QUALITY ASSURANCE IN LABORATORY TESTING FOR IEM

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ERNDIM Quantitative Amino Acids Method Survey, January 2018. Dr Rachel Carling and Professor Brian Fowler, Scientific Advisors.

A short questionnaire was distributed to all Erndim Quantitative Amino Acid (QAA) scheme participants in December 2017. The aim of the questionnaire was to establish an overview of the different methods in use to better understand variation in analytical performance and enable a review of the current method groups used in data analysis. A summary of the questionnaire responses is provided here and we would like to thank all those who participated.

A total of n=265 laboratories responded to the survey and provided information on their methodology which is summarised in Table 1.

Table 1: Summary of methodologies in use

Methodology	Number of labs (% of total)
Ion Exchange Chromatography (IEC)	143 (54.0)
Liquid Chromatography Tandem Mass Spectrometry (LCMS/MS)	62 (23.4)
Reverse Phase Liquid Chromatography (RPLC)*	37 (14.0)
Liquid Chromatography Mass Spectrometry (LCMS)	15 (5.7)
Other	8 (3.0)

* Reverse Phase Liquid Chromatography (RPLC) includes both High Performance Liquid Chromatography (HPLC) and Ultra Performance Liquid Chromatography (UPLC) with non-MS detection.

8 labs used methods not individually listed in the above table. These were reported as follows:

Gas Chromatography Mass Spectrometry (GCMS) (n=3), Gas Chromatography Flame Ionisation Detector (GCFID) (n=1), Gas Chromatography Tandem Mass Spectrometry (GCMSMS) (n=1), FIAMSMS (n=1), Ion Exchange Chromatography Fluorescence detection (n=1) and Nuclear Magnetic Resonance (n=1).

1. Ion Exchange Chromatography

n=143 participants reported using IEC and were asked further questions about choice of internal standard, type and frequency of calibration.

138/143 (96.5%) laboratories responded when asked about their choice of internal standard:

15/138 (10.9%) labs reported using no internal standard.

109/138 (79.0%) labs reported using a single internal standard.

14/138 (10.1%) labs reported using two internal standards.

Two internal standards

Of the labs who reported using two internal standards, 11/14 listed the internal standards used. The most popular combination was 2-amino-ethyl-L-cysteine and D-Glucosaminic acid. 1/14 reported using three internal standards.

Table 2: Choice of internal standards for two internal standard IEC methods

Internal standard	Number of labs
Norleucine	2
2-amino-ethyl-L-cysteine	9
D-Glucosaminic acid	6
Acetyl-lysine	1
Norvaline	2
Homocysteic acid	1
Homoserine	1
Di-amino butyric acid	1

One internal standard

Of the labs who reported using a single internal standard, 93/109 (85.3%) listed the internal standard used. The most frequently used internal standard was norleucine, followed by 2-amino-ethyl-L-cysteine.

Table 3: Choice of internal standard for single internal standard IEC methods

Internal standard	Number of labs
Norleucine	44
2-amino-ethyl-L-cysteine	36
Di-amino butyric acid	6
Glucosaminic acid	2
4-Chlorophenyl-alanine	2
Methioninesulfone	1
Vigabatrin	1
Phosphoethanolamine	1

Calibration

130/138 (94.2%) participants reported using an aqueous calibrator. Only 8/138 (5.6%) used a spiked plasma calibrator. 6/138 did not respond to this question.

114/138 (81.9%) participants responded to detailed questions about their calibration process.

101/138 participants use a single point calibration standard

5/101 use single point calibration at 100uM

3/101 use single point calibration at 200uM

4/101 use single point calibration at 250uM

7/101 use single point calibration at 500uM

82/101 did not report the calibrator concentration

7/138 participants use a two point calibration curve

1/138 participants use a four point calibration curve

4/138 participants use a five point calibration curve

1/138 participants use a nine point calibration curve

101/143 (78.6%) laboratories reported that the source of amino acids used in their calibration standards was Sigma. 7/101 (6.9%) specifically stated they used Sigma TraceCert as the source of the calibration material.

When asked to state the frequency with which they calibrated, 106/138 participants responded as summarised in table 4 below:

Table 4: Frequency of Calibration for IEC methods

Frequency of calibration	Number of laboratories
Daily/with each batch	30
Twice weekly	7
Weekly	17
Monthly	12
6 monthly	6
With change of ninhydrin	19
As necessary	15

2. Liquid Chromatography Tandem Mass Spectrometry

n=62 participants reported using LCMS/MS and were asked further questions about choice of internal standard, type and frequency of calibration.

29/62 LCMS/MS users perform analysis with in-house methods, 22/62 users perform analysis with a kit method and 11/62 did not respond to this question. The majority of kits in use are derivatised (18/22).

Table 5a: Derivatised vs non derivatised for kit LCMS/MS methods

Kit methods	Method	Number of labs
Waters AccQ-Tag	Derivatised	6
Sciex aTRAQ	Derivatised	9
Waters MassTrak	Derivatised	1
Zivak technologies	Derivatised	1
Not specified	Derivatised	1
Jasem	Underivatised	3
SpotOn	Underivatised	1

Table 5b: Derivatised vs non derivatised for in-house LCMS/MS methods

In house methods	Number of labs
Derivatised	11
Underivatised	16
Not specified	2

Internal standards

There is a variety of stable isotope internal standards in use for the LCMS/MS methods. 20/62 labs stated they were using stable isotope internal standards, 5/62 stated stable isotope internal standards and specified these were either 13C or 15N labels, 9/62 labs are using internal standards from a kit and 7/62 are using isotopes from Cambridge Isotopes Laboratory (CIL). Only 3/62 labs reported using a single analogue internal standard.

Calibration

33/62 participants reported using an aqueous calibrator. 10/62 used a spiked plasma calibrator and 18/62 did not respond to this question.

33/62 laboratories reported that the source of amino acids used in their calibration standards was Sigma. 1/62 specifically stated they used Sigma TraceCert as the source of the calibration material. 4/62 sourced calibration standards from Recipe ClinChek, 14/62 did not answer this question. 5/62 used alternative sources (Wako, Jeol, Fluka and Jasem).

46/62 participants responded to detailed questions about their calibration process.

Table 6: Number of calibration standards used by LCMS/MS method group

Number of calibration standards	Number of labs
Isotope dilution	1
One	8
Two	6
Three	3
Four	2
Five	10
Six	8
Seven	5
Eight	1
Nine	2
Not specified	16

When asked to state the frequency with which they calibrated, 40/62 participants responded as summarised in the table below:

Table 7: Frequency of Calibration for LCMS/MS methods

Frequency of calibration	Number of laboratories
Daily/with each batch	30
Twice weekly	1
Weekly	4
Fortnightly	2
Monthly	1
As necessary	2

3. Other Methods

n=37 participants reported using RPLC with non MS detection. 8/37 participants used high performance liquid chromatography (HPLC), 13/37 participants used ultra high performance liquid chromatography (UPLC) and 16/37 did not specify. Given that the particular survey question was specific to HPLC, it is probable that 24/37 participants are using HPLC. A limitation of this survey was the failure to request more detail about the methods of detection with the RPLC group.

n=15 participants reported using LCMS. 4/15 participants stated they were using Waters AccQ-tag method and 11/15 did not provide additional details. 8/15 participants were derivatising samples, 5/15 were not derivatising and 2/15 did not state.

4. Summary of Survey Findings

Ion exchange chromatography (IEC) remains the most commonly used method, with 55% (n=143) of laboratories utilising this technology. However, there has

been a change in recent years with an increasing number of participants moving to LCMS/MS; in 2007, 86% (147/178) of participating laboratories utilised IEC and only 3% (5/178) used LCMS/MS. By 2017 the number of laboratories using LCMS/MS had increased to 23% (62/265) whereas those using IEC had decreased to 54% (143/265).

Generally speaking, participants using LCMS/MS calibrate more frequently than those using IEC. 75% (30/40) of LCMS/MS users calibrate daily/with each batch of samples compared with only 28% (30/106) of IEC users. Likewise, single point calibration curves are used by 73% of IEC users (101/138) but only by 12% (8/63) of LCMS/MS users. The concentration at which a single point calibration is performed varies from anywhere between 100uM to 500uM for IEC, and up to 1500uM for LCMS/MS. It should be noted that only a small number of laboratories chose to provide calibrator concentrations.

Given the long analysis time for IEC, typically around 2 hours per sample, these differences are not necessarily unexpected. However, in view of the increasingly stringent performance criteria that laboratories must adhere too for accreditation to ISO 15189 and the transition in recent decades from IEC being considered a highly specialised piece of equipment to a routine analytical technique, it may be timely for laboratories to review their existing calibration procedures.

More than half of laboratories source their calibration material from Sigma (n=146 participants, 55.1%) although only a small number of laboratories appear to be using Sigma TraceCert, an aqueous certified reference material (CRM) containing 17 different amino acids. The majority of laboratories are preparing in house calibration standards from the Sigma material and this, combined with the absence of a CRM for a number of amino acids, means that absolute accuracy is an issue. The interlaboratory variation seen in the Erndim scheme in 2017 is most likely a direct reflection of this. The Horwitz equation (ref 1) can be used to predict interlaboratory variation on the basis of analyte concentration alone; it is independent of method, matrix and analyte. For most amino acids in the concentration range 10–500uM, this would equate to a target interlaboratory variation of approximately 10%. The inter laboratory variation (all method groups) seen for phenylalanine in 2017 is summarised below and it is evident that laboratories are struggling to achieve this with CVs significantly in excess of 10% at concentrations < 100μM. The magnitude of the variation seen at these concentrations will be influenced by several factors. In principle, instrument sensitivity should be adequate and so we hypothesise that use of single point calibrations at values well removed from 100μM and failure to include a zero calibrator are likely contributing factors. The latter will result in an overestimate at concentrations higher than the calibration point and an underestimate at concentrations below.

Distribution	%CV (mean)*	Spiked value (µM)
2017.01	11.3% (912)	1000
2017.02	20.8% (103)	100
2017.03	11.4% (372)	400
2017.04	73.5% (29)	20
2017.05	87.6% (29)	20
2017.06	8.8% (373)	400
2017.07	10.5% (916)	1000
2017.08	9.9% (102)	100

* *No outliers have been excluded which is why figures may differ from those in the annual report.*

Prior to the start of the 2018 scheme, the method groups which participating laboratories could select were updated to include the following:

- Ion exchange no internal standard
- Ion exchange 1 internal standard
- Ion exchange 2 internal standards
- Liquid Chromatography Tandem Mass Spectrometry
- Reverse Phase Liquid Chromatography (HPLC and UPLC with non MS detection)
- Liquid Chromatography Mass Spectrometry
- Other (to include GC, GCMS, GCMS, FIAMSMS etc)

5. Review of 2017 Returns by Method Group

A statistical analysis of the 2017 returns was undertaken to determine whether there were significant differences in accuracy and precision between the method groups.

Data was analysed using GraphPad Prism7. Outliers were removed using the ROUT method prior to calculating the following parameters on the cleaned data set:

Mean of method group

SD for method group

SEM for method group

% CV for method group

One sample T test (against theoretical mean)

% Bias (against theoretical mean*)

*NB. The theoretical mean was taken to be the spiked value. It should be noted that this is an inherent limitation of the data analysis because there is no absolute point of reference for the spike in terms of accuracy.

For the one sample T Test a p value <0.05 was taken as significant.

ANOVA and Bartletts test were then used to determine whether there were significant differences between the method group means and SDs respectively. A significant p value for ANOVA indicates there is a statistically significant difference in the means of the method groups e.g bias. A significant p value for Bartletts test indicates there is a significant difference in the SD of the 6 method groups e.g. precision.

Summary by analyte (only couple of examples shown here).

See Appendix for summary of data.

Taurine: 7/8 samples showed significant differences in the method group SD; IEC 2 IS was the least precise followed by LCMSMS. 6/8 samples showed a significant difference in the mean values of the method groups with LCMSMS having a negative bias relative to the others (average bias =-4%).

Arginine: 8/8 samples showed significant differences in the method group SD; Other and LCMSMS were the least precise. 4/8 samples showed a significant difference in the mean values of the method groups with LCMSMS having a negative bias relative to the others.

Citrulline: 7/8 samples showed significant differences in the method group SD; Other and LCMSMS were the least precise. IEC was the most imprecise in the low concentration pair, conversely LCMSMS was more imprecise at the higher concentrations with the method groups behaving similarly at the 200/700uM concentration. This is likely a reflection of infrequent single point calibration (IEC) vs daily multi point calibration (LCMSMS).

5/8 samples showed a significant difference in the mean values of the method groups with LCMSMS having a possible negative bias relative to the others, more evident as higher concentrations.

References

Horwitz W, Albert R. The Horwitz ratio (HorRat): A useful index of method performance with respect to precision. J AOAC Int. 2006 Jul-Aug;89(4):1095-109.

Data Appendix.

TAURINE SAMPLE PAIRS 2018.

2017.01	IEC 0 IS	IEC 1 IS	IEC 2 IS	Tandem MS	RP HPLC	Other
Number of values	26	120	13	30	30	13
Mean	103.8	100.2	107.4	95.5	100.8	104.3
Std. Deviation	11.39	9.54	12.88	11.45	10.10	10.13
Std. Error of Mean	2.23	0.87	3.57	2.09	1.84	2.81
Coefficient of variation	10.97%	9.52%	11.99%	11.99%	10.02%	9.71%
P value (two tailed)	0.1036	0.8198	0.0611	0.0406	0.6499	0.1527
Significant (alpha=0.05)?	No	No	No	Yes	No	No
Discrepancy	3.77	0.20	7.38	-4.48	0.85	4.29
% Bias against spike	3.77	0.20	7.38	-4.48	0.85	4.29
Bartletts Test						
P value	0.5579					
P value summary	ns					
Are SDs significantly different (P < 0.05)?	No					
ANOVA						
P value	0.0051					
P value summary	**					
Significant diff. among means (P < 0.05)?	Yes					
2017.07	IEC 0 IS	IEC 1 IS	IEC 2 IS	Tandem MS	RP HPLC	Other
Number of values	27	128	13	30	30	14
Mean	101.8	101.1	105.7	98.35	101.4	99.05
Std. Deviation	9.0	8.0	11.0	11.4	7.8	6.7
Std. Error of Mean	1.736	0.7087	3.044	2.083	1.423	1.798
Coefficient of variation	8.86%	7.93%	10.39%	11.60%	7.72%	6.73%
P value (two tailed)	0.3095	0.1264	0.0876	0.4343	0.3295	0.6075
Significant (alpha=0.05)?	No	No	No	No	No	No
Discrepancy	1.8	1.09	5.662	-1.651	1.417	-0.9464
% Bias against spike	1.8	1.1	5.7	-1.7	1.4	-0.9
Bartletts Test						
P value	0.0693					
P value summary	ns					
Are SDs significantly different (P < 0.05)?	No					
ANOVA						
P value	0.1888					
P value summary	ns					
Significant diff. among means (P < 0.05)?	No					

2017.02	IEC 0 IS	IEC 1 IS	IEC 2 IS	Tandem MS	RP HPLC	Other
Number of values	26	114	12	30	29	12
Mean	302.9	294.3	321.2	283.2	292.8	289.2
Std. Deviation	29.34	18.75	40.48	35.64	23.75	38.01
Std. Error of Mean	5.75	1.76	11.68	6.51	4.41	10.97
Coefficient of variation	9.68%	6.37%	12.60%	12.58%	8.11%	13.14%
P value (two tailed)	0.5002	0.009	0.0834	0.022	0.1695	0.3892
Significant (alpha=0.05)?	No	Yes	No	Yes	No	No
Discrepancy	3.94	-4.67	22.25	-15.75	-6.22	-9.84
% Bias against spike	1.32	-1.56	7.44	-5.27	-2.08	-3.29
Bartletts Test						
P value	<0.0001					
P value summary	****					
Are SDs significantly different (P < 0.05)?	Yes					
ANOVA						
P value	0.0009					
P value summary	***					
Significant diff. among means (P < 0.05)?	Yes					
2017.08	IEC 0 IS	IEC 1 IS	IEC 2 IS	Tandem MS	RP HPLC	Other
Number of values	26	124	13	33	30	15
Mean	295.4	292.7	307.0	292.4	293.6	289.1
Std. Deviation	17.65	19.16	29.21	31.47	21.31	29.97
Std. Error of Mean	3.46	1.72	8.10	5.48	3.89	7.74
Coefficient of variation	5.98%	6.55%	9.51%	10.76%	7.26%	10.37%
P value (two tailed)	0.3063	0.0003	0.3419	0.2403	0.1772	0.2201
Significant (alpha=0.05)?	No	Yes	No	No	No	No
Discrepancy	-3.62	-6.34	8.02	-6.55	-5.38	-9.93
% Bias against spike	-1.21	-2.12	2.68	-2.19	-1.80	-3.32
Bartletts Test						
P value	0.0005					
P value summary	***					
Are SDs significantly different (P < 0.05)?	Yes					
ANOVA						
P value	0.3482					
P value summary	ns					
Significant diff. among means (P < 0.05)?	No					

2017.03	IEC 0 IS	IEC 1 IS	IEC 2 IS	Tandem MS	RP HPLC	Other
Number of values	26	120	13	30	32	11
Mean	58.46	56.61	59.62	53.43	57.07	55.26
Std. Deviation	5.703	5.435	12.81	8.076	6.122	3.414
Std. Error of Mean	1.118	0.4961	3.553	1.474	1.082	1.029
Coefficient of variation	9.76%	9.60%	21.49%	15.10%	10.73%	6.18%
P value (two tailed)	<0.0001	<0.0001	0.0201	0.0289	<0.0001	0.0005
Significant (alpha=0.05)?	Yes	Yes	Yes	Yes	Yes	Yes
Discrepancy	8.36	6.51	9.52	3.39	6.97	5.16
% Bias against spike	16.7	13.0	19.0	6.8	13.9	10.3
Bartletts Test						
P value	<0.0001					
P value summary	****					
Are SDs significantly different (P < 0.05)?	Yes					
ANOVA						
P value	0.0312					
P value summary	*					
Significant diff. among means (P < 0.05)?	Yes					
2017.06	IEC 0 IS	IEC 1 IS	IEC 2 IS	Tandem MS	RP HPLC	Other
Number of values	27	127	13	32	30	14
Mean	56.2	56.4	58.5	54.7	56.6	56.59
Std. Deviation	5.68	5.51	5.08	10.60	5.90	4.2
Std. Error of Mean	1.09	0.49	1.41	1.87	1.08	1.134
Coefficient of variation	10.11%	9.77%	8.67%	19.38%	10.43%	7.50%
P value (two tailed)	<0.0001	<0.0001	<0.0001	0.02	<0.0001	<0.0001
Significant (alpha=0.05)?	Yes	Yes	Yes	Yes	Yes	Yes
Discrepancy	6.14	6.33	8.43	4.60	6.49	6.493
% Bias against spike	12.26	12.63	16.81	9.18	12.95	12.957494
Bartletts Test						
P value	<0.0001					
P value summary	****					
Are SDs significantly different (P < 0.05)?	Yes					
ANOVA						
P value	0.5916					
P value summary	ns					
Significant diff. among means (P < 0.05)?	No					

2017.04	IEC 0 IS	IEC 1 IS	IEC 2 IS	Tandem MS	RP HPLC	Other
Number of values	28	126	13	28	32	14
Mean	154.2	152.4	160.0	145.2	148.0	152.7
Std. Deviation	15.36	15.44	21.44	15.63	10.08	12.60
Std. Error of Mean	2.90	1.38	5.95	2.95	1.78	3.37
Coefficient of variation	9.96%	10.13%	13.41%	10.76%	6.81%	8.26%
P value (two tailed)	0.1628	0.0888	0.1198	0.119	0.2695	0.442
Significant (alpha=0.05)?	No	No	No	No	No	No
Discrepancy	4.16	2.36	9.96	-4.76	-2.00	2.67
% Bias against spike	2.78	1.57	6.64	-3.17	-1.34	1.78
Bartletts Test						
P value	0.0298					
P value summary	*					
Are SDs significantly different	Yes					
ANOVA						
P value	0.0381					
P value summary	*					
Significant diff. among means (	Yes					
2017.05	IEC 0 IS	IEC 1 IS	IEC 2 IS	Tandem MS	RP HPLC	Other
Number of values	26	124	13	30	32	15
Mean	153.8	150.7	157.1	145.7	149.6	146.9
Std. Deviation	12.26	10.92	18.16	14.13	13.09	11.23
Std. Error of Mean	2.40	0.98	5.04	2.58	2.31	2.90
Coefficient of variation	7.97%	7.25%	11.56%	9.70%	8.75%	7.65%
P value (two tailed)	0.1307	0.4989	0.1818	0.1093	0.8662	0.2982
Significant (alpha=0.05)?	No	No	No	No	No	No
Discrepancy	3.76	0.67	7.14	-4.26	-0.39	-3.13
% Bias against spike	2.50	0.44	4.76	-2.84	-0.26	-2.09
Bartletts Test						
P value	0.0693					
P value summary	ns					
Are SDs significantly different	No					
ANOVA						
P value	0.0438					
P value summary	*					
Significant diff. among means (	Yes					

ARGININE SAMPLE PAIRS 2018.

2017.01	IEC 0 IS	IEC 1 IS	IEC 2 IS	Tandem MS	RP HPLC	Other
Number of values	25	123	13	42	31	16
Mean	772.8	763.7	817.7	744	763.9	733.3
Std. Deviation	66.17	51.27	68.53	128.9	67.96	149.2
Std. Error of Mean	13.23	4.622	19.01	19.89	12.21	37.29
Coefficient of variation	8.56%	6.71%	8.38%	17.33%	8.90%	20.34%
P value (two tailed)	0.0434	<0.0001	0.3978	0.0066	0.0049	0.0894
Significant (alpha=0.05)?	Yes	Yes	No	Yes	Yes	No
Discrepancy	-28.22	-37.28	16.66	-56.95	-37.11	-67.72
% Bias against spike	-3.5	-4.7	2.1	-7.1	-4.6	-8.5
Bartletts Test						
P value	<0.0001					
P value summary	****					
Are SDs significantly different (P < 0.05)?	Yes					
ANOVA						
P value	0.0661					
P value summary	ns					
Significant diff. among means (P < 0.05)?	No					
2017.07	IEC 0 IS	IEC 1 IS	IEC 2 IS	Tandem MS	RP HPLC	Other
Number of values	27	128	13	39	29	16
Mean	779.4	765.2	797.7	733.3	777.3	742.6
Std. Deviation	44.9	49.0	80.8	76.9	33.2	62.5
Std. Error of Mean	8.634	4.333	22.41	12.32	6.173	15.61
Coefficient of variation	5.76%	6.41%	10.13%	10.49%	4.28%	8.41%
P value (two tailed)	0.0189	<0.0001	0.8867	<0.0001	0.0007	0.002
Significant (alpha=0.05)?	Yes	Yes	No	Yes	Yes	Yes
Discrepancy	-21.62	-35.82	-3.262	-67.74	-23.67	-58.39
% Bias against spike	-2.7	-4.5	-0.4	-8.5	-3.0	-7.3
Bartletts Test						
P value	<0.0001					
P value summary	****					
Are SDs significantly different (P < 0.05)?	Yes					
ANOVA						
P value	0.0005					
P value summary	***					
Significant diff. among means (P < 0.05)?	Yes					

2017.02	IEC 0 IS	IEC 1 IS	IEC 2 IS	Tandem MS	RP HPLC	Other
Number of values	24	121	12	40	30	13
Mean	101.6	99.66	107.5	100.2	97.51	87.99
Std. Deviation	5.525	6.383	7.959	11.91	6.8	27.45
Std. Error of Mean	1.128	0.5802	2.298	1.884	1.242	7.613
Coefficient of variation	5.44%	6.40%	7.41%	11.89%	6.97%	31.20%
P value (two tailed)	0.5773	0.0227	0.017	0.6589	0.0088	0.1131
Significant (alpha=0.05)?	No	Yes	Yes	No	Yes	No
Discrepancy	0.6376	-1.339	6.456	-0.838	-3.488	-13.01
% Bias against spike	0.6	-1.3	6.4	-0.8	-3.5	-12.9
Bartletts Test						
P value	0.0032					
P value summary	**					
Are SDs significantly different (P < 0.05)?	Yes					
ANOVA						
P value	0.0007					
P value summary	***					
Significant diff. among means (P < 0.05)?	Yes					
2017.08	IEC 0 IS	IEC 1 IS	IEC 2 IS	Tandem MS	RP HPLC	Other
Number of values	26	125	13	43	29	16
Mean	99.4	100.3	102.2	98.9	100.1	98.3
Std. Deviation	8.05	5.87	6.38	11.33	5.36	17.33
Std. Error of Mean	1.58	0.53	1.77	1.73	0.99	4.33
Coefficient of variation	8.10%	5.86%	6.24%	11.46%	5.35%	17.62%
P value (two tailed)	0.3237	0.1642	0.5217	0.2311	0.3853	0.5462
Significant (alpha=0.05)?	No	No	No	No	No	No
Discrepancy	-1.59	-0.74	1.17	-2.10	-0.88	-2.68
% Bias against spike	-1.57	-0.73	1.16	-2.08	-0.87	-2.65
Bartletts Test						
P value	<0.0001					
P value summary	****					
Are SDs significantly different (P < 0.05)?	Yes					
ANOVA						
P value	0.7758					
P value summary	ns					
Significant diff. among means (P < 0.05)?	No					

2017.03	IEC 0 IS	IEC 1 IS	IEC 2 IS	Tandem MS	RP HPLC	Other
Number of values	27	124	13	42	32	13
Mean	245.5	243.2	262.8	239.9	248.5	241.9
Std. Deviation	21.22	17.25	37.73	33.12	19.83	18.87
Std. Error of Mean	4.085	1.55	10.46	5.111	3.505	5.234
Coefficient of variation	8.65%	7.10%	14.35%	13.81%	7.98%	7.80%
P value (two tailed)	0.1876	<0.0001	0.2797	0.0351	0.4871	0.106
Significant (alpha=0.05)?	No	Yes	No	Yes	No	No
Discrepancy	-5.53	-7.83	11.85	-11.14	-2.47	-9.15
% Bias against spike	-2.2	-3.1	4.7	-4.4	-1.0	-3.6
Bartletts Test						
P value	<0.0001					
P value summary	****					
Are SDs significantly different (P < 0.05)?	Yes					
ANOVA						
P value	0.04					
P value summary	*					
Significant diff. among means (P < 0.05)?	Yes					
2017.06	IEC 0 IS	IEC 1 IS	IEC 2 IS	Tandem MS	RP HPLC	Other
Number of values	26	128	13	41	29	17
Mean	242.4	242.5	254.5	238.1	246.2	239.6
Std. Deviation	22.48	15.28	20.33	28.17	18.45	21.0
Std. Error of Mean	4.41	1.35	5.64	4.40	3.43	5.089
Coefficient of variation	9.28%	6.30%	7.99%	11.83%	7.49%	8.76%
P value (two tailed)	0.0614	<0.0001	0.5469	0.0055	0.1749	0.0392
Significant (alpha=0.05)?	No	Yes	No	Yes	No	Yes
Discrepancy	-8.64	-8.52	3.50	-12.90	-4.77	-11.43
% Bias against spike	-3.56	-3.51	1.44	-5.32	-1.97	-4.715347
Bartletts Test						
P value	<0.0001					
P value summary	****					
Are SDs significantly different (P < 0.05)?	Yes					
ANOVA						
P value	0.006					
P value summary	**					
Significant diff. among means (P < 0.05)?	Yes					

2017.04	IEC 0 IS	IEC 1 IS	IEC 2 IS	Tandem MS	RP HPLC	Other
Number of values	28	126	13	40	32	15
Mean	26.3	26.6	27.2	27.5	26.0	28.3
Std. Deviation	5.01	2.80	2.69	3.13	4.33	7.40
Std. Error of Mean	0.95	0.25	0.75	0.49	0.76	1.91
Coefficient of variation	19.08%	10.54%	9.88%	11.37%	16.63%	26.18%
P value (two tailed)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0006
Significant (alpha=0.05)?	Yes	Yes	Yes	Yes	Yes	Yes
Discrepancy	6.45	6.76	7.40	7.69	6.22	8.45
% Bias against spike	32.56	34.12	37.35	38.84	31.39	42.69
Bartletts Test						
P value	<0.0001					
P value summary	****					
Are SDs significantly different	Yes					
ANOVA						
P value	0.2861					
P value summary	ns					
Significant diff. among means	No					

2017.05	IEC 0 IS	IEC 1 IS	IEC 2 IS	Tandem MS	RP HPLC	Other
Number of values	27	123	13	42	31	16
Mean	26.5	26.6	26.2	28.0	26.2	25.5
Std. Deviation	4.92	2.68	2.03	2.90	2.58	5.16
Std. Error of Mean	0.95	0.24	0.56	0.45	0.46	1.29
Coefficient of variation	18.60%	10.08%	7.74%	10.36%	9.86%	20.25%
P value (two tailed)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0005
Significant (alpha=0.05)?	Yes	Yes	Yes	Yes	Yes	Yes
Discrepancy	6.66	6.81	6.42	8.20	6.39	5.65
% Bias against spike	33.63	34.38	32.43	41.41	32.27	28.54
Bartletts Test						
P value	<0.0001					
P value summary	****					
Are SDs significantly different	Yes					
ANOVA						
P value	0.0597					
P value summary	ns					
Significant diff. among means	No					

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2017.01	IEC 0 IS	IEC 1 IS	IEC 2 IS	Tandem MS	RP HPLC	Other
Number of values	25	122	13	39	28	15
Mean	16.04	15.69	17.04	16.79	15.59	15.53
% Bias against spike (15.3uM)	4.9	2.5	11.4	9.7	1.9	1.5
P value (two tailed)	0.1863	0.3683	0.1694	0.0181	0.5872	0.6186
Significant (alpha=0.05)?	No	No	No	Yes	No	No
Std. Deviation	2.73	4.762	4.292	3.758	2.789	1.76
Std. Error of Mean	0.546	0.4312	1.19	0.6018	0.5272	0.4543
Coefficient of variation	17.02%	30.35%	25.19%	22.39%	17.89%	11.33%
Bartletts Test						
P value	<0.0001					
P value summary	****					
Are SDs significantly different (P < 0.05)?	Yes					
ANOVA						
P value	0.1921					
P value summary	ns					
Significant diff. among means (P < 0.05)	No					

2017.07	IEC 0 IS	IEC 1 IS	IEC 2 IS	Tandem MS	RP HPLC	Other
Mean	15.45	15.46	16.59	16.66	16.38	15.14
% Bias against spike (15.3uM)	1.0	1.0	8.4	8.9	7.0	-1.1
P value (two tailed)	0.8623	0.5733	0.3848	0.0003	0.0221	0.8303
Significant (alpha=0.05)?	No	No	No	Yes	Yes	No
Std. Deviation	4.235	3.159	4.698	2.046	2.394	2.969
Std. Error of Mean	0.8645	0.2803	1.416	0.3363	0.4446	0.7421
Coefficient of variation	27.41%	20.44%	28.32%	12.28%	14.62%	19.61%
Bartletts Test						
P value	0.2279					
P value summary	ns					
Are SDs significantly different (P < 0.05)?	No					
ANOVA						
P value	0.0007					
P value summary	***					
Significant diff. among means (P < 0.05)	Yes					

2017.02	IEC 0 IS	IEC 1 IS	IEC 2 IS	Tandem MS	RP HPLC	Other
Mean	726.2	719.3	780.7	685.4	722.4	705.7
% Bias against spike (751uM)	-3.3	-4.2	3.9	-8.7	-3.8	-6.0
P value (two tailed)	0.0281	<0.0001	0.0995	<0.0001	0.0124	0.0402
Significant (alpha=0.05)?	Yes	Yes	No	Yes	Yes	Yes
Std. Deviation	51.77	56.95	57.12	90.74	57.58	74.29
Std. Error of Mean	10.57	5.221	16.49	14.53	10.69	19.86
Coefficient of variation	7.13%	7.92%	7.32%	13.24%	7.97%	10.53%
Bartletts Test						
P value	0.0032					
P value summary	**					
Are SDs significantly different (P < 0.05)?	Yes					
ANOVA						
P value	0.0007					
P value summary	***					
Significant diff. among means (P < 0.05)?	Yes					

2017.08	IEC 0 IS	IEC 1 IS	IEC 2 IS	Tandem MS	RP HPLC	Other
Mean	719.7	722.2	740.3	702.4	711	710.9
% Bias against spike (751uM)	-4.2	-3.8	-1.4	-6.5	-5.3	-5.3
P value (two tailed)	0.0012	<0.0001	0.3837	<0.0001	<0.0001	0.1349
Significant (alpha=0.05)?	Yes	Yes	No	Yes	Yes	No
Std. Deviation	42.66	42.38	40.88	65.25	45.16	101.4
Std. Error of Mean	8.531	3.79	11.8	10.07	8.386	25.36
Coefficient of variation	5.93%	5.87%	5.52%	9.29%	6.35%	14.27%
Bartletts Test						
P value	<0.0001					
P value summary	****					
Are SDs significantly different (P < 0.05)?	Yes					
ANOVA						
P value	0.1921					
P value summary	ns					
Significant diff. among means (P < 0.05)?	No					

2017.03	IEC 0 IS	IEC 1 IS	IEC 2 IS	Tandem MS	RP HPLC	Other
Mean	1890	1861	1973	1633	1972	1908
% Bias against spike (2000uM)	-5.5	-7.0	-1.3	-18.3	-1.4	-4.6
P value (two tailed)	0.0258	<0.0001	0.6382	<0.0001	0.4022	0.1298
Significant (alpha=0.05)?	Yes	Yes	No	Yes	No	No
Std. Deviation	235.6	200.8	201.6	383.5	188.2	213.3
Std. Error of Mean	46.2	18.26	55.91	58.48	33.26	57.01
Coefficient of variation	12.46%	10.79%	10.22%	23.48%	9.54%	11.18%
Bartletts Test						
P value	<0.0001					
P value summary	****					
Are SDs significantly different (P < 0.05)?	Yes					
ANOVA						
P value	<0.0001					
P value summary	****					
Significant diff. among means (P < 0.05)?	Yes					

2017.06	IEC 0 IS	IEC 1 IS	IEC 2 IS	Tandem MS	RP HPLC	Other
Mean	1853	1837	1952	1737	1901	1900
% Bias against spike (2000uM)	-7.4	-8.2	-2.4	-13.2	-5.0	-5.0
P value (two tailed)	0.0039	<0.0001	0.3317	<0.0001	0.0045	0.2044
Significant (alpha=0.05)?	Yes	Yes	No	Yes	Yes	No
Std. Deviation	235.6	187.8	171.1	262.9	176.2	312.7
Std. Error of Mean	46.2	16.53	47.46	41.56	32.16	75.84
Coefficient of variation	12.71%	10.22%	8.77%	15.14%	9.27%	16.46%
Bartletts Test						
P value	0.0044					
P value summary	**					
Are SDs significantly different (P < 0.05)?	Yes					
ANOVA						
P value	0.006					
P value summary	**					
Significant diff. among means (P < 0.05)?	Yes					

Significant (alpha=0.05)?	No	No	No	Yes	Yes	No
Std. Deviation	24.6	17.01	20.92	29.34	22.08	20.26
Std. Error of Mean	4.733	1.515	5.802	4.582	3.904	5.064
Coefficient of variation	9.90%	6.81%	8.16%	12.41%	9.14%	8.11%
Bartletts Test						
P value	0.0005					
P value summary	***					
Are SDs significantly differen	Yes					
ANOVA						
P value	0.0057					
P value summary	**					
Significant diff. among mean	Yes					
2017.05	IEC 0 IS	IEC 1 IS	IEC 2 IS	Tandem MS	RP HPLC	Other
Mean	251.6	246.3	252	247.6	246.5	237.6
% Bias against spike (250ul	0.6	-1.5	0.8	-1.0	-1.4	-4.9
P value (two tailed)	0.6917	0.0044	0.6636	0.6209	0.3301	0.0377
Significant (alpha=0.05)?	No	Yes	No	No	No	Yes
Std. Deviation	19.56	14.16	16.06	31.22	19.67	21.71
Std. Error of Mean	3.913	1.267	4.455	4.817	3.533	5.428
Coefficient of variation	7.78%	5.75%	6.38%	12.61%	7.98%	9.14%
Bartletts Test						
P value	<0.0001					
P value summary	****					
Are SDs significantly differen	Yes					
ANOVA						
P value	0.3134					
P value summary	ns					
Significant diff. among mean	No					