Australian Government
Department of Industry, Science, Energy and Resources

National Measurement Institute

Proficiency Test Final Report
AQA 21-11
Heroin

January 2022

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

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

SUMMARY

AQA 21-11 Heroin commenced in June 2021. Sets of heroin hydrochloride, each containing three test samples, were sent to 32 laboratories, with two laboratories requesting two sample sets to be analysed independently by different analysts. All 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 averages of participants' results.

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

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

- *Assess the proficiency of participants measuring heroin in samples typical of a routine seizure.*

Participant performance was assessed by z-scores and E_n-scores.

Of 102 z-scores, 88 (86%) returned $|z| \leq 2.0$, indicating a satisfactory performance.

Of 102 E_n-scores, 93 (91%) returned $|E_n| \leq 1.0$, indicating agreement of the participant's result with the assigned value within their respective expanded uncertainties.

Laboratories **2**, **5**, **6**, **10**, **11**, **13**, **14**, **15**, **16**, **17**, **18**, **19**, **20**, **21**, **22**, **23**, **24**, **25**, **27**, **28**, **31**, **33** and **34** returned satisfactory z-scores 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.*

Of 102 reported results, 99 (97%) were reported with an associated expanded measurement uncertainty. The magnitude of reported uncertainties was within the range 0.3% to 24% relative.

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

Sample S1 was cut with phenacetin, Sample S2 was cut with glucose, and Sample S3 was cut with sucrose.

Twenty-nine participants (85%) reported on the identity of the cutting agents. Laboratories **3**, **11**, **14**, **18** and **28** correctly reported all cutting agents used.

Phenacetin had a significantly higher reporting rate as compared to the sugars (glucose and sucrose).

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

The test samples produced for this study are homogeneous and well characterised. Surplus samples are 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;
- per- and polyfluoroalkyl substances in water, soil, biota and food;
- 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 participants 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;
- test the ability of participants to identify cutting agents commonly found in controlled drug preparation; and
- produce materials that can be used in method validation and as control samples.

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

1.3 Study Conduct

The conduct of NMI 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 17043:2010,[1] and The International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories.[4]

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 study is within the scope of NMI's accreditation.

2 STUDY INFORMATION

2.1 Study Timetable

The timetable of the study was:

Invitation issued: 7 June 2021

Samples dispatched: 11 August 2021

Results due: 29 November 2021

Interim report issued: 4 January 2022

2.2 Participation and Laboratory Code

Thirty-two laboratories registered to participate, with two laboratories requesting two sets of samples each to be analysed independently by different analysts. All participants were assigned a confidential laboratory code number for this study. All participants returned results.

2.3 Test Material Specification

Three test samples were prepared in July 2021. The starting material was heroin hydrochloride, approximately 75% base (m/m) supplied by the Australian Federal Police. Phenacetin and sucrose purchased from Sigma-Aldrich, and glucodin (glucose) purchased from a local pharmacy were used as cutting agents. Sample S1 was cut with phenacetin, Sample S2 was cut with glucose, and Sample S3 was cut with sucrose.

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 71% heroin base (m/m).

Sample S2 was prepared to contain approximately 35% heroin base (m/m).

Sample S3 was prepared to contain approximately 35% heroin 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 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.

2.5 Sample Dispatch and Receipt

Sets of three test samples, with each sample containing approximately 150 mg of material, were dispatched to participants on 11 August 2021.

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 emailed to participants.

2.6 Instructions to Participants

Participants were instructed as follows:

- Analyse each sample for amount of heroin base by your routine test method.
- Identify the diluent(s) and/or adulterant(s) in all samples if this is within your normal scope of analysis.
- For each sample, report % m/m heroin as base. Report this figure as if reporting to a client.
- For each result, report an estimate of your expanded uncertainty as % m/m heroin as base.
- Report the identity of diluent(s)/adulterant(s) in the 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 sheet and return by email to jenny.xu@measurement.gov.au.
- Results are to be returned by 11 October 2021. Late results may not be included in the study report.

The results due date was extended to 29 November 2021 for all participants due to delivery delays to international participants.

2.7 Interim Report

An interim report was emailed to all participants on 4 January 2022.

The interim report release was delayed due to significant sample delivery issues affecting a small number of international participants, as well as a distributor issue which required further investigation.

3 PARTICIPANT LABORATORY INFORMATION

3.1 Test Methods Reported by Participants

Participants' reported test methods are presented in Table 1. Responses may 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	Chloroform	2,2,2-Triphenylacetophenone	1	GC	FID	HP5
2	Chloroform containing 10 % (v/v) of methanol	2,2,2-acetophenone	1	GC	FID	HP5
3	Acetonitrile	N/A	6	UPLC	DAD	Acquity UPLC BEH C18 1.7 µm 2.1 x 100 mm
4	75% ACN: 25% Water	benzocaine	3	UPLC	DAD	Acquity UPLC BEH C18 1.7um (2.1 x 100mm)
5	acetonitrile / water	none	1	HPLC	UV/Vis	Kromasil
6	Methanol	NO (External Standard)	7	UPLC	DAD	Poroshell 120 EC-C18 (4.6x150mm, 2.7 microns pore size)
7	Ethanol	Eicosane	6	GC	FID	HP-5
8	Acetonitrile/ Methanol (95:5)	Pholocodine 1mg/ml	3	UPLC	PDA	ACQUITY C-18
9	Ethanol	N/A	4	UPLC	UV/Vis	Lichrocart 125-4 RP18
10	Methanol	none	5	HPLC	DAD	Kinetex C-18-XB
11	acetonitrile/ water (86/14)	none	4	HPLC	UV	NH2
12	ACN/MeOH/ H2O	Analog of heroin	7	UPLC	MSMS	C-18 column
13	Methanol	Nil	1	LC	DAD	Hypersil-5-ODS
14	Acetonitrile	Strychnine	6	GC	FID	HP1
15	Methanol	Methadone	4	GC	FID	Agilent HP-5 30mx0.32mm x0.25µm
16	Methanol	Mepivacaine	4	HPLC	DAD	C18
17	Chloroform	Octacosane	5	GC	FID	HP5
18	Ethanol	Triphenylacetophenone (TPAP)	3	GC	FID	HP1-MS

Lab. Code	Extraction Solvent	Internal Standard	Calib. Points	Technique	Detector	Column
19	ethanol: dimethyl-formamide (9:1)	tribenzylamine	6	GC	FID	HP1
20	Chloroform and Methanol	β-Benzopinacolone	1	GC	FID	HP5
21	Chloroform and Methanol	2,2,2-Triphenylacetophenone	1	GC	FID	HP5
22	Chloroform	2,2,2-triphenylacetophenone	4	GC	FID	HP1
23	Chloroform	2,2,2-Triphenylacetophenone	1	GC	FID	HP5
24	Chloroform, methanol	Benzapinacolone	1	GC	FID	HP5
25	Chloroform	2,2,2-Triphenylacetophenone	1	GC	FID	HP5
26	Ammonium Formate, pH 3	No	4	HPLC	MS	Ascentis Express Phenyl-Hexyl (2.7 µm)
27	Methanol	Nil	3	LC	DAD	Hypersil-5-ODS
28	acetonitrile	nil	6	UPLC	PDA	Acquity UPLPC BEH C18 1.7um 2.1x100 mm
29	water/ acetonitrile/ 2.5M sulphuric acid 90:10:1	None	3	HPLC	Diode Array	Shimpack XR-ODS
30	Acetonitrile/ Water	None	5	HPLC	UV	Kinetex 5u C18
31	Chloroform	β-Benzopinacolone	1	GC	FID	HP5
32	Ethanol	Propyl Paraben	8	UPLC	DAD	BEH Shield RP18
33	Methanol	Diazepam	6	GC	FID	128-5512 DB-5ms
34	Chloroform: Methanol (9:1)	Benzopinacolone	1	GC	FID	HP5

3.2 Details of Participant Calibration Standards

Participants' responses regarding their calibration standard used are presented in Table 2. Responses may have been modified so that the participant cannot be identified.

Table 2 Participant Calibration Standard

Lab. Code	Reference Standard	Purity (%)
1	Toronto Research Chemicals	98
2	Toronto Research Chemicals	98
3	NMI	99.4
4	NMI	99.3
5	LIPOMED	99.6
6	LIPOMED	99.95
7	Alcaliber	101
8	NMI	99.3
9	NMI	99.4
10	LGC	99.7
11	Lipomed	99.912
12	Lipomed	100
13	Lipomed	99.091±0.079
14	NMI	99.3
15	LGC	1.02 mg/mL
16	Lipomed	99.6 +/- 0.020
17	NMI	99.3
18	NMI	99.3
19	LGC	99.4
20	TRC-CANADA	98
21	Toronto Research Chemical Inc.	98
22	in lab synthesis	99.14
23	Toronto Research Chemicals	98
24	Toronto Research Chemicals	98
25	Toronto Research Chemicals	98
26	Local pharmaceutical supplier	100
27	Lipomed	99.091±0.079
28	NMI	99.4
29	LGC	99.7
30	Johnson Matthey	99.5
31	Toronto Research Chemicals	98
32	NMI	99.4
33	Lipomed	99.1
34	Toronto Research Chemicals	98

3.3 Reported Basis of Participants' Measurement Uncertainty Estimates

Participants were requested to provide information about their basis of measurement uncertainty (MU). Responses are presented in Table 3. Responses may have been modified so that the participant cannot be identified.

Table 3 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	Standard deviation of replicate analyses multiplied by 2 or 3			
2	Top Down - precision and estimates of the method and laboratory bias	Duplicate analysis	Instrument calibration Standard purity	Eurachem/CITAC Guide
3	Top Down - precision and estimates of the method and laboratory bias	Control samples - In-house control Duplicate analysis	Instrument calibration Homogeneity of sample Masses and volumes Standard purity	NATA GAG Estimating and Reporting Measurement Uncertainty of Chemical Test Results
4	Top Down - precision and estimates of the method and laboratory bias	Control samples - RM Duplicate analysis	Masses and volumes Standard purity	Eurachem/CITAC Guide
5	Standard deviation of replicate analyses multiplied by 2 or 3	Control samples - RM		ISO/GUM
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
7	Top Down - precision and estimates of the method and laboratory bias	Control samples - RM Duplicate analysis	Matrix effects	ISO/GUM
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	Uncertainty Budget Method	Control samples - CRM Duplicate analysis	Instrument calibration Homogeneity of sample Masses and volumes Standard purity	Internal SOP Document
10		Control samples - RM Duplicate analysis	Instrument calibration Masses and volumes Recoveries of SS Standard purity	Eurachem/CITAC Guide

Lab. Code	Approach to Estimating MU	Information Sources for MU Estimation*		Guide Document for Estimating MU
		Precision	Method Bias	
11	Top Down - precision and estimates of the method and laboratory bias	Control samples - RM	Laboratory bias from PT studies Standard purity	ISO 11352 and V03-110
12	Top Down - precision and estimates of the method and laboratory bias	Control samples - RM		
13	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
14	Top Down - precision and estimates of the method and laboratory bias	Control samples - CRM Duplicate analysis	Instrument calibration Masses and volumes Matrix effects Recoveries of SS Standard purity	ISO/GUM
15	Standard deviation of replicate analyses multiplied by 2 or 3	Duplicate analysis	Masses and volumes	ISO/GUM
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 ILAC 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
18	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
19	Top Down - precision and estimates of the method and laboratory bias	Control samples - RM	Standard purity	
20	Top Down - precision and estimates of the method and laboratory bias	Duplicate analysis	Instrument calibration Standard purity	Eurachem/CITAC Guide
21	Top Down - precision and estimates of the method and laboratory bias	Duplicate analysis	Standard purity	NARL
22	Standard deviation of replicate analyses multiplied by 2 or 3			

Lab. Code	Approach to Estimating MU	Information Sources for MU Estimation*		Guide Document for Estimating MU
		Precision	Method Bias	
23	Top Down - precision and estimates of the method and laboratory bias	Duplicate analysis	Instrument calibration Recoveries of SS	Eurachem/CITAC Guide
24	Top Down - precision and estimates of the method and laboratory bias	Duplicate analysis	Masses and volumes Recoveries of SS	Eurachem/CITAC Guide
25	Top Down - precision and estimates of the method and laboratory bias	Duplicate analysis	Instrument calibration Homogeneity of sample Masses and volumes Standard purity	Eurachem/CITAC Guide
26	Top Down - precision and estimates of the method and laboratory bias	Control samples Duplicate analysis	Laboratory bias from PT studies	Nordtest Report TR537
27	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
28	Top Down - precision and estimates of the method and laboratory bias	Control samples - in-house control Duplicate analysis	Standard purity	NATA GAG Estimating and Reporting Measurement Uncertainty of Chemical Test Results
29	Standard deviation of replicate analyses multiplied by 2 or 3	Control samples - CRM Duplicate analysis	Instrument calibration Standard purity	ISO/GUM MuM determined from multiple injections of reference material. 3x (Std Dev/mean)x100.
30	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
31	Top Down - precision and estimates of the method and laboratory bias	Duplicate analysis	Instrument calibration Standard purity	ISO/GUM
32	No uncertainty reported			
33	Estimating Measurement Uncertainty by black box with pairs of values	Standard deviation from PT studies only		ISO 21748
34	Top Down - precision and estimates of the method and laboratory bias	Control samples - RM	Matrix effects Standard purity	ASCLD/LAB Guidance on the Estimation of Measurement Uncertainty, AL-PD-3061

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

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

Table 4 Participant Comments

Lab. Code	Participants' Comments	Study Co-ordinator's Response
2	Do continue	We intend to coordinate a heroin PT study annually.
4	Heroin, acetylcodeine and monoacteylmorphine detected.	
6	Qualitative analysis was carried out by GC-MS	
18	Methodology: Dicloromethane (30mL/L of ethanol) was used to dissolve the TPAP	
28	Conversion to free base done on calibration standard. Sample S2 FTIR results D-Glucose/Dextrose - dextrose reported. Acetylcodeine also detected and quantitated.	
29	Analysis for Inert cutting agents not undertaken as part of standard analytical procedure. No analysis undertaken for inert bulking agents.	
7	It would be much better if there were samples covering the whole range. S2 and S3 are from the same range and there is no sample in the low range. This also happened in AQA-19-18 (Cocaine) where sample S3 was identical to Sample S2 (duplicate).	A range of drug purities are selected to cater for the needs of different laboratories. Occasionally we also produce blind duplicate samples to assess participants' performance, as was the case in AQA 19-18, or we produce samples with similar concentrations but different cutting agents to look at effects from the matrix, as was the case in this study. We will take your suggestions into consideration for future studies.
11	We would like to receive 3 samples of very different concentration for example 3%, 30% and 80%	
34	Purity of our routine seized heroin samples received in the laboratory is around 3-5%. This trend seen for heroin cases in our region. It will be good to have one of the vial with low purity heroin.	

4 PRESENTATION OF RESULTS AND STATISTICAL ANALYSIS

4.1 Results Summary

Participant results are listed in Tables 5 to 7 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.

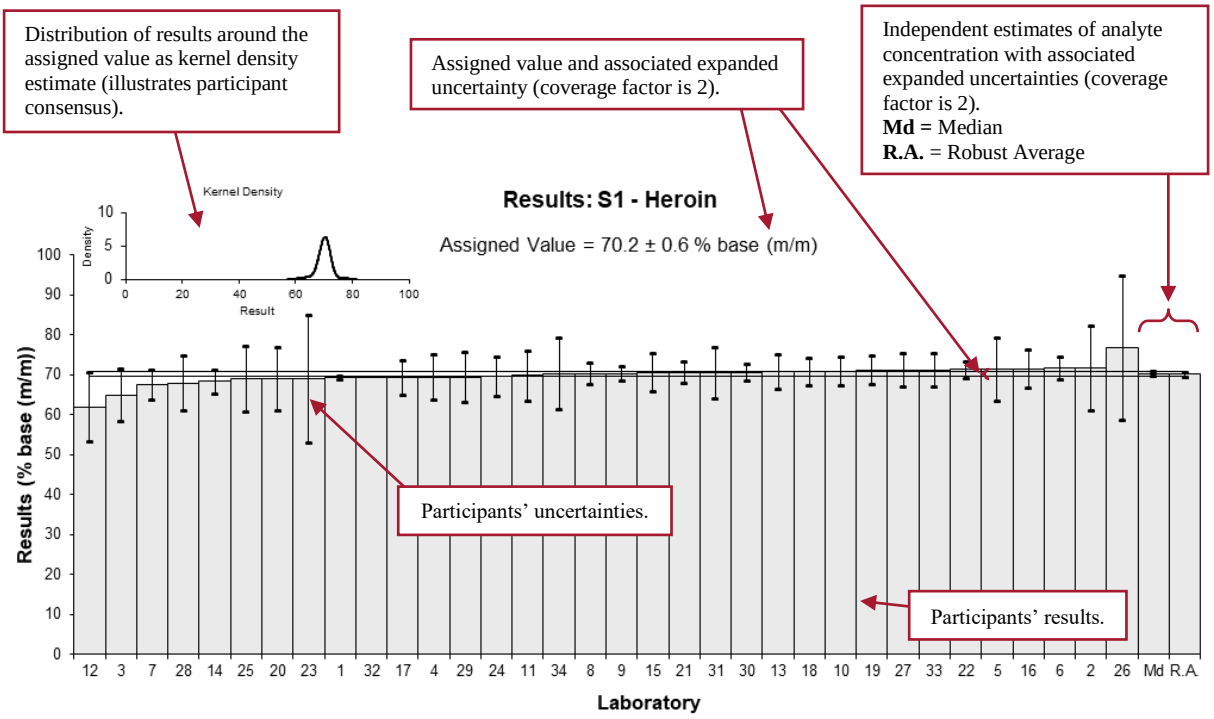


Figure 1 Guide to Presentation of Results

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-laboratory 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 and 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.

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

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; and
- $|z| \geq 3.0$ 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.

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

where:

E_n is E_n-score

χ is a participant's result

X is the assigned value

U_χ is the expanded uncertainty of the participant's result

U_X is the expanded uncertainty of the assigned value

For the absolute value of an E_n-score:

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

4.8 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

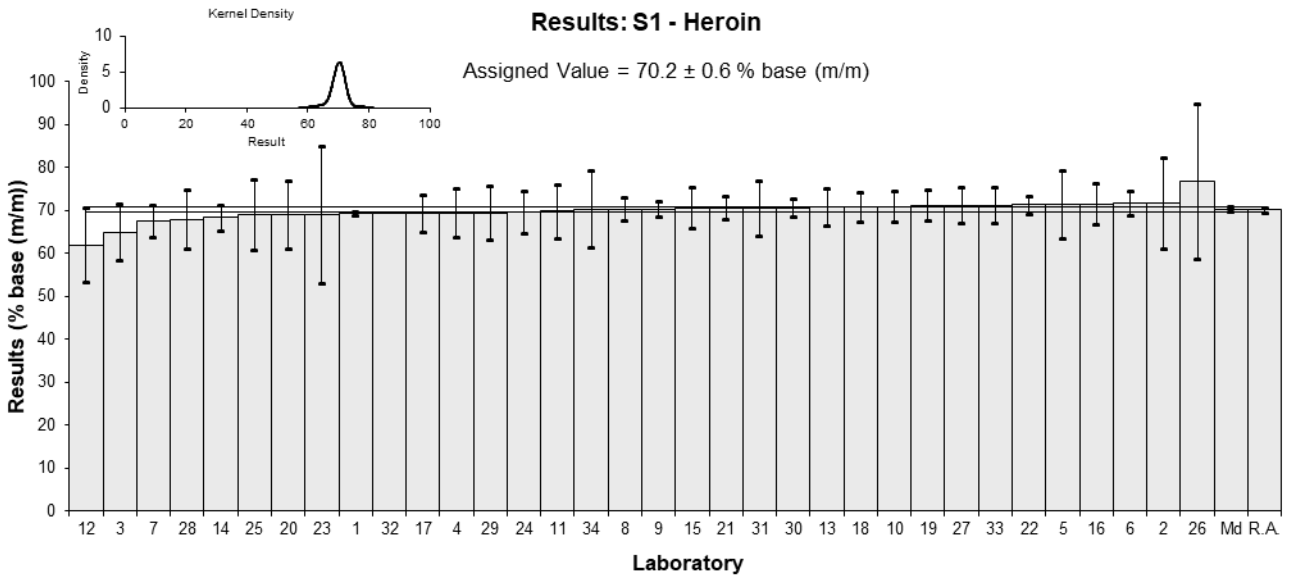
Sample No.	S1
Matrix	Powder
Analyte	Heroin
Units	% base (m/m)

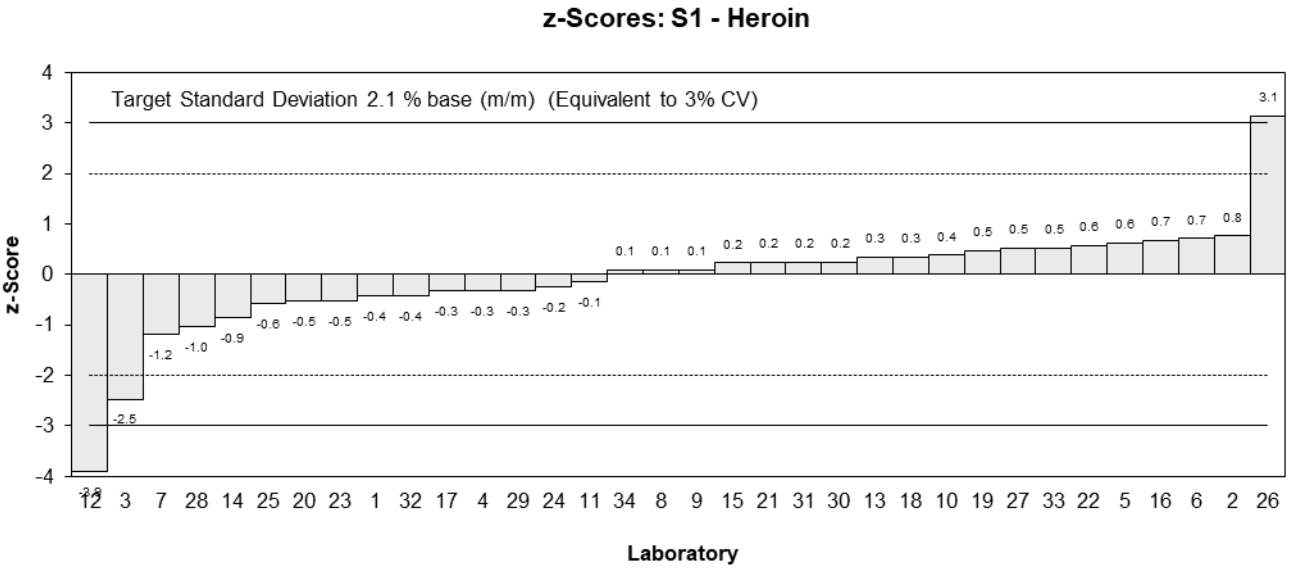
Participant Results

Lab. Code	Result	Uncertainty	z-Score	E_n-Score
1	69.3	0.4	-0.43	-1.25
2	71.8	10.6	0.76	0.15
3	65	6.5	-2.47	-0.80
4	69.5	5.7	-0.33	-0.12
5	71.5	7.9	0.62	0.16
6	71.7	2.9	0.71	0.51
7	67.7	3.7	-1.19	-0.67
8	70.4	2.80	0.09	0.07
9	70.4	1.7	0.09	0.11
10	71	3.6	0.38	0.22
11	69.89	6.29	-0.15	-0.05
12	62	8.7	-3.89	-0.94
13	70.9	4.3	0.33	0.16
14	68.4	3	-0.85	-0.59
15	70.68	4.69	0.23	0.10
16	71.6	4.7	0.66	0.30
17	69.5	4.3	-0.33	-0.16
18	70.9	3.5	0.33	0.20
19	71.2	3.6	0.47	0.27
20	69.1	7.8	-0.52	-0.14
21	70.7	2.8	0.24	0.17
22	71.4	2.1	0.57	0.55
23	69.1	16	-0.52	-0.07
24	69.7	4.8	-0.24	-0.10
25	69	8.2	-0.57	-0.15
26	76.8	18.0	3.13	0.37
27	71.3	4.3	0.52	0.25
28	68	6.8	-1.04	-0.32
29	69.53	6.15	-0.32	-0.11
30	70.71	2.12	0.24	0.23
31	70.7	6.4	0.24	0.08
32	69.3	NR	-0.43	-1.50
33	71.3	4.3	0.52	0.25
34	70.4	8.9	0.09	0.02

Statistics

Assigned Value	70.2	0.6
Robust Average	70.2	0.6
Median	70.4	0.5
Mean	70.0	
N	34	
Max.	76.8	
Min.	62	
Robust SD	1.4	
Robust CV	1.9%	


Results: S1 - Heroin
Kernel Density
Density
Result
Assigned Value = 70.2 ± 0.6 % base (m/m)
Results (% base (m/m))
12 3 7 28 14 25 20 23 1 32 17 4 29 24 11 34 8 9 15 21 31 30 13 18 10 19 27 33 22 5 16 6 2 26 Md R.A.
Laboratory


z-Scores: S1 - Heroin
Target Standard Deviation 2.1 % base (m/m) (Equivalent to 3% CV)
z-Score
12 3 7 28 14 25 20 23 1 32 17 4 29 24 11 34 8 9 15 21 31 30 13 18 10 19 27 33 22 5 16 6 2 26
Laboratory

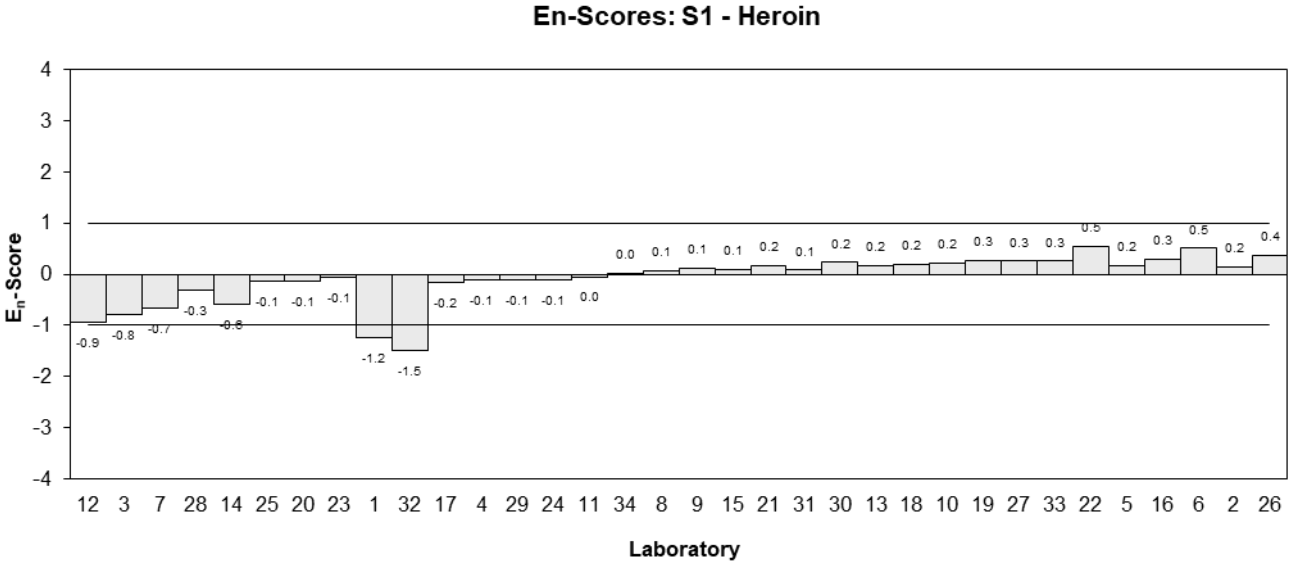

En-Scores: S1 - Heroin
En-Score
12 3 7 28 14 25 20 23 1 32 17 4 29 24 11 34 8 9 15 21 31 30 13 18 10 19 27 33 22 5 16 6 2 26
Laboratory

Figure 2

Table 6

Sample Details

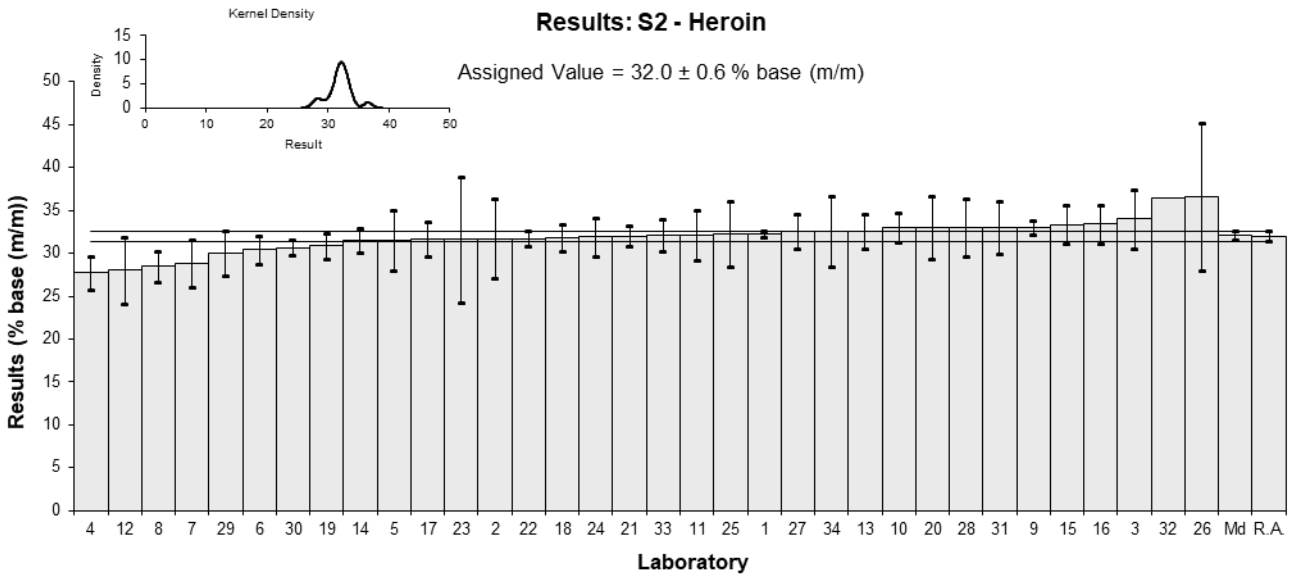
Sample No.	S2
Matrix	Powder
Analyte	Heroin
Units	% base (m/m)

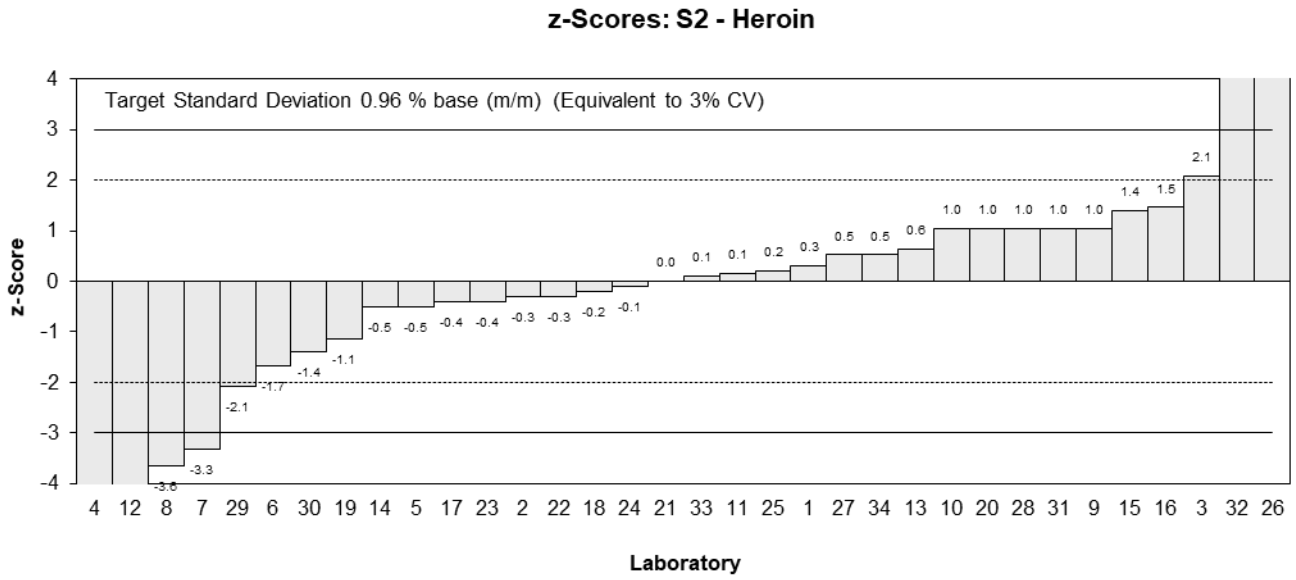
Participant Results

Lab. Code	Result	Uncertainty	z-Score	E_n-Score
1	32.3	0.4	0.31	0.42
2	31.7	4.6	-0.31	-0.06
3	34	3.4	2.08	0.58
4	27.7	2.0	-4.48	-2.06
5	31.5	3.5	-0.52	-0.14
6	30.4	1.6	-1.67	-0.94
7	28.8	2.8	-3.33	-1.12
8	28.5	1.80	-3.65	-1.84
9	33.0	0.8	1.04	1.00
10	33	1.7	1.04	0.55
11	32.14	2.89	0.15	0.05
12	28	3.9	-4.17	-1.01
13	32.6	2.0	0.63	0.29
14	31.5	1.4	-0.52	-0.33
15	33.35	2.21	1.41	0.59
16	33.4	2.2	1.46	0.61
17	31.6	2	-0.42	-0.19
18	31.8	1.6	-0.21	-0.12
19	30.9	1.5	-1.15	-0.68
20	33.0	3.7	1.04	0.27
21	32.0	1.2	0.00	0.00
22	31.7	0.9	-0.31	-0.28
23	31.6	7.3	-0.42	-0.05
24	31.9	2.2	-0.10	-0.04
25	32.2	3.8	0.21	0.05
26	36.6	8.6	4.79	0.53
27	32.5	2.0	0.52	0.24
28	33	3.3	1.04	0.30
29	30	2.66	-2.08	-0.73
30	30.66	0.92	-1.40	-1.22
31	33.0	3.0	1.04	0.33
32	36.5	NR	4.69	7.50
33	32.1	1.9	0.10	0.05
34	32.5	4.1	0.52	0.12

Statistics

Assigned Value	32.0	0.6
Robust Average	32.0	0.6
Median	32.1	0.5
Mean	31.9	
N	34	
Max.	36.6	
Min.	27.7	
Robust SD	1.4	
Robust CV	4.4%	


Results: S2 - Heroin
Kernel Density
Density
Result
Assigned Value = 32.0 ± 0.6 % base (m/m)
Results (% base (m/m))
4 12 8 7 29 6 30 19 14 5 17 23 2 22 18 24 21 33 11 25 1 27 34 13 10 20 28 31 9 15 16 3 32 26 Md R.A.
Laboratory


z-Scores: S2 - Heroin
Target Standard Deviation 0.96 % base (m/m) (Equivalent to 3% CV)
z-Score
4 12 8 7 29 6 30 19 14 5 17 23 2 22 18 24 21 33 11 25 1 27 34 13 10 20 28 31 9 15 16 3 32 26
Laboratory

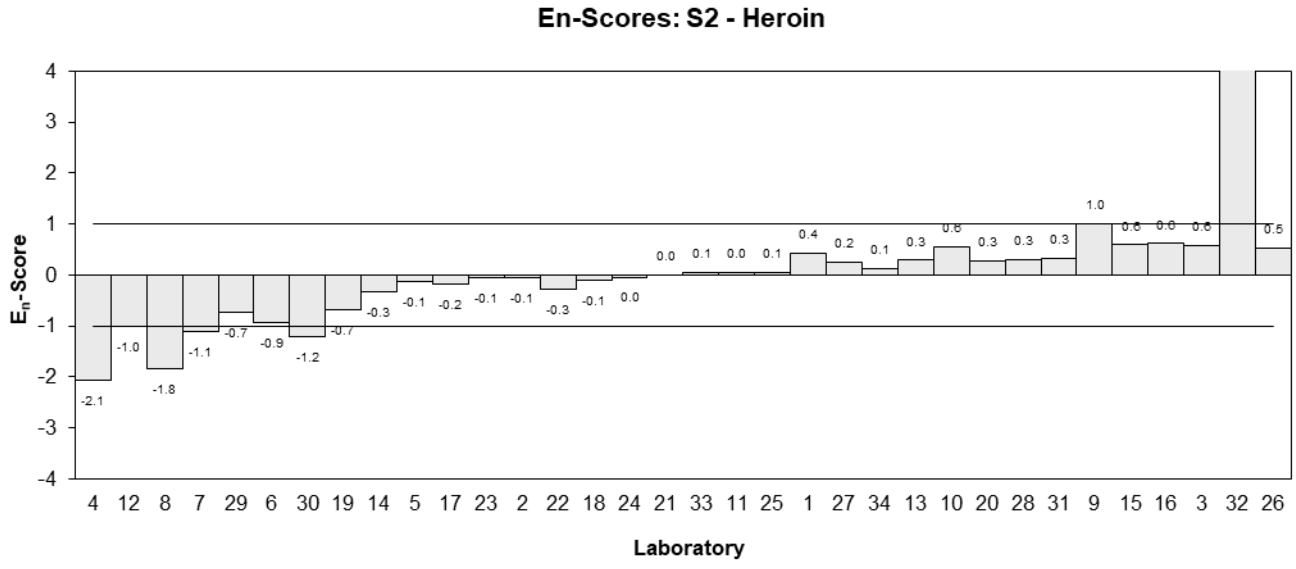

En-Scores: S2 - Heroin
En-Score
4 12 8 7 29 6 30 19 14 5 17 23 2 22 18 24 21 33 11 25 1 27 34 13 10 20 28 31 9 15 16 3 32 26
Laboratory

Figure 3

Table 7

Sample Details

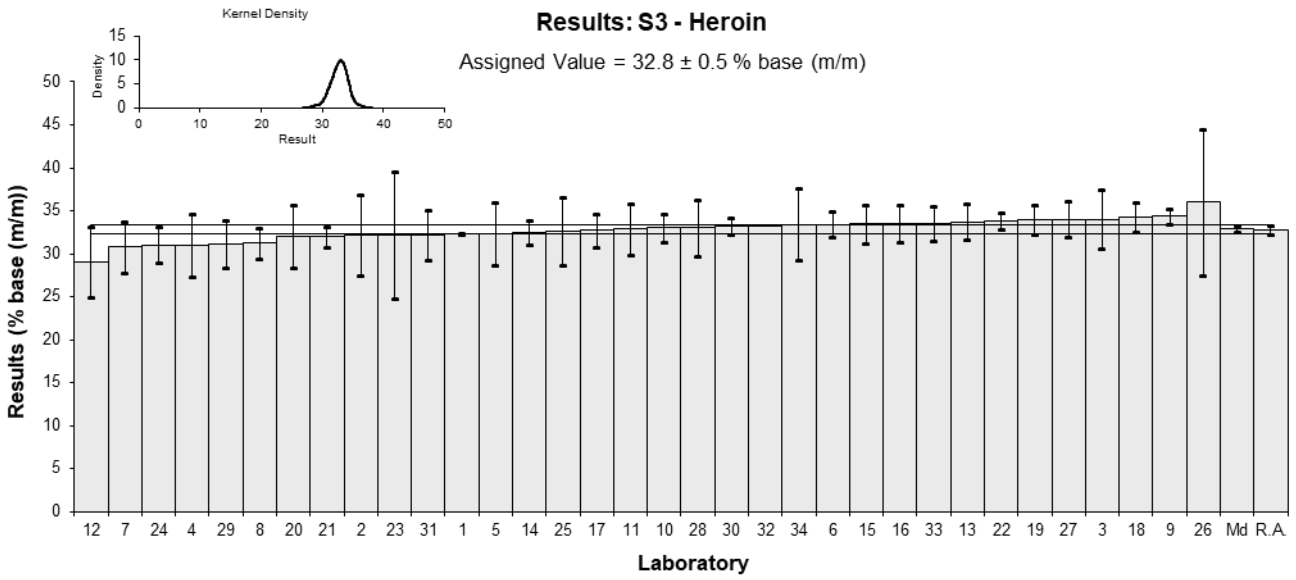
Sample No.	S3
Matrix	Powder
Analyte	Heroin
Units	% base (m/m)

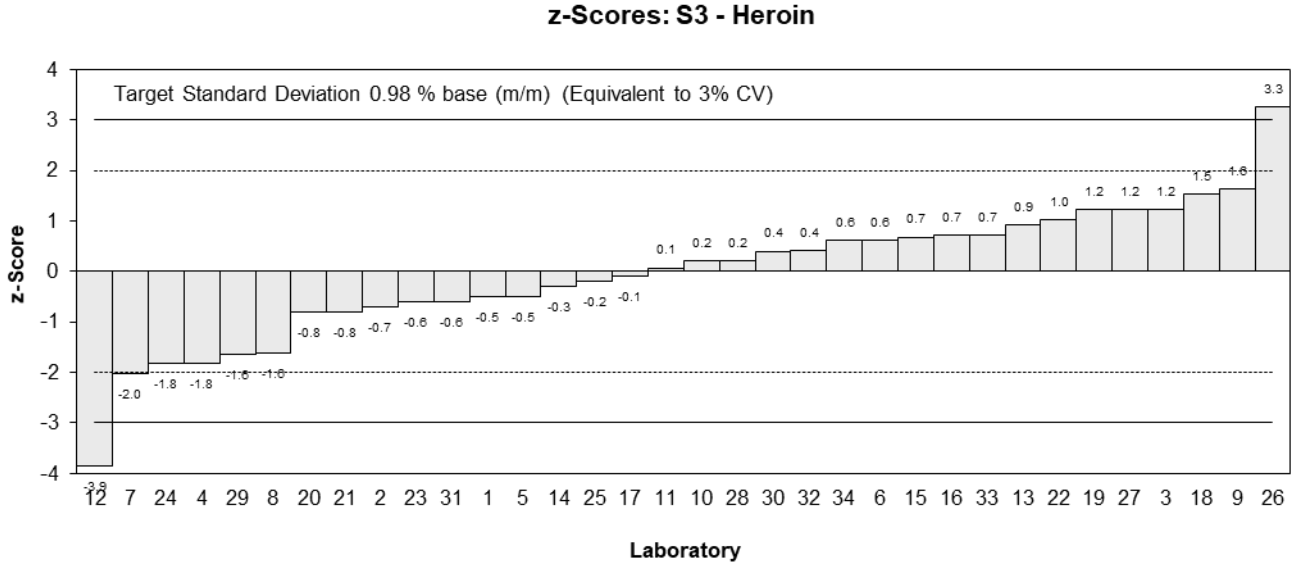
Participant Results

Lab. Code	Result	Uncertainty	z-Score	E_n-Score
1	32.3	0.1	-0.51	-0.98
2	32.1	4.7	-0.71	-0.15
3	34	3.4	1.22	0.35
4	31.0	3.7	-1.83	-0.48
5	32.3	3.6	-0.51	-0.14
6	33.4	1.5	0.61	0.38
7	30.8	3.0	-2.03	-0.66
8	31.2	1.80	-1.63	-0.86
9	34.4	0.9	1.63	1.55
10	33	1.7	0.20	0.11
11	32.86	2.96	0.06	0.02
12	29	4.1	-3.86	-0.92
13	33.7	2.1	0.91	0.42
14	32.5	1.4	-0.30	-0.20
15	33.46	2.22	0.67	0.29
16	33.5	2.2	0.71	0.31
17	32.7	2	-0.10	-0.05
18	34.3	1.7	1.52	0.85
19	34	1.7	1.22	0.68
20	32.0	3.6	-0.81	-0.22
21	32.0	1.2	-0.81	-0.62
22	33.8	1.0	1.02	0.89
23	32.2	7.4	-0.61	-0.08
24	31.0	2.1	-1.83	-0.83
25	32.6	3.9	-0.20	-0.05
26	36.0	8.5	3.25	0.38
27	34.0	2.1	1.22	0.56
28	33	3.3	0.20	0.06
29	31.19	2.76	-1.64	-0.57
30	33.19	1	0.40	0.35
31	32.2	2.9	-0.61	-0.20
32	33.2	NR	0.41	0.80
33	33.5	2.0	0.71	0.34
34	33.4	4.2	0.61	0.14

Statistics

Assigned Value	32.8	0.5
Robust Average	32.8	0.5
Median	32.9	0.4
Mean	32.8	
N	34	
Max.	36.0	
Min.	29	
Robust SD	1.2	
Robust CV	3.7%	


Kernel Density
Density
Result
Results: S3 - Heroin
Assigned Value = 32.8 ± 0.5 % base (m/m)
Results (% base (m/m))
12 7 24 4 29 8 20 21 2 23 31 1 5 14 25 17 11 10 28 30 32 34 6 15 16 33 13 22 19 27 3 18 9 26 Md R.A.
Laboratory


z-Scores: S3 - Heroin
Target Standard Deviation 0.98 % base (m/m) (Equivalent to 3% CV)
z-Score
12 7 24 4 29 8 20 21 2 23 31 1 5 14 25 17 11 10 28 30 32 34 6 15 16 33 13 22 19 27 3 18 9 26
Laboratory

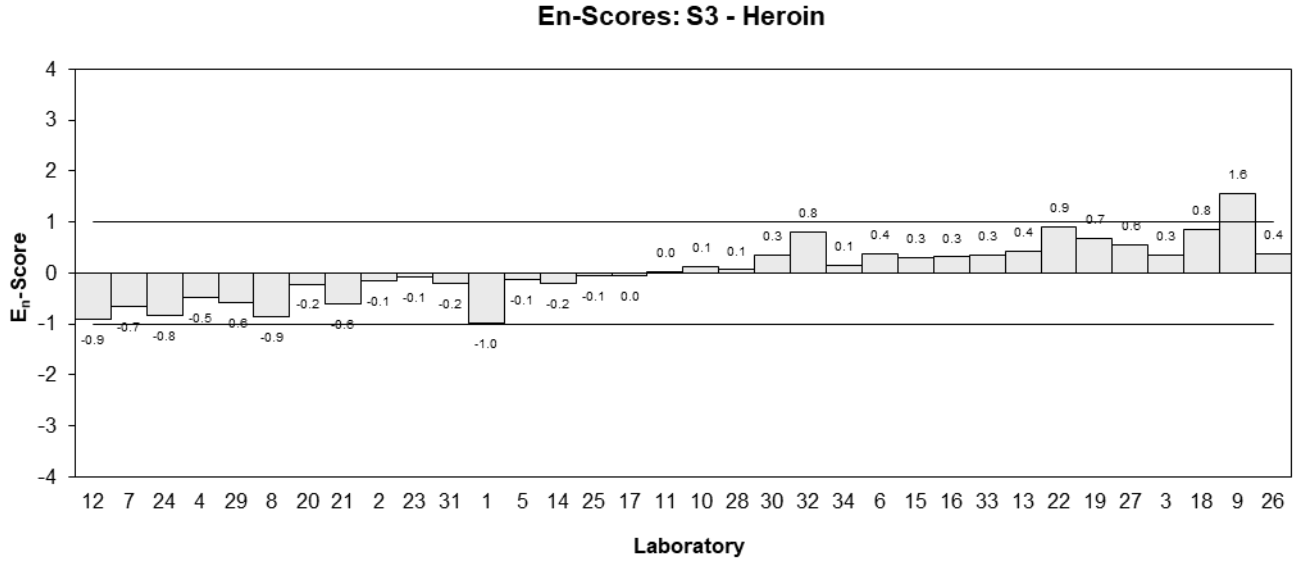

En-Scores: S3 - Heroin
En-Score
12 7 24 4 29 8 20 21 2 23 31 1 5 14 25 17 11 10 28 30 32 34 6 15 16 33 13 22 19 27 3 18 9 26
Laboratory

Figure 4

Table 8 Reported Cutting Agents*

Lab. Code	Cutting Agents		
	S1	S2	S3
Preparation	Phenacetin	Glucose	Sucrose
1	Not Detected	Not Detected	Not Detected
2	lactose	lactose	sugar
3	Phenacetin	Dextrose	Sucrose
4	Phenacetin		
5			
6	Phenacetin	NA	NA
7	Phenacetin		
8	Phenacetin		
9	Not detected	Not detected	Indications of sucrose
10	MAM, acetylcodeine, phenacetin	Acetylcodeine, (MAM)	Acetylcodeine, (MAM)
11	phenacetin, monoacetylmorphine, acetylcodeine	dextrose, monoacetylmorphine, acetylcodeine	sucrose, acetylcodeine
12	Phenacetin	none	none
13	Phenacetin	-	-
14	Phenacetin	Glucose	Sucrose
15	None identified	None identified	Sucrose
16	Phenacetin		
17	phenacetin		
18	Phenacetin	Glucose	Sucrose
19	phenacetin (not quantified)		
20	Not Detected	Not Detected	Not Detected
21	Phenacetin	Not Detected	Not Detected
22	phenacetin	none detected	sucrose
23	Not Detected	Not Detected	Not Detected
24	Phenacetin	Not Detected	Not Detected
25	Lactose	Lactose	Lactose
26	Phenacetin		
27	Phenacetin	-	-
28	Phenacetin	Dextrose	Sucrose
29	Phenacetin, Acetylcodeine	Acetylcodeine	Acetylcodeine
30	Phenacetin	None detected	Sucrose
31	Not Detected	Not Detected	Not Detected
32	Phenacetin : 5.9 %		
33	6-Monoacetylmorphine, Acetylcodeine, phenacetin	6-Monoacetylmorphine, Acetylcodeine	6-Monoacetylmorphine, Acetylcodeine
34	Phenacetin	Not Detected	Not Detected

* Responses may have been modified so that the participant cannot be identified.

6 DISCUSSION OF RESULTS

6.1 Assigned Value

The robust average of participants' results was used as the assigned value for each scored analyte. The robust averages and associated expanded uncertainties were calculated using the procedure described in ISO 13528:2015.[5] Results less than 50% and greater than 150% of the robust average were removed before the calculation of the assigned value, if applicable.[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 3). Three participants reported using the NATA GAG Estimating and Reporting MU as their guide; NATA no longer publishes this.[9]

It is a requirement of ISO/IEC 17025:2017 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 102 reported results, 99 (97%) were reported with an associated expanded MU. Laboratory **32** did not report any uncertainties; this participant reported that they were not accredited.

The magnitude of reported uncertainties was within the range 0.3% to 24% relative. Of 99 expanded MUs, 64 (65%) were between 3% and 10% relative to the result. Participants reporting MUs smaller than 3% or larger than 10% relative may need to reconsider whether these estimates are realistic or fit for purpose for the routine measurement of illicit drugs.

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. 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 69.89 ± 6.29%, the recommended format is 69.9 ± 6.3%.[8]

6.3 z-Score

Target SDs equivalent to 3% PCV was used to calculate z-scores. CVs predicted by the Thompson-Horwitz equation,[6] target SDs and between-laboratory CVs obtained in this study are presented for comparison in Table 9.

Table 9 Comparison of Thompson-Horwitz CVs, Target SDs and Between-Laboratory CVs

Sample	Analyte	Assigned value (% base (m/m))	Thompson-Horwitz CV (%)	Target SD (as PCV) (%)	Between-Laboratory CV (%)
S1	Heroin	70.2	1.2	3	1.9
S2	Heroin	32.0	1.8	3	4.4
S3	Heroin	32.8	1.7	3	3.7

Of 102 results for which z-scores were calculated, 88 (86%) returned a z-score with $|z| \leq 2.0$, indicating a satisfactory performance.

Twenty-six participants: **1**, **2**, **5**, **6**, **9**, **10**, **11**, **13**, **14**, **15**, **16**, **17**, **18**, **19**, **20**, **21**, **22**, **23**, **24**, **25**, **27**, **28**, **30**, **31**, **33** and **34** returned satisfactory z-scores for all three samples. Eight participants returned at least one questionable or unsatisfactory z-score.

Laboratory **12** returned unsatisfactory z-scores for all reported results, with all being lower than the assigned value (negative bias). Laboratory **26** returned unsatisfactory results for all reported results, with all being higher than the assigned value (positive bias). These participants may need to investigate the source of these biases.

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

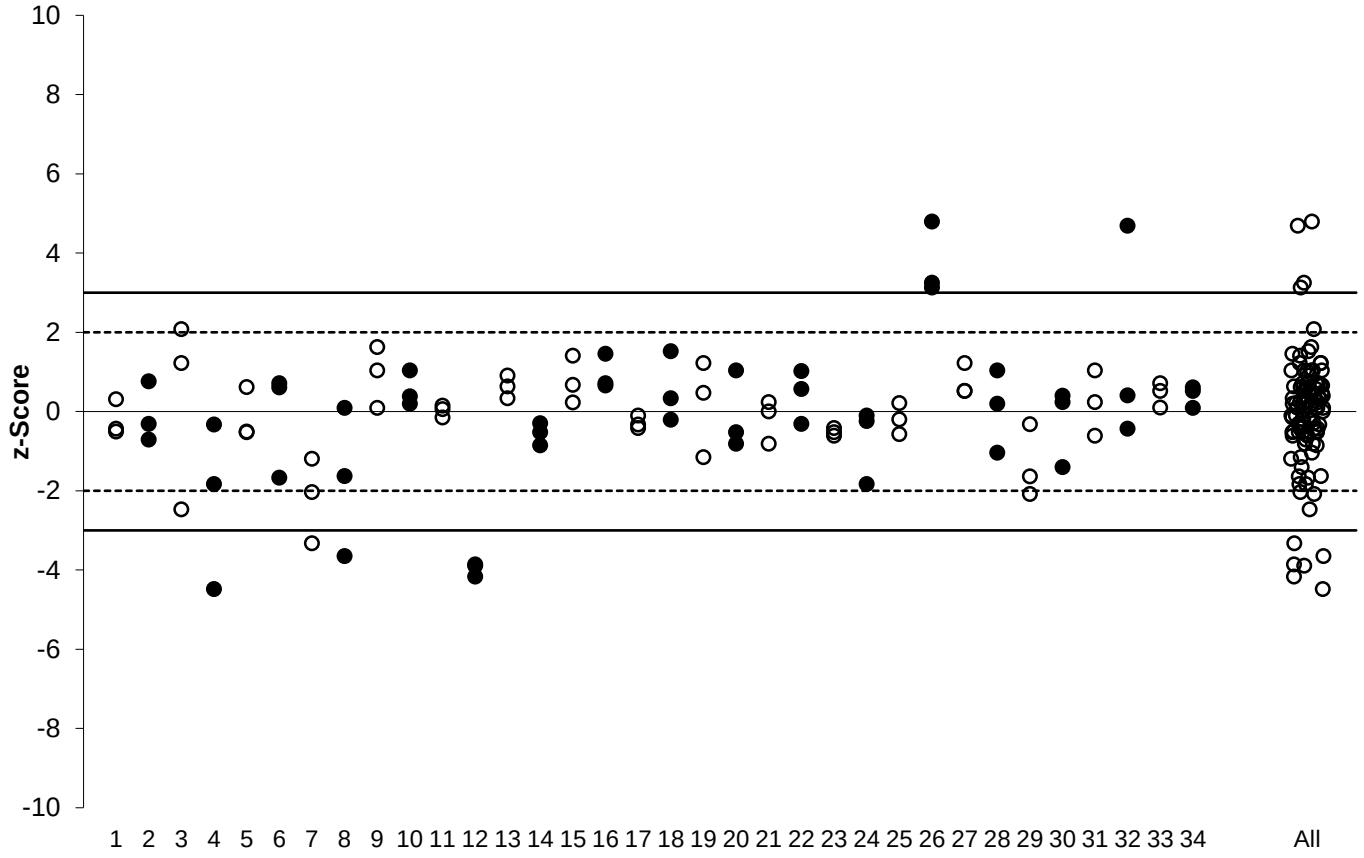


Figure 5 z-Score Dispersal by Laboratory

6.4 E_n-Score

E_n-scores should be interpreted in conjunction with z-scores; an unsatisfactory E_n-score can either be caused by an inappropriate measurement or uncertainty, or both. If a participant did not report an uncertainty with a result, an expanded uncertainty of zero (0) was used to calculate the E_n-score.

Of 102 results for which E_n-scores were calculated, 93 (91%) 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-six laboratories: **2**, **3**, **5**, **6**, **10**, **11**, **13**, **14**, **15**, **16**, **17**, **18**, **19**, **20**, **21**, **22**, **23**, **24**, **25**, **26**, **27**, **28**, **29**, **31**, **33** and **34** returned satisfactory E_n-scores for all three samples. Eight laboratories returned at least one unsatisfactory E_n-score.

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

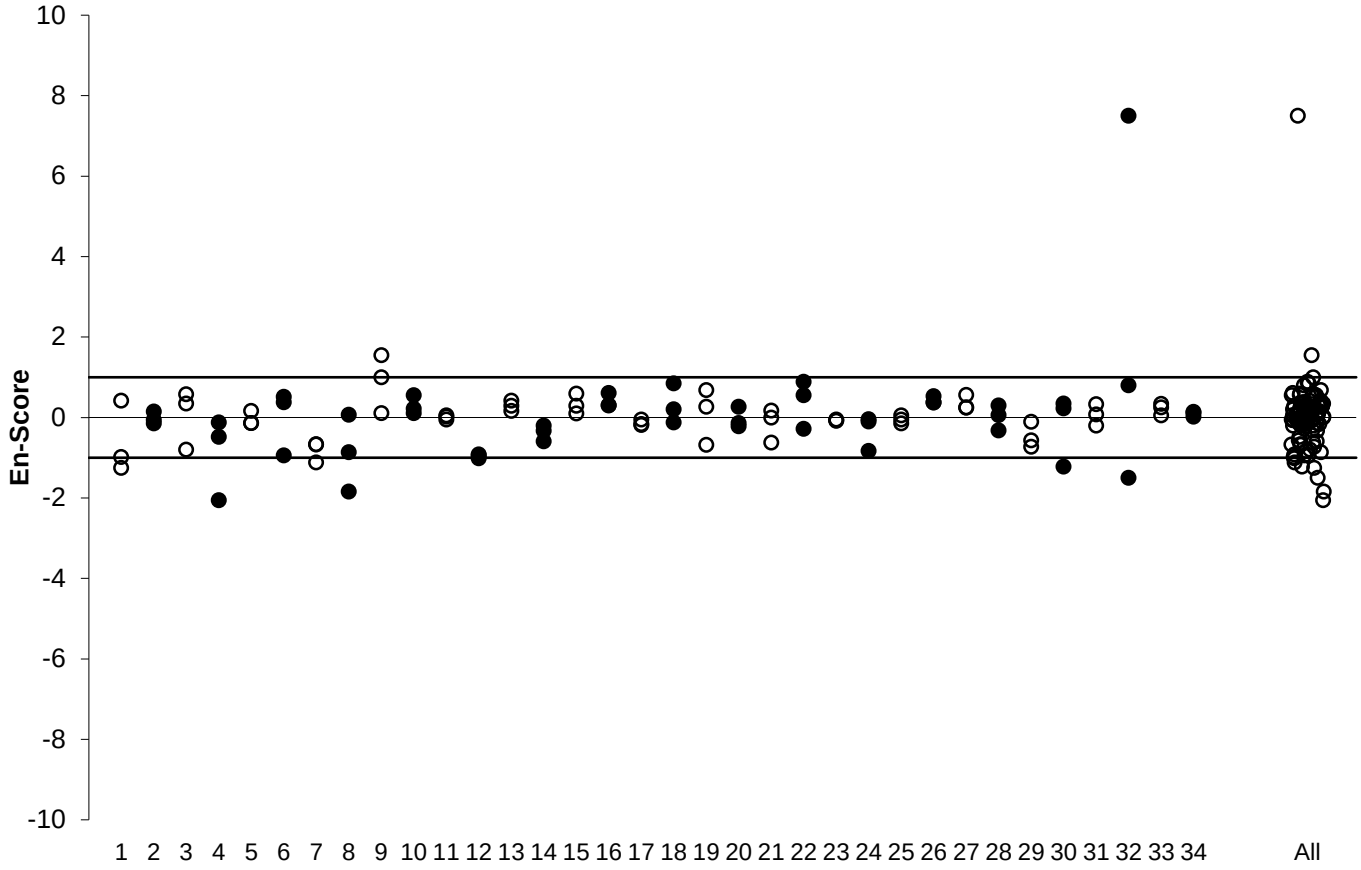


Figure 6 E_n-Score Dispersal by Laboratory

6.5 Identification of Cutting Agents

The test samples were prepared by adding phenacetin (Sample S1), glucose (Sample S2) and sucrose (Sample S3) to heroin hydrochloride.

Twenty-nine participants (85%) reported on the identity of at least one sample's cutting agent. Results reported by participants are presented in Table 8.

Laboratories **3**, **11**, **14**, **18** and **28** correctly reported all cutting agents used.

Twenty-five participants correctly reported phenacetin as the cutting agent in Sample S1. The rate of reporting for the sugar cutting agents in Samples S2 and S3 were much lower, with only five and nine participants correctly identifying glucose and sucrose respectively. Another participant reported 'sugar' in Sample S3; this participant may have been referring to sugar compounds generally and that they were not able to identify the exact cutting agent, or they may have been referring to table or regular sugar (i.e. sucrose, which is correct).

Laboratories **2** (Samples S1 and S2) and **25** (Samples S1, S2 and S3) reported lactose as a cutting agent, which was not added to any of the samples. Participants should take care to avoid any potential cross-contamination with other samples at their laboratory.

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 10.

Table 10 Summary of Participants' Analytical Methods

		Lab. Code
Accreditation	Yes to ISO/IEC 17025	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 27, 28, 29, 31, 33, 34
	Not accredited	26, 30, 32
Average Sample Mass Used per Analysis (mg)	< 10 mg	7, 26, 33
	11 – 30	1, 2, 3, 5, 6, 8, 10, 11, 13, 14, 15, 18, 19, 20, 21, 23, 24, 25, 27, 28, 29, 30, 31, 34
	31 – 50	4, 9, 12, 16, 17, 32
	> 101 mg	22
Conversion to base?	Yes	1, 2, 3, 4, 6, 7, 16, 17, 19, 20, 21, 22, 23, 24, 25, 26, 28, 31, 33, 34
	No	5, 8, 9, 10, 11, 12, 13, 14, 15, 18, 27, 29, 30, 32
Instrument Used for Quantification	HPLC-DAD	10, 16, 29
	HPLC-UV/Vis	5, 11, 30
	HPLC-MS	26
	UPLC-DAD	3, 4, 6, 8, 28, 32
	UPLC-UV/Vis	9
	UPLC-MS/MS	12
	LC-DAD	13, 27
	GC-FID	1, 2, 7, 14, 15, 17, 18, 19, 20, 21, 22, 23, 24, 25, 31, 33, 34
Solvent	Acetonitrile	3, 14, 28
	Acetonitrile/Other	4, 5, 8, 11, 12, 29, 30
	Chloroform	1, 17, 22, 23, 25, 31
	Chloroform/Methanol	2, 20, 21, 24, 34
	Ethanol(/Other)	7, 9, 18, 19, 32
	Methanol	6, 10, 13, 15, 16, 27, 33
	Ammonium formate buffer	26
Source of Calibration Standard	NMI	3, 4, 8, 9, 14, 17, 18, 28, 32
	Lipomed	5, 6, 11, 12, 13, 16, 27, 33
	LGC	10, 15, 19, 29
	Toronto Research Chemicals	1, 2, 20, 21, 23, 24, 25, 31, 34
	Other	7, 22, 26, 30

Plots of z-scores vs various methodology parameters are presented in Figures 7 to 11.

In this study, participants using methanol as their extraction solvent were generally biased slightly high across all samples (though all were still within satisfactory z-scores).

One participant reported using a calibration standard from a local pharmaceutical supplier, and their methodology was extraction with an ammonium formate buffer and analysis with HPLC-MS; all their reported results were significantly biased high with z-scores greater than 3.0 for all. Another participant reported using UPLC-MS/MS and all reported results were significantly biased low, with z-scores less than -3.0 for all. These participants may need to review if their methodology introduced bias to their measurements.

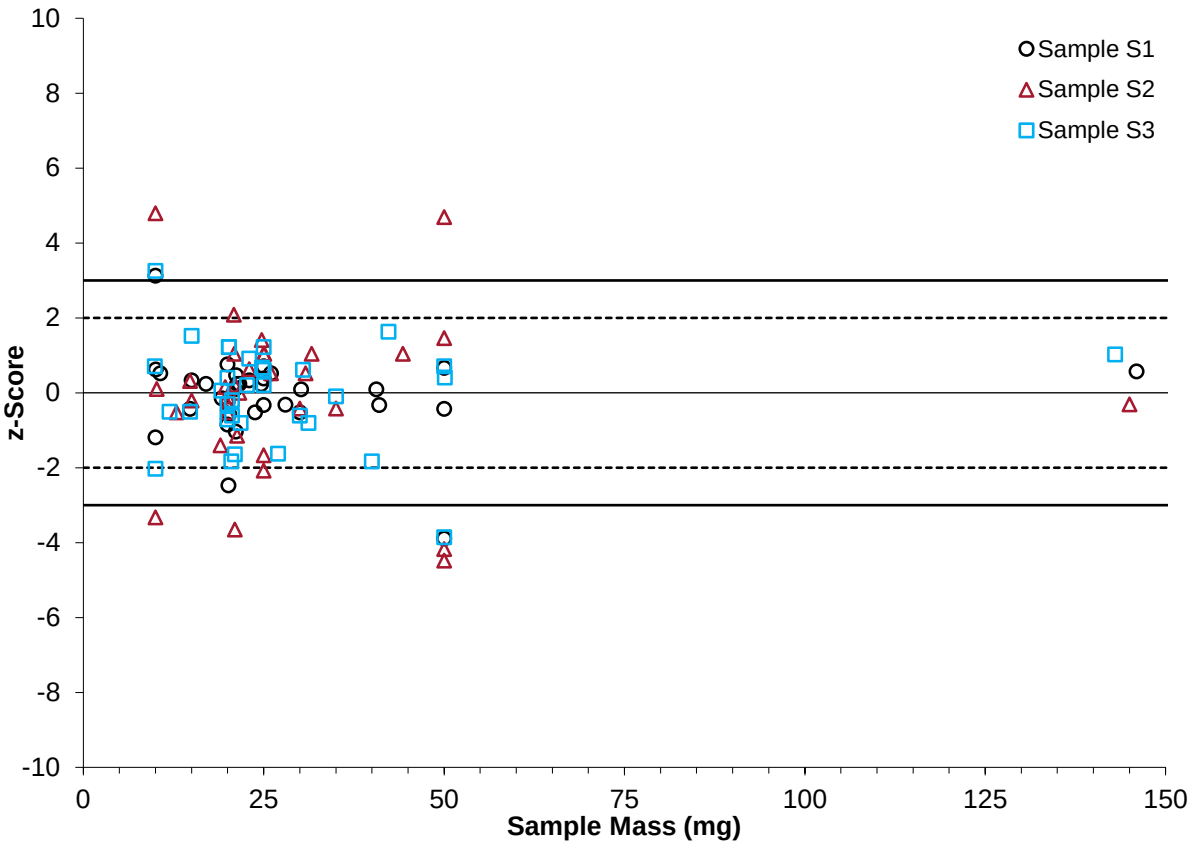


Figure 7 z-Score vs Sample Mass Used per Analysis

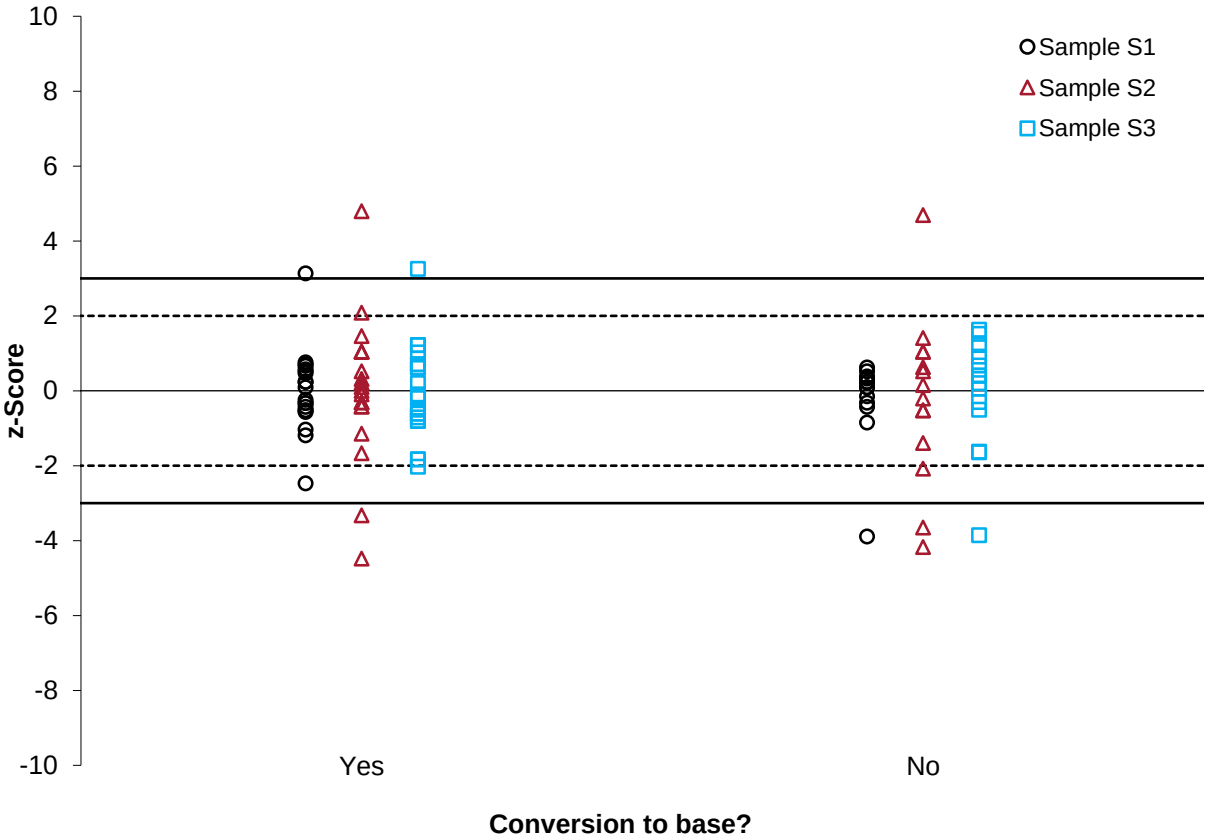


Figure 8 z-Score vs Sample Processing

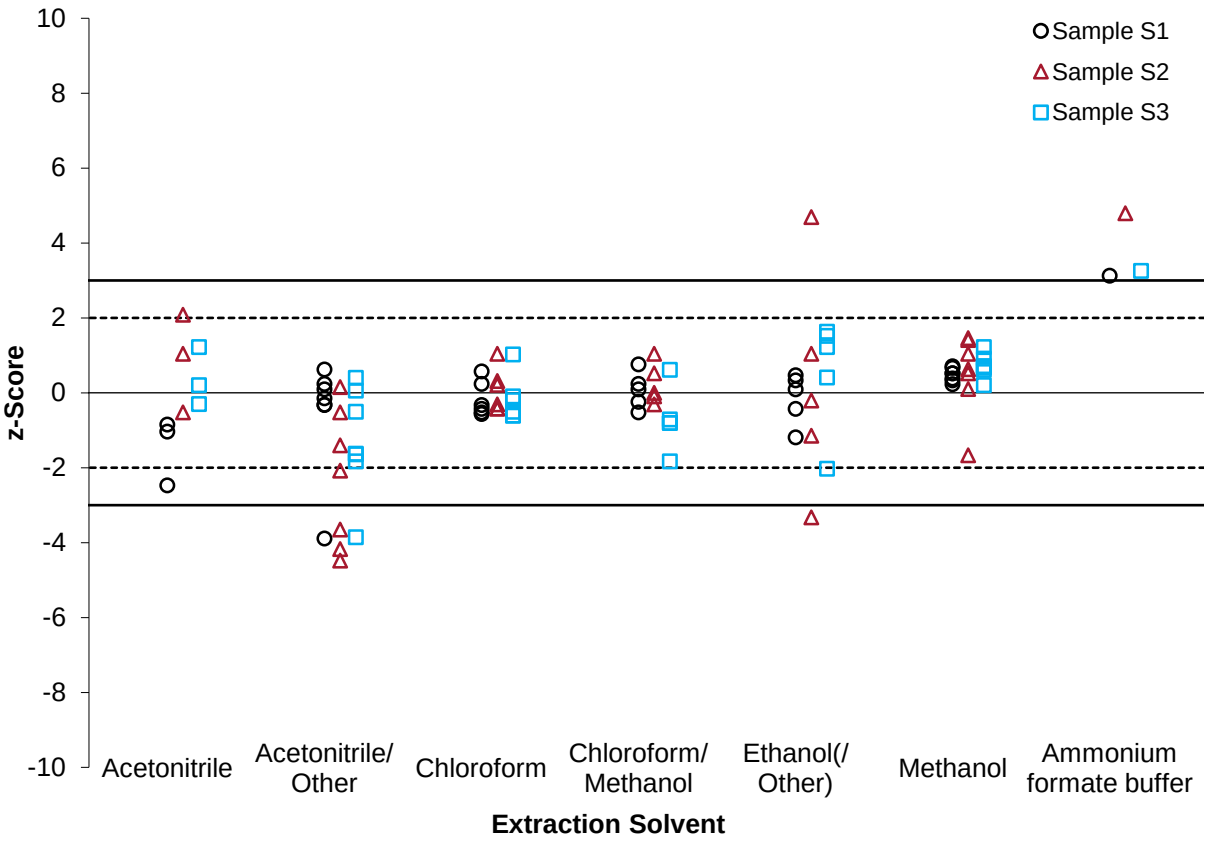


Figure 9 z-Score vs Extraction Solvent

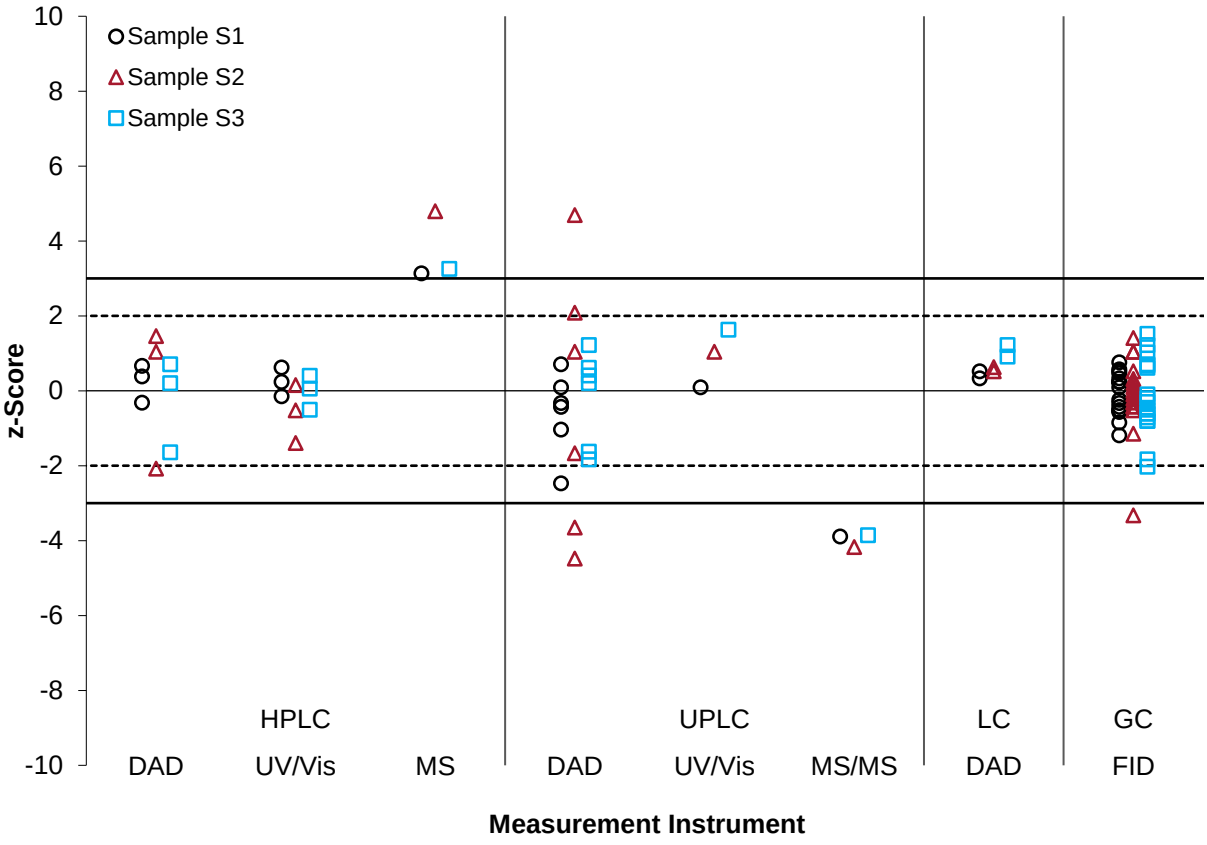


Figure 10 z-Score vs Measurement Instrument

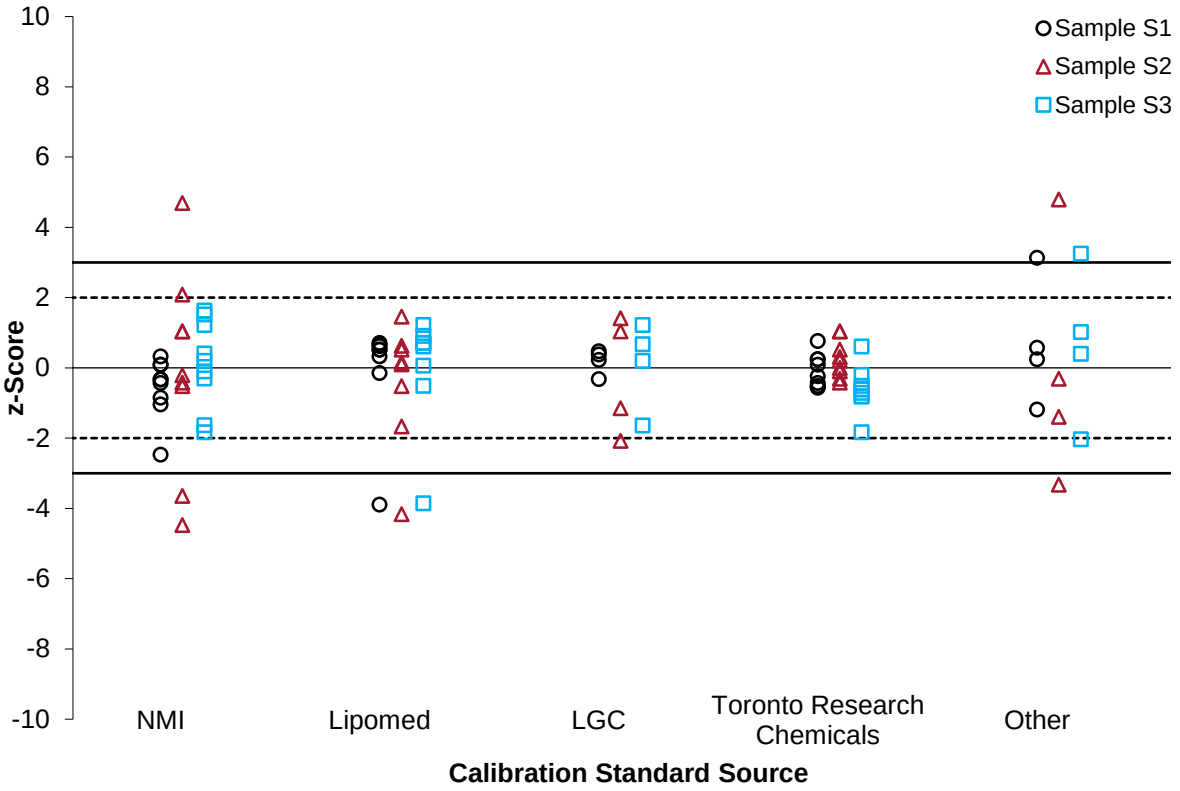


Figure 11 z-Score vs Calibration Standard Source

6.7 Comparison of Results and Date of Analysis

As there were delays with sample delivery to some participants, the test samples were analysed over the course of approximately 4 months. There was no evidence of sample degradation over this period, with no trend being found between when the samples were analysed and the results obtained by participants (Figure 12).

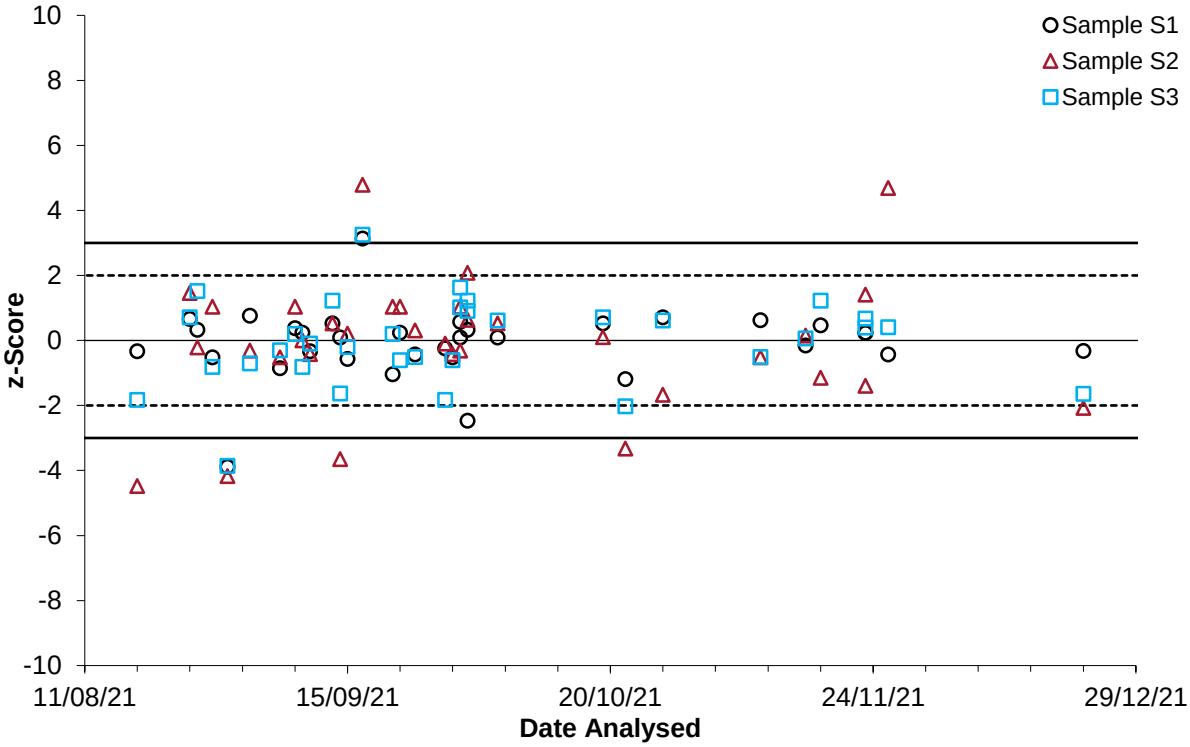


Figure 12 z-Score vs Sample Analysis Date

6.8 Matrix Effects

Samples S2 and S3 were prepared to contain the same proportion of heroin but with different cutting agents, so that potential effects of different sample matrices could be considered. Sample S2 was cut with glucose while Sample S3 was cut with sucrose. A summary of participants' Samples S2 and S3 results are presented in Figure 13.

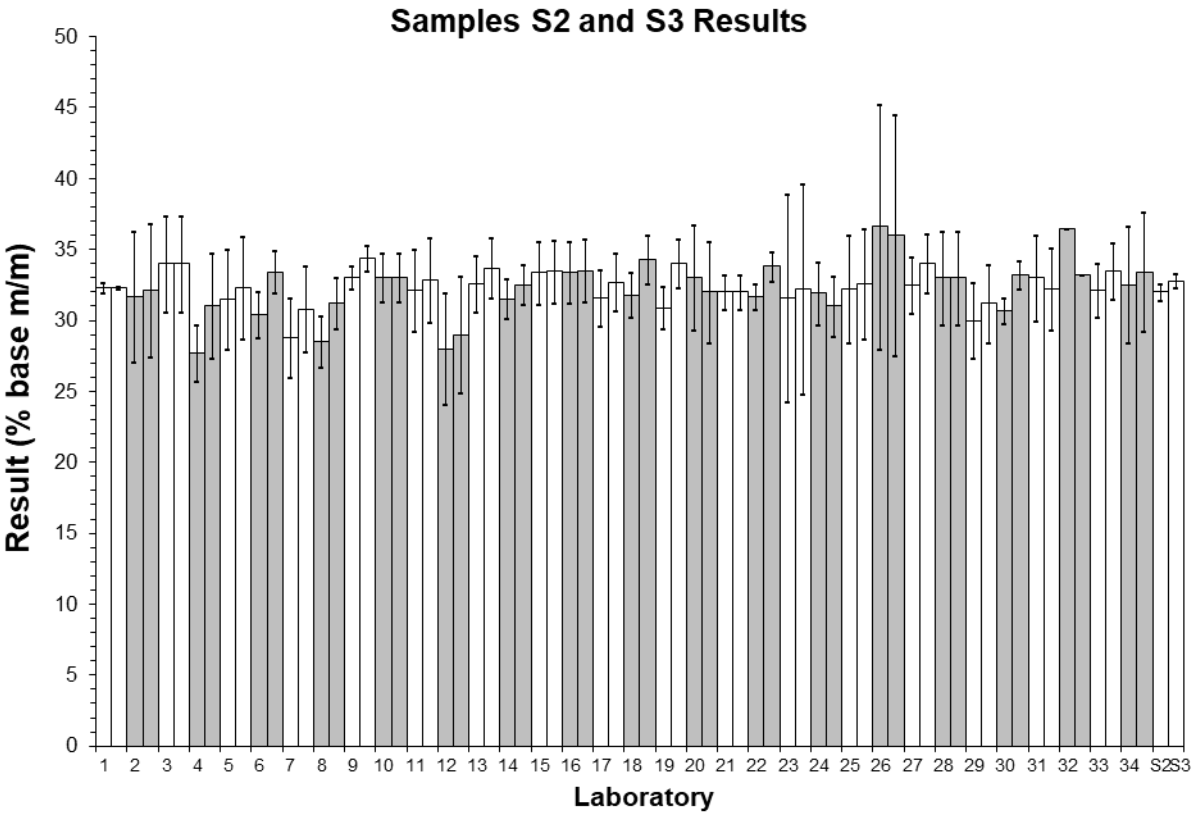


Figure 13 Summary of Samples S2 and S3 Results

The assigned values for Samples S2 and S3 were in agreement with each other within their respective uncertainties (32.0 ± 0.6 and 32.8 ± 0.5% base (m/m) respectively). Most participants also reported similar results for these samples, with all participants except Laboratories **22**, **30** and **32** reporting results that matched within their respective uncertainties.

It was observed in this study that the variability of participants' results for the glucose cut Sample S2 was greater than the sucrose cut Sample S3.

6.9 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 2012–2021 (last ten studies) is presented in Figure 14. The proportion of satisfactory z-scores and E_n-scores over this period on average is 81% and 80% respectively. While each PT study has a different group of participants, taken as a group, the performance over this period has improved.

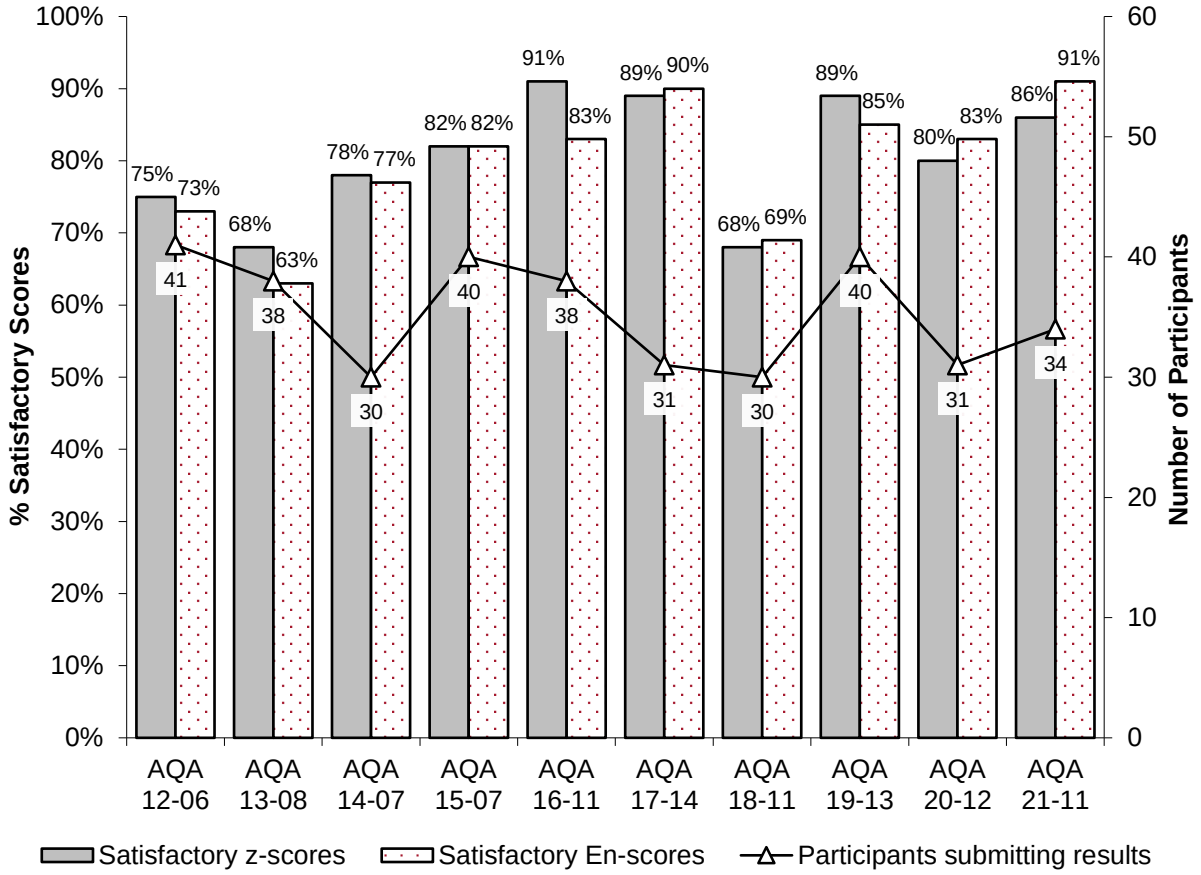


Figure 14 Summary of Participants' Performance in Heroin PT Studies

A number of participants have consistently participated in NMI heroin PT studies, and 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.

A summary of individual laboratory's performances over the last ten studies is presented in Figures 15 and 16 for Australian and international laboratories respectively. Two Australian and three international laboratories have achieved satisfactory z-scores across all samples in all heroin PT studies participated in over this period.

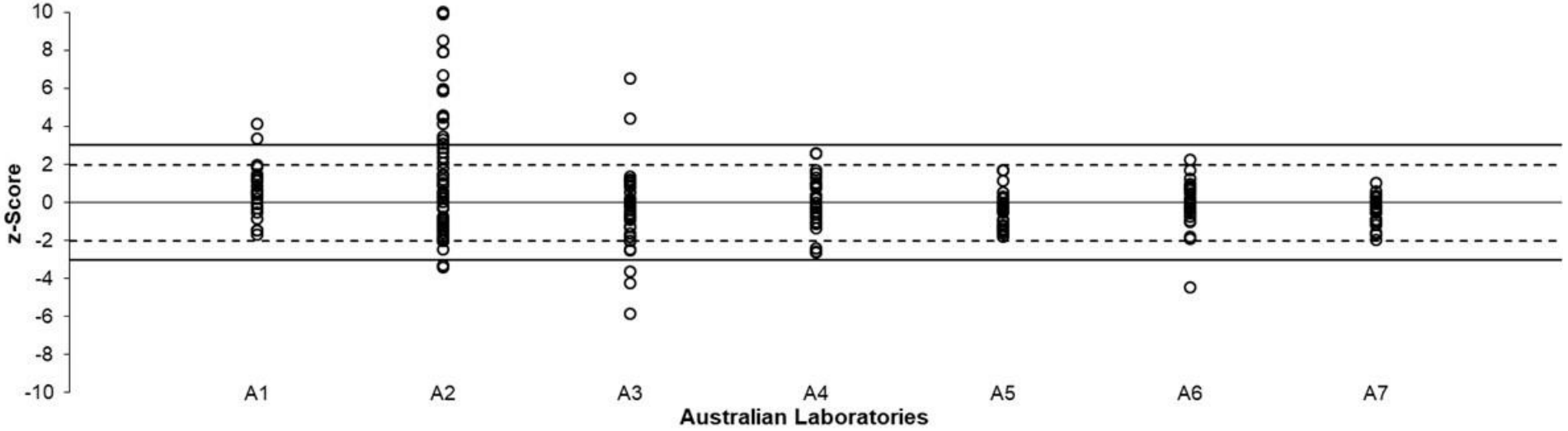


Figure 15 Summary of Australian Participants' z-Scores in Heroin PT Studies

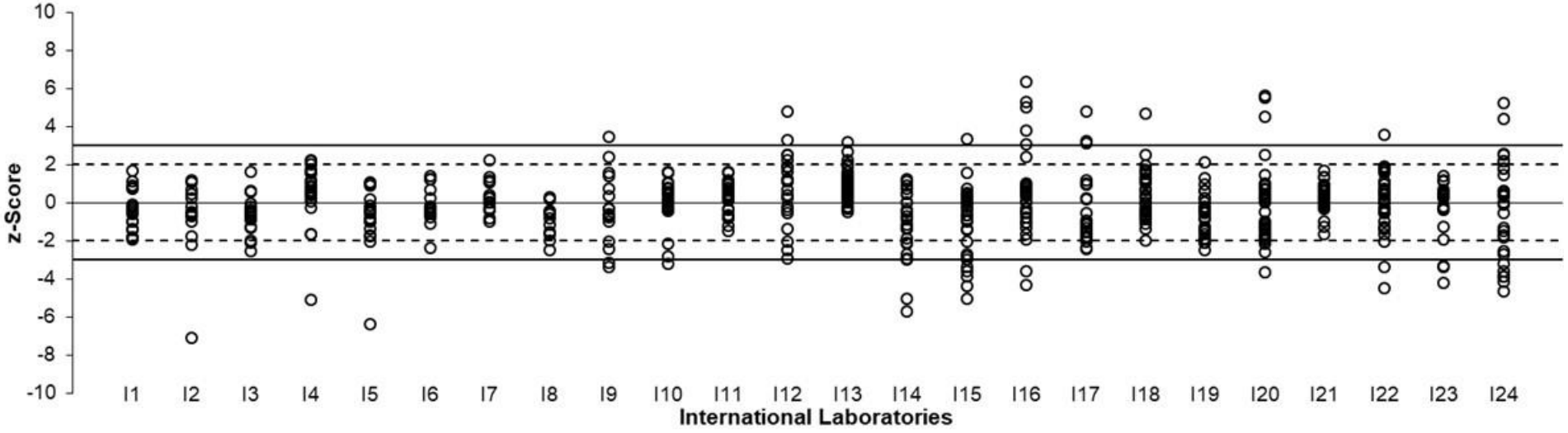


Figure 16 Summary of International Participants' z-Scores in Heroin PT Studies

A comparison of all results from Australian and international laboratories in heroin PT studies over the last ten studies is presented in Figure 17. Overall, both groups have performed very similarly, with both achieving 81% satisfactory z-scores over this period.

Figure 17 Comparison of Australian and International Laboratories in Heroin PT Studies

7 REFERENCES

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

[2] NMI, 2021, *Study Protocol for Proficiency Testing*, viewed January 2022, <https://www.industry.gov.au/sites/default/files/2020-10/cpt_study_protocol.pdf>.

[3] NMI, 2021, *Chemical Proficiency Testing Statistical Manual*, viewed January 2022, <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 CG 4, QUAM:2012.P1, *Quantifying Uncertainty in Analytical Measurement*, 3rd ed., viewed January 2022, <http://www.eurachem.org/images/stories/Guides/pdf/QUAM2012_P1.pdf>.

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

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

A1.1 Robust Average and Associated Uncertainty

Robsut 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 ($U_{rob\ av}$) 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 11.

Table 11 Uncertainty Estimate for Robust Average of Sample S1

Number of Results (p)	34
Robust Average	70.2% base (m/m)
$S_{rob\ average}$	1.4% base (m/m)
$u_{rob\ average}$	0.3% base (m/m)
k	2
$U_{rob\ average}$	0.6 % base (m/m)

Therefore, the robust average for Sample S1 is 70.2 ± 0.6% base (m/m).

A1.2 z-Score and E_n-Score Calculations

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

A worked example is set out below in Table 12.

Table 12 z-Score 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
69.3 ± 0.4	70.2 ± 0.6	3% as PCV, or: 0.03 × 70.2 = 2.1% base (m/m)	z-Score = $\frac{69.3-70.2}{2.1}$ = -0.43	E_n-Score = $\frac{69.3-70.2}{\sqrt{0.4^2+0.6^2}}$ = -1.25

APPENDIX 2 – 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
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
Md	Median
Min.	Minimum
MS	Mass Spectrometry
MS/MS	Tandem Mass Spectrometry
MU	Measurement Uncertainty
N	Number of numeric results
NATA	National Association of Testing Authorities, Australia
NMI	National Measurement Institute, Australia
NR	Not Reported
PCV	Performance Coefficient of Variation
PDA	Photodiode array
PT	Proficiency Test
R.A.	Robust Average
RM	Reference Material
SD	Standard Deviation
SI	International System of Units
SS	Spiked Samples
UPLC	Ultra Performance Liquid Chromatography
UV/Vis	Ultraviolet/Visible

END OF REPORT