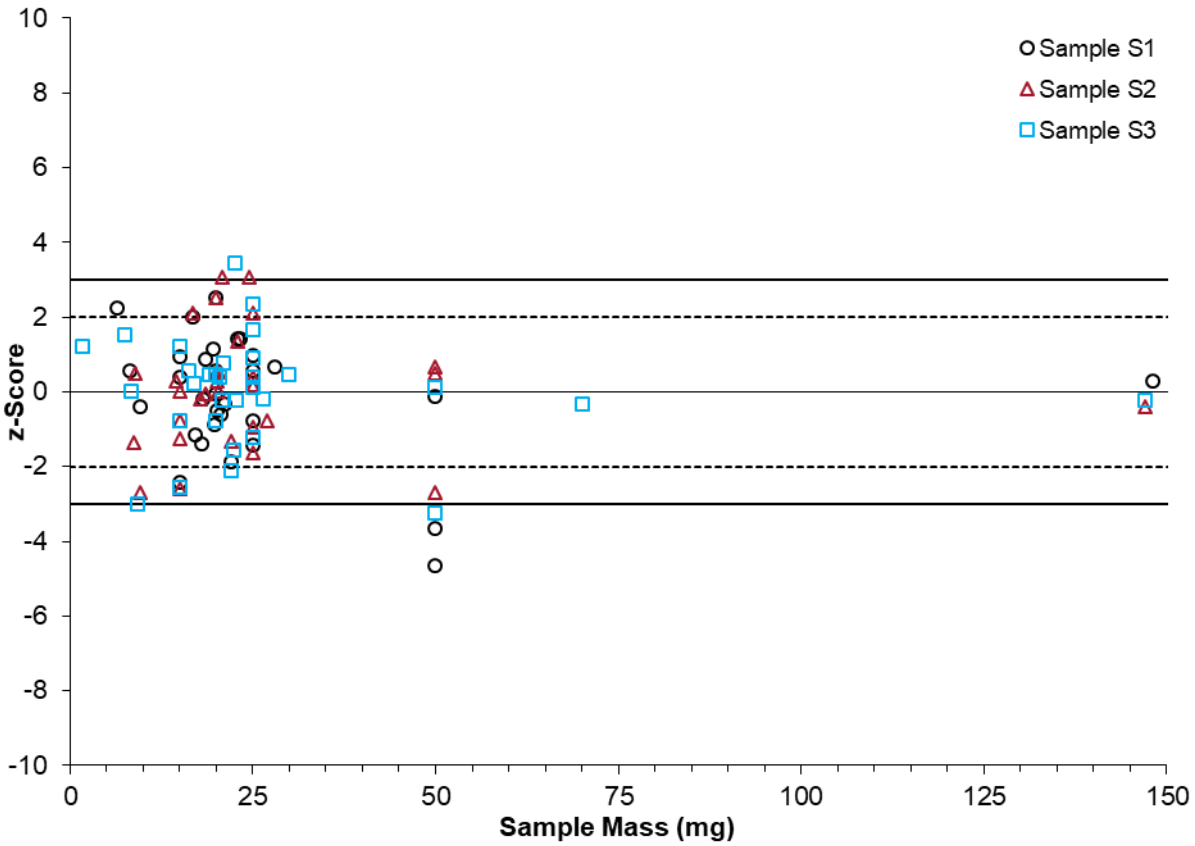


Figure 7 z-Score vs Sample Mass Used per Analysis

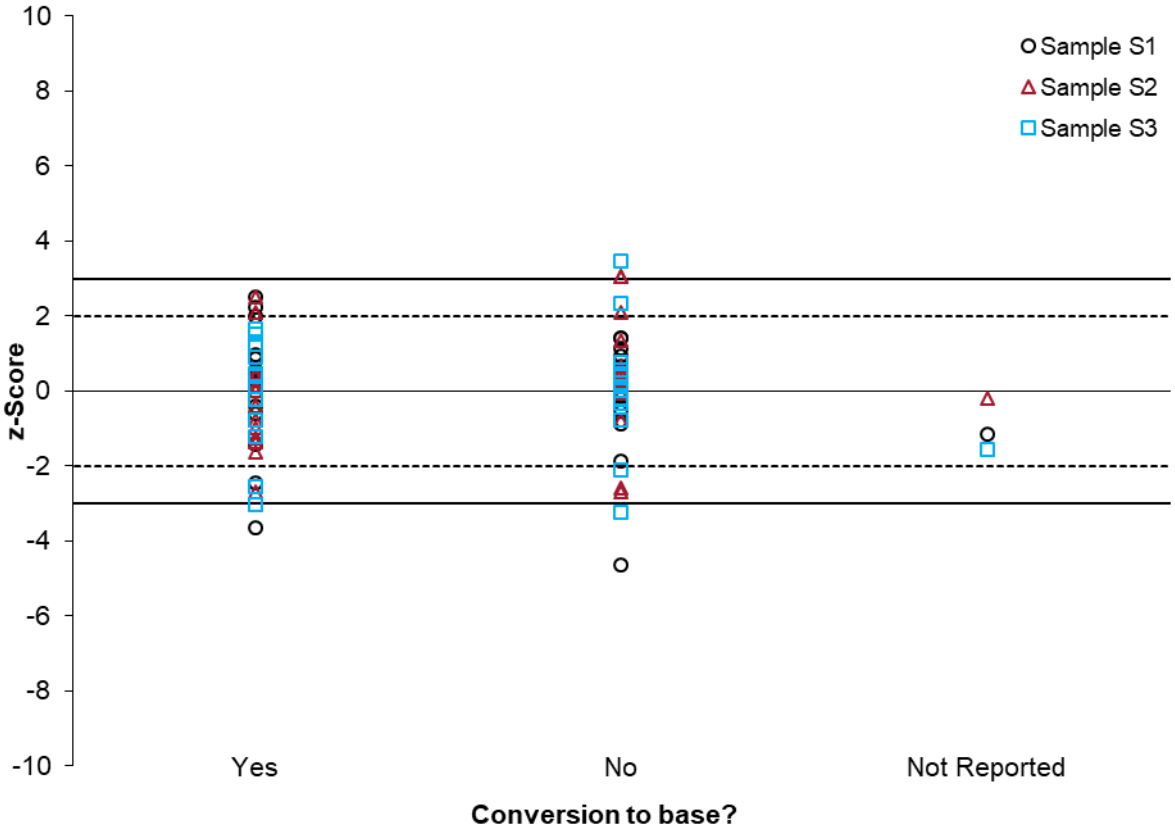


Figure 8 z-Score vs Sample Processing

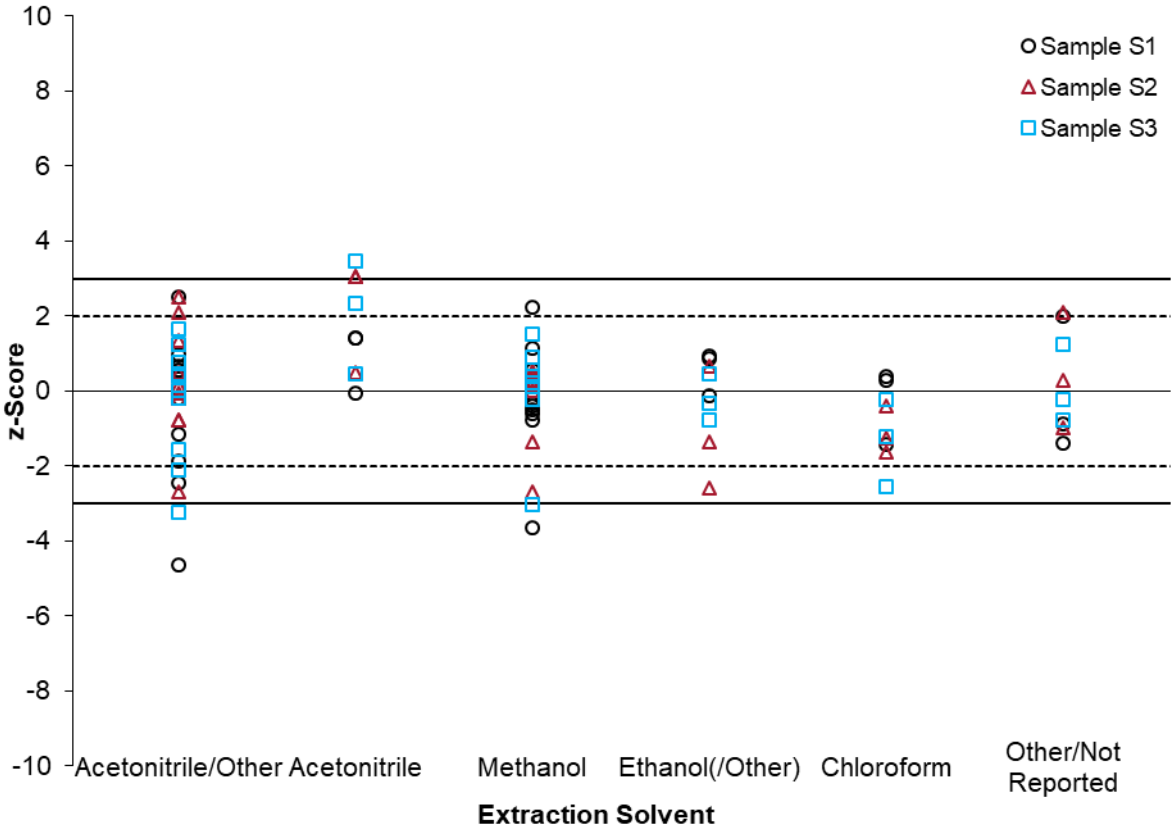


Figure 9 z-Score vs Extraction Solvent

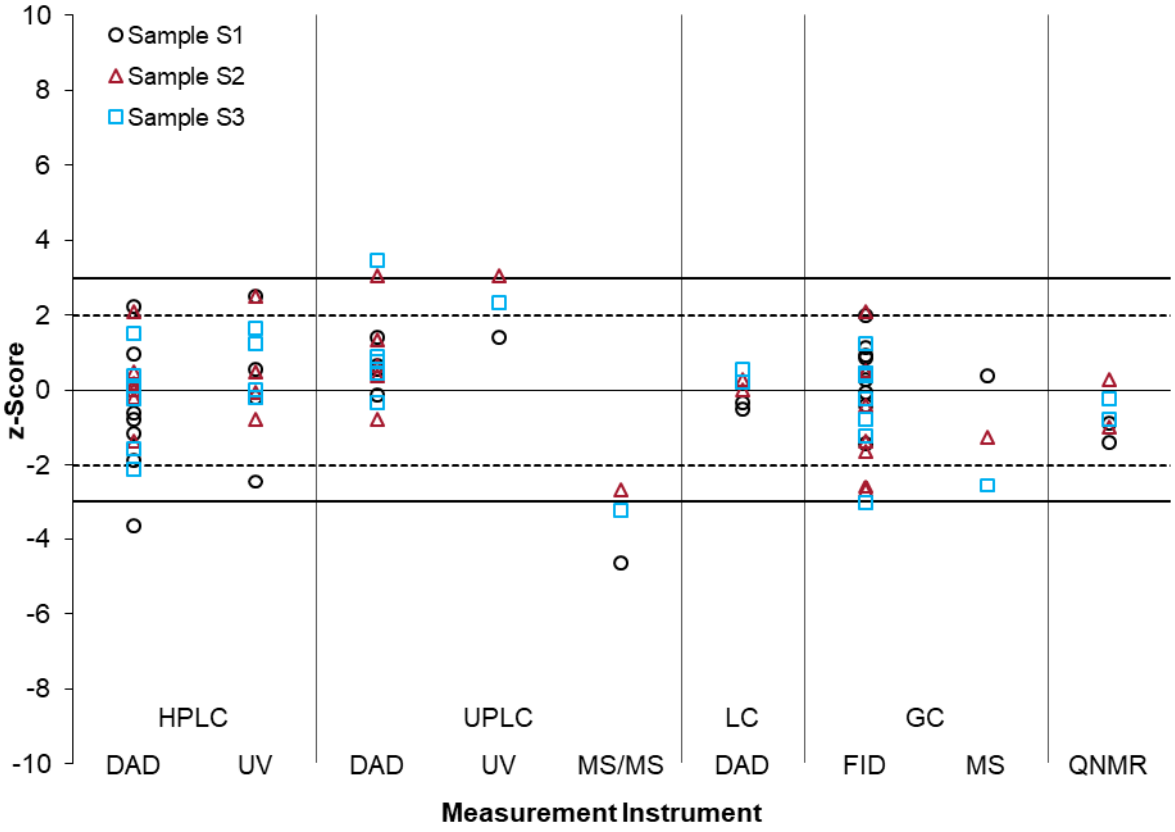


Figure 10 z-Score vs Measurement Instrument

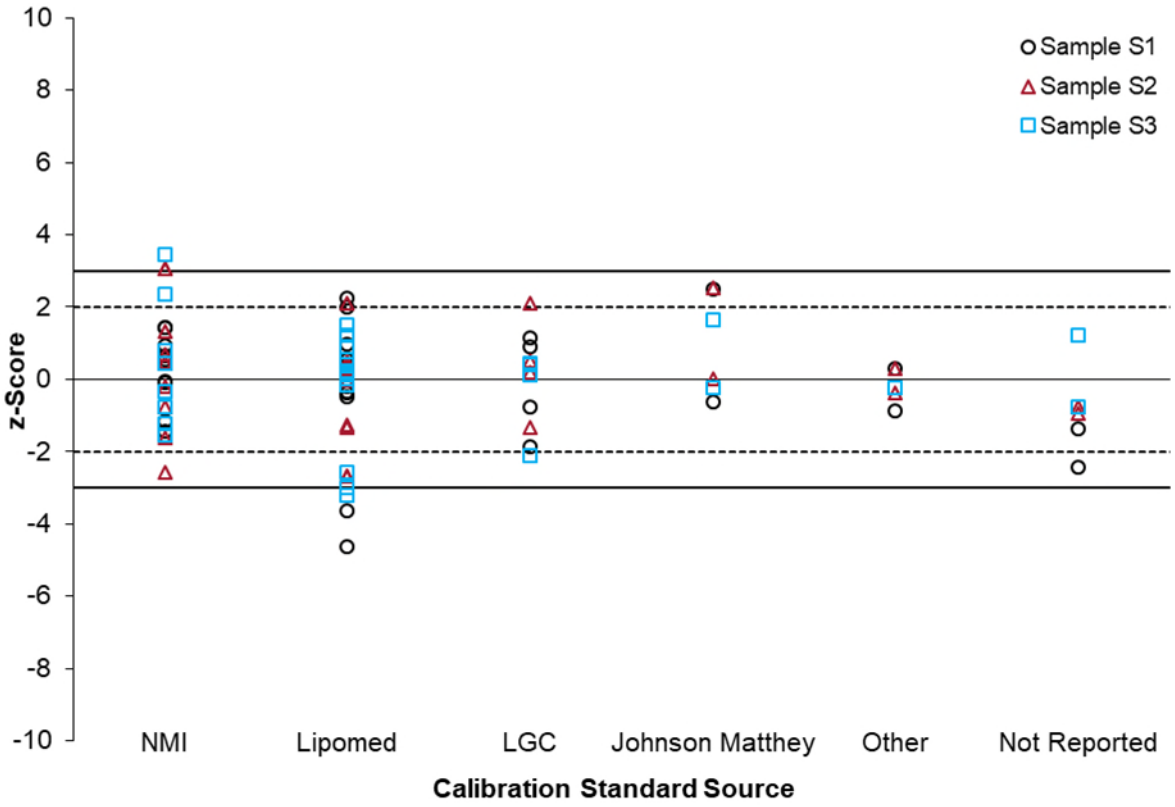


Figure 11 z-Score vs Calibration Standard Source

As there were delays with sample delivery to some participants, the samples were analysed by participants over the course of approximately 3 months. No trend was found between when the samples were analysed and the results obtained (Figure 12).

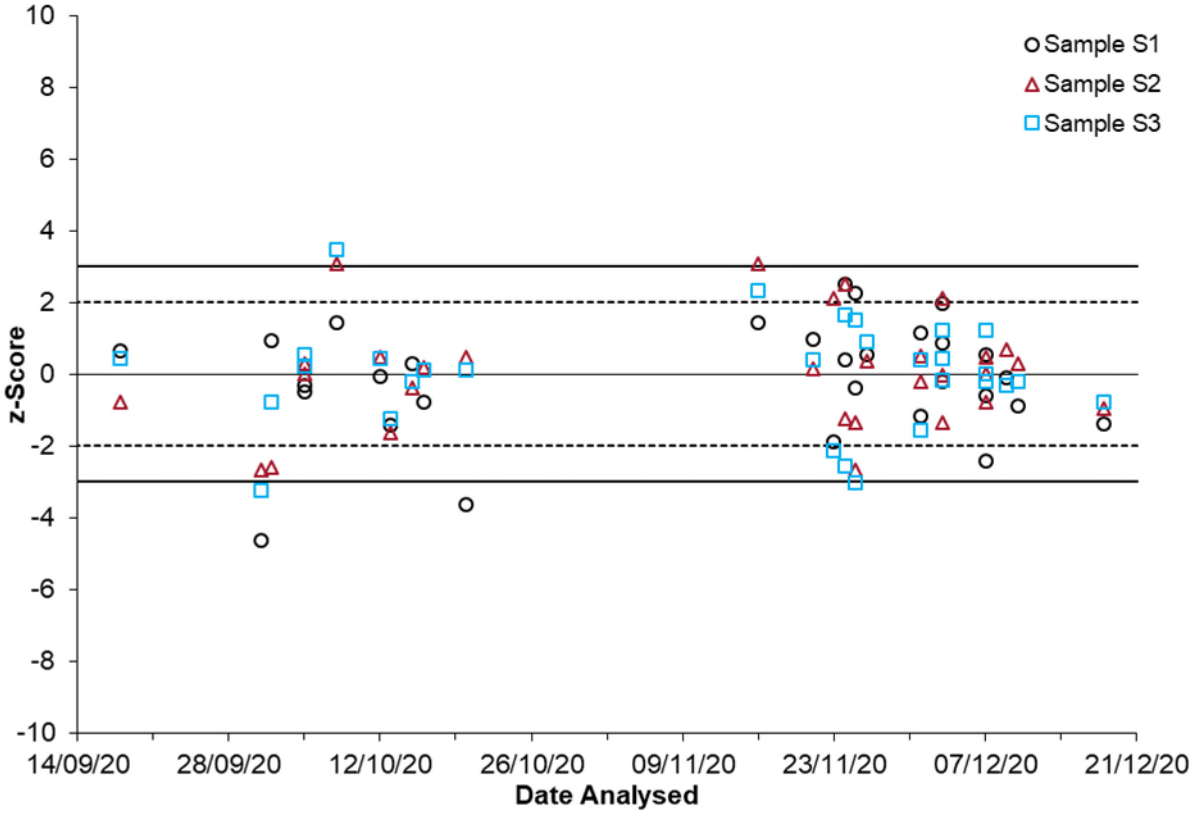


Figure 12 z-Score vs Sample Analysis Date

6.7 Comparison with Previous Heroin PT Studies

To enable direct comparison with previous Heroin 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 participants from 2011 – 2020 (last 10 studies) is presented in Figure 13. The proportion of satisfactory z-scores and E_n-scores over this period on average is 78% and 76% respectively. While each PT study has a different group of participants, taken as a group, the performance over this period has improved.

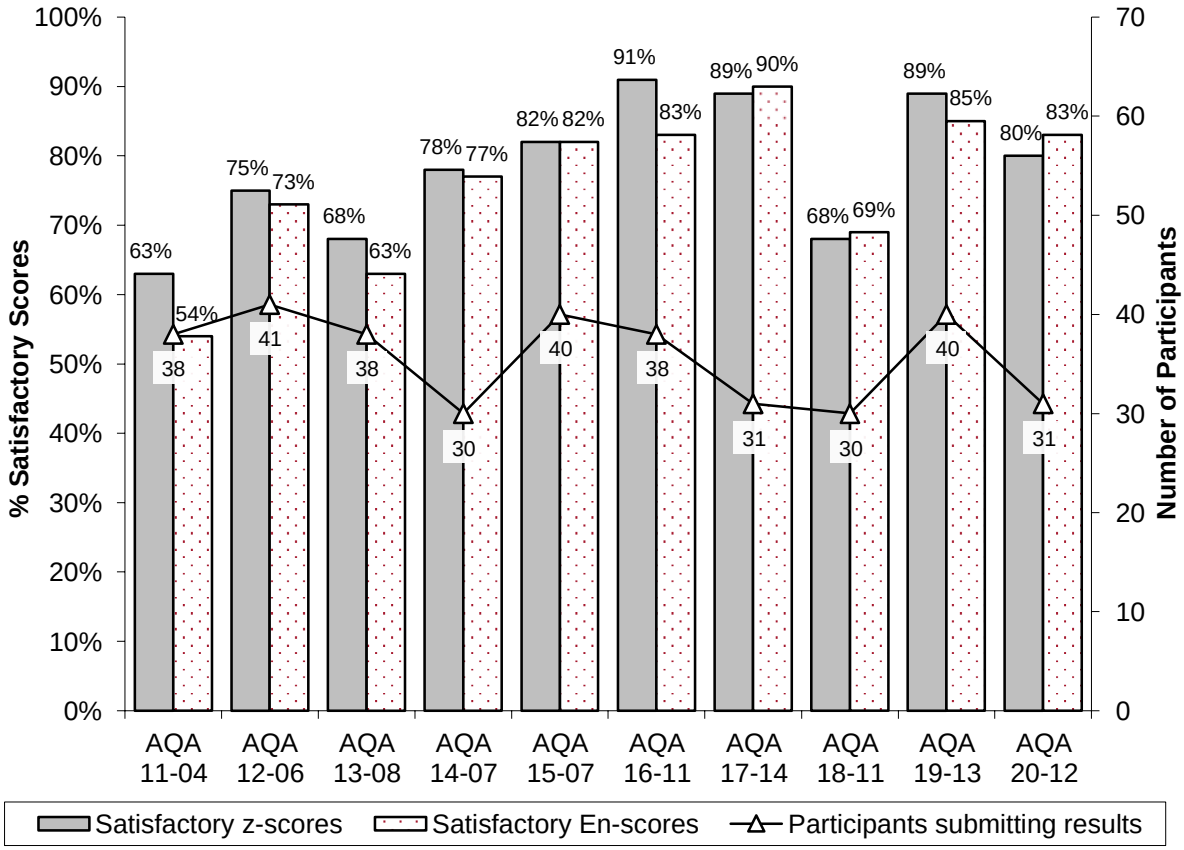


Figure 13 Summary of Participants' Performance in Heroin PT Studies

Individual performance history reports are emailed to each participant at the end of the 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, *Study Protocol for Proficiency Testing*, viewed July 2020, <https://www.industry.gov.au/sites/default/files/2019-07/cpt_study_protocol.pdf>.

[3] NMI, *Chemical Proficiency Testing Statistical Manual*, viewed July 2020, <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 comparisons*.

[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 2012, *Quantifying Uncertainty in Analytical Measurement*, 3rd ed., viewed July 2020, <http://eurachem.org/images/stories/guides/pdf/quam2012_P1.pdf>.

APPENDIX 1 – ROBUST AVERAGE AND ASSOCIATED UNCERTAINTY, Z-SCORE AND E_N-SCORE CALCULATIONS

A1.1 Robust Average and Associated Uncertainty

The robust average is calculated using the procedure described in ISO 13528:2015 Annex C.[5] The associated uncertainty is estimated as:

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

where:

$u_{rob\ average}$	is the standard uncertainty of the robust average
$S_{rob\ average}$	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 2 at approximately 95% confidence level.

A worked example is set out below in Table 12.

Table 12 Uncertainty of Robust Average of Sample S1 as % base (m/m)

No. results (p)	31
Robust Average	60.4
$S_{rob\ average}$	2.6
$u_{rob\ average}$	0.6
k	2
$U_{rob\ average}$	1.2

Therefore, the robust average for Sample S1 is 60.4 ± 1.2% base (m/m).

A1.2 z-Score and E_n-Score Calculations

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

A worked example is set out below in Table 13.

Table 13 z- and E_n-Score Calculation for Sample S1 Result Reported by Laboratory 1

Participant Result (% base (m/m))	Assigned Value (% base (m/m))	Target Standard Deviation	z-Score	E_n-Score
58.3 ± 5.9	60.4 ± 1.2	3% as PCV, or: 0.03 × 60.4 = 1.812% base (m/m)	z-Score = $\frac{58.3-60.4}{1.812}$ = -1.16	E_n-Score = $\frac{58.3-60.4}{\sqrt{5.9^2+1.2^2}}$ = -0.35

APPENDIX 2 – ACRONYMS AND ABBREVIATIONS

AFP	Australian Federal Police
ANAB	ANSI (American National Standards Institute) National Accreditation Board
ASCLD	American Society of Crime Laboratory Directors
CITAC	Cooperation on International Traceability in Analytical Chemistry
CRM	Certified Reference Material
CV	Coefficient of Variation
DAD	Diode Array Detector
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
ISO	International Organization for Standardization
LC	Liquid Chromatography
Max.	Maximum value in a set of results
Md	Median
Min.	Minimum value in a set of results
MS	Mass Spectrometry
MS/MS	Tandem Mass Spectrometry
MU	Measurement Uncertainty
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
RM	Reference Material
SD	Standard Deviation
SI	International System of Units
SS	Spiked Samples
UPLC	Ultra Performance Liquid Chromatography
UV	Ultraviolet

END OF REPORT